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# Editorial Board

# FOREWORD

I am pleased to put into the hands of readers Volume-3; Issue-3: May-Jun 2018 of **"International Journal of Environment, Agriculture and Biotechnology (IJEAB) (ISSN: 2456-1878)",**an international journal which publishes peer reviewed quality research papers on a wide variety of topics related to **Environment, Agriculture and Biotechnology**. Looking to the keen interest shown by the authors and readers, the editorial board has decided to release issue with DOI (Digital Object Identifier) from CrossRef also, now using DOI paper of the author is available to the many libraries. This will motivate authors for quick publication of their research papers. Even with these changes our objective remains the same, that is, to encourage young researchers and academicians to think innovatively and share their research findings with others for the betterment of mankind.

I thank all the authors of the research papers for contributing their scholarly articles. Despite many challenges, the entire editorial board has worked tirelessly and helped me to bring out this issue of the journal well in time. They all deserve my heartfelt thanks.

Finally, I hope the readers will make good use of this valuable research material and continue to contribute their research finding for publication in this journal. Constructive comments and suggestions from our readers are welcome for further improvement of the quality and usefulness of the journal.

With warm regards.

Editor-in-Chief
Date: June, 2018

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# Reproductive phenology of *Carapa guianensis* Aubl. (Meliaceae) in two forest areas of the Central Amazon

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***Abstract—*** *This article presents the phenological study of Carapa guianensis Aubl species from 1974 to 2000, in ADFR and TFES forests stations research in Central Amazon, Brazil. The objective was to analyze and compare the phenological pattern (flowering and fructification) and the influence of the climatic factors. The flowering in the TFES started in a higher precipitation season; meanwhile at ADFR it was irregular. The fruiting in both areas occurred more frequently rainiest season, but in the ADFR the mature fruits were more irregular. The frequency of occurrence was annual from "flower bud" to "immature fruit" phenophases in TFES, but was over-annual only in "mature fruits". But in ADFR, was annual from "flower bud" to "anthesis" and was over-annual in immature fruit" and "mature fruit", both with irregular pattern and duration from intermediate to prolonged. The duration of the floral bud phenophase and anthesis was similar in the two areas; however, 'immature fruits" in the TFES, in general, was higher than in the ADFR. But "mature fruits" were higher in ADFR. The phenophases did not occurred at same time in all trees studied, possibly due the influence of the intraspecific genetic variability in interaction with the environment.*

***Keywords— crabwood, flowering, fruiting, raining season.***

## I. INTRODUCTION

The long-term phenological study began in 1963 in the Adolph Ducke Forest Reserve (ADFR) and in 1974 at the Tropical Forest Experiment Station (TFES), both located in the region of Manaus, Amazonas, Brazil [1, 2].

In these areas, there is a major investigation of phenological events of about 120 species of tropical forests to generate subsidies to the management and reforestation plans in the Amazon forest. This research has generated several publications on the phenology of trees in the Central Amazon, which analyze the data collected in the ADFR. The former studied the phenology of Amazonian forest species occurred in a period of seven years (1962-1968) and the latter analyzed the phenology of forest species in upland tropical rainforests in the Central Amazon [1, 2, 3, 4, 5, 6, 7, 8].

In what concerns to the population of a single species or family, studies present results on the phenology of *Aniba rosaeodora* Ducke (Lauraceae) for a period of eleven years (1968-1978) [3]. The phenology of *Copaifera multijuga* Aubl (Fabaceae) was evaluated in seven years (February 1979 to December 1985), and there in relation to weather elements [4]. Also, was analyzed the phenological behavior of *Diplotropis purpurea* Rich. (Fabaceae) in six years (1980-1985) [5]. Among the studies on families, two must be highlighted: the phenology of five species of Lecythidaceae over an eleven-years period (1978-1988) [6] and the phenology analysis of five species of Sapotaceae in one twenty-one-year period (1970-1990) [7].

The species *Carapa guianensis* Aubl. (Meliaceae), commonly known as crabwood or andiroba in Brazil, was selected for the analysis of phenological behavior, due to its economic, social and ecological importance, also abundant in the Amazon region. The trees of andiroba can reach up to thirty meters high with a cylindrical trunk, straight and buttresses at the base [8,9]. It is a species of multiple uses, having a high-quality wood, which can be used in carpentry, construction, shipbuilding, boards and plywood, furniture, beams, interior works, pencils, masts and others. Another extraordinary use is the oil extracted from its seeds, which is currently one of the most important products in the regional market. Andiroba oil is a clear and transparent liquid, that at temperatures below 25°C it solidifies as vaseline [9,11, 12].

The oil can be used in the manufacture of soaps, candles, in the composition of cosmetics and in different medicines because andiroba oil is a rich source of essential fatty acids, including oleic, palmitic, myristic and linoleic acids, and contains no fatty components such as triterpenes, tannins, and alkaloids. The bitter taste of

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the oil is attributed to a group of terpene chemicals called meliacins, which are very similar to the bitter antimalarial chemicals. Recently, one of these meliacins, called gedunin, was documented to have pest control properties and antimalarial effects equal to that of quinine. A chemical analysis of andiroba oil identified the anti-inflammatory named andirobina, which has healing and insect repelling properties that are attributed to the presence of limonoids. The interest in using andiroba oil in cosmetics has increased significantly, especially after the patenting of a cream by Yves Rocher, from France, that has moisturizing and anticellulite properties based on this oil. [13].

The phenological study of this species is essential, as it enables the determination of the regularity and predictability in the supply of this natural resource, which allows more rational use in the Amazon.

The phenological patterns would be most affected by the intrinsic characteristics (genetic, physiological and reproductive) of the species and by ecological factors (pollination, predation, competition) and not only by climate variables [7]. Researchers also report the influence of climatic elements (precipitation, solar radiation, evaporation, relative humidity) basis on phenological studies on *Copaifera multijuga* and five Sapotaceae species [4, 7].

Phenology studies were installed in ADFR in 1963 and in TFES in 1970. Since then, phenophases of flowering, fruiting and leaf change have been studied. However, the ADFR is no longer surrounded by native forests, because all the perimeter has been deforested by the urban expansion in Manaus city. While the EEST is 43 km away from the nearest town and surrounded by native forest. Consequently, the climatic conditions and the interaction with other biotic components, especially pollinators, predators and dispersers, are under different conditions and, therefore, may influence the patterns of occurrence of the phenophases studied in this work.

This study aimed to determine the patterns of flowering (flower bud and anthesis) and fruiting (mature and immature), of *C. guianensis* and compare the phenological events in order to determine whether this species has similar phenological behavior in two distinct areas of upland forest (ADFR and TFES) and if it responds to the climatic factors over time between the years from 1974 to 2000. It is important to determine the pattern of flowering and fructification of phenophases to characterize the ecological group of forest succession (climax) to which these species belong [14]. The knowledge of the phenological pattern allows more specific studies on the reproduction of the species and also to provide basic information to support the planning of silvicultural projects for species plantations, timber and oil production and for the recovery of degraded areas, since there are good growth results in experimental plantations [15,16,17]. Therefore, the hypothesis of the study is that the climatic changes affect the phenophases of the Amazonian species and impair the production of fruits, thus reducing the supply of seeds for trees' reproduction and reducing the source of food for animals, changing the forest's ecological balance.

## II. MATERIALS AND METHODS

The studies were conducted in the ADFR, located 26 km north of Manaus, on AM-010 Road, measuring 10,072 ha in an upland rainforest at 59º52'40" to 59º52'00" west longitude and 03º00'00" to 03º08'00" south latitude [18] and in the TFES, located at approximately 45 km north of Manaus, on BR-174 Road, measuring 21,000 ha, at 2º37' to 2º38' south latitude and 60º09' to 60º11' west longitude [19].

According to the Köppen classification system, local climate is designated *Afi*: *A* - tropical climate with virtually no winter, the average temperature for the coldest month is never lower than 18ºC; *f* - rains throughout the year; *i* - indicating isotherm, that is, the annual average temperature fluctuations do not reach 5ºC; there is no winter or summer [19]. Climatological data used in this study were provided by the Coordination of Research on Environmental Sciences of the National Institute of Amazonian Research (INPA) and collected at the climatological station of the ADFR for the two experimental areas, which is located approximately 30 km away from the TFES.

The Figure 1 shows annual rainfall and minimum, average and maximum temperatures from in twenty-seven years data (1974-2000).

The driest month was August with 101 mm and the month with the highest average precipitation was April with 304.34 mm. The average monthly temperature ranged from 25.5°C to 26.7ºC. The average of minimum temperatures that predominated were around 22°C. Maximum temperatures ranged from 31.3ºC to 33.4ºC at the end of the dry season, whereas the lowest values were observed in the rainy season 22,1ºC.

The frequent rainfall which extend from November to May, called rainy season, reached monthly averages over 263 mm and lower average temperatures of 25,8º C. There is also a less humid period between June and October, with less constant rainfall, but no water deficit. In this period, there was an average rainfall of 121 mm per month and higher temperatures of 32,5 º C, considered as the dry season.

The predominant vegetation in the region was classified as a tropical upland rainforest, characterized by a great diversity of tree, shrub and herbaceous species [20,21].

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The forest that covers the areas studied in this work is part of the Amazon Moist Forest [22] which is always green, as the trees never lose all the foliage, at the same time, and has a large number of tree species that are usually divided into three distinct strata.

The upper or dominant stratum is formed by large trees with DBH (diameter at breast height) greater than 1 m and height sometimes reaching 45 m or more, as happens with *Cedrelinga catenaeformis* Ducke (Mimosoideae) and *Dinizia excelsa* Ducke (Fabaceae). The intermediate stratum (vegetation layer) is composed of smaller trees, whose DBH may exceed 1 m, but their height is usually below 45 m, as happens with *Enterolobium schomburgkii* Bth (Fabaceae), *Aniba duckei* Kostermans (Lauraceae), and palm trees such as *Euterpe oleracea* Mart. (Arecaceae) and *Mauritia aculeata* H.B.K. (Arecaceae). The lower stratum consists of species that develop in heavy shade conditions, such as *Geonoma deversa* (Poit) Kunth. (Arecaceae), *Manicaria saccifera* Gaertn (Arecaceae) and other shrubs and herbaceous plants [23].

The trees of the phenological study were previously selected in the forest according to their habitat, height, DBH and stem form [1]. Five *C. guianensis* individuals were sampled from the ADFR and five from the TFES. Subjects were observed with the aid of binoculars to record the phenological phases. Monthly observations were carried out in this study. The following phenophases were analyzed: flowering and fruiting. The flowering was divided in "flower buds" (appearance) and "anthesis" (early flowering). The Fruiting, divided into "immature fruits" (appearing new fruits) and "mature fruits" (presence of ripe fruits). The analysis was done from data collected monthly to verify the frequency of events, from 1974 to 2000 [2].

Phenological patterns are described according to "frequency" - number of cycles with and without phenophases per year, "regularity" - variation in the time of occurrence and, "duration of cycles or phases" - time in months that an individual remains in a phase or cycle [23,24].

The repetitive occurrence of phenological events in the year is called "annual frequency". According to the annual frequency of flowering and fruiting, the species are classified as: "sub-annual" (more than one event per year), "annual" (one event each year) and "over-annual" (events at intervals of two years or more) [24].

The phenological data of ADFR and TFES were stored in DBASE III software and analyzed in FENOLOG, which is a software developed at the Coordination of Research on Tropical Forestry of INPA. The relationships between phenological data and climate variables was calculated by the non-parametric analysis of Spearman's correlation coefficient, considering the monthly average values of climate variables [25].

## III. RESULTS

### 3.1 – Occurrence and pattern of phenophases

#### 3.1.1 "Flower bud" phenophase

The "flower bud" phenophase of *C. guianensis* in TFES presented annual frequency, normally occurring at the beginning of the rainy season and positive correlation with the minimum temperature ($r_s$ = 0.11, p <0.05) and day length ($r_s$ = 0.24, p <0.01).

The greatest number of trees (3-4) with "flower bud" per month occurred in the years 1975 (Jan), 1976 (Dec), 1977 and 1978 (Nov), 1979 (Set), 1980 (Oct), 1983 (Jan), 1984 end 1986 (Oct), 1987 (Dec) and 1988 (Nov). In the years 1981, 1985, 1989, 1990 and 1991, there were no trees with flower buds. Only in 1992 two trees produced bud flower, but only one tree in the years 1982, 1992 until 1999 (Fig 2A).

The peaks of occurrence (three or more trees per year) were registered in 1975 (Jan and Nov), 1976 (Nov and Dec), 1977 and 1978 (Nov), 1979 (Feb, Sep and Oct), 1980 (Oct and Dec), 1983 (Jan), 1984 and 1986 (Oct), 1987 (Dec), and 1988(Nov) (Fig 2A).

The "flower bud" phenophase of *C. guianensis* in ADRF presented annual frequency occurring at the beginning of the rainy season, and positive correlation with the minimum temperature ($r_s$=0,12; p<0,05) and day length ($r_s$=0,21; p<0,01).

The greatest number of trees (3-5) with "flower bud" per month occurred in the years 1976 (Jan, Nov and Dec), 1977 (Nov and Dec), 1979 (Feb, Ago, Sep and Oct), 1989 (Nov), 1980 (Oct) But without flower bud, were observed in 1975, 1978, 1980, 1986, 1988, 1990, 1991, 1992, 1994, 1997 e 1998. Only in 1985 (Jun), 1995 (Nov and Dec) and 1999 (Sep) two trees produced bud flower, but only one tree in the years 1974 (Jul),1976 (Jan), 1979 (Aug, Sep and Oct), 1981 and 1982 (Nov), 1983 (Oct and Nov), 1984 (Feb, Oct, Nov and Dec), 1987 (Jul), 1993 (Aug), 1996 (Oct), 1999 (Oct and Nov) and 2000 (Nov) (Fig 2B).

The peaks of occurrence were registered in 1976 (Jan, Nov and Dec), 1977 (Nov and Dec), 1979 Feb, Aug, Sep an Oct), 1984 (Feb, Oct, Nov and Dec), 1989 (Nov), 1995 (Nov and Dec), 1999 (Sep, Oct and Nov) (Fig 2B).

#### 3.1.2 - Anthesis phenophase

The Anthesis of *C. guianensis* in TFES, had the tendency of usually starting during the higher precipitation season and presented a positive correlation with minimum temperature ($r_s$ = 0.14; p < 0.01).

The greatest number of trees (3-5) per month occurred the anthesis in the years 1975 (Jan and Feb), 1976 (Dec), 1977 (Nov and Dec), 1978 (Nov), 1979 (Sep

and Oct), 1980 (Oct, Nov and Dec), 1983 (Jan and Feb), 1984 and 1986 (Oct and Nov), 1988 (Jan, Nov and Dec). But without anthesis were observed in 1981, 1985, 1988, 1990 and 1991. Only in 1978 (Dec) and 1979 (Jan) two trees they were in anthesis, but only one tree in the years 1975 (Nov and Dec), 1976 (Nov), 1979 (Sep and Nov), 1982 (Sep and Oct), 1992 (Mar), 1993 (Jan, Sept and Oct), 1994, 1995 and 1997 (Oct and Nov), 1998 (Sep and Oct) and 1999 (Feb) (Fig 3A).

The peaks of occurrence were registered in 1975 (Jan, Feb, Nov and Dec), 1976, 1977 and 1978 (Nov and Dec), 19779(Jan, Feb, Sep, Oct and Nov), 1980 (Oct, Nov and Dec), 1983 (Jan and Feb), 1984 and 1986 (Oct and Nov), 1987 (Dec), 1988 (Jan, Nov and Dec), 1992 (Mar, Apr and Dec) and 1993 (Jan, Sep and Oct) (Fig 3A).

The greatest number of trees (3-5) per month in ADFR occurred the anthesis in the years 1976 (Jan and Nov), 1977 (Jan and Dec), 1978 (Jan), 1982 and 1983 (Jan), 1989 (Nov) and 2000 (Jan). But without anthesis were observed in 1974 to 1975, 1980, 1986, 1988, 1991 to 1992, 1994, 1997 to 1998. Only in 1985 (Jun), 1995 (Dec) and 1999 (Oct) two trees they were in anthesis, but only one tree in the years 1976 (Feb), 1979 (Feb, Sep, Oct, Nov and Dec), 1981, 1982, 1983 (Jan, Nov and Dec), 1984 (Feb, Mar, Oct, Nov and Dec), 1985 (Jan, Jun and Jul), 1987 (Jul and Aug), 1990 (Jan), 1993 (Ago and Sep), 1996 (Oct, Nov and Dec), 1999 (Dec) and 2000 (Nov and Dec) (Fig 3B).

The peaks of occurrence were registered in 1976 (Jan, Feb and Nov), 1977 (Jan and Dec), 1978 (Jan), 1979 (Feb, Sep, Oct, Nov and Dec), 1982 and 1983 (Jan, Nov and Dec), 1984 (Feb, Mar, Oct, Nov and Dec), 1985 (Jan, Jun and Jul), 1989 (Nov), 1996 and 1999 (Oct, Nov and Dec) and 2000 (Jan, Nov and Dec) (Fig 3B).

### 3.1.3 - Immature fruit phenophase

The production of immature fruits in the TFES presented highest annual frequency, usually occurring during rainy season (Dec, Jan and Feb) (Fig. 4A). It showed significant positive correlation with precipitation ($r_s = 0.19$; $p < 0.01$) and minimum temperature ($r_s = 0.20$; $p < 0.01$), and significant negative correlation with maximum temperature ($r_s = - 0.24$; $p < 0.01$).

The greatest number of trees (3-5) per month the immature fruit occurred in the years 1975 (Mar), 1979 (Nov and Dec), 1980 (Jan), 1981 (Jan and Feb), 1983 (Mar), 1986 (Dec), 1987 (Jan), 1988 (Feb and Mar). But without immature fruit was observed in 1985, 1990, 1991 and 2000. Only in 1975 (Apr), 1977 (Jan), 1978 and 1989 (Jan and Feb), two trees had immature fruit, but in only one tree in the years 1976 and 1978 (Dec),1982 (Nov and Dec), 1984 (Dec), 1992 (May, Jun, Jul, Aug, Sep and Oct), 1993 (Mar, Apr, May, Nov and Dec), 1994 (Dec and Jan), 1995(Jan, Feb, Mar and Dec), 1996 (Jan and Feb), 1997 (Jan, Feb, Mar, Apr, May and Dec), 1998 (Jan, Feb, Nov and Dec), 1999 (Mar) (Fig 4A.

The peaks of occurrence were registered in 1975 (Mar and Apr), 1978 (Jan, Feb and Dec), 1979 (Feb, Nov and Dec), 1980 (Jan), 1981 (Jan and Feb), 1983 (Mar), 1986 (Dec), 1987 (Jan), 1988 (Feb and Mar), 1989 (Jan and Feb), 1992 (May, Jun, Jul, Aug, Sep and Oct), 1993 (Mar, Apr, Nov and Dec), 1995 (Jan, Feb, Mar and Dec), 1997 (Jan, Feb, Mar, Apr, May and Dec) and 1998 (Jan, Feb, Nov and Dec) (Fig 4A).

In the ADFR, the phenophase "immature fruit" presented an over-annual pattern that occurred during rainy season.

The greatest number of trees (3-5) per month in ADFR occurred the "immature fruit" in the years 1974 (Jan), 1976 (Mar), 1982 (Feb) and 1983 (Mar and Apr). But without "immature fruit" was observed in 1975, from 1977 to 1981, 1986, 1988, 1989, 1991, 1992, 1994, 1995, 1996 and, from 1998 to 2000. Only in 1982 (Mar), two trees had "immature fruit", but only one tree in the years 1984 (May), 1985(Jan), 1987 (Sep, Oct), 1990 (Mar), 1993 (Out) and 1997(Jan and Feb) (Fig 4B).

The peaks of occurrence were registered in 1974 (Jan), 1976 (Mar), 1982 (Feb and Mar), 1983 (Mar and Apr) (Fig 4B).

The longest intervals were observed from 1977 to 1981, in a 5-years period, and from 1994 to 1996 as well as from 1998 to 2000, in a 3-years period (Fig. 4B).

### 3.1.4 - Mature fruit phenophase

The phenophase "mature fruits" in the TFES presented an annual pattern and happened during rainy season (Fig. 5A). In this area, the occurrence of mature fruits was considered rare. Nonetheless, it tended to occur during rainy season, and showed significant positive correlation with precipitation ($r_s = 0.22$; $p < 0.05$) and minimum temperature ($r_s = 0.11$; $p < 0.05$).

The greatest number of trees (3) per month the mature fruit occurred in the years 1983 (Mar), 1987 (Feb) and 1988 (Apr). Only in 1974 (Mar), two trees had "mature fruit". But without immature fruit was observed from 1975 to 1982, in 1984, 1986, from 1989 to 1993 and 1996. Only in one tree occurred in 1985 (Jan), 1994 (Mar), 1995 (Apr), 1997 (Jun), 1998 (Mar) and 1999 (Apr) (Fig 5A).

The peaks of occurrence were registered in 1983 (Mar), 1987 (Feb) and 1988 Apr) (Fig 5A).

The phenophase "mature fruits" in the ADFR presented annual frequency irregular (Fig. 5B). It tended to happen during rainy season and showed a significant positive correlation with precipitation ($r_s = 0,12$; $p < 0,05$).

The greatest number of trees (3-5) per month the mature fruit occurred in the years 1974 (Feb) and 1983

(May and Jun). But without mature fruit was observed in 1975, from 1977 to 1981, 1984, 1986, 1988, 1989, 1991, 1992, from 1994 to 1996, and 1998 until 2000. Only in 1976 (April), two trees had mature fruit. Only in one tree in 1982 (Apr), 1985 (Feb), 1987 (Oct and Nov), 1990 (Apr), 1993 (Nov) and 1997 (Mar, Apr, May and Jun) (Fig 5B).

The peaks of occurrence were registered in 1974 (Feb), 1983 (May, Jun) and 1997 (Mar, Apr, May, Jun) (Fig 5B).

**3.2. Duration of phenophase**

The variation of "flowering" phenophase duration (flower buds and anthesis) in *C. guianensis* in TFES and ADFR, was lower than that of "fruiting" phenophase. While "flower buds" ranged from one to three months in TFES, in ADFR it was one to four months. The "anthesis" had no differences and the variation was from one to five months. However, "immature fruits" in the TFES ranged from one to six months and in ADFR ranged from one to two months. "Mature fruits" did not change in the TFES and occurred in one month, but in the ADFR the variation was one to four months (Table 1).

The differences in phenophases duration, mainly fruiting of *C. guianensis*, between the two areas may be related to the adaptation strategies to the environment, especially herbivory by mammals and insects, since the area of the ADFR is limited by the urban expansion of Manaus city, concentrating the action of predators, whereas in TFES with larger area, it does not have physical barriers with other parts of the native tropical forest in the Manaus region.

## IV. DISCUSSION

**4.1 – Occurrence and pattern of phenophases**

The flowering (flower buds and anthesis) of *C. guianensis* in the TFES and ADFR tends to happen in October, November, December and February. Similarly, flowering of *Couepia edulis* Prance, (Chrysobalanaceae) was observed in areas of the cities of Coari and Tefé, in the State of Amazonas, between February and March (rainy season), but found no differences in the phenological pattern of both study areas [26].

For the species *Caryocar villosum* Aubl (Caryocaraceae) within the ADFR, it was observed the occurrence of flowering during July and August (dry season). The same species being observed at Curuá-Una, another Experimental Station in the State of Pará, showed bloom in September and October, and also in the dry season [1, 27].

The fruiting (immature and mature fruits) in the TFES and ADFR tended to start in the period of most precipitation (November to May), what evidences the tendency of fruit production during the rainy season in agreement with what was verified for most of the species assessed in the ADFR [2] and for species assessed in an area near Belém - Pará [28]. However, the production of mature fruits of *C. guianensis*, in both study areas, showed intervals between the occurrences, which suggest a predation tendency of the fruit before maturation. These data are in agreement with the studies carried out on the evaluation of the production of mature fruits by species of the Amazonian forest [2, 8].

Meanwhile, the flowering and fruiting of *C. guianensis*, in twenty-seven years, occurred at the maximum in four among five trees, except the fruiting in the year 1974 in the ADFR. These result shows that the species can flowering and fruiting annually, but not all adult trees of the species flower and fruit each year.

**4.2. Duration of phenophases**

In the TFES, the highest frequency of flowering and immature fruits was two months, whereas of mature fruits was one month. In the ADFR, the highest frequency of flowering was two months and of mature and immature fruits was one month.

Different authors reported similar results. Flowering intervals ranging from one to seven months for in twenty-seven species were evaluated in the ADFR, and the most frequent duration was observed in twelve years of the study, it was three months. For fruiting, the intervals ranged from one to nine months, and the most frequent duration was five months [2]. An average fruiting duration ranging from three to six months was observed in a study of five species of Lecythidaceae in ADFR [6]. The fruiting period was the phenological event with the longest duration in the populations of *Psychotria nuda* (Cham. & Schltdl.) *Wawra* (Rubiaceae) and *P. brasiliensis* Vell. (Rubiaceae), and which more than 50% of its individuals presented mature fruits during most of the fruiting, which shows a high synchrony between the two populations. [29]. A study of *Guatteria australis* St. Hill (Annonaceae) in lowlands and sandbank forests reported duration of fruiting ranging from four to five months. [30].

We also observed that the duration of the phenophase "immature fruits" of *C. guianensis* in the TFES was longer than the duration of the flowering and mature fruits, whereas in the ADFR, the total duration of fruiting was longer than the duration of the flowering. Such observations are in agreement with other studies that verified a longer duration of fruiting (seven months) than flowering (six months) in a study of *Diplotrapis purpurea* [5].

The extended fruiting period of tropical plants might as well be explained by the fact that; besides dispersion, angiosperms must also have the strategy to defend their fruits from damages caused by herbivores

[31]. Plants can reduce the exposure time of ripe fruits by remaining with their unripe fruits for several months and ripening them little by little during fruiting [32]. Andiroba fruits are a source of primary food for rodents, armadillos, wild pigs, deer, etc. [33]. The Seeds can also be predated by insects (*Hypsipyla ferrealis* Hampson (Lepidoptera: Pyralidae) [34]. The area of the ADFR, surrounded by Manaus city may have influenced the local microclimate and the interaction with plants and animals and the modifications in regularity of phenophases.

## V. CONCLUSIONS

The frequency of occurrence was annual from "flower bud" to "immature fruit" phenophases of *C. guianensis* in TFES, but was over-annual only in "mature fruits". But in ADFR, was annual from "flower bud" to "anthesis" and was over-annual in immature fruit" and "mature fruit".

The "flowering" in the TFES started in a higher precipitation season; meanwhile at ADFR it was irregular. The "mature and "immature fruits" in both areas occurred more frequently in rainiest season, but in the ADFR the "mature fruits" were more irregular.

The duration of the "flower bud" phenophase and "anthesis" were similar in areas; however, "immature fruits" in the TFES, in general, was higher than in the ADFR. But "mature fruits" were higher in ADFR.

The annual flowering and fruiting of *C. guianensis* did not occur at the same time in all trees of the species, possibly due do the influence of the great intraspecific genetic variability in native forests in interaction with the environment.

## DISCLOSURE STATEMENT

The authors declare no conflict of interest. They also declare that the founding sponsors had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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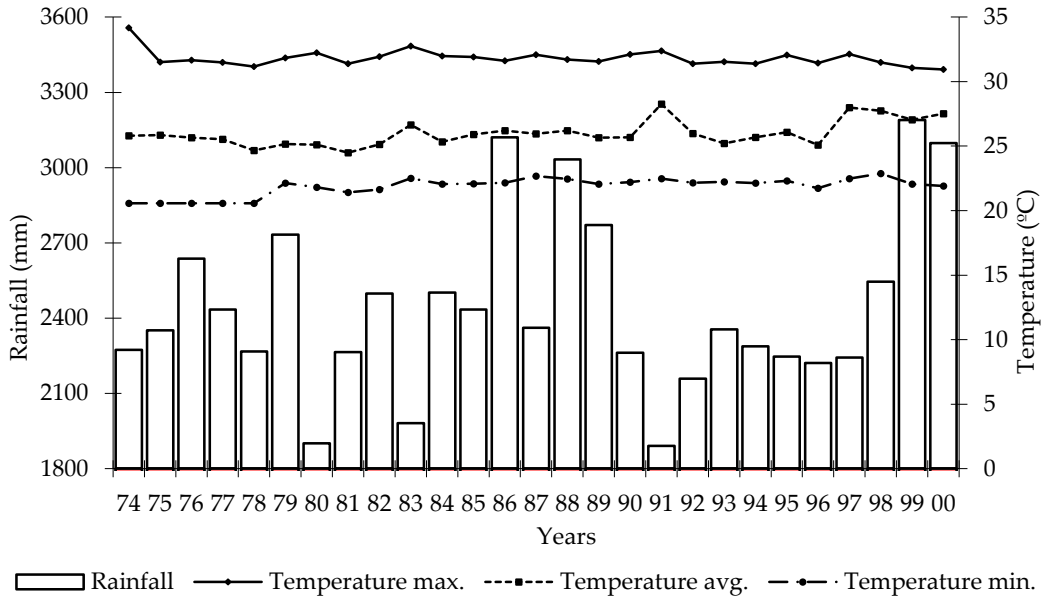


*Fig.1: Annual rainfall, minimum, average and maximum temperatures between years 1974 to 2000 in Manaus, Central Amazon Brazil.*

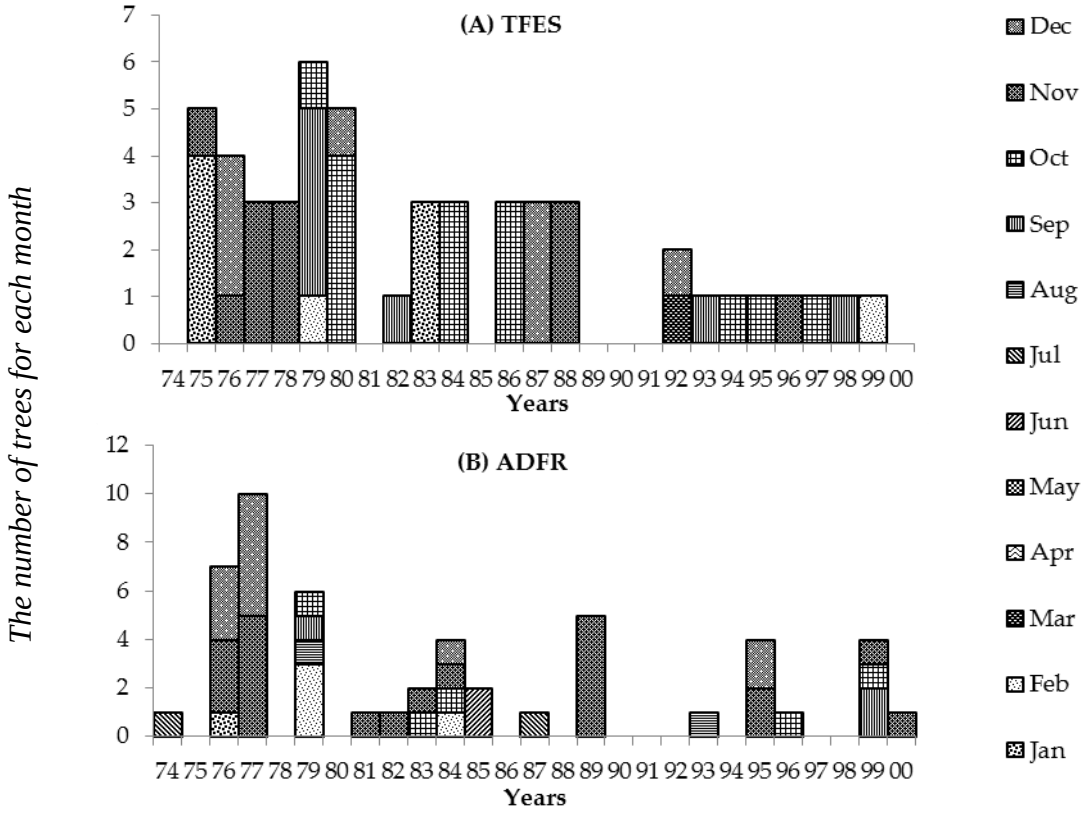


*Fig.2: Flowering pattern (Flower bud) of Carapa guianensis in number of trees with flowers buds per month, in each year of observation. (A) Tropical Forestry Experimental Station – TFES (n=5) and (B) Adolpho Ducke Forest Reserve - ADFR (n=5) belonging to the National Institute of Amazonian Research - INPA, Manaus, Central Amazon Brazil.*

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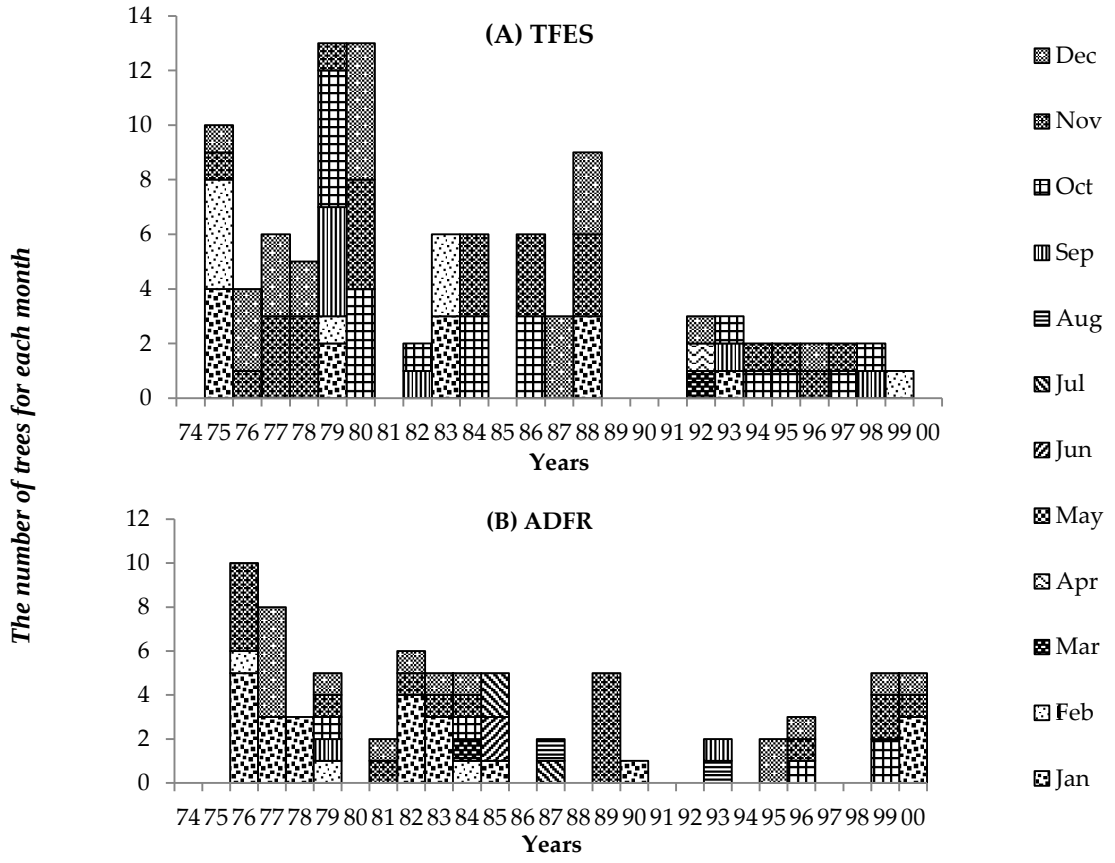


*Fig.3: Flowering pattern (anthesis) of Carapa guianensis in number of trees flowering per month, in each year of observation. (A) Tropical Forestry Experimental Station – TFES (n=5) and (B) Adolpho Ducke Forest Reserve - ADFR (n=5) belonging to the National Institute of Amazonian Research - INPA, Manaus, Central Amazon Brazil.*

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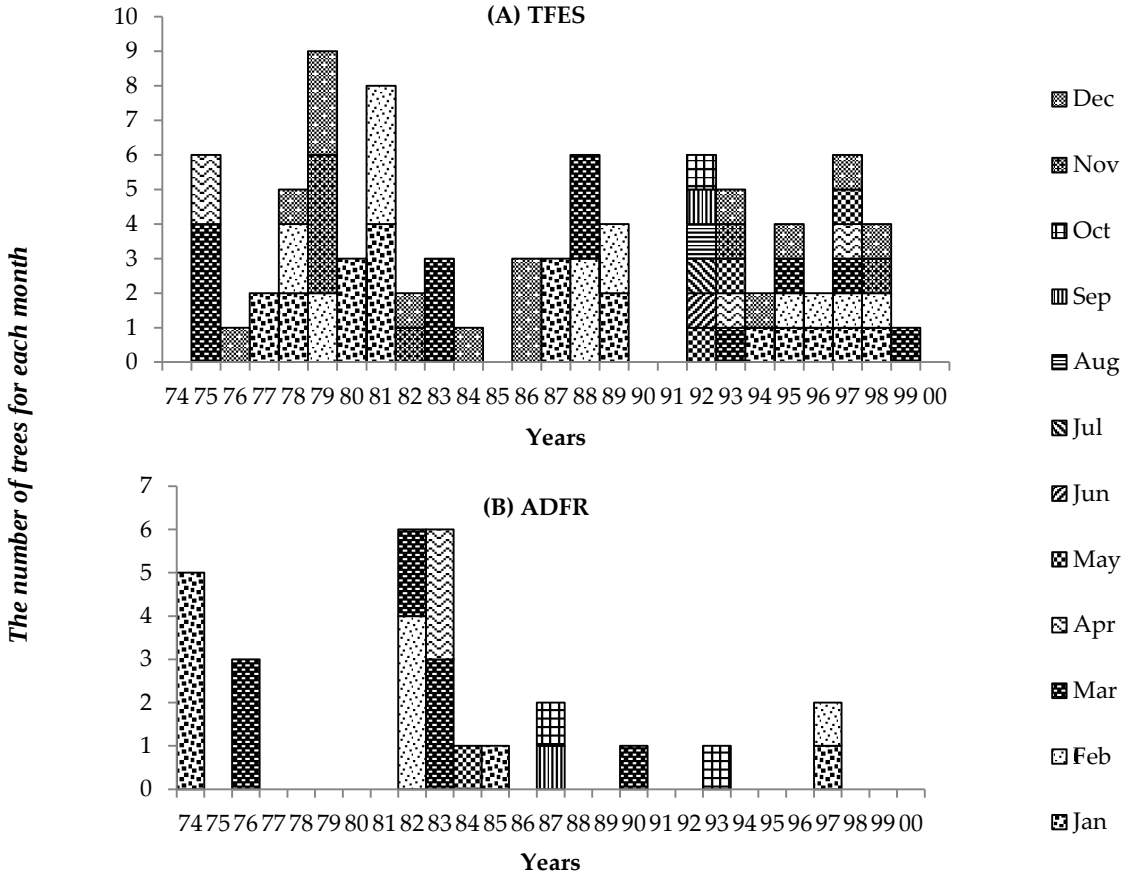


Figure 4. - Fruiting pattern (immature fruits) of *Carapa guianensis* in number of fruiting trees per month, in each year of observation. (A) Tropical Forestry Experimental Station – TFES (n=5) and (B) Adolpho Ducke Forest Reserve - ADFR (n=5) belonging to the National Institute of Amazonian Research - INPA, Manaus, Central Amazon Brazil.

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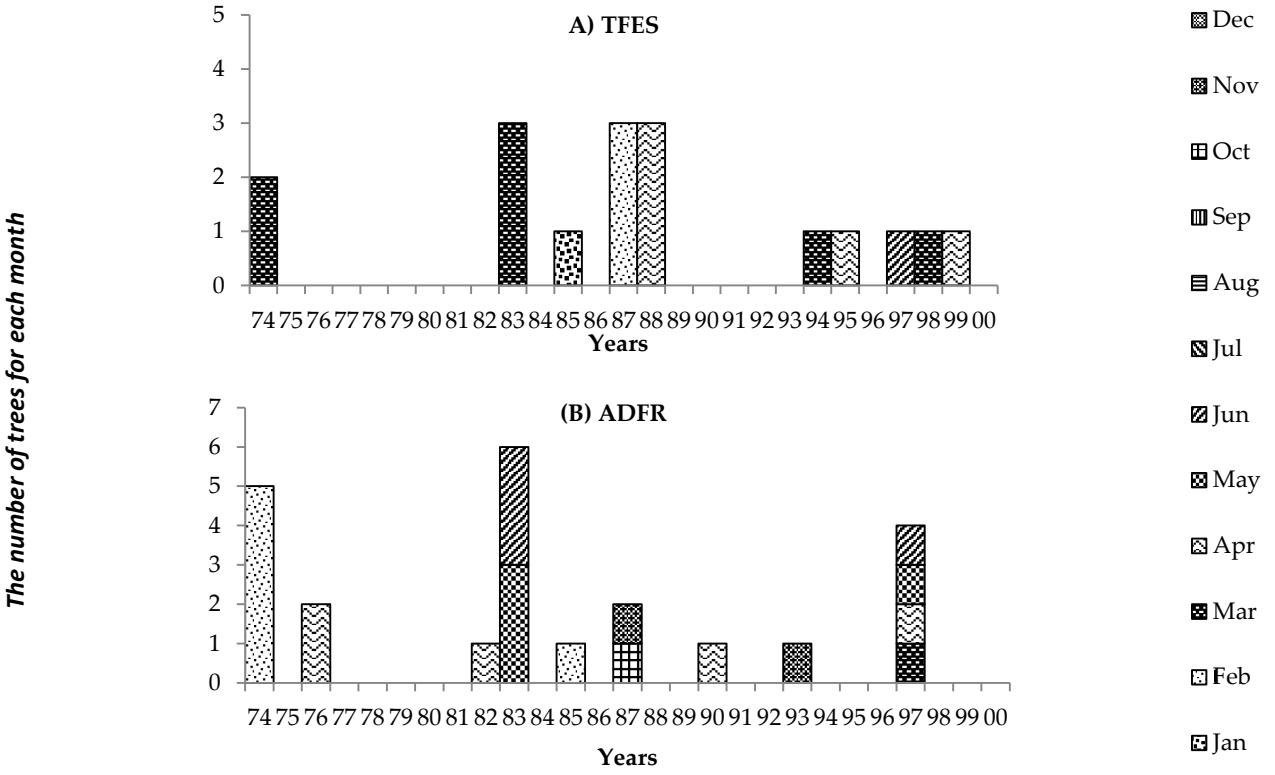


*Fig.5: Fruiting pattern (mature fruits) of Carapa guianensis in number of trees fruiting per month, in each year of observation. (A) Tropical Forestry Experimental Station – TFES (n=5) and (B) Adolpho Ducke Forest Reserve - ADFR (n=5) belonging to the National Institute of Amazonian Research - INPA, Manaus, Central Amazon Brazil.*

*Table.1: Duration (months) of Flower buds, Anthesis, Immature fruits and Mature fruits phenophases of Carapa guianensis Aubl. (Meliaceae) in Tropical Forest Experimental Station (TFES) and Adolpho Ducke Forest Research (ADFR) in Manaus, Central Amazon Brazil.*

| Local study | Phenophase | | | |
|---|---|---|---|---|
| | Flower buds | Anthesis | Immature fruits | Mature fruits |
| TFES | 1-3 | 1-5 | 1-6 | 1 |
| ADFR | 1-4 | 1-5 | 1-2 | 1-4 |

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# Assessing indicators of runoff and erosion by rain simulation in the Ben Ahmed watershed (Central Morocco)

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***Abstract—*** *The objective of this study was to investigate the risks of runoff and erosion of soils in the Ben Ahmed watershed, it's located in the region of casa-settat, 70 km south-east of Casablanca, and characterized by a semi-arid climate. The study consists of measuring on 1 $m^2$ plot, the volumes of runoff and sediments, under the influence of rainfall generation (60mm/30 min). Soil samples were collected from each plot to determine texture, organic matter and humidity. Results obtained show that the detachability varies between 19 and 34 g/l, infiltrability oscillate between 15 and 37 $mm.h^{-}$. Pearson correlation test shows that infiltration was negatively correlated with runoff and soil detachability (R=-0.99, R=-0.87 respectively). It's significantly correlated with the proportions of sand(R=0.69), silt (R= -0.98) an clay (R= 0.92), however, is weakly correlated with organic matter (R=-0.32). Infiltration and detachability were significantly correlated with humidity (R = -0.99, R = -0.63respectively).*

***Keywords— detachability, runoff, infiltration, rain simulation, Ben Ahmed watershed, central Morocco.***

# Recherche d'indicateurs de ruissellement et d'érosion au moyen de simulation de pluie dans le bassin versant de Ben Ahmed (Maroc central)

***Résumé—*** *L'objectif de ce travail est d'étudier les risques de ruissellement et d'érosion dans le bassin versant de Ben Ahmed, au moyen d'un simulateur de pluie. Le bassin se situe dans la région de Casa-Settat, à 70 km au Sud-Est de Casablanca, caractérisé par un climat de type semi-aride. L'étude consiste à mesurer sur une parcelle de $1m^2$ les volumes d'eau ruisselés et les quantités des sédiments érodés sous l'influence d'une averse générée avec une intensité érosive de 60 mm pendant 30 min. Ainsi, des échantillons de sol ont été prélevés de chaque parcelle, pour déterminer la texture, la matière organique et l'humidité. Les résultats obtenus montrent que la détachabilité varie entre 19 et 34 $g.l^{-1}$, l'infiltrabilité oscille entre 15 et 37 $mm.h^{-1}$. Le test de corrélation de Pearson montre que l'infiltration est négativement corrélée avec le coefficient de ruissellement et la détachabilité (R= - 0.99, R= -0.87 respectivement). Elle est corrélée significativement avec les proportions de sable (R= 0.69), limon (R= -0.98) et avec celle de l'argile (R= 0.92), par contre une faible corrélation est observée avec la matière organique (R=-0,32). L'infiltration et la détachabilté étaient significativement corrélées avec l'humidité (R= -0.99, R= - 0.63 respectivement).*

***Mots clé — détachabilite, ruissellement, infiltration, simulation de pluie, Ben Ahmed, Maroc central.***

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## I. INTRODUCTION

Le sol est un milieu vivant issu de l'altération physique et chimique de la roche mère sous l'action des agents climatiques (température, précipitation, humidité…) et biologiques. Il assure plusieurs fonctions écologiques (Thiombiano, 2015) : fonctions biologique, fonction de stockage et de support et fonction alimentaire.

La dégradation des terres peut résulter de la fragilité des écosystèmes des terres, qui, sous la pression humaine excessive ou des changements drastiques dans l'utilisation des terres, réduisent leur productivité et leur résilience (Turkelboom *et al.,* 2008). Pour la plupart des sols, l'érosion hydrique est le processus le plus commun entrainant la dégradation des sols. (Stocking et Niamh, 2000).

L'érosion des sols associé à la dégradation des terres sont des phénomènes spatio-temporels qui prennent de l'ampleur dans un plusieurs pays du monde (Hoyos, 2005; Pandey *et al.*, 2009 ).

L'érosion des sols en termes réels met en danger la sécurité alimentaire, la productivité subsistance du sol, la surface stockage de l'eau, la qualité de l'eau de surface, la beauté du paysage et l'équilibre écologique naturel. Sa solution réside dans l'adaptation des pratiques de conservation (Toumi, 2013).

Depuis les années trente, l'érosion des sols a reçu une attention importante des chercheurs et aménagistes. Ceci a permis de bien comprendre et de quantifier les processus de l'érosion dans différents environnements pédologiques, climatiques et culturales. En outre, un grand nombre de techniques de quantification ont été développées et adaptées à ces différents environnements.

Les méthodes utilisées dans la quantification de l'érosion varient en fonction des objectifs, des moyens et des échelles d'étude. La simulation de pluie constitue l'une des méthodes les plus fréquemment utilisée sur terrain pour déterminer, à une petite échelle correspondant à la surface élémentaire représentative d'une parcelle (cultivée ou non) et sous diverses conditions de pluie et de sol, certaines caractéristiques hydrodynamiques des sols, et mesurer le ruissellement et les pertes en sol induites. Plusieurs types de simulateur de pluie existent et peuvent arroser des surfaces allant d'un mètre carré à une cinquantaine de mètres carrés (Benkhelil *et al.,* 2004). Ces simulateurs de pluie présentent l'avantage d'être des dispositifs mobiles, d'avoir la capacité de produire des averses avec les fréquences, les intensités et les quantités de pluies semblables à des pluies naturelles ou à des évènements rares.

Notre objectif est l'étude de l'érosion et du ruissellement des sols au niveau du bassin versant de Ben Ahmed. Une étude quantitative basée sur une campagne de simulation de pluie, a été réalisée dans le bassin versant de Ben Ahmed, situé à 70 km au sud-est de Casablanca. Les expérimentations consistent à mesurer sur des sites expérimentaux les volumes d'eau ruisselés et les quantités des sédiments érodés sous l'influence d'une averse générée par un simulateur de pluie.

## II. MATERIEL ET METHODE

**Zone d'étude**

La zone d'étude se située dans la région de Casa-Settat, à 70 km au Sud-Est de Casablanca, au centre du Maroc (33°06'43''N, 7°24'21'' W), sur une superficie de 545 ha. Le climat est de type semi-aride, influencé par l'océan Atlantique, avec des hivers tempérés et des étés chauds. L'étude des séries chronologiques des précipitations fournies par l'Agence de Bassin Hydraulique de Bouregreg (ABHBC), qui couvre une période de 40 ans (1968 à 2010), nous a permis de constater que le bassins versant jouisse dans l'ensemble d'une pluviométrie moyenne pour des latitudes semi aride. De l'Ouest à l'Est, les exutoires de ce bassin reçoivent annuellement en moyenne 328 mm.

**Simulation de pluie**

L'étude des phénomènes de ruissellement et de transports solides a été menée par méthode expérimental sur terrain pour deux types de sol du bassin versant de Ben Ahmed. La méthode employée consiste à provoquer du ruissellement sur des parcelles de $1m^2$, à l'aide d'un simulateur de pluie de type ORSTOM.

Les mesures de l'intensité du ruissellement et des transports solides ainsi provoqués, correspondent à une intensité d'averse de 60 mm pendant 30 minutes.

Les éléments de la structure du simulateur sont **1/** la tête du simulateur, suspendue dans la partie supérieure de la tour et constitue le moteur de l'asperseur, **2/** un manomètre qui permet le contrôle de la pression de l'eau, **3/** une bâche pour isoler le simulateur des effets du vent ou d'une éventuelle pluie naturelle, **4/** des tuyaux d'arrivée et de sortie de l'eau et **5/** des câbles de commande de la tête (G).

Ce simulateur de pluie est constitué d'un système d'arrosage fixé au sommet d'une tour pyramidale de 2 m de haut. L'aspersion est assurée par un gicleur calibré monté sur un bras mobile. L'angle de balancement du bras permet d'ajuster l'intensité de pluie nécessaire tombant sur la parcelle d'étude.

Les variations de cet angle modifient la surface arrosée et, de ce fait, l'intensité de la pluie sur la parcelle d'un mètre carré, étudiée.

L'alimentation en eau est assurée par une motopompe. L'installation d'un manomètre dans la tour permet de régler la pression d'admission de l'eau (0,8-0,9 bar) au gicleur.

La parcelle étudiée est limitée par un cadre métallique d'un mètre carré enfoncé dans la terre jusqu'à une

profondeur de 10 cm. Un système de recueil des eaux, constitué d'une gouttière collectrice limite la parcelle à sa base et reçoit l'eau ruisselée et les sédiments. Pour chacune des micro-parcelles, une séquence de pluie érosive de 60 mm/h a été simulée pendant 30 min. Les volumes de ruissellement ont été prélevée pendant chaque minute grâce à un système gouttières installé au niveau des micro-parcelles. Les volumes des charges solides ont été prélevés durant chaque 5 min.

D'autre paramètres ont été mesurés et calculés lors de la simulation de pluie, notamment :

-Les lames ruisselées) (LR en mm);

- Les lames infiltrées, calculées comme suit: Linf=Pluie-LR (en mm);

-Les coefficients d'écoulement : Ke = (LR/pluie) ×100) (%);

-Les concentrations en sédiments de l'eau de ruissellement (Conc) (en g. $l^{-1}$);

**Echantillonnage du sol**

Des échantillons de sol ont été prélevés de chaque site expérimental à une profondeur de 0-20 cm. Ces échantillons ont été séchés à l'air libre et analysés après au laboratoire d'analyse des sols de l'INRA de Rabat pour déterminer leur teneur en matière organique, texture et l'humidité.

La distribution granulométrique a été déterminée en utilisant la méthode de pipette Robinson, alors que la teneur en matière organique a été estimée en utilisant la méthode de walkley-Black (Walkley et Black 1934).

**Tests statistiques**

Un moyen de variance a été utilisé pour déterminer la différence entre les sols étudiés (ANOVA). Les relations entre les différents paramètres du sol étaient déterminées par le test de corrélation de Pearson. Tous les tests ont été réalisés à l'aide du logiciel SPSS.

## III. RESULTATS

**Granulométrie**

L'analyse granulométrique montre qu'il y a une différence de texture des sols entre les sites expérimentaux. L'argile et le limon sont les fractions granulométriques les plus représentatives dans la couche arable sur les quatre sites. Le sable reste la fraction la moins représentée avec moins de 20 % de terre fine (Tableau. 1) . Selon les limites des classes granulométriques utilisées dans le système USDA/FAO, les sols étudiés entrent dans les classes « Argileuse » pour les deux premiers sites «Limono-Argileuse » pour le site3, Limoneuse pour le site 4.

*Tableau.1 : Résultats de l'analyse granulométrique*

| Sites | Sols | Granulométrie | | | CaCo3(%) | Texture |
|---|---|---|---|---|---|---|
| | | Argile(%) | Limon(%) | Sable(%) | | |
| 1 | Peu Evolués d'Apport | 47.1 | 36.2 | 16.7 | 2.2 | Argileuse |
| 2 | Rendzines | 47.1 | 26.1 | 26.8 | 1.4 | Argileuse Limono |
| 3 | Peu Evolués d'Apport | 41.7 | 45.1 | 13.2 | 3.5 | Argileuse |
| 4 | Rendzines | 22.6 | 61.7 | 15.7 | 4 | Limoneuse |

**Matière organique et humidité**

Les résultats de la matière organique (%) et l'humidité (%) sont présentés dans le tableau 2. Les teneurs en matière organique mesurés sur l'ensemble des échantillons du sol prélevés de chaque site expérimental, sont compris entre 1.2 % 3.3%. La teneur la plus faible a été trouvée dans les sols du site 2, tandis que le site 4 enregistre une teneur de 3% (Tableau.2). Ces résultats montrent que les sites d'expérimentation sont caractérisés par des sols pauvres à moyennement pourvus en matières organique.

Les valeurs de l'humidité varient de 31 à 44,5% et la valeur la plus élevée (44%) est observée dans les rendzines (sol du site 4), tandis que la valeur la plus faible de l'humidité caractérise le sol peu évolué d'apport (site 3). Les tests de comparaison des moyennes effectuées à travers les analyses statistiques indiquent une différence significative entre les sols pour la teneur en matière que pour l'humidité.

*Tableau.2 : Résultats de la matière organique*

| Sites | Sols | MO% | | H% | |
|---|---|---|---|---|---|
| | | M | SD | M | SD |
| 1 | Peu Evolués d'Apport | 2.9c | 0.15 | 33a | 1.00 |
| 2 | Rendzines | 1.2a | 0.10 | 38.3c | 0.57 |
| 3 | Peu Evolués d'Apport | 1.9b | 0.10 | 31b | 0.16 |
| 4 | Rendzines | 3.3d | 0.10 | 44,5d | 0.50 |

Mo matière organique (%) H humidité (%), M moyen , SD coefficient de variance

a, b, c, d : les valeurs suivies de la même lettre ne sont pas significativement différentes ($p< 0,05$)

**Infiltration et ruissellement**

Le ruissellement est mesuré pendant chaque minute et l'infiltration est calculée à partir d'eau par soustraction du ruissellement de l'eau apportée. On observe, en général, en fin d'expérience une stabilisation de cette valeur que l'on nomme infiltrabilité finale (Figure 1) et dont les valeurs observées sont très variables selon les sols (Tableau 3). Les sols les plus infiltrants sont les sols peu évolues d'apport (de 33 à 37 $mm.h^{-1}$) et les moins infiltrants sont les Rendzines ( 15 à 24 $mm.h^{-1}$).

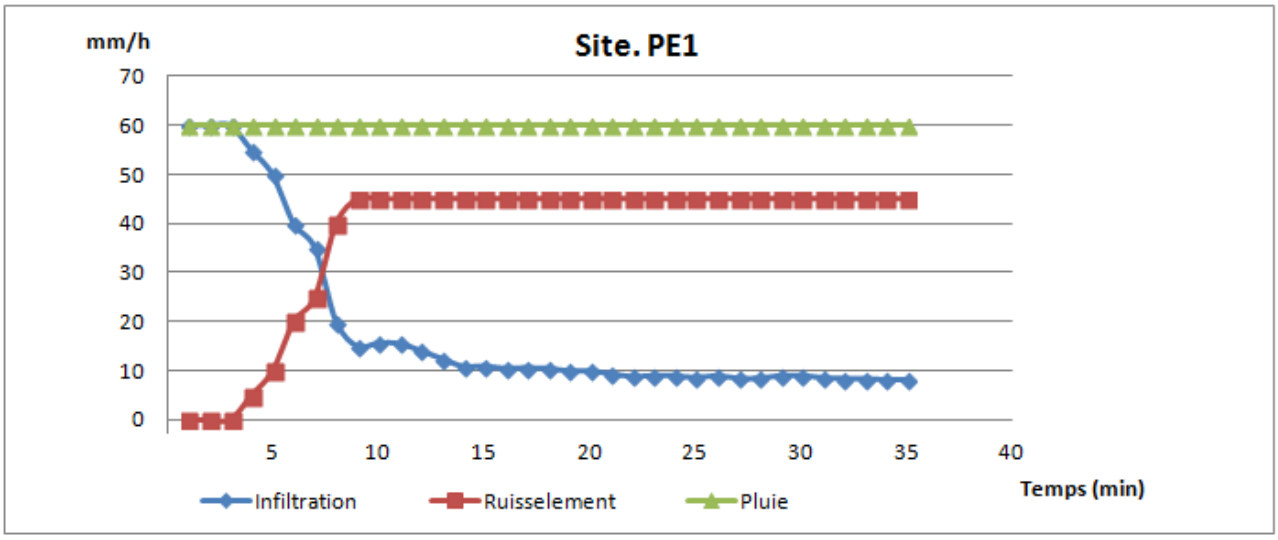


*Fig.1: Évolution du ruissellement et de l'infiltrabilité durant un test de simulation de pluie*

*Tableau.3 : Le ruissellement et l'infiltration lors de la simulation de pluie*

| Sites | Sols | LR(mm) | | Ke(%) | Inf (mm/h) | |
|---|---|---|---|---|---|---|
| | | M | SD | | M | SD |
| 1 | Peu Evolués d'Apport | 27b | 1 | 45 | 33c | 1 |
| 2 | Rendzines | 36c | 1 | 60 | 24b | 1 |
| 3 | Peu Evolués d'Apport | 22a | 1 | 37 | 37d | 1 |
| 4 | Rendzines | 43d | 0,58 | 72 | 15a | 1 |

LR : lame ruisselé Ke : coefficient d'écoulement Inf : infiltration

Ainsi, avec une pluie de 60 mm durant 30 minutes sur le sol peu évolué d'apport (site 1), le ruissellement est plus important et atteint 27 mm, indiquant que 45% de pluie se transformait en ruissellement. Par contre, la lame infiltrée est de l'ordre de 33mm. Pour les rendzines (site2) la lame ruisselée est de l'ordre de 36 mm indiquant que 60% de pluie se transformait en ruissellement. Pour le sol peu évolué d'apport (site 3), la lame ruisselée est de l'ordre de 23 mm/h, la lame infiltrée atteint 37 mm/h, ce qui implique que 38% de pluie se transformait en ruissellement. En ce qui concerne le site 4 (Rendzines), plus de 70% de pluie se transformait en ruissellement avec un débit ruisselé de 43mm/h.

**Détachalité**

La collecte des particules solides à l'exutoire de la parcelle permet de quantifier l'érosion, bien qu'on ne puisse pas parler d'érosion à l'échelle d'une parcelle d'un mètre carré. Valentin (1981), propose le terme de détachabilité et la définie comme l'aptitude d'un sol à être fractionner en particules susceptibles d'être transportées.

Le tableau 4 illustre une différence significative entre les sols des quatre sites expérimentaux, les valeurs moyennes de la détachabilité pour chaque type de sol, obtenues sur les 30 minutes du test de simulation de pluie, varient de 19 g.L-1 au niveau des sols Peu Evolués d'Apport (site 3), à 34,4g.L-1 sur les Rendzines du site 2 (Tableau 4).

*Tableau 4. Evolution de la détachabilité des sols*

| Sites | Sols | CS (g/l) | |
|---|---|---|---|
| | | M | SD |
| 1 | Peu Evolués d'Apport | 30.4d | 0.72 |
| 2 | Rendzines | 34.4b | 0.81 |
| 3 | Peu Evolués d'Apport | 19a | 1 |

| | | | |
|---|---|---|---|
| 4 | Rendzines | 27c | 1 |

CS : concentration des sédiments ($g.l^{-1}$)

**Relation entre les paramètres hydrologique et les paramètres de sol**

L'étude de la relation entre les paramètres déterminés (Tableau 5) montre que l'infiltration est négativement corrélée avec le coefficient de ruissellement et la détachabilité (R= - 0,99, R= -0.87 respectivement). La texture montre également des corrélations significatives avec l'infiltrabilité. On observe dans le tableau 5 une corrélation positive avec la proportion de sable (R= 0.69), argile (R= 0.92) et négative avec celle de limon (R= -0.98). Ces relations significatives sont dues en partie au fait que les sols observés ont des textures très contrastées et que la corrélation n'est pas masquée par d'autres facteurs.

Le coefficient de ruissellement, d'autre part, est négativement corrélé avec l'infiltration (R= -0.99) et positivement corrélé avec la détachabilité (R= 0.90).

La détachabilité était positivement corrélée avec le coefficient de ruissellement (R=0.90) et négativement corrélée avec l'infiltration (R=-0.87).

L'infiltration et la détachabilité étaient corrélés négativement avec l'humidité (R=-0.99, R= -0.63 respectivement). Par contre, le coefficient de ruissellement est corrélé positivement (R= 0.99). Ces résultats montrent que l'humidité est un facteur qui influence l'infiltration et l'apparition du ruissellement au niveau du sol dans le basin versant de ben Ahmed. Les paramètres hydrodynamiques et la matière organique étaient faiblement corrélés.

*Tableau 1. Corrélation de Person entre les paramètres hydrologiques et les paramètres de sol*

| | Paramètres de sol | If (mm/h) | Ke(%) | D ($g/m^2/h$) |
|---|---|---|---|---|
| Paramètres | If | 1 | | |
| | Kr | -0,99** | 1 | |
| | D | -0,87** | 0,90** | 1 |
| | $H_{20}$ | -0,99* * | 0,99** | -0,63* |
| Paramètre physico-chimique | MO | -0,32 | -0,34 | 0,37 |
| | A | 0, 92** | -0,47 | 0,43 |
| | S | 0, 69* | 0,58* | 0,021 |
| | L | -0,98** | 0,03 | 0,21 |

If : infiltration (mm/h) ; Ke : coefficient (%) d'écoulement ; D : détachablité ($g/m^2$) ; H : humidité (%) ; Mo : matière organique (%) ; S : sable (%) ; L : limon (%), A : argile (%).

## IV. DISCUSSION

Nos résultats montrent que la détachabilité du sol varie entre 19 et 34 g/l, ces valeurs sont faibles par apport à celles trouvées par Maïga-Yaleu (18,4 -177,1g/l) (Maïga-Yaleu *et al.*, 2015) en Sahel, et sont supérieures aux valeurs trouvées en moyen de 12g/l par Mathys (Mathys *et al.*, 2005) et par Martınez-Mena (1,29-18,09 g/l) (Martınez-Mena *et al.*, 2001) dans une zone semi-aride d'espagne.

Les tests de corrélation montrent que la détachabilité était positivement corrélée avec le coefficient de ruissellement (R=0,90) et négativement corrélée avec l'infiltration (R=-0,87). Nous remarquons aussi que l'infiltration est négativement corrélée avec le coefficient de ruissellement et la détachabilité (R= - 0,99, R= -0,87 respectivement). Nos résultats sont similaires avec ceux obtenus par Mehilo *et al.*, 2017, qui ont réalisés des tests d'infiltrabilité dans le bassin versant d'Ourika, Haut-Atlas (Maroc).

L'infiltration est déterminée principalement par la texture du sol, qui détermine correctement la perméabilité du profil en l'absence d'intervention déterminante de la structure du sol, ce qui concorde avec les résultats de Marston et Dolan (1999) obtenus à partir de simulations de pluie et par Cheggour *et al.* (2008) qui ont réalisé des tests d'infiltrabilité dans le bassin versant du Rhéraya (Haut-Atlas occidental, Maroc). En revanche, aucune relation significative n'a été observée par Sabir *et al.* (2004) qui ont réalisé aussi des tests d'infiltrabilité sur des sols peu évolués du Rif central au Maroc. Contrairement à ce qui est signalé par Sabir *et al.* (2007), l'infiltabilité est faiblement corrélée avec la matière organique (R=-0,32).

Il est intéressant de noter que l'infiltration et la détachabilté étaient également corrélées significativement avec l'humidité (R= -0,99, R= - 0, 63 respectivement). Ces résultats concordent avec ceux de Kouamé Antoine *et al.* (2015) et de Mehilo *et al.* (2017).

Les auteurs qui ont étudié l'infiltrabilité et la détachabilité en relation avec les caractéristiques du sol, ont noté que

les relations prépondérantes ne sont pas toujours les mêmes. Ces différences peuvent s'expliquer en partie par le fait que ces expériences n'ont pas toujours été faites avec les mêmes dispositifs, et donc avec un possible effet de mode opératoire. Toutefois, ces variations dans les relations observées sont aussi dues aux différences entre les milieux étudiés; chaque contexte étant l'objet de processus dont l'importance relative varie, les processus dominants masquant les autres dans leur relation aux indicateurs mesurés.

## V. CONCLUSION

Les résultats de cette étude montrent que l'infiltration permet d'identifier les facteurs responsables du ruissellement et donc de l'érosion hydrique. Grace au dispositif expérimental utilisé, les résultats obtenus sont utiles pour comprendre les risques de ruissellement et d'érosion dans le bassin versant de Ben Ahmed.

Ainsi, ces résultats montrent que l'infiltration est négativement corrélée avec le coefficient de ruissellement et la détachabilité (R= - 0,99, R= -0,87 respectivement). De la même manière elle est corrélée significativement avec l'humidité (R= -0,99) et les proportions de sable (R= 0 ,69), limon (R= -0,98) et avec celle de l'argile (R= 0,92).

Ces relations permettent une première analyse des processus dominants de l'érosion et une hiérarchisation des facteurs dans le bassin versant de Ben Ahmed.

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# Screening of sweet potato (*(Ipomea batatas [L.] Lam.)* cultivars for drought tolerance

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***Abstract***— *The effect of drought on most agricultural crops results inmany problems for the producers in Nigeria and even other parts of the world. These problems include reduced vegetative parameters and yield loss which consequently lead to reduced income for the growers of the crops. The most direct way of avoiding drought is to discover or create drought tolerant varieties of sweet potato. Sweet potato is a crop which is part of the Nigerian diet due to its perceived nutritive values. A field experiment was carried out in Bowen University, Iwo to evaluate different cultivars of sweet potato for drought tolerance. The experimental design was laid in Randomized Complete Block Design with three replicates and three treatments including the mild water stress (32 days of drought), severe water stress (from the day of drought till harvest) and nowater stress (control). Results showed that under the control treatment, the highest yield was from the Local variety 1 with 127.63 g while the lowest yield under control was from Local variety 2 with 39.20 g. Under the mild water stress, the highest yield was from Introduced variety 1 with 272.46 g while the lowest yield was from Local variety 2 with 59.66 g. Under the severe water stress, the highest yield was from Local variety 1 with 41.15 g while the lowest yield was from Introduced variety 1 with 0 g. The highest yield among the three treatment methods was under the mild water stress treatment from Introduced variety 1 with 272.46 g.Therefore, variety 3, the local variety, is recommended under severe drought based on the above reason but under moderate drought,the Introduced variety i.e. variety 1 (orange fleshed sweet potato) is preferred because it had the highest yield and is also of high nutrient content compared to the other varieties.*

***Keywords— drought, field experiment, sweet potato, tolerance.***

## I. INTRODUCTION

Sweet potato (*Ipomea batatas* [L.] Lam.) is an economically important crop in the world and particularly in Nigeria. Sweet potato occupies the position of seventh most important crop in terms of global production and in developing countries it ranks third in value of production and fifth in caloric contribution to the human diet [1]. Uganda, Nigeria, Tanzania, Angola, Burundi, Mozambique, Madagascar, Rwanda and Ethiopia, China, Indonesia, Viet Nam, India, USA and Japan are the top 15 sweet potato producers in the world [2]. It contributes significantly to the agricultural production of Sub Saharan Africa countries with roughly 3.2 million hectares and a production estimated at 13.4 million tons of tubers in 2005 [3]. A lot of root tubers are harvested per unit area and per unit time during relatively short periods of rain, meaning that it can withstand occasional drought, and is much more productive in less fertile soil than crops such as maize [4]. Sweet potato is considered as one of the major sources of food, animal feed and industrial raw materials. It has a significant contribution as an energy supplement and a phytochemical source of nutrition. It provides strong nutrients and ultimately good health to those who eat it. It possesses anti-carcinogenic and cardiovascular disease preventing properties [5].

Sweet potato is one of the main foods cultivated and consumed by most Nigerians.It is not too difficult to grow and is of great potential industrially and economically and due to its significance and importance, sweet potato is increasing in Nigeria's agriculture and food systems [6]. According to the survey conducted in six States in Nigeria by [7], the different forms of sweet potato utilization are boiling and eating with stew/palm oil, slicing and frying, roasting, boiling and eating as snack; boiling and pounding alone or with boiled yam/garri for eating with soup; cooking alone or with another crop to make pottage; slicing and sun-drying for milling into flour; feeding of vines and leaves to livestock; small tuberous roots as livestock feed; made into fufu like cassava; fresh leaves and young shoots consumed as vegetable. Also, in some African countries like Kenya, the storage roots are boiled and eaten, or chipped, dried and milled into flour which is then used to prepare snacks and baby weaning foods [8].

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Sweet potato is considered as one of the major sources of food, animal feed and industrial raw materials. It has a significant contribution as energy supplement and phytochemical source of nutrition. It provides strong nutrients and thereby good health to those who eats it and possesses anti-carcinogenic and cardiovascular disease preventing properties [9]. Sweet potato varieties are outstanding source of vitamin C, B2, B6 and E, as well as dietary fiber, potassium, copper, manganese and iron, and are low in fat and cholesterol. The root parts of sweet potato contain 25-30% carbohydrates and 2.5-7.5% protein. In addition to this, it also supplies 200-300 mg 100 $g^{-1}$ of potassium, 0.8 mg 100 $g^{-1}$ of iron (Fe), 11 mg 100 $g^{-1}$ of calcium (Ca) and 20-30 mg 100$g^{-1}$ of vitamin C of its dry matter [10]. Industrially, Sweet potato yield starch, natural colorants, and fermented products such asbutanol, acetone, ethanol, wine, and lactic acid [11,12]. Leaves, stems, roots of sweet potato serve as livestock feed [13]. Leaf protein content of sweet potato contains twice that from the storage roots[14].

In spite ofthe high nutritious and economic potential of sweet potato, it faces with a lot of challenges and abiotic and biotic constraints such as drought, low soil nutrients, weeds, pests, diseases, lack of post-harvest storage facilities and improved varieties [15,16]. With climate change whose signs are already visible, agricultural production is facing alarming threats which can lead to serious problems of food insecurity [17] and unprecedented extreme hunger. Moreover, [18] reported that, Africa and especially West Africa will be seriously affected by the deleterious effects of climate change. The variability of climate change and the prevalence of extreme events, including drought, are a harsh reality for small farmers in Africa and in Nigeria who depend exclusively on rain-fed agriculture. Over the last decade, environmental stresses have become more frequent and are exacerbated by a rapid change in climate. It constitutes perhaps the most momentous environmental challenge of our time and poses serious threats to sustainable development worldwide and chiefly in most developing countries [19]. It has been estimated that drought is the most important environmental stresses and represents 70% of yield losses of cereal crops worldwide [20]. In addition, drought is regarded as environmental factors that leads to about 75% yield loss each year in the world [19]. The 2011 Texas drought has caused a record $5.2 billion in farming losses, for example, making it the most costly drought on record [21]. Among different abiotic stresses, drought is by far the most complex and devastating worldwide [22].

It has been demonstrated that sweet potato crop is sensitive to water shortage in the course of establishment, vine development and storage initiation[23]. [24] also reported that the water scarcity during critical periods of growth leads to irreparable consequences on yield. According to [25] drought is the chief production limitation of sweet potato in the areas where agriculture mainly dependents on rainfall. [26] revealed that water stress in sweet potato reduces vegetative and yield parameters in terms of quantity and quality. A variety is considered as drought resistant when it can produce high yield under water stress [27].[28]showed that the yield of most crops has been used as indicator for drought tolerance. Henceforth, sweet potato varieties tolerant to water stress should be able to produce more quantity and quality yields under drought conditions. This could be discovered only through screening of sweet potato genotypes under managed water stress conditions [29]. Thus, identification of cultivar performance under drought conditions is thus considered to be of vital importance. Therefore, the aim of this study is to improve stability and increase production of sweet potato in Nigeria through the development of drought tolerant cultivars. More specifically, the objectives are to (1) Evaluate sweet potato cultivars for drought tolerance under field conditions and (2) identify sweet potato cultivars withhigh yield and high quality.

## II. MATERIALS AND METHODS

### Description of the experimental site

The field experiment was carried out on sweet potato at Bowen University Teaching and Research Farm Iwo, Osun State, Nigeria. Iwo is a City in Osun State, Nigeria. The City formerly part of old Oyo State was later separated and became one of the major townships in Osun State, Nigeria. It has a latitude of 7° 38' 6.97" N and a longitude of 4° 10' 53.62" E. Rainfall and temperatures data were recorded daily from the date of planting till harvest.

### Plant material

The material used in this study consisted of four (4) sweet potato cultivars. Two sweet potato cultivars (local variety 1 and 2) were obtained from Iwo farmers and the two other cultivars were newly introduced (introduced variety1 and 2). The introduced variety 1 is orange-fleshed cultivar which has been recognized as good sources of β-carotene, a precursor of vitamin A.

### Experimental design and water stress

The soil was prepared, ploughed, harrowed and ridged. A Randomized Complete Block Design was used for the drought experiment. The experimental block unit was 10m by 2m with twelve beds. Each bed in a block measured 2m and the space between rows was 90cm and the space within a row was 30cm. There were three experimental blocks in total with 36 beds for the experiment and four cultivars of sweet potato. Sweet potato vines were cut to 30cm long each and planted on the 30$^{th}$ of November,

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2016 at the rate of six (6) vines per experimental unit with a depth of 15cm at a spacing of 30cm. The soil was thoroughly watered before planting.

For the firm establishment, sweet potato plants were watered daily in the evenings for about a month and 11 days i.e. from 1st of December 2016 to 11th of January 2017.

From January 12th 2017, there was imposition of water stress i.e. no watering of treatment 1 and treatment 2 while the treatment zero which served as control was watered daily in the evening until harvest. T1 was the mild drought stress and T2 was the severe moisture stress (no water was applied till harvest though there was some rainfalls toward the end of the experiment). In the mild moisture stress, drought was imposed for about a month and 6 days that is from January 12th to February 17th 2017. On the evening of February 17th, the watering of only T1 (mild drought stress) resumed again, therefore T0 (Control) and T1 (mild-drought stress) were the only treatments being watered till the date of harvest which was the 10th of April, 2017.

**Measurement of vegetative and reproductive parameters**

Data were collected on the following parameters;

- Vine length- The length of two most vigorous vines were taken using a measuring tape. The length was measured from the point of soil contact to the apical tip. The vines were straightened so as to get accurate reading.
- Petiole length- the stalk of the leaf was measured from the base of the leaf to the point of attachment to the stem.
- Leaf length- The length was measured from the tip of the leaf to the base or bottom of the leaf
- Leaf breadth- This was the measurement of the width of the leaf. The widest part of the bottom was measured from side to side.
- Internode length- This was obtained by measuring the distance between the nodes of the vines.
- Plant height- This was measured with a carpenters measuring tape, done by putting the tape on the ground and elongating the tape to check the height without straightening of the vine.
- Fresh weight of the vines per plant: it is the weight of above ground biomass before drying in the oven using a scale
- Dry weight of the vines after drying in the oven set at $85^0$C for 4 days was also taken using a scale
- Fresh weight of the roots harvested: it is the weight of all storage roots at harvest per plant
- Dry weight of the root: it is weight recorded with a weighing balance after drying in the oven
- Total fresh weight (total yield): It is the total weight of storage roots
- Leaf tissue is most commonly used for RWC3 determination, measured as follows. A sample of leaf tissue was taken and the fresh weight was immediately determined, followed by flotation on distill water for up to 4 hours according to methods of Smart and Bingham (1974). The turgid weight was then recorded after the 4 hours, and the leaf tissue was subsequently oven-dried to a constant weight at about $75^0$ C for 48 hours. RWC was calculated by following formula:

$$RWC\ (\%) = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Turgid weight} - \text{dry weight}} \times 100$$

**Statistical analysis**

All data recorded were subjected to statistical analysis using "R" software to identify significant difference among the sweet potato cultivars used under the three treatments. ANOVA was performed for the assessment of the variation at 0.05 level of probability using Newman-Keuls Multiple Comparison-PostHOC test. In addition, Pearson correlation coefficient between traits measured was computed.

## III. RESULTS

The temperature of Iwo in Osun state was recorded daily from the 12th of January till 10th of April as shown in Figure 1.From January to March, the period was very hot without recording any single rainfall.

**Plant height**

The mean plant height readings under the non-stress treatment are presented in Figure 2. The means for control ranges from 21.3 to 29.9 cm. The lowest 21.3 cm was recorded in variety 1 (introduced variety 1) and the highest 29.9 cm was from variety 2. Under the mild water stress, the readings vary between 20.67 and 29.4 cm. The lowest 20.67 cm was obtained from variety 4 (Local variety 2) and the highest 29.4 cm was from variety 2 (introduced variety 2). Under the severe water stress, the values range between 16.1 and 25.1. The lowest was 16.1 which was of variety 3 (Local variety 1) and the highest was 25.1 which was variety 2 (introduced variety 2). Overall, the mean plant height values range between 16.1 and 29.9 cm. The lowest value was under the severe water stress while the highest value was under the control treatment. There was no significant difference between the values obtained from the control and the mild water stress, but in the severe water stress there was a significant difference as there was a reduction in the mean values. Except in the case of variety 2 (introduced variety 2) which had a value of 25.1 cm.

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**Leaf width and length**

Table 1 below shows the mean leaf width and length under non stress, mild water stress and severe water stress conditions. The mean leaf width values under the control ranges from 6.95 to 9.5 cm. The lowest value 6.95 cm was recorded in variety 4 (Local variety 2) and the highest was 9.5 cm from variety 3 (Local variety 1). Under the mild water stress conditions, the values range between 6.67 and 8.75 cm; the lowest was 6.67 from variety 4 (Local variety 2) and the highest was 8.75 cm which is variety 3 (Local variety 1). Under the severe water stress, the values vary from 6.35 to 8.37 cm. The lowest was 6.35 observed in variety 4 (Local variety 2) and the highest was 8.37 cm variety 3 (Local variety 1). In general, there was a slight decrease in leaf width under drought stress.

There was no significant difference amongst treatments but significant differences was observed between varieties as shown by ANOVA. The mean leaf length values under the control ranges from 8.1 to 11.6 cm, the lowest value was 8.1cm for Local variety 2 and the highest value was 11.6 cm obtained from Local variety 1. Under the mild water stress, the values range from 8.33 to 11.15 cm, the lowest value 8.33 was of Local 2 and the highest value 11.15 was from Local 1. Under the severe water stress, the values vary between 8.13 and 10.92 cm, the lowest value 8.13 was from Local2 and the highest value 10.92 cm was of Local 1. Overall, between the three treatments the mean leaf length values ranges between 8.1-11.6 cm the lowest value 8.1 is under the control treatment and the highest value 11.23 was also under the control treatment.

**Internode and vine length performance**

The Table 2 below shows the Mean Internode and vine length performance under non stress, mild water stress and severe water stress conditions. The mean internode values under the control treatment ranges between 4.13 and 7.17 cm, the lowest value 4.13 was from Local variety1 and the highest value 7.17 cm was from Introduced variety 1. Under mild water stress, the mean values varies from 3.88 to 6.70 cm, the lowest value 3.88 was recorded in Local variety 1and the highest value 6.70 was obtained from Introduced variety 1. Under severe water stress the mean values range between 3.35 and 4.60 cm, the lowest value 3.35 was from Introduced variety 2 and the highest value 4.60 was from Local variety 2. In general, the mean value between the three treatments ranges from 3.35 to 7.17 cm, the lowest value 3.35 was recorded under severe moisture stress while the highest value 7.17 was under the control treatment. There was significant difference in the values between varieties.

The mean vine length under non stress, mild water stress and severe water stress conditions are presented in Table 5. There was significant differences ($P< 0.05$) both among the four varieties and the three treatments. Local variety 2 and introduced variety 2 did better under drought compared to the other two varieties.

**Petiole length**

The Figure 3 below shows the Mean petiole length under non stress, mild water stress and severe water stress conditions The Figure reveals that as the moisture stress increases, there is a decrease in petiole length. The mean value between the three treatments ranges from 6.97 to13.2 cm, the lowest value 6.97 cm was under the severe water stress and the highest value 13.2 was under the control.

**Plant fresh weight**

There were significant differences ($P< 0.01$) between varieties and treatments (Table 3) and as shown in ANOVA table in appendix 4. It was observed that as the period of drought increases, there was decrease in plant fresh weight. Under the moderate water stress the mean values ranges between 300 and 733.33 g, the lowest value 300 was obtained from the Introduced variety 1 and the highest value 733.33g was recorded in Introduced variety 2. Under the severe water stress, the mean values vary from 233.33 to 633.33 g, the lowest value 233.33 was for Introduced variety 1 and the highest value 633.33 was for Introduced variety 2.

The table below shows the Mean plant dry weight under no water stress, mild water stress and severe water stress conditions. The average value of dry weight decrease in all the drought treatments except in the introduced variety 2 between the control and the moderate moisture control. Under the severe water stress, the mean values ranges from 50 to 133.33.g, the lowest value 50 was recorded in the Introduced variety 1 and the highest value 133.33 was obtained from the Local 2.

**Effect of drought of sweet potato yield (total root fresh weight) and dry weight**

The results of fresh weight is shown in Table 4. No fresh weight was recorded for the introduced variety 1 under severe drought. These results of this table also reveal that there is increase in the yield of moderate moisture stress compared to the control for the introduced variety 1 and introduced variety 2 and the local variety 2. Though the fresh weights of these three varieties significantly reduced at severe drought stress. Under severe moisture stress, local variety 1 performed better (189.00 g) than others followed by local variety 2 and the introduced variety 2.

There was significant difference between the total dry weights under no drought stress, the lowest value 32.63 was of the introduced variety 1 and the highest value 215.13 was obtained from Local 1. Under mild water stress, the values range between 70.83 and 128.50, the lowest value 70.83g was recorded from introduced variety 1 and the highest value 128.50 was from Local 1. There

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was no significant difference between introduced variety 1 and Local 2 and there was also no significant difference between introduced variety 2 and Local 1. Under severe water stress, the values range between 0g and 54.47g.

**Effect of drought on sweet potato yield per plant (fresh weight) and dry weight**

The introduced variety 1 performed much better than other in term of fresh root weight per plant and was highly significant than the others. Though no fresh weight was obtained under severe stress. As observed with the total fresh weight under severe stress, local variety 1 performed better than others followed by local variety 2 and the introduced variety 2. The table below shows the effect of drought of sweet potato yield per plant (fresh weight) under control, mild water stress and severe water stress conditions.

**Effect of drought on Relative water content**

The analysis of table 6 reveals that in the four cultivars used, it was observed as the drought period increases the relative water content decreases. But this decline in relative water content was not pronounced in variety 3 and variety 4. Though the ANOVA that there was no significant different amongst different treatment.

**Relationship between eleven traits related to drought tolerance in sweet potato**

Table 7 shows the correlation coefficient of the morphological and yield parameters measured. Total fresh root weight (total yield) and total dry root weight were significantly and positively correlated to the following traits: leaf length ($r = 0.34$, $P < 0.05$), and plant height ($r = 0.35$, $P < 0.05$) but negatively correlated with vine length ($r= 0.15$, $P> 0.05$). Fresh root weight per plant and dry root weight were positively and significantly correlated with Total fresh root weight ($r= 0.70$, $P<0.01$) total dry root weight ($r = 0.60$, $P < 0.01$), and internode length ($r= 0.40$, $P< 0.05$) while there were positive and not significant correlation with vine length, petiole length, leaf length, leaf width and plant height.Leaf length was positively and significantly correlated with leaf width ($r= 0.60$, $P< 0.01$)

## IV. DISCUSSION

As the effect of climate change get exacerbated and water resources become more restrictive for agricultural uses, the creation of drought-tolerant cultivars isof paramount importance[30]. Henceforth, part of the objectives of this study was to identify sweet potato cultivars that could be less affected by drought stressthat is with water use efficiency and without a significant loss of fresh roots i.e. yield and without losing the merchant quality and nutrition.

As shown in Figure 1 the temperature ranges between $30^0$C and $38^0$C and occasionally $40^0$C between the month of January and April. The relative humidity (data not shown) was not high an indication of drier air which could have led to high evapo-transpiration and as a result this could affect the availability of water in soil for crop production. Therefore, the period of this study was characterized by scorch sunlight, drier air and significant evapo-transpiration.

There was no significant difference between the values obtained from the control and the mild water stress though slight differences were noted, but in the severe water stress there was significant differences as there was a reduction in the mean values of aboveground and underground parameters. This results are similar to [31] who reported that significant differences in aboveground biomass amongst genotypes were observed, which indicates that genotypes differed significantly in their tolerance to drought conditions. For instance, the effect of drought on plant height of the four varieties used decreased across different moisture conditions. But introduced variety 2 and local variety 2 did better compared to the other 2 varieties.

No significant difference across the three treatments i.e. the control, mild water stress and the severe water stress was observed. This could be explained by the fact that water stress did not significantly affect leaf width. Therefore it can be hypothesized that the higher leaf width of variety 1 and variety 3 could help in sunlight interception for better photosynthesis and thus to high dry matter production. Meanwhile, there was a significant difference in leaf width between the four varieties, indicating that sweet potato varieties respond different to water stress.

There were no significant differences between the leaf length, internode length and petiole length values recorded in control, moderate or mild water stress and severe moisture stress, though a slight differences were observed at severe drought level. This illustrates that drought did affect the three vegetative parameters but there were not significantly affected. [32] reported that biomass and morphological parameters such a main stem length, internode diameter and length, leaf area and number decreased in response to drought stress. Moreover, the study of [33] carried out in South Africa revealed that the internode diameter was reduced by 12% to 50% across the sweet potato accessions used.

The vine length revealed a decrease in vine length especially under the severity of water stress. Under moderate water stress the lowest vine length value was 52.83 from Introduced variety 2 the highest value was 125.92 cm for the Introduced variety 1. Under the severe water stress, the lowest value was 38.3 of the Introduced variety 2 and the highest value was 109.13 of Local 2. This demonstrates that Local variety 2 performed better under

severe moisture stress when compared to other varieties. Our results are consistent with those of [32] and [33] observed that the reduction in stem length (relative to the control) of 15 accessions exposed to drought stress varied considerably from 16.1% to 46.0%.

Highly significant and positive correlations were observed between the 11 characters studied under drought conditions. Table 7 shows total yield and yield per plant, the ultimate indicator for abiotic tolerance, was positively and significantly correlated with leaf length ($r = 0.34$, $P < 0.05$), and plant height ($r = 0.35$, $P < 0.05$) and positively correlated with plant weight, leaf width, petiole length, and internode length. This illustrates the importance of these parameters in breeding program for drought tolerance.

The results obtained from the plant fresh weight indicated that the introduced variety 2 was higher than other varieties which indicates that the varieties responded differently and some are more sensitive than other under drought stress. The results from plant dry weight illustrate that Local variety 2 and introduced variety 2 accumulated more dry matter than the other varieties. The reduction in plant fresh weight and plant dry weight obtained in this study is consistent with those of [33] and [32].

Under moderate drought stress all the four varieties performed well, indicating that they can only tolerate mild stress. Under severe drought, variety 1 did not produce any tubers. This indicates that this variety was more sensitive than other varieties under severe drought. Therefore, variety 1 can only cope under moderate moisture stress. This study is similar to [34] who reported that water stress sensitiveness of Orange-Fleshed Sweet Potato is considered as one of the major drawbacks of this crop type and currently available varieties do not allow sustainable and enduring production in drought prone regions. Variety 3 performed better in term of yield than other varieties under severe drought. [35] and [33] indicated that storage root drymass is correlated positively with vegetative growth. Similarly, [36] reported a reduction in root dry mass under stress conditions.

The accumulation of dry matter four all the four varieties was excellent under moderate drought stress. Under severe drought, the highest root dry matter was recorded in variety 3 followed by introduced variety 2 and variety 4 under severe moisture stress. [23] reported a reduction in root dry mass under water stress condition. The variation in dry matter content can also be dependent on various factors such as soil type, pest, diseases, cultivar and climate [36].

## V. CONCLUSION

Overall, taking all these above data into consideration and looking at the ones which are least affected by drought to most of the factors variety 1 was least affected by drought on total dry weight under moderate drought stress, while variety 2 was least affected by drought on, plant height, petiole length and plant fresh weight. Variety 3 was least affected by drought on, leaf width, leaf length, tuber fresh weight (total yield), sweet potato yield per plant (fresh weight/plant) and dry weight. Variety 4 was least affected on internode, vine length, and plant dry weight. Variety 3, the local variety, is recommended under severe drought based on the above reason but under moderate drought Introduced variety i.e. variety 1 (orange fleshed sweet potato) is preferred based on the fact that it had the highest yield and also is of high nutrient content compared to other varieties.

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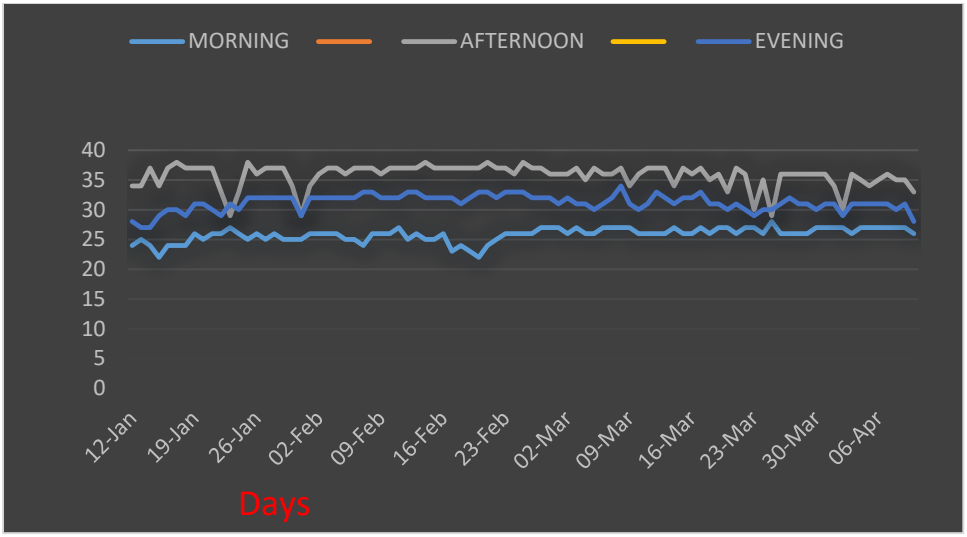


*Fig.1: Daily temperature in Iwo in the course the experiment*

*Table.1: Mean leaf width and length under non-stress, mild water stress and severe water stress (Average ± Standard deviation)*

| | Leaf width | | | Leaf width | | |
|---|---|---|---|---|---|---|
| Varieties | Control | Mild water stress | Severe water stress | Control | Mild water stress | Severe water stress |
| Variety 1 | 9.22 ±0.45 | 8.6 ±0.49 | 8.1 ±1.26 | 9.77 ±0.97 | 9.65 ±0.55 | 8.83 ±0.82 |
| Variety 2 | 7.63 ±0.46 | 6.82 ±0.33 | 6.62 ±1.01 | 11.2 ±0.28 | 11.02 ±0.51 | 10.6 ±0.33 |
| Variety 3 | 9.5 ±1.43 | 8.75 ±0.66 | 8.37 ±2.15 | 11.6 ±0.13 | 11.15±1.19 | 10.9 ±3.03 |
| Variety 4 | 6.95 ±0.63 | 6.67 ±0.63 | 6.35 ±0.28 | 8.1 ±0.75 | 8.33±0.42 | 8.13 ± 0.17 |

Different letters in the same column show significant difference at 0.05 probability level for vine length.

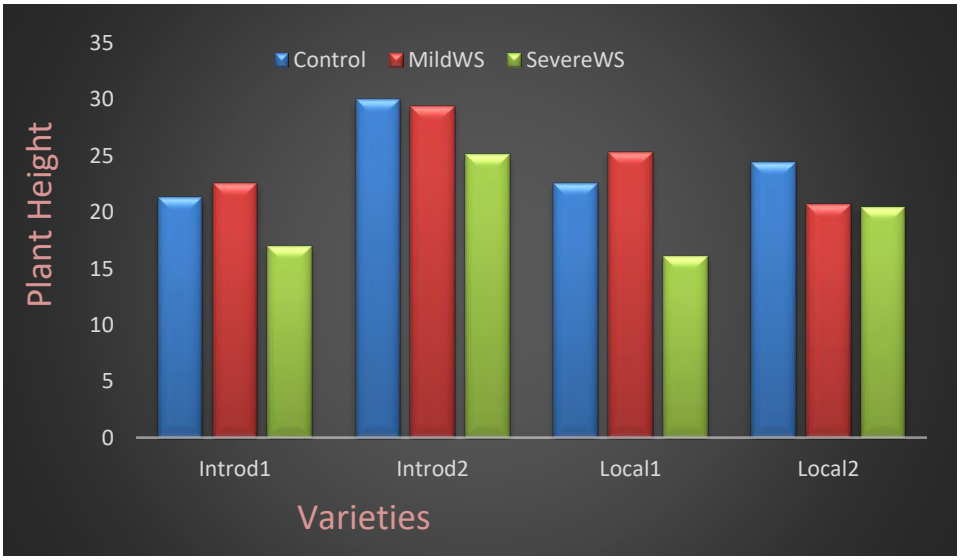


*Fig.2: Mean plant height under non-stress, mild water stress and severe water stress*

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*Table.2: Mean performance of Internode and vine length under non-stress, mild water stress and severe water stress*

| | Internode | | | Vine length | | |
|---|---|---|---|---|---|---|
| Varieties | Control | Mild water stress | Severe water stress | Control | Mild water stress | Severe water stress |
| Variety 1 | 7.17 ± 3.57 | 6.70 ±3.32 | 4.48 ±0.75 | 131.0±39.98 a | 125.92± 51.11 a | 50.53 ± 18.84 b |
| Variety 2 | 4.82 ±0.50 | 4.35 ±0.31 | 3.35 ±0.51 | 69.37±7.30 c | 52.83± 18.84 c | 38.3 ± 5.79 b |
| Variety 3 | 4.13 ± 0.10 | 3.88 ±1.40 | 3.97 ±0.43 | 101.53± 25.82 b | 95.77± 29.92 b | 55.7 ± 29.92 b |
| Variety 4 | 5.58 ± 0.98 | 5.10 ±0.13 | 4.60 ±0.65 | 131.48 ± 60.30 a | 113.45± 43.31 a | 109.13 ± 26.23 a |

Different letters in the same column show significant difference at 0.05 probability level for vine length.

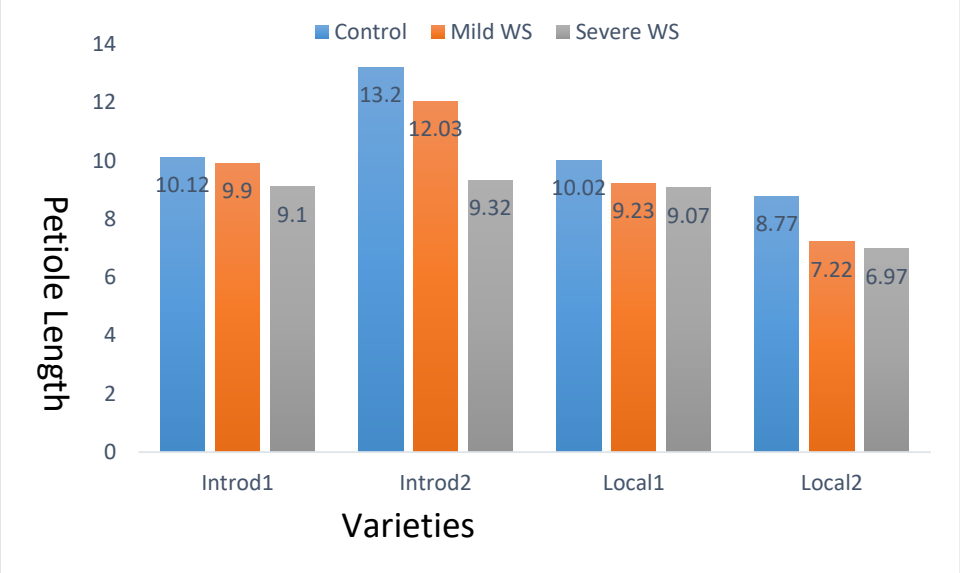


*Fig.3: Mean petiole length under non-stress, mild water stress and severe water stress*

*Table.3: Mean plant fresh weight and dry weight under no water stress, mild water stress and severe water stress*

| | Plant fresh weight | | | Plant dry weight | | |
|---|---|---|---|---|---|---|
| | Control | Mild water stress | Severe water stress | Control | Mild water stress | Severe water stress |
| Variety 1 | 333.33[a] | 300[a] | 233.33[a] | 100 | 83.33 | 50 |
| Variety 2 | 866.67[b] | 733.33[b] | 633.33[b] | 116.66 | 116.66 | 100 |
| Variety 3 | 600[b] | 500[c] | 366.66[c] | 183.33 | 116.66 | 66.66 |
| Variety 4 | 666.66[b] | 566.66[c] | 533.33[b] | 216.67 | 166.66 | 133.33 |

Different letters in the same column show significant difference at 0.05 probability level for plant fresh weight.

*Table.4: Effect of drought of sweet potato yield (fresh weight and dry weight)*

| | Total Root fresh weight (yield) | | | Total Root dry weight | | |
|---|---|---|---|---|---|---|
| Varieties | Control | Mild water stress | Severe water stress | Control | Mild water stress | Severe water stress |
| Variety 1 | 132.83c | 294.10a | 0d | 32.63c | 70.83b | 0b |
| Variety 2 | 324.87b | 397.60a | 87.00b | 120.63a | 115.27a | 34.30a |
| Variety 3 | 607.667 a | 339.77a | 189.00a | 215.13a | 128.50a | 54.47a |
| Variety 4 | 182.00c | 238.63a | 96.00b | 63.63bc | 83.33b | 32.200a |

Different letters in the same column show significant difference at 0.05 probability level

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*Table.5: Effect of drought of sweet potato yield per plant (fresh weight) and dry weight*

| Varieties | Root fresh weight per plant | | | Root dry weight/plant | | |
|---|---|---|---|---|---|---|
| | Control | Mild water stress | Severe water stress | Control | Mild water stress | Severe water stress |
| Variety 1 | 66.417bc | 272.456a | 0d | 16.32a | 66.26a | 0b |
| Variety 2 | 80.798b | 99.40b | 23.32b | 29.94a | 28.82a | 9.42a |
| Variety 3 | 127.63a | 67.953b | 41.15a | 45.45a | 25.70a | 11.88a |
| Variety 4 | 39.20c | 59.66b | 19.20b | 13.67a | 20.83a | 6.44a |

Different letters in the same column show significant difference at 0.05 probability level

*Table.6: Mean performance of Relative water content under drought condition*

| Varieties | Control | Mild water stress | Severe water stress |
|---|---|---|---|
| Variety 1 | 86.31a | 80.51a | 73.31a |
| Variety 2 | 81.55a | 78.11a | 72.32a |
| Variety 3 | 78.72a | 78.70a | 76.21a |
| Variety 4 | 77.03a | 75.24a | 74.09a |

*Table.7: Correlation coefficient among the 11 characters*

| | vlg[a] | petlg | leafL | leafw | intL | plhg | plwg | TotFrW | TotDrW | FrWP | DrWP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| vlg | 1 | | | | | | | | | | |
| petlg | -0.16 | 1 | | | | | | | | | |
| leafL | -0.15 | 0.49** | 1 | | | | | | | | |
| leafw | 0.24 | 0.13 | 0.6** | 1 | | | | | | | |
| intL | 0.29 | -0.01 | -0.15 | 0.17 | 1 | | | | | | |
| plhg | -0.34* | 0.13 | 0.27 | -0.05 | -0.4 | 1 | | | | | |
| plwg | -0.28 | 0.39* | 0.20 | -0.33* | -0.15 | 0.44** | 1 | | | | |
| TotFrW | -0.02 | 0.06 | 0.34* | 0.17 | 0.03 | 0.35* | 0.21 | 1 | | | |
| TotDrW | -0.06 | 0.02 | 0.34* | 0.16 | -0.03 | 0.38* | 0.26 | 0.99** | 1 | | |
| FrWP | 0.22 | 0.02 | 0.19 | 0.22 | 0.40* | 0.04 | -0.07 | 0.69** | 0.59* | 1 | |
| DrWP | 0.16 | 0.03 | 0.24 | 0.21 | 0.33 | 0.14 | 0.01 | 0.80** | 0.73** | 0.98** | 1 |

*P<0.05, ** P<0.01

[a]Vlg= vine length, petlg= petiole length, leafL= leaf length, leafw= leaf width, intL=internode length, plhg= plant height, plwg= plant weight, TotFrW= total root fresh weight, TotDrW = total root dry weight, FrWP= average root fresh weight/plant, DrWP= average root dry weight/plant

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# Evaluation of Viability Encapsulation of Probiotic Cuko Pempek

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***Abstract*—** *The purpose of this research made Cuko Pempek as functional food by supplementing BAL to produce Cuko pempek probiotic. The existence of anti-microbial and anti-bacterial Cuko pempek components became obstacles, therefore it needed strategy to answer two main issues that was first, still allowe the existence of capsaicin and alisin which was caracter impact of Cuko pempek; and second, to protect BAL in order to survive. The strategy was the encapsulation prepared according to Sheu and Marshall, (1993) and the preparation of Cuko pempek modified from ID, (2012). The result showed that the encapsulation of Cuko pempek probiotic with cold storage at temperature of 12ºC produced viability with the average number of cells reaching the range of $10^9$, $10^8$, and $10^7$ and the shelf life until the 20th day even some units until the 30th day. The encapsulation of Cuko pempek probiotic with storage at temperature of 27ºC produced viability with the average number of cells reaching the range of $10^9$, $10^8$, and $10^7$ and the shelf life until the 10th day even some units reaching the 20th day, but in the 8th day there was contamination in 5 experimental units, on the 10th day increased 5 contaminated units, and on the 12th day increased 3 units and on the 13th day occurred Sacharomyces contaminant on all experimental units.*

***Keywords*— Encapsulation Probiotic, BAL, Cuko pempek.**

## I. INTRODUCTION

Pempek is a typical culinary Palembang, South Sumatra, Indonesia, made from wheat flour and tapioca, and fish. At this time it has become a culinary industry that development so rapidly therefore must be balanced with the provision of equipment distribution and presentation of a safety, healthy, and comfortable. Cuko pempek is a companion sauce to eat pempek. But Cuko pempek has specific characteristics, especially its cuka acid content, tooth decay (dental caries). This is in line with those proposed by Hoppenbrouwers and Driessens (1988) that acetic acid damages teeth twice as strongly of lactic acid. In addition acetic acid is anti-microbial (Lodovico et al., 2002; Snyder, 1997).

However, the anti-microbial characteristic possessed by Cuko pempek components that is capsaicin and alisin include weak category (Skrinjar and Nemet, 2009). Although, Zeyrek and Oguz, (2005) states, capsaicin can act as an effective bactericide. But the study of Farag *et al*., 1995 concluded that capsaicin from irradiated chilies was still overgrown with 4,2 x $10^3$/g; 14.3 x $10^3$/g; and 9,2 x $10^5$/g.

Cuko pempek is a food product that has potential to be functional food by making Cuko pempek probiotik (Dunne et al., 2001). Cuko pempek probiotic is cuko pempek containing BAL, and is expected to improve its functionality (Gardiner et al., 2001; Naito *et al*., 2008). Probiotics are supplementary foods that contain living micro-organisms that provide either human or animal host benefits by balancing the microorganisms in the digestive tract (Fuller, 1989). Further Senok *et al*., (2005) probiotics are living microorganisms when arranged in certain amounts will provide benefits for the health of its host.

Encapsulation is the process of forming a matrix-shaped layer in which the inner-shaped interior resembles a capsule wall acting as a cloaking (Vidhyalakshmi *et al*., 2009). Gbassi and Vandamme (2012) call the term Probiotic Encapsulation Technology (PET), in which microbes can be widely immobilized using semipermiabel and biocompatible materials that govern the delivery of microbial cells. (Vidhyalakshmi et al., 2009) encapsulation tends to stabilize cells, potentially increasing cell viability and stability during production, storage, and handling.

BAL encapsulation techniques use phase separation techniques from Sheu and Marshall (1993) and use alginate ingredients (Anal and Singh, 2007) were selected to conduct a study of Cuko pempek probiotic.

## II. MATERIALS AND METHODS

### Lactic Acid Bacteria and Media

*L. bulgaricus* and *S. thermopylus* were obtained from Balai Besar Vateriner Bogor. Lactobacilus was transferred to media of broth MRSAgar while Streptococus to media of broth Blood Agar Base. Then spread in the media agar of petri dish and incubated at 37°C. BAL was harvested after 18 hours incubation to obtain a BAL culture concentration with a range of $10^{11}$sel/mL.

### Preparation of BAL Encapsulation

The preparation of encapsulation used alginate natrum (Sheu and Marshall, 1993; Sultana *et al*., 2000) was 1% ($A_1$), 2% ($A_2$), and 3% ($A_3$) then mixed with BAL *L.*

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*bulgaricus* ($B_1$) and *S. thermopylus* ($B_2$) culture solution with 4: 1 ratio. After a flat stirring, the mixture was dropped using a 5 mL syringe into a 0.2% tween 80 solution in vegetable oil in a 1000 mL beaker glass. It was then poured 0.05M CaCl solution as much as 250 mL rapidly through the edge of the glass wall and left for 30 minutes. The capsule granules would descend and the tween 80 solution, the vegetable oil and the remaining CaCl solution were removed by pouring slowly. The capsule granules were centrifuged at 350x for 15 minutes and then poured into a filter dish and washed with aquades. The preparation of Probiotic encapsulation was with three replications.

**The Preparation of Cuko pempek**

The preparation of Cuko pempek was according to ID (2012), brown sugar, garlic, cayenne and red chili mashed, and salt. Sour source was used yakult. Chili and salt blend and then mixed yakult and fermented for one week (7 days). Then water and sugar were heated to boil, removed and filtered. Then the fermented chili and yakult were fed into the filtered sugar water mixture, plus the fine garlic. The mixture was heated to boiling and cooled, then Cuko pempek was produced.

**The Preparation of Encapsulation of probiotic Cuko pempek**

500mL cuko pempek put in a container of plastic cans size 2000mL as much as the amount of treatment with three replications. Then encapsulation probiotic BAL inserted into Cuko pempek. Some were stored at a temperature of 12ºC and some were at temperature of 27ºC.

**Observation of viability Encapsulation of probiotic Cuko pempek**

Proactive observation of probiotic Cuko pempek encapsulation during storage on 1st day, 10th day, 20th day, and 30th day. Other parameters; shelf life and pH based on time.

## III. RESULTS AND DISCUSSION

### A. BAL Viability of Cuko Pempek in Cold Temperature of 12ºC

The results of diversity analysis of encapsulation of probiotic Cuko pempek at storage temperature of 12ºC, that alginate concentration, BAL type, interaction, and treatment combination had no significant effect on BAL cell viability. While the group was very significant effect. This meant that alginate concentration did not affect BAL cell viability and was reliable for the encapsulation of probiotic Cuko pempek (Sheu and Marshall, 1993; Lotus et al., 2000; Mokarram et al., 2009; Lotfipour et al., 2012; Wikstrom, 2013), as well as the types of BAL (Speck and Myers, 1946; Drakes et al., 2004; Denou et al., 2008; Jimenez et al., 2010). The average number of BAL cells on the first day was $10^9$ for L. bulgaricus ($B_1$) and $10^8$ for S. thermopylus ($B_2$), then the average number of BAL cells both $B_1$ and $B_2$ on the 10th day, the 20th day, and the 30th day is sequential $10^8$, $10^7$ serta $10^7$ and $10^6$. The average number of BAL cells was eligible to act as probiotic requiring an average number of BAL cells before consumption of $10^7$. As, Ishibashi and Shimamur, 1993) suggests, called probiotic food, should contain probiotic cells before consumption of $\geq 10^7$ cells per gram or per-mL of the product. Meanwhile, Lee and Salminen, (1995) require for probiotic drink products to contain as big a cell $\geq$ 105 per mL of product. While FAO/WHO, (2002) requires the number of probiotic cells before consumption by 106 - 107 CFU/g or CFU/mL.

For storage of Cuko Pempek at cold temperature of 12ºC, BAL viability decreased linearly in ten days observation down by one log as in $A_1B_1$, $A_2B_1$, and $A_2B_2$ was obtained on observation the 30th day of the average number of BAL cells by $10^6$. This was caused by two things, first, because the BAL cells were stored in calcium alginate capsules; second, BAL cells were better protected from less favorable environmental influences such as acidity, the presence of capsaicin components of chili and the alisin component of garlic. As Sheu and Marshall, (1993) assert that encapsulated BAL cells have viability for up to 2 weeks and their viability is 40-45% higher than un-encapsulated BAL cells; Sultana et al., (2000), Encapsulation improves viability for up to 8 weeks. Wikstrom, (2013), that encapsulation provides cell viability capability for long periods of time. Furthermore, Gbassi and Vandamme, (2012) there are two reasons for encapsulation, first, to ensure the viability of encapsulated probiotic cells; and second, to ensure the release of probiotic cells when consumed and within the digestive tract. On the other hand, Lotfipour et al., (2012) explains that BAL encapsulation made from alginate provides better viability in acidic conditions. This confirms why the viability of BAL cells in Cuko pempek encapsulation had good viability.

Pattern of BAL cell viability reduction that was encapsulated with calcium alginate ingredients in cold storage temperature of 12ºC, was shown in Figure 1, that $A^3B^1$ (alginate 3% and L. bulgaricus) had the highest viability on average in the four consecutive observation points in sequence $4{,}60x10^9$; $1{,}09x10^8$; $4{,}96x10^6$; and $2{,}43x10^7$; while the lowest was in $A^1B^2$ (alginate 1% and *S. thermopylus*) with average at the four in sequence $6{,}55x10^8$; $4{,}07x10^7$; $4{,}18x10^7$; and $7{,}16x10^7$. The average number of cells met the probiotic requirements before consumption.

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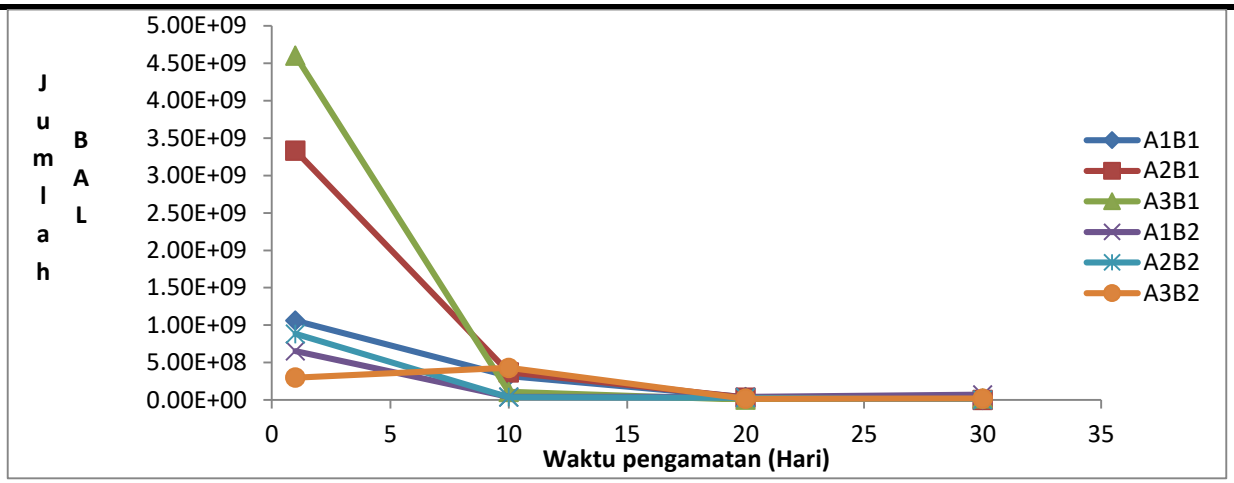


*Fig.1: Graph of BAL Viability at Temperature Storage 12ºC*

However, storage at cold temperatures of 12ºC supported the viability of BAL cells. As the result of the research of Sheu and Marshall, (1993) that the encapsulation of BAL cells stored at cold temperatures had better viability. This pattern of decreased viability was similar to that described by Iyer and Kailasapathy, (2005) that BAL viability stored at cold temperatures decreases from $10^8$ to $10^7$ at the 2nd week and to $10^6$ and $10^7$ at the 4th week.

Furthermore, the results of the analysis of diversity with observations on the 1st day, 10th day, 20th day and 30th day, alginate concentration (A) and probiotic type (B) to pH encapsulation probiotik Cuko pempek at storage temperature of 12ºC, that the concentration alginate and its interactions had no significant effect, the type of probiotics, combinations of treatments and groups had a very significant effect. This was due to the presence of probiotic activity over time which results in changes in pH.Roberts et al., (1994) B. longum BB-79 encapsulation after 10 days had a pH of 3,9 - 4,2; Iyer and Kailaspathy, (2005) encapsulation of L. acidophilus had a pH of 4,6; L. *plantarum* pH 5,6 (Ayama *et al*., 2014).

To look at the degree of difference in each treatment that had a very significant effect on the pH of further tests, it was shown in Figure 2 that the pH encapsulation of probiotic Cuko pempek was very stable until the 10th day, and relatively stable until the 20th day and partly until the 30th day. This showed that during the period of time until the 10th day there was no significant microbiological activity, then until the 20th day there was little microbiological activity and increased activity until the 30th day. As expressed Robert et al., (1994) that in the encapsulation BAL probiotics begin to change pH after day 10.

**B. BAL Viability of Cuko Pempek at Room Temperature of 27ºC**

The result of diversity analysis of alginate concentration, BAL type, combination of treatment and its interaction had no significant effect on cell viability of BAL encapsulation of probiotic Cuko pempek at storage temperature of 27ºC, while the group was very significant. The viability on the first day was $10^8$, then the average number of BAL cells both L. bulgaricus (B1) and S. thermopylus ($B_2$) on the 10th, 20th, and 30th days was sequential $10^7$, $10^6$ and $10^5$. This decrease in BAL cell viability appears to be associated with a decrease in pH, since room temperature induced microbiological activity that affects pH. Noland and Aryana (2012) observes BAL viability in yogurt, BAL viability decreases when the pH falls below pH 4.3. However, the average number of BAL cells still qualifies as probiotics requiring a pre-consumption amount of $10^7$ (Ishibashi and Shimamur, 1993; Lee and Salminen, 1995; FAO / WHO, 2002). However, the number of eligible BAL cells was only until the 10th day.

Figure 3 about the decreased pattern of BAL cell viability of encapsulation of probiotic Cuko pempek at storage temperature of 27ºC, that $A_3B_1$ (3% alginate treatment and *L. bulgaricus*) had the highest viability and lowest available in $A_3B_1$ (3% alginate treatment and *S. thermopylus).* The viability of BAL cells at storage temperature of 27ºC occured in a lower log pattern decrease. This appears to be the condition of the room temperature caused the growth rate and activity of probiotic take place.

Furthermore, the results of the analysis of diversity, that the concentration of alginate, BAL type, combination of treatment and its interaction had no significant effect on pH encapsulation of probiotic Cuko pempek at storage temperature of 27ºC. To see the level of difference of group effect continued test shown in Figure 4. The pH pattern from high condition started pH 5 on the 1st day, then fell to its lowest point on the 10th day of 3,73 and then up to the the 20th day of 3,87 – 3,9 and up again until the 30th day of 4. But the increase did not go beyond pH on the first day.

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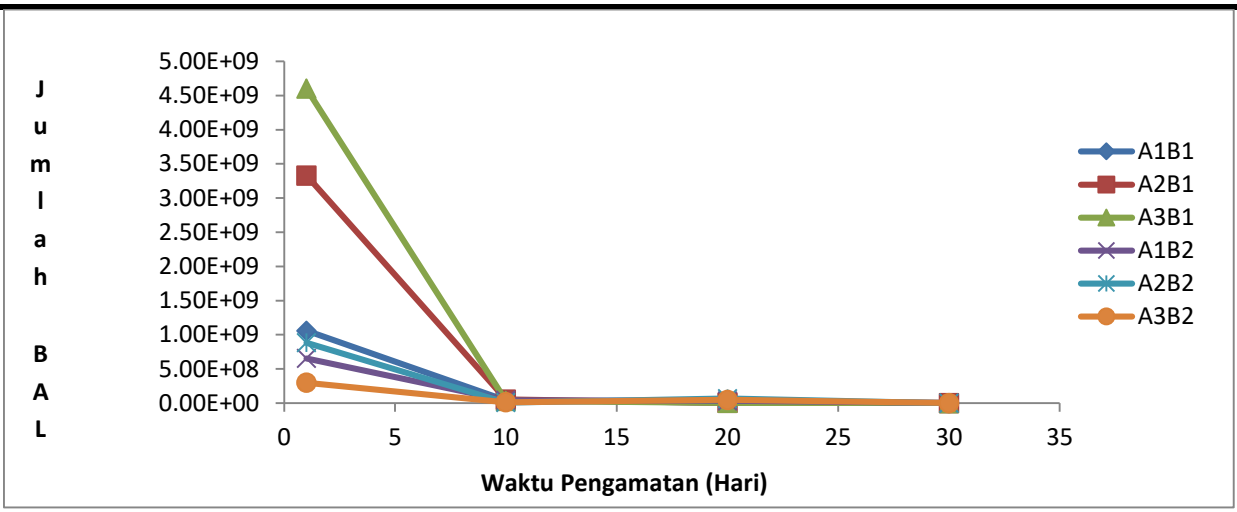



*Picture 3. Graph of VAL Viability at storage temperature of 27°C*

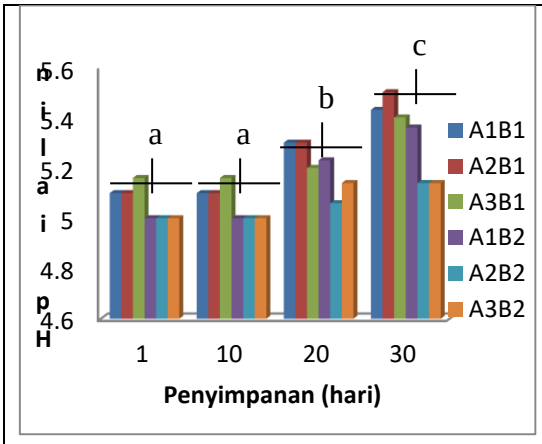
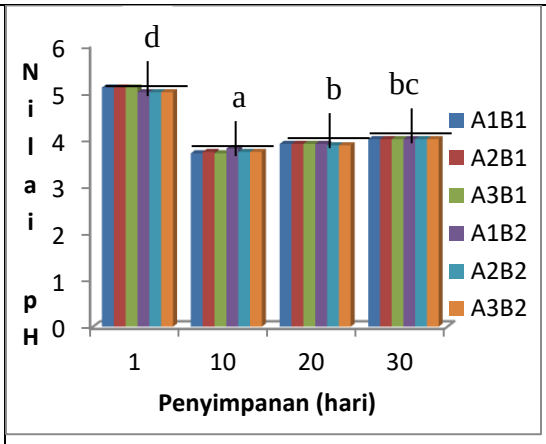



*Figure 2. Graph of decreasing pattern of pH encapsulation of probiotic Cuko pempek at storage temperature of 12°C*




*Gambar 4. Graph of decreasing pattern of pH encapsulation of probiotic Cuko pempek at storage temperature of 27°C*

Unlike storage at cold temperatures of 12°C, storage at temperature of 27°C in addition to caused the fermentative rates to produce decreasing pH, also provided a great chance of contamination. On the 8th day, the units $A_1B_1$(I), $A_1B_2$(II), $A_2B_1$((I), $A_2B_1$(III), and $A_3B_1$(III) were contaminated. Then on the 10th day the contamination increased in unit $A_1B_1$(III), $A_2B_1$(II), $A_3B_1$(II), $A_3B_1$(III), and $A_2B_2$(II), so that on the 10th day all experimental units using *L. bulgaricus* ($B_1$) had been contaminated *Saccharomyces*.

On the 12th day of the experimental unit using *S. thermopillus* ($B_2$) which on the 10th day was contaminated one of $A_2B_2$(II), three units were added, namely $A_1B_2$(I), $A_1B_2$(II), and $A_1B_2$(III). Then, on the 13th day all the experimental units were contaminated with *Saccharomyces*. This phenomenon indicated that acidic of Cuko pempek and stored at 27°C prone to contaminated and overgrown with *Sacharomyces*. This confirms the results of the study of Narendranath *et al*. (2001),that *Saccharomyces* grows in a minimum medium containing acetic acid and lactic acid at temperature of 30° C. Furthermore, Thomas *et al*. (2002) explains that Saccharomyces grows on a minimum medium containing lactic acid when its pH is 4.5.

## IV. CONCLUSIONS

1. The encapsulation of probiotics Cuko pempek with storage temperature of 12°C produced viability with average number of cells reaching $10^9$, $10^8$, and $10^7$and shelf life until the 20th day and some units until the 30th day, with a relatively constant pH ranging from 5,07 – 5,25.
2. Encapsulation of Cuko pempek probiotic with storage temperature 27°C produced viability with average

number of cells reaching $10^8$, $10^7$, dan $10^6$and shelf life up to the 10th day and some units reaching the 20th day, with decreasing pH from the 1st day, the $10^{th}$ day, the 20th day and the 30th consecutive day pH 5,0 – 5,1; pH 3,70 – 3,80; pH 3,87 – 9,0; and pH 4.

3. For encapsulation of probiotic Cuko pempek with storage temperature of 27ºC on the 8th day arose contaminant at 5 unit experiment, on the 10th day added 5 unit and on the 12th day arose in three other unit and on the 13th day arose contaminant in the form *Sacharomyces* on all experimental units.

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# Evaluating The Effect of Integrated Use of Farm Yard Manure and Urea on the Socio economic Performance of Tomato (*Lycopersiconesculentum Mill)* at Tselemti Woreda, North western Tigray, Ethiopia

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***Abstract—*** *As compared to the potential productivity & national average yield of the crop, farmers in Tselemti 'woreda 'are not getting as much tomato yield /profit as expected, because of the low soil fertility & un proper soil management practices. To mitigate the problem farmers commonly use a blanket recommendation of inorganic fertilizers. But currently most farmers are not applying inorganic fertilizers at recommended rates,because of the high price of inorganic fertilizers. Hence, use of FYM would be un avoidable, particularly for resource poor farmers. However FYM alone may not be enough to meet the nutrient requirements of high yielding tomato varieties.So, integrated use of organic and inorganic plant nutrient sources help to overcome problems with the sole application and have moreeconomic profit. This study was therefore conducted with the objective of evaluating the economic feasibility of combined use of FYM & Urea nutrient sources in tomato production & assessing farmer's perception on the use & advantage for sustainable &better tomato production, in Tselemtiwereda, May ani site during the 2012/13 off season time. Organic (FYM) and inorganic (Urea) nutrient sources was integrated in different proportions to supply 60Kgha$^{-1}$ of Nitrogen (N) from both sources at different ratios. The treatment combinations are T1 (control or with no fertilizer), T2 (100%IF), T3 (25% FYM+75%IF), T4 (50%FYM+50IF), T5 (75%FYM+25%IF) & T6 (100%FYM).Three 'kebeles' and 72 farmers was surveyed for the perception data.The partial budget analysis revealed that maximum net of return (59902.45) Birr/ha was recorded in treatments that receive 25% N from FYM in conjunction with 75% N from IF sources followed by 50%N from FYM and 50%IF with a net return of 56,386birrha$^{-1}$and 23,862.45 and 15,896.5 net return over the control. The Perception of the respondent farmers also indicates that 94.4% of the answered farmers use inorganic fertilizers in tomato production, but 48.61% of them were responded use of inorganic fertilizer in tomato production is not economically feasible and 44.4% use an integration of both nutrient sources for better tomato productivity. The overall study revealed that a combined application of FYM with Urea at (25:75 and 50:50 ratios) significantly increased economic profit in tomato production. Therefore, it is recommended for tomato producers of Tselemti wereda for profitable & acceptable tomato production.*

***Keywords— FYM, Inorganic, Integrated, Organic, Net return, Tomato, Urea.***

## I. INTRODUCTION

In terms of profitability, evidence of positive returns is often found for integrated mineral organic system (Sedaf and Qasimkhan, 2010). The integration of FYM and inorganic fertilizer on maize in Zimbabwe resulted in a return to the labor of about $ 1.35 per day, while the best single fertilizer or manure treatment yielded only $0.25 (Mekuria and Waddington, 2002). The profitability of alternative nutrient input sources depends not only on yield gains but also on market conditions (Place *et al.*, 2003).

Through the conventional understanding the best way to improve the productivity of resource poor farmers is the use of high-yielding variety of crops and chemical fertilizer; however research evidences show that the resulting yield increases may not be sufficient to pay for these inputs (Christopher, 1994). The addition of any amount of fertilizer is interesting to farmers if and only if it is profitable through

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the enhancement of either yield or quality. Mostly, maximum profits are rare at maximum yields because the last increment of fertilizer to produce a little more yield may cost more than the yield increase is worth. Any new technology can be evaluated in terms of its impact on the productivity, profitability, acceptability and sustainability of farming systems. Integrated nutrient management practices are survival and risk avoidance strategies for farmers. Many farmers understand the role of FYM in improving soil quality and sustaining yield (Duncan *et al.,* 1990).

Tolesaa and Friesen (2001) reported that the application of 25% recommended inorganic NP fertilizers mixed with FYM resulted in the highest marginal rate of return in maize indicating that the integrated approach can enable to save up to 75% of commercial fertilizers. Likewise, Bayu*et al*. (2006) also reported the possibility of saving up to 50% of the recommended NP fertilizers due to amendment with 5-15 t $ha^{-1}$ FYM to sorghum crop without significantly affecting the optimum possible yield that can be obtained with the application of full dose of inorganic NP fertilizers alone.

Farmers' decisions to adopt a new technology in preference to other alternative technologies depend on complex factors (Tesfaye, 2003). One of the factors is the farmers' perception to the characteristics of the new technology. The typical characteristics of a technology are relative advantage, complexity, compatibility, risk and uncertainty. While farmers do not necessarily make conscious and sophisticated analysis of the degree of risk in adopting technology, they are aware of the implication of particular choices. If a farmer's actual experience with the innovation is satisfactory, his/her perceptions probably will become more favorable (Van den Ban and Hawkins, 1998).

Farmers' opinions towards the use of either organic or inorganic sources of plant nutrients are influenced by a variety of factors such as: information sources, ethical concerns about the environment, farmers' knowledge, economic considerations (cost and benefit aspects), marketing procedures, the rationale of the extension system and the like (Chouichom and Yamao, 2010). Many researchers reviewed lots of reasons that farmers are frustrated in using mineral fertilizer such as: the ever increasing price ofmineral fertilizer is becoming beyond the purchasing power of farmersand fear of burning effect by chemical fertilizers on crops when moisture is limiting (Hailu, 2010). Generally, the frustration of the smallholder farmers is to escape possible crisis when the prices of their farm products are too low or lost in the unpredictable rainfall situation. Therefore, farmers are inclined into locally available resources and technologies such as the use of FYM, Butit is not possible to obtain a higher crop yield(profit) by using organic manure alone due to their unavailability in excess amount and they contain a comparatively low quantity of nutrients compared to inorganic fertilizers.

However, no study has been done on the use of integrated fertilization on the economic profit & social perception of irrigated tomato so far in the study area.Therefore, the aim of this research was to study the impact of combined use of FYM and urea nutrient sources on the economic profitability & social aspects of irrigated tomato on Tselemtiworeda, Northern Ethiopia.

## II. MAREIALS AND METHODS

The field experiment was conducted at North Western Zone of Tigray, TselemtiworedaMay ani '*kebele'* on farmer fields during the off season of 2012/2013.

From the total 21 village '*kebeles*' of the '*woreda*' three kebeles (May ani, Medhanialem and Whdet) were selected purposively (Purposive Sampling) for the survey data, based on their accessibility, good potential and better experience in irrigated tomato production using Probability Proportional to Sample size (PPS) method.The target population was those farmers which have at least five years' experience in the production of irrigated tomato. A total of 544 farmers was selected from the three '*kebeles'* that fulfill the criteria with the assistance of the extension staffs of each '*kebele',* To make it have a sense of statistic, using the PPS method, 72 households were selected from these 544 households.

*Table.1: Method of sample size determination of the respondents using PPS*

| S/N | Kebele name | No Total HHs selected | Using PPS |
|---|---|---|---|
| **1** | Whdet | 280 | 37 |
| **2** | Medhanialem | 150 | 20 |
| **3** | Mai ani | 114 | 15 |

*Source: Tselemtiwereda office of agriculture and rural development(2005);PPS=Probablity proportional to size*

Descriptive survey design for data collection was adopted in the study. Primary data were collected from the respondents with the aid of a structured interview consisting of both open and close ended questions. The secondary data were

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also gathered from various sources including Tselemti office of agriculture and rural development, and Maitsebri Agricultural Research Center (MyARC).Besides, relevant literature, official reports were also consulted as a secondary data source. Primary data were collected from sampled farmers who had involved at least for five years in tomato production. Pre-tested interview schedule and checklists were employed as survey instruments.

Based on the 2007 national census conducted by the Central Statistical Agency (CSA, 2007) of Ethiopia, the '*woreda*' has a total population of 138,858 of whom 70,108 are men and 68,750 women.A total of 30,485 households resulting in an average of 4.55 persons per household. Various land use types i.e rain fed cultivated land, irrigated land, grazing land, forest land, home stead and the like are existing.The'*woreda'* has a total area of 70926ha with 30,365 ha arable land,7000ha irrigated land, 4577ha forest land, 14882 ha grazing and bush land and 14102ha gully and mountainous land (MyARC, 2010).

For economic evaluation of the cost and benefit in using the different ratios of organic and inorganic fertilizers, the Partial Budget Analysis (PBA), which includes the Dominance Analysis (DA) and Marginal Rate of Return (MRR), was used following the CYMMYT procedure (CIMMYT, 1998). In this study, the partial budget analysis was made to determine the most economically acceptable treatments (combinations) by estimating the costs and benefits based on the current market price of tomato fruit, inorganic fertilizers and the spreading costs of farmyard manure for the cropping season at the study area.

The varying labor costs were estimated based on the existing rate of payment to daily farm laborers. The fruit yield harvested from the experimental plots was converted into hectare bases. Then, the market value of the fruit yields was based on the prevailed market price. To estimate economic parameters, tomato was valued at an average open market price of 200.00 birr per quintal (100kg) of fruit. The price of 20 work days (WD) per hectare for collection and application of FYM (Astewel, 2010) at a wage rate of 50 Birr per workdays was used. Since the local market for the applied TSP as a source of phosphorus was not available the cost was calculated by changing to DAP equivalent. So, the price of inorganic fertilizers at the time of transplanting in the area was 1500.00Birr per quintal for DAP and 1250.00 Birr per quintal for Urea. Experimental yields are often higher than the yields that farmers could expect using the same treatments; hence in economic calculation yields of farmers are adjusted by 15% less than that of the research results (CIMMYT, 1998). Based on this principle the yield obtained from each treatment was changed to hectare basis and reduced by 15% for economic analysis and the analysis was undergone through the following stages:

**Net Income:** Estimate the net benefit arising from all alternative treatments. Net income (NI) is calculated as the amount of money left when the total variable costs of inputs (TVC) are deducted from the total revenue (TR).

**Dominance Analysis:** Before proceeding with the calculation of Marginal Rates of Return, an initial examination of the costs and benefits of each treatment, called dominance analysis is important. Dominance analysis is used to eliminate some of the treatments from further consideration in the MRR and thereby simplifying the analysis of MRR. i.e., those treatments which involve higher cost but do not generate higher benefits (called dominated treatments) are eliminated. The dominance analysis was carried out by first listing all the treatments in their order of increasing costs that vary (TVC) and their net benefits (NB) are then put aside. Any treatment that has higher TVC but net benefits that are less than or equal to the preceding treatment (with lower TVC but higher net benefits) is dominated *treatment* (marked as"D"). The dominance analysis illustrates that to improve farmers' income, it is important to pay attention to net benefits rather than yields, because higher yields do not necessarily mean higher net benefits.

**The Marginal Rate of Return:** is used to assess relative profitability among alternative treatments. It measures the percentage increase in net income in relation with each additional input of expenditure (ΔTVC) and the 100% rate of return was considered as a minimum value, which farmers could be willing to invest given their level of poverty and the fragile nature of the environment (CIMMYT, 1998). MRR was calculated as the ratio of change in return on the average of each replicated treatment to the change in total cost with regard to the control. It compares the increments in costs and benefits between pairs of treatments.

$$MRR = \frac{\Delta NI \quad X100}{\Delta TVC}$$

*Where:*

ΔNI = Change in Net Income;

ΔTVC = change in Total Variable Cost

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The marginal rates of return appear in between two treatments. It makes no sense to speak of the marginal rate of return of a particular treatment because the MRR is a characteristic of the change from one treatment to another.

**Identification of a candidate recommendation from among the non-dominated treatments:** This is the treatment which gives the highest net return and a marginal rate of return greater than the minimum considered acceptable to farmers.

The social data collected were analyzed with the aid of the descriptive statistical tools of frequency count and percentage.

Simple descriptive statistics such as simple measures of mean, standard deviation, frequency, percentages and cross tabulation were used for the survey data gathered from the sampled farm households. Statistical package for social science (SPSS) version 16 was employed to analyze the data.

## III. RESULTS AND DISCUSSION

**Effect of Integrated use of Organic and IF on Economic Performance**

The economic analysis revealed, how gross returns and net benefits were influenced by the integrated use of organic and inorganic plant nutrient sources on irrigated tomato productivity(Table 2). Based on the current market price of economic yield (output) and prevailing price of inputs during the production period, maximum net of return of Birr 59902.45ha$^{-1}$ was recordedin the plots that receive 25% N from FYM (4.5t/ha) in conjunction with 75% N from inorganic (97.5Kg/ha Urea)sources

Followed by 50% FYM combined with 50% of urea with an average net benefit of birr 56386birrha$^{-1}$. It was noted that the integrated use of organic manure with mineral fertilizer at 25:75 and 50:50 ratios were economically profitable and resulted in high net return. However the lowest net return (36040 Birr/ha) was received in the control treatment (Table 2). Similar results were reported by Alam*et al.,* (2004)who observed that higher profit was obtained when inorganic fertilizer was combined with organic manures.

*Table.2: TVC cost and NR of tomato as influenced by the integrated use of fertilizers*

| Trts | Combinations | | Gross return (birr ha$^{-1}$) | TVC | Net return (birrha$^{-1}$) | Net return over control |
|---|---|---|---|---|---|---|
| | FYM (tha$^{-1}$) | IF(Urea) (kgha$^{-1}$) | | | | |
| **T1** | 0 | 0 | 36040 | 0 | 36040 | - |
| **T2** | 0 | 130 | 55624 | 3687.5 | 51936.5 | 15896.5 |
| **T3** | 4.5 | 97.5 | 63818 | 3915.55 | 59902.45 | 23862.45 |
| **T4** | 9 | 65 | 60061 | 3675 | 56386 | 20346 |
| **T5** | 13.5 | 32.5 | 52411 | 4137.5 | 48273.5 | 12233.5 |
| **T6** | 18 | 0 | 47515 | 4600 | 42915 | 6875 |

*FYM=Farm Yard Manure; IF=Inorganic fertilizer; TVC=Total Variable Cost*

From the agronomic point of view, the best results were obtained from plots which received combined nitrogen (organic and inorganic) i.e.25%N from FYM with 75%N from inorganic fertilizers, yielded better than the rest of treatment combinations.(Table 3). lists the total costs that vary and the net benefits for each of the treatments in the integrated use of organic (FYM) and inorganic (Urea) plant nutrients for tomato production. This used to identify the inferior treatments i.e. those which involve higher costs but do not generate higher benefits (dominated treatment). To proceed through the dominance analysis, treatments were listed in order of increasing total costs that vary and their corresponding benefits were put aside. This illustrates that to improve farmers' income it is important to pay attention to net benefits, rather than yields. As indicated in (Table 3), $T_1$ showed theleast TVC (0) while $T_6$ showed the maximum TVC (6400 Birr) and all the remaining treatments were confined between these two ranges. As goes from treatment ($T_1$) to treatment ($T_4$) both the TVC and net profit increases, but for treatment ($T_2$) the total variable cost is increased but showed lower net benefits than treatment ($T_4$) (56386). No farmer would choose $T_2$in comparison with $T_4$, because $T_2$has higher costs that vary, but lower net benefits. Such a treatment is called a dominated treatment (marked with a "D" (Table 3).On the other hand as goes from treatment $T_2$ to treatment $T_3$ both the TVC and the net benefit are increased and considered for further analysis of MRR. The

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rest treatments ($T_5$ and $T_6$) are dominated were not considered for further analysis.

Therefore, from the total six treatments only three treatments ($T_1$, $T_3$ and $T_4$) were considered for analysis of Marginal rate of return (MRR) (Table 3). Marginal Rates of Return (MRR) appear in between two treatments. It makes no sense to speak of the marginal rate of return of a particular treatment; rather, the marginal rate of return is a characteristic of the change fromone treatment to another. Since dominated treatments were not included in the marginal analysis, the marginal rate of return was positive. The marginal rate of return indicates what farmers can expect to gain, on the average, in return for their investment when they decide to change from one practice (or set of practices) to another. From the present day experiment combined use of FYM and inorganic fertilizers at the rate of 25% N from FYM with 75% N from inorganic and 50% from FY M with 50% from inorganic could be considered to have an economic advantage over the use of other alternative combinations. So to improve farmers' income decision cannot be taken regarding these treatments without knowing what rate of return is acceptable to the farmers rather attention should be given to the net benefits because higher yield does not necessarily mean high net benefit

*Table.3: Economic analysis for integrated use of fertilizers in tomato*

| Trts | Combinations | | Yield ($Qlha^{-1}$) | GR (birr $ha^{-1}$) | TVC | NR ($birrha^{-1}$) | Dominance | MRR (%) |
|---|---|---|---|---|---|---|---|---|
| | FYM | IF | | | | | | |
| **T1** | 0 | 0 | 212.0 | 36040 | 0 | 36040 | - | |
| **T4** | 50 | 50 | 353.0 | 60061 | 3675 | 56386 | - | 553.6 |
| **T2** | 0 | 100 | 327.2 | 55624 | 3687.5 | 51936.5 | D | - |
| **T3** | 25 | 75 | 375.4 | 63818 | 3915.5 | 59902.4 | - | 609.4 |
| **T5** | 75 | 25 | 308.3 | 52411 | 4137.5 | 48273.5 | D | - |
| **T6** | 100 | 0 | 279.5 | 47515 | 4600 | 42915 | D | - |

*Trt= Treatment; TVC= Total Variable Cost; D= dominated; MRR= Marginal Rate of Return; FYM= Farm Yard Manure; IF=inorganic fertilizer (Urea);Qt=Quital;$ha^{-1}$=per hectare*

The first rate of return was recorded between treatment 1 and treatment 4 with a change in net income of 20346 Birr and a change in total variable cost of 3675 Birr. Therefore the Marginal rate of return was 553.6 % or 5.53 birr. This means that for every 3675 Birr investment of a farmer in using 9 t/ha of FYM and 65 kg of inorganic fertilizer (Urea), farmers could expect to recover the 3675 birr and Birr and obtain additional 23862.4 Birr and have an economic advantage over the use of other alternative combinations. So, this is important to farmers to improve their income.

**Perception of Farmers to Use FYM for Tomato production**

Despite the high number of livestock per household and the availability of cheap family labor that could be used for FYM collection and transportation the use of FYM for increased irrigated tomato productivity is not a common practice in the area. According to the respondents, FYM is used mostly for major rain fed crops such as Maize, obtain additional 20346 Birr. Similarly as goes from treatment 4 to treatment 3, Marginal Rate of Return (MRR) is 609.4% or 6.09 Birr which is relatively highest in this experiment. This means that for every 3915.5 Birr investment of a farmer in using 4.5t/ha of FYM and 97.7 kg of inorganic fertilizer (Urea), farmers could expect to recover the 3915.5

Sorghum and Finger millet. Even though livestock manure is highly respected by the farmers as a soil fertility improvement, the collection, storage and application method is not practiced properly and only used in the rain fed crops. The chi-square test indicated that there isa significant difference ($p<0.01$) among the three '*kebeles* 'in the use of the FYM in irrigated tomato production. Relatively more farmers in M/ani were used FYM for tomato production (Table 4). Even though farm yard manure was easily accessed to the farmers about 43.1% of them were not used for tomato production because of different reasons (Table 5

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*Table.4: Farmers experience in the use of FYM for irrigated tomato production*

| Question | Response | Kebele Name | | | Total | Percent | $X^2$ | significance |
|---|---|---|---|---|---|---|---|---|
| | | Whdet | M/alem | M/ani | | | | |
| **Do you use FYM for tomato production in your irrigation site?** | **Yes** | 15 | 16 | 10 | 41 | 56.9 | | |
| | **No** | 22 | 4 | 5 | 31 | 43.1 | | |
| | | | | | | | 8.97 | 0.011** |
| | **Total** | 37 | 20 | 15 | 72 | 100 | | |

*$X^2$=Chi-square test;**= significant p<0.01*

*Table.5: Main reasons of farmers for do not use FYM in tomato production*

| S/N | Main reasons | Frequency | percentage |
|---|---|---|---|
| 1 | Lack of know how | 18 | 42.8 |
| 2 | Labor shortage and far from the source | 13 | 31 |
| 3 | Priority to use IF | 10 | 23.8 |
| 4 | Disease and weed problems | 1 | 2.4 |
| | **Total** | **42** | **100** |

As depicted in table 5from the farmers which do not use FYM for irrigated tomato production, 42.8% were because of the lack of know how whether FYM is important for tomato production or not, 31% of the farmers responded that due to labor shortage and far from the source, 23.8% of the respondents give priority to inorganic fertilizers as a nutrient sources and the remaining 2.4% answered as due to diseases and weed problems of the FYM.

**Farmers' perception to use of Inorganic fertilizers in tomato production**

The results depicted in (Table 6) showed that all most all of the respondent farmers

(94.4%) in the study area were using inorganic fertilizer for tomato production. 5.6% of the respondent farmers were not used inorganic fertilizers because of different reasons. In terms of the economic feasibility of inorganic fertilizer for tomato production, (48.61%) of respondents argued that use of inorganic fertilizer is better to gain better profit, whereas more than half (51.39%) of the farmers respond that use of inorganic fertilizers is not economically feasible. From the 37(53.9%) respondent farmers who answered inorganic fertilizer is not economically feasible, 12.9% of them viewed because price of inorganic fertilizer is high, 83.88% of them respond because the current market the price of the vegetables (tomato) is below the initial price of the inputs used for production and the rest 3.2% of the respondent farmers answered that due to the burning effect of inorganic fertilizers especially after first harvest.

*Table.6: Farmers experience and perception on Inorganic fertilizer for tomato production*

| Questions | Response | Kebele Name | | | Total | % |
|---|---|---|---|---|---|---|
| | | Whdet | M/alem | M/ani | | |
| **Do you use IF to increase your tomato productivity?** | **Yes** | 35 | 18 | 15 | 68 | 94.4 |
| | **No** | 2 | 2 | 0 | 4 | 5.6 |
| | **Total** | **37** | **20** | **15** | **72** | **100** |
| **Do you really believe that use of IF in** | **Yes** | 18 | 5 | 14 | 37 | 51.39 |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **tomato is economically feasible?** | | **No** | 19 | 15 | 1 | 35 | 48.61 |
| | | **Total** | **37** | **20** | **14** | **72** | **100** |
| **If your answer is no why?** | High price of fertilizers(not feasible) | | 1 | 0 | 3 | 4 | 12.9 |
| | price of vegetables is below IF | | 15 | 10 | 1 | 26 | 83.9 |
| | Burning effect of IF after 1st picking | | 0 | 1 | 0 | 1 | 3.2 |
| | **Total** | | **16** | **11** | **4** | **31** | **100** |

**Perception of Judicious Use of FYM with Inorganic Fertilizers**

Farmers' perception to integrated use of organic and inorganic fertilizer for tomato production showed in (Table 7).The result indicates that the sole use of FYM and inorganic fertilizers were practiced by an equal percentage (27.8%) of respondents. Majorities (44.44%) of the respondents were using the integration of organic (FYM) and inorganic (Urea) nutrient sources for better tomato productivity. The main reasons why farmers are interested to use the integration of both nutrient sources for tomato production is concentrated on three reasons. As depicted in ( Table 7), 65% of the total respondent farmers in the study '*kebeles'* used integrated nutrient management for tomato for the advantage of early and better performance of the crop, 18.8% of the respondents use for soil fertility improvement and the remaining 15.5% used integrated nutrient management for sustainable crop production. This agrees with the findings of Charreau (1991), who reported, higher and sustainable crop yields are achieved with the same amount of nutrients when supplied through combined use of organic and inorganic fertilizers than mineral fertilizer or organic alone.

*Table.7: Farmers perception to integrated use of nutrients for tomato production*

| **Questions** | | **Kebele Name** | | | **Total** | **%** |
|---|---|---|---|---|---|---|
| | | Whdet | M/alem | M/ani | | |
| **Which type of plant nutrient sources is better for tomato productivity** | FYM only | 8 | 11 | 1 | 20 | 27.8 |
| | IF only | 14 | 1 | 5 | 20 | 27.8 |
| | Integration of both | 15 | 8 | 9 | 32 | 44.4 |
| | **Total** | **37** | **20** | **15** | **72** | **100** |
| **If your answer is integration of both why?** | Early and better performance of the crop | 10 | 5 | 6 | 21 | 65.6 |
| | Soil fertility improvement | 3 | 2 | 1 | 6 | 18.8 |
| | Sustainable crop production | 2 | 1 | 2 | 5 | 15.6 |
| | **Total** | **15** | **8** | **9** | **32** | **100** |

*IF=Inorganic Fertilizer;FYM=Farm Yard Manure*

## IV. CONCLUSION

Based on the current market price of inputs and outputs, the economic analysis indicates that all fertilized treatments (either integrated or lone) record higher net returns over the control treatment. From the present day experiment, treatments which received integration of FYM and inorganic fertilizers at the rate of 25% N from FYM with 75% N from inorganic sources resulted a maximum net of

return,i.e,15.4% more than the use of sole inorganic fertilizers and 39.6% more than the use of FYM only. Generally, in order to improve farmers' income decision cannot be taken regarding only the agronomic observations without knowing what rate of return is acceptable to the farmers rather; attention should be given to the net benefits because higher yield does not necessarily mean higher net benefit.

Based on the findings, regarding the social perceptions to the integrated use of organic and inorganic nutrient sources for irrigated tomato production, it can be concluded that, almost half of the respondents do not use FYM only for tomato production, because of lack of know-how, Priority to use inorganic fertilizers, labor shortage and disease and weed problems. Similarly, half of the respondent farmers perceive that, use of inorganic fertilizers is not economically feasible due to the high price of fertilizer, the low market price of tomato and burning effect of inorganic fertilizers especially after first picking. So farmers are interested on the integrated use of both nutrient sources for the advantage of early and better performance of tomato crops, soil fertility improvement and sustainable crop production.

To get better yield and higher economic benefit from irrigated tomato productions Every concerned stakeholder should give due attention to locally available nutrient resources (FYM) and the integrated use with Inorganic fertilizer for sustainable tomato production.

## CONFLICT OF INTEREST

The author(s) have not declared any conflict of interests.

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# Enhancing Productivity and Production of Onion (*Allium cepa L.*) Through the use of Improved Varieties at North Western Zoze of Tigray, Ethiopia

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***Abstract*—** *Field experiment was conducted to study the effect different varieties on yield, yield attributing character and postharvest storability of onion (Allium cepa L.) in Tselemti district, North western Zone of Tigray During 2007-2009off season time. Four improved onion varieties namly:Bombey red, Adama red, Nasik red &Nafis including Shendi (imported from Sudan),were tested in Randomized Complete Block Design (RCBD) with four replications. Accordingly, treatments were assigned randomly to the experimental plot within a block. The results showed that the difference in variety had significant effect on all characters except the non-significant effect of variety on neck thickness and bulb diameter. Nasik red variety gave significantlyhighest in plant height ,leaf number, leaf length bulb length and marketable bulb yield i.e 35588kg/ha that exhibited 18% and 36% advantages on the dominantly produced varieties bombey red and adama red respectively.The overall study revealed that growing Nasik red variety is not only significantly increased the marketable bulb yield , but also better shelf life. Therefore, it is recommended for onion producers of Tselemti wereda for profitable onion yield*

***Key words*— Onion, Storability, Variety, Yield.**

## I. INTRODUCTION

Onion (Allium cepa L.) is an important bulb crop, belonging to the family Alliaceae (Hanelt,1190).It is one of the most important and popular bulb crops cultivated commercially in nearly most parts of the world. Onions as food, medicine and religious object were known during the first Egyptian dynasty (3200 B.C.) (Ray and Yadav, 2005). ). It is important in the daily diets of human's in worldwide and Ethiopians as well (MoARD, 2006). Onion contributes significant nutritional value to the human diet and are primarily consumed for their distinctive flavor widely used in soups, meat dishes, salads, food dressings and sandwiches, medicinal purposes and is cooked alone as a vegetable. Its pungency is due to the presence of a volatile oil (Allyl propyl disulphide) (Malik, 1994).

Onion (Allium cepa L.) is a recently introduced bulb crop in the agriculture commodity of Ethiopia and it is rapidly becoming a popular vegetable among producers and consumers (Lemma and Shimeles, 2003; Dawit et al., 2004). It is more widely grown in Ethiopia for local consumption and for flower export (Lemma and Shimeles, 2003). It is valued for its distinct pungency or mild flavour and also consumed universally in small quantities and used in many homes almost daily, primarily as a seasoning for flavouring of dishes, sauces, soup, and sandwiches in many countries of the world (Geremew et al., 2010).

Onion is one of the most important vegetable crops in Ethiopia which is used almost daily as a spice and vegetable in the local dish regardless of religion, ethnicity, and culture (CSSE, 2006). The diverse agro-climatic conditions that prevail in the country provide the opportunity of producing onion bulb, seeds and cut flower for local use and export market (CSSE, 2006). Additionally, its higher yield potential, availability of desirable cultivars for various uses, ease of propagation by seed, high domestic (bulb and seed) and export (bulb, cut flowers) markets in fresh and processed forms is making the crop increasingly important in Ethiopia (Yohannes,1987).

Ethiopia has enormous potentials to cultivate the vegetable crops at small as well as large commercial scale. The

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country has high potential to benefit from onion production, and the demand for onion is increasing from time to time for its high bulb yields, seed and flower production potential (Lemma and Shimelis, 2003).Statistics indicated that, the production of onion in Ethiopia during 2012/2013 growing season was in about 21865ha of land yielding a total production of 219919 tons with an average yield (10.06 tons /ha) which is too low as compared to the world average of 19.31 tons/ha (FAOSTAT,2013).

There are a number of constraints that cause low productivity of onion in Ethiopia. The low yield of onion in the country is reported to be due to low fertility of soil, inappropriate fertilizer rate, lack of improved varieties, and poor management practices (Lemma and Shimelis, 2003).

The use of proper agronomic practice has an undoubted contribution in increasing crop yield. The optimum level of any agronomic practice like plant spacing, plant population, planting date, harvesting time can bring desired results. The optimum use of spacing or plant population has dual advantages. It also avoids strong competition between plants for growth factor such as water, nutrient and light. Conversely,optimum plant population enables efficient use of available crop land without wastage (Zubelidia and Gases, 1977).

The use of appropriate agronomic management practices is important to increase the productivity and production of the crop. However, in the country, intra-row spacing of 10 cm and inter-row spacing of 20 cm during transplanting to permanent field is used which was recommended before 20 years (FAO, 1995). But, plant spacing as an important economic consideration in the production of onion should have to depend on type of variety (plant architecture, growth habit etc.), agro-ecology, production system etc. Therefore, for onion production, it is very difficult to give general recommendation to be applied uniformly in all agro ecologies of the country (UAAIE, 2001). Gupta et al. (1994) and Lemma and Shimeles (2003) suggested that to optimize onion productivity, full package of information is required for each growing region of the country.

Similarly, the success of onion production is also depend on soil nutrients. Different levels of nutrients affect the yield and taste of the bulbs even within a variety.Application of nutrients play a major role in increasing productivity of onion. Onions are weaker than most other crop plants in extracting nutrients from the soil, especially the immobile types because of their shallow and unbranched root system; hence they require and often respond well to addition of fertilizer (Brewster, 1994). Nitrogen and phosphorus are often referred to as the primary macro nutrients because of the probability of plants being deficient in these nutrients and because of large quantities taken up from the soil relative to other essential nutrients(Marschner, 1995).

Generally, there are a number of constraints that cause low productivity of onion in Ethiopia. The low yield of onion in the country is reported to be due to low fertility of soil, inappropriate fertilizer rate, lack of improved varieties, and poor management practices (Lemma and Shimelis, 2003). Among these constraints, inappropriate use of mineral fertilizers, lack of improved and adaptable varieties and un proper plant spacing are the most limiting factors in our mandate areas.

Therefore the present study was conducted with general objectives of identifying adaptable onion varieties, assessing the effects of nitrogen and phosphorus fertilizer rates and different inter and intra row spacing on growth, yield and yield components of onion in the study area.

**Objective**

To investigate the performance of different varieties of onion & identify the best agro ecologically adaptive, high yielder and disease resistance onion varieties to the area

## II. METHODOLOGY

The experiment was conducted at Tselemti Wereda Shire-Maitsebri (SMARC) experimental Station during 2007-2009E.c off season periods. Four improved onion varieties namly: Bombeyred, Adamared, Nasik red & Nafis including Shendi (imported from Sudan),were evaluated for their yield performance for the last three years. The field experiment was laid out in Randomized Complete Block Design (RCBD) with four replications. Accordingly, treatments were assigned randomly to the experimental plot within a block.

A plot size of 2 x 3 m (6 $m^2$) was used. The blocks were separate by 1.5m, whereas plots within a block were 1m apart from each other. Each plot consists of 5 rows of 3m length, with a spacing of 40cm between rows & 10 cm between plants. Recommended amount of fertilizer (200kg/ha DAP & 100kg/ha Urea) were used. All management practices (ploughing, cultivation, watering, nursery and transplanting method, weeding and others) were applied uniformly to all plots as per standard recommendations for the crop. The experimental area was kept weed free by hand pulling throughout the cropping season. In addition to the field evaluation, simple postharvest evaluation also conducted for all the varieties. For this purpose 6kg representative bulbs were selected from each

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variety and kept under normal storage condition to compare their storability.

**Method of Agronomic Data Collection**

All data relating to yield and yield components were collected from the central three rows by excluding plants from either end of the rows. For the purpose of crop data collection two (2) plants/row or six(6) plants/plot were selected randomly from each plot and observations on growth, yield and yield components of the crop such as: plant height, average leaf length, average leaf number, average neck thickness, average bulb length, diameter &yield were recorded.

**Plant height (cm):** Plant height was measured from the ground level up to the tip of the
Longest leaf using ruler. Plant height of sex randomly selected plants were measured in the
central rows of each plot at physiological maturity stage of the crop and the average was
Computed.

**Days to physiological maturity:** It was registered on plot basis as the actual number of days from date of transplanting to when about 75% of the leaves fell down and 2/3 leaves had turned yellow

**Number of leaves per plant:** The number of fully developed leaves of six randomly selected plants was counted at the active green leaf stages and the average was computed to obtain number of leaves per plant.

**Leaf length (cm):** Leaf length was recorded as the average length of the longest leaves in six randomly selected plants at maturity.

**Bulb diameter (cm):** Bulb diameter was measured at right angles to the longitudinal axis at the widest circumference of the bulb of six randomly selected plants in each plot using veneer calliper (Saud et al., 2013) at harvest.

**Bulb length (cm):** Bulb length was the vertical average length of the matured bulb of six randomly selected plants in each plot which was measured by veneer calliper.

**Bulb neck thickness (cm):** The average neck thicknesses of six randomly selected plants in each plot were obtained by measuring the neck of bulbs at the narrowest point at the junction of bulb and leaf sheath using a veneer calliper.

**Marketable bulb yield (t /ha):** Marketable bulb yield was determined after discarding the unmarketable bulb, weight healthy bulbs and having nationally accepted marketable bulb weight of 60 g (Tegbew, 2011) at harvest in each plot and converted to t /ha.

For postharvest evaluation six kg of onion from each variety were weighed and stored in a conventional storage room. Weight loss and number of sprouted bulbs were recorded in every two weeks for about 3months, then finally at the end of the 3rd month weight and number of un sprouted bulbs were recorded and tried to compare the storability of each variety.

**Method of Data Analysis**

All crop data collected in this study were subjected to two way statistical analysis of variance (ANOVA) following a procedure appropriate to a randomized complete block design as suggested by (Gomez and Gomez, 1984).When the treatment were significant, least significance differences (LSD) by Dunken's multiple range comparison were used for mean separation at p=0.05.

## III. RESULTS & DISCUSSION

The data in (Table 1&2)showed a significant ($p<0.05$) variability between the varieties in most of the traits.

**Days to Bulb Maturity**

Days to maturity was significantly ($P < 0.05$) influenced by variety. Bombay Red and Shendi matured significantly earlier than the other varieties at about 124.9 & 127 and the next variety at about 134.2 days, whereas Nasik variety matured in about 16.8 days later than Bombey red (Table -1). The variation of maturity among onion varieties might be due to their genetic differences. Bombay Red variety was found to be the earliest,which matured 23 days earlier than Adama Red followed by Melkam which was earlier by 18 days than the Adama Red (Yemane et al., 2014).Similarly Azoom et al. (2014) also reported significant differences among eight onion varieties for days to bulb maturity. Bombay Red and Adama Red matured by less than 120 or/and in between 110 to 130 days, respectively (EARO, 2004).

**Leaf Length**

The analysis of variance revealed a significant ($P < 0.05$) effect of variety on leaf length. Both Nasik Red and Adama red showed the highest mean leaf length (40.27cm & 39.25cm) .Nasik red had significantly higher leaf length by about 12.2% than Bombay Red(currently dominant variety in the testing area (Table -1). The difference of varieties in leaf length might be due to their differences in genetic makeup. In agreement with the current result, Yemane et al. (2014) reported that Adama Red (40.75 cm) showed higher leaf length than Melkam (37.83 cm) and Bombay Red (35.17 cm). Similarly, Mondal et al. (1986), Ghafoor et al. (2003) and Jilani et al. (2010) also reported the differences among cultivars with respect to leaf length.

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**Plant height**

The analysis of variance showed that plant height was significantly (P < 0.05) affected by Variety. The plant heights of Nasik red, Nafis and Adama red varieties attained maximum height of (45.9,45.25 &42.9 cm ) respectively, which was significantly different than Bombay Red and Shendi variety(Table-1).

Although they have grown in the same environment the difference in plant height among the onion varieties could be due to the difference in their genetics make up that was differently influenced by the environment. Tegbew (2011) indicated the mean plant height of Adama Red (62.25 cm) cultivar was significantly higher than Bombay Red (56.04 cm) cultivar. The result was similar to the finding of Ghafoor et al. (2003) and Yemane et al. (2014) who indicated the presence of significant differences among onion cultivars in plant height.

*Table.1: Effect of variety on days to maturity, plant height, number of leafs per plant and leaf length of onion*

| S/N | Variety | DM | Pht (cm) | ALN | ALL(cm) |
|---|---|---|---|---|---|
| 1 | Nasik red | 141.7c | 45.90a | 10.881a | 40.27a |
| 2 | Shendi | 127.0a | 37.16b | 8.456 c | 33.56c c |
| 3 | Bombey red | 124.9a | 30.23c | 8.920bc | 28.08 d |
| 4 | Nafis | 134.2b | 45.25a | 10.057abc | 39.52ab |
| 5 | Adama red | 140.9c | 42.90a | 10.599ab | 36.69bc |
| **Mean** | | **133.73** | **40.29** | **9.78** | **35.62** |
| **CV (%)** | | **3.4** | **12.7** | **20.2** | **11.0** |
| **LSD** | | **6.402** | **7.309** | **2.816** | **5.594** |

*;Means with in the same column followed by the same alphabets do not differ significantly at the 5 % level of significance:DM=Days to maturity;pht=plant height;ALN=Average leaf number;ALL=Average leaf length;*

**Marketable bulb yield**

The variety had significant effect (P < 0.05) on marketable bulb yield. Nasik red had significantly higher marketable bulb yield (35588Kg/ha) than Bombay Red and Adama which are the commonly produced varieties in the area (Table-2). A cultivar may performs differently under diverse agro-climatic conditions and various cultivars of the same species grown even at the same environment with different management often yield differently due to the genetic makeup of the cultivars and the interaction effects of genotype x environment or genotype x management (Yemane et al., 2013). Gautam et al. (2006) indicated that yield of fresh onion bulb was significantly affected by varieties.In agreement with this finding Rajcumar (1997), Jilani (2004) and Geremew et al. (2010) also reported significant difference within varieties for marketable bulb yield in onion.

*Table.2: Effect of variety on Bulb diameter, Bulb length, Neck thickness and Marketable yield of onion*

| S/N | Variety | ANT(cm) | ABL(cm) | ABD(cm) | MY(Kg/ha) |
|---|---|---|---|---|---|
| 1 | Nasik red | 1.424 | 5.546a | 5.816 | 35588.0a |
| 2 | Shendi | 1.207 | 5.674a | 5.992 | 26119.6b |
| 3 | Bombey red | 1.323 | 4.899 b | 6.039 | 30005.1ab |
| 4 | Nafis | 1.319 | 5.637a | 5.863 | 26362.6b |
| 5 | Adama red | 1.322 | 5.436a a | 5.586 | 26099.4b |
| **Mean** | | **1.319** | **5.438** | **5.859** | **2883.94** |
| **CV (%)** | | **27.3** | **7.6** | **8.9** | **27.4** |

*ANT=Average neck thickness;ABL=Average bulb length;ABD=Average bulb diameter;MY=Marketable yield*

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**Postharvest performance of the varieties**

In addition to the agronomic performance simple postharvest evaluation was also carried out to evaluate the storability potential of each variety. For this purpose similar amount (weight) of onion from each variety was store for three months at a normal room temperature and the trial was repeated for two (2) years. Data like Number of sprouted bulbs, Weight of non -sprouted bulbs, Total weight loss e.t.c was recorded in every two weeks for about three months. The mean result showed that, there was a big difference in weight loss among the different onion varieties (Tabel-3&4).Maximum weight loss of (100%)& 97% was recorded at Adama red in the 1st and 2nd year respectively followed by bombey red. Among the tested varieties minimum weight loss

Was recorded from shendi. 60% in the 1st year and 40% in the seconder were loss from the total initial weight in the three month period. This indicates that varietal difference have a great role in the postharvest and quality of onion bulbs (Table-5&6).

*Table.3: simple postharvest performance of the different onion varieties (2007 cropping season)*

| S/N | Variety Name | Initial no.bulbs | Initial wt.(Kg) | Wt. No sprouted | | | Total Wt.loss | Total Wt.loss (%) |
|---|---|---|---|---|---|---|---|---|
| | | | | 1 ST M | 2 nd M | 3rd M | | |
| **1** | Shendi | **43** | **3.5** | **2.5** | **1.8** | **1.4** | **2.1** | **60** |
| **2** | Nafis | **38** | **3.5** | **2.7** | **1.4** | **0.29** | **3.21** | **92** |
| **3** | Adama red | **47** | **3.5** | **2.7** | **0.89** | **-** | **3.5** | **100** |
| **4** | Nasik red | **43** | **3.5** | **2.9** | **1.4** | **0.4** | **3.1** | **88.5** |
| **5** | Bombey red | **55** | **3.5** | **2** | **0.53** | **0.1** | **3.4** | **97** |

*Table.4: simple postharvest performance of the different onion varieties (2008 cropping season)*

| S/N | Variety Name | Initial no.bulbs | Initial wt.(Kg) | Wt. No sprouted bulb | | | Total Wt.loss | Total Wt.loss (%) |
|---|---|---|---|---|---|---|---|---|
| | | | | 1 ST M | 2 nd M | 3rd M | | |
| **1** | Shendi | 70 | 6 | 5.05 | 4.54 | 3.58 | 2.42 | 40 |
| **2** | Bomboy red | 62 | 6 | 5.35 | 4.56 | 2.03 | 3.97 | 66 |
| **3** | Adama red | 63 | 6 | 4.316 | 1.701 | 0.545 | 5.455 | 90 |
| **4** | Nasik red | 56 | 6 | 4.95 | 3.995 | 1.307 | 4.693 | 78 |
| **5** | Nafis | 60 | 6 | 4.2 | 2.28 | 0.88 | 5.12 | 85 |

## IV. SUMMARY AND CONCLUSION

Onion is widely recognized as an important vegetable condiment as a form of dry bulb and cash crop in Ethiopia. It is successfully produced under rained as well as irrigated conditions in different agro ecologies of the country by small holder farmers and commercial growers. However, the productivity of onion is not as expected due to many production constraints. The use of lower yielder and un adaptable varieties is among the many production constraints. So, selection of varieties that produce high yield is very critical to improve the yield of onion.Therefore; this study was conducted to evaluate the agronomic and postharvest performance of five onion varieties. The experiment was conducted at Shire-Maitsebri Agricultural Research center, Tselemtiworeda, North western Zone of Tigray during 2007-2009 under irrigation. The experiment was laid out in randomized complete block design (RCBD) with four replications. Data were collected for phenology of the crop, growth, yield and yield components and analyzed accordingly.

The analysis of variance revealed the significant effect of variety on all the parameters exceptneck thickness and bulb diameter. Nasik red variety had superior plant height, Leaf number, leaf length, bulb diameter and marketable bulb yield than the other tested varieties. Nasik red had higher marketable bulb yield by about 18%& 36.35% than the dominantly produced bombey red and Adama red varieties respectively.

Regarding to the postharvest performance of the onion varieties, Minimum weight loss was recorded from Shendi variety followed by Nasik red variety. Total weight loss at

the end the three month storage was recorded from the commonly produced varieties.

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# Production of Biogas from Organic Waste and its Utilization as an Alternative Energy Source

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***Abstract—*** *As a result of increase in human need for energy, the source of a new energy is necessary to replace the role of fossil fuel whose existence is beginning to scarce. The organic waste used for the production of biogas is an alternative energy, allowing reducing environmental pollution. The biogas test has been done by using digester fixed dome type made of fiberglass capacity of 5.5 $m^3$, equipped with an inlet for introduction of biogas raw material and an outlet for the release of residual biogas fermentation and an elbow iron mixing road. A soft PVC gas holder has capacity of 5.6 $m^3$. The digester is filled by organic wastes namely cow dung and grasses. Cow dung and water is added at the ratio of 1:2, then after methane production was stable, filling with grasses mixed with water. The results of the test show that organic waste get to produce biogas in blue flame, to be used as fuel to cook, to operate gas generator and for a infra red drying fuel. The Water Boiling Tests show that thermal efficiency is 57.9%, the fire power, 4.0173 watts, the burning rate 0.0688 gram/minute, the specific fuel consumption, 0.1248 kg/hour.*

***Keywords— agitated digester, biogas production, organic waste, power generation, infra-red dryer***

## I. INTRODUCTION

The increasingly less reserve of petroleum is leading to increase in prices of refined fuel oil. Given increase in prices of refined fuel oil attributable to the upsurge of the world oil costs, the government is encouraged to deal with energy issues. One of efforts to tighten the refined fuel oil is to seek renewable source of alternative energy.

Most of need for fuel for low income population is satisfied by firewood, dried ups, and they are repeatedly chopping down trees in off-limits forests, thereby making natural conservation around the forest area in gradually danger. Based on the matters, it is necessary to try to use a source of renewable alternative energy. Biogas is a source of alternative energy having been developed, made out of diverse organic wastes by means of an anaerobic decomposition process. In general, the type of the resulting waste may be divided into two fold : one is organic waste consisting of kitchen waste, traditional market rubbish, livestock feces, agroindustrial waste, garden rubbish, agricultural waste and plantation. The second type is organic waste in the form of glass bottles, paper, cans, and plastics. The organic waste volume is, on average, more than inorganic waste, covering 60-70% of total waste volume (1).

Based on information from the Ministry of Environment, each individual produces, on average, 0.8 kg of waste per day. The average waste per person will continue to increase with the improved well-being and lifestyle. Assuming 220 million people in Indonesia, the waste discharged is at the rate of 176,000 tons per day, some 105,600 – 123,200 tons of them are organic waste.

So far, the management of organic waste is even using conventional techniques such as open dumping system management to the landfill, making it compost, burning it, or dump it into the river. The management of waste using those techniques tends to be environmentally less friendly and economically less valuable. The management in the open dumping system frequently creates new problems; i.e., generate pollutant gases such as $H_2S$ and $NH_3$. The management of waste made into compost tends to be economically less valuable and burning it will cause the environmental pollution and respiratory troubles for humans. The management of waste by discarding it into the river will have direct impact being source of human diseases such as skin diseases and infectious diseases, while the indirect impact is a cause of the flood.

Looking at the drawbacks of such techniques, a more environmentally friendly management technique that able to produce products of high economic values is necessary. For this purpose, the management of organic waste as a source of alternative energy should be applied. A potential method is by applying anaerobic technology to the production of biogas.

Biogas technology was introduced in the 1980s; however, until now the development has not been encouraging, the existing obstacles include high construction costs, biogas digesters are not functioning due

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to leakage, requiring manual management (feeding/remove the stuffing of digester).

Biogas is gas produced from the fermentation of organic materials by anaerobic bacteria (bacteria being exist in anaerob conditions). The components of biogas are $CH_4$ 50-70%, $CO_2$ 30-45%, $N_2$ 0.2%, $H_2S$ 500 ppm, and $0_2$ < 2% (2). Biogas is a fuel such like LPG which can be used for cooking and for power energy plant. Since it has a calorific value about 5,000-6,513 kcal/$m^3$ (3), the biogas is a source of environmentally friendly and renewable energy. The biogas digester output is slurry or residual sludge of fermentation which is useful as organic fertilizer for agricultural or plantation activities.

This study is designed to apply the technology to the production of household-scale biogas by utilizing organic waste derived from garden waste.

## II. METHODOLOGY

### A. *Biogas Digester*

Fixed dome digester used is made of fiberglass, 2.2 meters in height, 1.8 meters in diameter, capacity of 5.5 $m^3$. Fixed dome is a most popular model in Indonesia, where the installation of digester 3/4 is embedded in the ground, allowing the conservation of space, maintains the stability of digester temperature, and support the growth of methane bacteria.

Digester is equipped with an inlet for the introduction of biogas raw materials and an outlet for discharging the fermentation of residual biogas, made of PVC pipe, inlet of 31 mm in diameter and 19 mm in height spliced to PVC pipe of 16.5 mm in diameter, 30 mm in height, outlet of 4" in diameter, 22 mm in length. The outlet is operated based on the principles of hydrostatic pressure equilibrium.

Digester is equipped with a mixer, made of angle iron, 2 meters in height, and 1.5 meters in diameter. The purposes of the mixing are to prevent scum from formation, to reduce sedimentation, and to improve productivity. In addition, the mixing is generating exactly contact between a substrate and a population of bacteria, and produce a homogeneous condition and keep solid matters in suspension (4). Digester 2/3 is embedded in the ground, allowing the conservation of the land, thereby making the charging of the raw material easier and the temperature more stable.

### B. *Agitated* Tank Biogas Material

Cow dung is collected in a plastic bag at capacity of 200 liters equipped with a mixer made of PVC pipe. The purpose of the mixing is to admix the whole organic waste – cow dung and water – to make the anaerobic digestive process faster. Manual mixing is made by spinning the mixer.

### C. *Safety* Valve

This digester is equipped with a safety valve for regulating the gas pressure in the digester. It is made of PVC pipe, 3" in diameter, and 22 mm in height. This safety valve is using the principles of T pipe. When the gas pressure in the pipeline is higher than the water column, the gas will be coming out through the T pipe, allowing the reduction of the pressure in the digester.

### D. *Gas* Holder

Gas holdet is made of soft PVC, 3.2 meters in length, 1.5 meters in diameter, and a capacity of 4 $m^3$. Digester is connected to the gas container by plastic tubing. Gas outlet is made of ½ inch plastic tubing. The gas container is placed on a height of 1.5 meters from the surface of the ground. The biogas is distributed by using the plastic tubing to the biogas stove. Furthermore, the biogas took in the gas container is distributed by using the plastic tubing to be used as fuel for cooking and generator to produce electricity.

### E. The Filling up of Raw Material Biogas

Digester is filled up by cow dung and water is added. The cow dung is coming from cattle breeding of SMK II (STMPER) and Cikole. The water is added to the cow dung at a ratio of 1: 2 in order to obtain a dry weight about 9%. After the full filling up of digester has been completed and methane has been produced, the digester is filled with grasses and water is added at a ratio of 1:2.

### F. *Measurement of Biogas Production*

The biogas produced is measured by a gas meter in specifications as follows: $Q_{max}$ 6$m^3$/h, $Q_{min}$ 40 $dm^3$/h, $P_{max}$ 50 kPa, V 0.7 $dm^3$. While the gas meter used to measure the use of biogas for biogas stove and gas generator are as follows: $Q_{max}$ 3 $m^3$/h, $Q_{min}$ 16 $dm^3$/h, temperature -20ºC + 50°C, $P_{max}$ 1.5 bar, V 1.2 $dm^3$.

### G. *Utilization of Biogas for Cooking*

The biogas produced is used as fuel for cooking by using biogas stoves. To take advantage of biogas as a fuel stove required air pump to increase the pressure biogas. Air pump used has the following specifications: LP 60; 220 V/240 V; frequency, 50-60 Hz; output, 70 liters/min; power, 60 watts; and pressure, 0.04 mPa, is necessary.

### H. *Utilization of Biogas to Run a Gas Generator*

The biogas produced is used to run a gas generator. Gas generator used has the following specifications : AJP 4000 E-type; rate voltage, 220 V; frequency rate, 50 Hz; rate output, 2.5 KVA; maximum output, 3.0 KVA; power generator, 3,000 watts.

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*I. Utilization of Biogas to Run The Far Infrared Dryer*

Biogas is used as fuel to run the dryer with the following specifications (5) : the type of far infrared dryer tray cabinet, dimension of length 2 meters; wide 2 meters; and height 2 meters.

Parts of wall made of styrofoam with a 40 mm thick insulating material to withstand the heat out of the dryer due to the heat transfer by conduction. Th inside styrofoam coated 304 stainless steel plate thickness of 1 mm as a reflector of electromagnetic radiation, while the outer walls using patterned alumunium plate orange peel with thickness of 0,8 mm. Floor section using T block with 20 mm thick and using the same layer as the walls. Dryers have two pieces of fan in the front and back, 1 piece exhaust fan, 2 piece of of intake air circulation holes in the door, 2 shelves and 1 control potel. Fans are used to flatten the hot air in the drying chamber, while the exhaust fan is used to absorb water vapor out of the drying chamber. As the type of heating used gasolec S8 as easily available and suitable for LPG and natural gas. Gasolec has a capacit 3,5 kw/hour with an operating pressure 350 – 1400 mbar (6). Infrared dryer is used to run the compressor with the following specifications maximum working pressure 9 kg/cm$^2$, water test pressure 14,7 kg/cm$^2$, capasity 22 l.

*J. Testing the methane content*

Testing the methane content is done simply by means of flame.

*K. Water Boiling Test*

The water boiling test is designed to determine the capability of biogas. The test is established in a biogas stove at room temperature using biogas to boil 2 liters of water in a pot. Once the water in the first pot is boiling, the test is done by replacing the pot in the second phase. The Water Boiling Test is delivering data on thermal efficiency, fire power, burning rate and specific fuel consumption (7).

*L. Thermal Efficiency Test*

Efficiency is the percentage of usable heat than the heat generated by a cookware during the test, the equation used is as follows (7) :

$$\eta_{overall} = \frac{(m_w.c_p + m_{pa}.c_{pa})(T_2 - T_1) + m_s.H_{fg}}{m_f.E}$$

Note:

$\eta$ = Overall efficiency of gas burner
$m_w$ = Mass of water under heat (kg)
$m_{pa}$ = Mass of pot of water under use (kg)
$c_p$ = Heat of water type (kj/kg)
$c_{pa}$ = Heat of pot type (kj/kg)
$T_2$ = Temperature of boiling water ($^o$C)
$T_1$ = Initial temperature of water ($^o$C)
$m_s$ = Mass of water under evaporation (kg)
$m_f$ = Mass of fuel under application (kg)
$H_{fg}$ = Latent heat of water evaporation ($^o$C)
E = Low caloric value of fuel (kj/kg.bb)

*M. Fire Power Test*

This test is designed to determine the amount of power generated by a stove to cook. The power is derived from the multiplication of the mass of the fuel by caloric value of the fuel divided by time. Thus, the power generated by a stove is derived from the mass of the fuel under application and the caloric value of fuel (biogas) and the length of time to cook (7).

To determine the amount of fire power, the following equation is used:

$$P = \frac{m_f.E}{\Delta t}(KW)$$

Note:

P = Fire power (KW)
$m_f$ = Consumption of fuel during time t (kg)
E = Low caloric value of fuel (kj/kg).
$\Delta t$ = Time of testing (second)

*N. Burning Rate*

This is a measure of the rate of fuel consumption while bringing water to a boil. It is calculate by dividing the equivalent fuel by the time of the test (7).

$$R_{cb} = \frac{f_{cd}}{\Delta t_c}$$

Note :

Rcb = Burning rate (grams / minute)
$f_{cd}$ = Biogas consumed (grams)
$\Delta t_c$ = Time to boil (minute))

*O. Specific fuel consumption*

Specific fuel consumtion can be defined for any number of cooking tasks and should be considered the fuel required to produce a unit output, wether the output is boiled water. In the case of the cold start high power Water Boiling Test, it is a measure of the amount of biogas required to produce one liter of boiling water starting with cold stove (7).

$$SC_h = \frac{f_{hd}}{P_{ht} - P}$$

Note :

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$SC_h$ = Specific fuel consumption (grams fuel / grams water)
$f_{hd}$ = Equivalent biogas consumed
$P_{ht}$ = Weight of pot with water before test (grams)
P = Weight of pot with water after test (grams)

## III. RESULTS AND DISCUSSION

Based on the results of the chemical analysis, cow dung have chemical composition as follows: pH, 8.0; the content of water, 76.25%; total nitrogen, 1.38%; total organic carbon, 34.42%; phosphate, 1.6%; and C/N ratio, 25. While the chemical composition of grasses as follows the content of water, 80,82 %; total nitrogen, 1.76 %; total organic carbon, 87,95 %; phosphate, 0,92 %; and C/N ratio, 49,9.

The optimum C/N ratio for the methane forming organism is 25-30 (8). If the C/N ratio in the material is high, it will be making the process of radical changes longer, leading to less production of methane. According Jewel (1982) when the amount of C in the material is very much high C/N ratio), the N will be ran out in advance, so that C are left in great quantities, thereby making the bacteria cease from active. The balance of carbon (C) and nitrogen (N) in the organic substance will be simply determining the microorganisms living and activities (9). A factor affecting the C/N ratio value of cow dung is the feeding of woof. To achieve ideal C/N ratio, the weeds need to be added. Carbon in carbohydrate and nitrogen in protein, nitric acid, ammonia, are the main substances for anaerobic bacteria. Carbon is used as energy and nitrogen to build structural cells of the bacteria.

The content of water in cow dung used as a raw material for production of biogas is 76.25 % and dried matter is 23.75%, while grasses the content of water is 80,82 % and dried matter is 19,12 %. In the processing of cow dung as input for digester, the cow dung and grasses is diluted at a ratio of cow dung / grasses and water = 1:2. The use of water is twice more than the amount of cow dung / grasses under mixing, because of the content of dried matter is high and methane bacteria needs water in large quantities for the process of biogas formation. According Kim (2011) in Triakuntini et al. (2012), the normal activity of the methane microbe requires about 90% of water and 7-10% of dried matter and fermentation input (10). This condition can be realized by using dilution by water at a ratio of 1:1 or 1:2. If the water is every little, the acetic acid would be accumulated; thereby inhibiting the fermentation process and a crust is formed and, in turn, hinder the gas formed into the surface.

pH of cow dung is 8.0, this value is qualifying for the production of biogas; according to Van Lier in Zhao (2011) the anaerobic microbe is active at optimum pH of 6.5 – 8 (11). If pH is below 6.5, it can be toxic to the methane forming bacteria; otherwise, it will be leading to the end product, $CO_2$, as the main product. Rather, when the pH is more than 8, it can be inhibiting microbe. The pH value during the observation, the inlet around 7 to 7.48, whereas at the outlet from 7 to 7.34.

### *A. The Temperatures of Digester and Ambient*

Temperature is one of important factors in the process of biogas fermentation, as the temperature affects the optimal development of biogas forming microorganism. As ESCAP argues it, the temperature is essential for the process of fermentation, as it is related to the ability of bacteria in processing biogas. Optimum ambient temperature is ranging from 30-35°C (8).

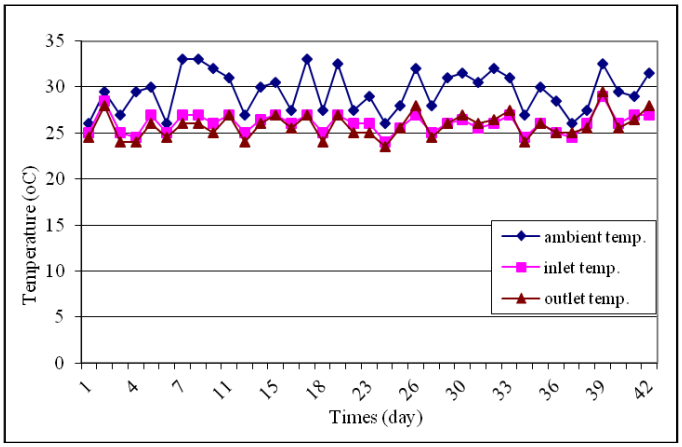


*Fig.1: Ambient temperature and the temperature of stuffing in the digester during observation*

As shown in Figure 1, the temperatures of inlet digester is in the range of 24.5-28.5°C, outlet, 24-28°C, and the ambient, 26-33°C, including the optimal temperature of digestion for biogas forming bacteria. According Rouf (2011), the optimum methanogenic bacteria are active at temperatures between 25-30°C (12). According Price (1981), microorganisms response to temperature changes in temperature cause in reaction speed, change in population of bacteria causes selection or mutation in the microorganism (13) .

### *B. Production of Biogas*

The production of biogas is starting at 1 day after the full charging of digester and gas may be burned; eventhough the flame is yellowish blue in color and the pressure of gas is still very small and, therefore, can not be used as fuel. Biogas can be used as fuel at day 4, on the condition of the gas holder contains half. This hows that the biogas containing methane gas is high enough to be used as fuel. The production of biogas shows an increase until day 12, as shown in Figure 2. On the 13rd day, the biogas has reduction. This reduction is indicative of the organic waste

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need to be charged into the biogas digester, resulting in longer increase biogas production, because the substrat that has been fermented to be replaced by new waste still fresh. Charging raw material once a week, in the first week until week three of charging using com manure. Charging grasses done from week 4 until stable biogas production. Production of biogas from cow dung is higher than the grasses. Production of biogas from cow dung in the second week of 10.156 $m^3$, in week 3 of 9.208 $m^3$. While production biogas from the grasses at week 4, 9.775 $m^3$, week 5 amounted to 9.904 $m^3$ and the 6 th week increase to 7.423 $m^3$. This is due to cow dung containing the C/N ratio better than the grasses at 25, while the grasses at 49,9. Production of biogas average per day to 1.383 m3 of cow dung, to grasses of 1.291 m3. Arifin research results (2011) conducted in boarding Saung Balong Majalengka showed that the production of biogas from cow dung of 1.51 $m^3$ per day (14).

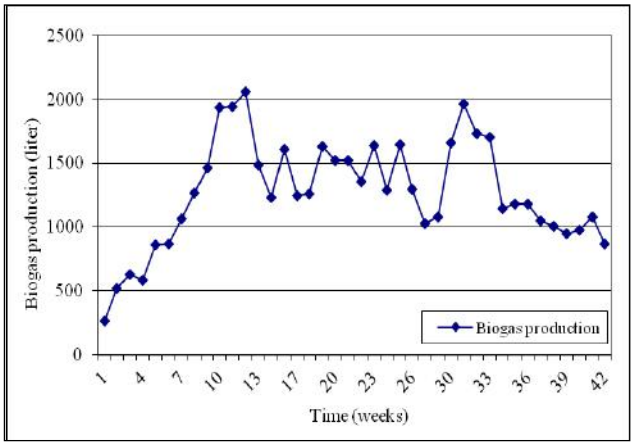


*Fig.2: Biogas production during the observation*

The content of methane is put to the simple flame test. The biogas may be burned properly if the content of methane has reached 57%, generating the blue flame (15). Meanwhile, according to Hessami in Alpen Steel (16), the biogas is well inflammable if the content of methane has reached at least 60%. According to Jewell (1982) the color of flame generated by the combustion of biogas may be used as an indicator to determine the content of $CO_2$ in the biogas (9). The blue flame is indicative of high methane. The yellow flame indicates the content of $CO_2$ is more than normal; i.e., it is more than 48%. When the gas is not inflammable, the content of $CO_2$ is extremely high.

The formation of biogas by the anaerobic fermentation is a process consisting of three stages: hydrolysis, acydogenesis, and methanogenesis. The first is the hydrolytic process by which the organic materials such as carbohydrate, lipid and protein are degraded by hydrolytic microorganisms to be such dissolved compounds as carboxylic acid, keto acid, hydroxyl acid, ketone, alcohol, simple sugar, amino acid, $H_2$ and $CO_2$. Organism having a role in the fermentation is *Bacteroides ruminicola*. The reactions are as follows (17) :

$$C_6H_{12}O_6 + 2H_2O \longrightarrow 2CH_3COOH + 2CO_2 + 4H_2$$
(acetic acid)

$$C_6H_{12}O_6 \longrightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$$
(butyric acid)

$$C_6H_{12}O_6 + 2H_2 \longrightarrow 2CH_3CH_2COOH + 2H_2$$
(propionic acid)

In the next stage, acydogenesis or acidification, the dissolved compounds are converted into fatty acid in short chain which are, generally, acetic acid and formic acids. Also, it is changing a low molecular compound molecular into alcohol, organic acid, amino acid, $CO_2$, and $H_2S$ by acydogenic microorganism of desulfovibrio genus. The reactions are as follows (18):

$$CH_3CH_2COOH \longrightarrow CH_3COOH + CO_2 + 3H_2$$
(acetic acid)

$$CH_3CH_2CH_2COOH \longrightarrow 2CH_3COOH + 2H_2$$
(acetic acid)

The last stage is methanogenesis, by which fatty acids in short chain is modified into $H_2$, $CO_2$, and acetate. Acetate will be undergoing decarboxylation and reduction of $CO_2$; afterwards, it is together with $H_2$ and $CO_2$ producing the end products, methane and carbon dioxide. Organisms having role are methanogenetic bacteria, for examples, *Metanobacterium formiciccum, Metanobacterium mobile, Metanobacterium ruminantium, Metanobacterium sohngenii, Metanobacterium prpionicum, Metanobacillus omelianski, Metanococcus mazaei, Maethanococcus vanbiellii, Metanosarcina metanica* (8). The reactions are as follows:

$$CH_3COOH \longrightarrow CH_4 + CO_2$$
$$2H_2 + CO_2 \longrightarrow CH_4 + 2H_2O$$

*C. Biogas application test as fuel for cooking*

To turn on biogas stove need to use air pump to suck the biogas into the gas burner. The specifications of air pump used are as follows: LP 60, 220 V/240 V, 50-60 Hz frequency, 70 liters/ min output, 60 watts power, 0.04 mPa pressure. The results of biogas test as a fuel for cooking shows that consumption of biogas for the use of gas burner is 580-800 liters per hour, depending on the size of the flame. Researches by Martono (19) showed that consumption of biogas for the use of gas burner is 200-450 liters/hour, while for the large gas burner is 1000-3000 liters/hour. Meanwhile, the researches by Widodo (20) suggest that a gas burner need for 300 liters/hour to flare up at a pressure of 75 mm $H_2O$. Biogas produced is blue in color, odorless, and does not emit smoke. This is evidence of shrinking the biogas accommodating plastic

representing decline in the composition of methane. Meanwhile, when the content of methane is high, the biogas accommodating plastic will be inflated and the burst of fire in the biogas stove is nice and blue in color.

*D. Water Boiling Test*

The Water Boiling Test is designed to determine the combustion power of biogas under test. The results of the Boiling Water Test suggest the consumption of biogas to boil 2 liters of water is 143 liters; the time required is 9 minutes and 14 seconds, from the initial temperature of 28°C to the final temperature of 99°C, no any soot in the bottom of pot. In light of the time to boil the water, the biogas can compete with other fuels such as kerosene. To cook 2 liters of water requires 9 minutes and 52 seconds, from the initial temperature of 21°C to the end temperature of 96°C, resulting in soot in the bottom of pot. Meanwhile, to cook the water using LPG take 6 minutes 29 seconds, from the initial temperature of 23°C to the final temperature of 98°C, no any soot in the bottom of pot. The Water Boiling Test generate thermal efficiency of 22.9% - 83.3%, the average is 57.9%, the fire power is 1.79 - 2.71 KW, the average is 2.21 KW, the burning rate is 0.0688 grams/minute, and specific fuel consumption of biogas is 0.359 - 1.078 kg/hour, the average is 0.1248 kg/hour. Syamsuri et al (2015) research result showed that the test using burner diameter of 2 to 4 was obtained power fire of 0,4744 to 0,55 KW, the fire power of 1,21 to 2,052 KW, thermal efficiency of 56,81 to 61,64 % (21). While Sudarmanta (2012) research results showed that the specific fuel consumption of 0,3451 kg/hour, the thermal efficiency of 50,591 % (22). Thermal efficiency is the magnitude of energy received by a pot as compared to energy released by the combustion of biogas. The thermal efficiency of biogas produces satisfactory results.

The gas burner power is heat supplied by the fuel during the test. The equation of power showed the consumption of fuel is directly proportional to the capacity. Therefore, when the biogas stove has a big power, the consumption of fuel is high, as well. Rather, when the biogas stove has small power, the consumption of fuel is, of course, low.

*E. Gas generator test*

Biogas can be used as fuel to run a gas generator. The gas generator being used has a capacity of 3000 watts; the consumption of biogas is 1053 liters per hour. Researches by Martono (18) showed that, to run a diesel machine per bhp requires biogas some 420 liters/hour. According to ESCAP (8), 1 $m^3$ of biogas can run a motor 2 HP for 2 hours and generate 1.25 kwh electricity.

*F. Infra Red dryer test*

The biogas produced can be used as fuel for Infra Red dryer. Infra Red dryer has a wavelength of 25-1,000 μm or approaching microwave. The drying process by Infra Red technology is very efficient because the radiation of heat is directed through inner molecule and breaks the bond of water molecules in the material molecules without any intermediary medium (air) as did in the processes of convection and conduction. In the Infra Red, the resultant product have high quality in the efficient process than drying by convection and conduction, the dried product did not experience a significant change in color, the aroma of product is even strong. The infra red dryer capable of drying 12 kg of cassava slices of water content 57,12 % to 14,865 % during 3 hours for drying at a temperature of 60 $^oC$. The consumption of biogas is 5,484 $m^3$, whereas, when LPG is used, the consumption of fuel is approximately 1.5 kg.

## IV. CONCLUSION

The conclusion that can be drawn from the use of organic waste for the

Production of biogas is as follows: the production of biogas is improved with the time of observation, the content of methane is quite high, the biogas stove can be fired up in a blue flame, the gas generator and the far infra red dryer might be ran. The consumption of biogas for lighting up the biogas stove is ranging from 580-800 liters per hour, for the gas generator is 1757 liters per hour at 3000 watts, and for the Far Infra Red dryer of 2 m in length, 2 m in wide, and 2 m in height to dry 12 kg of cassava slices required about 2.653 – 3 $m^3$ of biogas. Biogas can be an alternative substitute of kerosene for day-to-day purposes.

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# Effect of Substitution of *Artemia salina* Protein by Soya Protein in *Clarias gariepinus* Larvae Compounded Diets: Growth, Feed Efficience and Survival

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***Abstract—*** *Artemia salina, the main first-feeding protein source of the catfish Clarias gariepinus larvae is relatively scarce and very expensive in Côte d'Ivoire and it raises the cost of catfish fingerlings production. To reduce the feed cost, feeding trial was completed with five isonitrogenous (35%) diets formulated by substituting artemia protein in control diet by soya protein at 25% ($SB_{25}$), 50% ($SB_{50}$), 75% ($SB_{75}$) and 100% ($SB_{100}$) level. Clarias gariepinus larvae initial body weight 0.0064 ± 0.001 g were stocked at 1 larvae $L^{-1}$ and fed with the experimental diets three times daily ad libitum for 49 days. At the end of the growth trial, diets $SB_{25}$ and $SB_{50}$ present similar growth with the control diet. The low growth recorded from fish fed $SB_{75}$ and $SB_{100}$ highly affected final biomass despite the best survival rate recorded. Best values of feed conversion ratio were recorded from larvae fed control diet followed by $SB_{25}$, $SB_{50}$. High levels of soya proteins in diets affect feed palatability and larvae growth, vigour, motility and reactivity. Compounded feeds $SB_{25}$ and $SB_{50}$ can be used us low cost Clarias gariepinus larvae diets without adverse effects on growth and survival compared of artemia control diet.*

***Keywords— Clarias gariepinus, larvae, soybean meal, growth, survival.***

## I. INTRODUCTION

Availability of quality feeds, feeding strategies and control of cannibalism are essential in *Clarias gariepinus* larvae growth and survival [1], [2], [3]. The lack of available low cost larvae feeds has continued to be a major constraint to the competitive catfish culture in Côte d'Ivoire [1], [4]. In fact, Artemia nauplii capsulated cysts which is currently used as protein source in catfish *Clarias gariepinus* larvae feed remains the major constraint in larvae feeding [5], [6], [7]. This protein source is hardly available and locally expensive (210.57 USD $kg^{-1}$) and it raises the cost of catfish fingerlings production [8]. To reduce the feed cost, the use of *Artemia* in catfish larvae diets must be reduced as suggested by Siddiqui and Ahmed [9]. This can be achieved by replacing *Artemia salina* proteins with alternative highly available soybean meal. Soybean meal has 45-50% protein content and is the better plant protein ingredient used as alternative protein sources in fish diets [10], [11], [12]. It is also the primary plant protein used in catfish diets in Africa due to the fact that soya is widely used for vegetable oil production which increases the locally available of soybean meal for animal nutrition [13]. Also imported soybean meal has good availability and locally and imported soybean meal are reasonable price (0.60-0.95 USD $kg^{-1}$) compared to *Artemia salina* nauplii. Results of lot of feeding trial have shown considerable success in partial or total inclusion of soy bean meal in catfish *Clarias gariepinus* larvae and the fingerlings diets [14], [2]. According Francis et al. [15], vegetable protein can substitute fish meal to supply required protein needed for good growth. However, inclusion levels of vegetal protein in diet and their effective utilization by fish depending to species and growth stage due to the presence of high crude fiber content and antinutritional factors [16], [15], [17], [18]. Consequently, high inclusion of vegetal ingredients in fish diets could cause slower growth rates, poor performance and high mortalities [13], [17], [19], [20]. For effective substitution of *Artemia salina* by soybean meal in *Clarias gariepinus* larvae diets it's essential to determine the optimal level of replacement which promotes growth and survival. This study assesses the effect of gradual replacement of Artemia proteins by soybean proteins in *Clarias gariepinus* larvae diets on growth performances, feeds efficiency and survival.

## II. MATERIALS AND METHODS

### *2.1 Experimental diets*

For feeding trail, control diet was formulated at 35% protein with *Artemia salina* as the main diet protein source without soybean meal. Then, four isonitrogenous diets were formulated at 35% crude protein by substituting *Artemia salina* in control diet by soybean meal based on crude proteins as follows: $SB_{25}$ = 25% of soybean protein replaced *Artemia salina* proteins; $SB_{50}$ = 50% of soybean protein replaced *Artemia salina* proteins; $SB_{75}$ = 75% of soybean protein replaced *Artemia salina* proteins; $SB_{100}$ = 100 % of soybean protein replaced *Artemia salina* proteins (Table 1). All diets were composed and produced with methodology described by Ossey et al. [19]. Nutritional compositions of diets were determined and all diets were stored at -20°C until use.

### *2.2 Biochemical Analysis*

The proximate compositions of experimental diets were determined according to AOAC methods [21]. Dry matter (DM) was determined after drying 5g of sample in an oven at 105°C for 24 hours until constant weight; crude protein (N=6,25) was determined by Kjeldahl method; crude lipid of sample was obtained by Soxhlet extraction with hexane; Ash was measured by incineration at 550°C for 24 hours in a muffle furnace, crude fibre were measured by acid digestion following by ashing dry residue at 550°C muffle furnace for 4 hours, while nitrogen-free extract (NFE) was calculated by difference. The gross energy contents of the diets were calculated based on their crude protein, lipid and carbohydrate contents using the energy equivalents of 22.2, 38.9 and 17.15 kJ $g^{-1}$ respectively [22]. Ingredients and chemical composition of the compounds diets are presented in Table 1.

### *2.3 Experimental Fish and Feeding Trial*

The experimental was carried out at the hatchery of the Centre de Recherches Océanologiques (CRO), Abidjan, Côte d'Ivoire. Three days-age *Clarias gariepinus* larvae initial body weight 0.0064 ± 0.001 g were transferred in aquarium (39. 40 cm × 50. 20 cm × 27. 00 cm), capacity of 50 L and acclimated four (4) days prior to beginning of the growth trial. Fish were counted and stored at density of 1 larva $L^{-1}$ in each aquarium. Three replicates were constituted by diet and the feeding trial was conducted in 15 aquariums. Fish were fed three times daily (07:00, 12:00 and 17:00 hours) *ad libitum* for 49 days [19]. Every day, dead fish of each aquarium were removed and counted. Once a week, 15 larvae were randomly sampled in each aquarium for total length and wet weight measured. Then, all larvae were weighed and feed ratio was adjusted to reflect the new fish biomass. At the end of experiment, all survival fish were collected, weighted, measured and counted. Missing fish were presumed to have succumbed to the cannibalism [23]. During growth trial, the average water temperature, measured twice daily was 29.32 ± 0.50°C, average dissolved oxygen content of water was 04.65 ± 0.60 mg/L and average pH was 07.18 ± 0.30.

### *2.4 Growth Feed Efficiency Parameters*

The growth and nutrient utilization parameters were calculated for each treatment as follows: weight gain (WG) (g) = final body weight – initial body weight; daily weight gain (DWG) ($gday^{-1}$) = final body weight – initial body weight / number of feeding day; specific growth rate (SGR) (%/day) = [ln (final body weight) – ln (initial body weight)] × 100/ number of feeding day; biomass gain (BG) (g) = final biomass – initial biomass; feed conversion ratio (FCR) = total weight of feed consumed (g) / biomass gain (g); total weight of feed consumed is obtained by total feed distributed fewer uneaten food; survival rate (SR) (%) = (final number of larvae / initial number of larvae)×100; cannibalism rate (CR) (%) = (number of larvae missing/initial number of larvae)×100; mortality rate (MR) (%) = (number of dead larvae/initial number of larvae)×100.

### *2.5 Statistical Analysis*

Data analysis was performed using Statistica 7.1 software. All data are presented as mean ± standard deviation (SD). Results were compared using ANOVA one-way analysis followed by the Tukey's multiple range test to compare differences among treatment means. Significant differences were considered at $p < 0.05$.

## III. RESULTS

Growth and feed efficiency parameters, cannibalism, mortality and survival rate of *Clarias gariepinus* larvae fed control diet and diets $SB_{25}$, $SB_{50}$, $SB_{75}$, and $SB_{100}$ are presented in Table 2.

### *3.1 Growth*

At the end of the growth trial, final body weight, weight gain, daily weight gain and specific growth rate recorded were significantly ($p< 0.05$) influenced by the levels of soy bean meal inclusion in the control diet. These growth parameters were significantly highest from larvae fed control diet $SB_{25}$, and $SB_{50}$ which did not differ significantly ($p>0.05$) followed by the group of the fish fed $SB_{75}$. Larvae fed $SB_{100}$ recorded the significant lowest values of these growth parameters. The fish final biomass and biomass gains decreased with the soy bean meal inclusion level in control diet. The significant ($p< 0.05$) highest biomass gain value was recorded from fish fed control diet (82.21 ± 0.11g) followed by $SB_{25}$ (69.44 ±

0.12g), $SB_{50}$ (57.42 ± 0.14g), and $SB_{75}$ (47.73 ± 0.13g) and the lowest value was obtained by fish fed $SB_{100}$ (47.73 ± 0.13g).

### *3.2 Feed Efficiency*

Total quantity of feed used by aquarium and feed conversion ratio values recorded were affected by the level of *Artemia salina* replacement by soybean meal in control diet. Results showed that quantity of feed used decreased with the soybean meal inclusion level in diet conversely FCR significantly ($p< 0.05$) increased. The lowest value of FCR correlated with best feeds efficiency was recorded from fish fed control diet (01.88 ± 0.29) when the highest value of these parameters was obtained from fish fed diet $SB_{100}$ (3.30 ± 0.18).

### *3.3 Cannibalism, Mortality and Survival*

Cannibalism, mortality and survival rate values showed significant influence with the level of soy bean inclusion in control diet. Cannibalism rate values varied between 15.55 and 23.32%, mortality rate ranged between 1.25 and 2.21% while survival rate varied between 68.27 and 83.20%. The highest ($p< 0.05$) value of cannibalism rate was recorded from fish fed $SB_{25}$ (29.75 ± 0.75 %) and $SB_{50}$ (29.86 ± 0.66 %), followed by fish fed $SB_{75}$ (25.60 ± 0.40 %) and control diet (23.32 ± 4.28 %) when the lowest cannibalism rate was observed from fish fed $SB_{100}$ (15.55 ± 0.55 %).

Fish fed control diet recorded the highest mortality rate (02.21 ± 0.01) followed by those of fish fed $SB_{25}$ (01.65 ± 0.14) and $SB_{50}$ (01.87 ± 0.25), when the lowest mortality rate values were obtained from fish fed $SB_{75}$ (01.30 ± 0.01) and $SB_{100}$ (01.25 ± 0.12). The significant ($p< 0.05$) best value of survival rate was recorded from fish fed $SB_{100}$ (83.20 ± 0.04), followed by control diet (74.47 ± 5.46) and $SB_{75}$ (73.10 ± 0.15) and the lowest values of survival rate were observed from fish fed $SB_{25}$ (68.60 ± 0.60) and $SB_{50}$ (68.27 ± 0.03).

## IV. DISCUSSION

At the end of the growth trial, feeds which artemia protein was substituted by 25% ($SB_{25}$) and 50% ($SB_{50}$) of the soya protein present similar growth with the control diet. Up to 75% of soya protein inclusion, values of final fish growth, weight gain, and daily weight gain recorded were decreased. These results show that artemia protein can be substituted by soya protein at 25 to 50% without adverse effects on *Clarias gariepinus* larvae growth. In fact, high levels of soybean meal increase anti-growth substances and indigestible carbohydrates levels in diets which lead to slow growth and poor feed performances [24], [25].

Consequently, low growth recorded from fish fed $SB_{75}$ and $SB_{100}$ highly affected final fish biomass by aquarium. Quantity of fish feed used also decreased with the levels of soybean meal inclusion. However, best values of feed conversion ratio were recorded from larvae fed control diet followed by diets $SB_{25}$, $SB_{50}$ and $SB_{75}$ when diets $SB_{100}$ presents the lowest value of FCR. These results could show an increasing reduction of feed palatability, acceptability and digestibility when artemia proteins were gradually combined with soya protein in diet. Concerning cannibalism, several studies showed that it's intensified by increasing size differences, suitable feeding practices, inter individual contacts, competition of food and stress [26], [27], [28], [29], [30], [31]. The low cannibalism value recorded with fish fed $SB_{100}$ could confirm that soya protein diet $SB_{100}$ was not accepted and not palatable for larvae which consequently reduces quantity of feed use, inhibits competition of food and stress, and entails slows growth for all the fish in aquarium. In these conditions, cannibalistic behaviour of larvae was reduced consequently in the groups of fish fed $SB_{100}$ and these groups recorded the highest values of survival rate. Despite high survival rate recorded with $SB_{100}$, growth and feed efficiency values show that high levels of soya proteins in diets affect feeds palatability and larvae growth, vigour, motility and reactivity. In these conditions, 100% soya proteins diets are not recommended for *Clarias gariepinus* larvae growth. Conversely, survival rate (68%) obtained with feeds which artemia protein was substituted by 25% ($SB_{25}$) and 50% ($SB_{50}$) soya protein were similar to the survival rate (67-69 %) of the larvae *Clarias gariepinus* fed with commercial high proteins content (56-57%) diets reported by Yakubu et al. [3]. In addition, these two diets present similar growth results with control diets. In these conditions, artemia protein in 35% protein control diet can be replaced by 25 to 50% of soya protein for catfish *Clarias gariepinus* larvae growth.

## V. CONCLUSION

*Artemia* protein in *Clarias gariepinus* larvae 35% protein diet can be replaced by soya protein to 25 and 50% for reduce the feed cost. Compounded feeds $SB_{25}$ and $SB_{50}$ can be used us low cost nutritive C*larias gariepinus* larvae diets without adverse effects on growth and survival compared of Artemia dietary control diet.

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*Table. 1: Formulation and proximate composition of experimental diets*

| Ingredients composition (%) | Soybean meal inclusion | | | | |
|---|---|---|---|---|---|
| | **Control diet (0%)** | **$SB_{25}$ (25%)** | **$SB_{50}$ (50%)** | **$SB_{75}$ (75%)** | **$SB_{100}$ (100%)** |
| *Artemia salina* Meal | 57.80 | 44.00 | 30.00 | 15.00 | - |
| Soy bean meal | - | 20.90 | 40.00 | 60.00 | 81.00 |
| Maize flour | 24.56 | 18.00 | 12.76 | 08.00 | 02.00 |
| Maridav | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Palm oil | 02.00 | 02.00 | 02.00 | 02.00 | 02.00 |
| Lysine | 02.13 | 02.13 | 02.13 | 02.13 | 02.13 |
| Methionine | 01.61 | 01.61 | 01.61 | 01.61 | 01.61 |
| VITAMYNOLYTE Super prémix | 02.00 | 02.00 | 02.00 | 02.00 | 02.00 |
| Total | 100 | 100 | 100 | 100 | 100 |
| Proximate analysis | | | | | |
| Moisture (%) | 10.60 | 10.23 | 10.67 | 10.73 | 10.99 |
| Crude protein (% DM) | 35.13 | 35.63 | 35.44 | 35.16 | 35.15 |
| Total fat (% DM) | 04.76 | 06.43 | 07.95 | 09.58 | 11.25 |
| Ash (% DM) | 03.21 | 04.83 | 06.28 | 07.81 | 09.42 |
| Crude fiber (% DM) | 03.90 | 04.38 | 04.76 | 05.17 | 05.60 |
| Nitrogen free extract (%)[3] | 42.38 | 38.46 | 34.87 | 31.52 | 27.56 |
| Gross Energy ($kjg^{-1}$)[4] | 16.93 | 17.03 | 16.96 | 16.95 | 16.92 |
| P/E ($g.\ kJ^{-1}$)[5] | 20.74 | 20.93 | 20.90 | 20.74 | 20.77 |

*Table. 2: Growth, feed efficiency and survival rate of larvae C. gariepinus fed the experimental diets*

| Parameters | Experimental diets | | | | |
|---|---|---|---|---|---|
| | **Control diet (0%)** | **$SB_{25}$ (25%)** | **$SB_{50}$ (50%)** | **$SB_{75}$ (75%)** | **$SB_{100}$ (100%)** |
| Initial larvae number | 50 | 50 | 50 | 50 | 50 |
| Initial body weight (g) | 0.0064±0.01 | 0.0064±0.01 | 0.0064±0.01 | 0.0064±0.01 | 0.0064±0.01 |
| Final body weight (g) | $02.19±0.13^{c}$ | $01.89±0.12^{c}$ | $01.91±0.13^{c}$ | $01.32±0.01^{b}$ | $0.70±0.02^{a}$ |
| Weight gain (g) | $02.18±0.16^{c}$ | $01.88±0.31^{c}$ | $01.90±0.13^{c}$ | $01.31±0.01^{b}$ | $0.69±0.02^{a}$ |
| Daily weight gain ($gday^{-1}$) | $0.04±0.01^{b}$ | $0.04±0.001^{b}$ | $0.04±0.002^{b}$ | $0.02±0.001^{a}$ | $0.01±0.001^{a}$ |
| Specific growth rate ($\%day^{-1}$) | $11.85±0.05^{d}$ | $11.53±0.03^{c}$ | $11.43±0.10^{c}$ | $10.88±0.01^{b}$ | $09.57±0.03^{a}$ |
| Initial biomass of fish (g) | $0.32±0.001^{a}$ | $0.32±0.001^{a}$ | $0.32±0.001^{a}$ | $0.32±0.001^{a}$ | $0.32±0.001^{a}$ |
| Final biomass of fish (g) | $82.53±0.10^{e}$ | $69.76±0.20^{d}$ | $57.74±0.22^{c}$ | $48.05±0.15^{b}$ | $28.95±0.30^{a}$ |
| Biomass gain (g) | $82.21±0.11^{e}$ | $69.44±0.12^{d}$ | $57.42±0.14^{c}$ | $47.73±0.13^{b}$ | $28.63±0.15^{a}$ |
| Quantity of feed used (g) | $155.16±0.11^{e}$ | $147.89±0.10^{d}$ | $128.18±0.16^{c}$ | $110.90±0.17^{b}$ | $95.53±0.21^{a}$ |
| Feed conversion ratio | $01.88±0.29^{a}$ | $02.12±0.06^{a}$ | $02.22±0.07^{ab}$ | $02.31±0.01^{b}$ | $03.30±0.18^{c}$ |
| Cannibalism rate (%) | $23.32±4.28^{b}$ | $29.75±0.75^{c}$ | $29.86±0.66^{c}$ | $25.60±0.40^{b}$ | $15.55±0.55^{a}$ |
| Mortality rate (%) | $02.21±0.01^{c}$ | $01.65±0.14^{b}$ | $01.87±0.25^{bc}$ | $01.30±0.01^{a}$ | $01.25±0.12^{a}$ |
| Survival rate (%) | $74.47±5.46^{b}$ | $68.60±0.60^{a}$ | $68.27±0.03^{a}$ | $73.10±0.15^{b}$ | $83.20±0.04^{c}$ |

Mean values ± SD in the same row sharing the different superscript are significantly different ($p<0.05$)

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# Simulation Impact of REDD Policy: Case Study of Forest Area in Indonesia

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***Abstract*—***Indonesia's forests in different periods have been deforested at different levels. Deforestation caused carbon emissions. The purposes of this study were :1) to measure deforestation and carbon emissions in period of 2005-2010 in Indonesia and 2) to find out the incentive value to be paid by the government. One method for measuring emissions from deforestation and forest degradation is GeOSIRIS model. A modeled GeOSIRIS policy used a carbon payment system to incentivize emission reductions. Data used in this study were maps of forest cover in 2005 and 2010, map of deforestation 2005-2010, carbon and agricultural price and driver variables for deforestation such as slope, elevation, logarithmic distance to the nearest road, logarithmic distance to the nearest provincial capital, the amount of area per pixel included in a national park, a timber plantation. The result of this study showed rate of deforestation was 4.65 million ha/5 years. The REDD policy could decrease deforestation in Indonesia by 0.66 million ha (17.45 %). Assuming that international carbon price was US$ 10/tCO$_2$e, the change of emissions due to REDD was 24.75%, or reduced emissions by 1.09 million tCO$_2$e/5 years. Finally, Gross National Revenue from carbon payments (NPV 5 years) was US$ 10.917 billion, where incentivize emission reductions to sub-national entities (NPV, 5 years) was US$ 9.178 billion and net central government surplus from carbon payments was US$ 1.739 billion (NPV, 5 years).*

***Keywords*—deforestation, carbon emission, agricultural revenue, carbon payments, geosiris model**

## I. INTRODUCTION

Tropical forests and other vegetated landscapes like grasslands and wooded savannahs play a major role in the global carbon sequestration process and their conservation and protection offers immense potential for reducing greenhouse gas emissions and global warming [5]. Referring to [3] that clearing of primary forests also results in the destruction of unique tropical forest habitats, thus causing the loss of biodiversity.

Among tropical countries Indonesia experiences the second highest rate of deforestation. Therefore, accurate and up-to-date forest data are required to fight deforestation and forest degradation to support initiatives of climate change mitigation and biodiversity conservation policy [8]. Meanwhile [16] explained that the largest deforestation in Indonesia occurred in Kalimantan and Sumatra with a percentage of 36.32% and 24.49% respectively, followed by Sulawesi 11.00%, Java 9.12%, Maluku 8.30%, Bali-Nusa Tenggara 6.62%. Papua became the smallest area contributing to deforestation of 4.15%. It could be seen that deforestation in Indonesia until 2009 was concentrated in Kalimantan and Sumatra.

Out of the 15.79 Mha of forest cover loss in Indonesia, reported 38% (6.02 Mha) happened inside primary intact or damaged forests [10]. Meanwhile [11] said that over the study period annual primary forest cover loss increased with the highest total loss happened in 2012 (0.84Mha). The number was greater than the reported forest loss in Brazil (0.46Mha), which was the historical leader in the tropical forest clearing. Referring to [13], Borneo Island in the period 2000-2011 has deforestation amounted to 3.040 million ha, namely deforestation in peatland forests of 0.560 million (18.42%) and deforestation in mineral land (non-peatland) for 2,480 million (81.58%). Based on the period of time of deforestation, 48.5 % of deforestation occurred in the period 2006-2011, i.e. deforestation on peatland forests of 0.334 million ha (59.69%) and deforestation in mineral forests of 1.144 million (46.15%).

In Indonesia deforestation is usually linked with production of timber and expansion of settlement and agricultural area. When this existing trend continues without implementing any corrective measures, it is projected to result in a reduction of forest cover by 15% between 2015 and 2030, going from approximately 88,000,000 ha to 74,994,100 ha. On average, 830,000 ha of forest would be cleared for timber extraction or land conversion every year between 2015 and 2030. When the forest cover declines, so does the amount of carbon stored. The cumulative emissions from 2015 to 2030 due to forest loss would reach 2.5 billion tCO$_2$, which, assuming an average carbon price of USD 5 to USD 10 per ton (based on international average market prices), would translate in a cumulative loss of about USD 10 billion to USD 25 billion between 2015 and 2030 [4].

REDD is not directed at stopping planned conversion of forests to other economic uses, nor at stopping the use of forests for timber. REDD signifies a way to value natural

resource of carbon so that it can be considered along with other regular forest assets, when making decisions about land use and forest use [14].

In the calculation and modeling for carbon emissions, there are several methods and approaches. One model is the GeOSIRIS model developed by Jonah Busch at Conservation International. The GeOSIRIS model was originally developed as OSIRIS as a transparent decision support tool for REDD+ policy makers [7].

The GeOSIRIS modeler is different from the REDD modeler found in Land Change Modeler (LCM). The REDD modeler in LCM predicts how carbon emissions and deforestation would change if a certain reference area were shielded from deforestation. Meanwhile, the GeOSIRIS modeler adopts an alternate strategy. A carbon payment system is used by a modeled GeOSIRIS policy to give incentives to emission reductions. The policy can be governed at various administrative levels, such as province or district. Rather than defending a specific section of land from deforestation, scope of work for GeOSIRIS projects would be on regional or national scale, by setting a certain price to every ton of carbon dioxide emitted ($/t$CO_2$e). The GeOSIRIS model assumes forest users encounter a trade-off between the carbon revenue obtained by protecting the forests and the agricultural revenue obtained from deforesting the land. Given some variables such as a proposed carbon price and maps of previous deforestation, the model predicts how carbon emissions, deforestation, and agricultural and carbon revenues would change if such policy were implemented [7].

The model designs balance incentives to lower usually high deforestation emissions with incentives to keep usually low deforestation emissions. Approximations of emission reductions under REDD depend significantly on the degree to which demand for tropical agriculture in the borderline generates leakage. This emphasizes the potential importance to REDD of balancing strategies to supply agricultural needs outside the forest borderline [6].

The purposes of this study were to measure deforestation and carbon emissions in period of 2005-2010 in Indonesia and to find out the incentive value to be paid by the government.

## II. MATERIAL AND METHODS

### 2.1 Data used

This study used data from https://clarklabs.org/download/terrset-tutorial-data/, accessed on April 4, 2017, consisting of: (a) forest cover maps in 2005 and 2010, deforestation map 2005-2010 (see figure 1); (b) map of potential driver variables for deforestation, consisting of maps: slope, elevation, logarithmic distance to the nearest road, distance from the provincial capital, national park map, and plantation area map. These data are global data with spatial resolution of 3 km x 3 km. These data include global data that can be used for monitoring a large area (such as the whole Indonesia), due to the availability of sufficient data. However, for more specific planning, medium and detail scale data are needed to obtain more accurate results.

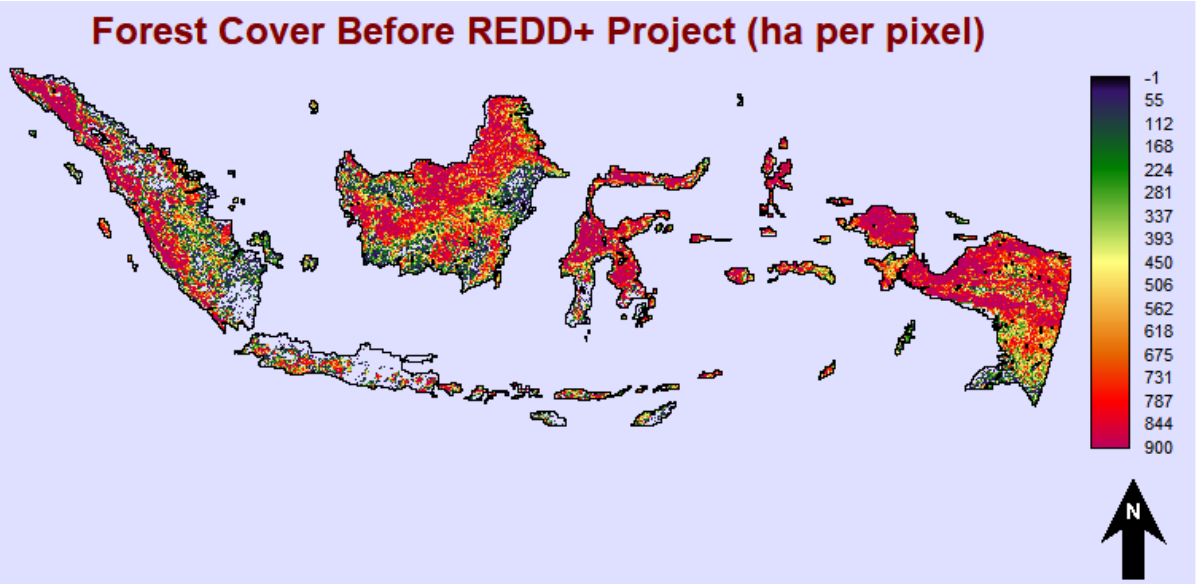


*Fig. 1: Forest Area in Indonesia.*

The disadvantage of these data is that the spatial resolution is too small (where one pixel represents an area of 900 ha). Therefore, areas with less than 900 ha (one pixel) will be combined into a more dominant class. The map actually covered the entire territory of Indonesia. For this study other than covering Indonesia, it was also cropped to cover Kalimantan and Sumatra Islands.

The GeOSIRIS model in REDD impact calculations is based on an enhanced OSIRIS model [7]. The flow chart of the GeOSIRIS modeling stage is presented in figure 2.

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In general, GeOSIRIS model has two main steps: (1) regression analysis, where the regression coefficient(s) and Effective Opportunity Cost image are calculated, and (2) calculations of proportional national change in agricultural price, output images (deforestation and emission), output image on administrative level decisions then the summary Excel spreadsheet is generated.

**2.2. Regression Analysis**

Stage of activity in this research refers to Eastman [9]. The regression step of the GeOSIRIS modeler calculates the correlation between deforestation and some individual variables (14 variables), including agricultural revenue. There are several options to classify this regression, where GeOSIRIS will run a separate regression for several different classes. These classes can be based on the amount of preexisting forest cover or geographic regions, such as provinces or districts (for geographic stratification).

This study is based on geographic regions, for Indonesia such as provinces (33 provinces) or districts (426 districts), For Sumatra Island such as provinces (13 provinces) or districts (131 districts) and for Kalimantan Island, such as provinces (5 provinces) or districts (55 districts).

The regression model used in this study is Poisson regression, in which the deforestation is counted by assuming that each pixel is composed of smaller subsections which may be individually deforested [9]. The Poisson regression uses the following formula:

$$E(Y/X) = e^{\sum_{i=0}^{i=N} Bt.Xt} \quad \ldots\ldots. (1)$$

$E(Y|X) = m\sum BiXi\ i=N\ i=0$

where:

$E(Y|X)$ = the expected count of deforestation (Y) given certain input conditions ($X$)

$Xi$ = independent variable (X0=1 for the constant term)

$Bi$ = variable coefficients (or parameters)

The model parameters consist of external variables (economic variables) and parameters that affect the price of agricultural products. Net Present Value formula:

$$NPV = \sum_{t=1}^{T}(Bt - Ct)(1-i)^{-t} \quad \ldots\ldots\ldots(2)$$

where:

Bt = total revenue generated in year t,

Ct = total costs in year t,

i = interest rate

T = expected lifetime (5 years)

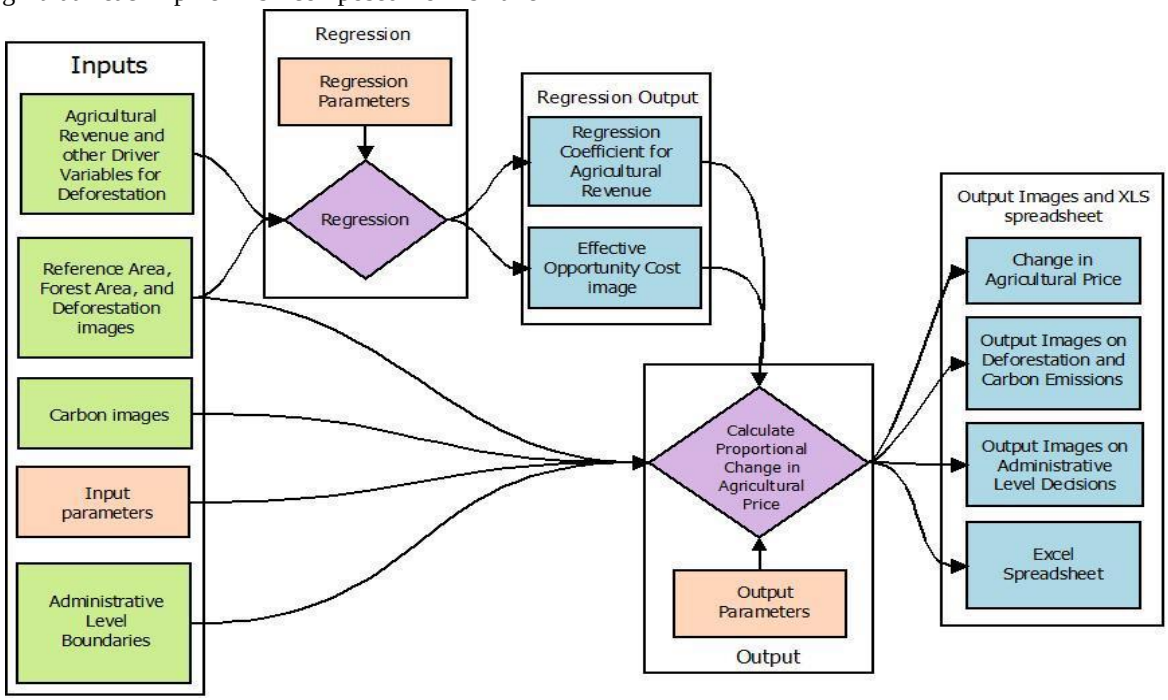


*Fig. 2: Flowchart Stage of Research Activities*

The GeOSIRIS model can be applied at different administrative levels, such as the district or provincial level. Image files are inputted in the administrative levels table of the input image files panel. The emission factor map is used to calculate the amount of $CO_2$ (in tons) that will be emitted per hectare of deforestation. There are three components for the emission factor in the GeOSIRIS model: soil carbon, above and below-ground carbon, and peat. The calculations of the emission factor for each pixel are:

$E = (A\text{B}+\text{SC}*fs)*3.67$ where peat P=0 ……(3)

$E = A\text{B}*3.67+fp$ where peat $P$>0 …..……(4)

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where:
$E$ = emission factor ($t$CO2e/ha)
$AB$ = above and ground carbon
$SC$ = soil carbon
$fs$ = soil carbon factor
$fp$ = emission factor for peat soil

### 2.3 Calculating the Proportional Change in Agricultural Price

The GeOSIRIS model compares two consecutive values of changes in agricultural product price to see whether the value is appropriate. The model will keep on going until either the precision model or the maximum number of iterations is exceeded. The last iteration value obtained will be used for final calculation. Analysis of changes in agricultural prices, where proportional changes in agricultural prices are calculated, the image as a result of the analysis, and summary of the calculation results (in Excel worksheet) are then generated.

The final proportional change in the price of agricultural product is calculated in the output parameters panel. An iterative loop and two input parameters, which are model precision and maximum number of iterations, are used in this calculation. The price change is then calculated as the sum of endogenous change and exogenous change.

Change in Agricultural Price = endogenous change (independent) + exogenous changes …… (5)

Endogenous Change

$$= \left[\frac{\text{Deforestation without REDD}}{\text{Deforestation with REDD}}\right]^{e} \text{..... (6)}$$

where:
the exponent e = price elasticity

The model compares two successive values in the change of agricultural price to see if they are within the model precision value. If they are, then the most recent iteration value is used for the final calculations. The model will continue to run until two successive values meet the model precision criteria, or the maximum number of iterations is exceeded, in which case the model terminates without performing any final calculations.

The model parameters are economic and those affecting the price of agriculture products. The price elasticity is a measure on how sensitive the agriculture production price is to the change in deforestation. The external factors causing the increase in agricultural price (exogenous change) is a part of the final change in agricultural price as shown in figure 3.

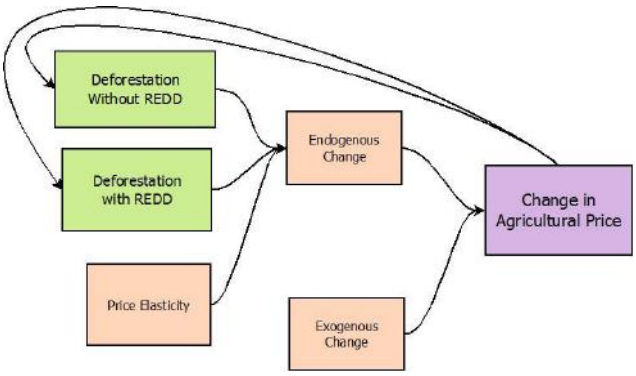


*Fig. 3: The Exogenous Increasing in Agricultural Price.*

## III. RESULTS AND DISCUSSION

### 3.1 Deforestation

The total forest area in Indonesia in 2005 was 93.02 million ha or about 53.58 % of the whole Indonesia. It consisted of 13.04 million ha (14.02 %) peatland forest and of 79.98 million ha (85.98 %) non-peatland forest or mineral forest. Based on forest type, it consisted of primary forest and secondary forest. In the period of 2005-2010 deforestation in Indonesia was 4.65 million ha, comprising 1.70 million ha (36.56 %) of peatland forests and 2.95 million ha (63.44 %) of mineral forests. The rate of deforestation at forest area was 4.99 %, at peatland forest was 13.03 % and at non-peatland forest was 3.68, as presented in table 1 and figure 4.

Deforestation that occurred at mineral forests was higher than at peatland forests because people prefer to utilize forests in mineral land first, where accessibility is easier and the existence of forests is also wider. Reduced forests in mineral land would then trigger people to take advantage of peatland forests.

The deforestation was relatively similar to the results of [13]. Refer to [13] deforestation at Indonesia in the period 2006-2011 amounted to 3.84 million ha (5.04%), namely deforestation at peatland forests of 1.28 million ha (33.29 %) and deforestation at mineral land (non-peatland) of 2.61 million ha (66.71 %).

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*Tabel.1: Results of Deforestation Estimation Year 2005-2010 in Indonesia (million ha)*

| No | Parameter | Indonesia | | | Sumatra | | | Kalimantan | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | a | b | c | a | b | c | a | b | c |
| 1 | Land area (million ha) | 173.59 | 27.76 | 145.83 | 43.70 | 8.75 | 34.95 | 52.05 | 8.18 | 43.87 |
| 2 | Starting forest area (million ha) | 93.02 | 13.04 | 79.98 | 18.70 | 3.11 | 15.59 | 29.32 | 4.03 | 25.29 |
| 3 | Deforestation without REDD (million ha/5 years) | 4.65 | 1.70 | 2.95 | 1.63 | 0.82 | 0.81 | 1.42 | 0.35 | 1.07 |
| 4 | Deforestation without REDD (modeled; million ha/5 years) | 3.79 | 1.21 | 2.58 | 1.28 | 0.57 | 0.71 | 1.02 | 0.30 | 0.72 |
| 5 | Deforestation with REDD (modeled; million ha/5 years) | 3.13 | 0.83 | 2.30 | 0.89 | 0.24 | 0.64 | 0.85 | 0.21 | 0.64 |
| 6 | Reduction in deforestation (million ha/5 years) | 0.66 | 0.38 | 0.28 | 0.39 | 0.32 | 0.06 | 0.17 | 0.09 | 0.09 |
| 7 | Change in deforestation due to REDD (percent) | -17.45 | -31.77 | -10.73 | -30.39 | -56.97 | -9.08 | -16.70 | -28.52 | -11.80 |

Remark: a= all land, b= peatland, c=non peatland

Meanwhile deforestation at Sumatra in the period 2006-2011 amounted to 1.92 million ha (7.75%), namely deforestation at peatland forests of 0.64 million ha (33.17 %) and deforestation at mineral land (non-peatland) of 1.28 million ha (66.83 %). In Kalimantan island in the period 2006-2011 also amounted to 1.48 million ha (4.20%), namely deforestation at peatland forests of 0.34 million ha (22.60 %) and deforestation at mineral land (non-peatland) of 1.15 million ha (77.40 %). Although the amount of deforestation is not exactly the same, but show a relatively similar pattern.

The rate of deforestation in Sumatra was higher than both deforestations occurred in Kalimantan Island and Indonesia over the same period. Deforestation in Sumatra Island was 8.74 % while in Indonesia was 4.99 % and in Kalimantan Island was 4.84 %. The same condition also occurs at the rate of deforestation in peat forests and also mineral forests.

Based on type of forest, the rate of deforestation at peatland forest in Sumatra (26.26 %) was higher than Kalimantan Islands (8.68%) and Indonesia (13.01%).

The condition is triggered by the conversion of forests as oil palm plantations and also industrial plantations (pulp) in the center on the island of Sumatra and also the island of Kalimantan.

Furthermore, Refer [11] declared deforestation of primary forest at Kalimantan in 2000-2012 amounted to 2.377 million ha, comprising of deforestation at wetland forest 0.897 million ha and at dryland forest 1.390 million ha. The rate of deforestation of total primary forest was 7.92%, at wetland forest was 5.25%, and at dryland forest. Meanwhile at Sumatra Sumatra Island experienced intensive forest clearance which resulted in the conversion of 70% of the island's forest area until 2010.

Research conducted by [11], in the period 2000-2009, on the island of Sumatra deforestation occurred of 3.71 million ha or 23.92% of deforestation that occurred in Indonesia. The largest contributor to deforestation on Sumatra Island is Riau Province at 31.42%, while Bengkulu Province is the region with the lowest deforestation of 3.53%.

The rate of deforestation at Kalimantan and Sumatra Islands varied depending on the level of spatial resolution of data sources used. Research used Landsat Image data, therefore he got larger amount of deforestation. This was because spatial resolution of the image was 30 m, more meticulous than the global data used in this study with spatial resolution of 3 km [11].

The deforestation in 2005-2010 happened as a result of government policy in the development of agricultural areas, the development of oil palm plantations and industrial plantations.

This is in line with the findings of study of expansion of agricultural policy, timber extraction and infrastructure expansion [9]. The main reasons of forest cover deficit in Kalimantan were related to the expansion of worldwide markets for pulp, wood and palm oil [15,17]. While Margono [12] asserted that in the period of 2000-2010 the cause of deforestation was the expansion of agricultural areas, especially palm oil plantations, expansion of pulp and paper plantation industrial areas and industrial forest clearance. Based on the figure 4 areas with relatively flat up to undulating topography and relatively easy accesibility (with existing rivers), it is a priority area for forest exploitation, thus causing the area to have higher deforestation rates (yellow to red).

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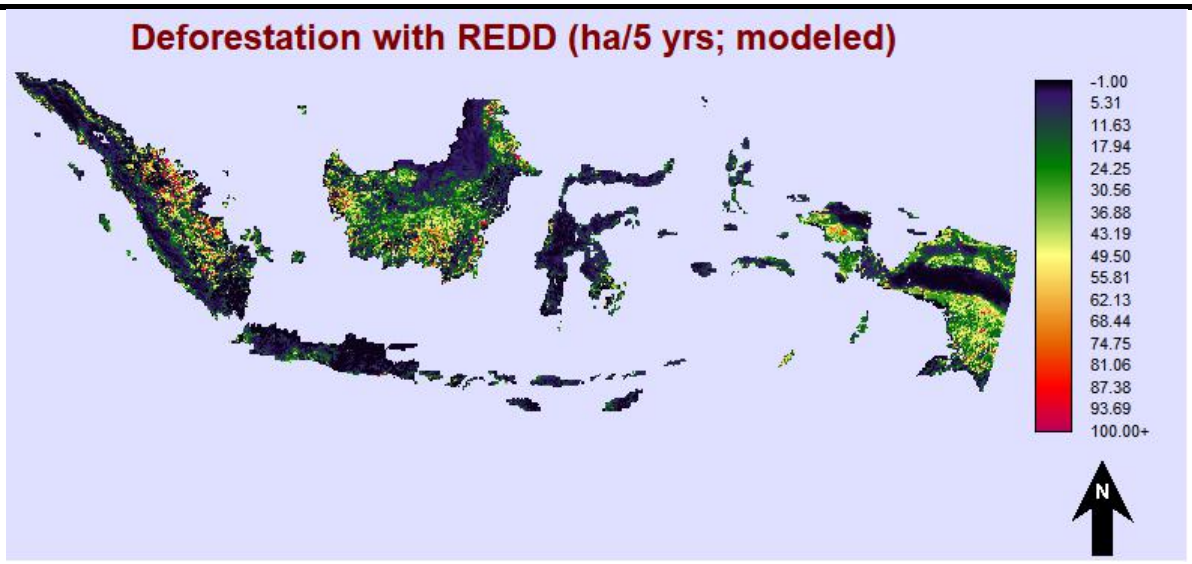
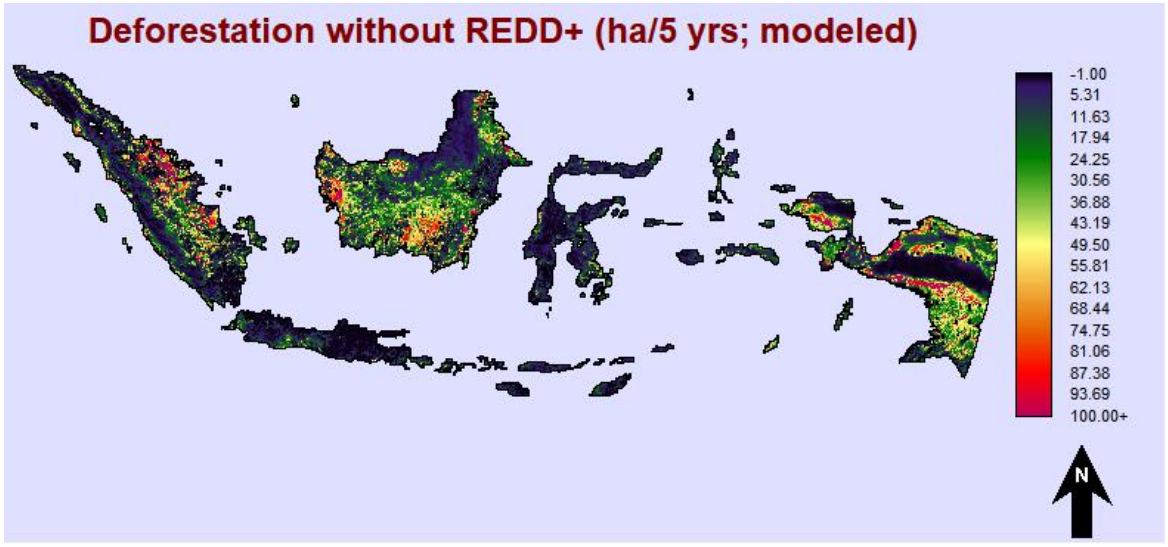



*(a) with REDD*



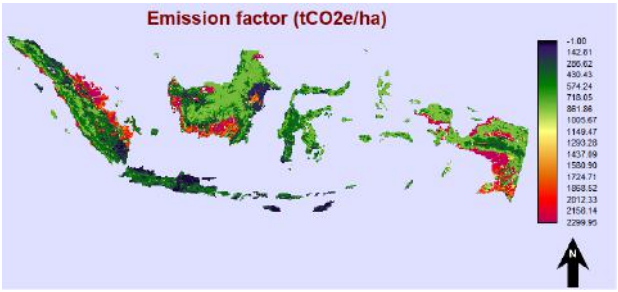

*(b) Without REDD*

*Fig. 4: Map of Deforestation at Indonesia Country.*

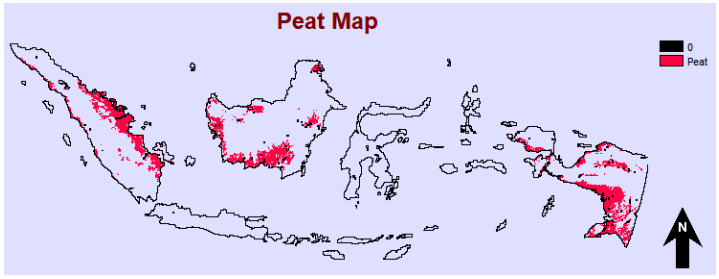
While areas with topographic hills to mountains (the existence of roads is very limited), then the area of forest is still relatively not yet logged, so rate of deforestation is relatively lower (blue to green).

**3.2 Carbon Emissions The impact of REDD**

Implementation of REDD policies, which have an impact on reducing forest degradation, also directly impact on reductions of carbon emissions. Based on the variables affecting deforestation, carbon emissions and peat swamp factors (figure 5) have a strong effect to deforestation.




*a Emission Factor*




*b. Peat swamp*

*Fig. 5 : Emission Factor and Peat Swap at Indonesia Country*

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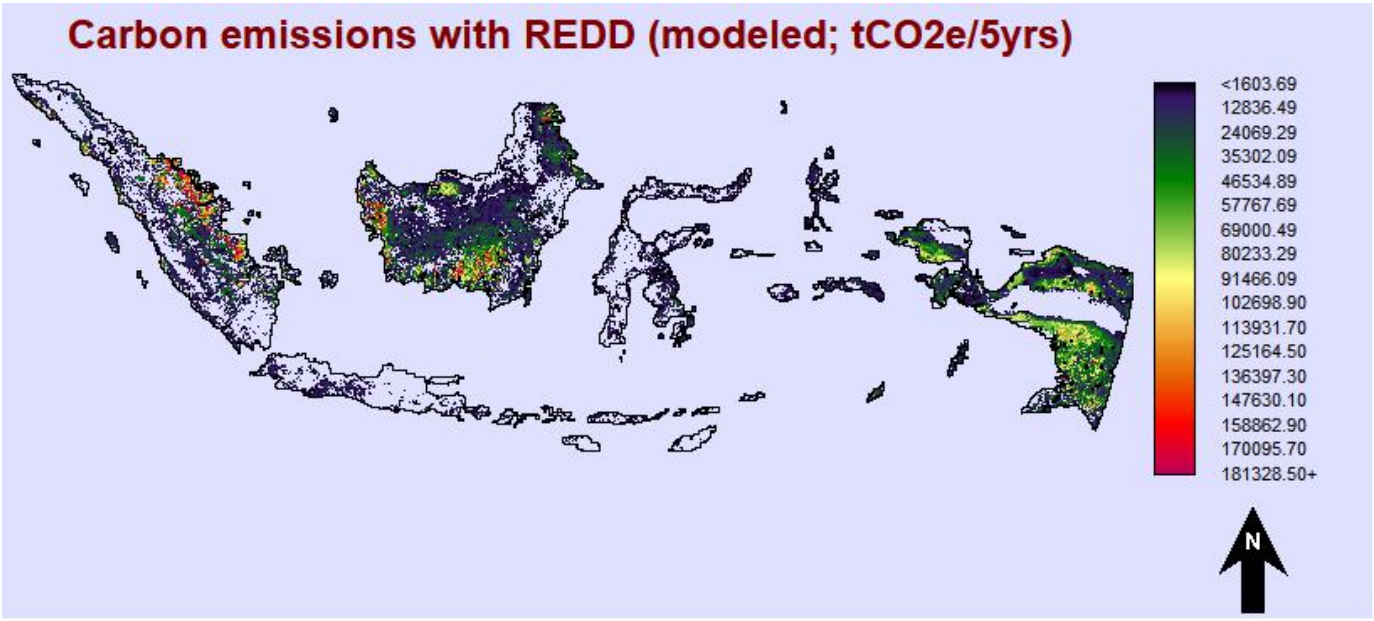
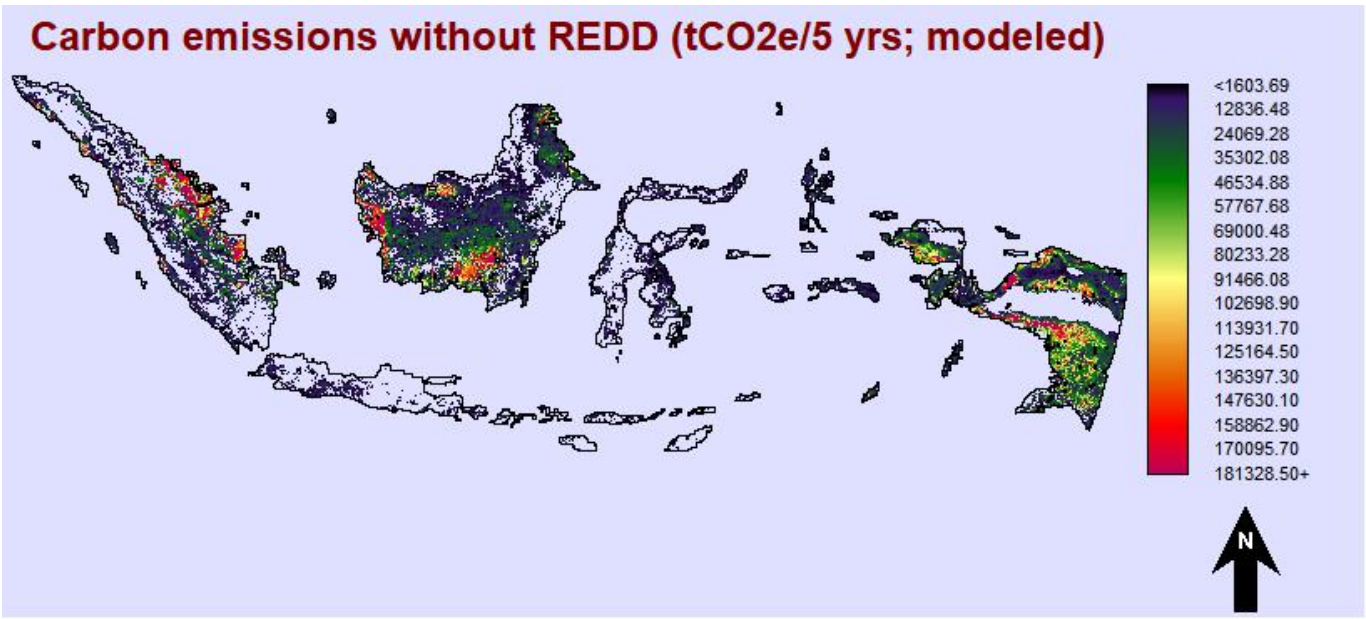
Based on figure 5, at Sumatra island, emission factors in Riau Province, Riau Islands, South Sumatra and Bangka Belitung have relatively higher value compared to other provinces. This is related to the existence of large peatland forest located in the area. Conversion of peatland forest into palm oil plantations causes the carbon emission factor to be higher. Meanwhile at Kalimantan island, carbon emission factors in West Kalimantan Province and Central Kalimantan have relatively higher value compared to other provinces.

This is related to the presence of large peatland forest located in this area, while peatland is the highest contributor to emissions. Implementation The REDD policy at Indonesia, Sumatra island and Kalimantan island as presented in figure 6 and table 3.




*a. With REDD*




*b. Without REDD*

*Fig. 6: Map of Carbon Emission in Indonesia*

*Tabel.3: Result of carbon emission expectation year 2005- 2010 in Indonesia (x million ha)*

| No | Parameter | Indonesia | | | Sumatra | | | Kalimantan | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | a | b | c | a | b | c | a | b | c |
| 1 | Emittable $CO_2$ from forest carbon stock (estimated; t Mg $CO_2$) | 84.64 | 28.50 | 56.14 | 16.79 | 6.80 | 9.99 | 27.16 | 8.76 | 18.40 |
| 2 | Emissions without REDD (estimated; $tCO_2$/5 years) | 5.65 | 3.72 | 1.93 | 2.31 | 1.82 | 0.50 | 1.39 | 0.74 | 0.65 |
| 3 | Emissions without REDD (modelled; $tCO_2$/5 years) | 4.41 | 2.65 | 1.76 | 1.66 | 1.25 | 0.41 | 1.10 | 0.64 | 0.46 |
| 4 | National reference level of emissions ($tCO_2e$/5 years) | 4.41 | - | - | 1.66 | - .00 | - .00 | 1.10 | - .00 | - .00 |
| 5 | Emissions with REDD (modelled; $tCO_2$/5 years) | 3.32 | 1.79 | 1.53 | 0.88 | 0.53 | 0.35 | 0.85 | 0.46 | 0.40 |
| 6 | Gross emission reductions ($tCO_2e$/5 years) | 1.10 | 0.86 | 0.24 | 0.79 | 0.72 | 0.07 | 0.27 | 0.19 | 0.07 |
| 7 | Gross emission increases ($tCO_2e$/5 years) | 0.004 | 0.00 | 0.00 | 0.01 | 0.00 | 0.01 | 0.02 | 0.01 | 0.01 |
| 8 | Net emission reductions ($tCO_2e$/5 years) | 1.09 | 0.86 | 0.23 | 0.78 | 0.72 | 0.05 | 0.25 | 0.18 | 0.06 |
| 9 | Credited emission reductions ($tCO_2e$/5 years) | 1.09 | | | 0.78 | | | 0.25 | | |
| 10 | Change in emissions due to REDD (percent) | -24.75 | -32.42 | -13.22 | -46.83 | -57.79 | -13.17 | -22.29 | -28.30 | -14.02 |

Remark: a= all land, b= peatland, c=non peatland

Refer table 3, the forest emissions (emitable $CO_2$) at Indonesia was 84.64 million $tCO_2e$, donation from peat land forest 28.50 million $tCO_2e$ (33.37%) and from mineral forest 56.14 million tCO2e (66.33%). Based on spatial distribution, the forest emissions (emitable $CO_2$) at Sumatra Island was 16.79 million $tCO_2e$, donation from peatland forest 6.80 million $tCO_2e$ (40,48%) and from mineral forest 9.99 million $tCO_2e$ (59,52%). Meanwhile the forest emissions (emitable $CO_2$) at Kalimantan Island was 27.16 million $tCO_2e$, donation from peatland forest 8.76 million $tCO_2e$ (32.25%) and from mineral forest 18.40 million $tCO_2e$ (67.75%)

Impact of REDD policy in Indonesia targeted carbon emissions of 4.41 million ha. Meanwhile, the gross emission reduction that could be obtained was 3.32 million $tCO_2e$, and emission that could be absorbed by forests was 1.09 million tCO2e. Distribution on Sumatra island, targeted carbon emissions of 1.66 million ha. Meanwhile, the gross emission reduction that could be obtained was 0.88 million $tCO_2e$, and emissions that could be absorbed by forests was 0.79 million $tCO_2e$.

Meanwhile implementation REDD policy at Kalimantan island, targeted carbon emissions of 1.10 million ha. The gross emission reduction that could be obtained was 0.85 million $tCO_2e$, and emissions that could be absorbed by forests was 0.27 million tCO2e.

Both islands (Kalimantan and Sumatra) contribute carbon emissions as much as 69.14%. Meanwhile, according to [1] stated that Indonesia had various emission levels from deforestation on each island. The highest emissions came from Sumatra, which were almost 56% of all emissions, and the second was Kalimantan with 28%, thus total for both islands was 84%. Therefore, it is important to focus on these two islands in implementing emission reduction strategies. The high emissions from Sumatra and Kalimantan were caused by the high deforestation rate on both islands, reaching 77% of Indonesia's total deforestation.

Meanwhile [2] Deforestation in Sumatra contributed the greatest importance of the existing focus on clearance of peatland forest.

The REDD policy was capable of reducing carbon emissions at Indonesia by 1.09 million $tCO_2e$ (24.753%). Meanwhile, the reduction of carbon emission in peatland forest area was 0.86 million $tCO_2e$ (28.30%) and in mineral soil forest area was 0.23 million $tCO_2e$ (14.02 %).

The REDD policy was capable of reducing carbon emissions at Sumatra Island by 0.78 million $tCO_2e$

(46.83%). Meanwhile, the reduction of carbon emission in peatland forest area was 0.72 million $tCO_2e$ (28.30%) and in mineral soil forest area was 0.05 million tCO2e (14.02 %).

The REDD policy was capable of reducing carbon emissions at Kalimantan Island by 0.25 million $tCO_2e$ (22.29%). Meanwhile, the reduction of carbon emission in peatland forest area was 0.18 million $tCO_2e$ (28.30%) and in mineral soil forest area was 0.06 million $tCO_2e$ (14.02 %).

The reduction in carbon emission levels at Indonesia (24.75 %) was lower than the reduced emission carbon that occurred at Sumatra Islands (46.83 %, but it was higher than the reduced emission carbon that occurred at Kalimantan Islands (22.29 %) in the same period.

The carbon emission reduction at Indonesia was 1.091 million $tCO_2e$ (24.75%), comprising of 858 million (32.42%) at peatland forests and a decrease in mineral soil carbon emissions of 233 million $tCO_2e$ (13.22%).

Meanwhile, the decline in carbon emissions in Sumatra island was 0.78 million $tCO_2e$ (46,83 %), consisting of 0.72 million $tCO_2e$ (57.78 %) at peatland forest and 0.05 million $tCO_2e$ (13.17%) at mineral soil. The decline carbon emissions in Kalimantan island was 245 million $tCO_2e$ (22.29%), consisting of 180 million $tCO_2e$ (28.52%) at peatland forest and 64 million $tCO_2e$ (14.22%) at mineral soil.

Changes in carbon emissions due to REDD were proportional to the rate of deforestation that occurred. The relatively smaller peatland forest area compared to the mineral forests caused the reductions deforestation rate (percentage of deforestation) in peatland forests to be greater than the rate of deforestation in mineral forests, with the same forest area.

Assuming that world carbon price was US $ 10 / $tCO_2e$, impact of REDD Policy at Indonesia, Kalimantan Island and Sumatra were that the gross national revenue from carbon payments and allocation for local government presented in Table 4.

*Tabel.4 : Economic Revenue Impact of REDD Policy at Indonesia (US$ billion)*

| No | Economic Revenue | Indonesia | Sumatra | Kalimantan |
|---|---|---|---|---|
| 1 | Gross national revenue from carbon payments ( NPV -- 5 yrs) | 10.917 | 7.75 | 2.45 |
| 2 | Carbon payments to sub-national entities ( NPV -- 5 yrs) | 9.178 | 6.78 | 2.15 |
| 3 | Net central government surplus/deficit from carbon payments ($, NPV -- 5 yrs) | 1.739 | 0.97 | 0.30 |
| 4 | Participan (number) Province ( %, number) | 70 (23) | 58 (7) | 80 (4) |
| | Distric ( %, number ) | 66 (281) | 64 (84) | 78 (43) |

Refer table 4, if the REDD policies are applied to the territory of Indonesia, it will be gross national revenue from carbon payments (NPV, 5 years) would be $ 10.917 billion, with allocation for local government (provincial and district) as incentives (NPV, 5 years) was $ 9.178 billion (84.07%). Net government surplus originating from carbon payments was US $ 1.739 billion (NPV, 5 years.

For the REDD policies are applied to Sumatra island, it will be gross national revenue from carbon payments (NPV, 5 years) would be $ 7,75 billion, with allocation for local government as incentives (NPV, 5 years) was $ 6.78 billion (87.48%). Net government surplus originating from carbon payments was US $ 0.97 billion (NPV, 5 years).

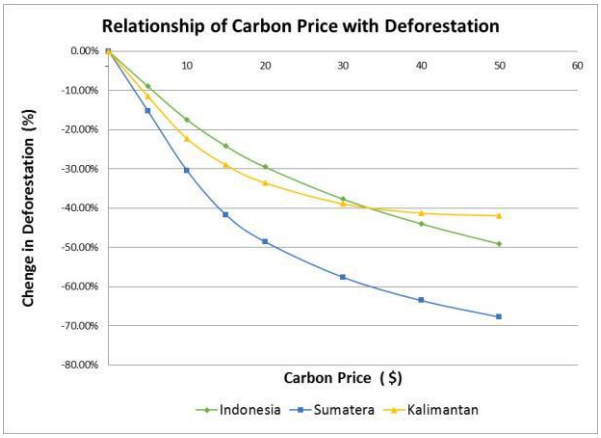
If the REDD policies are applied to the territory of Kalimantan island, it will be gross national revenue from carbon payments (NPV, 5 years) would be $ 2.45 billion, with allocation for local government as incentives (NPV, 5 years) was $ 2.15 billion (87.56%). Net government surplus originating from carbon payments was US $ 0.30 billion (NPV, 5 years).

Results of the study [2] that calculated carbon emissions in Bolivia, GeOSIRIS could also be used to evaluate how much reduction of deforestation could be achieved with the price of alternative carbon. Refer [1] with international $CO_2$ price of US$ 5-50 /$tCO_2$, we can simulation relationship carbon price with deforestation and emission at the Kalimantan Island, Sumatra and Indonesia as show on figure 7.

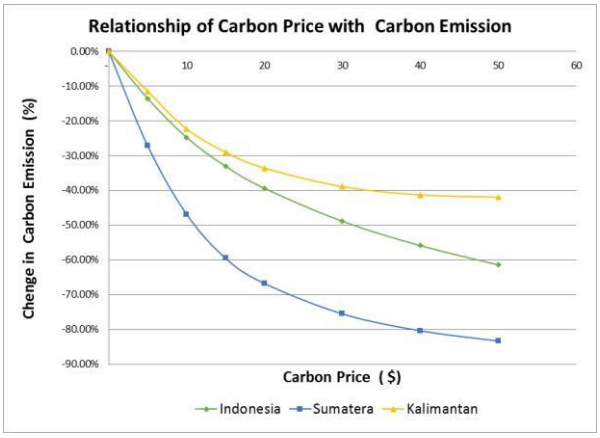
Based on figure 7, with a price of $10 it could be reduced by about 17 % - 30% and at $50 by around 40 % - 70%. The increase in carbon prices will spur activities to protect the forests so that the forests will be better

protected and deforestation will also occur. Conversely, if there is an increase in price of agricultural products, then the rate of deforestation will also increase, because more forest areas will be cultivated into agricultural areas. The relationship between carbon prices to deforestation and carbon emissions has the same pattern (refer to fig 7). The impact of rising carbon prices leads to increased deforestation as well as carbon emissions. The impact of rising carbon prices on forest areas in Sumatra has a bigger impact than deforestation on the average of Indonesia and also forests in Kalimantan.

Similarly, a success in reducing deforestation is linearly related to reduction of carbon emissions. The more forests that can be protected from logging, the more economically beneficial they will be




***a.*** *Price with Deforestation*




***b.*** *Price with Carbon Emission*

*Fig.7: Relationship of Carbon Price with Deforestation and Carbon Emission*

## IV. CONCLUSION

In the period 2005-2010, deforestation at Indonesia was 4.65 million ha (4.99 %). The simulation result, impact of REDD policy could reduce deforestation at Indonesia by 0.66 million ha (17.45%). With assumption that international carbon price of US$ 10/tCO2e, the change of emissions due to REDD was 24.75%, or reduced emissions by 1.09 million $tCO_2e$/5 years. Finally, Gross National Revenue from carbon payments (NPV 5 years) was US$ 10.917 billion, where incentivize emission reductions to sub-national entities (NPV, 5 years) was US$ 9.178 billion and net central government surplus from carbon payments was US$ 1.739 billion (NPV, 5 years).

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# Effects of Socio-Economic factors of Loan Administrators on Recovery Rate among Agricultural Cooperatives in Benue State, Nigeria

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***Abstract—*** *This study was undertaken to analyze the effect of socio-economic factors of loan administrators on loan recovery rate among agricultural co-operatives in Benue State of Nigeria. A purposive and simple random sampling technique was used to select 130 respondents. Data were collected using structured questionnaire and was analyzed using descriptive statistics and multiple regression. The result showed that majority of the respondents were male (58.46%), married (67.69%), educated (63.01%), with mean cooperative experience of 14.39 years (86.92%) and household size between 1 - 5 members. The result also showed that respondents were averagely young (36 years) and were relatively low income earners (₦ 2,480,000 per annum). The result shows that loan size was the only variable that significantly and positively affected loan recovery rate. The coefficients of salary, age, years in education, household size, cooperative experience, marital status and sex had no effect on recovery rate. It was recommended that administrators should give higher portfolio size loans as these will trigger them to carefully look at business activities in their coverage areas that are capable of repaying loans from precede of sales and cash flow.*

***Keywords— Agricultural loan, Agricultural Cooperatives, default prevention, and loan recovery rate, Benue State, Nigeria.***

## I. INTRODUCTION

Loan recovery is an important service that helps to both maintain clients and free up money for lending again. It is a strategic process that is a key to generating good habits and a payment culture among clients. It is a business activity whose primary objective is to generate returns for the institution, converting losses into income (Teskiewicz, 2007). The process requires significant interaction with the client, beginning with a careful analysis of the client's situation and continuing through timely and frequent contact over the duration of the loan. A good loan administration requires that the loans be timely released, acquired at least cost and the process less cumbersome. Timely release of loans prevents loan diversion, while low cost and ease of the loan encourage patronage, and help to minimize loan defaults. This study was therefore, specifically designed to examine the socio-economic characteristics of loan administrators of the Agricultural Cooperatives in Benue State, Nigeria and their effects on recovery rate. The knowledge of these factors will provide useful information for lending institutions, as well as for credit policy formulation in Benue State in particular, and Nigeria in general.

**Hypotheses**

The hypothesis postulated for testing was that the socio-economic factors of loan administrators have no significant effect on loan recovery rate.

## II. METHODOLOGY

The study was conducted in Benue State of Nigeria. The study adopted multistage sampling techniques. The first stage involves a purposive selection of one Local Government Area (LGA) each from the three agricultural zones based on the high concentration of Co-operatives, and include Ukum, Makurdi and Otukpo. In the second stage, a sampling frame for each cooperative institution was developed and a sample proportion of 25% across board was respectively used to obtain a sample size of 130 respondents. The data collection instrument was the structured questionnaire. The collected data were analyzed using descriptive statistics and multiple regression analysis.

Multiple regression analysis was used to determine the socio-economic characteristics of loan administrators affecting recovery rate. Four functional forms were tried linear, semi-log, Cobb Douglas and exponential, and the best (semi-log) was chosen based on the highest $R^2$ and *a priori*

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*expectation.* The a priori expectation was that $\beta_1, \beta_2, \beta_3, \beta_4, \beta_5, \beta_6, \beta_7,$ and $\beta_8 > 0$.

It is expressed as follows:

Y = Loan recovery rate (Ratio of amount recovered over the ration of amount lend of the administrators)

$X_1$ = Age (years)

$X_2$ = Sex (1= male, 0= Female)

$X_3$ = Marital Status (1 = Married, Others = 0)

$X_4$ = Household size (numbers of dependants)

$X_5$ = Education (years)

$X_6$ = Annual salary (₦)

$X_7$ = Cooperative experience (years)

$X_8$ = Size of portfolio in terms of amount authorized to give out as loan to borrowers (years)

$\beta_1 - \beta_8$ are parameters to be estimated.

## III. RESULTS AND DISCUSSION

### Socio-economic characteristics of loan administrators

Analysis of the socio-economic characteristics of loan administrator is presented in Table 1. The result indicates that majority of the respondents (58.46%) were male whereas 41.43% were female. This is attributed to the work life in-balance between male and female in the financial institutions. Females especially married ladies, found it difficult to combine working in financial institution with their family chores and single ladies may not likely to marry as work in financial institution limit their social life. This agrees with the finding of Oliver (2016), that the male dominated financial institutions due to insufficient flexible working options and stigma for using them, insufficient support for family's responsibilities, persistent source of low inclusion in culture such as invisible unconscious biases and traditional assumptions limiting women counterpart.

The mean age of the loan administrators was found to be approximately 36 years. This finding shows that most of the respondents were young people who are energetic enough to withstand the stress involved in lending and recovery. This is in line with the findings of Ikandi (2013) that young people are innovative and active at work as the older ones are weak and no longer in their productive stage.

The results further revealed that majority of the respondents (67.69%) were married. This shows that financial institutions in the study area were dominated by individuals who are married. This result could be attributed to the fact that married people work harder and are more strategic in actions. This is because married people are more responsible, and cater for family needs like hospital bills, school fees, shelter etc. This confirmed with the finding of Bradford (2015) that marriage transforms men's social worlds, as they spend less time with friends and more time in work places, as well as go to bars less. Thus, activities involving recovering and prevention of default can be effectively undertaken by this group.

The mean household size of the respondents was found to be four. This could be due to the fact that a small family is necessary to keep ecological hazards, economic problems at bay, and essential to guarantee a better quality of life. This finding concurs with Hollander (2001) that a small family promises well-nourished and health family affiliates and more attentions will be given to loan recovery and prevention strategies. Specially, majority of the respondents (81%) had household size of 1 - 5, 18% , 6 - 10. while 1% had a household size of 11 - 15.

The mean number of years spent on formal education by the respondents was 9.78 years. This could be due to the fact that the minimum requirement for any employee in the financial institution is first degree or its equivalence. This gives room for effective communication, and adoption of modern technology. High level of education determines quality of skills of loan administrators (Moyib *et.al., 2013:* Girei *et. al., 2014*). Specifically, 63.1% of the respondents spent 4 - 9 years in formal education, 13.85% spent 10 – 15 years, 22.30% spent 16 - 21 years, while only 0.77% spent 22 to 27 years. The finding shows that respondents are all educated in the study area.

The years of respondents' cooperative experience was found to be 14.39. This implies that most of the respondents are well experienced in financial services. Increase in the years of cooperative experiences can be translated into good loan management by administrators, as well as their ability to manage loan and prevent it from going into default (Osuntogun, 1980 & Okorie *et al,* 2011). Majority of the respondents (86.92%) had about 11 - 19 years of cooperative experience, 10.77% had about 2 - 10 years, while 3.30% had about 20 - 28 years of banking experience.

The mean annual salary of loan administrators was ₦ 2,480,000.07. The annual salaries of the administrators were relatively poor to carter for economic activities despite the small household size of respondents. This could be attributed to cutting cost measures (salary slash) adopted by financial institutions to reduce the impact of the loans loss on the performance and financial health of banks. The financial sector appears to be having its own fair share of the effect of economic recession, as a number of banks are experiencing poor asset quality and increase in non-performing loans, resulting in downsizing of staff, and reduction in staff salary (Umar, 2016).

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*Table.1: Socio-economic characteristics of loan administrators (n = 130)*

| Socio-economic variable | Number of Respondents | Percentages | Means |
|---|---|---|---|
| **Sex** | | | |
| Male | 76 | 58.46 | |
| Female | 54 | 41.43 | |
| **Age (yrs)** | | | |
| 21-30 | 44 | 34.85 | |
| 31-40 | 58 | 44.62 | 35.63 |
| 41-50 | 25 | 19.23 | |
| <50 | 3 | 2.30 | |
| **Marital Status** | | | |
| Married | 88 | 67.69 | |
| Single | 42 | 32.30 | |
| **Household size** | | | |
| 1-5 | 105 | 80.77 | |
| 6-10 | 23 | 17.69 | 4.14 |
| 11-15 | 2 | 1.54 | |
| **Formal education (yrs)** | | | |
| 4-9 | 82 | 63.07 | |
| 10-15 | 18 | 13.85 | 9.78 |
| 16-21 | 29 | 22.30 | |
| 22-27 | 1 | 0.77 | |
| **Cooperative experience (yrs)** | | | |
| 2-10 | 14 | 10.77 | |
| 11-19 | 113 | 86.92 | 14.39 |
| 20-28 | 3 | 2.30 | |
| **Annual income (₦'000)** | | | |
| 50-700 | 5 | 3.84 | |
| 701-1351 | 10 | 7.69 | |
| 1352-2002 | 15 | 11.53 | |
| 2003-2653 | 42 | 32.30 | 2,480.07 |
| 2654-330 | 12 | 9.23 | |
| 3305-3955 | 23 | 17.69 | |
| 3956-4606 | 19 | 14.61 | |

Source: Field survey data, 2017

**The effect of socio-economic characteristic of loan administrators on recovery rate**

The result of effects of socio-economic characteristics of loan administration on recovery rates is presented on Table 2. The result shows that the coefficient of determination ($R^2$) was 0.342, indicating that 34.2% of the variations in recovery rate was explained by the explanatory variables in the model. The result also showed that F-statistic (7.84) was statistically significant ($p<0.05$), indicating the goodness of fit of the model and the overall significant of variables used in the model. The result shows that portfolio size was the only variable that significantly and positively affected loan recovery rate. The coefficient of portfolio size (1265.63) also increased recovery rate by 1265.63. This could be attributed to the size of client business. The higher the size of business, the higher the chances of loan recovery. Portfolio size of an administrator is depending on the size of borrowers' business in a particular region. This result agrees with the findings of Piet (1995) that the top-down approach to portfolio allocation involves first, the decision as to how much to allocate to each broad asset category; and second, a decision on an optimal strategy within each asset category. The result also showed that the coefficient of salary, age, education, household size, banking experience, marital status

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and sex were insignificant, and therefore, have no significant effect on recovery rate.

*Table.2: The effect of socio-economic characteristic of loan administrator on recovery Rate.*

| Variables | Linear | Semi-log+ | Double-log | Exponential |
|---|---|---|---|---|
| Constant | 1822.20(3.47) | -5461.45*** | 4.65(6.19) | 7.57(32.29) |
| Sex | -39.35(-0.38) | -72.71(-0.49) | -0.30(-0.45) | -.01(-0.36) |
| Marital Status | -37.02(-0.34) | -49.09(-0.31) | -0.16(-0.23) | -0.01 (-0.22) |
| Salary | 0.85(-0.99) | 16.48(0.15) | -0.10(-0.20) | -8.62E-005(-0.06) |
| Age | -6.78(-0.46) | -213.60(-0.87) | -012(-1.11) | -0.004(-1.24) |
| Numbers of years in education | -4.59(-0.01) | -25.62(-0.-26) | -0.01(-0.31) | -0.002(-0.48) |
| Household Size | -0.46(-0.82) | -7.81(-0.70) | -0.00(-0.06) | 0.001(0.05) |
| Banking Experience | -12.54(-0.82) | -190.54(-0.93) | -0.08(-0.95) | -0.006(-0.83) |
| Portfolio size | 0.75(7.34) | 1265.63(7.50)*** | 0.53(7.06) | 0.000(6.79) |
| $R^2$ | 0.330 | 0.342 | 0.31 | 0.30 |
| Adjusted-$R^2$ | 0.29 | 0.298 | 0.27 | 0.26 |
| F statistic | 7.461 | 7.847*** | 7.093 | 6.533 |

Source: Data analysis, 2017.

*** significant at 1%, + lead equation, Figures in the parenthesis are t statistic.

## IV. CONCLUSION AND RECOMMENDATIONS

The result of this study has shown that portfolio size was the only variable that significantly and positively affected loan recovery rate. The higher the size of business, the higher the chances loan recovery. Portfolio size of an administrator is depending on the size of borrowers' business. The result also find out that the coefficient of salary, age, education, household size, and banking experience marital status and sex had no significant effect on recovery rate.

It is therefore, recommended that loan administrators should be given higher portfolio size loan as these will trigger them to carefully look at business activities in their coverage areas that are capable of repaying loans from proceeds of their sales and cash flow. If loan are given to viable business ventures with good business fortunes, loan monitoring will be easier and administrators will be more focused.

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# Physicochemical and Nutritional Properties of Varieties of Carrot (*Daucus carota*) grown in Region of Korhogo, North of Côte d'Ivoire

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***Abstract*—***Very popular for its therapeutic and nutritional virtues, culture of carrot (Daucus carota) has developed in temperate zones of Asia and Europe but also in some tropical regions of Africa including Côte d'Ivoire. Agronomic factors, commercial and food requirements require selection of varieties with good nutritional values. In this study, physicochemical properties and nutritional values of four carrot varieties namely Amazonia, Bahia, Madona and Pamela+ were compared after cultivation and harvest in region of Korhogo. Results showed that, Amazonia, the control carrot variety stands out for its acidity and minerals levels. Bahia is the richest variety with high levels of carbohydrate and protein. Madona is the most basic, wettest and fatest carrot. For a long storage, Pamela is most interesting variety. To our knowledge, it is the first time that physicochemical and nutritional parameters of carrot varieties in region of Korhogo have been studied. Future research on these carrot varieties will be studied of their post-harvest conservation, their sensory analysis and their transformation.*

***Keywords*—***Carrot varieties, Daucus carota, Korhogo, nutritional values, physicochemical properties.*

## I. INTRODUCTION

Carrot (*Daucus carota*) is a bi-annual herbaceous plant of the Apiaceae family grown for its taproot which is edible fresh or cooked. Developed as a spare and unbranched organ (in loose soil, without obstacle), its root is fleshy, brittle, pigmented (but rarely white) with pleasant taste. Carrot is well known to be rich in carotene, vitamins, proteins, sugar and minerals (Le Clerc, 2001; Reduron, 2007). Carrot is the most important economically species in the Apiaceae family (Rubatzky *et al.*, 1999). It is one of most popular root vegetables grown in the world and most important source of food carotenoids in Western countries, including United States of America (Block, 1994; Torronen *et al*., 1996). This root vegetable is much consumed in the world because of its nutritional value, its simple and various modes of consumption (Chaux & Foury, 1994). Carrot is one of the ten most important worldwide vegetable crops for area of production and market value (Simon *et al.*, 2008). In addition to its consumption, carrot is also used as a dye plant to color butter or certain cheeses (Reduron, 2007). There are various colors of carrot (yellow, pink, purple, white, etc.) related to difference in carotenoid content (Clotault *et al.*, 2008; Clotault, 2009). However, orange carrot gradually supplanted all other colors because of its more desirable hue, especially after cooking (Reduron, 2007). This type of carrot is generally the most rich in total carotenoids. The two major carotenoids in orange carrot are β-carotene and α-carotene. It also contains a low proportion of lutein (Nicolle *et al.*, 2004; Clotault, 2009). Many orange cultivars have appeared over time with in particular a diversification of root forms. Thus, the vegetable carrot has diversified into local varieties to respond to crop patterns and various situations (Pitrat & Foury, 2003; Doré & Varoquaux, 2006). The food interest of the root of carrot concerns its taste, its color, but also its nutritional characteristics (Aubert & Bonnet, 1977; Tirilly & Bourgeois, 1999). Interest is more and more also focused on nutraceutical compounds of this root because of their importance for good health. Indeed carrot is an interesting food for its content of antioxidant compounds, mainly anthocyanins or chlorogenic acid and carotenoids (Sun *et*

*al.*, 2009). β-carotene or provitamin A is the carotenoid which is transformed by human metabolism into vitamin A (Dreosti, 1993; Lecomte, 2013). The consumption of carrot contributes to a healthy and balanced diet (Shankara *et al.*, 2005). Very popular for its therapeutic and nutritional virtues, culture of carrot has developed in temperate zones of Asia and Europe but also in some tropical regions of Africa including Côte d'Ivoire. In addition to agronomic factors (precocity, high yields, pest resistance), commercial and food requirements require the selection of varieties with good nutritional values.

Objective of this study is to compare physicochemical and nutritional properties of four hybrid varieties of carrots, namely Amazonia, Bahia, Pamela+ and Madona, grown in northern Côte d'Ivoire.

## II. MATERIALS AND METHODS

### 2.1. Zone of study

Study was conducted in commune of Korhogo, located in northern Côte d'Ivoire. Geographic coordinates of this area are 9° 26' North Longitude and 5° 38' West Latitude. Climate is Sudanese, very hot, very dry and characterized by an alternation of two main seasons: the dry season and rainy season. Dry season runs from November to April and rainy season from May to October. Maximum rainfall is achieved in August and September and varies between 255 and 267 mm, with an annual average of about 1200 mm. The dry season of this climate is marked by harmattan which is a hot and dry wind from Northeast whose peak is between December and January. Average temperatures vary between from 24 to 33 °C with a monthly average humidity of 20%. Soil profile of region is characterized by the very large predominance of ferralitic soils. In general, these soils have very variable saturation levels between 20 and 50%. Relief is generally flat and dotted with inselbergs. Average annual duration of sunstroke in this geographic zone is 2500 hours. Monthly average is about 205 hours in the dry season compared to nearly 140 hours during months of July and August who are the most watered (Koffie & Yéo, 2016).

### 2.2. Plant material

Plant material is composed of four hybrid varieties of carrot (*Daucus carota* sub. sp. *Sativus*), belonging to the Kuroda type. These varieties are known by their vernacular names. Variety namely Amazonia is the most cultivated in the region of Korhogo. It has been used as control in this study because its agronomic characteristics are well known. After 90 to 95 days of cultivation, the pivoting roots of Amazonia can reach 16 to 18 cm long (Technisem, 2017).

### 2.3. Conduct of experimentation

Test was conducted using a completely randomized block device of Fisher with four (4) treatments and four (4) repetitions. Study consisted of sixteen (16) elementary plots. Each sub-plot consisted of seventy-two (72) plants, transplanted on six (6) lines according to spacings of 25 cm x 8 cm (25 cm between two lines and 8 cm between two plants in the same line). Each elementary plots had an area of 2 $m^2$ each and the blocks, were respectively separated by a distance of 50 cm and 80 cm. Whole plot consisted of one thousand one hundred and fifty-two (1152) plants on a total area of around 60 $cm^2$. Test was conducted in dry season (off-season period). Two (2) kilograms (kg) of fully decomposed chicken manure were added to each basal plot as background fertilizer two weeks prior to carrot seeding. After sowing, mulching of plots was done to maintain sufficient soil moisture after watering operations. Thinning was done at the stage of appearance from 3 to 5 leaves (22 to 35 days after emergence of plants). Purpose of thinning was to maintain spacing of 8 cm between plants on same line. Weeding has been regularly carried out to eliminate weeds and ensure good aeration of soil. A first mineral fertilization was carried out on 46$^{th}$ day after sowing with mineral fertilizer NPK (formula: 12-11-18+2.7MgO+8S+B+Fe+Zn+Mn) at rate of 50 g per elementary plot. A second mineral fertilizer (formula: 15.4N+25.6CaO+0.3B) was applied 3 weeks after first amendment of the soil, at a rate of 30 g per elementary plot. Preventive treatments against insects were applied on the plots once a week from 8 leaves (66 days after emergence of plants) with the product "Cypercal" provided by Callivoire (Côte d'Ivoire) at rate of 1 l/ha. This dose is equivalent to a mixture of 6.6 ml of the product and 2.5 l of water per elementary parcel. The carrot roots were harvested from 90$^{th}$ day after emergence of plants (3 months and week after sowing).

### 2.4. Analytical procedures

Root samples of four (4) varieties of carrots from experimental test were cleaned in the laboratory to remove foreign elements. These roots were crushed using an electric grinder (Clatronic KM 3648, France) with perforated disk of 10 µm of diameter. The grind of each variety of carrot was put carefully in closed bottle and stored in refrigerator at 4 °C before their use for biochemical and nutritional analyzes.

Physicochemical parameters of samples of each variety of carrot were determined according to the official methods of analysis of Association of Official Analytical Chemists (AOAC, 1990). Moisture content of samples was determined by desiccation using the method of De Knegt & Brink (1998). A clean platinum dish was dried in an oven (Memmert UN 110, Allemagne) and cooled in a

desiccator and weighed. From each sample, 10 g was weighed and spread on the dish. Then the dish containing the sample was weighed. It was then transferred into the air oven at 105 °C to dry until a constant weight was obtained and the loss in mass was determined. In order to obtain the pH of the samples, 10 g of each sample was weighed and suspended in 10 ml of distilled water. The pH was determined with a digital pH-meter (Hanna EUTECH INSTRUMENTS PH 700, Espagne). Titratable acidity of samples was determined by titration with 0.1 N of sodium hydroxide solution, using phenolphthalein as indicator. The results are calculated in citric acid equivalent and expressed in g/l of acid (Abbas & Khoudi, 2016).

For nutritional parameters, carbohydrate was determined according to phenol sulfuric acid method (Dubois *et al.,* 1956). A standard curve was obtained using the following concentration of sucrose in (mg/ml) 2.5 2.0, 1.25, 1.0, 0.5 g of each sample with 9 ml of distillated water was measured into test-tube. 2 ml of phenol solution (1%) and 1 ml of concentrated H2SO4 solution were added. This was shaken for 15 min and boiled for 30 min. It was then allowed to cool. The absorbance was then read off a spectrophotometer (UV-Visible, type 7315) at 700 nm. The sugar concentration was then obtained by extrapolation from the standard curve. Protein was analyzed by the Microkjedhal nitrogen method, using a conversion factor of 6.25 according method described by Hamon *et al*. (1990). Lipid content was obtained by Soxhlet extraction as described by Lecoq (1965). Ash was determined according to the standard methods described by the Association of Official Analytical Chemists (AOAC, 1990). Five (5) g of sample of carrot crushed was ashed in a muffle furnace Pyrolabo, France) at 550 °C. Percentage of residues obtained after incineration corresponds to ash content.

### 2.5. Statistical analyzes

Data collected in triplicates from these studies were analyzed using Statistical Analysis Software XL-STAT version 7.5.3. Data were expressed as means, giving relative standard deviations. The Student Newman Keuls test (SNK) with 5% of signification was used to discriminate the means. Correlations and a principal component analysis (PCA) were realized in order to detect differences that discriminate the carrot varieties.

## III. RESULTS AND DISCUSSION

Results obtained for physicochemical and nutritional parameters of four carrot varieties are presented in Table 1. Variance analysis for each parameter studied revealed significant differences between four carrot varieties according to SNK test at 5 %.

### 3.1. Physicochemical properties

Moisture content of carrot varieties varies from 86.3 to 87.2% (SNK, 5%). Analysis of these data shows that carrot Palema+ is a variety which has the highest moisture content with 87.2% compared to other varieties (86.96 – 86.21%). These values revealed that carrot is a wet root. Moreover, these values corroborate those of other authors which showed that moisture content of carrots varies from 86 to 89% (Gopalan *et al*., 1991; Arscot & Tanumihardio, 2010). However, studies of Cohen *et al*. (2009) showed that moisture content of carrot is 89%, while those of Holland *et al*. (1991) reported a value of 88.80%.

Varieties of carrots analyzed are pH values ranging from 6.51 to 6.60. There are slightly acidic according to pH scale of the products (Anonyme, 2009). Analysis of variance (SNK, 5%) revealed significant difference between these pH. Control variety Amazonia and variety Pamela+ have approximate pH values of 6.51 and 6.47 respectively. These values are significantly lower than those of Bahia and Madona varieties who are also neighbors with respectively pH of 6.63 and 6.60. These results are almost similar to those of Abbas and Khoudi (2016) who reported a pH value of 6.53 for carrot puree. The studies of Argha and Gavin (2016) revealed however average value of pH of the carrot between 4.9 and 5.2. Indeed, according to Anonyme (2009), pH of some products may vary with varietal characteristics, growing conditions and others factors.

For titratable acidity, Analysis of variance showed significant difference between varieties of carrot studied. The highest acidity has been observed in control variety Amazonia with 0.192 g/l while Bahia is the least acidic variety with 0.156 g/l. The other two varieties, Madona and Pamela+ presented intermediary values with respectively acidities of 0.169 and 0.171 g/l. However, studies of Abbas and Khoudi (2016) on carrot puree reported titratable acidity of 0.2 g/l. Result obtained by these authors show that varieties of carrot of our study are slightly acidic and must be pleasant to eat.

### 3.2. Nutritional properties

Carbohydrate contents between 5.62 and 6.71% are observed with carrot varieties studied. Bahia is carrot variety with highest carbohydrate content (6.71%) while control variety gave the lowest (5.62%). Madona and Pamela+ varieties showed respective intermediate rates of 6.45 and 6.29%. Our results are quite similar to those of Cohen *et al*. (2009) who reported a value of 6.7%. However, these rates obtained in our study are lower than those of carrot varieties studied by Arscot and Tanumihardio (2010) with 7%.

For protein, contents of carrot varieties are ranged between 2.71 and 3.66%. Analysis of variance (SNK, 5%)

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revealed significant difference between these values. Variety Amazonia, as control showed the lowest protein level with 2.71%, followed by Madona which contains 2.89%. Varieties Pamela+ and Bahia revealed high proportions of protein with respectively 3.15 and 3.66%. These protein levels are well above those obtained through work on other carrot varieties. These protein levels are well above those obtained by Gopalan *et al*. (1991), Holland *et al*. (1991) and Cohen *et al*. (2009) on others varieties of carrots. The work of all these authors, indicated protein proportions in carrot ranging from 0.7% to 1.1%.

Lipid contents of varieties of carrot analyzed are between 0.79 % and 0.84 %. These values are significantly different according to SNK test at 5%. Lowest content of lipid is observed with Pamela+ carrot variety (0.79 %) while variety Bahia showed highest level of 0.84 %. Madona and Amazonia varieties presented intermediary and approximate contents of lipid with respectively 0.83 % and 0.82 %. Lipid contents of carrot varieties studied are higher than those of Gopalan et *al*. (1991), Holland et *al*. (1991) and Cohen *et al*. (2009) who respectively obtained lipid levels of 0.2%, 0.5% and 0.3% with other carrots. These results show that carrots of our study are rich in lipids.

Ash contents of carrots studied varies between 0.89% and 1.3%. These results showed a significant difference between varieties analyzed according to SNK test at 5%. Amazonia variety gave highest ash content with value of 1.3% followed by Madona who showed 1.24% of ash. The two others carrot (Bahia and Pamela+) presented lowest ash rates with same value (0.89%). On average, ash content of carrot studied is similar to that of Gopalan et *al*. (1991) with a rate of 1.1%.

### 3.3. Correlations between parameters

According to Pearson test, analysis of results revealed significant correlations between some parameters (Table 2). Most significant positive correlations are between moisture and lipids ($R^2$ = 0.92) and lipids and pH ($R^2$ = 0.93). Most negative correlations are between titratable acidity and carbohydrates ($R^2$ = -0.989).

### 3.4. Discrimination of carrot varieties

Two axes F1 and F2, allowed to express 95.71% of variability of observations (Table 3 and Fig. 1). Axis 1 (F1) contributed to 58.67% of observed variance and axis 2 (F2) to 37.03%. The parameters of carrot varieties studied which have best express on F1 axis are titratable acidity level (which is positively correlated with it), pH, carbohydrate and protein contents (negatively correlated to this axis). For second axis (F2), lipid content, ash and moisture levels are variables positively correlated. The two main axes F1 and F2 described four quarters of plans. Top right quarter above F1 axis and right of F2 axis, containing Amazonia variety. Upper left quarter of plan, above F1 axis and to left of F2 axis with Madona variety. Right lower quadrant, below F1 axis and to right of F2 axis with Pamela+ variety. Lower left quarter of plan which is located below F1 axis and to left of F2 axis including Bahia variety.

Distribution of variables in overall plan constituted by axes F1 and F2 was following. Ash level and titratable acidity are the two variables which appear in top right quarter of plan. pH, moisture and lipid contents are located in upper left quarter of plan. Carbohydrate and protein levels are shown in lower left quarter plan. No variable do not appears in lower right quarter of plan.

So Bahia variety is characterized by carbohydrate and proteins contents while Amazonia, a control variety is marked by ash and titratable acidity levels. pH, lipid and moisture levels were most important determinants of Madona variety. No parameters are characteristic of Pamela+ variety.

## IV. CONCLUSION

This study showed a significant variation in physicochemical and nutritional characteristics of the four carrot varieties grown in region of Korhogo. Investigations closed that, Amazonia, the control carrot variety stands out for its acidity and minerals levels. Nutritionally, Bahia is the richest variety with high levels of carbohydrate and protein. Madona is the most basic, wettest and fatest carrot. For a long storage, Pamela is most interesting variety. To our knowledge, it is the first time that physicochemical and nutritional parameters of carrot varieties in region of Korhogo have been studied. Future research on these carrot varieties will be study of their post-harvest conservation, their sensory analysis and their transformation. These studies will provide scientific data but also advice carrot varieties meeting requirements of growers and consumers in this region.

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## TABLES

*Table 1: Physicochemical and nutritional parameters of varieties of carrots*

| | | Moisture (% ) | pH | Acidity (g/l) | Carbohydrate (% ) | Protein (% ) | Lipid (% ) | Ash (% ) |
|---|---|---|---|---|---|---|---|---|
| Varieties | **Bahia** | 86.437$^{a}$ | 6.631$^{a}$ | 0.156$^{b}$ | 6.712$^{a}$ | 3.656$^{a}$ | 0.841$^{a}$ | 0.890$^{c}$ |
| | **Madona** | 86.210$^{a}$ | 6.603$^{a}$ | 0.169$^{b}$ | 6.459$^{a}$ | 2.886$^{c}$ | 0.831$^{ab}$ | 1.244$^{b}$ |
| | **Pamela+** | 87.200$^{b}$ | 6.472$^{b}$ | 0.171$^{b}$ | 6.293$^{a}$ | 3.146$^{b}$ | 0.786$^{b}$ | 0.888$^{c}$ |
| | **Amazonia*** | 86.692$^{a}$ | 6.514$^{b}$ | 0.192$^{a}$ | 5.623$^{b}$ | 2.715$^{d}$ | 0.820$^{ab}$ | 1.300$^{a}$ |

**Control*

*Table.2: Correlation between parameters (Pearson (n)) :*

| Variables | Moisture | pH | Acidity | Carbohydrate | Protein | Lipid | Ash |
|---|---|---|---|---|---|---|---|
| Moisture | **1** | 0.8915 | -0.2649 | 0.3237 | 0.0287 | 0.9209 | 0.4521 |
| pH | 0.8915 | **1** | -0.6307 | 0.6464 | 0.4785 | 0.9328 | 0.0087 |
| Acidity | -0.2649 | -0.6307 | **1** | **-0.9893** | -0.8742 | -0.3340 | 0.7189 |
| Carbohydrate | 0.3237 | 0.6464 | **-0.9893** | **1** | 0.7938 | 0.3358 | -0.6426 |
| Protein | 0.0287 | 0.4785 | -0.8742 | 0.7938 | **1** | 0.2760 | -0.8552 |
| Lipid | 0.9209 | 0.9328 | -0.3340 | 0.3358 | 0.2760 | **1** | 0.2602 |
| Ash | 0.4521 | 0.0087 | 0.7189 | -0.6426 | -0.8552 | 0.2602 | **1** |

*Values in bold are most significant correlations*.

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*Table 3: Values of variables along the axes*

| Parameters | F1 | F2 |
|---|---|---|
| Moisture | -0.5504 | 0.8275 |
| pH | -0.8596 | 0.5099 |
| Acidity | 0.9312 | 0.3220 |
| Carbohydrate | -0.9172 | -0.2593 |
| Protein | -0.8281 | -0.4765 |
| Lipid | -0.6524 | 0.7132 |
| Ash | 0.4959 | 0.8605 |

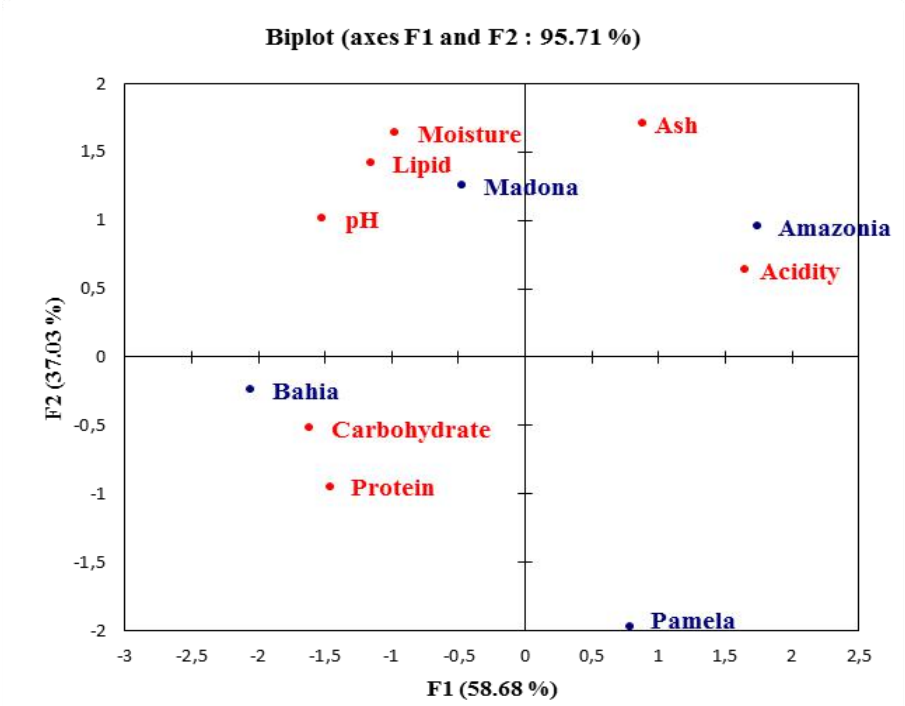


*Fig. 1: Plan biplot of varieties of carrots and variable scores (PCA)*

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# Performance of combined tillage equipment and it's effect on soil properties

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***Abstract*—***An experiment was conducted to evaluate the performance of a locally manufactured combined tillage implement (moldboard plow + ripper) in one of the fields of the kufa university faculty of agriculture. The experiment was included two factors , the first factor is combing the ripper to mold board plow in five level these are fixing the combined ripper shanks while the shanks points oriented in two different levels with and opposite to the plowing direction , two levels of different ripper depths the same depth and 5 cm above the depth of mold board plow share and the fifth level is control treatment ( mold board plow alone ) .The second factor was the plowing operation speed at five levels (1.4, 2.0 , 3.6 and 4.7) Km.hr $^{-1}$ . the experiment was conducted as a factorial experiment with RCBD , the LSD test at 5 % was used to compare between means .The results of the research were showed that combining the locally manufactured ripper implement to mold board plow resulted in significant increase in the number of soil clods with the desired diameter (5-10 cm ) very low number of soil block with diameter larger than 10 cm , more even soil roughness and the actual productivity has not decreased to the extent that it affects the efficient performance of the tillage process compared to the use at the mold board plow alone .*

***Keywords*—combination ,tillage, soil, roughness, ripper , moldboard.**

## I. INTRODUCTION

Now a days, the conventional tillage practices are very expensive in cost , more time consuming and higher number of passes which cause soil compaction. Furthermore; conventional tillage is considered to be one of the low fuel-efficient operation. Digman 2012 mentioned that only 20% of diesel fuel energy is available at the tractors drawbar, however, only 4% out of that energy is converted in to breaking up the soil . Therefore , it is so important to find out an early way leading to get the most out of tillage operation , one way to bypass these problems is the use of combined tillage implements in one field operation. This practice is useful only for those who prefer the use of conventional tillage practices such as the Iraqi farmers. Manjeet et al. 2016 defined the combined tillage is the way in which two or more implements operates at the same time in order to manipulate the soil. In general sense, combined tillage means integrated management of resources such as time, energy, fuel, labor, soil and water conservation, on the other hand, increasing yield and better utilize of natural resources. It also contributes and sustained agriculture production. Nasr et al 2016divided the combined agricultural implements intofive groups these are:1-Soil preparation.2-soil preparation andfertilizing. 3-soil preparation ,fertilizing and seeding. 4-soil preparation and seeding. 5-fertilizing and seeding. For each of thefive groups mentioned they suggested two or more operations e.g the third group mentioned above has three operations these are plowing , fertilizing and seeding ;tilling , fertilizing and seeding and cultivating fertilizing and seeding. Grisso et al (2012) revealed that combined implements operations reduce fuel consumption, time and labor requirements by limiting at least one individual trip over the field. Javadi et al. 2006 , Asgill 2008 and Manjeet et al 2016 revealed that combination tillage implements were more energy efficient , higher tillage performance index (TPI) and saving nearly 50% in cost and 50 _55% in timecompared with the same single passive tillage implement .

Moitzi et al. 2014 revealed that the area – specific fuel consumption increased linearly with working depth for moldboard plow and short disc harrow , but disproportionately for subsoil . Wheel slip was also found to increase fuel consumption and decrease field capacity performance at all depth . The concept of combination tillage practice was entered into force for the primary and secondary tillage operations since hundred year ago (Shafee 1995), but is still not widespread even in places where this application is needed , such as rice cultivation in southern Iraq . Theobjectives of this research were to investigate the performance of the developed combined implement in terms of it effect on :

1- Improving soil refined and surface uniformity .
2- Saving time and fuel consumption .

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## II. MATERIAL AND METHOD

**Description of the developed implement:**

A combination implement was developed at the faculty of agriculture university of Kufa in order to meet the seedbed requirements by sufficient loosening field soil breaking clods and gaining a uniform soil bed in a single pass and least time . Fig 1 shows the developed implement which was combined from moldboard plow and locally manufactured heavy duty ripper . It had 6 shanks fixed into a heavy rectangular frame . The long sides of the rectangle form the two rows of the ripper and the shanks placed in equal and interlaced spaces .

However for optimum performance the spaces was set equal to 40 cm in the same row . The unique ripper is attached to the end of the moldboard plow chassis so that the extension of the pull line passes through the center of the ripper tool . The plow and the ripper work as one unit , when the moldboard plow raised hydraulically it picks up the ripper too .


*Fig 1 Top: the shanks position behind the bottoms of the moldboard plow , bottom :the combined implements (moldboard plow + ripper )*

During plowing the front shanks of the ripper hit with the center of the inverted slices that are usually formed by moldboard the result is dismantling and breaking the slices and displacing some of the clods and soil to the sides. Here comes the role of the rear row shanks in disassembling the rest of slices and handle the big clods that were displaced by the front shanks . Moreover the developed implement makes the ground more even . The ripper shanks were designed to be easily adjusted in depth , however the adjustment was set according to the moldboard plow depth . The ripper shanks points ( shovels) were made from heavy long wearing metal with dimensions of 15 cm length 2 cm thickness . Each point was supplied with two slotted holes so it can be fitted in the required center .

**Test procedure** :

An experiment was conducted at one of the agriculture field at the faculty of agriculture University of Kufa to test and evaluate the performance of the developed implement in term of breaking and pulverizing the field soil in one tractor pass .

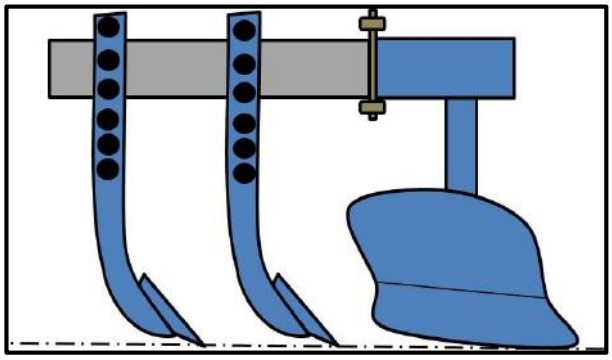
The combined implement was pulled byMassy Fergusson tractor with nominate power 82 kw and total mass 3250 kgm. The test was performed according to 5x5 factorial split plots design with the randomized complete block design with three replicators . the experiment contain the following two factor:

1-Fixing thecombined ripper shanks while shanks points oriented in two different ways and two different depths taking in to consideration the depth of moldboard plow and control treatment (moldboard plow alone ) , these are :

a- Fixing the ripper shanks with shanks points orientedtoward the direction of tillage operation with two depths: same depth as plowing share (S1D1) and 5 cm above plowing depth(S1D2) fig 2

b- Fixing the ripper shanks with shanks points oriented opposite the direction of the tillage operation with two depths : same depth as plowing share depth (S2D1) and 5 cm above plowing depth (S2D2) fig 2 .

c- Control treatment , without the use of the ripper implement MB (moldboard plow alone ) .



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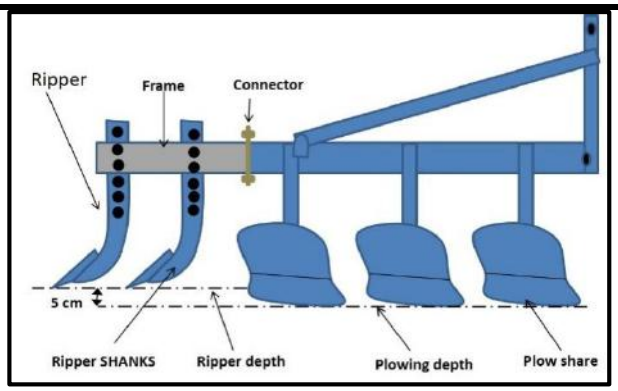


*Fig 2 moldboard plow + ripper with two directions and two depths .*

2- Tillage operation field speeds with five averaged levels : 1.4 , 2.0 , 3.6 , 4.2 and 4.7 km . $hr^{-1}$ .

The quality of the tillage and operation performance were evaluated through the estimation of the following parameters :

1 – Soil refinement : it was determined by using two different size opining sieves . The substance of this test is to pass the soil of an area 50 * 50 cm picked randomly from the tillage treated soil through a 10 * 10 cm opining then through a 5 * 5 cm opining sieve .

2- Soil surface roughness index : it was determined by using the following formula :

$$SD = \sqrt{\frac{\Sigma(di-d)^2}{n}} \text{.....cm} \quad \text{.....(1)}$$

Where :SD : soil roughness index , di : soil ripple height , which determined by use a wooden rectangle triangle with a 50 cm height and 2 m base length . before sampling the triangle was leveled and samples were taken every 10 cm .

3 – Actual Productivity (ha. $hr^{-1}$ ) :

A.P. =0.1 * W * VP * FE (ha. $hr^{-1}$ ) ….(2)

Where : A.P.:W: active working width (m) , VP: tillage operation speed (km. $hr^{-1}$ ) and FE : field efficiency ( 60%) .

3- Slippage % :

$$S\ \% = \frac{VT-VP}{VT} * 100\ \% \text{ ……... (3)}$$

Where : S : slippage % , VT: theoretical speed (km. $hr^{-1}$ ) ,

4- Fuel ConsumptionFu.C **(** L .$ha^{-1}$):

Fu.C=(Qd*10000) / (W*D ) …….(4)

Where : Qd : treatment fuel consumption ( L) , D: treatment length (m)

## III. RESULTS AND DISCUSSION

**Soil colds larger than 5 cm in diameter**

Table1shows that combining the manufactured ripper with moldboard plow to perform plowing operation has a significant effect on the size of soil clods larger than 5 cm in diameter compared to the use of moldboard plow alone (MP) and these are true results for all the combined implement treatments levels . These results are consistent with the published resultedby Manjeet et . al (2016) . However the treatment of fixing the ripper shanks with the skanks points oriented opposite to the direction of plowing at the shallow depth (S2D2) exceeded the other treatments by achieving a number of 69.8 clods . $m^{-2}$ followed by the treatments with the greatest depth (S2D1) and the same implement shanks points orientation , while treatment of using moldboard plow alone recorded the least number of clods that was 32.1 clods . $m^{-2}$.

*Table 1: Soil colds larger than 5 cm (clods . $m^{-2}$)*

| TRT | V( km .$hr^{-1}$) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 1.4 | 2.0 | 3.6 | 4.2 | 4.7 | |
| MB | 29 | 30 | 32.5 | 36 | 33 | 32.1 |
| S1 D1 | 31.5 | 33.5 | 35.5 | 38.5 | 40.5 | 35.9 |
| S1 D2 | 34.5 | 38.5 | 41 | 43.5 | 47.5 | 41.0 |
| S2 D1 | 39.5 | 42.5 | 45 | 46 | 53.5 | 45.3 |
| S2D2 | 64.5 | 66 | 70 | 75 | 73.5 | 69.8 |
| | 39.8 | 42.1 | 44.8 | 47.8 | 49.6 | |
| LSD TRT=2.25 LSD TRT.V =3.12 LSDV=1.319 | | | | | | |

Therefore the use of combining implement gave an excellent results in term of improving the size of soil clods with the suitable diameters .The results also revealed that the plowing speed has a significant effect on soil clod larger than 5 cm in diameter the number of soil clods increased as plowing speed increased . This is due the fact that increased the speed of plowing increased the impact speed of the plow bottoms and the ripper shanks against the soil slices formed by the plowshare , as a result increased the process of fragmentation of the soil blocks into smaller soil clods , similar results were found by Abo-herbageet.al.(2010) when they tested a chisel plow at different speed .The interaction between operational speed and the combined implement at different shanks points orientation and different depths was significant so that the synthesis between the ripper combination and the high field speed at shallow depth and shanks points oriented opposite to plowing direction gave the highest number of soil clods.

**Soil clods larger than 10 cm**

The results in table 2and figure 3 indicate that the use of the combined implement (moldboard plow + ripper) has influenced the quality of the tillage operation , the clod size distribution was fairly appropriate where the high number of clods larger than 5 cm were found in table (2) and the low number of clods greater than 10 cm table 3 and the absence of clods greater than 20 cm in all combined implement treatment and has appeared in the moldboard plow treatment . Similar results were published by Servadio et. al (2016) .

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Based on the advanced findings we can conclude that combining the manufactured ripper behind the moldboard plow has worked to break the soil slices formed by the plow share and reduces the soil clods and increased their numbers compared to the treatment of moldboard plow alone .

*Table.2: Soil colds larger than 10 cm (clods . m-²)*

| TRT | V( km .hr-¹) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 1.4 | 2.0 | 3.6 | 4.2 | 4.7 | |
| MB | 11.24 | 10.4 | 10.8 | 10.2 | 9.07 | **10.32** |
| S1 D1 | 5.91 | 4.7 | 4.4 | 3.7 | 3.91 | **4.54** |
| S1 D2 | 8.18 | 7.3 | 6.9 | 5.7 | 5.60 | **6.74** |
| S2 D1 | 6.64 | 5.7 | 5.8 | 4.4 | 3.95 | **5.31** |
| S2D2 | 4.03 | 6.5 | 5.2 | 5.7 | 3.41 | **4.95** |
| | 7.20 | 6.9 | 7.0 | 6.0 | 5.19 | |
| LSD TRT=0.064 LSDTRT.V= 0.113 LSDV=0.0057 | | | | | | |

*Fig.3: top :clods after the use of MP . bottom:clods after the use of combined implement.*

Despite the emergence of a number of soil clods greater than 10 cm in the moldboard plow treatment (MB) table 3 but the apparent sign in this treatment is the surface of the plowing area large soil blocks and in most cases the soil slices remained intact and did not break into parts .It was also noticed the highest number of clods greater than 10 cm was at the treatment when the ripper shanks points oriented opposite to the direction of plowing (S2D2) with a number of clods equal to 13.5clods . m-² and in diameter of 15 to 20 cm the reason for this result was the ripper shanks worked efficiently in breaking the large masses of soil inverted by the moldboard plow , then followed by the treatment with the largest depth and at same direction (S2D1) while the least number of soil clods recorded by the treatment when the ripper shanks point oriented with direction of plowing (S1D1) and its value was 4.5 clods . m-². The results in table 3 indicated that the speed had not significant in this parameter and it seems that the presence of large soil masses with large diameter counted on block increased the large disparity of the parameter between replicates with in the single treatments . As shown in table 3 and figure 2. that the interaction between the combined implement and filed speed did not show a significant effect in this parameter due to the same reasons explained before .

**Soil surface roughness**

The results in table 3 and fig 4 illustrated that the treatment moldboard plow alone (MB) was the Highest variability of the soil surface roughens . This is very naturel result because of the stirring action of the plow and the dismantling soil to masses of different sizes which make the soil surface is more winding and uneven. However the use of the combined implement contributed to increased the leveling of soil surface . this was clear when the ripper shanks point oriented toward plowing direction in treatment (S1D2) which achieved the best degree of surface leveling compared to the rest of the treatments. That was happened due to the right shanks distribution behind soil layers formed by moldboard plow and the continues pushing of the soil in front of the shanks which have led to increased surface leveling . this treatment did not differ from the treatment when the ripper shanks point oriented opposite to the direction of plowing (S2D2) which achieved preference in surface roughens . this is due to the relatively high pulverizing efficiency of this treatment relative to the rest of treatments which contribute acquisition of a more even soil surface compared to the other treatment

*Table 3: Soil surface roughens (cm)*

| TRT | V( km .hr-¹) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 1.4 | 2.0 | 3.6 | 4.2 | 4.7 | |
| MB | 10 | 11 | 8.5 | 8 | 7.5 | **9** |
| S1 D1 | 3.5 | 6.5 | 6 | 3.5 | 3 | **4.5** |
| S1 D2 | 8.5 | 7 | 6 | 9.5 | 6.5 | **7.5** |
| S2 D1 | 12 | 8 | 9.5 | 4.5 | 6 | **8** |
| S2D2 | 11 | 10.5 | 22 | 9 | 14.5 | **13.5** |
| | 9 | 8.6 | 10.4 | 6.9 | 7.5 | |
| LSD TRT= NSLSD TRT.V= NS LSDV= NS | | | | | | |

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*Fig.4 : soil surface before and after combined implement* **.**

The results also showed that the highest speed caused the lowest surface roughness indicator this is may be due to the increased in velocity accompanied by an increase in the fragmentation of the soil matter into smaller fines . The binary interaction between the investigated factors showed that the treatment of the ripper shanks points projected opposite to the plowing direction (S1D1) with highest speed had the best level of surface roughest compared to the other treatment . Combining the ripper implement with moldboard plow during plowing process increased the tractor slippage percentage in all treatment tested . However this increased in slippage show variable differences relative to the shanks points orientation and the calibrated depth . Despite to this finding the result revealed that the combined implement when the ripper shanks point oriented opposite to the direction of plowing with the

**Tractor slippage**

shallow depth (S2D2) has achieved nearly close slippage percentage as compared with treatment of moldboard plow alone (MB) .The interpretation for these results were the ripper shanks had impacted directly soil slices which already cut and inverted by moldboard plow loosening them and lowering their resistance which made the shanks penetrate and sweep through easily.

However when the shanks depth increased to the depth of moldboard plow share (S2D1) the slippage of the tractor increased as a result of the excessive load . The results also showed that the use of the ripper with the shanks points oriented with the direction of plowing gave the highest tractor slippage specially when the shanks points fixed at the same depth as the moldboard plow share depth , the tractor slippage at this treatment reached at most 27% the big masses of soil and in front of the shanks obstruct the shanks movement which increased the load on the tractor lowering the tractor speed and hence the tractor slippage increased .

*Table 5: Tractor Slippage %*

| | ***TRT*** | ***V( km .hr-¹)*** | | | | | ***Mean*** |
|---|---|---|---|---|---|---|---|
| | | *1.4* | *2.0* | *3.6* | *4.2* | *4.7* | |
| | *MB* | *12.3* | *13.47* | *14.0* | *14.0* | *15.43* | ***13.84*** |
| | *S1 D1* | *20.0* | *23.94* | *24.6* | *28.2* | *36.35* | ***26.61*** |
| | *S1 D2* | *16.1* | *23.46* | *25.0* | *27.6* | *35.1* | ***25.43*** |
| | *S2 D1* | *15.1* | *17.52* | *18.7* | *19.4* | *19.82* | ***18.13*** |
| | *S2D2* | *13.3* | *13.81* | *14.5* | *15.5* | *16.92* | ***14.80*** |
| | | *15.4* | *18.44* | *19.3* | *21.0* | *24.72* | |
| *LSDTRT= 0.40 LSD TRT .V=0.38 LSD V =0.21* | | | | | | | |

The speed factor is the other factor which has a significant effect in the percentage of the slippage so as plowing speed increased the slippage percentage increased for all the treatment tested the reasons for this were the power required to break the soil increased and the impact speed that happened between the soil slices and ripper shanks was also increased which increased the actual time required to perform the work relative to the theoretical time which increased the tractor slippage . The slippage values in all combined implements treatment were out of the permitted limits except the treatment in which the shanks pointes oriented opposite to the tillage direction (S2D2) where the slippage within the permissible limits and this is true until the speed reached 4.2 km/hr . the interaction between the main factors was significant even though each factor has effected the slippage parameter independently which gave dam priority to (S2D2) treatment to get lowest slippage value (13.27%) at the lowest speed.

**The actual productivity**

The actual productivity values and means of the studied factors have been tabulated in table 5. Despite the significant results of the actual productivity however a quick view of the results it can be concluded that the differences in productivity between the use of the moldboard plow (MB) alone or the combination with the ripper were not great enough to affect the efficient performance of the plowing process . The difference was 0.003 ha.hr$^{-1}$ between the use of the moldboard plow alone and the highest value achieved when the ripper combined with moldboard plow . It is considered very simple in comparison with applying another agriculture operation such as field cultivator or disc harrow to complete the seedbed preparation .

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*Table.6: Actual Productivity ( ha.hr-1)*

| TRT | V( km .hr-1) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 1.4 | 2.0 | 3.6 | 4.2 | 4.7 | |
| MB | 0.10 | 0.16 | 0.29 | 0.35 | 0.42 | 0.26 |
| S1 D1 | 0.09 | 0.14 | 0.25 | 0.29 | 0.31 | 0.22 |
| S1 D2 | 0.09 | 0.14 | 0.25 | 0.29 | 0.32 | 0.22 |
| S2 D1 | 0.09 | 0.15 | 0.27 | 0.32 | 0.40 | 0.25 |
| S2D2 | 0.09 | 0.16 | 0.29 | 0.34 | 0.41 | 0.26 |
| | 0.09 | 0.15 | 0.27 | 0.32 | 0.37 | |
| LSDTRT= 0.005 LSD TRT .V=0.003 LSD V =0.002 | | | | | | |

The speed factor has a significant effect in determining the actual productivity of the operation at all levels of this study . The highest productivity rate was shown at 4.7 km.hr-1 and it was 0.37 ha.hr-1 The reason for this results is due to the fact that the actual productivity is directly proportional to field speed so that the increased in the field speed has led to increase in the actual productivity and vice versa . the interaction between the two factor had a significanteffect in the actual productivity and that was clear when the combined implement used with shanks points oriented opposite to the plowing direction at the shallow depth (S2D2) with highest speed which has achieved an actual productivity equal to 0.409 ha.hr-1 (table 5 ) .

**Fuel consumption**

Recently; fuelconsumption has dominated the interest of the researchers due to the steady rise in fuel prices it is not easy term to rate because the tractor fuel consumption based on kg of pull as compared with other vehicles which were rated in km. hr-1 traveled . Eliminating one operation of the seedbed preparation by combining one light tillage tool can usually save amount of fuel suppose to be consumed by the eliminated operation . the noticed trend of signifies of some related researches showed that the fuel consumption varies with plowing speed and depth among other factor however the correct set up equipment's appropriate counter weight diesel quality correct tire pressure tractor maintenance are the other technical factors that effect consumption . Anyway in this study the fuel consumption test was performed to compare between the moldboard plow when used separately and when it was combined with the ripper in respect to all of the treatment tested . it seems very clear from the fig 4 that the process of plowing using the moldboard plow separately require about43 L.ha -1 however , when using the combined implement (moldboard plow + ripper ) with the shanks points oriented opposite to the plowing direction at the shallow depth the amount of fuel consumed was 49.69 L.ha-1 which means that the combined implement has saved about 7.67 L.ha-1 compared to the processing of plowing and cultivating each one separately.

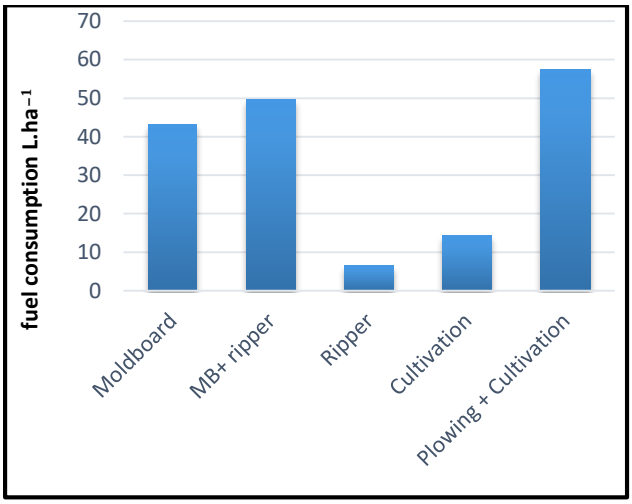


*Fig 5: fuel consumption(L.Hec-1)*

## IV. CONCLUSION

In addition to the mentioned advantages the fuel saving is one of the most important features of the combined implement, the direction of the ripper shanks has great influence on the performance of the combinedimplement .Generally the best performance was found when the ripper shanks were oriented opposite to the direction of plowing . The speed of plowing operation had a significant effect on the results in all the parameters studied whether in the use of combined implement or the use of mold board plow alone . In relation to the things that havealready been mentioned the combined implement can a achieve something's as a result such as reduced the number of traffic shortening the time of agricultural process lowering the agricultural production cost as well as reducing fuel consumption .

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# Chitosan for Plant Growth Promotion and Disease Suppression against Anthracnose in Chilli

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***Abstract*—** *Chitosan is naturally occurring compound potentially used in sustainable agriculture to control plant diseases and enhance growth. An attempt was made to control anthracnose or ripen fruit rot of chilli caused by Colletotrichum capsici in the field under inoculated condition and to increase the growth and yield of chilli by different concentrations of chitosan as seed treatment and foliar application methods. C. capsici isolate "So" was found to be the most virulent against chilli at the time of pathogenicity test. Chitosan at 1% concentration was found to be most effective against the radial growth of C. capsici. Subsequently, seed treatment or foliar spray was done with C. capsici spore suspension ($5\times10^6$ $ml^{-1}$) and different concentrations of chitosan as per requirement of the treatments. Anthracnose or ripen fruit rot of chilli and post-harvest disease incidence (DI) and percent disease index (PDI) were significantly lowest in the treatment $T_8$, where seeds were treated with 1% chitosan combination with foliar spray of chitosan (0.5%) in pathogen inoculated condition. On the contrary, anthracnose or ripen fruit rot of chilli and post-harvest DI and PDI were significantly highest in the treatment $T_1$, where seeds were treated with C. capsici. Germination percentage, growth promoting components, yield and thousand seed weight (TSW) were also highest in treatment $T_8$ compared to all other treatments. As a result, the combined use of chitosan as seed treatment (1%) and foliar spray (0.5%) appeared to be most effective in controlling anthracnose of chilli and increased yield and yield contributing characters.*

***Keywords*— Anthracnose, biological control, chitosan, Colletotrichum capsici, yield.**

## I. INTRODUCTION

Chilli (*Capsicum frutescence* L.) is an important spice and cash crop that is grown throughout tropical, subtropical and temperate regions. In Bangladesh, 102.06 thousand hectares of cultivable land is under chilli cultivation and the country produced 1.30 lakh tons of chilli in 2015-2016 with an average yield 1.27 t/ha (Annon., 2017). Diseases are one of the main constrains for the low yield and quality of this crop (Poonpolgun and Kumphai, 2007). Anthracnose or ripen fruit rot of chilli caused by *Colletotrichum capsici* is one of the most devastating disease of chilli. Anthracnose is mainly a problem on mature fruits, causing severe losses due to both pre-and post-harvest fruit decay (Hadden and Black, 1989).

At present diseases are mainly managed by the use of chemicals such as fungicides. The continuous and indiscriminate use of chemicals to manage the crop diseases results in accumulation of harmful chemical residues in the soil, water and grains. The indiscriminate use of chemicals is not only hazardous to living being but also breaks the natural ecological balance by killing the beneficial and antagonistic microorganisms. In addition, over usage of synthetic fungicide has facilitated the development of fungicide resistance among some pathogenic population (Rhouma *et al*., 2009). Therefore, in the absence of anthracnose resistant cultivars, bio-pesticides, such as chitosan offer a more sustainable approach for the control of diseases in fruits and vegetables (Bautista-Baños *et al*., 2006; Ali *et al*., 2010; Maqbool *et al*., 2010). Chitosan is a linear polysaccharide composed of randomly distributed –β-(1→ 4)-linked D-glucosamine (deacetylated unit). It is made by treating the

chitin shells of shrimp and other crustaceans with an alkaline substance, like sodium hydroxide. It is considered as a biodegradable and biocompatible material with no toxicity or side effects (Rodriguez-Pedroso *et al*., 2009). Over the last decade, chitosan polysaccharide has taken on enormous importance in the control of pathogenic microorganisms. The presence of amino groups ($-NH_2$) in its chemical structure gives chitosan unique and ideal food conservation and security properties which are exploited through the development of biodegradable edible coatings and films containing natural antimicrobials; it also has elicitor properties that enhance the natural defenses of fruit, vegetables and grains (Bautista-Baños *et al*., 2016). Vasudevan *et al*., (2002) suggested that application of chitosan formulation can increase root and shoot length and grain yield. It also increases the growth of nursery-raised plants such as cucumber, pepper and tomato etc. Chitosan coatings can control several fungal diseases in plants such as table grapes (Romanazzi *et al*., 2006), delay the ripening mechanisms in banana fruits (Maqbool *et al*., 2010) and induce resistance against many pathogens (Wilson *et al*.,1994; Benhamou, 1998). It has been hypothesized that the interaction of chitosan with negatively charged molecules on the fungal cell surface causes leakage of proteinaceous compounds (Leuba and Stossel, 1986). It is also believed that the interaction between chitosan and fungi leads to the inhibition of mRNA and protein synthesis (Hadwiger, 1999). However, using chitosan very little is known about disease control and enhancement of growth and yield of chilli in Bangladesh. For that reason, this study was undertaken to select the most effective dose of chitosan in reducing the mycelial growth of *C. capsici* and to evaluate the effect of chitosan in controlling the disease and progress growth and yield of chilli.

## II. MATERIALS AND METHODS

### 2.1 Experimental site and materials

A field experiment was carried out at Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh, during the period from March 2016 - August 2017. Seeds sample of chilli variety "BARI Morich 3" was collected from the Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh.

### 2.2 Collection, isolation and identification of *C. capsici*

Three isolates of *C. capsici* were collected from infected region of chilli fruits following standard phytopathological procedures (Dasgupta, 1981; Agostini and Timmer, 1992). Disease samples were collected from the field and carried to the laboratory in polythene bags. The infected plant parts were cut into small pieces (1 $cm^2$) in advance lesion margin where healthy and disease tissues remain together. The cut plant parts and fruit tissues were surface sterilized by 1% sodium hypochloride (NaOCl) solution for 2-3 minutes then washed three times with sterile deionized water to remove the NaOCl. The cut pieces were then placed onto sterilized agar (20 ml) in glass petridishes and incubated at room temperature ($25 \pm 2$ ℃) until acervuli formation. Conidia produced in acervuli came out in the form of ooze and were placed onto PDA and incubated at room temperature for seven days. The fungal pathogen was identified by observing the morphological feature, acervuli formation, presence or absence of setae, cultural and conidial characters under compound microscope (Barneet and Hunter, 1972).

### 2.3 Preparation of spore suspension

Ten days old culture grown on PDA was flooded with 10 ml of sterilized distilled water. Acervuli and conidia along with mycelial mass were separated from the substratum by scrapping with a narrow edge sterilized glass slide (Sharif and Bhuiyan, 2005). The suspension was sieved through double layer cheese cloth to discard acervuli and mycelial mass. The spore suspension was adjusted to $5x10^6$ $ml^{-1}$ by adding sterilized distilled water and counting under compound microscope using counting slide (haemocytometer). Seeds were submerged in spore suspension with gentle stirring for 5 minutes. The wetted seeds were air dried in a sterile cabinet and prepared for further treatment.

### 2.4 Pathogenicity test for the selection of virulent isolate of the test pathogen in pot culture

Three isolates of *C. capsici* named as So, Mo and Ba were evaluated for their pathogenicity test in the pot culture experiment under the shade condition. Each earthen pot was filled with 2.0 kg sterilized soil. Twenty-five pieces of chilli seeds for each pot were inoculated with spore suspension of So, Mo and Ba isolate of *C. capsici.* Then, the inoculated seeds were sown in each pot and in control pot healthy seeds were sown without inoculation. Disease development was observed regularly and recorded at 10 to 20 days after sowing to estimate the effect of pathogens in causing pre-emergence and post-emergence seedling mortality. The causal agent of seedling mortality was confirmed after re-isolation of the pathogen from un-germinated seeds.

### 2.5 Pathogenicity test of the test pathogen on detached chilli fruit

Pathogenicity test with three selected isolates of the pathogen *C. capsici* was conducted by inoculating harvested chilli fruit. Each isolate of *C. capsici* was grown on PDA plates separately. Inoculation was made by puncturing the tissue with small sterilized needle and then spore suspension ($5\times10^6$ spore $ml^{-1}$) of *C. capsici* sprayed over the chilli fruit. After

inoculation, chilli fruits were kept in a separate transparent polyethylene bag to avoid drying. Inoculated chillies were incubated for four days at room temperature. After four days, percent disease index (PDI) was rated following 0-4 scale, where 0=No visible sign or symptoms, 1=1-25%, 2=26-50%, 3=51-75% and 4=76-100% infection on chilli fruits (Abraham *et al*., 1996).

**2.6 Collection of chitosan**

Chitosan was collected from Bangladesh Atomic Energy Commission (BAEC), Dhaka, Bangladesh. Chitosan was derived from the shell of quick growing sea shrimp. The solution was extracted from sea shrimp and then it was irradiated with γ-ray (20 kD) which acts as a plant growth promoter.

**2.7 *In-vitro* evaluation of chitosan for their inhibitory effect on the radial growth of *C. capsici***

Several preliminary evaluations of chitosan were done with different concentration of chitosan such as 0.4%, 0.6%, 0.8%, 1% and 1.2% on PDA plate against the *C. capsici*. Plates were individually challenged at the center with equal agar plug (5 mm in diameter) taken from 7 days old culture of the pathogen and incubated at $25^0$C. Mean colony diameter was measured when the control plate (without chitosan) reached full growth. The radial growths of *C. capsici* in three replications were recorded separately and there averages were taken. The percent inhibition of the radial growth was calculated as described by Sundar*et al.*, (1995).

$$\% \text{ inhibition over control} = \frac{X - Y}{X} \times 100 \ (equation\ 1)$$

Where,

X = Mycelial growth of pathogen without chitosan (control),

Y = Mycelial growth of pathogen with chitosan

**2.8 Seed treatment with chitosan and rising of seedlings**

Seeds of chilli were surface sterilized by immersion of 1% NaOCl. Then seeds were thoroughly rinsed in sterile distilled water and immersed into each of the chitosan solution ($p^H$ 5.5-6.0) at concentrations ranging from 0.6-1%. After gentle stirring, seeds were submerged for 1 hour. Finally, the wetted seeds were air dried in a sterile cabinet and sown in the tray containing soil that was well prepared through mixing fertilizers and cow dung. Chitosan (0.5%) was applied as foliar spray at three times such as 10 days after transplanting, at vegetative stage and fruiting stage.

**2.9 Land preparation and transplanting of seedlings**

Land was prepared with well tilth using a tractor driven disc plough and harrow. After land preparation the whole experimental area was divided into three blocks, representing three replications. The unit plot size was 3.0 m X 2.0 m. Distance between block to block was 1.0 m and that plot to plot in a block was 0.50 m. Drains were made surrounding the each unit plots and the excavated soil was used for raising plots 15 cm high from the general soil surface. Ten different treatments were allotted randomly to each block. Thirty-five days aged apparently healthy chilli seedlings of variety 'BARI Morich 3" was collected from the tray. A total of 12 seedlings were planted in each plot on November 2016. Weeding, irrigation and other intercultural operations were done as and when necessary until the maturity of plants.

**2.10 Treatments of the experiment**

The treatments of the field experiment were as follows:

$T_0$= Untreated healthy seeds without pathogen (Control 1)

$T_1$= Seed treated with *C. capsici* (Control 2)

$T_2$= Seed treated with *C. capsici* + 0.6% chitosan

$T_3$= Seed treated with *C. capsici* + 0.8% chitosan

$T_4$= Seed treated with *C. capsici* + 1% chitosan

$T_5$= Seed treated with *C. capsici* + Foliar spray (0.5%) of chitosan

$T_6$= $T_2$ + Foliar spray (0.5%) of chitosan

$T_7$= $T_3$ + Foliar spray (0.5%) of chitosan

$T_8$= $T_4$ + Foliar spray (0.5%) of chitosan

$T_9$= Seed treated with *C. capsici* + 0.1% Bavistin 50 WP

**2.11 Observation of disease development**

Chilli plants were observed regularly after transplanting of seedlings to record the incidence of post emergence seedling mortality, anthracnose diseases on fruits. The disease incidence was recorded two times at 90 and 120 day after transplanting (DAT). Observations were made by selecting six plants randomly from each plot. Flowerings of chilli plants were started at 40-45 DAT. Plants were produced flowers and fruits continuously up to 3-4 months after transplanting. But all fruits didn't mature and ripe at a time. The ripened fruits were harvested and weighed and it was continued up to 4 months after transplanting. Post-harvest disease incidence and PDI were recorded in the laboratory at 135 days after harvesting (DAH).

**2.12 Data recording and disease assessment**

Data were taken on germination percentage, mortality percentage, root length and shoot length, root fresh weight and shoot fresh weight, root dry weight and shoot dry weight, plant height and number of branch, disease incidence and percent disease index (PDI) and yield. The following formulae were used for calculation (Rahman *et al*., 2013).

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100 \ (equation\ 2)$$

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$$\text{Disease index} = \frac{\text{Summation of all rating of fruits observed}}{\text{Number of fruits observed} \times \text{Maximum rating}} \times 100 \ (equation\ 3)$$

Percent disease index (PDI) was rated by following 0-4 scale, where 0=No visible sign or symptoms, 1=1-25%, 2=26-50%, 3=51-75% and 4=76-100% infection on chilli fruits.

**2.13 Experimental design and data analysis**

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Data recorded on various parameters were analyzed statistically using the Statistix 10 statistical computer programme after proper transformation whenever necessary. The means were compared following LSD (Least significance Differences) test (Gomez and Gomez, 1984).

## III. RESULTS

**3.1 Pathogenicity test of *C. capsici* isolates against chilli seedlings in pot culture and detached chilli fruits**

The pathogenicity test of the three selected isolates of *C. capsici* against chilli seedlings variety BARI Morich-3 were conducted in pot containing sterilized soil to find out most virulent isolate of the test pathogen. All the isolates of the test pathogen were virulent but variable in causing total seedling mortality of chilli ranged from 28.32-63.00% (Table 1 and Fig. 1-2). The *C. capsici* isolate So was appeared to be the most virulent causing the highest 63.00% total seedling mortality followed by isolate Mo caused 43.00% mortality. Significantly the lowest 28.32% total seedling mortality was observed with the isolate Ba. No pre-emergence and post-emergence seedling mortality was observed in the untreated control pot. Furthermore, every isolates were sowed pathogenic reaction to the detached chilli fruit (Table 1 and Fig. 3). All the three isolates were able to develop characteristics symptom such as sunken necrotic lesion with concentric ring of acervuli in chilli fruits. Chilli fruit which was inoculated with the isolate So showed highest PDI (83.33%). The virulence of the isolates in detached fruits was supported by the virulence of the same isolates in the earlier pot culture.

**3.2 Effect of chitosan on mycelial growth of virulent isolate of *C. capsici***

The mycelial growth of the test pathogen was significantly reduced with all the five selected concentrations viz., 0.4%, 0.6%, 0.8%, 1% and 1.2% of chitosan as compared to untreated control (Table 2 and Fig. 4). All the five concentrations of chitosan are significantly variable in reducing the mycelial growth of *C. capsici*. Significantly the highest 100.00% reduction of the mycelial growth of *C. capsici* over the control PDA plate was observed at both 1% and 1.2% of chitosan amended with PDA plate followed by the second highest 0.8% of chitosan with 87.79% reduction of mycelial growth. Significantly the lowest 28.56% reduction of the mycelial growth of *C. capsici* was observed at the lowest 0.4% concentration of chitosan amended with the PDA plate. Based on the *in-vitro* evaluation 0.6%, 0.8% and 1% chitosan concentrations were selected for the field trial.

**3.3 Effect of chitosan on germination percentage and post-emergence seedlings mortality**

In addition, to know the effect of chitosan on germination and post-emergence seedling mortality chilli seeds were treated with 0.6%, 0.8% and 1% of chitosan. For positive control Bavistin (0.1%) 50 WP was used and there was no treatment in untreated control seeds. These seeds were sown in plastic tray after required treatments and data were recorded up to complete germination. All treatments increased the germination percentage compared to the treatment where seeds treated with *C. capsici* (Table 3). The range of germination percentage was 56.37-92.10%. Significantly the highest germination percentage 92.10% was in the treatment where seeds were treated with *C. capsici* and 1% chitosan and significantly the lowest germination percentage 56.37% was in the treatment where seeds were treated with *C. capsici* without chitosan or any fungicides such as Bavistin (0.1%). In case of seedling mortality all treatments reduced post-emergence seedling mortality compared to the treatment where seeds were treated with *C. capsici* (Table 3). Significantly the lowest 7.67% post-emergence seedling mortality was found in the treatment where seeds were treated with *C. capsici* and 1% chitosan. No statistical difference was found among the treatments where seeds were treated with 0.6% chitosan, 0.8% chitosan and 0.1% Bavistin. This experiment showed that seed treatment with 1% chitosan was most effective to increase germination and to control post-emergence seedling mortality of chilli.

**3.4 Effect of chitosan and Bavistin on growth of chilli seedlings**

Thirty-five days after sowing growth promoting components such as shoot length, root length, fresh shoot weight, fresh root weight, dry shoot weight and dry root weight were increased in the treatments where seeds were treated with chitosan in pathogen inoculated condition comparison to the treatment where seeds were treated with *C. capsici* (Table 4). Seed treatment with 1% chitosan showed the highest shoot length (14.84 cm), root length (8.09 cm), fresh shoot weight (0.63 g), fresh root weight (0.045 g), dry shoot weight (0.084 g) and dry root weight (0.0098 g) in pathogen inoculated condition. The lowest shoot length (9.48 cm), root length

(4.68 cm) and fresh shoot weight (0.46 g) were recorded in the treatment where seeds were untreated and the lowest fresh root weight (0.019 g), dry shoot weight (0.055 g) and dry root weight (0.0020 g) were recorded in the treatment where seeds were treated with *C. capsici*. In the field, plant height and number of branches were observed at mature stage of chilli plant. All the applications of chitosan as seed treatment or foliar spray increased the plant height and number of branches in comparison to the treatment $T_1$ where seeds were treated with *C. capsici* (Table 5). The highest plant height (58.57 cm) at flowering stage (45 DAT) and (60.83 cm) at fruiting stage (60 DAT) were recorded in the treatment $T_8$ where seeds were pathogen inoculated condition and treated with 1% chitosan and foliar spray (0.5%) of chitosan. The lowest plant height (45.52 cm) at flowering stage and (47.93 cm) at fruiting stage were recorded in the treatment $T_1$ where seeds were treated with *C. capsici*. In the same way, the highest number of branches (17.55) at flowering stage and (21.59) at fruiting stage were recorded in the treatment $T_8$ where seeds were pathogen inoculated condition and treated with 1% chitosan and foliar spray (0.5%) of chitosan. The lowest number of branches (11.30) at flowering stage and (13.18) at fruiting stage were recorded in the treatment $T_1$ where seeds were treated with *C. capsici*.

**3.5 Effect of chitosan and Bavistin on disease incidence (DI) and percent disease index (PDI)**

Disease incidence and percent disease index (PDI) were reduced in the treatments where chitosan or Bavistin were used as seed treating agent or foliar spray of chitosan over pathogen treated plots. Disease incidence and PDI were recorded two times before first and second harvesting of fruits. Significantly the highest DI (64.67%) at 90 days after transplanting (DAT) and (90.33%) at 120 DAT and PDI (3.34%) at 90 DAT and (4.96%) at 120 DAT were recorded in the treatment $T_1$ where seeds were treated with *C. capsici* (Table 6). Significantly the lowest DI (10.08%) at 90 DAT and (22.63%) at 120 DAT and PDI (0.51%) at 90 DAT and (1.29%) at 120 DAT were recorded in the treatment $T_8$ where seeds were pathogen inoculated condition and treated with 1% chitosan and foliar spray (0.5%) of chitosan.

**3.6 Effect of chitosan and Bavistin on the yield and thousand seed weight (TSW) of chilli**

Results of the present study indicates that by the application of different treatments of chitosan yield and yield contributing components were significantly increased in all the treatments over the treatment $T_1$ where seeds were treated with *C. capsici* (Table 7). Significantly the highest yield (7.03 t/ha) was recorded in the treatment $T_8$ where seeds were treated with *C. capsici*, 1% chitosan in combination with foliar spray (0.5%) of chitosan. Significantly the lowest yield (2.86 t/ha) was recorded in the treatment $T_1$ where seeds were treated with *C. capsici*. Total three harvests were done at different times as chilli is an indeterminate crop and fruits are not mature and ripen at the same time. Moreover, applications of chitosan significantly increased TSW compare to pathogen inoculated condition. Significantly highest TSW (5.04 g) was recorded in the treatment $T_8$ where seeds were pathogen inoculated condition and treated with 1% chitosan and foliar spray (0.5%) of chitosan. On the contrary, significantly the lowest TSW (4.08 g) was recorded in the treatment $T_1$ where seeds were treated with *C. capsici*.

**3.7 Effect of chitosan and Bavistin on post-harvest disease incidence (DI) and percent disease index (PDI)**

Post-harvest disease incidence and PDI were recorded in the laboratory at 135 days after harvesting (DAH). Post-harvest disease incidence and PDI were reduced in those treatments where chitosan or Bavistin were used as seed treating agent or foliar spray (0.5%) of chitosan in the field compare to pathogen inoculated condition (Table 8). Significantly the highest post-harvest DI (32.61%) and PDI (0.165%) were recorded in the treatment $T_1$ where seeds were treated with *C. capsici*. Significantly the lowest post-harvest DI (8.58%) and PDI (0.026%) were recorded in the treatment $T_8$ where seeds were treated with 1% chitosan combined with foliar spray of 0.5% chitosan in pathogen inoculated condition.

## IV. DISCUSSION

The anthracnose or ripen fruit rot of chilli is a seed borne as well as soil borne disease caused by *Colletotrichum capsici* that occurs every year with intensities and imposes considerable quantitative and qualitative losses of the crop in the field as well as in the storage. This disease is mostly difficult to control as *Colletotrichum* species naturally produce micro-sclerotia to allow dormancy in the soil during the winter or when subjected to stressful conditions and these micro-sclerotia can survive for many years (Pring *et al*., 1995) and subsequently attack the crop resulting poor yield. In recent times, chitosan has been extensively used to control fungal diseases in many fruits and vegetables. In this study, all the tested concentrations of the chitosan were found effective against *C. capsici* which was a dose dependent manner. The complete reduction of the mycelial growth of *C. capsici* was got at 1% of chitosan. The result is supported by the reports of Chookhongkga *et al*., 2013 where chitosan was found to reduce the mycelial growth of *Rhizopus* sp., *C. capsici*, *C. gloeosporioides* and *Asperillus niger*. Chitosan shows direct toxicity to pathogens, can form physical barriers around the penetration sites of pathogens, preventing them from spreading to healthy tissues and is known to induce reactions locally and systemically that entail signaling

cascades, and the activation and accumulation of defenses-related antimicrobial compounds and proteins. It is also reported to induce a decline in malondialdehyde content, alter the relative permeability of the plasma membrane and increase the concentrations of soluble sugars and proline, and of peroxidase and catalase activities. Chitosan is often used in plant disease control as a powerful elicitor rather than a direct antimicrobial or toxic agent (Algam *et al*., 2010).

We observed highest seedling mortality, disease incidence (DI) and percent disease index (PDI) and lowest seed germination, growth, yield and thousand seed weight (TSW) of chilli in pathogen inoculated condition. Application of chitosan increased seed germination, growth promoting components, yield and TSW and reduced seedling mortality, DI and PDI. The highest post-emergence mortality was found in chilli caused by *C. capsici*. The pre-emergence and post-emergence mortality of different vegetable crops including chilli caused by the tested pathogen are also reported by Simi, 2017. Significantly the highest germination percentage and the lowest post-emergence seedling mortality were found in the treatment where seeds were treated with *C. capsici* and 1% chitosan. The results are supported by Photchanachai *et al*., 2012 and Nitu *et al*., 2016 that chitosan increased germination percentage and reduced post-emergence seedling mortality of different crops. Moreover, we have observed highest shoot length, root length, fresh shoot weight, fresh root weight, dry shoot weight and dry root weight in the treatments where seeds were treated with chitosan in pathogen inoculated condition compared to the treatment where seeds were treated with *C. capsici.* The results of the present study supported by the observation of Benhamou *et al*., 1994, O'Herlihy *et al*., 2003; Guan *et al*., 2009 who observed the enhancement of growth promoting components including shoot length, root length etc. of different crops by chitosan. Chitosan has also been extensively utilized as a foliar treatment to control the growth, spread and development of fungal diseases and to increase yield and quality in many crops. Consequently, we found that foliar application of chitosan increased the plant height and number of branches. The highest plant heights and number of branches and the lowest DI and PDI were recorded in the treatment $T_8$ where seeds were treated with 1% chitosan combination with foliar spray of 0.5% chitosan in pathogen inoculated condition. These results are supported by Mondal et *al*., (2013) that foliar application of chitosan increased most of the morphological characters such as plant height, leaf number per plant and growth such as total dry mass per plant, absolute growth rate, relative growth rate with increasing concentration of chitosan in okra. Moreover, Faoro *et al*., (2008) showed that the use of chitosan applied as a foliar spray on barley reduced locally and systemically the infection by powdery mildew pathogen *Blumeria graminis* f. sp. *hordei*. The results of the current study justify the statement of Benhamou *et al*., (1994); O'Herlihy *et al*., (2003) and Edirisinghe *et al*., 2014 that chitosan has been considered as alternate to chemical fungicides to control diseases in different crops. However, significantly highest yield and TSW of chilli were recorded in the treatment $T_8$ and these results are supported by Walker *et al*., (2004) and Mondal et *al*., (2013) that chitosan increased yield and yield attributes with increasing concentration of chitosan in different crops. Edirisinghe *et al*., (2014) and Sivakumar *et al*., (2016) also examined that chitosan could become a promising substance to control postharvest diseases in different crops. Accordingly, post-harvest DI and PDI were reduced by all the chitosan treatments over the treatment $T_1$ and significantly lowest post-harvest DI and PDI were found by combined use of chitosan as seed treatment and foliar spray. Edirisinghe *et al*., (2014), Berumen-Varela *et al*., (2015), Romanazzi & Feliziani (2016) and Sivakumar *et al*., (2016) reported that chitosan increases phenyl ammonia lyase, chitinase (endo and exochitinases) and β-1,3-glucanase activities in numerous treated tropical and temperate fruits. They also found that chitosan application induces fruit disease resistance during fungal infection through regulation of reactive oxygen species (ROS) levels, antioxidant enzymes, and the ascorbate-glutathione cycle. The present study revealed that combined use of chitosan as seed treatment and foliar spray could provide excellent protection against anthracnose or ripen fruit rot of chilli, had influenced on growth promoting components, yield, TSW and reduced post-harvest DI and PDI.

## V. CONCLUSION

The present study showed that seed treatment with 1% chitosan combined with foliar spray of 0.5% chitosan was appeared to be excellent in controlling post-emergence seedling mortality, anthracnose or ripen fruit rot of chilli, post-harvest disease incidence (DI) and percent disease index (PDI) with the significant increase of growth, yield and thousand seed weight. Moreover, Chitosan at 1% concentration completely inhibited the mycelial growth of *C. capsici* in *in vitro* evaluation. Growers can adopt eco-friendly control measures against anthracnose or ripen fruit rot of chilli through the seed treatment with 1% chitosan combined with foliar spray of 0.5% chitosan as an alternative to chemical fungicides.

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*Table.1. Pathogenicity of Colletotrichum capsici isolates on seedlings and fruits of chilli*

| *Colletotrichum capsici* isolates | Seedlings | | | Fruits |
|---|---|---|---|---|
| | Pre-emergence mortality (%) | Post-emergence mortality (%) | Total mortality (%) | PDI |
| So | 17.00 | 46.00 | 63.00 a | 83.33 a |
| Ba | 9.66 | 18.66 | 28.32 c | 56.25 c |
| Mo | 8.30 | 34.70 | 43.00 b | 62.50 b |
| Untreated Control | 0.00 | 0.00 | 0.00 d | 0.00 d |

**Means within the same column having a common letter(s) do not differ significantly (P=0.05) by LSD.*

*Table.2. Effect of chitosan on mycelial growth of C. capsici*

| Treatment | Average mycelial growth after 10 days (mm) | % mycelial growth inhibition over control |
|---|---|---|
| Control | 90.00 a | - |
| 0.4% chitosan | 64.30 b | 28.56 |
| 0.6% chitosan | 36.70 c | 59.22 |
| 0.8% chitosan | 11.00 d | 87.78 |
| 1% chitosan | 0.00 e | 100.00 |
| 1.2% chitosan | 0.00 e | 100.00 |

**Means within the same column having a common letter(s) do not differ significantly (P=0.05) by LSD.*

*Table 3. Effect of chitosan and Bavistin on germination percentage and post-emergence seedling mortality*

| Treatment | % germination | % increase over control | % post-emergence mortality | % reduction over control |
|---|---|---|---|---|
| Control | 73.00 c | 29.50 | 13.28 b | 46.88 |
| Seed treated with *C. capsici* | 56.37 d | - | 25.00 a | - |
| Seed treated with *C. capsici* + 0.6% chitosan | 82.00 b | 45.00 | 10.50 bc | 58.00 |
| Seed treated with *C. capsici* + 0.8% chitosan | 85.00 b | 45.47 | 9.47 bc | 62.12 |
| Seed treated with *C. capsici* + 1% chitosan | 92.10 a | 63.38 | 7.67 d | 67.32 |
| Seed treated with *C. capsici* + Bavistin (0.1%) 50 WP | 81.57 b | 44.70 | 11.21bc | 55.16 |

**Means within the same column having a common letter(s) do not differ significantly (P=0.05) by LSD.*

*Table.4: Effect of chitosan and Bavistin on growth parameters of chilli seedlings at 35 days after sowing (DAS)*

| Treatments | Shoot length (cm) | Root length (cm) | Fresh shoot weight (g/plant) | Fresh root weight (g/plant) | Dry shoot weight (g/plant) | Dry root weight (g/plant) |
|---|---|---|---|---|---|---|
| Control | 9.48 c | 4.68 c | 0.46 cd | 0.029 b | 0.063 c | 0.0064 b |
| Seed treated with *C. capsici* | 10.12 c | 5.13 c | 0.53 bc | 0.019 b | 0.055d | 0.0020 c |
| Seed treated with *C. capsici* + | 12.90 b | 6.59 b | 0.43 d | 0.025 b | 0.066 c | 0.0067 b |

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| | | | | | | |
|---|---|---|---|---|---|---|
| 0.6% chitosan | | | | | | |
| Seed treated with *C. capsici* + 0.8% chitosan | 13.20 b | 7.14 ab | 0.57 ab | 0.032 ab | 0.078 b | 0.0092 a |
| Seed treated with *C. . capsici* + 1% chitosan | 14.84 a | 8.09 a | 0.63 a | 0.045 a | 0.084 a | 0.0098 a |
| Seed treated with *C. capsici* + Bavistin (0.1%) 50 WP | 12.85 b | 6.46 b | 0.44 d | 0.031 b | 0.067 c | 0.0071 b |

**Means within the same column having a common letter(s) do not differ significantly (P=0.05) by LSD.*

*Table.5: Effect of chitosan and Bavistin on plant height and number of branches of chilli*

| Treatment | 45 DAT | | 60 DAT | |
|---|---|---|---|---|
| | Plant height (cm) | No. of branching | Plant height (cm) | No. of branching |
| $T_0$ | 46.47 h | 13.58 h | 49.68 ef | 16.16 g |
| $T_1$ | 45.52 i | 11.30 i | 47.93 f | 13.18 h |
| $T_2$ | 47.30 g | 14.50 f | 49.46 ef | 17.40 e |
| $T_3$ | 48.70 f | 14.57 f | 50.83 e | 17.65 e |
| $T_4$ | 53.78 d | 15.05 e | 56.52 c | 17.72 e |
| $T_5$ | 52.26 e | 16.68 c | 54.04 d | 19.42 c |
| $T_6$ | 53.93 c | 15.64 d | 57.71 bc | 18.41 d |
| $T_7$ | 57.54 b | 16.90 b | 59.49 ab | 19.79 b |
| $T_8$ | 58.57 a | 17.55 a | 60.83 a | 21.59 a |
| $T_9$ | 47.76 g | 13.76 g | 51.18 e | 16.74 f |

**Means within the same column having a common letter(s) do not differ significantly (P=0.05) by LSD.*

$T_0$= Untreated healthy seeds without pathogen (Control 1), $T_1$= Seed treated with *C. capsici* (Control 2), $T_2$= Seed treated with *C. capsici* + 0.6% chitosan, $T_3$= Seed treated with *C. capsici* + 0.8% chitosan, $T_4$= Seed treated with *C. capsici* + 1% chitosan, $T_5$= Seed treated with *C. capsici* + Foliar spray (0.5%) of chitosan, $T_6$= $T_2$ + Foliar spray (0.5%) of chitosan, $T_7$= $T_3$ + Foliar spray (0.5%) of chitosan, $T_8$= $T_4$ + Foliar spray (0.5%) of chitosan, $T_9$= Seed treated with *C. capsici* + 0.1% Bavistin 50 WP

*Table 6. Effect of chitosan and Bavistin on disease incidence (DI) and percent disease index (PDI) of chilli*

| Treatment | 90 DAT | | | | 120 DAT | | | |
|---|---|---|---|---|---|---|---|---|
| | % DI | % Reduction over $T_1$ | PDI | % Reduction over $T_1$ | % DI | % Reduction over $T_1$ | PDI | % Reduction over $T_1$ |
| $T_0$ | 39.37 b | 39.12 | 1.30 b | 59.68 | 67.83 b | 24.90 | 2.6 b | 47.58 |
| $T_1$ | 64.67 a | - | 3.34 a | - | 90.33 a | - | 4.96 a | - |
| $T_2$ | 28.09 c | 56.56 | 1.20 c | 64.00 | 58.95 c | 34.74 | 2.36 cd | 52.42 |
| $T_3$ | 27.07 c | 58.14 | 1.13 c | 66.07 | 48.67 d | 46.12 | 2.25 d | 54.64 |
| $T_4$ | 16.06 e | 75.15 | 0.77 e | 77.04 | 40.33 e | 55.35 | 1.94 e | 60.89 |
| $T_5$ | 16.20 e | 74.95 | 0.79 e | 76.45 | 34.22 fg | 62.12 | 1.90 e | 61.69 |
| $T_6$ | 15.13 ef | 76.60 | 0.74 e | 77.75 | 34.44 f | 61.87 | 2.02 e | 59.27 |
| $T_7$ | 13.33 f | 79.60 | 0.71 e | 78.74 | 31.49 g | 65.24 | 1.79 f | 63.91 |
| $T_8$ | 10.08 g | 84.41 | 0.51 f | 84.83 | 22.63 h | 74.95 | 1.29 g | 73.40 |
| $T_9$ | 20.37 d | 68.50 | 0.92 d | 72.46 | 43.13 e | 52.25 | 2.24 d | 54.84 |

**Means within the same column having a common letter(s) do not differ significantly (P=0.05) by LSD.*

$T_0$= Untreated healthy seeds without pathogen (Control 1), $T_1$= Seed treated with *C. capsici* (Control 2), $T_2$= Seed treated with *C. capsici* + 0.6% chitosan, $T_3$= Seed treated with *C. capsici* + 0.8% chitosan, $T_4$= Seed treated with *C. capsici* + 1% chitosan, $T_5$= Seed treated with *C. capsici* + Foliar spray (0.5%) of chitosan, $T_6$= $T_2$ + Foliar spray (0.5%) of chitosan, $T_7$= $T_3$ + Foliar spray (0.5%) of chitosan, $T_8$= $T_4$ + Foliar spray (0.5%) of chitosan, $T_9$= Seed treated with *C. capsici* + 0.1% Bavistin 50 WP

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*Table 7. Effect of chitosan and Bavistin on yield and thousand Seed weight (TSW) of chilli*

| Treatment | Yield t/ha | % increased yield over $T_1$ | TSW (g) | % increased TSW over $T_1$ |
|---|---|---|---|---|
| $T_0$ | 3.62 i | 26.57 | 4.28 g | 4.90 |
| $T_1$ | 2.86 j | - | 4.08 h | - |
| $T_2$ | 4.06 g | 41.91 | 4.33 g | 6.13 |
| $T_3$ | 4.36 f | 52.45 | 4.49 f | 10.05 |
| $T_4$ | 4.80 e | 67.83 | 4.61 de | 12.10 |
| $T_5$ | 5.60 d | 95.80 | 4.56 e | 11.76 |
| $T_6$ | 5.98 c | 109.09 | 4.75 c | 16.42 |
| $T_7$ | 6.20 b | 116.78 | 4.88 b | 19.61 |
| $T_8$ | 7.03 a | 145.80 | 5.04 a | 23.52 |
| $T_9$ | 3.94 h | 37.76 | 4.67 d | 14.46 |

**Means within the same column having a common letter(s) do not differ significantly (P=0.05) by LSD.*

$T_0$= Untreated healthy seeds without pathogen (Control 1), $T_1$= Seed treated with *C. capsici* (Control 2), $T_2$= Seed treated with *C. capsici* + 0.6% chitosan, $T_3$= Seed treated with *C. capsici* + 0.8% chitosan, $T_4$= Seed treated with *C. capsici* + 1% chitosan, $T_5$= Seed treated with *C. capsici* + Foliar spray (0.5%) of chitosan, $T_6$= $T_2$ + Foliar spray (0.5%) of chitosan, $T_7$= $T_3$ + Foliar spray (0.5%) of chitosan, $T_8$= $T_4$ + Foliar spray (0.5%) of chitosan, $T_9$= Seed treated with *C. capsici* + 0.1% Bavistin 50 WP

*Table.8: Effect of chitosan and Bavistin on post-harvest disease incidence (DI) and percent disease index (PDI) of chilli at 135 days after harvesting*

| Treatment | % DI | % reduction over $T_1$ | PDI | % reduction over $T_1$ |
|---|---|---|---|---|
| $T_0$ | 24.83 b | 23.86 | 0.140 b | 15.15 |
| $T_1$ | 32.61 a | - | 0.165 a | - |
| $T_2$ | 17.08 c | 47.62 | 0.065 c | 60.60 |
| $T_3$ | 15.62 d | 52.10 | 0.061 d | 63.03 |
| $T_4$ | 12.08 f | 62.96 | 0.055 e | 66.67 |
| $T_5$ | 14.54 e | 55.41 | 0.057 e | 65.45 |
| $T_6$ | 12.78 f | 60.81 | 0.045 f | 72.73 |
| $T_7$ | 12.33 f | 62.19 | 0.042 f | 74.55 |
| $T_8$ | 8.58 g | 73.69 | 0.026 g | 84.24 |
| $T_9$ | 14.89 de | 54.34 | 0.057 e | 65.45 |

**Means within the same column having a common letter(s) do not differ significantly (P=0.05) by LSD.*

$T_0$= Untreated healthy seeds without pathogen (Control 1), $T_1$= Seed treated with *C. capsici* (Control 2), $T_2$= Seed treated with *C. capsici* + 0.6% chitosan, $T_3$= Seed treated with *C. capsici* + 0.8% chitosan, $T_4$= Seed treated with *C. capsici* + 1% chitosan, $T_5$= Seed treated with *C. capsici* + Foliar spray (0.5%) of chitosan, $T_6$= $T_2$ + Foliar spray (0.5%) of chitosan, $T_7$= $T_3$ + Foliar spray (0.5%) of chitosan, $T_8$= $T_4$ + Foliar spray (0.5%) of chitosan, $T_9$= Seed treated with *C. capsici* + 0.1% Bavistin 50 WP

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*Fig. 1: Pathogenicity test of C. capsici isolates against chilli seedling*

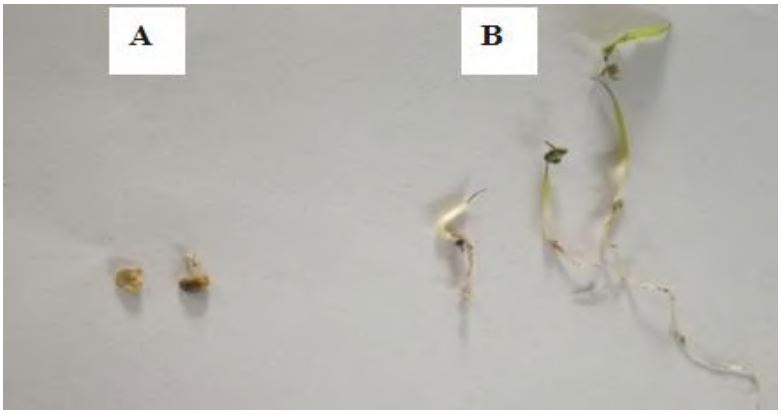


*Fig. 2: Pre-emergence (A) and post-emergence (B) seedling mortality*


*Fig. 3: Pathogenicity test of C. capsici isolates on chilli fruit*

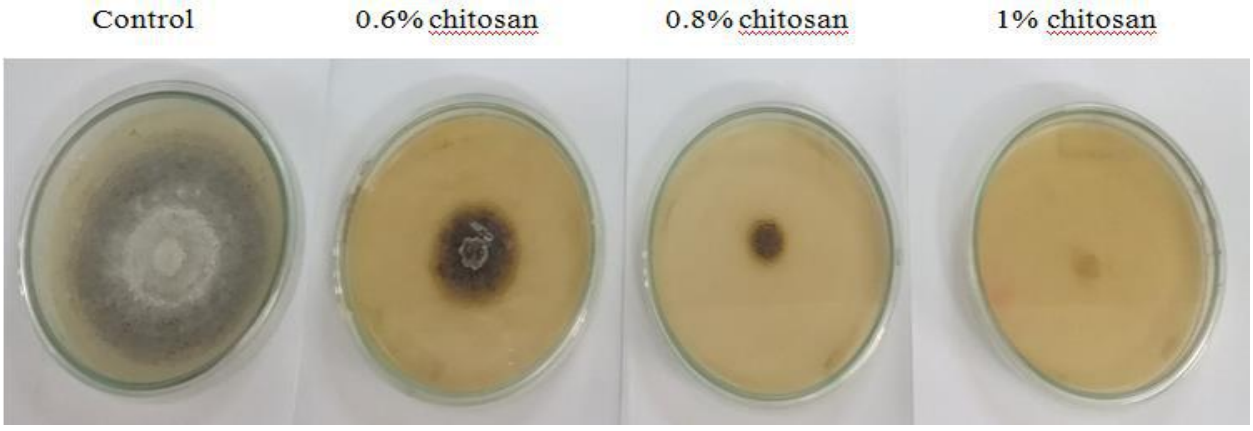


*Fig. 4. Mycelial growth inhibition of C. capsici by chitosan on PDA*

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# Effects of Spacing, Cutting Height and Cutting Interval on Fodder Yield and Nutritional Value of *Cajanus Cajan*

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***Abstract*—** *Forage production is one of the ways of sustaining ruminant animal production in Nigeria as these animals depend largely on plant-based feed. Hence, Cajanus cajan pasture was established to evaluate the effects of planting space, cutting height and interval at harvest on fodder yield and nutritional value of C. cajan. Pre-planting operations (bush clearing, ploughing, harrowing and ridging) were carried out on a hectare of land, sectionalized into fifteen equal portions. 2 - 3 seeds of C. cajan were planted using five different planting spaces (40x60 cm, 60x60 cm, 80x30 cm, 100x30 cm and 120x30 cm) of three replicates per treatment. Post-planting operations (thinning, supplying and weeding) were done to ensure uniform plant stands, nursed to maturity and harvested at different cutting heights (50, 100 and 150 cm) with cutting intervals from 2, 3 and 4 weeks respectively for five consecutive times to calculate the initial, total and average yield per plot. Air-dried samples of harvested forages were analysed for proximate composition; and data generated were subjected to statistical analysis. Results showed that; C. cajan sown using 40x60 cm planting space, cutting height of 50 cm and cutting interval of 4 weeks had the best fodder yield both at the initial (8.95 kg) and cumulative (3.60 kg) compared to other treatments. Crude protein, crude fibre and nitrogen free extract contents were significantly ($p>0.05$) influenced; and could adequately support the growth of ruminant animals. Thus, it can be concluded that C. cajan could be established using 40x60 cm planting space, harvested at 4 weeks interval and cutting at 50 cm height for maximum fodder yield with the aim of feeding ruminant animals.*

***Keywords*— Cajanus cajan, cutting height, plant spacing, cutting interval, fodder yield.**

## I. INTRODUCTION

*Cajanus cajan* (pigeon pea) is a perennial member of family Fabaceae and is one of the most common legumes of the tropics and sub-tropics with a wide adaptability to poor soil conditions than most tropical legumes and drought tolerant (Akinola and Oyejola, 1994; Speranza *et al*., 2007, Crop Trust, 2014). *Cajanus cajan* is a glandular-pubescent, short-lived perennial (1-5 years) shrub, usually grown as an annual plant, 0.5-4 m high, with roots up to 15 - 20cm deep; stems up to 15 cm in diameter; branches are many and slender. It is emerging as common domestic forage plants as they are raised in traditional home gardens in many parts of Nigeria. Pigeon peas are evergreen and could be used as a potential source of forage for ruminant animals year round. Pigeon pea is a multi-purpose nitrogen fixing plant that provides food, fuel wood, fodder and shelter material to subsistence farmers. The seeds can be used as animal feed and post-harvest products such as haulms, leaf and young stems have good fodder value. Pigeon pea has a prolonged cooking time and low food value for human when compared to cowpea (Amaefule and Obioha, 2001). Amaefule and Onwudike (2000) reported that pigeon pea is rich in nitrogen (21-30% CP), thus qualifies it as a suitable protein source of ruminant animal feed. These attributes have elicited interest among Animal Nutritionist on the need to explore and exploit further use of the plant/crop as an alternative source of plant protein for ruminants (Ahamefule *et al*., 2006). Seeds are sown where desired, in pure stands at about 9–22 kg/ha for rows, but sometimes it is broadcast. Seed germinates in about 2 weeks. Quite frequently (in India) pigeon pea is grown mixed with other crops are grown in alternate rows with rows of sorghum, groundnuts, sesame, cotton, pineapples, millets or maize. For pure crops pigeon pea should be sown 2.5–5 cm deep in rows 40–120 cm by 30–60 cm. When sown as a mixture, it should be sown in widely spaced rows ranging

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from 1.2–2.1 m depending on the associated crop. About 3–4 seeds may be planted in each hill, and later thinned to 2 plants per hill. Plants show little response to fertilizers, e.g., mixed plantings with millet in India showed negative responses to N. For the first month, pigeon pea shares the inter-cultivation of the main crop. In the tropics, 20–100 kg/ha phosphoric acid is recommended. S, with or without P, can significantly increase seed yield and nitrogen fixation. Early CVs start podding in 12 weeks, but maturation requires 5–6 months. Late CVs require 9–12 mos. The crop may be ratooned for forage or let persist for 3–5 years. Seed yields drop considerably after the first year, and disease build-up may reduce stand. *Cajanus cajan* is a short-lived perennial leguminous shrub that usually grows to a height of about 1-2 m, but can reach up to 2-5 m high. The stems are woody at the base, angular and branching. The leaves are alternate and trifoliate while the leaflets are oblong and lanceolate. Leaves and stems are pubescent. The flowers are papilionaceous and generally yellow in colour. They can also be striated with purple streaks. The fruit is a flat, straight and pubescent pod, 5-9 cm long x 12-13 mm wide. It contains 2-9 seeds that are brown, red or black in colour, small and sometimes hard-coated (FAO, 2016a; Bekele-Tessema, 2007). According to FAO, 2016b, World production of *C. cajan* was 4.85 million tonnes in 2014. The main producers were India (3.29 million tonnes, 65% of world production), Myanmar (0.57 million tonnes), Malawi (0.3 million tonnes), Kenya (0.28 million tonnes) and Tanzania (0.25 million tonnes). Forage yield ranges from 20-40 t DM/ha. Levels as high as 24 tonnes DM/ha of fodder and stalks have been reported from the Sahel, and it has been suggested that there should be further study on the use of pigeon pea as a forage plant in this area (FAO, 2016a). Up to 40 tonnes DM/ha could be expected under optimal conditions (ILRI, 2013). Pigeon pea is used as a contour hedge in erosion control (Bekele-Tessema, 2007). An N-fixing legume, it does not need inoculation before sowing. It was reported to fix 40-97 kg N/ha/year in Africa and up to 235 kg N/ha/year in Florida, 88% being used for pods and seed formation. Pigeon pea cultivation could provide 40-60 kg N/ha to the following crop (Valenzuela, 2011). The extensive root system of *Cajanus cajan* improves soil structure by breaking plow pans, and enhancing water holding capacity of the soil (Crop Trust, 2014; Mallikarjuna *et al*., 2011). Its deep taproot is able to extract nutrients (e.g. P) from the lower layers of the soil and deposit them in upper layers where they can benefit other crops (Valenzuela, 2011). The leaves and immature stems can be cut and used as a green manure (OAF, 2015). Fallen leaves act as a mulch and are estimated to return about 40 kg N/ha to the soil. They also return organic matter, which helps in preventing erosion due to heavy rains, and reduces soil temperature (Ecocrop, 2016). Thus, the crux of this study is to evaluate the appropriate planting space, cutting interval and height of *C. cajan* for maximum fodder yield.

## II. MATERIALS AND METHODS

The study was carried out at the Crop section of the Teaching and Research Farm and laboratory analyses were carried out at the Animal Production and Health Nutrition Laboratory of the Federal University of Technology, Akure (Latitude 70 18" and Longitude 50 10" E) (Aro *et al*., 2008) between March – July, 2016. A hectare of land was acquired to establish *Cajanus cajan* pasture. The seeds were gotten from the Ministry of Agriculture, Ado-Ekiti, Ekiti state; the land was ploughed and harrowed, two to three seeds of pigeon pea were planted per hole using planting spaces of 40x60 cm, 60x60 cm, 80x30 cm, 100x30 cm and 120x30 cm. After germination, thinning and supplying were done to ensure uniform plant stand per hole; and were managed for 3-4 months prior to the flowering stage. The commencement of harvest was done using different cutting heights (50, 100 and 150 cm) and cutting intervals (2, 3 and 4 weeks) on the allotted plots for five consecutive times. The forages were weighed per plot. Sub-samples of air-dried harvested forage were bulked and taken to the Nutrition Laboratory for proximate analysis (moisture content, dry matter, crude protein, crude fibre, ash and nitrogen free extract) according to the procedures of A.O.A.C. (2002). The experimental design was 3x3 factorial arrangements in a completely randomized design and data generated were subjected to statistical analysis using SAS (2008) and the means were compared using Duncan Multiple Range Test of the same package.

## III. RESULTS AND DISCUSSION

The fodder yield of *Cajanus cajan* was significantly ($p>0.05$) influenced by the planting space, cutting height and cutting interval. The initial yield ranged from 0.34 kg (120x30 cm planting space, 150 cm cutting height and 2 weeks cutting interval) to 8.95 kg (40x60 cm planting space, 50 cm cutting height and 4 weeks cutting interval). It was observed that the longer the period /interval of cutting and at a shorter cutting height, the more the biomass yield (Table 1). Hence, the same was the trend across the planting space. Though, higher yield was recorded at 40 x 60 cm planting space plot, especially at 50 cm cutting height. However, the regeneration ability of the plant is very high and could persist and rejuvenate easily if left for four (4) weeks before harvesting

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(ICRAF, 1992; Akinola and Oyejola, 1994). Yield attributes per plant were significantly lower with narrow spacing (because of the competition between plants) when compared to the yield attributes per plant recorded with wider spacing. These results were in accordance with the findings of Telgate *et al*. (2004). Pavan *et al*. (2009) and Mula *et al*. (2011). The least fodder yields (total and average yield) irrespective of cutting intervals were obtained on experimental plots of 120x30 cm planting space and cutting height of 150 cm. The initial cutting (8.95 kg), total yield (3.60 kg) and average yield (0.78 kg) per experimental plot of *C. cajan* at 40 x60 cm planting space, 50 cm cutting height and at 4 weeks interval had the highest yield compared to other experimental plots.

*Table.1: Biomass yield (kg) of Cajanus cajan sown and harvested at different intervals and heights*

| | | **2 weeks cutting interval** | | | **3weeks cutting interval** | | | **4weeks cutting interval** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Planting space(cm) | Cutting height(cm) | Initial yield | Total yield | Average Yield | Initial yield | Total yield | Average yield | Initial yield | Total yield | Average Yield |
| | 50 | $8.47^{a}$ | $1.46^{a}$ | $0.30^{a}$ | $8.85^{a}$ | $2.40^{a}$ | $0.47^{a}$ | $8.95^{a}$ | $3.60^{a}$ | $0.78^{a}$ |
| | 100 | $4.48^{e}$ | $1.02^{c}$ | $0.21^{bc}$ | $4.81^{f}$ | $1.63^{c}$ | $0.34^{c}$ | $4.85^{h}$ | $2.55^{b}$ | $0.48^{c}$ |
| 40 x 60 | 150 | $1.83^{k}$ | $0.61^{f}$ | $0.12^{ef}$ | $2.92^{k}$ | $0.87^{g}$ | $0.18^{g}$ | $4.29^{j}$ | $1.85^{f}$ | $0.36^{f}$ |
| | 50 | $6.24^{b}$ | $1.15^{b}$ | $0.23^{b}$ | $6.34^{b}$ | $1.88^{b}$ | $0.38^{b}$ | $6.69^{c}$ | $2.53^{b}$ | $0.52^{b}$ |
| | 100 | $2.96^{i}$ | $0.97^{d}$ | $0.19^{c}$ | $4.03^{i}$ | $1.48^{d}$ | $0.29^{ef}$ | $4.88^{h}$ | $2.35^{c}$ | $0.49^{c}$ |
| 60 x 60 | 150 | $2.29^{j}$ | $0.87^{e}$ | $0.16^{d}$ | $0.99^{n}$ | $0.89^{g}$ | $0.18^{g}$ | $3.35^{l}$ | $1.53^{g}$ | $0.33^{g}$ |
| | 50 | $5.49^{c}$ | $0.62^{f}$ | $0.13^{e}$ | $5.55^{d}$ | $1.49^{d}$ | $0.30^{e}$ | $7.55^{b}$ | $2.35^{c}$ | $0.47^{d}$ |
| | 100 | $5.20^{d}$ | $0.64^{f}$ | $0.14^{de}$ | $5.24^{e}$ | $0.80^{h}$ | $0.16^{h}$ | $6.74^{d}$ | $2.14^{e}$ | $0.44^{e}$ |
| 80 x 30 | 150 | $2.16^{j}$ | $0.58^{fg}$ | $0.13^{e}$ | $2.28^{l}$ | $0.56^{k}$ | $0.12^{j}$ | $3.58^{k}$ | $1.45^{h}$ | $0.29^{h}$ |
| | 50 | $3.83^{f}$ | $0.64^{f}$ | $0.13^{e}$ | $5.69^{c}$ | $1.45^{e}$ | $0.29^{ef}$ | $6.17^{e}$ | $2.40^{c}$ | $0.47^{d}$ |
| | 100 | $3.66^{g}$ | $0.49^{h}$ | $0.10^{f}$ | $4.23^{i}$ | $0.67^{i}$ | $0.14^{i}$ | $5.98^{f}$ | $2.25^{d}$ | $0.45^{e}$ |
| 100 x 30 | 150 | $1.14^{m}$ | $0.41^{i}$ | $0.08^{g}$ | $1.15^{m}$ | $0.50^{l}$ | $0.10^{jk}$ | $1.39^{m}$ | $1.15^{i}$ | $0.24^{i}$ |
| | 50 | $3.43^{h}$ | $0.55^{g}$ | $0.10^{f}$ | $4.53^{g}$ | $1.36^{f}$ | $0.28^{f}$ | $5.25^{g}$ | $2.30^{d}$ | $0.45^{e}$ |
| | 100 | $2.24^{j}$ | $0.29^{j}$ | $0.06^{h}$ | $4.30^{h}$ | $0.60^{j}$ | $0.13^{ij}$ | $4.55^{i}$ | $1.87^{f}$ | $0.36^{f}$ |
| 120 x 30 | 150 | $0.34^{l}$ | $0.27^{j}$ | $0.05^{h}$ | $0.55^{o}$ | $0.38^{m}$ | $0.08^{k}$ | $0.84^{n}$ | $0.85^{j}$ | $0.16^{j}$ |
| | SEM | 0.04 | 0.02 | 0.01 | 0.02 | 0.02 | 0.01 | 0.03 | 0.05 | 0.01 |

a,b,c…m = means within the same row with different superscripts are significantly ($p<0.05$) different

As shown in Table 2, cutting interval had a significant effect on the percentage content of all the components measured, with dry matter percent and crude fiber percent increasing as the cutting interval increased from week 2 to 4. By comparison, crude protein and ash all showed a decrease as the cutting interval increased. This report agreed to the assertion of Pipat *et al.* (2014) who investigated the effect of cutting interval and cutting height on yield and chemical composition of king napier grass (*Pennisetum purpureum x Pennisetum americanum*). Consequently from Table 2, the harvested forage could serve as a means of feeding ruminant animals either as hay, haylage or silage, as the nutritive value of the forage could meet the nutrient requirements by ruminant for growth and maintenance purpose. The disparity in the crude protein and fibre contents could be attributed to the age at cutting and the leaf to stem ratio of the forage. Hence, the forage is considerably lignified. However, ruminants can utilize them with the help of the fibrolytic enzymes which will attack the fibre during ruminal fermentation for effective degradation. Moreso, the variation in the nutrient compositions of the foliage at different cutting intervals could be attributed to the period of cut. Perhaps, it contributed to its high lignifications of the stem and hence, could inhibit digestion.

*Table.2: Proximate composition of Cajanus cajan forage harvested at different stages and heights*

| | ***C. cajan* forage (DM basis)** | | | |
|---|---|---|---|---|
| **Parameters (%)** | **2 weeks** | **3 weeks** | **4 weeks** | **SEM** |
| Dry matter (DM) | 86.34 | 86.77 | 87.09 | 0.12 |
| Crude protein | 15.53[a] | 15.01[b] | 14.97[b] | 0.05 |
| Crude fibre | 27.43[c] | 28.88[b] | 30.55[a] | 0.06 |
| Ether extract | 1.80 | 1.78 | 1.80 | 0.01 |
| Ash | 4.03 | 4.01 | 3.97 | 0.01 |
| Nitrogen free extract | 37.55[a] | 37.09[ab] | 36.01[b] | 0.03 |
| *Gross energy (KJ/100gDM) | 14.12 | 14.19 | 14.26 | 0.02 |

abc = means within the same row with different superscripts are significantly ($p<0.05$) different
*Calculated as described by Ekanayake *et al*. (1999).

## IV. CONCLUSION AND APPLICATION(S)

*Cajanus cajan* pasture could be established to serve a multi-purpose – fixing atmospheric nitrogen into soil, drought tolerant, and could bridge the gap of malnutrition affecting ruminant animals. By extrapolation, if *C. cajan* pasture is fully established on an hectare of land using 40 x 60 cm planting space, more fodder yield would be obtained at 4 weeks cutting interval and 50 cm cutting height. Thus, encourage pasture establishment for improved ruminant production especially in Nigeria to curb the incidence of Fulani herdsmen imbroglio.

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# Effects of Chemical, Biological and Botanical for the Management of Alternaria Leaf Spot Disease of Radish for Healthy Seed Production

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***Abstract—*** *An experiment was conducted to evaluate the effect of different treatments viz. SAAF (mancozeb +carbendazim) (2g $l^{-1}$), Acorous calamus root extract (50 %), Trichoderma harzianum ($10^6$ conidia $ml^{-1}$) aqueous suspension and two radish varieties (Mino Early and Pyuthaney Rato) against Alternaria leaf spot disease (Alternaria brassicae) of radish in research plot of Department of Plant Pathology AFU, Chitwan, Nepal during winter season of 2015. Foliar applications of the treatments were used for six times from 40 DAS to 90 DAS at 10 days interval. All treatments were found significant during all observations for per cent disease index (PDI) and yield. Lowest PDI was recorded in application of SAAF (carbendazim 12% + mancozeb 63%) (52.33 % and 34.33 %), which was statiscally at par and followed by foliar application of T.harzianum (56.17 % and 39.5 %) in both vegetative growth stage (60 DAS) and reproductive stage (90 DAS) of radish. There was significant difference in PDI between the varieties, Mino Early (43.58 %) followed by Pyuthaney Rato (49.92 %) in 90 DAS. Similarly in case of seed yield, highest yield was recorded from SAAF (0.6 t ha1) followed by Trichoderma harzianum (0.49 t $ha^{-1}$). Also in case of variety significant difference in yield was recorded, highest yield was recorded from Mino Early (0.57 t ha-1) followed by Pyuthaney Rato (0.30 t $ha^{-1}$).*

***Keyword— Acorous calamus, alternaria leaf spot, fungicides, trichoderma.***

## I. INTRODUCTION

Crucifer vegetables are the important winter season cash crop grown in Nepal. Around 70% of Nepal's total household is involved in vegetable farming (CBS 2010). Radish (*Raphanus sativus* L.) is one of the top five vegetables produced in Nepal, which covers 7.47% of total vegetable production area of Nepal with an average productivity of 14.45 mt $ha^{-1}$ (VDD 2013). Radish crop has easy cultivation practice, wider climatic adaption and extensive use and thus it is popular among the farmers (Shrestha and Shakya 2004). Also, radish is the most important seed crop in terms of high demand of quality commercial seed (HVAP 2011).

The most common and destructive diseases of Brassicaceae crops worldwide are those caused by four species of Alternaria viz., *A. brassicae* (Berk.) Sacc, *A. brassicicola* (Schwein.) Wiltsh., *A. raphani* Groves and Skolko, and *A. alternata* (Fr.) Keissl which are seed borne pathogen. *A. brassicae* (Berk.) Sacc, *A. brassicicola* (Schwein.) Wiltshire are responsible for a serious grey and dark leaf spot disease on those crops (Fazal et al. 1994; Verma and Saharan 1994). At least 20% of agricultural spoilage is caused by Alternaria species; most severe losses may reach up to 80% of yield (Shrestha and Chaudhary 1999). Among the root crops like radish, turnip, beet root and carrot, maximum infection (10-60%) has been found in radish, and the disease has widely spread in all the growing areas of Nepal (Shrestha 1990). Yield reduction up to 45% in radish has been reported from Kathmandu and Chitwan (Shrestha 1996). Average yield losses in the range of 32-57% due to *Alternaria* have been reported from Nepal (Shrestha et al. 2005). Due to high plant parasitic nature of this pathogen it has become major problem in production of seed as it affect most during pod formation stage of the crop affecting seed quality by reducing seed size, seed discoloration and reduction in oil content (Prasad and lallu 2006).

Application of chemical fungicide is the arguably easiest and most effective method for the management of the disease. Chemical fungicide inhibits the spore germination and penetration of the pathogen in host but pathogen can generate resistance against the fungicide if not used in proper dose and interval of time (Namada et al. 2004; Kirik et al. 2005) and cause environmental pollution (Tisdale et al. 1985). These kinds of health and

environment issues have created deception and fear mongering situation towards the use of chemical pesticides and thus there are strict rules and regulation towards the judicial use of pesticide around the globe. Thus, this study was mainly focused on identifying the effect of different varieties of radish, botanical extract and bio-control agent and chemical fungicide for the management of Alternaria leaf spot of radish and quality seed production.

## II. MATERIALS AND METHODS

***Preparation of the treatments***

Trichoderma selective media was used for isolation of Trichoderma sp. from soil brought from the nearby forest in Rampur, Chitwan (Elad et al. 1981). Morphological observations were made from cultures grown on PDA plates at 24°C under ambient laboratory conditions of diffuse daylight. The microscopic characteristics were observed for the more complex conidiophores developing from the characteristic tufted or postulate areas of condition, usually 3 to 5 days after inoculation. Identifications were performed by using the identification keys provided by Rifai 1969 and Bissett 1984, 1991a, 1991b. Examination of the shape, size, arrangement and development of conidiophores or phialides were done and hence identified as *Trichoderma harzianum*. Concentration of $10^6$ conidia $ml^{-1}$ water was prepared from freshly grown *Trichoderma harzianum* isolates using haemocytometer. Chemical fungicide SAAF (carbendazim 12% + mancozeb 63%) @ 2g $l^{-1}$ water was prepared for. Fresh roots of *Acorus calamus* (sweet flag) from healthy plants were collected, surface sterilized for 2 min in 70% ethanol, and washed in three changes of distilled water. 100 gram of root sample was weighed and grinded with mortar and pestle and finally paste was filtered in sterile double layered muslin cloth. The volume of filtrate collected was later mixed with 100 ml sterilized distilled water to make stock solution, later it was made 50% by adding more sterilized distilled water (stock solution: sterilized distilled water as 1:1 proportion). Foliar application of all these treatments was done at 40 DAS for 6 times at 10 days interval. First application was done 40 days after planting.

*Table.1: Details of treatments combination*

| SN | Treatment combination | Concentration |
|---|---|---|
| 1 | Mino Early + SAAF | 2g $l^{-1}$ |
| 2 | Mino Early + *Acorus calamus* root extract | 50 % ( 1part root extract in 2 part distilled water) |
| 3 | Mino Early + *Trichoderma harzianum* | $10^6$ conidia $ml^{-1}$ |
| 4 | Mino Early + control (distilled water) | - |
| 5 | Pyuthane Rato + SAAF | 2g $l^{-1}$ |
| 6 | Pyuthane Rato + *Acorus calamus* root extract | 50 % |
| 7 | Pyuthane Rato + *Trichoderma harzianum* | $10^6$ conidia $ml^{-1}$ |
| 8 | Pyuthane Rato + control (distilled water) | - |

***Disease incidence and Severity***

Observation of disease was done from ten sample plants randomly selected and tagged for further observations. First scoring was done 40 days after sowing (DAS), respectively; two more scoring at an interval of 10 days were done for foliar severity assessment. Similarly, first stem and pod disease scoring was done at 70 DAS and other two scoring was done at an interval 10 days. Disease scoring was done in 0-5 scale (0= no infection, 1= 1-5% infection, 2= 6-10% infection, 3= 11-20% infection, 4= 21-30% infection, 5= 31-100% infection (Shrestha et al. 2005). The disease severity of foliar and stem and pods diseases at each disease scoring was calculated using the formulae (Ayyanagar 1928).

$$\text{Percent disease index (PDI)} = \frac{\text{sum of all ratings}}{\text{total number of plants observed} \times \text{maximum disease rating scale}} \times 100$$

## III. RESULTS AND DISCUSSIONS

Disease severity of Alternaria leaf spot at 40, 50, 60, 70, 80 and 90 DAS of radish varied significantly among the treatments and also between the two varieties except for 60 DAS. At 40 DAS lowest percent disease index (PDI) was recorded in application of SAAF (35.00%) which was followed by foliar application of *Trichoderma harzianum* (46.50%). At 50 DAS lowest percent disease index (PDI) was recorded in application of SAAF (51.83%) which is statically at par with foliar application of *Trichoderma harzianum* (55.50%). At 60 DAS lowest percent disease index (PDI) was recorded in application of SAAF (52.33%) which was statically at par with foliar application of *Trichoderma harzianum* (56.17%). Similarly in case of varieties during vegetative stage, at 40 and 50 DAS lowest percent disease index (PDI) was

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recorded in Mino early (43.20% and 53.42%) followed by Pyuthaney Rato (53.42% and 60.25%) whereas at 60 DAS there was not any significant difference between the varieties.

There was abrupt decline in PDI in all treatments including untreated control due to defoliation of the plant. Soon after that severity percentage increased with the development of the reproductive part of the plants. At 70, 80 and 90 DAS lowest percent disease index (PDI) was recorded in application of SAAF (21. 33%, 25.17% and 34.33%) which was followed by foliar application of *Trichoderma harzianum* (27.50%, 33.67% and 39.50%) respectively. Similarly in case of varieties, at 70, 80 and 90 DAS lowest percent disease index (PDI) was recorded in Mino Early (24.50%, 33.00% and 43.58%) followed by Pyuthaney Rato (33.75%, 43.60% and 49.92%) respectively.

Highest yield was recorded from SAAF (0.6 t $ha^{-1}$) followed by *Trichoderma harizanum* foliar application (0.49 t ha $^{-1}$) at 90 DAS. And in case of variety also the significant difference in yield was recorded, highest yield was recorded from Mino Early (0.57 t $ha^{-1}$) and lowest from Pyuthaney Rato (0.30 t $ha^{-1}$) at 90 DAS.

*Table.2: Effect of foliar spray on the severity of Alternaria leaf spot of radish in Horticulture farm at AFU, Rampur, Chitwan during 2014-2015*

| Factors | PDI at Vegetative Stage (Leaf formation ) | | | PDI at Reproductive stage (Pod and Stem formation) | | | Yield (t $ha^{-1}$) |
|---|---|---|---|---|---|---|---|
| | 40 DAS | 50 DAS | 60 DAS | 70 DAS | 80 DAS | 90 DAS | |
| Variety | | | | | | | |
| Mino Early | 43.20 | 53.42 | 57.50 | 24.50 | 33.00 | 43.58 | 0.57 |
| Pyuthaney Rato | 50.10 | 60.25 | 62.20 | 33.75 | 43.60 | 49.92 | 0.30 |
| LSD value at α 0.05 | 6.51 | 3.32 | Ns | 3.43 | 5.22 | 3.16 | 0.06 |
| SEM value± | 2.15 | 1.09 | 1.66 | 1.13 | 1.72 | 1.04 | 0.02 |
| Treatments | | | | | | | |
| SAAF @ 2gm $lit^{-1}$ | 35.00$^{c}$ | 51.83$^{c}$ | 52.33$^{c}$ | 21.33$^{c}$ | 25.17$^{d}$ | 34.33$^{d}$ | 0.60a |
| *Acorous calamus* root extract @ 50 % | 47.83$^{b}$ | 58.00$^{ab}$ | 61.33$^{b}$ | 29.17$^{b}$ | 42.67$^{b}$ | 46.50$^{b}$ | 0.39c |
| *Trichoderma harzianum* @$10^{6}$ conidia $ml^{-1}$ | 46.50$^{c}$ | 55.50$^{bc}$ | 56.17$^{bc}$ | 27.50$^{b}$ | 33.67$^{c}$ | 39.50$^{c}$ | 0.49b |
| Control | 57.33$^{a}$ | 62.00$^{a}$ | 69.67$^{a}$ | 38.50$^{a}$ | 51.67$^{a}$ | 66.67$^{a}$ | 0.26d |
| LSD (=0.05) | 9.21 | 4.69 | 7.12 | 4.85 | 7.39 | 4.48 | 0.08 |
| SEm (±) | 3.04 | 1.54 | 2.35 | 2.26 | 2.44 | 1.47 | 0.02 |
| Coefficient of variation (%) | 15.94 | 6.67 | 9.60 | 13.45 | 15.58 | 7.70 | 16.15 |
| Grand mean | 46.7 | 56.83 | 59.90 | 29.12 | 38.29 | 46.75 | 0.43 |

Figures in column with same letter are not significantly different (p=0.05). LSD= Least significant difference. SEm= standard error of mean

In present study among the fungicide, biocontrol agent and botanical, SAAF @ 0.2g $l^{-1}$ was most effective followed by foliar application of *Trichoderma harzianum* @ $10^{6}$ conidia $ml^{-1}$ and foliar application of *Acorous calamus* root extract @ 50% in reducing PDI of alternaria leaf spot disease and increasing the seed yield. The finding of this study was similar to the findings of Ansari et al. (1990) where the best control of alternaria blight was from the foliar application of mancozeb based fungicide viz. Dithane M 45. Furthermore, Arifuzzaman et al. (2007) reported spray of Mancozeb (0.3%) and carbendazim (0.1%) reduced disease severity by 52.27% and 70.95% in leaf where as 54.99% and 78.11% in pod. The fungi toxic and inhibitory effect of the chemical fungicide SAAF may be the reason behind minimum disease severity. The yield were significantly higher in foliar application of *Trichoderma harzianum* (Thakur et al. 2017). Mycoparasitic behavior of the *Trichoderma sp.* is reported to give systemic protection against many seed borne foliar diseases (Hanson 2000) which may be the probable reason behind the lowest disease incidence with the use of *Trichoderma harzianum*. The disease causes black spots on leaves, stems and pods resulting in the loss of both yield and quality of seed of radish. Incidence of Alternaria blight and its adverse effect on seed yield had been reported by (Meenu and Hundal 2004). Bhandari (2008) reported that 0.2 % Mancozeb spray was the best for controlling the disease and had highest yield (0.73 t/ha) which is similar to the findings of present study. The higher yield in SAAF sprayed plot was may be due to

lower disease severity in SAAF sprayed plot in both vegetative and reproductive stage of radish plant. In case of variety Mino Early was better performing than Pyuthaney Rato, this was may be due smooth leaf surface of the Pyuthane Rato which was favorable for spore germination of the pathogen (*Alternaria brassicae*) leading to higher disease severity and lower yield of the radish seed.

## IV. CONCLUSIONS

From the present study it is clear that foliar spray of SAAF (mancozeb and carbendazim) @ 2 g $l^{-1}$ is the best control measure for controlling alternaria leaf spot of radish and increasing seed yield. However we cannot ignore environmental and health hazards due to the use of chemical fungicide. Foliar application of *Trichoderma harzianum* @$10^6$ conidia $ml^{-1}$ was also found to be significant in reduction of disease and increasing seed yield. In conclusion, foliar spray of *Trichoderma harzianum* @$10^6$ conidia $ml^{-1}$ could be used for the control of alternaria leaf spot disease of Radish as it is quite good method of bio-control, affects a wide range of plant pathogens, acts as good mycoparasite, produces antibiotics and has an enzyme system to inhibit growth of pathogen and more importantly ecofriendly. However, further studies on efficacy of different conidial concentration of different Trichoderma isolates can be done for further identification of best concentration of the different isolates.

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# Will Growth of Technology Lift up the Economic Status of Farmers?

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***Abstract*****—** *As a very hot news that spread through out the state Tamilnadu, India that the formers who produced tomatos are dumped aside of road without bringing to market for domestic cooking consumption. Usually, the agricultural zaid commodities are not brings expected profit to the producers due to perishable character and gets low price at the markets in any situation. Tomato is zaid crop but it availing in all seasons and price of it will be also differing Moreover, crop in the zaid season gets very less price in the market but the level of production is high. Due to stable demand and substitute commodities are the cause for emerging low price in the market. As a remedy for this problem, recent research found that the rotten tomatos is good source to generate electricity. Hence, This empirical report brought out the real situation of zaid crop tomato in the market and plights of formers due to decreased price. Moreover, report suggests to the government that have to follow a procurement price for zaid crops also, to empower the economic status of formers followed by the execution of technology for alternative purposes.*

***Keywords— Tomato, Production, Zaid, Price, Electricity.***

Prevailing problems in the marketing of agricultural products are dumping the producers (farmers) in the deep ocean of tears which not paves way to escape from the economic burden and gives afflicts to survive as farmers prolong is being common trend in every crops season. Generally, agricultural productions can be divided in to three types as the seasons forthcoming every year. Thus, June-september (4 months) crops are kharif, October-march (6 months) crops are rabi and April-june (3 months) crops are called Zaid [1].

*Month wise availability of Tomato in India*

| Period of Harvest | Areas |
|---|---|
| January-March | Bihar, Eastern UP, MP, Orissa, foot hills of Uttarakhand, Andhra Pradesh, Assam |
| April- May | Haryana, Punjab, Karnataka, Rajasthan |
| June-July | H.P. Uttarakhand, Tamil Nadu, Punjab, Gujarat |
| August-September | Andhra Pradesh, Himachal Pradesh, Uttarakhand, Maharashtra, Gujarat |
| October-November | Chattisgarh, Tamil Nadu |
| December | Andhra Pradesh, Rajasthan, Chattisgarh, Madhya Pradesh, Orissa |

Source: http://agriexchange.apeda.gov.in/Market%20Profile/one/TOMATO.aspx [2]

As being the problems of marketing of zaid crops in tamilnadu and other states of India farmers are economically affected too and many of them feeling shame about being as an agricultural products producers due to falling a price of the productivity that holding them lest they improve economically and to be under debt. Tomatto as zaid crop that price is fallen from the range of Rs.30-60 to Rs.1-3 and now is being in the markets at very cheap cost which not meet the minimum production cost of the producer and directs further production trend to be declined for short period. Moreover, as a recent incident proclaimed that the tonnes of tomatos are dropped into lakes and ponds without commercial and further production purposes. A writer sreedevi jeyarajan says that lakes and ponds in tamilnadu are becoming red beds due to price falling of tomato and Dheiva sigamani, State President, Tamil farmers union said that Rayakottai, Krishnagiri district and Valappadi in Salam district are having great tomato markets but tomatoes are dumped into nearby lakes as an agitation of farmers [3]. Because, price falling of tomato is being unrecoverable to obtain harvesting costs to manage the production function for long time.

**Why the price is fallen?**

Quantity and quality of the products also determines the price along with market demand of the same to meet maximum profit and recover harvesting costs. Dheiva sigamani says that farmers are inspired to produce maximum level of tomato production ratio due to it is

short season crop, existence of high market price that was few months ago, limited water supply required for irrigation purpose in the production process of tomato are indirectly led to bring excess production [3]. Demand for tomato was not increased to increase the production level and it will not increased if horizontal level of supply not decreased. As we know the zaid crops are wants minimum level of water to be supplied and climates should be cool. Hence, farmers are forwarded to increase the level of tomato production without prediction of future outcomes. Thus, minimum demand and over supply has brought the price to be fallen for tomato in the market..

**Public Opinion**

Why dose the Minimum Support Price (MSP) is not been helpful to farmers?. As we know the MSP is usually implemented for two types of crops such as Kharif and rabi because these are long time season crops and having sustainable demand for it in the market. But, zaid crops are apart from the characters of the remain because products are available much in the short season and there is no possible way of leading sustainable production trend. So, India's agricultural price policy neglectes MSP system for such a crops comes under zaid. Although, some states are forwarding to bring MSP for zaid crops also as Rs.225 for one quvindal in Karnataka, Rs.5 per kg in Hyderabad and Rs.6 per kg in tamilnadu etc. Moreover, very practically, S. Narayan, former Economic Adviser to the Prime Minister, said that "Rice and wheat are standard items and the government has been procuring them over a long period of time. But the case of potato and tomato is different with so many variants. To standardise and give the correct price would be difficult" and also stated United Progressive Alliance (UPA) regime between 2004 and 2014 was regular increase in the minimum support price and farmers were better off. But, after 2014, the MSP was not increased. But, the cost has gone up to farmers distress as an instances [4].

**Way to recover the loss**

Why does the farmers have dumped?. Here two reasons are visualize that the farmers are agitates against government due to use less MSP in the short season and if tomatoes put forth to store, rotten one will make rotten all so better be thrown out instead of to be sold. Hence, Truths about farmers is that they are innocent, illiterate and also being zero awareness about technical growth and global advances. But, rest of farmers what steps has been taken to bear their economic loss and burden even we have sufficient potential to further process is still questionable.

'A team of scientists is exploring an unusual source of electricity — damaged tomatoes that are unsuitable for sale at the grocery store. Their pilot project involves a biological-based fuel cell that uses tomato waste left over from harvests in Florida.The researchers present their work at the 251st National Meeting & Exposition of the American Chemical Society (ACS). ACS, the world's largest scientific society, is holding the features of more than 12,500 presentations on a wide range of science topics.

"We have found that spoiled and damaged tomatoes left over from harvest can be a particularly powerful source of energy when used in a biological or microbial electrochemical cell," says Namita Shrestha, who is working on the project. "The process also helps purify the tomato-contaminated solid waste and associated waste water." Shrestha is a graduate student in the lab of Venkataramana Gadhamshetty, Ph.D., P.E., at the South Dakota School of Mines & Technology. They are collaborating on this project with Alex Fogg, an undergraduate chemistry major at Princeton University. Other project collaborators include Daniel Franco, Joseph Wilder and Simeon Komisar, Ph.D., at Florida Gulf Coast University.

Tomatoes are a key crop in Florida, notes Gadhamshetty. He stresses that the project is important to the state because Florida generates 396,000 tons of tomato waste every year, but lacks a good treatment process. Gadhamshetty began working on the topic as a professor at Florida Gulf Coast University. "The project began a few years ago when Alex visited lab in Fort Myers, Florida, said that he was interested in researching a local problem, especially local tomatoes grown in our state and the large waste treatment issue," Gadhamshetty says. "We wanted to find a way to treat this waste that, when dumped in landfills, can produce methane — a powerful greenhouse gas — and when dumped in water bodies, can create major water treatment problems." So, the team developed a microbial electrochemical cell that can exploit tomato waste to generate electric current. Shrestha explains, "Microbial electrochemical cells use bacteria to break down and oxidize organic material in defective tomatoes." The oxidation process, triggered by the bacteria interacting with tomato waste, releases electrons that are captured in the fuel cell and become a source of electricity. The natural lycopene pigment in tomatoes, the researchers have found, is an excellent mediator to encourage the generation of electrical charges from the damaged fruits. Some of their results proved to be counterintuitive. "Typical biotechnological applications require, or at least perform better, when using pure chemicals, compared to wastes," Gadhamshetty notes. "However, we found that electrical performance using defective tomatoes was equal or better than using pure substrates. These wastes can be a rich source of indigenous redox mediators and carbon, as well as

electrons."At the moment, the power output from their device is quite small: 10 milligrams of tomato waste can result in 0.3 watts of electricity. But the researchers note that with an expected scale up and more research, electrical output could be increased by several orders of magnitude.

According to calculations by Shrestha, there is theoretically enough tomato waste generated in Florida each year to meet Disney World's electricity demand for 90 days, using an optimized biological fuel cell. "Our research question at this time is to investigate the fundamental electron transfer mechanisms and the interaction between the solid tomato waste and microbes," Gadhamshetty notes. They plan to improve the cell by determining which of its parts — electrode, electricity-producing bacteria, biological film, wiring — are resisting the flow of electricity. Then they will tweak or replace that part.The team acknowledges funding from the National Science Foundation, National Aeronautics and Space Administration, Electric Power Research Institute and the Office of Research & Graduate Studies at the Florida Gulf Coast University.The American Chemical Society is a nonprofit organization chartered by the U.S. Congress. With more than 158,000 members, ACS is the world's largest scientific society and a global leader in providing access to chemistry-related research through its multiple databases, peer-reviewed journals and scientific conferences. Its main offices are in Washington, D.C., and Columbus, Ohio [5].

**Government contribution to recover the loss**

As a government contribution to standardise the economic level of farmers, necessary steps need to be taken to prevent such dumping energy potential crops. While MSP is not been effective, that is good if better technical powers adopted to the appropriate field. Sufficient awareness need to forwarded among farmers regarding technical advances in supporting primary sector. Steps need to taken for collect all solid waste (vegetables) or damaged and rotten tomatoes in preparing energy for future use. When short time price falling accumulates in the market, farmers need to approach the technical filed which ready to collect agri-wastes to the energy production. Appropriate price need to fix for rotten tomato that should be purchased from farmers as it should be a way of recovering losses in the market. And also technical filed should be motivated in this function and sufficient support should be delivered to the same. Price determination for rotten tomatoes should be based on the value of further alternative energy but not compared with the price of tomatoes that is in the markets. Thus, if policy makers brings technological growth to the primary sector to protect it value for future purposes, farmers also be able to develop themselves economically and many problems regarding production and marketing of agricultural products will be solved.

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# How Heavy Metal contamination is contributing pollution in Delhi

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***Abstract*—***Inexpensive and environmentally supportable corrective choices are compulsory to reinstate polluted lands so as to decrease the connected risks, make the land resource available for agricultural production, enhance food security, and scale down land tenure problems. In evolving countries with great population density and scarce funds available for environmental restoration.*
*Comparative study of all the important metals according to Region, Population, Public places in Delhi's Soil.*
***Keywords—Heavy Metal, Contamination, Delhi's Soil, Polluted site, NCR zone.***

## I. INTRODUCTION

The acquisitive use of biosolids for polluting soils with heavy metals has caused great concern about their uses in cultivation[1]. Heavy metals most usually found in biosolids are Pb,As, Zn, Cd, Fe and Ni, and the metal mass percentage in soil ratio are responsible for its cultivation the mass ration changes by the countryside and the intensity of the industrial commotion, as well as the type of procedure employed during the biosolids handling[2]. Under different circumstances, metals added to soils in applications of biosolids can be percolated downwards through the soil profile and can have the probable to contaminate groundwater[3]. Recent studies on some New Zealand soils treated with biosolids have shown increased concentrations of Cd, Ni, and Zn in drainage leachates which is also in sighted in the present research in Delhi's soil[4,5].

## II. MATERIAL & METHOD

When plasma energy is given to an analysis sample from outside, the component elements (atoms) are excited. When the excited atoms return to low energy position, emission rays (spectrum rays) are released and the emission rays that correspond to the photon wavelength are measured. The element type is determined based on the position of the photon rays, and the content of each element is determined based on the rays' intensity. Plasma is the forth state of matter, next to the solid, liquid and gaseous state. In the ICP-OES the plasma is generated at the end of a quarts torch by a cooled induction coil through which a high frequency alternate current flows. As a consequence, an alternate magnetic field is induced which accelerated electrons into a circular trajectory. Due to collision between the argon atom and the electrons ionization occurs, giving rise to a stable plasma. The plasma is extremely hot, 6000-7000 K. In the induction zone it can even reach 10000 K.

$$\textbf{\% Relative Standard Deviation (\%RSD)} = \frac{\text{Standard Deviation}}{\text{Mean Value}} \times 100$$

## III. INTENSITY OF SPECIFIC METAL IN SOIL OF PUBLIC TRANSPORTATION

*Table.6.4: Public Transportation*

| | D99 | D101 | D72 | D45 | D29 | D31 | D35 | D36 | D37 |
|---|---|---|---|---|---|---|---|---|---|
| As 188.979 | -2.9 | -3.5 | -3.9 | -6.5 | -0.2 | 4.3 | 2.1 | -4 | -4.8 |
| Cd 228.802 | 14 | -5.4 | 16 | -1.1 | 13 | 11.7 | 6.2 | 14.1 | 8.3 |
| Fe 238.204 | 1068870.4 | 973691.3 | 1134006.1 | 1350420.1 | 1138255.6 | 1023822.2 | 1127767.4 | 988009 | 1417410.1 |
| Ni 231.604 | 580.9 | 665.5 | 601.2 | 635.7 | 576.7 | 517.1 | 503.6 | 473.8 | 489.4 |
| Pb 220.353 | 108.7 | 119.5 | 103.7 | 66.1 | 81 | 96 | 50.7 | 72.9 | 71.7 |
| Zn 206.200 | 4484.8 | 2595.4 | 24859.6 | 1947.4 | 3087.9 | 4400.4 | 1271.6 | 1695.7 | 3174.3 |

**1-Public Transportation -Some Specific Metals**

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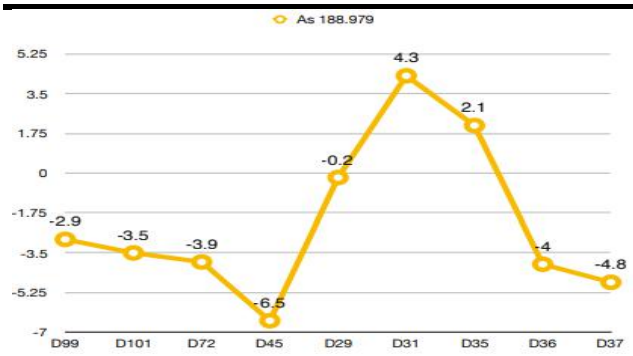


*Fig.1.1: Arsenic*

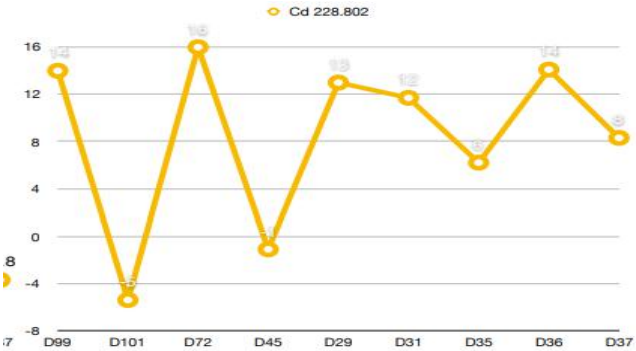


*Fig.1.2: Cadmium*

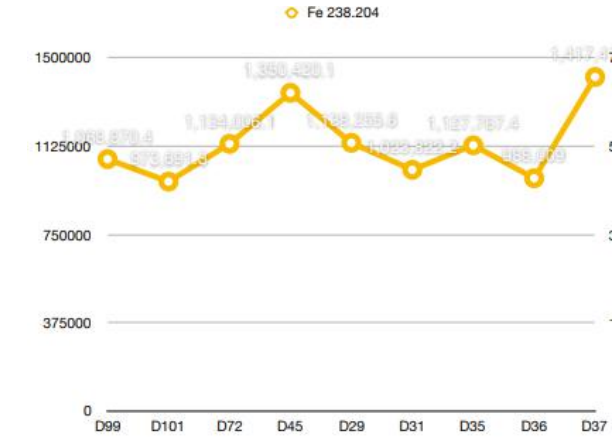


*Fig.1.3: Iron*

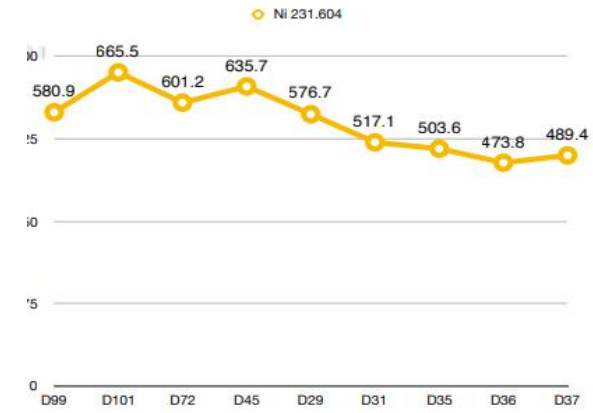


*Fig.1.4: Nickel*

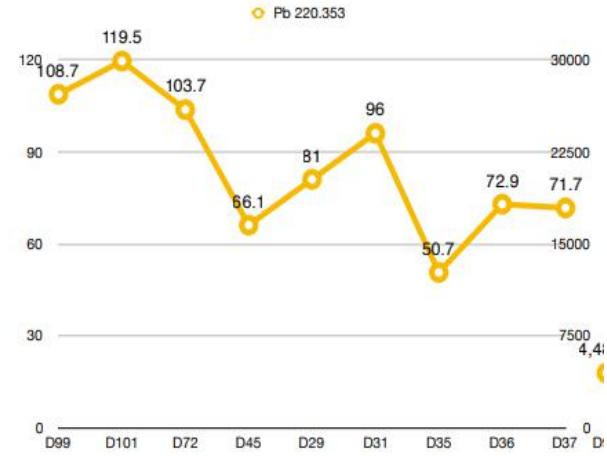


*Fig.1.5: Lead*

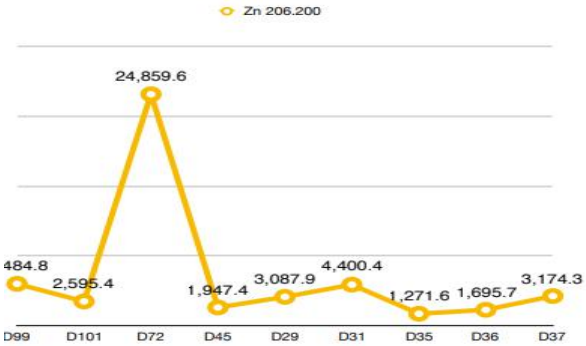


*Fig.1.6: Zinc*

## IV. DISUSSION

1. **Arsenic (Fig.1.1)**-The magnitude of as was found maximum in (D31) & least in (D45), it suggest most anthropogenic in these areas.
2. **Cadmium (Fig.1.2)-** Similar trend was observed in Cd concentration was least in (D101) & most in (D72), in rest of the places reported the same in rest of the areas.
3. **Iron (Fig.1.3)-**Iron being vital metal for the soil, abundantly present in soil, was found most Fe rich area for soil.
4. **Nickel (Fig.1.4)-**Maximum intensity of Ni was observed in all the areas of public transport which is evident for mechanical or automotive activities.
5. **Lead (Fig.1.5)-** The presence of Lead (Pb) was almost equally and abundantly reported in all the soil samples, Maximum intensity of Pb was observed in all the areas of commuting ,it tells the use of Automobiles & fuels is almost equal in all the places.
6. **Zinc (Fig.1.6)-**Zinc should be more likely present in the soil, for the nutrient value of soil ,Zn was least reported in all the places except (D72) having exceptionally high concentration in soil.

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## V. CONCLUSION

*Table.5.1: Public Transportation*

| S.No. | Metal | Public Transportation |
|---|---|---|
| **1** | **As 188.979** | The magnitude of As was found most near AIIMS Hospital & least Delhi's International Airport. |
| **2** | **Cd 228.802** | Similar trend was observed in Cd concentration was least on Kashmiri gate ISBT. & most in Anand Vihar Metro. |
| **3** | **Fe 238.204** | Iron being vital metal for the soil, abundantly present in soil, was found most Fe rich area for soil. |
| **4** | **Ni 231.604** | Maximum intensity of Ni was observed in all the areas of public transport which is evident for mechanical or automotive activities. |
| **5** | **Pb 220.353** | Maximum intensity of Pb was observed in all the areas of commuting |
| **6** | **Zn 206.200** | Anand Vihar being on outskirts of Delhi, having exceptionally high concentration in soil. |

Heavy metal contamination of soil is carriage of dangers and hazards to human beings and the biological network through: straight incorporation or contact with contaminated soil, the food chain (soil-plant-human or soil-plant-animal human), drinking of contaminated ground water, decrease in food quality (safety and marketability) via phytotoxicity, lessening in land usability for agricultural production causing food insecurity, and land occupancy difficulties [5,6,7].

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# Consumers' Food Value Attributes on Ghana's Local Market; Case Study of Berekum Municipality.

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***Abstract—*** *This paper investigates consumers' price perceptions at Ghana's local markets. By analyzing the questionnaire survey results, it identifies the relative importance local customers placed on weights and measures in comparison to other food attributes in purchasing agricultural products. In determining customers' decision-making behaviors, we applied Kahneman's (2012) prospect theory and developed picture-based scenarios for the customers to express their value perceptions at the market. We also asked questions to see what food attributes are important for them. We wanted to find out if such attributes as weights and measures are important for local Ghanaian customers other than more well-recognized ones such as food quality and price. The results indicate that our respondents decide to buy agricultural products like vegetables, eggs, and rice, on the basis of four attributes: (1) weights and measures, (2) health values, (3) safety, and (4) affordability. As previous studies on Western consumers tend to show high importance on food quality, our results suggest that customers' choices may differ more likely by socio-cultural backgrounds. This conclusion can be buttressed by another part of our survey that shows that our respondents are mostly middle-class, educated nuclear families in this region. About 80percent of them agreed that traditionally prescribed weights and measures were important to understand the value of agricultural products at the market.*

***Keywords— consumer behavior, Ghana, traditional weights and measure, local market, price perception.***

## I. INTRODUCTION

Consumer behaviors at ubiquitous and vibrant local markets of Africa like Ghana are among seemingly uncontrollable but important variables retailers should want to understand better. There, consumers employ various standards in deciding to buy a product. However, studies on consumer behaviors toward food products have focused mostly on such product quality cues as freshness, taste, health values, and origin, for purchasing. In examining food value cues, for example, scholars in the United States and other developed countries have shown good interests in organic products (Yiridoe, K.E., Bonti-Ankomah, S., & Martin, C.R, 2005). Consumers become either willing to accept or willing to pay for a product base on their perceptions of price. Prices at the market, therefore, are the representation of the product value (Zeithaml, 1988).

Several studies on price perceptions have argued that quality and value, though important in understanding consumer behaviors, are erratic and volatile. Zeithaml (1988) argues that there is limitation in the meaning of quality and value. There are inconsistent measurement procedures that limit the meaning of these concepts. Quality and value are not clearly defined, leading to the disparately using the concepts (Yiridoe et al, 2005; Acebrónand Dopico, 2000; Doorn and Verhoef, 2011; Zeithaml, 1988).

Lusk and Briggeman (2009) argue that consumers have a set of stable beliefs associated with food price and consumption. These beliefs play important roles in explaining consumer choices and illustrate the core underlying values that motivate their purchasing decisions. Lusk (2011) relates food values to consumers' purchasing decisions of organic products. He argues that consumers prefer organic products to inorganic ones because the former are more traditional and environmentally friendly even though some customers are concerned about relatively high price. Another study finds that, in general, product appearance tends to be less important among consumers with a high preference for organic and pesticide-free products (Yiridoe et al., 2005).

This paper attempts to understand if these generalized attributes also help understand local consumers in Ghana. In other words, we examine if other seemingly less important attributes such as weight and measures influence the consumers. If so, to what extent? To investigate this question, we designed and applied a questionnaire to ask local consumers at one of popular

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vegetable markets in Ghana. In the discussion below, we first briefly introduce our study area, and then elaborate on our survey method. In the final section, we discuss the result of our survey. The result shows that local Ghanaian consumers emphasize the importance of weights and measures more than quality attribute.

## II. METHODS

### 2.1 Background

The field study was carried out in Berekum Municipality of Ghana. This Municipality lies in the northwestern part of the Brong Ahafo Region with a land area of 863.3km$^2$. It has a population of 129,628. About two thirds (67.3%) of the population was economically active. More than half of the labour force is involved in agriculture especially crop farming (GSS, 2014).

The most economic market within the Municipality is operated weekly. During this period all wholesalers, retailers and other actors in and out of the Municipality meet to engage in diverse agriculture related businesses. The proximity of the Municipality to Cote D'Ivoire is another remarkable feature, which promotes economic and commercial activities between the Municipality and Cote D'Ivoire during this weekly market day.

### 2.2 Research Methodology

The field study was carried out during the periodic market (Thursday) for three weeks between December 2016 and January 2017. A consumer survey was conducted to clarify food attributes that influence consumers' purchasing decisions and to investigate if weights and measures influence their purchasing behaviors. Using random sampling technique, we interviewed 60 regular consumers at this market. The respondents were mostly women. This dominance by female indicates the general gender expectation in procuring food at market.

Our questionnaire survey had two components. The first component was to identify respondents' socio-economic backgrounds, including household size, income, occupation, age, and educational level. In the second part the respondents were asked to indicate their preference between two-basic scenarios. These scenarios are designed to determine consumers' preferences and thus pricing attributes. To help the respondent at the market to better understand these scenarios, questions were translated into the local language, Twi, and scenarios were presented with simplified pictures. Those respondents who had time and literate, filled the questionnaire themselves. It was later collected by a field officer. Of the surveyed individuals, 59 individuals filled all portions of the survey questions, implying a response rate of 98percent.

The questionnaire designs draw upon the idea of food value scale and the prospect theory. The questions meant to elicit consumer's food values in relation to prices. Some questions meant to identify consumers' perceptions about food product value attributes to determine the importance of weight and measure. Following Lusk's research (2011), we selected five general food values that motivate consumer choices: quality, quantity, health, trust and origin.

In designing scenarios in the questionnaire, ideas from Kahneman's (2012) prospect theory was applied. This theory evaluates our decision-making processes by using our notions of losses and gains. Like the paired comparison method, it measures the extent to which an individual recognizes preferences over the other in the business world. In other words, it was akin to a game of gamble. To help us understand consumers' decision making at local market, this notion was used to examine if consumers consider their losses and gains when they buy food crops with a standard or without any standard measurement. In addition, this will help explain the factors or criteria consumers use at the local market.

The scenarios were based on three operating components: food scale values, price and weight. The respondents answered seven questions in the form of choosing A or B option. For instance, one question established a scenario, in which there are two packages of eggs with the same weight. Package A contains four big eggs, whereas package B contains 8 small eggs. Four big egg costs GH¢ 1 and eight small egg cost GH¢1.5.

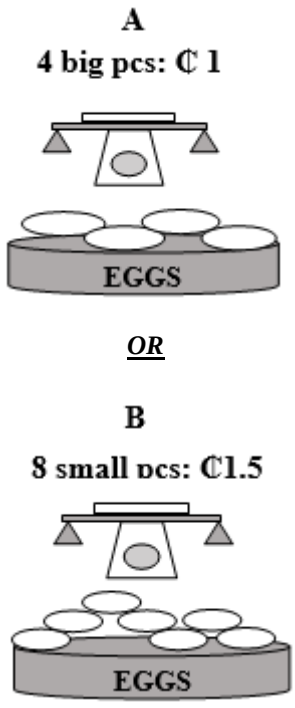


*Fig.1: Scenario A and B which do you Prefer?*

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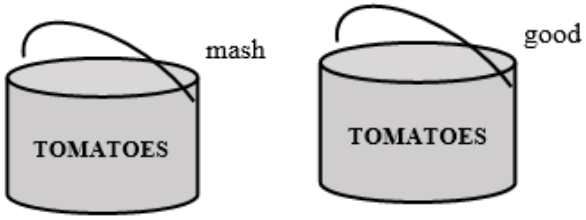
This scenario seeks to examine if consumers examine quantity, size and weight. Respondents were asked to circle their preference and explain their reasons for their selection.

In another scenario below design under the food value quality, we tried to examined consumers' preferences for quality, what they mean by quality and in so doing do they consider the weight.




Bucket A (5kg) Bucket B (4.5kg)

***SAME PRICE***

*Fig.2: Bucket A and Bucket B, circle the one you prefer?*

To measure consumer's food values, we used the paired comparison method. This method allows an analysis on the relative importance of available options. Consumers were asked to circle the highest preference and to describe their reasons. The probability that a consumer chooses A is greater than B, and reasons for this decision may differ. In addition, it focused on observations, explanation and the general assumption of consumers on the local market.

## III. RESULTS AND DISCUSSION

### 3.1 Social Characteristics of respondents

Table 1 demonstrates the results regarding our first part of the survey: the respondents' socio-economic variables. The majority (73%) was female. As mentioned above, this probably was because in Ghana shopping for food is traditionally the responsibility of women.

*Table.1: Characteristics of participants*

| Variable | | Frequency | Percentage (%) |
|---|---|---|---|
| Age | >10 years | 0 | 0 |
| | 11-20 years | 0 | 0 |
| | 21-30 years | 32 | 53 |
| | 31-40 years | 22 | 37 |
| | < 40 years | 6 | 10 |
| Gender | Male | 16 | 27 |
| | Female | 44 | 73 |
| Education | Primary | 3 | 5 |
| | Junior high | 11 | 18 |
| | Senior High | 23 | 38 |
| | Tertiary | 23 | 38 |
| | None | 0 | 0 |
| Household annual income (Ghana cedi) | 0-999 | 3 | 5 |
| | 1,000-1,999 | 2 | 3 |
| | 2,000-2,999 | 6 | 10 |
| | 3,000-3,999 | 17 | 28 |
| | <4,000 | 32 | 53 |
| Household size | 1 to 4 persons | 30 | 50 |
| | 5 to 8 persons | 27 | 45 |
| | 9 to 12 persons | 3 | 5 |

The age group and household size variables show some distinctive social characteristics of customers at the local market in Berekum. About 53percent of them were aged between 21 and 30. This means that mostly young female adults came shopping. About 50 percentof them had one to four persons at their homes, likely showing some recent trend of expanding nuclear families. Consumers with large household size, which consisted of another 50 percentare normally price sensitive to feed everyone. In our interviews, we observed that these people placed relatively high importance on quantity or convenience.

The annual income of respondents shows that about 53percent had income above 4,000 Ghana cedis ($US 1 equals about 4.4 Ghana cedis). According to the World Bank, Ghana's GDP per capita in 2016 was US$1,513.5 (World Bank, 2017). This means that more than half of those who came to the Berekum weekly market were relatively well-off. This condition is attributed partly to the fact that most respondents work for government institutions and trade. The average annual income of these workers is above the average GDP per capita or about 4,300 Ghana cedis (Field survey, 2016). Farmers and service providers earned between 3,000 and

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3,999 Ghana cedis in 2015 (Field survey, 2016). The low-income categories largely represent students who likely came to the market alone.

With respect to education, all respondents had some form of schooling. About 38 percentof consumers had some schooling until the 18th year and 38percenthad schooling beyond their 18th year. This is not surprising since the Municipality has the high literacy rate. Moreover, the female population had more schooling than the male one. In addition, it reflects the general interest of food related topics.

**2.2 Food values**

In the second part of the survey, we attempted to identify how the respondents place importance on different food value attributes in deciding to buy products at this market. Table 2 shows the result. In answering the questionnaire for this part, respondents had multiple choices. The results reveal that about 15.5 percent of the respondents placed weight as an important food scale. In other words, weight is the most important factor for the respondents to decide in purchasing food items at the market.

*Table.2: Food value attributes that influence consumers' choices*

| Variables | Frequency | Percentage (%) |
|---|---|---|
| Weight | 65 | 15.5 |
| Health benefit | 61 | 14.5 |
| Safety | 54 | 12.9 |
| Affordability | 52 | 12.4 |
| Bargain | 40 | 9.5 |
| Shelf life | 31 | 7.4 |
| Appearance | 29 | 6.9 |
| Taste | 27 | 6.4 |
| Bulkiness | 23 | 5.5 |
| Naturalness (organic) | 18 | 4.3 |
| Price (cheap) | 6 | 1.4 |
| Origin | 3 | 0.7 |
| Value for money | 1 | 0.2 |

Following weight, other important food attributes for the respondents were health benefit (14.5 %)safety (12.9 %)),and affordability (12.4 %). Altogether these four attributes consist about 55 percent. Variables that are relevant to measures (including sizes) are "appearance," "weight," and "bulkiness." These amounted to about 28percent. Price-related variables such as "value for money," "price (cheap)," "affordability," and "bargain" amounted to about 23.5percent. Quality-related variables, such as "health benefit," "taste," "origin," and "naturalness," amounted to 25.9percent.

This result shows a stark contrast to the argument Lusk (2011) made. In the 2011 study, he found that food safety was the most important food value attribute. On the contrary, our survey found that about 13percent of the respondents found it important. This discrepancy may mean that consumers' food value choices may differ by country, region, or society. Further studies may clarify social and regional impacts on price perceptions.

Another salient aspect of consumers' price perceptions is the interconnection between price and quality. Quality in general appears to influence the market. Olson (1977) emphasizes the inter-relationship between price and perceived quality although other studies have shown mixed results. He further argues that the price-quality relationship becomes less important when other indicators are factored. This may be the case in our survey as quality related attributes amounted to only 25.9 percent.

*Table.3: Consumers' notions about the use of weights and measures*

| Statements | Yes (%) | No (%) |
|---|---|---|
| Traditional buckets better to know the value of vegetables | 92 | 8 |
| Helpful to use the same weight/measure | 80 | 20 |

Another aspect of this survey asked the respondents about their notions on weights and measures. We asked if traditional buckets that are commonly used at the market help better understand the value of vegetables. We also asked them if it would help that marketers use the same weight and measure in selling products. The results in Table 3 show that about 92 percent of consumers agreed with the use of traditional weights. This suggests that though traditional weights vary sizes and do not give standardized measurements, consumers recognize the importance of using a standard that has been practiced traditional ways for identifying food values. Similarly, about 80 percent of the respondents agreed with using the same weights or measures. These results suggest that consumers at the Berekum market largely prefer the use of standardized measure, either traditional or conventional forms, in purchasing vegetables.

## IV. CONCLUSION

This survey attempted to better understand the extent to which weights and measures are key factors in determining consumers' purchasing behaviors. The results show that consumers' product purchasing at Ghana's local market was based on four important food value scales: (1) weights and measures, (2) health values,

(3) safety, and (4) affordability. The least important food values were value for money, price, origin and natural. However, consumers' perceptions on product values appeared to differ by socio-economic backgrounds and the cultural value system. The study also found that food values are significantly influenced by consumers' traditional perceptions on product values. In addition, consumers' food value choice can differ by regions. The survey indicates the significance of weights and measures related to price decisions of consumers in Berekum, Ghana, and about 80 percent of consumers agree to use the same weights and measurements in pricing of agricultural products. These suggest that consumers at the local market prefer the use of a standardized measure either conventional or traditional.

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# Effects of Organic Turmeric on Liver Integrity and Oxidative Stress of the Brain in Rabbits Exposed to Ultraviolet Radiation

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***Abstract—*** *This project investigated the effects of organic turmeric on the liver and oxidative stress of the brain in rabbit acutely exposed to ultraviolet radiation. Thirty five weaned rabbits between 8-10 weeks of age, randomly allocated to control (A) and five (5) treatments: B, C, D, E and F were used for this experiment. Treatment A: fed organic feed without turmeric inclusion and not radiated, Treatment B: fed diet supplemented with 2% turmeric as its constituents but not radiated, Treatment C: fed organic feed without turmeric inclusion before but after radiation, Treatment D: fed organic feed without turmeric inclusion before and after radiation. Treatment E: fed diet supplemented with 2% turmeric before but not after radiation. Treatment F: fed diet supplemented with 2% turmeric before and after radiation. There were significant ($p<0.05$) differences in superoxide dismutase, catalase and melondialdehyde. Histological studies reveals that the treatments radiated showed structural differences from the control. Liver section in Treatment D showed portal triad infiltrated by lymphocytes with less vacuolar degeneration. The liver damage was mild in Treatments C and E. The histology of rabbits in treatment A and F revealed normal liver hepatocytes and portal triad. It was concluded that ultraviolet (UV) radiation resulted in oxidative stress in the brain and feeding of 2% organic turmeric supplemented diet before and after exposure to radiation seen to have be effective against oxidative damages caused by ultraviolet radiation. Also, UV radiation has detrimental effect on the liver and organic turmeric had hepatoprotective and antioxidant properties.*

***Keywords—Brain, Liver, Oxidative Stress, Organic Turmeric, Rabbits, Ultraviolet Radiation***

## I. INTRODUCTION

Rabbit (*Oryctolagus cunniculus*) is one of the animals that can be reared successfully at family level (FAO, 1996). It is characterized by short gestation interval and very good source of protein with balanced amino acid profile. Its meat is appreciated for its properties in particular through its high protein/energy ratio. It has high essential fatty acid content and low cholesterol (Xiccato, 1999). The daily weight gain is high in proportion to the body weight which gives them a rapid growth rate, and sexual maturity is early (Ajayi *et al.*, 2005).

The stratosphere ozone layer forms a thin shield in the upper atmosphere, protecting life on the earth from the sun's ultraviolet (UV) radiation. In the 1980s, scientist found evidence that the ozone layer was depleted (EPA, 2010). Depletion of ozone layer results in increased UV radiation reaching the earth's surface. UV is invisible and does not produce immediate reaction. UV radiation leads to the damage of cellular constituents, resulting in a complex cell response that includes induction of genes and perturbation of a variety of signaling pathways (Bender *et al*., 1997).

Oxygen is a highly reactive atom that is capable of becoming part of potential damaging molecules commonly called "free radicals". Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to ageing and to degenerative diseases of

ageing such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction (Sies *et al.*, 1999). Certain organs system in humans is susceptible to greater level of oxidative stress. The brain, which accounts for only 2% of the body weight, consumes 20% of the total oxygen inspired. The brain processes much $O_2$per unit tissue mass. The nervous system is rich in both unsaturated fatty acid and iron, the high lipid content of nervous tissue, coupled with its high aerobic metabolic activity makes it susceptible to oxidative alteration (Bauer and Bauer, 1999).

Liver is a vital organ present in vertebrates and some other animals. It has a wide range of functions, including detoxification, protein synthesis and production of biochemicals, necessary for digestion. Excessive iron deposition in the liver can lead to further injury such as hepatocellular necrosis, inflammation, fibrosis and in some cases even to carcinoma (Zhao *et al*., 2005). In recent years, several hundred plants have been examined for their use in a wide variety of liver disorders. These plants include *Silybum marianum* (milk thistle), *Picrorhiza kurroa* (kutkin), *Camellia sinensis* (green tea) and *Glycyrrhiz aglabra and Curcuma longa* (Turmeric) (Luper, 1999). Curcumin is the principle active ingredient in turmeric, a spice derived from the rhizome of *Curcuma longa*. Turmeric is used in both Chinese and Indian traditional medicines (Aggarwal *et al*., 2006), with applications as an anti-inflammatory agent, for peptic ulcer and dyspepsia, in skin diseases and wound healing and in liver and urinary tract diseases (Luper *et al*., 1999). Even very high level of curcumin and related curcuminoids had low toxicity at oral doses up to 12 g per day in humans (Lao *et al*., 2006). Curcumin is also effective in preventing chemically-induced liver damage and prevented carcinogenic effects of the hepatocarcinogens afflatoxin or nitrosodiethylamine (Sharma *et al*., 2005). It reduced liver fibrosis in a rat model of nonalcoholic steatohepatitis and in rats given thioacetamide (Leclercq *et al*., 2004).

## II. MATERIALS AND METHODS

### 2.1 Experimental Site

This experiment was carried out at the rabbitry unit of the Teaching and Research farm, Ladoke Akintola University of Technology (LAUTECH) Ogbomoso. Ogbomoso is located in the derived savannah zone of Nigeria. It lies on longitude $4^0$ $15^1$ East greenwish meridian and latitude $8^0$ $15^1$ North at the equator. The altitude is between 300 and 600m above sea level while the mean temperature and annual rainfall are $27^0$ C and 1247mm respectively

### 2.2 Experimental Materials

Three wooden cages with 24 hutches in each cage were used for this experiment. The dimension of the hutches was 51cm length by 62cm breath, large enough to contain 5 rabbits of age 8-10 weeks at a time. However, two rabbits were placed in each hutch. Thirty five (35) weaned rabbits of average weight 1200-1300g purchased from reliable source were used for this experiment. The does and the bucks were separated, so as to prevent mating.

200kg of Turmeric rhizome was purchased from Euro bridge farm Odogbolu, Ogun State, and was separated from all forms of attached soil before being boiled and sliced to increase the surface area. The sliced samples were air dried to reduce moisture content. The dried turmeric was ground into powder with an electric grinder and sieved so as to have uniform size of powder.

Organic turmeric → boiling→ slicing → drying → grinding

Exposure to UV radiation was done by putting them in a wooden artificial radiation box. 3 florescence UV bulbs were used and exposure was done for ten minutes for 10consecutive days with 5 rabbits at a time.

### 2.3 Feed Formulation and Feeding

Two diets were formulated, turmeric was not included as one of the constituents of the diet 1 but was included in the diet 2. Turmeric was added at 2% inclusion in the feed. Garlic, *Asparagus racemosus* and *Moringa oleifera* were included as premix to take care of ethnoveterinary requirement. The rabbits were fed ad-libitum. The gross composition of the experiment diets is presented in TABLE 1.

### 2.4 Treatments

Thirty five rabbits were allocated into a control and five treatment groups as follow: Control A: Eight rabbits, fed organic feed without turmeric inclusion and not radiated. Treatment B: Seven rabbits, fed diet supplemented with 2% turmeric as its constituents but not radiated. Treatment C: Five rabbits, fed organic feed without turmeric inclusion before but turmeric after radiation. Treatment D: Five rabbits, fed organic feed without turmeric inclusion before and after radiation. Treatment E: Five rabbits, fed diet supplemented with 2% turmeric before but not after radiation. Treatment F: Five rabbits, fed diet supplemented with 2% turmeric before and after radiation.

### 2.5 Data Collection

At the end of the third week of feeding after radiation, the rabbits were sacrificed through cervical dislocation. The brains were removed and apportion of each brain was homogenized with 3-sucrose buffer solution of 0.25mls with pH of 7.4 in a mortar and a pestle. The homogenates were stored in ice-block and later processed for oxidative stress determination with three different oxidative stress markers.

### 2.6 Assay Methods

#### 2.6.1 Determination of Lipid Peroxidation (MDA)

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Principles: Assessment of lipid peroxidation was carried out based on the principle of Varshney and Kale (1990). Estimation of lipid peroxidation was based on the reaction of melondialdehyde with thiobarbituric acid (TBA) forming a MDA-TEAR adduct that absorb light strongly at 532mm.

Procedure: 0.4ml of reaction mixture i.e. sample already quenched with 0.5ml of 30%. TCA was added to 1.6ml of Trihydroxymethyl methylamine potassium chloride at PH 7.4. 0.5ml of 8% TBA was added and incubated for 45minutes at 300C to produce a pink coloured reaction mixture which was centrifuge at 1400rpm for 15minutes. The absorb area of the clear supernatant was then read at 532nm.

$$\text{MDA (units)} = \frac{\text{Absorbance X Volume of Mixture}}{\text{E532 X Volume of Sample}}$$

**2.6.2 Determination of Superoxide Dismutase (SOD) Activity**

SOD activity was determined by the method described by Misra and Fridorich (1972)

Principle: the ability of superoxide dismutase to inhibit the auto oxidation of epinephrine at PH 10.2 to adrenochrome makes this reaction a basis for a simple assay of dismutase.

Procedure: 1ml of sample was diluted in 9ml of distilled water to make a 1 in 10 dilutions. An aliquot of the diluted sample was added to 25ml of 0.05M carbonate buffer pH 10.2 to equilibrate in the spectrophotometer and the reaction was initiated by adding 0.3ml of adrenaline. The change in absorbance was monitored at 430nm for 5 minutes.

**2.6.3 Determination of Catalase (CAT) Activity**

Catalase activity was determined according to the method described by Aebi (1983).

**2.8 Statistical Analysis**

All data were subjected to Analysis of Variance (ANOVA) using SAS (2008). Duncan Multiple Range Test of the same

Principle: Catalase catalyses the decomposition of hydrogen peroxide ($H_2O_2$) to water and oxygen. Hydrogen peroxide is formed in the eukaryotic cells as a by-product of various oxidase and seperoxide reactions. Hydrogen peroxide is highly deleterious to the cells and its accumulation causes oxidation of cellular targets such as DNA, protein and lipid leading to mutagenesis and cell death. Removal of the $H_2O_2$ from the cell by catalase provides protection against oxidative damage to the cell.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + 2O_2$$

Procedure: 0.1ml of the sample was pipette into curette containing 1.9ml of 50MM phosphate buffer PH 7.0. The reaction was initiated by addition of 0.1ml of freshly prepared 30% (w/v) hydrogen peroxide. The rate of decomposition of hydrogen peroxide was measured spectrophotometer at 240nm

**2.7 Histology**

The livers of 35 matured rabbits (25 males and 10 females) were used for this study after which they were sacrificed via cervical dislocation at the Animal Production and Health Laboratory (LAUTECH). The eviscerated liver of the rabbits was weighed and transferred into containers containing formal saline fixative. The livers were dehydrated through varying concentration of alcohol and cleared with xylene. The tissues were subsequently impregnated and embedded in paraffin wax. They were then cut at 3-5 microns, dewaxed and stained with haematoxylin and eosin. The slides were reviewed and photomicrographs taken with a digital camera attached to a light microscope.

statistical package was used to separate the mean among treatment.

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*Table.1: Feed Formulation Table*

| Ingredients(kg) | Diet 1(No Turmeric) % | Diet 2 (Turmeric) % |
|---|---|---|
| Maize | 18 | 18 |
| Maize bran | 10.8 | 8.8 |
| Wheat offal | 12 | 12 |
| P.K.C | 45 | 45 |
| Fish meal 72% | 2 | 2 |
| Bone meal | 2 | 2 |
| Oyster shell | 1.5 | 1.5 |
| Salt | 0.25 | 0.25 |
| *Moringa oleifera* | 0.2 | 0.2 |
| Ginger | 0.09 | 0.09 |
| Garlic | 0.08 | 0.08 |
| *Asparagus racemosus* | 0.08 | 0.08 |
| Tumeric | —— | 2 |
| Total | 100 | 100 |
| **Calculated Proximate Composition of Diet** | | |
| Energy | | 2360.07 ME cal/kg |
| Crude protein | | 18.17% |
| Ether extract | | 4.75% |
| Lysine | | 0.68% |
| Methionine | | 0.33% |
| Crude fibre | | 8.46% |
| Calcium | | 1.44% |
| Phosphorus | | 0.49% |

*Table.2: Level of Oxidative Stress in the Brain of Male Rabbits by Different Markers*

| Treatment | SOD (nmol/L) | CAT (nmol/L) | MDA (nmol/L) |
|---|---|---|---|
| A | $22.36\pm1.08^{a}$ | $14.21\pm3.18^{a}$ | $41.02\pm2.72^{a}$ |
| B | $16.52\pm0.69^{c}$ | $14.95\pm1.49^{b}$ | $32.14\pm6.16^{b}$ |
| C | $16.98\pm1.16^{c}$ | $14.40\pm1.61^{a}$ | $16.44\pm1.05^{d}$ |
| D | $15.50\pm1.25^{b}$ | $11.50\pm1.32^{a}$ | $26.15\pm1.50^{a}$ |
| E | $16.90\pm1.66^{c}$ | $13.21\pm1.39^{a}$ | $30.30\pm2.32^{b}$ |
| F | $22.32\pm0.72^{c}$ | $15.30\pm1.10^{b}$ | $42.50\pm2.42^{c}$ |

$^{a,\ b,\ c}$ *=means on the same column but with different superscripts are statistically (p<0.05) significant. SOD = Superoxide Dismutase; CAT = Catalase; MDA = Malondialdehyde*; A = fed organic feed without turmeric inclusion and not radiated; B = fed diet supplemented with 2% turmeric as its constituents but not radiated; C =fed organic feed without turmeric inclusion before but turmeric after radiation; D =fed organic feed without turmeric inclusion before and after radiation; E = fed diet supplemented with 2% turmeric before but not after radiation; F = fed diet supplemented with 2% turmeric before and after radiation

## III. RESULTS

### 3.1 Oxidative Stress of the Brain

Level of oxidative stress in the rain is showed in TABLE 2. There were significant ($p<0.05$) differences in superoxide dismutase (SOD) in which treatment A (those fed organic feed without turmeric and not radiated) had the highest (22.36±1.08nmol/L) value while treatment D (those fed organic feed without turmeric before and after radiation) had the least (15.50±1.25nmol/L) value of SOD. The significant differences observed in catalase indicated that rabbit fed organic feed with turmeric before and after radiation (Treatment F) appears to have the highest (15.30±1.10nmol/L) values while the least (11.50±1.32nmol/L) was recorded for Treatment D (fed organic feed without turmeric before and after radiation). There were significant ($p<0.05$) observed in melondialdehyde (MDA), highest(42.50±2.42nmol/L) and the least (16.44±1.05nmol/L) values of MDA were recorded in treatment F (those fed organic feed with turmeric before and after radiation) and C (those fed diet supplemented with 2% turmeric after radiation) respectively.

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**3.2 Histology Study**

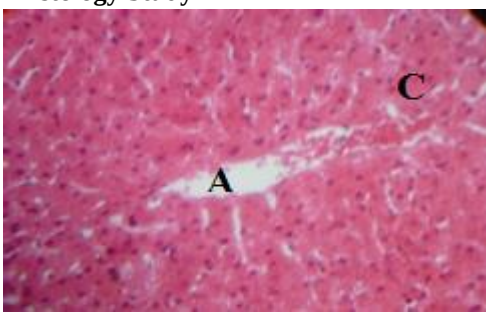


*Fig.1: Photomicrograph of Liver from rabbit fed organic feed without turmeric inclusion and not radiated (Control A) showing normal liver architecture; normal hepatocyte (C) and portal vein (A) (Mag x 400).*

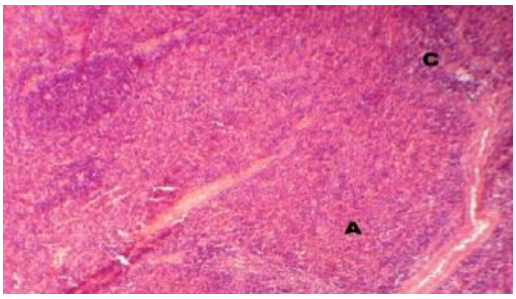


*Fig.2: Photomicrograph of Liver from rabbits fed organic feed with inclusion of turmeric throughout and they are not radiated (Treatment B). It shows mild vacuolar degeneration (A) with focal necrosis of hepatocyte especially around central veins (C) (Mag x 100), which may due to environmental factors.*

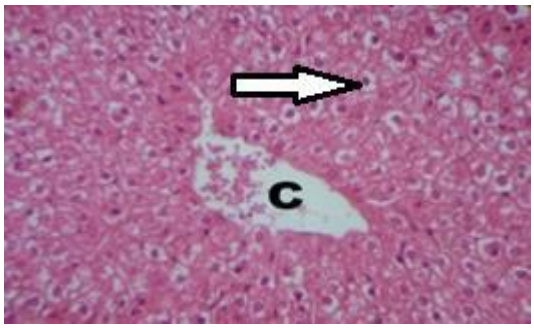


*Fig.3: Photomicrograph of Liver from rabbits fed organic feed without turmeric inclusion before but after radiation (Treatment C). It shows normal hepatic cells each with well-defined cytoplasm, prominent nucleus, nucleolus (Arrow) and well brought out central vein (C) (Mag x 400).*

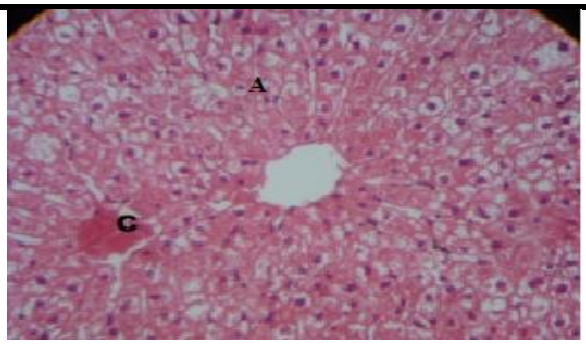


*Fig.4: Photomicrograph of Liver from rabbits fed organic feed without inclusion of turmeric before and after radiation (Treatment D) showing portal triad infiltrated by lymphocytes (C) with less vacuolar degeneration (A) (Mag x 400).*

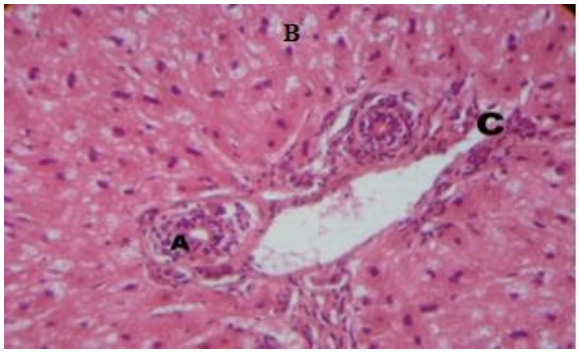


*Fig.5: Photomicrograph of Liver from rabbits fed turmeric supplemented diet before radiation but no turmeric after radiation (Treatment E) showing diffuse mild vacuolar degeneration (B), mild inflammation of portal triad (A) and normal venule (C) (Mag x 400).*

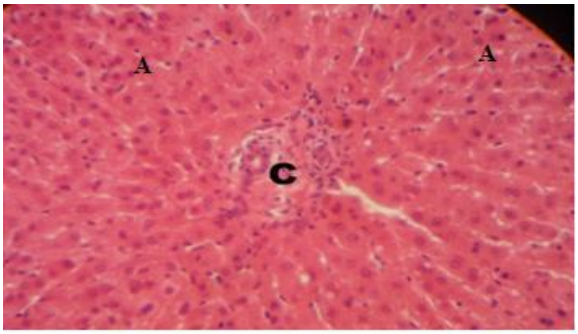


*Fig.6: Photomicrograph of Liver from rabbits fed organic turmeric supplemented diet before and after radiation (Treatment F) showing normal hepatocytes (black arrow) and portal triad (C) (Mag x 400).This confirm the hepatoprotective role of curcumin.*

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## IV. DISCUSSION

Free radical have been implicated in many disease processes including cancer, while superoxide dismutase (SODs) are the main enzymes responsible for the elimination of superoxide radicals and are considered to be key antioxidants in aerobic cells (Hileman *et al.*, 2001). The observed value for the treatment D (fed organic feed without turmeric before and after radiation) indicated that SOD and catalase revealed oxidative stress in the brain of pubertal rabbits exposed to ultraviolet radiation when compared with treatment A. This implied that ultraviolet radiation exposed to, reduced the activities of antioxidant enzymes. Nagai *et al.* (2008) reported that superoxide dismutase catalyses the dismutation of superoxide into oxygen and hydrogen peroxide. Similar report was given by Chelikani *et al.* (2004) who reported catalase catalyses the decomposition of hydrogen peroxide to water and oxygen. Therefore, reduction in the number of these antioxidants in the brain indicates damaging effect of ultraviolet radiation. The significant differences observed in treatment F (rabbits fed organic feed with organic turmeric before and after radiation) for SOD and catalase indicated that turmeric fed before and after radiation ameliorates the effect of ultraviolet radiation. Similar reports were given by Chattopadhyay *et al.* (2004) who indicated that curcuminoids can reduce free radical compounds such as hydroxyl radicals and superoxide radicals in biological system. Melondialdehyde, one of the several molecular weight end products formed via decomposition of certain primary and secondary lipid peroxidation products (Janero and Burghardt, 1988). Melondialdehyde (MDA) revealed oxidative stress in the brain of pubertal rabbits exposed to radiation and fed turmeric supplemented diet after (treatment C). This indicated that turmeric fed after radiation could not effectively prevent damages caused by ultraviolet radiation. High level of MDA observed in treatment F indicated that turmeric had prophylactic and therapeutic effects against ultraviolet radiation.

Histology revealed that the treatments that were radiated have structural differences compared to the control. Liver section of rabbit, control (treatment A) as seen in Fig. 1 exhibited normal hepatic cells, each with well-defined cytoplasm, prominent nucleus and nucleolus and well brought out central vein. These features gave an indication of normal hepatic architectural integrity. In treatment B, rabbits were fed organic turmeric supplemented diet but were not radiated. Liver section shows mild vacuolar degeneration with focal necrosis of hepatocyte especially around central veins as displayed in Fig. 2. Some rabbits in this group may susceptible to disease due to environmental factors.

Histology of the Rabbits that fed organic feed without turmeric inclusion before but turmeric after radiation as pictured in Fig. 3 shows normal hepatocytes around portal triad with well-defined cytoplasm, prominent nucleus and nucleolus. This confirmed the hepatoprotective role of turmeric. Similar reports were given by Singh *et al.* (2011). Liver histology of rabbits, fed organic feed without inclusion of turmeric before and after radiation (Fig. 4) shows portal triad infiltrated by lymphocytes with less vacuolar degeneration. The damage caused by ultraviolet radiation is more severed because T-Lymphocyte is the main mononuclear cell infiltrating portal triad as in primary biliary cirrhosis (Whiteside *et al.*, 1984). The histology of the liver of the rabbit in this treatment confirms that ultraviolet radiation may be responsible for the liver damage (Zhao *et al*., 2005).Histology of rabbits fed organic feed with inclusion of 2% turmeric before radiation but no turmeric after radiation i.e. treatment E (Fig. 5) and those fed turmeric supplemented diet before and after radiation i.e. treatment F (Fig. 6) shows diffuse mild vacuolar degeneration and normal hepatocytes and portal triad respectively. The effect of the ailment on the liver is mild and not significant in treatment E. This means that the turmeric that was fed to the rabbits before radiation in treatment E ameliorate the damages caused by radiation on the liver. When compared the histology of treatment D (Fig. 4) to that of treatment C (Fig. 3); it was observed that the damages caused by radiation on the liver in rabbits of treatment D (Fig. 4) was more pronounced compare to that of treatment C (Fig. 3). Treatment F (Fig. 6) shows normal hepatocytes and normal portal triad which is similar to control while treatment E shows diffuse mild vacuolar degeneration. This finding correlates with the work of Kuttan (1985) who reported the use of curcumin in wound healing, liver ailments, hepatitis, urinary tract disease and as a cosmetic compound. Also, Feroz and Nahida (2013) reported that roots of *Paeonia officinalis Linn* serve as hepatoprotective. Therefore, the hepatoprotective and antioxidant role of turmeric on the liver was confirmed.

## V. CONCLUSION

It was concluded that Ultraviolet (UV) radiation resulted in oxidative stress in the brain which could be detrimental to the brain function. Feeding of 2% organic turmeric supplemented diet before and after exposure to radiation seen to have be effective against oxidative damages caused by ultraviolet radiation. Ultraviolet radiation caused a damaging effect on the liver through vacuolar degeneration and portal triad infiltration by lymphocytes of radiated rabbits and organic turmeric has hepatoprotective and antioxidant effects.

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# *Ex Vitro* Propagation of Rubber Tree (*Hevea Brasiliensis)* using Stem Cuttings

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***Abstract—*** *Stem cutting propagation preserves the genetic traits and leads to transfer of superior and genetically similar traits of parent plants to progenies. This method is also used to propagate recalcitrant, nonviable and difficult to germinateseeds. Stem cutting in tree species is used to address phenological and intraclonal problems. The use of rubber cuttings as planting material is a feasible option, worthy of investigation. There has been little or no research studies into the usage of Hevea brasiliensis stem cuttings as an alternative vegetative propagation method for an in vivo propagation of rubber tree in Ghana. Propagation of H. brasiliensis by stem cutting techniques was used to study alternative procedures for mass production of rubber planting materials. Brown and green rubber stem cuttings of Clone I and Clone II were soaked for 6 hours in 0.0-22.5g/L Naphthalene Acetic Acid (NAA) followed by propagation in a nursery bag filled with nutrient-rich soil. Only the brown stem cuttings of H. brasiliensis survived. The percent survival, length of shoots, number of roots as well as length of roots of Clone II was significantly (P<0.05) higher than Clone I. Rubber stem cuttings treated with 15.0g/L NAA significantly (P<0.05) developed higher shoots (83.33%),number of roots (6.167), length of shoots (15.38cm) and length of roots (6.00cm) than the remaining treatments. There was significant (P<0.05) effects of NAA and Clone II in sprouting and rooting growth of the brown stem cuttings. Successful in vivo propagation of rubber tree(H. brasiliensis)was achieved.*

***Keywords—Brown and Green, Hevea brasiliensis Clones, Naphthalene Acetic Acid, Sprouting and Rooting Growth, Stem Cuttings.***

## I. INTRODUCTION

Rubber seeds are not only very recalcitrant but also of low quality, low vigour potency and low germination frequency. Low quality seed and poor germination rates affect the availability of planting stocks (Palanisamy and Subramanian, 2000). The time taken to raise seedlings of *H. brasiliensis* in nursery before transplantation can be shortened by stem cut nursery techniques. In addition, cost and effort of raising planting materials can be minimised by putting tree parts into mass propagation during the seed-off year or even during peak seasons of rubber trees by utilizing the stems being cut after budding successes (Corpuz, 2013).

*Hevea brasiliensis*can be propagated by various methods including *in vitro* techniques, seedlings from nursery, budding and grafting all of which have their own merits and demerits in production of the plant species. Vegetative propagation facilitates rapid and large scale production of planting materials not only in rubber but also other tree species and can play a key role in tree improvement programmes for multiplication of superior clones or tested plus trees (Palanisamy and Subramanian, 2000).

Intra-clonal heterogeneity emanates from different genetic variations imposed by both the rootstocks and scion-stock of the budded/grafted plantlets. *Hevea brasiliensis* is currently propagated by grafting and budding techniques. Budding and grafting of different plants or clones cause intra scion-rootstock variability. Thus, for genetic stability the budding technique should be avoided. In view of this, stem cuttings and micropropagation are recommended for production of planting materials. A stem cutting inherits all the traits of the donor plant and it is a good substitute for budding and

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grafting. A stem cut inherits all the traits of the tree source (prototype) (Corpuz, 2013).

Propagation by rooted cuttings has been tried as an alternative to overcome the stock/scion effect. Satchuthananthavale, (1973) has reported that cuttings grow better than budding. Furthermore, stem cuttings require limited space. The technique is also rapid, inexpensive and does not require special techniques used in grafting or budding. There is no problem of compatibility with rootstocks or of poor graft unions. The most common treatment to enhance rooting in stem cuttings is the use of growth regulators (Fowler, 2010). These substances enhance cell differentiation, starch hydrolysis, sugar and nutrient mobilization to the basal end of the cuttings resulting in root initiation (Das *et al.*, 1997).

There is rapid root formation and development and higher percentage of roots on cuttings treated with auxins (Leakey, 2004). Of all the growth regulators (auxins, cytokinins, gibberellins, abscisic acids and ethylene), auxins have the greatest effect on root formation in cuttings. Auxins are not only involved in rooting but also enhance stem growth, lateral bud inhibition, abscission of leaves and fruits and activation of cambial cells. Besides, the naturally occurring indole-3-acetic acids (IAA), there are more effective synthetic auxins such as indolebutyric acid-IBA, naphthaleneacetic acid-NAA (Fowler, 2010) which is used in rooting of rubber stem cuttings.

Since juvenile rubber shoots are very sensitive to desiccation and high temperature, they require immediate use of their cuttings for propagation. Rubber is a seasonal tree species which does not produce fruits regularly, thus production of planting materials is seriously hindered.There has been little or no research studies into the application of stem cuttings of *Hevea brasiliensis* as an alternative vegetative propagation method for an*in vivo* propagation of rubber tree in Ghana.The ability to successfully regenerate planting materials from *Hevea* through *in vivo* locally would go a long way in rubber improvement and not only of rubber trees but also stimulate interest in attempts at *in vivo* propagation of some tropical woody species in the country.

The use of stem cuttings will resolve the problem associated with the shortage of rubber planting materials usually in the off-season and lead to the large-scale production of rubber propagules affordable to rubber outgrowers.Moreover, production of rubber planting materials via stem cuttings with their own root system could counteract the major setback associated with budding/grafting which is intra-clonal variation due to stock-scion interaction.

This study is aimed at using rubber stem cuttings influenced by clonal types, stem types and growth regulator as an alternative to budding and grafting technique for planting material development. Specific objectives are to:

- determine the survival rate of lignified (brown) and non-lignified (green) stem cuttings of rubber as affected by clones and NAA levels;
- determine the sprouting (shoot development) and rooting growth of *H. brasiliensis* stem cuttings as influenced by the clones and the rooting compound;
- determine the survival rate, length of sprouts and length of roots among *H. brasiliensis* stem cuttings as influenced by the clones and the NAA at varying concentrations.

## II. MATERIALS AND METHODS

### 2.1. Collection of *Hevea brasiliensis*stem cuttings

Rubber cuttings of two *Hevea* clones (*Clone I* and *Clone II*) were used in this study. They were collected from rubber outgrowers in the Western Region of Ghana.

### 2.2. Propagation of *Hevea brasiliensis* by stem cuttings

One hundred and twenty (120) lignified (brown) and non-lignified (green) rubber stem cuttings of each of the two *Hevea* clones (*Clone I* and *Clone II*) were prepared from harvested stems. Each cutting was trimmed to a uniform length of 40cm with each cutting consisting of 4 or 5 nodes. The basal portions of the cuttings were then immersed in 0.0, 7.5, 15.0 or 22.5g/L NAA for 6 hours and thereafter planted in nursery bags filled with sandy-loam soil mixed with manure in a ratio of 3:1. The experiment was arranged in a 2x4x5 factorial experiment with three (3) replications. The number of stem cuttings of each clone was determined as (2 clones x 4 treatments x 3 replications x 5 cuttings per treatment =120).

Stem cuttings were considered potentially sprouted when their buds ruptured or became visible. The number of cuttings that sprouted was counted at 20-day intervals after planting for four times (20, 40, 60 and 80 days). The number of cuttings that developed shoots (survival), number of roots and number of shoots were counted. The length of shoots as well as the length of roots measured using a metre rule 80 days after planting.

### 2.3. Data analysis

Data was subjected to analysis of variance (ANOVA) using the Statgraphics® Centurion XVI. Fisher's least significant difference (LSD) procedure was used for the separation of means where appropriate at 5%.

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## III. RESULTS

### 3.1. Effects of clonal types and NAA concentrations on shoot development

None of the green stem cuttings survived irrespective of the clonal types and the concentrations of NAA suggesting the stem types have an influence on sprouting or shoot development. Contrarily, *Hevea brasiliensis* brown stem cuttings (Fig. 1) survived irrespective of the clonal types and NAA levels. A higher significant difference was observed in the survival rate (cuttings that did not die), sprouting and rooting potentials between brown and green stem cuttings of *Hevea brasiliensis* clones. Total number of brown stem cuttings planted=120. Number of brown stem sprouted=87.This implies that percentage of brown stem cuttings sprouted:$\frac{87}{120}$**x100%**

Therefore, percentage of *H. brasiliensis* brown stem cuttings sprouted was**72.5%.**

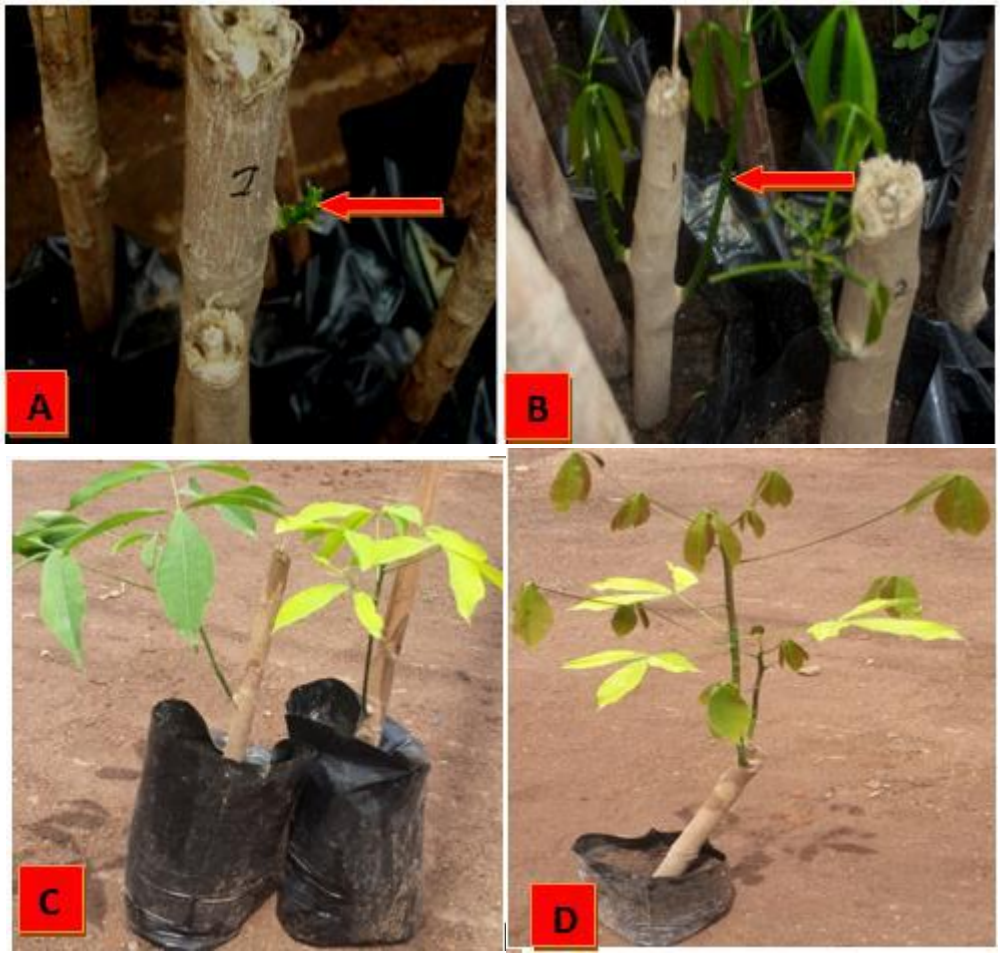


*Fig.1: H. brasiliensis brown stem cuttings used for propagation*

*Fig.2 (A-E): Morphological characteristics, growth and developmental stages of stem cuttings of two H. brasiliensis clones treated with NAA.*

**A-**Stem cutting of *H. brasiliensis* showing shoots 20 days after planting
**B-** Stem cutting of *H. brasiliensis* showing shoots 40 days after planting
**C-** Rubber stems cuttings showing well-developed shoots 60 days after planting
**D-** Rubber stem cuttings showing well-developed shoots 80 days after planting
**E-**Uprooted rubber stem cuttings with root and shoot systems

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**3.2. Effects of types of clone on sprouting growth, survival rate, length of sprouts, number of roots and length of roots of *H. brasiliensis*stem cuttings**

Table 1 depicts effect of types of clone on shoot development, survival rate, length of sprouts, length of roots and number of roots of *H. brasiliensis* stem cuttings 80 days after planting (DAP). The clonal types in 20 (Fig. 2A) and 60 (Fig. 2C) days after planting had no significant effects on the sprouting growth of the *Hevea brasiliensis* stem cuttings between the clones (Table 1). Although, the clonal types on the 40th (Fig. 2B) and 80th (Fig. 2D) days after planting had no significant effect on the sprouting growth of the stem cuttings, *Clone II* showed higher performance than *Clone I* (Table 1).

The clonal types, however, showed a significant difference ($P<0.05$) in the survivability of the brown stem cuttings (Table 1). *Clone II* showed a higher mean number of survived stem cuttings representing more than half of the number of brown stem cuttings that survived (Table 1). The survivability of cuttings is very important in the establishment of planting materials since the growth and rooting depend on the ability of the cuttings to survive.

After 80 (Fig. 2D) days of propagation, brown stem cuttings of *Clone II* of *Hevea brasiliensis* had a higher survival percentage of 98.333% as compared to 55.000% of *Clone I*. Thus, there was a significant difference ($P<0.05$) between *Clone I* and *Clone II* (Table 1).

*Table.1: Effect of types of clone on the sprouting growth/shoot development, survival rate, length of sprouts, number of roots and length of roots of brown H. brasiliensisstem cuttings 80 days after planting (DAP)*

| TYPE OF CLONES | NUMBER OF CUTTINGS SPROUTED DAYS AFTER PLANTING | | | | PERCENT SURVIVAL (%) | SHOOT LENGTH/CM | ROOT NUMBER | ROOT LENGTH/CM |
|---|---|---|---|---|---|---|---|---|
| | 20-DAY | 40-DAY | 60-DAY | 80-DAY | | | | |
| ***CLONE I*** | 0.500±0.905[a] | 0.500±0.905[a] | 0.667±0.778[a] | 1.333±0.985[a] | 55.000±33.166[a] | 6.167±3.228[a] | 2.917±0.996[a] | 4.000±1.414[a] |
| ***CLONE II*** | 0.500±0.522[a] | 0.500±0.522[a] | **1.250±1.055[a]** | **2.000±0.853[a]** | **98.333±5.774[b]** | **11.950±7.113[b]** | **5.000±2.663[b]** | **5.667±2.146[b]** |

Means with the same letter superscript are not significantly different ($P\geq0.05$)

Regardless of the concentration of NAA, almost all the survived brown stem cuttings showed significant ($P<0.05$) length of shoots/sprouts between the two clones of *Hevea brasiliensis*. With the length of shoots measured after 80 (Fig. 2D) days of propagation, *Clone II* of the brown stem cuttings performed significantly higher in mean length of 11.950cm than that of *Clone I* in mean length of 6.167cm (Table 1). Therefore, this signifies that *Clone II* brown stem cuttings grow and develop faster in shoot length up to 80 days of planting (Table 1).

Clonal types had a significant difference ($P<0.05$) in both the number of roots and the length of roots of the brown stem cuttings. The number of roots developed varied significantly as influenced by the clones of the brown stem cuttings. The *Clone II* brown stem cuttings statistically were higher in the mean number of roots (5.000) compared with (2.917) in *Clone I* brown stem cuttings (Table 1).

In the case of the length of roots, *Clone II* of the brown stem cuttings once again was statistically longer in mean length of 5.667cm compared to the 4.000cm of the *Clone I* brown stem cuttings (Table 1).

**3.3. Effects of NAA concentrations on sprouting growth, survival rate, length of shoots, length of roots and number of root of *H. brasiliensis*stem cuttings**

Table 2 shows the effects of concentration of NAA on shoot development, survival rate, length of sprouts, length of roots and number of roots of *H. brasiliensis* stem cuttings 80 days after planting (DAP). The concentration of NAA did not have significant effects on the sprouting of the brown stem cuttings 20th, 40th, 60th and 80th days after planting (Table 2). Similarly, no significant difference ($P\geq0.05$) was observed in all the days after planting among the NAA treatments in the sprouting growth of the brown stem cuttings of *H. brasiliensis* (Table 2).

Though, 15.0g/L NAA was higher (1.000) in sprouting of the brown stem cuttings in both the 20th (Fig. 2A) and 40th (Fig. 2B) days after planting, statistically, it showed no significant difference ($P\geq0.05$) with the other NAA concentrations. At 60 (Fig. 2C) days, both 7.5g/L and 22.5g/L NAA had similar number of cuttings sprouted (1.333) which were not

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significantly different (P≥0.05) from the remaining treatments (Table 2).

Although at 80 days, no significant difference existed among the treatments, the trend increased with the control treatments and the 7.5g/L NAA showing a higher and equal number of sprouted cuttings of 1.833. At 80 days after planting, almost all the living cuttings sprouted (Fig. 2D) and at this propagation time, a large number of stem cuttings sprouted (Table 2).

*Table.2: Effect of NAA levels applied on the sprouting growth /shoot development, survival rate, length of sprouts, number of roots and length of roots of H. brasiliensisstem cuttings 80 days after planting (DAP)*

| CONC. OF NAA (g/L) | NUMBER OF CUTTINGS SPROUTED DAYS AFTER PLANTING | | | | PERCENT SURVIVAL (%) | SHOOT LENGTH/CM | ROOT NUMBER | ROOT LENGTH/CM |
|---|---|---|---|---|---|---|---|---|
| | 20-DAY | 40-DAY | 60-DAY | 80-DAY | | | | |
| **Control** | 0.333±0.516$^{a}$ | 0.333±0.516$^{a}$ | 0.500±0.548$^{a}$ | **1.833±1.472$^{a}$** | 63.333±42.739$^{a}$ | 3.067±2.013$^{a}$ | 1.833±0.753$^{a}$ | 2.167±0.408$^{a}$ |
| **7.5** | 0.333±0.516$^{a}$ | 0.333±0.516$^{a}$ | **1.333±0.516$^{a}$** | **1.833±0.753$^{a}$** | 76.667±32.042$^{a}$ | 6.833±2.406$^{ab}$ | 3.833±1.169$^{ab}$ | 5.833±1.169$^{b}$ |
| **15.0** | **1.000±1.095$^{a}$** | **1.000±1.095$^{a}$** | 0.667±0.516$^{a}$ | 1.333±0.516$^{a}$ | **83.333±32.042$^{a}$** | **15.383±6.656$^{c}$** | **6.167±2.787$^{c}$** | **6.000±1.673$^{b}$** |
| **22.5** | 0.333±0.516$^{a}$ | 0.333±0.516$^{a}$ | **1.333±1.633$^{a}$** | 1.667±1.033$^{a}$ | **83.333±23.381$^{a}$** | 10.950±4.418$^{bc}$ | 4.000±1.414$^{b}$ | 5.333±1.366$^{b}$ |

Means with the same letter superscript are not significantly different (P≥0.05)

The level of NAA concentrations showed no significant difference (P≥0.05) in the survivability (cuttings that did not die) of the brown stem cuttings. The controls are significantly lower in survival (63.333%) compared with 76.667%, 83.333% and 83.333% of 7.5g/L, 15.0g/L and 22.5g/L NAA respectively (Table 2).

The concentration of NAA had effect on length of shoots/sprouts and this effect was highly significant (P<0.05) (Table 2) suggesting NAA had a significant influence on shoot development. The length of shoots ranged from 15.383cm to 3.067cm with 15.0g/L NAA having the highest significant mean length of shoots (Table 2).

Similarly, the concentration of NAA had effect on the root growth of brown stem cuttings and the effect was highly significant (P<0.05) (Table 2). The effect of NAA on the length of roots varied significantly (P<0.05) between the controls and the NAA treatments (Table 2). The 15.0g/L NAA significantly had a higher mean number of roots (6.167) and root length (6.000cm) compared to the control treatments with number of roots (1.833) and mean length of roots (2.167cm) (Table 2).

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**3.4. Effects of clonal types and NAA levels on sprouting growth, survival rate, length of sprouts, and number of root and length of roots of *H. brasiliensis* stem cuttings**

Table 3 depicts the effects of type of clones and level of NAA applied on shoot development, survival rate, length of sprouts, length of roots and number of root of *H. brasiliensis* brown stem cuttings 80 days after planting (DAP). The interaction between *Clone I* and NAA levels statistically showed no significance (P≥0.05) in the sprouting of the brown stem cuttings in the number of days after planting (Table 3).

Also, brown stem cuttings showed no significant difference (P≥0.05) in the number of cuttings sprouted in all the days after planting in the interaction between *Clone II* and NAA levels (Table 3). On the 20th (Fig. 2A) and 40th (Fig. 2B) day after planting, though no significant difference existed between 15g/L NAA and the other treatments, it significantly (P<0.05) showed high number of sprouted stem cuttings (1.333).

On the 60th (Fig. 2C) and 80th (Fig. 2D) days after planting, 22.5g/L NAA and the control treatments significantly had a high number of sprouted stem cuttings of 2.000 and 2.667 respectively (Table 3). It was observed that a few number of cuttings sprouted in the first three data collection days. In both clones, a large number of stem cuttings sprouted on the 80th day after planting (Table 3).

*Table.3: Effect of types of clone and level of NAA applied on the sprouting growth/shoot development, survival rate, length of sprouts, number of roots and length of roots of H. brasiliensis stem cuttings 80 days after planting (DAP)*

| TYPE OF CLONES | CONC. OF NAA (g/L) | NUMBER OF CUTTINGS SPROUTED DAYS AFTER PLANTING | | | | PERCENT SURVIVAL (%) | SHOOT LENGTH/CM | ROOT NUMBER | ROOT LENGTH/CM |
|---|---|---|---|---|---|---|---|---|---|
| | | 20-DAY | 40-DAY | 60-DAY | 80-DAY | | | | |
| ***CLONE I*** | **0.0** | 0.333±0.577$^{ab}$ | 0.333±0.577$^{ab}$ | 0.333±0.577$^{ab}$ | 1.000±1.732$^{abc}$ | 33.333±41.633$^{a}$ | 2.633±2.715 | 1.667±0.577$^{a}$ | 2.000±0.000$^{a}$ |
| | **7.5** | 0.000±0.000$^{a}$ | 0.000±0.000$^{a}$ | **1.333±0.577$^{abcd}$** | **1.667±0.577$^{bcd}$** | 53.333±30.551$^{ab}$ | 5.333±2.454 | 3.333±0.577$^{bc}$ | **5.000±1.000$^{bc}$** |
| | **15.0** | **1.333±1.528$^{abcd}$** | **1.333±1.528$^{abcd}$** | 0.333±0.577$^{ab}$ | 1.333±0.577$^{abcd}$ | **66.667±41.633$^{abc}$** | **9.567±1.701** | **3.667±0.577$^{cd}$** | 4.667±0.577$^{b}$ |
| | **22.5** | 0.333±0.577$^{ab}$ | 0.333±0.577$^{ab}$ | 0.667±1.155$^{abc}$ | 1.333±1.155$^{abcd}$ | **66.667±23.094$^{abc}$** | 7.133±1.589 | 3.000±1.000$^{abc}$ | 4.333±1.155$^{b}$ |
| ***CLONE II*** | **0.0** | 0.333±0.577$^{ab}$ | 0.333±0.577$^{ab}$ | 0.667±0.577$^{abc}$ | **2.667±0.577$^{d}$** | 93.333±11.547$^{bc}$ | 3.500±1.479 | 2.000±1.000$^{ab}$ | 2.333±0.577$^{a}$ |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **7.5** | 0.667±0.577$^{abc}$ | 0.667±0.577$^{abc}$ | 1.333±0.577$^{abcd}$ | 2.000±1.000$^{cd}$ | **100.000±0.000$^{c}$** | 8.333±1.305 | 4.333±1.528$^{cd}$ | 6.667±0.577$^{d}$ |
| | **15.0** | 0.667±0.577$^{abc}$ | 0.667±0.577$^{abc}$ | 1.000±0.000$^{abc}$ | 1.333±0.577$^{abcd}$ | **100.000±0.000$^{c}$** | **21.200±2.524** | **8.667±0.577$^{e}$** | **7.333±1.155$^{d}$** |
| | **22.5** | 0.333±0.577$^{ab}$ | 0.333±0.577$^{ab}$ | **2.000±2.000$^{cd}$** | 2.000±1.000$^{cd}$ | **100.000±0.000$^{c}$** | 14.767±1.607 | 5.000±1.000$^{d}$ | 6.333±0.577$^{cd}$ |

Means with the same letter superscript are not significantly different (P≥0.05)

Statistical analysis showed significant interactions between clones and NAA on the survivability of the brown stem cuttings. Although, no significant difference (P≥0.05) existed among the NAA concentrations within *Clone I*, 15.0g/L and 22.5g/L NAA showed an equal and higher survival rate (66.667%) of the brown stem cuttings as compared with the control treatments producing the least rate of 33.333% (Table 3).

Also, no significant difference (P≥0.05) was shown in *Clone II* among NAA concentrations. However, 7.5, 15.0 and 22.5g/L NAA showed equal and higher brown stem cutting survivability (100.000%) with the control treatments having lower survival rate of 93.333% (Table 3).

The interaction between clones and NAA on the shoot length of the *H. brasiliensis* brown stem cuttings was statistically different (P<0.05) (Table 3). In both clones, there was a significant difference (P<0.05) in the concentration of NAA on the length of shoots of *H. brasiliensis* brown stem cuttings with 15.0g/L NAA showing the highest shoot length (9.567cm and 21.200cm) (Table 3). This was followed by 22.5g/L NAA of shoot length whilst the control treatments showing the least shoot length of 2.633cm (Table 3).

For the number of roots (Fig. 2E) produced, significant difference (P<0.05) existed in the interaction between the two clones and NAA treated brown stem cuttings with 15.0g/L NAA providing high number of roots (3.667 and 8.667). The control treatments had lower number of roots (Table 3). The interaction between the two clones and NAA concentrations showed significance (P<0.05) in the root length (Fig. 2E) with both 7.5g/L and 15.0g/L NAA producing high mean length of roots of 5.000cm and 7.333cm. The lowest length of shoots was provided by the control treatments (Table 3).

## IV. DISCUSSION

Vegetative propagation provides the best opportunity to ensure the multiplication of valuable trees for production (Mialoundama *et al*., 2002). A particular importance is the use of stem cuttings which impact significantly on the production of this important economic tree species. The use of stem cutting results in the production of true to type planting materials (clones) with genetic constitution similar to that of the mother plant and thus reliably clone plants with desirable traits (Enslin, 2006). Additionally, the technique leads to early maturity of new plants and the production of large scale planting materials within a short time for field establishment.

### 4.1. Effects of clonal types and NAA concentrations on shoot development

None of the green stem cuttings survived even under the same propagation conditions of the brown stem cuttings. Most of the brown stem cuttings of *Hevea* sprouted regardless of the concentration of NAA and the type of clones. For the surviving brown stem cuttings, there was significant difference (P<0.05) between the two clones in the number of roots, length of roots, length of shoots, survival rate and shoot growth. With the large number of both brown and green stem cuttings propagated more than half of them did not survive. Also, of the two clones studied, *Clone II* had a higher survival rate, number of shoots and roots as well as length of shoots and roots than *Clone I*. This could be attributed to both biotic and abiotic stresses especially an extremely high temperature and a very low relative humidity during the early stages of planting as well as fungal attacks on the cuttings. The reason for the successful sprouting and growth of brown stem cuttings could be due to the hydrolysis

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and availability of carbohydrates stored within the stem tissues of hardwood cuttings (Leakey, 2004).

Cuttings from green stems because they are slightly lignified and succulent are extremely susceptible to attack by soil-borne pathogens (Nestel, 1976). Fungal attack could be a serious threat to the failure and dying of the rubber stem cuttings. Rubber production, as is in the case of other crops is affected by various plant physiological conditions and pathogenic diseases such as South American Leaf Blight (SALB) and *Microcyclus ulei* (Le Guen *et al*., 2003).Differences in soil moisture caused by ions such as Sodium, Calcium and Magnesium, aeration; pH and temperature could affect physical properties, absorption rates, and breakdown of soil as well as plant injury or death (Hartmann and Kester, 1975).

### 4.2. Effects of NAA concentrations on sprouting growth, survival rate, length of shoots, length of roots and number of root of *H. brasiliensis*stem cuttings

The concentrations of NAA had significant influence on length of shoots and roots and the number of roots, except the survival rate and shooting growth of the brown rubber stem cuttings. The treatment of stem cuttings chemically by the application of auxins such as NAA or other growth regulators and fungicides strongly influence the ability of stem cuttings to develop roots. Root initiation and elongation are influenced by genetic, physiological and environmental factors (Leakey, 1985).

It is established that stem cuttings with a single node are better propagules or planting materials for large and successful survival plum. A large sour plum cutting which response to hormone application in small effects could be as a result of high endogenous auxins within the cutting tissues which could have negative interaction with the applied NAA growth regulator (Owuor *et al*., 2009). Alpha naphthalene acetic acid (ANAA) has been found to be reliable in rooting cuttings (Corpuz, 2013) and there are compounds within stem cuttings such as phenolic that interact with auxins to promote rooting and increase root length (Hartmann *et al*., 1997).

Root development differs between tree species and among plants within clones. Thus, variation in rooting may be attributed to lack of endogenous auxins, phenolic or other rooting co-factors in the cutting or lack of enzymes or their activators for synthesis of auxin-phenol complexes (Leakey, 1985).

Also, although some showed signs of growth in the early stage, they could not complete the entire planting duration which could be attributed to lack of root to translocate nutrients and moisture through the stem tissues. Some cuttings sprouted vigorously for a long time but later wilted or died due to lack of root formation and rotting of the basal part of the cuttings. Uncontrolled heat and light which cause an increase in temperature may cause plants to suffer from heat injury. Also, insufficient supply of water and drought are detrimental to all plants. Over-supply of water suffocates the plant roots and can cause diseases such as root rot, damping off and collar rot (Enslin, 2006).

Auxins, NAA when applied at high rates to *Oxalis* plants as a foliar spray was revealed to be phytotoxic (Holt and Chism, 1988). The capacity of the stem cuttings to form roots can be determined by the percentage of cutting rooted, the number of roots per rooted cutting and the speed with which roots emerge and grow (Leakey, 1985).

### 4.3. Effects of types of clone on sprouting growth, survival rate, length of sprouts, number of roots and length of roots of *H. brasiliensis*stem cuttings

Comparatively, *Clone II* significantly developed more shoots and roots than *Clone I*. In addition, the length of sprouts and roots in *Clone II* was significantly longer than *Clone I*. The difference in performance between the two clones could be due to the drastic environmental changes which had adverse effect on *Clone I* than *Clone II*. The type of clone or genotype of stem cutting under propagation has a great influence on the survival and the growth of plant species. Stem cutting, the most frequent propagation method for woody and herbaceous plants is usually faced with challenging factors resulting from mother plant status/source, media, type of cuttings, plant growth regulators and environmental conditions (Hassanein, 2013). Irrespective of the method of propagation, the rooting percentage of plant species is influenced by different genotypes (clones) (Yang, 2009).

## V. CONCLUSION

The sprouting and rooting growth success of the *Hevea brasiliensis* clones, particularly *Clone II*, out-performed and survived significantly better and higher than *Clone I* of the brown stem cuttings. Also, there was higher significant ($P<0.05$) survival rate of the *Hevea brasiliensis* brown stem cuttings than the green stem cuttings of which none sprouted. In the case of the levels of NAA influence on the sprouting and rooting growth on the *Hevea brasiliensis* brown stem cuttings, 15g/L of NAA out-performed better and higher than all the other NAA treatments and the control treatments. The interaction effects between *Clone II* and 15.0g/L NAA levels statistically ($P<0.05$) performed higher and better than the

other interactions. Therefore, an alternative method to ensure rapid and mass propagation of *Hevea brasiliensis* planting materials for plantation is established. In using stem cuttings for the propagation of *Hevea brasiliensis* trees, these findings can be considered to shape the propagation procedures.

Propagation methods such as marcottage which can promote effective rooting on stem cuttings could be tried.The growth medium (soil), fungicide, season and timefor the collection and propagation of *Hevea brasiliensis* stem cuttings must be taken into consideration since they influence survivability and growth.

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# Effect of *IN-OVO* injection with Nano Iron - Particles on Physiological Responses and Performance of Broiler Chickens under Saini Conditions

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***Abstract—*** *A total of 600 fertile eggs, in a completely randomized design were used to investigate the effects of Iron nano-particles IN-OVO injection on productive performance, immune status and physiological responses in broiler chickens. The eggs were divided into 6 groups that assigned as: T1 (control; without injection), T2 (injected with 0.1 ml saline 9.0%; sham control), T3; (injected with 0.1 ml of 20 ppm Fe-NPs organic, T4 (injected with 0.1 ml of 20 ppm Fe-Nano inorganic), T5 (injected with 0.1 ml of 20 ppm Fe organic) and T6 (injected with 0.1 ml of 20 ppm Fe-inorganic). At 7th day of incubation, the corresponding doses were in- ovo injected in 0.1 ml solution into the air sac.*

*The results showed that: Hatchability was highly significant (P< 0.01) in T1, 0.1 ml of 20 ppm Fe-NPs, 0.1 ml of 20-ppm Fe-NPs-Alimet chelate, 0.1 ml of 20 ppm Fe-Aliment chelate and 0.1 ml of 20-ppm Fe-Aliment chelate. The egg weight was higher (P< 0.01) in T2. There was an increase (P< 0.01) in chick weight in controls, other Fe-NPs organic or Fe-NPs-inorganic and Fe organic in comparison with other treatments. In addition, chick body weight to egg weight ratio in controls, Fe-Nano organic and FeNPs- inorganic was higher (P < 0.01) than in the other groups. T3 has shown the highest (P< 0.01) relative weight compared to the other treatments. Serum Fe content and liver function were (P< 0.01) higher in by using Fe-NPs, Fe-NPs alimet inorganic and Fe-organic than other treatments. The treatments of Fe-NPs- organic and Fe-Aliment chelate, chickens' blood hemoglobin increased significantly compared with the other treatments. These results suggest that Fe-NPs, Fe-NPs-Alimet chelate and Fe-Alimet chelate improved embryonic growth and development.*

***Keywords— Broiler chicken, hatchability, in- ovo, iron nano-particles, immunity.***

## I. INTRODUCTION

Minerals play a vital role for maintaining homeostatic conditions in living organisms. Nanotechnology (the use of nano-particles of diameters between 1 and 100 nm) is nowadays applied in science, engineering, and agriculture (Scott and Chen, 2002 and Oberdorster and Donaldson, 2007). Nano-particles activities depend on their physical and chemical characteristics. Nanoparticles can show unique biological behavior, yet, the main mechanism of their action is still unknown (Shimizu, *et al.,* 2009). These particles have features, such as large surface area (increasing physical, chemical, and biological activities) and higher solubility and mobility (Diman*et al.,* 2018 and Toyooka, *et al.,* 2009). High surface to volume ratio allows the functionalizing of nanoparticles with different ligands, coatings and other useful tools for lots of biomedical applications. High surface to volume ratio allows the functionalizing of nanoparticles with different ligands, coatings and other useful tools for lots of biomedical applications. Thus, it allows the functionalizing of nanoparticles with different ligands, coatings and other useful tools for lots of biomedical applications. However, the new physical and chemical properties of novel engineered nanoparticles make them extremely attractive for use in applications like medical sciences (Park, *et al*., 2010). Nano-particles have many novel properties compared with the bulk materials. Thus, inorganic nano-particle elements are widely used to enhance the productive performance of livestock, Ma *et al.,* (2006).

Embryonic development relays upon the availability of the required nutrients within the egg. Nutrient management *in-ovo* may provide an alternative method for poultry industry to increase hatchling weight. Chicks are affected by the nutrients in yolk remaining in the peritoneal cavity post hatching (Romanoff, 1960). Thus, a continually and precisely regulated supply of trace elements derived from stores within the egg is essential to ensure avian embryonic survival.

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However, the high-metabolic rate, fast-growing rate of chicken embryos could be liable tomineral deficiency that lead to metabolic disorders (Tona*et al.*, 2004).

On the other hand, embryonic development relays upon the availability of the required nutrients within the egg. Nutrient management *in-ovo* may provide an alternative method for poultry industry to increase hatchling weight. Chicks are affected by the nutrients in yolk remaining in the peritoneal cavity post hatching (Romanoff, 1960). Thus, a continually and precisely regulated supply of trace elements derived from stores within the egg is essential to ensure avian embryonic survival. The high-metabolic rate, fast-growing rate of chicken embryos could be liable tomineral deficiency that lead to metabolic disorders (Tona*et al.*, 2004).

Iron (Fe) is essential for a variety of physiological processes in livestock (e.g. DNA synthesis, oxygen transport, etc.) as illustrated by Lozoff*et al.*, (2006);Whitnall and Richardson, (2006) and Li and Zhao, (2009). NRC (1994) recommended 50-120 ppm daily intake of iron for poultry. Iron in the form of nano-particles has been reported to be less toxic than inorganic iron salts (Nikonov*et al.*, 2012). Additionally, they have prolonged effects on biological activities (Kovalenko and Folmanis, 2006). Iron nano-particles are more stable in air and have the ability to be degraded or metabolized in vivo, making them excellent candidates for a large number of applications (Bronstein *et al*., 2007).

Iron oxide nanoparticles (IONPs) are frequently used in biomedical applications, yet their toxic potential is still a major concern. While most studies of biosafety focus on cellular responses after exposure to nanomaterials, little is reported to analyze reactions on the surface of nanoparticles as a source of cytotoxicity. Results showed that IONPs had a concentration-dependent cytotoxicity on human glioma U251 cells, and they could enhance $H_2O_2$-induced cell damage dramatically. However, many studies have been conducted to evaluate the potential toxicity of iron oxide nanoparticles, Das, *et al.*, (2007).

The goal of present study was to investigate the effects of *in- ovo* injection of iron, iron nanoparticle and iron chelates nanoparticles methionine during broiler embryonic devolvement on productive performance, physiological and immunological responses and the absorption of iron.

## II. MATERIALS AND METHODS

### Experimental Design and Management

A total of 600 fertile broiler eggs obtained from cobb500™ parent stock were randomly divided into six equal groups. Eggs were individually weighed with an average of 60.83± 0.80g. Eggs were set in the hatchery and injection site was disinfected with ethyl alcohol, sealed with wax after injection then transferred to hatching baskets. The eggs were divided into 6 groups that assigned as: T1 (control; without injection), T2 (injected with 0.1 ml saline 9.0%; sham control), T3; (injected with 0.1 ml of 20 ppm Fe-NPs organic, T4 (injected with 0.1 ml of 20 ppm Fe-Nano inorganic), T5 (injected with 0.1 ml of 20 ppm Fe organic) and T6 (injected with 0.1 ml of 20 ppm Fe-inorganic). At 7$^{th}$ day of incubation, the corresponding doses were *in-ovo* injected in 0.1 ml solution into the air sac. Iron oxide nano-particles were prepared according to Reimers and Khalafalla (2011), suspended in Kno DMEM cell culture medium and dispersed by an ultrasonic bath. The injection was performed at day 7 of incubation into the air sac. Eggs were candled on 7$^{th}$ day of hatchery and 17$^{th}$ day to remove infertile eggs. Aliment according to HMTBA, Novus International, Inc., Charles, MO, USA. Iron Aliment Chelate according to Predieri*et al*. (2005), Fe-Nano Alimet Chelate Based on Marinescu *et al.* (2006).

Post- hatch, a total number of 360 one-day-old chicks were randomly distributed into six equal| (n = 60 / treatment) groups with three replicates (20 chicks/ each) according the corresponding treatments.

Experimental chicks were kept under similar managerial, hygienic and environmental conditions. The chicks were housed in cages from hatch up to 5 weeks of age. Average of indoor ambient temperature (AT, $^{o}$C) and Relative Humidity (RH, %) were recorded using electronic digital thermo-hygrometer. Average of AT and RH was 35.7 ±0.98$^{o}$ C and 24.2 ±1.32 %, respectively. Feed was offered *ad libitum* according to NRC (1994) recommendations. Fresh water was made available all the daytime. Live body weight and feed intake were recorded weekly before offering feed. At the end of the trial, five broiler chicks from each group were picked randomly for blood sampling.

Blood samples (n= 30) were randomly withdrawn from 5 chicks immediately before slaughtering of chicks (at day 35) from the (brachial) wing vein into tubes containing EDTA as anticoagulant and centrifuged at 3000 rpm for 20 minutes for the separation of plasma and kept at (−20°C) until further analysis.

Experimental traits:

1. Hatchability percentage and ratio of chick weight to egg weight.
2. Weekly body weight, body weight gain, feed consumption and feed conversion ratio.
3. Hematological parameters: Red blood cells count, and hemoglobin concentration were measured immediately after blood collection.

4. Blood metabolites: Total protein (TP), albumin (AL), total lipids (TL), Triglycerides (Tg), cholesterol, iron, TIBC and ferritin, liver enzymes (alanine transaminase (ALT), aspartic transaminase (AST)), plasma immunoglobulin IgG and IgM concentration, creatinine (Cr) and globulin and albumin ratio (A/G ratio) were calculated. Blood metabolites were determined calorimetrically by using commercial kits (Bio Systems S.A. Costa Brava 30, Barcelona. Spain, Barcelona).
5. Blood hormones: Triiodothyronine (T3) hormoneswas measured by ELISA technique using IMMUNOSPEC kits supplied by (Immunospec Corporation, 7018 Owensmounth Ave. Suite 103 Canoga Park, CA 91303, USA).

Statistical analysis was carried out using General Linear Model (GLM) procedures by SAS (2010) using simple one-way analysis of variance. Significant differences among treatment groups were tested using Duncan's multiple range tests, Duncan, (1955)**.**

## III. RESULTS AND DISCUSSION

***Effect of ovo injection by Fe-Nano, Fe-Nano-Alimet chelate, Fe-Aliment chelate and Fe-Aliment chelate on hatchability traits***

Table (1) shows the egg performance when injected with different forms of supplementary Iron. . There was a significant difference (P<0.01) between control and sham control with respect to hatchability percent. There seem to be a need for NaCl solution because of a deficiency of this mineral in the egg, which might explain the positive effect of saline injection. Sodium chloride is a mineral salt and it seemed to close a gap in the requirements of egg growth to this mineral. It might also have a positive effect with respect to buffering the medium inside the egg, which led to facilitating the growth performance, livelihood of the embryo and therefore the hatchability percent improved as a result. It seems that these explanations are logic since there was no significant difference between sham control (saline solution injection) and injection of different forms of Iron either as in nano particle form or not and the form of being organic or inorganic. The different forms of Iron in nano particle or in the organic or inorganic forms showed the same significant difference as the saline solution injection did. The same explanations might, therefore, apply. The check weight/egg weight ratio of control and sham control were not significantly different (74.5 and 74.8 for control and sham control, respectively). The injection of different forms of Iron positively enhanced this ratio. The ratios were 85.2, 4.6 and 84.4 for T3, T4 and T5, respectively).The inorganic form of Iron (T6) was similar to both controls. Saki *et al.* (2014) found no significant effects on hatchability percent among the groups fed 50 and150 ppm Fe-Aliment chelate relative to control one. This may be explained by the deficiencies or excesses of individual trace elements that can cause impaired growth, abnormal development, thus, affecting all of the major organ systems and in extreme cases, death of the embryo (Richards and Steele, 1987). Appropriate amounts of each trace element are required to support embryonic growth and development, Richards, (1989). In mammals, Fe link to amino acids increased the transfer of Fe across the placenta and into the embryo, Ashamead and Graff, (1982)**.**

The form of nano Fe in any form depends on the presence of protein and it would be interesting to investigate the relationship between protein and Fe atoms. Foye, *et al*., (2006) found that Fe atoms adhered easily to protein and that the co-existing system of protein and iron could directly scavenge ROS (OH•, O•− 2 and H2O2). Nano-particles can evade conventional physiological ways of nutrient distribution and transport across tissue and cell membranes, as well as protect compounds against destruction prior to reaching their targets. *In-ovo* administration of nanoparticles, may be seen as a new method of nano-nutrition, providing embryos with an additional quantity of nutrients.

**Growth performance at 7 day of age:**

Effects of *in-ovo* injection of nano forms of Fe-Nano particles (either organic or inorganic) on average weight gain and feed efficiency ratio of broiler during the first week of age are shown in table (2). Body weight (gm) values during the first week gradually increased significantly (P<0.01). The control group showed the lowest body weight over the period of first seven day period (90.55 gm). Sham control showed higher significant body weight (120.5 gm) over this period compared to regular control. It was lower than the treatment of the injection of nano-Iron in either form (132.4 and 123.9 gm for T3 and T4, respectively). The injection of regular Fe salt in both forms (organic and inorganic) showed lower (105.99 and 118.9 gm for organic and inorganic forms of regular Fe injection, respectively) body weight than both controls. Therefore, the percent increments of T2, T3 and T4 were 33.68, 46.26 and 36.83%, compared to T1, respectively. Therefore, the weight gains of T2, T3 and T6 were significantly (P<0.01) higher compared to other treatments. They increased by 65.69, 65.69 and 58.01 % than T1. With regard to feed intake, T2, T3, T4 increased by 59.03, 37.9, 6.19 %, respectively, than that of the T1 control. Results of feed conversion ratio (gm feed/gm gain) revealed a highly significant difference (P<0.01) among the experimental treatments (97.75, 155.45, 134.8, 103.8, 112.75, and 155.75 for T1, T2, T3, T4, T5 and T6,

respectively). It is observed that T3, T4, T2 and T5 recorded the best FCR and this may be due to the increase in feed intake and reduction of daily weight gain. This explained was introduced byFoye, *et al*., (2006) who noted that, *in-ovo* injection could lead to improved digestive capacity, increased growth rate and feed efficiency. Uni.*et al.,* 2005 and Foye, *et al*., (2006) reported that the breast weight percentage was not significantly different among all treatments.

**Growth performance at 35 day of age:**

Effects of *in-ovo* injection by nano forms of Fe-Nano particles on average weight gain and feed efficiency ratio of broiler during the experimental period (0-5 weeks of age) are shown in table (3). The weight gain (gm) of the T2, T3, T4, T5 and T6 (2101.94, 2118.94, 2124.6, 2049.67 and 2003.47 gm, respectively) significantly (P<0.05) increased than T1 (1855.23 gm). They increased by 13.29, 14.2, 10.27 and 10.48% than T1. It is clear that T2, T3, T4 were increased feed intake by 21.43, 15.03, 2.45 and 3.38%, respectively, than that of the T1 treatment. Results of feed conversion ratio (FCR) (gm feed/gm gain) revealed a significant difference (P<0.01) among the experimental treatments. It was monitored in this study, that T2, T3, T4, T5 and T6 recorded the best FCR; these results match up the increase in feed intake and reduction of daily weight gain.

**Blood analysis.**

The effects of *in-ovo* injection of broiler eggs on plasma iron definitions in chicks on 35day of age are shown in Table (4). The results indicate that the effect of *in-ovo* injection of broiler eggs with nano forms of Fe-Nano, Fe-NPs-Alimet chelate, Fe-Aliment chelate and Fe-Aliment chelate recorded significant increased (P<0.01) the values of WBC's, HGB, MCHC and HCT, while it was insignificant in RBC's and MCV , MCH, RDWCV and RDWSD compared to control treatment (Table 3).

On the other view, it was found through the results in table 4 that the iron injection significantly (P<0.01) enhanced different blood parameters for T2 and T1 compared to other treatment (T3, T4, T5 and T6). Which the reduction value were 25.72, 40.7, 57.76, 32.63 and 30.5% compared to T1, respectively. There were significant (P<0.01) decrease in T2, T3, T4, T5 and T6 in TIBC. This decrease were by 47.8, 97.89. 84.6, 3.86%, respectively. The same trend was observed in feretin, where there were significant deferent between T2, T3, T4 and T5 compere to T1 (by 49.45, 49.12, 33.42 and 37.87%, respectively). This data was synchronized with the data showed of hematological parameters in table 3, especially in RBC's, Hb and HTC.

The treatments 25 ppm Fe-NPs, 100 ppm Fe-NPs-Alimet chelate and 150 ppm Fe-Alimet chelate have shown higher Fe content in serum and liver compared with those in other treatments. Seo*et al*. (2008) concluded that iron content of broiler meat could be effectively enriched by supplementation of 200 ppm of Fe as Fe-Aliment chelate for 5 weeks. The results was demonstrated iron concentrations in the liver and kidney (Bertechini, *et al.,* 2012) and chickens for fattening (Shinde, *et al.,* 2011).The greatest mean increase was +22% and +31.9% for broiler muscle and liver, respectively. In addition, hemoglobin in two treatments of 100-ppm Fe-NPs- Alimet chelate and150-ppmFe-Alimet chelate significantly increased compared with other treatments.

The results of Warner *et.al.* (2006) indicated that the absolute amount of iron per liver increased steadily up to hatching time. Their results showed that the highest liver weight was observed in treatment having 25 ppm of Fe-NPs. The treatments 25 ppm Fe-Nano, 100 ppm Fe-NPs-Alimet chelate and 150 ppm Fe-Alimet chelate have shown higher Fe content in serum and liver compared with those in other treatments.

Effects of *in-ovo* injection on broiler eggs on plasma iron definitions in chicks on 35 day of age are shown in Table (5) .The data showed major variations in TP for T1, T2, T3 and T5 compared to T4 and T6, where, they were increased by (11.8, 12.57 and 2.96 %) related to T1, while the lowest value was for T6 (by 1.97 %) and no significant difference. The same trend was observed in A, G and A/G ratio. Since the albumen is synthesized mainly in liver, that liver function was enhanced by the injection of Iron in its different forms, and that the albumin is a main source for amino acid formation, the protein synthesis increased leading to more formation of muscles, which in turn leads to increased final body weight. This is clearly manifested in the results obtained in this study (Table 3).

## IV. CONCLUSION

These results suggest that under semi-arid conditions, the *in-ovo* injection of 20-ppm iron nanoparticles (Fe-NPs), 20-ppm iron nanoparticles Alimet chelate (Fe-NPs-Alimet chelate) and 20-ppm Fe-Alimet chelate as injection contributed to embryonic growth development. Iron nanoparticles and Alimet chelate form, as the active in gradient of feed additives, premixes, and compound feed, due to the high surface activity and penetration into cell can actively influence the intracellular metabolism by stimulating various processes.

The nano form of Fe are not harmful to the embryo (injected with 20 ppm) and can be used to improve the post-hatch performance of broiler.

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*Table.1: Mean ± SE of egg weight, checks hatching weight, ratio between egg and checks weights and hatchability percent as affected byiIn ovoinjection*

| Items | Egg weight (g) | Hatch weight of chicks (g) | Ratio between chicks weight to egg weight % | Hatchability % |
|---|---|---|---|---|
| T1 | 60.83$^{a}$±0.80 | 45.32$^{b}$ ± 0.80 | 74.52$^{b}$± 0.78 | 92.01 $^{b}$ ± 4.11 |
| T2 | 60.81 $^{a}$ ±0.79 | 48.56$^{ab}$ ± 0.75 | 74.81$^{b}$±0.90 | 96.36 $^{a}$ ±3.08 |
| T3 | 60.91 $^{a}$ ±0.78 | 51.90$^{a}$±0.94 | 85.22$^{a}$±1.02 | 95.05 $^{a}$ ±2.15 |
| T4 | 60.81 $^{a}$ ±0.79 | 51.43$^{a}$ ± 0.77 | 84.58$^{a}$±0.83 | 94.21 $^{a}$ ±3.57 |
| T5 | 60.80 $^{a}$ ±0.91 | 51.33$^{a}$ ± 0.84 | 84.42$^{a}$±0.90 | 95.15 $^{a}$ ±2.5 |
| T6 | 60.82 $^{a}$ ±0.91 | 47.43$^{b}$ ± 0.77 | 77.88$^{ab}$±0.90 | 94.92$^{a}$±0.90 |
| Sig. | n. s | * | * | * |

a, b: Means within a column with different superscripts are significantly different (P< 0.01).
Sig. = Significance, * (P< 0.01), n. s = not significant.

*Table.2: Effect of ovov injection on broiler eggs on final weight, weight gain, feed intake and feed efficiency ratio at 7 day of age*

| Items | Chick Weight (g) | Body weight (g) | Weight gain (g/period) | Feed intake (g/period) | Feed conversion ratio |
|---|---|---|---|---|---|
| T1 | 45.32$^{c}$±0.80 | 90.55$^{c}$±22.82 | 45.23$^{b}$±21.89 | 97.75$^{c}$±28.22 | 2.15$^{c}$± 0.09 |
| T2 | 48.56$^{ab}$±0.75 | 120.50$^{a}$±24.55 | 74.94$^{a}$±23.08 | 155.45$^{a}$±30.01 | 2.08$^{b}$±0.17 |
| T3 | 51.90$^{a}$±0.94 | 132.44$^{a}$±26.78 | 74.94$^{a}$±25.66 | 134.80$^{a}$±32.05 | 1.80$^{a}$±0.24 |
| T4 | 51.43$^{a}$±0.77 | 123.90$^{ab}$±25.91 | 54.67$^{b}$±26.14 | 103.8$^{a}$±27.08 | 1.89$^{a}$±0.11 |
| T5 | 51.33$^{a}$±0.84 | 105.99$^{b}$±25.91 | 54.67$^{b}$±24.14 | 112.75a$^{b}$±29.23 | 2.05$^{b}$±0.17 |
| T6 | 47.43$^{b}$±0.7 | 118.90$^{b}$±25.91 | 71.47$^{b}$±25.14 | 155.75$^{ab}$±27.25 | 2.18$^{bc}$±0.17 |
| Sig. | * | * | * | * | * |

a,b: Means within a column with different superscripts are significantly different (P< 0.01).
Sig. = Significance,* (P< 0.01), n.s = not significant

*Table.3: The effect of ovo injection of broiler on final weight, weight gain, feed intake and feed efficiency ratio at 35 day of age.*

| Items | Chick Weight (g) | Body weight (g) | Weight gain (g/period) | Feed intake (g/period) | Feed conversion ratio |
|---|---|---|---|---|---|
| T1 | 45.32$^{c}$±0.80 | 1900.5$^{b}$±22.82 | 1855.23$^{b}$±21.89 | 3691.75$^{c}$±28.22 | 1.99$^{a}$±0.09 |
| T2 | 48.56$^{ab}$ ±0.75 | 2150.50$^{a}$±24.55 | 2101.94$^{a}$±23.08 | 2900.45$^{a}$±30.01 | 1.38$^{b}$±0.17 |
| T3 | 51.90$^{a}$± 0.94 | 2170.44$^{a}$±26.78 | 2118.94$^{a}$±25.66 | 3136.80$^{a}$±32.05 | 1.48$^{b}$±0.24 |
| T4 | 51.43$^{a}$±0.77 | 2175.90$^{a}$±25.91 | 2124.67$^{a}$±26.14 | 3059.8$^{a}$±27.08 | 1.44$^{b}$±0.11 |
| T5 | 51.33$^{a}$±0.84 | 2100.99$^{ab}$±25.91 | 2049.67$^{ab}$±24.14 | 3566.75$^{ab}$±29.23 | 1.74$^{ab}$± 0.09 |
| T6 | 47.43$^{b}$±0.70 | 2050.90$^{ab}$±25.91 | 2003.47$^{ab}$±25.14 | 3766.75$^{ab}$±27.25 | 1.88$^{ab}$±0.17 |
| Sig. | * | * | * | * | * |

a, b, c: Means within a column with different superscripts are significantly different (P< 0.01).
Sig.= Significance,** (P< 0.01), n.s= not significant

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*Table.4: Effect of ovo injection on broiler eggs on plasma iron definitions in chicks on 35day of age.*

| TR | T1 | T2 | T3 | T4 | T5 | T6 |
|---|---|---|---|---|---|---|
| WBCS ($10^9$/l) | 108.70$^{b}$±8.45 | 144.77$^{a}$±9.55 | 142.33$^{a}$±8.24 | 104.23$^{b}$±7.99 | 121.27$^{ab}$±7.85 | 113.33$^{b}$±10.00 |
| L1% | 60.00$^{ab}$±2.20 | 60.00$^{ab}$±2.30 | 61.33$^{a}$±2.40 | 52.00$^{c}$±2.00 | 56.67$^{abc}$±2.33 | 59.67$^{ab}$±2.22 |
| N1% | 31.67$^{abc}$±1.96 | 31.67$^{abc}$±2.01 | 29.67$^{c}$±1.92 | 38.00$^{a}$±2.12 | 35.33$^{abc}$±1.89 | 30.33$^{bc}$±1.89 |
| M1% | 5.00$^{a}$ ±0.45 | 5.00$^{a}$±0.51 | 5.33$^{a}$±0.53 | 6.00$^{a}$±0.49 | 5.00$^{a}$±0.60 | 6.00$^{a}$±0.44 |
| E1% | 3.33$^{a}$±0.50 | 3.33$^{a}$±0.61 | 3.67$^{a}$±0.61 | 4.00$^{a}$±0.55 | 3.00$^{a}$±0.70 | 4.00$^{a}$±0.50 |
| HB ( g/l) | 10.60$^{a}$±0.65 | 11.57$^{a}$±2.30 | 10.27$^{a}$±1.85 | 10.70$^{a}$±1.45 | 10.37$^{a}$±2.61 | 10.60$^{a}$±1.35 |
| RBCS ($10^{/6}$µl) | 3.12$^{a}$±0.38 | 2.78$^{a}$±0.21 | 2.94$^{a}$±0.29 | 2.44$^{a}$±0.19 | 2.56$^{a}$±0.20 | 3.09$^{a}$±0.33 |
| HCT % | 34.80$^{a}$±4.16 | 35.50$^{a}$±5.56 | 35.30$^{a}$±3.96 | 31.67$^{a}$±4.47 | 31.30$^{a}$±6.12 | 34.27$^{a}$±6.32 |
| MCV (µm, fl) | 90.63$^{b}$±8.52 | 127.53$^{a}$±9.18 | 121.67$^{a}$±12.25 | 119.67$^{a}$±10.25 | 122.00$^{a}$±9.98 | 82.33$^{b}$±14.59 |
| MCH (pg) | 35.67$^{ab}$±5.52 | 44.13$^{a}$±6.72 | 38.87$^{a}$±4.56 | 38.57$^{a}$±4.95 | 39.57$^{a}$±4.04 | 29.63b$^{c}$±6.07 |
| MCHC (µm, fl) | 30.90$^{ab}$±6.42 | 32.30$^{ab}$±4.12 | 30.37$^{ab}$±4.25 | 29.67$^{b}$±5.21 | 32.53$^{ab}$±5.51 | 33.63$^{a}$±4.95 |
| RDW_CV | 15.23$^{a}$±0.91 | 12.17$^{b}$±0.74 | 15.50$^{a}$±0.81 | 14.97$^{a}$±0.81 | 15.50$^{a}$±0.84 | 15.50$^{a}$±0.75 |
| RDW_SD | 33.37$^{a}$±4.01 | 45.97$^{a}$±3.85 | 37.93$^{a}$±4.13 | 40.53$^{a}$±3.95 | 34.70$^{a}$±4.21 | 37.93$^{a}$±4.00 |

a, b, c Means within the same row with no common superscript differ significantly.
** P≤ 0.01, NS= non-significant

*Table.5: Effect of ovo injection on broiler eggs on plasma iron definitions in chicks on 35 day of age.*

| TR | T1 | T2 | T3 | T4 | T5 | T6 |
|---|---|---|---|---|---|---|
| Iron(µg/L) | 360.33$^{a}$± 37.98 | 267.67$^{ab}$± 38.10 | 213.67$^{b}$± 38.2 | 163.00$^{b}$± 37.99 | 180.33$^{b}$ ±38.22 | 147.33$^{b}$± 38.15 |
| TIBC(µg/L) | 158.00$^{b}$± 25.21 | 276.33$^{a}$± 26.50 | 312.67$^{a}$± 26.19 | 291.67$^{a}$± 25.22 | 276.00$^{a}$ ±26.19 | 343.67$^{a}$± 25.21 |
| Ferritin (µg/L) | 51.17$^{ab}$± 9.77 | 76.47$^{a}$± 10.02 | 76.30$^{a}$±9.99 | 68.27$^{ab}$± 9.89 | 47.53$^{ab}$ ±9.94 | 40.20$^{b}$± 10.10 |

a, b, c Means within the same row with no common superscript differ significantly.
** P≤ 0.01, NS= non-significant

*Table.6: Effect of ovo injection on broiler eggs on blood analysis at 35 day of age*

| TR | T1 | T2 | T3 | T4 | T5 | T6 |
|---|---|---|---|---|---|---|
| TP (g/dL) | 2.73$^{ab}$±0.34 | 3.05$^{a}$±0.40 | 2.67$^{ab}$±0.39 | 2.40$^{b}$±0.37 | 2.96$^{ab}$±0.35 | 2.99$^{b}$±0.39 |
| Alb (g/dL) | 1.33$^{ab}$±0.18 | 1.70$^{a}$±0.22 | 1.50$^{ab}$±0.21 | 1.35$^{b}$±0.20 | 1.60$^{ab}$±0.19 | 1.57$^{ab}$±0.18 |
| Gl (g/dL) | 1.40$^{a}$±0.24 | 1.35$^{a}$±0.21 | 1.17$^{b}$±0.20 | 1.05$^{b}$±0.25 | 1.36$^{a}$±0.23 | 1.42$^{a}$±0.22 |
| A/g | 1.05$^{ab}$±1.01 | 0.79$^{ab}$±1.10 | 0.78$^{ab}$±0.98 | 0.78$^{b}$±0.99 | 0.85$^{ab}$±1.99 | 0.91$^{a}$±1.00 |
| ALT (g/dL) | 103.67$^{a}$±4.33 | 135.67$^{a}$±3.90 | 94.67$^{b}$±2.22 | 84.33$^{b}$±2.22 | 83.33$^{b}$±2.22 | 75.67$^{c}$±4.97 |
| AST (g/dL) | 13.27$^{a}$±2.28 | 14.17$^{a}$±1.80 | 12.53$^{a}$±2.10 | 13.50$^{a}$±1.90 | 15.40$^{a}$±2.08 | 14.40$^{a}$±1.98 |
| Urea (g/dL) | 12.33$^{a}$±1.57 | 15.67$^{a}$±1.55 | 13.00$^{a}$±1.45 | 13.33$^{a}$±1.77 | 14.33$^{a}$±1.65 | 16.33$^{a}$±1.57 |

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| | | | | | | |
|---|---|---|---|---|---|---|
| Uric Acid (mg/dL) | $4.36^{b}$±0.79 | $4.30^{b}$±0.87 | $6.17^{a}$±0.77 | $6.24^{a}$±1.00 | $4.56^{b}$±0.95 | $4.44^{b}$±0.99 |
| Cr (mg/dL) | $0.52^{b}$±0.06 | $0.5^{b}$±0.05 | $0.66^{ab}$±0.07 | $0.81^{a}$±0.08 | $0.59^{b}$±0.05 | $0.57^{b}$±0.04 |
| Ch (mg/dL) | $148.33^{b}$±5.98 | $129.67^{c}$±5.29 | $178.00^{a}$±6.19 | $162.67^{a}$±6.49 | $163.00^{a}$±5.99 | $156.67^{b}$±6.29 |
| Tg(mg/dL) | $267.67^{b}$±13.38 | $191.67^{c}$±14.38 | $323.33^{a}$±15.18 | $299.67^{b}$±13.28 | $317.00^{a}$±12.38 | $166.67^{c}$±14.38 |
| HDL (mg/dL) | $60.01^{abc}$±3.78 | $68.01^{a}$±3.48 | $44.67^{c}$±3.89 | $63.67^{ab}$±4.00 | $49.33^{bc}$±4.08 | $56.5^{bc}$±3.98 |
| LDL (mg/dL) | $46.66^{a}$±4.98 | $49.33^{a}$±5.58 | $34.01^{b}$±4.78 | $42.01^{ab}$±5.01 | $33.33^{b}$±4.58 | 44.55±4.68 |
| T L (mg/dL) | $229.01^{d}$±18.83 | $315.33^{bc}$±19.93 | $453.67^{a}$±18.93 | $368.33^{b}$±20.03 | $294.01^{c}$±19.03 | $295^{c}$±20.20 |
| A P (U/dL) | $27.03^{a}$±0.99 | $25.82^{ab}$±1.00 | $22.89^{ab}$±0.99 | $18.84^{b}$±0.89 | $25.17^{ab}$±0.98 | $24.9^{ab}$±0.99 |
| T3 (nmol/L) | $1.38^{a}$±0.18 | $1.44^{a}$±0.19 | $1.46^{a}$±0.17 | $1.23^{ab}$±0.18 | $1.07^{b}$±0.17 | $1.25^{ab}$±0.19 |

a, b, c Means within the same row with no common superscript differ significantly.

** P≤ 0.01, NS= non-significant

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# Molecular Cloning of Sucrose Isomerase Gene and *Agrobacterium*-Mediated Genetic Transformation of Potato (*Solanum tuberosum* L.) Plants

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***Abstract*—***Potato (Solanum tuberosum L.) is one of the most common and important food sources on the planet, and they essential as a staple dietary item for much of the world's population. Potatoes contain carbohydrates, which lead to high blood sugar. Palatinose (isomaltulose, 6-O-alpha-D-glucopyranosyl-D-fructose) is a functional isomer of sucrose its non-cariogenicity low calorific value and it is an ideal sugar substitute to use in food production. The sucrose isomerase*(*palI*) gene that is obtained from Erwinia rhapontici *is one of the most common genes that can convert sucrose into palatinose. In present study,pQE-30- palI construct wassucceffuly transformed and expression into* E. coli. *Sucrose isomerase* (*palI*) gene *was cloned and overexpressed into a plant expression vector* pBinAR-*palI contains sucrose isomerase gene (palI) fused to proteinase inhibitor II signal sequence under CaMV-35S promoter and Octopine Synthase (*OCS*) terminator. Expression of the protein was verified by western blot assay. Also, expression of the palI gene within the apoplast of transgenic tubers under control of a tuber-specific patatin class I B33 promoter instigated quantitative conversion of sucrose into palatinose.Tuber extracts from potatocv. Désiréewere analyzed for their soluble carbohydrate composition using HPLC.*

***Keywords*—*Potato; Agrobacterium; Sucrose isomerase; Palatinose; B33 promoter; Expression; HPLC.***

***Abbreviations:*SP**- signal peptide of the proteinase inhibitor II gene ; **MS**- Murashige and Skoog medium; **BAP**-Benzyl Amino Purine; **ZR**- Zeatin Riboside; **IAA**-Indole Acetic Acid; **IBA**-3-Indole Butyric Acid;**TE**-Transformation Efficiency; **OCS**- polyadenylation signal of the octopine synthase gene;**ER**- Endoplasmic Reticulum; **B33**- promoter of the class I patatin gene B33; palI: *Erwinia rhapontici* sucrose isomerase gene; EcoRI,Asp718,BamHI,SalI,HindIII- restriction cleavage sites.

## I. INTRODUCTION

Potato (*Solanum tuberosum* L.) is a staple food and is considered the most important economic tuber crop around the world (Joseph *et al*., 2015). Sucrose substitutes vary greatly in degree of sweetness, volume, texture and stability under different conditions and sweetener is not a perfect substitute for sucrose in all applications. In some cases high amounts of sucrose over long periods was found to cause cancer diseases (Price *et al*., 1970). Some microbes contain Isomaltulose synthase (*PalI*), catalyzes the isomerization of sucrose to produce isomaltulose (palatinose,α-D-glucopyranosyl-1, 6-D-fructofuranose) and trehalulose (α-D-glucopyranosyl-1, 1-D-fructose), as the main products with residual amounts of glucose and fructose (Börnke et al., 2001; Zhang et al., 2003; Watzlawick and Mattes, 2009). Isomaltulose is a naturally occurring isomer of sucrose (α-D-glucopyranosyl-1,2-D-fructofuranose) that is valued as an acariogenic sweetener (Takazoe, 1989). Palatinose is a nutritional sugar it is digested more slowly than sucrose and has health advantages for diabeticsand nondiabetics (Lina *et al*., 2002). Also, it is the first non-cariogenic sugar, similar physico-chemical properties as sucrose (taste, texture, and mass) but shows a slower rate of release of monosaccharides into blood (Goda and Hosoya, 1983; Minami *et al*., 1990).They described the report onthe cloning and characterization of a bacterial sucrose isomerase (*palI*) gene from *Erwinia rhapontici* which catalyses the conversion of sucrose into palatinose and its function has been tested by heterologous expression in *Escherichia coli*. This enzyme is strictly substrate-specific toward sucrose and the reaction is essentially irreversible. The yield of palatinose formed from sucrose ranged from 85 and 15% for trehalulose, respectively (Cheetham, 1984). Expression of a chimeric sucrose isomerase (*palI*) gene within the apoplasm of transgenic tobacco plants and accumulated considerable amounts of non-cariogenic sucrose isomer palatinose (Xuguo et al. 2016). However,

conversion of sucrose into the non-metabolizable isomer palatinose caused severe growth retardations in these plants most likely due to the depletion of a carbohydrate source for sink development (Börnke *et al*., 2002).

In this study, description the cloningand characterization of a chimeric sucrose isomerase (*palI*) gene from *Erwinia rhapontici* and introduced palI gene with different promoter into potato plants were described.In addition to, Expression of the *palI* gene which conversion of sucrose into palatinose within the apoplast of transgenic tubers was studies.

## II. MATERIALS AND METHODS

**Isolation and cloning of sucrose isomerase gene.**

The coding region of the sucrose isomerase (*palI*) was cloned by polymerase chain reaction (PCR).Genomic DNA from *E. rhapontici* was isolated by a standard protocol and used as a template. Amplification was carried out using the following specific gene primers 5′-GGGATCCTCACCGTTCAGCAATCA3′and 5′-GTCGACCTACGGATTAAGTTTATA-3′, which were obtained from sucrose isomerase sequence (GenBank Acc. No.: AF279281) and signal peptide of proteinase inhibitor II gene (Keil*et al*.,1986), which was fused via a linker with the sequence ACC GAA TTG GG to the *Erwinia rhapontici* sucrose isomerase gene, which comprises the nucleotides 109 to 1803. Thus, a signal peptide of a plant protein, which is required for the uptake of proteins into the endoplasmic reticulum (ER) was fused N-terminally to the sucrose isomerase sequence.

**Plasmid Construction and bacterial strain**.

The *Erwinia rhapontici* sucrose isomerase gene and expression vector pQE-30 (Qiagen Inc. Valencia, CA) were digested with restriction enzymes *BamHI/SalI* respectively. The digested products were separated using agarose electrophoresis and the bands were extracted. The purified sucrose isomerase gene (*palI*) and the linear vector were ligated overnight at 16°C with T4 DNA ligase followed by transformed into *E.coli* JM109 competent cells. The transformation mixture was plated on (Luratia Broth) LB agar plates containing ampicillin (100$\mu$g/mL$^{-1}$). The plates were incubated for 16h at 37°C. The desired recombinant plasmid pQE-30- *palI* was confirmed by PCR and restriction enzyme digestion with *BamHI/SalI* and DNA sequencing (Invitrogen).

Construction of the sucrose isomerase overexpression construct the coding region of the *palI* gene, ranges from codons 109 to 1,803bp was amplified from *Erwinia rhapontici* by PCR. The *palI* gene fused to proteinase inhibitor II signal sequence (SP) was inserted in sense orientation between the CaMV-35S promoter and Octopine synthase (*OCS*) terminator within a binary vector plasmid pBinAR by the restriction endonuclease Asp718 (Höfgen and Willmitzer, 1990; Börnke *et al*., 2001). This plasmid is a derivative of the binary vector pBin19 (Bevan, 1984). The *A. tumefaciens* strain LBA4404 harbouring recombinant binary vector plasmid pBinAR-palI was maintained on LB medium (Chilton *etal*.,1974) supplemented with 25 mg L$^{-1}$rifampicin and 50 mg L$^{-1}$ kanamycin and incubated overnight at 28°C in an incubator shaker at 90 rpm/min before using in transformation (Fig. 1).

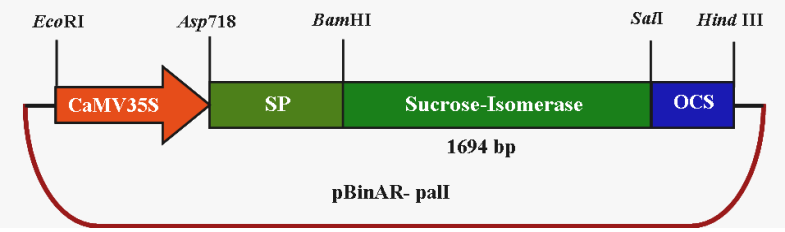


*Fig.1: Structure of palI expression construct used to transformed potato. The palI coding region ranging from 109 to 1,803bp was inserted between the CaMV-35S promoter and* OCS *terminating region of vector pBinAR using BamHI and Sal I restriction sites, respectively. The signal sequence for the proteinase inhibitor II (SP) was inserted in front of the palI coding region.*

The 35S promoter was removed from the pBinAR vector using the restriction endonucleases EcoR I and Asp718. A fragment with a length of about 1526 by comprising the tuber-specific promoter of the class I patatin gene (B33-promoter) of potato and inserted into the pBinAR vectorby EcoR I and Asp718. This resulted in the plant expression vector pBin33-Kan.The palI gene was introduced in the sense orientation into the plant transformation vector pBin33-Kan by *Asp*718/*Xba*I between the patatin B33 promotor and the octopine synthase polyadenylation signal (Fig. 2).

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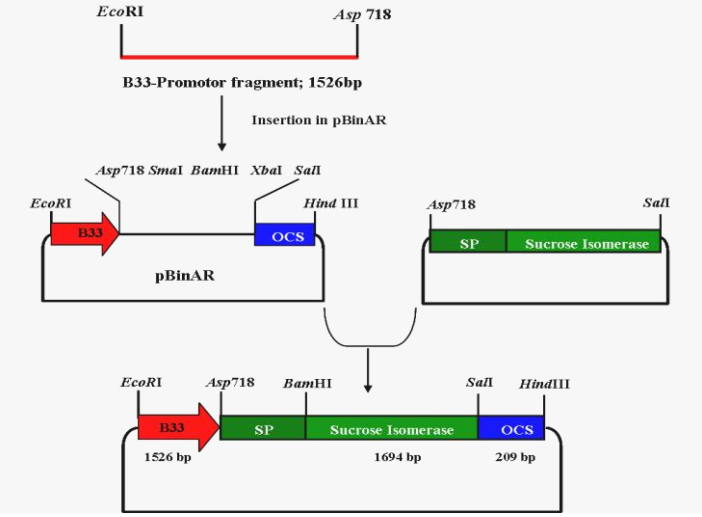


*Fig.2:Schematic representation of the construct pBin33, which mediates the expression of sucrose Isomerase from E. rhapontici of transgenic Potato tubers. The gene for the Sucrose-Isomerase, merged with the signal peptide of the proteinase inhibitor I gene (Schaewen et al., 1990), the tubers-specific B33 promoter of Patatin class I gene was between ASP 718 / EcoRI fragment and the OCS terminator cloned into the vector pBin19.The obtained construct pBin33was then used for the production of transgenic potato plants.*

**In vitro regeneration and tuber formation ofpotato**(*Solanum tuberosum* L.)

Leaf explants were collected from 4 to 6 week-old *in vitro* grown potato (*Solanumtuberosum* L.)cv. Désiréeand cultured on MS (Murashige and Skoog, 1962) medium supplemented with 100 mg $L^{-1}$, myo-inositol, 30 g $L^{-1}$ sucroseand different growth regulators IAA(0.05, 0.1, 0.5 mg $L^{-1}$)in combination with zeatinriboside (1.0, 2.0, 3.0, 4.0, 0.5 mg $L^{-1}$) for six weeksfor direct regeneration.The cultures were incubated at 25±2.0°C with a 16/8 h light/dark photoperiod provided by cool-white fluorescent lamps (40-50 µmol $m^{-2}s^{-1}$). The shoots regenerated were cultured MS medium containing 100 mg/l, myo-inositol, 1.0 mg $L^{-1}$ BAP, 0.5 mg/l kinetin and 2.5 g $L^{-1}$ phytagel with different concentrations 5%, 6%, 7%, 8%, 9% and 10% of sucrose for *in vitro* tuberization in potato. The plantlets were incubated under dark condition at 25±2.0°C. Microtubers wereharvested after eight weeks of incubation. Data wererecorded on mean number of shoots/explant, mean length of shoots/explant (mm), regeneration percentage, microtuber formation per explant, percent of explant formed microtuber, number of microtuber/ explant and average weight of microtuber/ explant.

***Agrobacterium*-mediated transformation of potato**.

The leaf explants were immersed in the *Agrobacterium* suspension containing either the binary vector pBinAR-palI or the binary vector pBin33-Kan for 20 min. Afterinfection, the leaf explants were placed on MS medium- free hormone at 25±2°C for co-cultivation of 48h. And then, the cultures were transferred on shoot induction medium (MS plus 100 mg $L^{-1}$kanamycin and 300 mg $L^{-1}$ cefotaxime to inhibit further bacterial growth) for six weeks and then cultures were incubated at 25±2°C with a 16/8 h light/dark photoperiod provided by cool-white fluorescent lamps. Shoots were transferred to MS medium 1.0 mg $L^{-1}$ BAP, 0.5 mg $L^{-1}$ kinetin, 10% sucrose with100 mg $L^{-1}$ kanamycin and 300 mg $L^{-1}$ cefotaxime at 25±2.0°C under darkfor micro tubers formation. Transformation frequency was calculated by multiplying the percentage of explants that produced plants by the percentage of plants that were confirmed to be transgenic by PCR.

**Expression of *palI* gene in *E. coli*.**

*E. coli* XL-1Blue cells were transformed with recombinant plasmid pQE-30- *palI* extracted from JM 109. The bacteria cells were grown in 5 ml LB liquid medium at 37°C with 100 $\mu$g/mL$^{-1}$ ampicillin for 4-6 h at 220 rpm till to an absorbance of 0.6 at 600 nm. Expression of the fusion protein was induced by isopropyl-β-D-thiogalactopyranoside (IPTG) with a final concentration of 0.5 mM for 5 h at37°C. These samples were harvested by centrifugation at 13.000 rpm for 1min. The pellet was resuspended in 1 ml of 30 mM HEPES-KOH (pH 7.5) for preparation of palatinose. The suspension was centrifuged at 15.000 rpm for 2 min at 4°C, and the supernatant was used for enzyme measurements.The expression of *palI* protein was analyzed by SDS-PAGE. For conversion of sucrose into palatinose by *E. Coli* cell suspension, the E. coli cells (1 g wet wt.) expressing the *palI* gene were washed two times with 50 mmol $L^{-1}$ PBS (pH 6.0). The cell pellets were resuspended in the same solution at the desired concentrations. Reactions were conducted in 50 ml flasks containing 10 ml 550 g $L^{-1}$ sucrose solution at 30 °C and shaken at 150 r/min for about 5 h. Aliquots of the reaction mixture were sampled and analyzed for the amounts of palatinose formed. The reactions were

terminated by heating the flasks for 10 min in a boiling water bath.

**Antibody preparation and western blot assay.**

The sucrose isomerase (*palI*) gene subcloned in expression vector pQE-30 which resulted pQE- *palI* and introduced into *E. coli* XL-1Blue. The protein over expressed in *E. coli* was purified to form antibodies against sucrose isomerase (*palI*)by immunization rabbit. The plant proteins were separated by SDS-PAGE and transferred to the porablot and incubated with antibodies against the *palI* protein from transgenic potato plants.

**HPLC analysis**

Reaction products were filtered through 0.22 µm membrane filters before HPLC analysis (Agilent 1200, USA system equipped with a refractive index detector). The samples were diluted 10-fold and 20 µl of diluted sample was injected onto a Rezex RCM-Monosaccharide $Ca^{++}$ column (Phenomenex, USA) for measurement of the sugar composition. The mobile phase was water with a flow rate of 0.5 ml/min at 80 °C. Glucose, fructose, sucrose and palatinose were used as standard sugars. HPLC analysis of soluble carbohydrate composition of tuber extracts was carried out as previously described (Börnke *et al*., 2002). The samples extracted with 80% ethanol at 80°C for one hour.

**Gel preparation and electrophoresis**

All plasmid, restriction digestion and amplified PCR products were loadedonto 1.5 % agarose gel. The purity and concentration of amplified product waschecked from the band in agarose gels. Concentration of the DNA wasestimated using a 1Kb DNA Ladder.

**Statistical analysis**

The experiments were three replicates for each treatment, each treatment contain 30 Jars (ten jar for each replicate), and four explants were cultured in Jar. Analysis of variance (ANOVA) was applied to data using Costat Software (2006). The differences among means for all treatments were tested for significance at 5% level by Duncan (1955).All values are reported as means ± standard deviation.

## III. RESULTS AND DISCUSSION

***In vitro* regeneration ofpotato (***Solanum tuberosum* L.) **plants**.

The study of the effect of growth regulators on the shoot induction of potato cv. Désiréeafter six weeks is shown in **table 1**. The highest value of mean number of shoots per explant (39.65) and mean length of shoots per explant (33.5 mm) was recorded on MS medium containing 0.1 mg $L^{-1}$ IAA and 3.0 mg $L^{-1}$ ZR and also this treatment had the highest regeneration rates (82 %) after six weeks compared to other treatments (Fig.3 A and B). The mean number of shoots and regeneration percentage was increased in parallel with increasing concentration of ZR up to 3.0 mg $L^{-1}$and then mean number of shoots per explant (Fig.3 C and D) while mean length of shoots per explant was decreased under 4 and 5 mg $L^{-1}$ ZR treatment.Shoots were cut and transferred to the root induction medium containing 1mg $L^{-1}$IBA. After rooting, the plantlets were transferred to greenhouse for acclimization and grown in soil. These results agreement with Gustafson *et al*. (2006) that used NAA in combination with trans-zeatin,the highest mean number of shoots per explant (27.3) and regeneration rate (71%) of *Solanum tuberosum* L. cv. Shepody. Webb *et al*. (1983) investigated the regeneration of shoots from potato leaf discs cultured on different concentrations of NAA and BA. While, Park *et al*. (1995) reported that regenerated shoots from callus developing from edges of leaf explants of four North Dakota potato genotypes on MS medium containing 3.5mg $L^{-1}$IAA and 3.0-4.0 mg $L^{-1}$ZR. Also, Gustafson *et al*. (2006) obtained 67% regeneration frequency in stem explants of potato from MSmedium supplemented with 0.1 mg $L^{-1}$IAA, 0.1 mg $L^{-1}$ZR. While, shoot regeneration from leaf petioles explants of commercial cultivars was obtained on MS medium supplemented with 3.0 mg $L^{-1}$ BA, 2.0or 0.5mg $L^{-1}$IAA and 1.0 mg $L^{-1}$ GA3 (Yee *et al*. 2001).

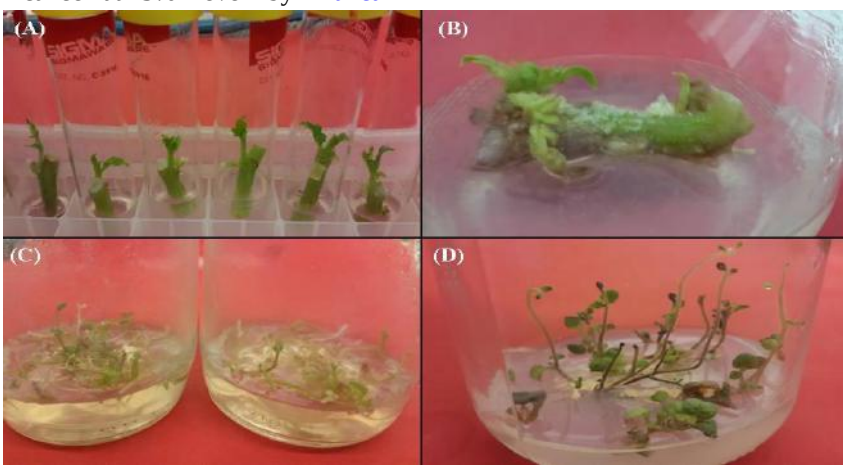


*Fig.3:In vitro regeneration of shoots from leaf explants of cv. Désirée.Leaf explants were collected from 4 to 6 week-old in vitro grown potato (A); shoots regenerated directly from explants on MS medium containing 0.1 mg $L^{-1}$ IAA and 3.0 mg $L^{-1}$ ZR (B); Rooting is concomitant with shoot elongation. Some 4-6 weeks after the subculture plantlets (cv. Désirée) have a well development root system (C); Branching by activation of axaillary buds also starts, but at higher internodes (D).*

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**Microtuber formation ofpotato (***Solanum tuberosum* L.) **cv. Désirée**.

The effect of sucrose on microtuber production was presented in **Table 2**. The results showed that the percentage of plants producing microtuber increased with the increase of sucrose concentrations. The highest number of microtuber was (5.25) detected under 10% sucrose whereas the lowest number was (2.35) at 5% sucrose. Also, the highest average weight of microtuber was (397 mg) observed on 10% sucrose as shown in **Figure 4**. The present investigation was tended to find out the effect of sugar level on microtuberization and found that microtuberization increased with the increasing sugar level and the optimum concentration was 10% sucrose which was similar to observed previously by Saha *et al.* (2013). Potato tubers are modified shoots closely associated with stolons from which they develop (**Fig. 4**). Tubers and stolons differ by planes of cell division which in stolons promote elongation while, in tubers increase their thickness. Signal that the plant is competent to produce tubers generated in leaves is transmitted to other plant parts by the phloem system. Signal induces a change in the plane of the cell elongation and division. Cell division plates become parallel to the elongation axis of stolons promoting radial growth. At the sub cellular level, transition in the plane of cell divisions is connected with the arrangement of microtubules (Efstathios*et al*., 2012). Under *in vitro* conditions, the change in the microtubules arrangement in the subapical zone of stolon outgrowth can be observed on the tuberization medium after 50 days (Sanz *et al*., 1996).

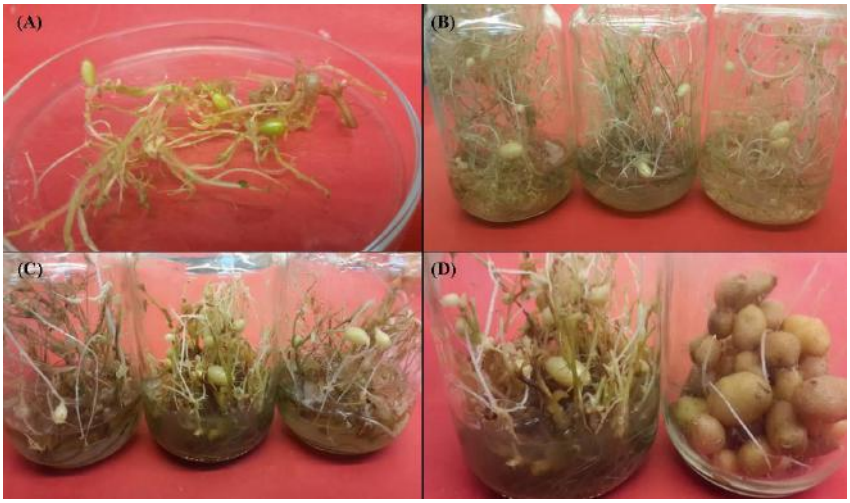


*Fig. 4: Microtuber formation of cv. Désirée. Tubers usually form on the tip of stolons, tuber bearing shoots (A); Microtuber formation at 5% sucrose (B); Microtuber formation at 8% sucrose (C); Biggest size of microtuber production under dark condition due to application of MS + 10% sucrose + 1.0 mgL$^{-1}$BAP and 0.5 mg L$^{-1}$ kinetin (D).*

*Table.1: The effect of plant growth regulators on adventitious shoot from leaf of potato (Solanumtuberosum L.) cv. Désirée after six weeks.*

| Growth regulators (mgL$^{-1}$) | | Mean number of shoots/explant | Mean length of shoots/explant (mm) | Regeneration (%) |
|---|---|---|---|---|
| **ZR** | **IAA** | | | |
| 1.0 | 0.05 | 12.45±0.65$^{k}$ | 11.0±0.59$^{j}$ | 20 |
| 1.0 | 0.1 | 19.50±0.87$^{h}$ | 12.5±0.46$^{i}$ | 38 |
| 1.0 | 0.5 | 23.48±0.93$^{g}$ | 17.4±0.63$^{g}$ | 45 |
| 2.0 | 0.05 | 15.47±0.69$^{j}$ | 10.2±0.57$^{k}$ | 28 |
| 2.0 | 0.1 | 17.15±1.21$^{i}$ | 13.5±0.28$^{h}$ | 34 |
| 2.0 | 0.5 | 19.42±0.82$^{h}$ | 11.0±0.49$^{j}$ | 39 |
| 3.0 | 0.05 | 27.55±0.96$^{f}$ | 19.5±0.92$^{f}$ | 65 |
| 3.0 | 0.1 | 39.65±0.58$^{a}$ | 33.5±0.49$^{a}$ | 82 |
| 3.0 | 0.5 | 34.26±0.85$^{b}$ | 25.5±0.94$^{b}$ | 79 |
| 4.0 | 0.05 | 29.83±0.79$^{e}$ | 20.8±0.85$^{e}$ | 68 |
| 4.0 | 0.1 | 31.75±0.68$^{d}$ | 23.6±0.73$^{d}$ | 74 |
| 4.0 | 0.5 | 33.38±0.88$^{c}$ | 24.8±0.59$^{c}$ | 76 |

Each treatment had 5 replications (plates) with 10 explants per replication. Values followed by the same letter are not significantly different from each other.

*Table.2: Effect of different sucrose concentrations on in vitro microtuber production of potato (Solanum tuberosum)cv. Désirée.*

| Avg. wt. (mg) of microtuber | No. of microtuber per explant | Percent (%) of explant formed microtuber | No. of culture Showing microtuber | No. of explant culture | Sucrose concentration |
|---|---|---|---|---|---|
| 220±0.95$^{f}$ | 2.35±0.34$^{f}$ | 40 | 12 | 30 | 5% |
| 253±0.78$^{d}$ | 2.60±0.29$^{e}$ | 67 | 20 | 30 | 6% |
| 289±0.83$^{a}$ | 3.25±0.54$^{d}$ | 77 | 23 | 30 | 7% |
| 340±1.03$^{e}$ | 3.75±0.42$^{c}$ | 80 | 24 | 30 | 8% |
| 365±0.55$^{c}$ | 4.25±0.39$^{b}$ | 90 | 27 | 30 | 9% |
| 380±0.71$^{b}$ | 5.25±0.56$^{a}$ | 97 | 29 | 30 | 10% |

Each treatment had 5 replications (plates) with 10 explants per replication. Values followed by the same letter are not significantly different from each other.

**Expression of sucrose isomerase (*palI*) genein *E. coli*.**

Cloning of the *palI* genefrom *Erwinia rhapontici*by PCR using specific primer; 5′-GGGATCCTCACCGTTCAGCAATCA3′ and 5′-GTCGACCTACGGATTAAGTT TATA-3′. The amplified*palI* genewas inserted into the expression plasmid pQE-30using *Bam*HI and *Sal*I recognition sites into the sequences (underlined), respectively. The recombinant plasmid pQE-30- *palI* was transformed into bacteria (JM 109)and restriction endonuclease digestion with *Bam*H I/*Sal* I(Fig.5). The recombinant expression vector plasmid pQE-30- *palI* extracted from JM 109 and then transformed into *E. coli* XL-1Blue cells.To conform thegene expression of protein product, the *palI* gene was expressed in *E. coli* under the control of an IPTG-inducible promoter.Enzymatic activity was assayed by incubation ofa crude cell extract prepared from the expressor strain withsucrose solution and by subsequent sugar analysis via HPLC.Chromatograms of bacterial extraction indicated the presence of additional peaks in the reaction mixture. In comparison with the standards, the results indicated this major peak of palatinose (Fig. 6). This clearly demonstrates the sucrose isomerase activity of PalI gene to convert sucrose to palatinose. The conversion of sucrose into palatinose drastically affected the sucrose content of *E. coli*. These results were agreed with (Börnke *et al*., 2001). The appearance of palatinose in the bacterium extract indicated the sucroseisomerase activity of the recombinant PalI proteinand the ability of the palI gene to convert sucrose to palatinose. Glucose and fructose as by-products of the reaction has been described previously (Cheetham, 1984). He also mentioned that the optimum pH for isomerase activity was between 6.0 and 6.5 and optimum temperature was 30°C, which is in good agreement with the finding that the enzyme is localized to the periplasmic space of *E. rhapontici* cells.

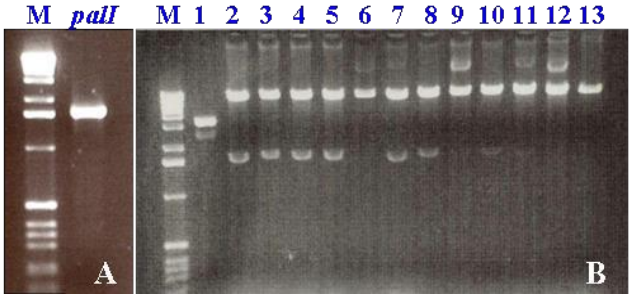


*Fig.5: Cloning of the palI genefrom Erwinia rhaponticiby PCR (A) .Plasmid pQE-30 digested withBamHI and Sal (B). Lane palI: palI gene, Lanes (1-13): samples from selected colonies, Lane M: DNA marker (1kb plus DNA ladder).*

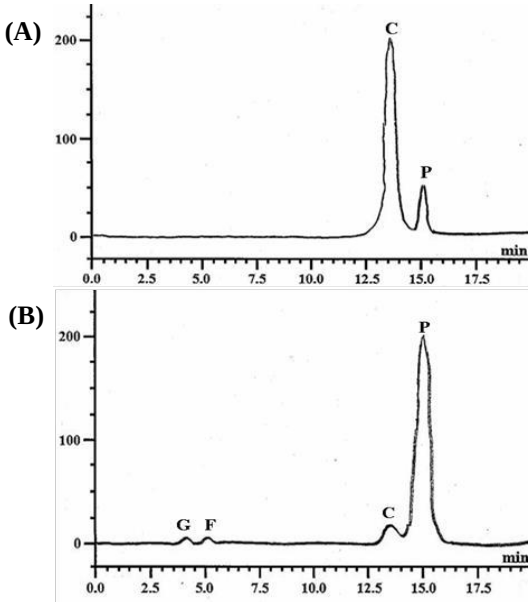


*Fig.6: HPLC analysis of the E. coli (pQE-30- palI) culture supernatant: The palatinose peak was assigned from culture supernatant after 45 min. (A) and after 150 min. (B) comparison to a standard.S; sucrose, P; palatinose, G; glucose, F; fructose.*

**Transformation of potato (*Solanum tuberosum* L.)cv. Désirée.**

**Binary vectorspBinAR-palIand pBin33-Kan.**

*A. tumefaciens* colonies transformed with pBinAR-palIor pBin33-Kan wereanalyzed by colony PCR using palIgene-specific primers. The expectedband size of 1803 bp was observed in selected colonies. This confirmed the presence of the vector in the colonies.Presence of the pBinAR-palIor pBin33-Kan plasmid was verified in PCRpositive colonies by digestion with *Asp*718/*Xba*I. A 1804 bp fragment wasreleased from DNA extracted from the colonies (Fig.7). These results confirm successful cloning of the expression vector pBinAR-palIor pBin33-Kan into *A. tumefaciens*.

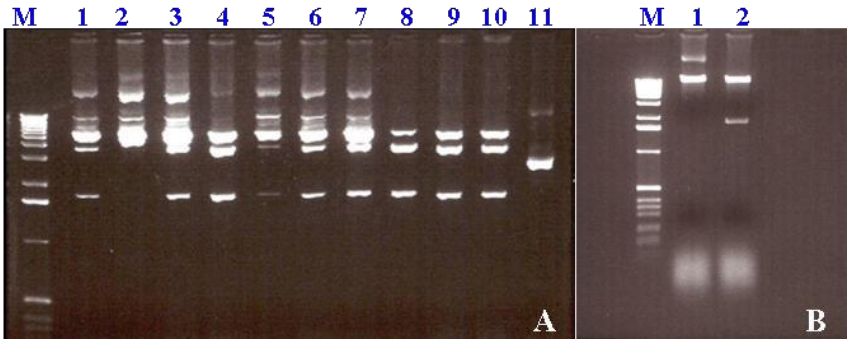


*Fig.7: A. tumefaciens colonies transformed with* pBinAR-*palIor pBin33-Kan wereanalyzed by colony PCR using palIgene-specific primers. (A):* pBinAR-*palI digested with* Asp*718/*Xba*I to release the vector DNA (upper band) along with the insert DNA (1,803 bp, lower band) and (B): pBin33-Kan digested with* Asp*718/*Xba*I. Lane M: DNA marker (1kb plus DNA ladder).*

***Agrobacterium*-mediated transformation**.

Leaves excised from 4 to 6 week-old *in vitro*grown potatocv. Désiréeas explants and incubated with *Agrobacterium* strain LBA4404 carrying either the binary vector pBinAR-palI or the binary vector pBin33-Kan for 20 min. Explants were co-cultivated with *Agrobacterium* for 48 h on the MS medium-free of hormones. After co-cultivation in the dark, the explants were transferred to the shoot induction medium containing 0.1 mg $L^{-1}$ IAA and 3.0 mg $L^{-1}$ZR with 300 mg $L^{-1}$cefotaxime and 100 mg $L^{-1}$ kanamycin to select for transformed cells for six weeks. And then cultures were incubated at 25±2°C with a 16/8 h light/dark photoperiod provided by cool-white fluorescent lamps. Shoots were transferred to a rooting medium containing 1mg $L^{-1}$ IBA and 50 mg $L^{-1}$ kanamycin and then the plantlets were transferred to greenhouse and grown in soil. At this time, the transformation efficiency was evaluated for further analysis with *Agrobacterium* harboring the binary vector pBinAR-palI. For in *vitro* tuber formation, the transgenic potato shoots were cultured on MS medium containing 1.0 mg $L^{-1}$ BAP, 0.5 mg $L^{-1}$ kinetin, 10% sucrose with 50 mg $L^{-1}$ kanamycin and then shoots were incubated under dark condition at 25±2.0°C. Transgenic potato plantlets were checked by PCR and Western analyses. Potato leaf discs were transformed using *Agrobacterium*-mediated gene transfer under patatin promoter gives rise to tuber-specific expression (Rocha-Sosa *et al*., 1989). This result is also in accordance with Sarker and Mustafa (2002) where histological *GUS* assay showed the expression of *GUS* gene in the leaf tissues of transformed shoots. While, recent study, Veale *et al.* (2012) used *In vitro* potato explants were infected with *Agrobacterium* LBA4404 strain harbouring the binary vector pSPUD5 carrying the *cry1Ia1* gene under the transcriptional control of the (ocs) promoter and the *nptII* gene, cultured on the pre-culture medium with 50 µM acetosyringone.

**PCR-detection**

To confirm the stable transformation in the genome, DNA was isolated using genomic DNA isolation kit (Bio Basic, Canada) from putative transformed plants of high dose (100 mg $L^{-1}$) kanamycin exposure plants. After transformation with *Agrobacterium* strain LBA4404 carrying either the binary vector pBinAR- *palI* or the binary vector pBin33-Kan contains sucrose isomerase gene (*palI*) fused to the signal peptide of proteinase inhibitor II gene (SP), isolated DNA was quantified and amplified by PCR using the specific primers. The quality of the plant DNA was confirmed by a positive control PCR reaction. Sixty putatively transformed plants were tested for *palI* gene. PCR analysis revealed that putative potato transformed plants displayed expected 1803 bp size band (size 1694 bp of *palI* gene and size 109 bp of signal peptide of proteinase inhibitor II gene) as shown in (Fig.8). The genomic DNA from non transformed control potato plants did not appear any band in PCR reaction. Transformation efficiency (TE) was calculated as percentage number of PCR positive events compared with

the total number of regenerated plants infected with *A. tumefaciens* carrying the binary vector pBinAR- *palI*. Analysis of variance showed that the highest observed 32.8% against *palI* gene in potatocv. Désirée. This result is in agreement with the findings of Beaujean *et al*. (1998), Gustafson *et al*. (2006) and Molla *et al*. (2011).

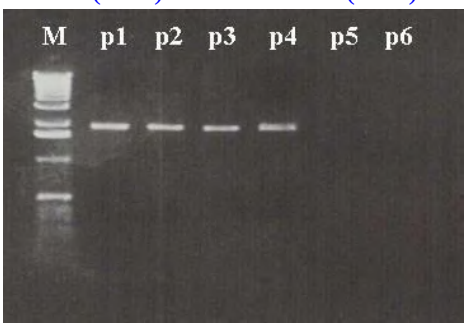


*Fig.8: PCR analysis revealed that putative potato transformed plants displayed expected 1803 bp size band (size 1694 bp of palI gene and size 109 bp of signal peptide of proteinase inhibitor II gene). Lane M: DNA marker (1kb plus DNA ladder), Lanes (p1-p4): transformed potato plants, Lanes (p5-p6): nontransformed plants.*

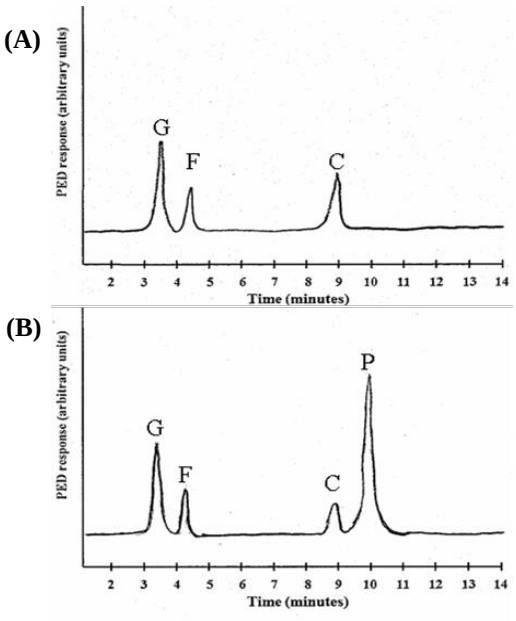


*Fig.9: HPLC analysis of soluble carbohydrates of tuber extracts of potato cv. Désirée (A) tuber extract from nontransgeic plants, and (B) tuber extract from transgenic plants. S; sucrose, P; palatinose, G; glucose, F; fructose, PED; pulsed electrochemical detector.*

**Expression of sucrose isomerase (*palI*) gene in transgenic potato plants.**

Potato transgenic plants were transformed with *Agrobacterium*harbouring recombinant binary vector plasmid pBinAR- *palI* contains sucrose isomerase gene (*palI*) fused to proteinase inhibitor II signal sequence (SP) under CaMV-35S promoter and Octopine synthase (*OCS*) terminator (Börnke *et al*., 2002). 60 regenerated potato shoots were screened for expression of *palI* protein *in vitro* plantlets by Western Blotting assay using a polyclonal antibody raised examined leaves of the palI-expressing potato. Total proteins were descriptively isolated by SDS-PAGE and blotted the protein with antibodies specific for *palI*-proteins. Our results showed that palI protein was expressed in all samples of transgenic potato plants compared to the nontransgenic potato control. This is in contrast to results obtained with constitutive expression of *palI* in transgenic potato plants. The detection of *palI*protein can be performed using any part of potato plantlets, which was expressed mainly under the regulation of the CaMV-35S promoter. In addition the antibody obtained will provide a basic detector of genetically modified potato plants. Rui-juan*et al*. (2016) used western blot assay for detection PMI protein in genetically modified rice and showed that PMI was expressed in all samples except anther, indicating the constitutive expression of PMI in genetically engineered rice.

**HPLC analysis**

Sucrose isomerase expression under the control of the tuber-specific patatin class I B33 promoter leads to *in vivo* conversion of sucrose into palatinose, tuber extracts from potatocv. Désiréewere analyzed for their soluble carbohydrate composition using HPLC. The chromatograms assay indicated that there was an additional major peak that was not present in the control and compared to the standard this peak could be set to palatinose(Fig.9). An additional minor peak eluted close to the sucrose signal in extracts from transgenic tubers.Quantitative analysis of non-structural carbohydrates of transgenic tubers has shown accumulation of palatinose in the range of 2.4 mol $g^{-1}$ FW to 19.5 mol $g^{-1}$ FW while, sucrose and glucose content was only 1.4 mol $g^{-1}$ FW and 0.48 mol $g^{-1}$ FW, respectively, whereas the non-transgenic tubers contained 16.8 mol $g^{-1}$ FW sucrose and 4.85 mol $g^{-1}$ FW glucose. The conversion of sucrose into palatinose drastically affected the sucrose and glucose content of transgenic tubers. These results indicate almost quantitative conversion of sucrose into palatinose via *palI* expressing potato tubers. These results are compatible withRocha-Sosa *etal*. (1989), they have the sucrose isomerase gene expression under the control of the tuber-specific patatin class I B33 promoter in transgenic potato plants. This protein was combined with the signal peptide of the proteinase inhibitor II, which governs secretion of the enzyme into the apoplasmic space (Von Schaewen *et al*., 1990).The patatin class I B33 promoter it seems inactive during early tuberization (Tauberger *et al*., 1999). The apoplasmic localization of the sucrose isomerase leads to accumulation of palatinose. This indicates the presence of sucrose within the apoplast even in later stages of tuber development which have been interpreted as a result of

leakage of recipient receptor cells rather than an obligatory step in phloem unloading (Oparka *et al*., 1992). This is consistent with the results obtained from transgenic potato tubers expressing yeast invertase within apoplast where a reduction in sucrose content was observed accompanied by increased palatinose content (Sonnewald *et al*., 1997; Hajirezaei *et al*., 2000).

## IV. CONCLUSION

The results demonstrate that*in vitro* regeneration on leaf explants cv. Désirée, in a single step regeneration procedure on MS medium supplemented with 0.1 mg $L^{-1}$ IAA and 3.0 mg $L^{-1}$ZR and shoots regenerated directly from explants without an intervening callus stage. Microtuber production formed on MS medium containing 10% sucrose with 1.0 mg $L^{-1}$ BAP and 0.5 mg $L^{-1}$ kinetin under dark condition. Also, we were able to genetically modified potato plants to produce non-cariogenic, low-calorie sucrose isomer palatinose. Palatinose production was achieved by expression of a sucrose isomerase isolated from *Erwinia rhapontici*. Sucrose isomerase overexpression construct contains the coding region of the *palI* gene fused to proteinase inhibitor II signal sequence under CaMV-35S promoterof screening for expression of *palI* protein *in vitro* plantlets by Western Blotting using a polyclonal antibodyalso, our use tuber-specific patatin class I B33 promoter leads to in vivo conversion of sucrose into palatinose with potato cv. Désirée tubers, tuber extracts from potatowere analyzed for their soluble carbohydrate composition using HPLC.

## ACKNOWLEDGEMENTS

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# Geochemical Partitioning of Some Heavy Metals in Bottom Sediment of River Delimi in Jos, Nigeria

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***Abstract—*** *The determination of total metal content is usually insufficient to fully assess the environmental impacts of contaminated sediments. In order to differentiate metals of lithogenic from those of anthropogenic origin and assess their bioavailability and potential toxicity, detail information on their partitioning to various geochemical fractions of the sediment is necessary. In recent times there has been a lot of concern on the rate at which River Delimi ecosystem deteriorate as it passes through Jos city. This study was aimed at determining the geochemical partitioning of Cd, Cu, Pb and Zn in River Delimi sediment with a view to determining the extent to which they might be remobilized and affect the quality of the river ecosystem. The study was conducted at three sites along River Delimi and one control site at Lamingo Dam all within Jos city. The total metals content in sediment were extracted using aqua-regia. A five-step sequential extraction procedure was used to determine the partitioning of the metals into different geochemical fractions of the sediment. The metals extracted were determined using Atomic Absorption Spectrophotometer (AAS). The total metals content in sediment were generally higher at the study compared to the control site. The mean values of Cd in sediment (1.330mg/kg, 1.515mg/kg, 1.301mg/kg and 0.900mg/kg) respectively for stations I, II, III and IV were all above the limit of 0.68mg/kg recommended by USEPA. Sediment samples obtained from River Delimi had more metals associated with the non-residual fractions compared to those from Lamingo Dam. The high amount of these metals recovered in non – residual fractions mean that the metals are in potentially available forms and could pose a serious threat to the river ecosystem. Measures should therefore be put in place by the relevant authorities to curtail indiscriminate dumping of domestic and industrials wastes into the river.*

***Keywords— Bioavailability, Heavy metals, Partitioning, Sediment, Toxicity.***

## I. INTRODUCTION

The excess accumulation of heavy metals in sediment could have significant impacts on the river water quality and on the local communities. Studies on the distribution of heavy metals in sediment can provide evidence on human impact inflicted on the aquatic ecosystem and will in a way assist in determining the risk associated with dumping of domestic wastes and discharge of industrial effluents into the ecosystem [1].

It should be noted however that determining the total metal content is usually insufficient to fully assess the environmental impacts of contaminated sediments [2]. Detail information on the geochemical partitioning of the metals in sediments as pointed out by [3] is necessary to fully understand the different sediment sources, element distribution pattern and evaluating the environmental conditions existing in an area.

Metal partitioning into various geochemical fractions is also very useful for determining the extent to which they might be remobilized into the environment as well as differentiating metals of lithogenic from those of anthropogenic origin [4]. Another report by [5] states that delineating various forms of elements and their speciation in sediments is essential for assessing their bioavailability and toxicity.

River Delimi is one of the major inland water bodies in Nigeria serving as habitat to numerous species of plants and animals. In recent time however there has been a lot of concern on the rate at which the quality of the river deteriorate due to dumping of industrial and domestic wastes. Earlier studies by [6] revealed that the river water contained excess amount of metal pollutants. However, there is insufficient information on the geochemical partitioning of the metals in the river sediment. This study

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was therefore aimed at determining the partitioning of Cd, Pb, Cu and Zn in various geochemical fractions of bottom sediment obtained from River Delimi with a view to determining the magnitude of human impact on the river ecosystem

## II. MATERIAL AND METHODS

### 2.1 Study Area

The study was conducted at designated points along River Delimi in Jos city (Figure 1) in north-central Nigeria. The city is about 1250mm above sea level located on Lat. 90 52'59''N and Long. 8054'26''E. The average monthly temperatures range between 21 and 25$^0$C. The mean annual rainfall ranges from 2000 – 3250 mm between May and September [7]. River Delimi is located in the north central and north eastern parts of Nigeria. It mainly originates from Delimi village located few kilometers to the southern outskirt of Jos Plateau State. The river is characterized by low base flows in dry season and relatively high peak flows in rainy season, thus responds very quickly to rainfall in its catchments' area. High peak flow results in flooding of the river, which is mainly due to the geomorphological composition of the river system. The river covers a distance of about 15 km as it passes through Jos and serves as the major source of water for vegetable crop irrigation in the city.

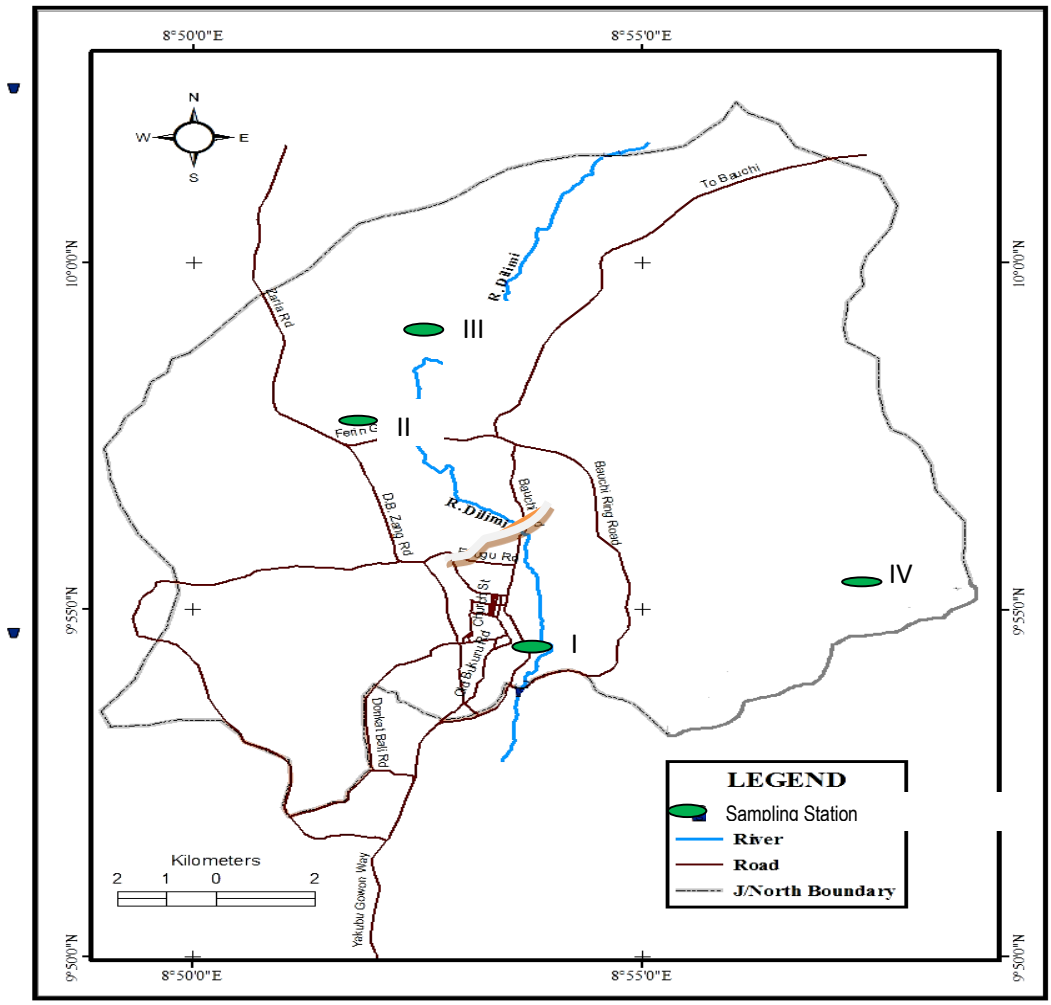


*Fig.1: Map of Jos metropolis showing the sampling stations. Source: University of Jos (2012)*

### 2.2 Sampling strategy

Sediment samples (top 10cm) were collected using soil auger at 3 different sampling stations along the river (study site) as follows:

- Station I = Gangare (Lat. 90 53' 22''N, Long. 80 52'57''E )
- Station II = Farin Gada (Lat. 90 57'36''N, Long. 80 52'27''E )
- Station III = University of Jos main campus (Lat. 90 58'52''N, Long. 80 52'54''E )
- Station IV (Control Site) = Lamingo Dam (Lat. 90 53'07'' Long. 80 54'55'')

**2.3 Samples Preservation and Pretreatment**

Sediment samples were collected in a polythene bags and kept on ice. The samples were subsequently transported to the laboratory and kept at low temperature ($4^0$C). Sediment samples meant for total metal analysis were placed in porcelain crucibles and oven dried at 80°C for 24 hours. The dried samples were ground using a previously acid washed porcelain mortar and pestle. The samples were then kept in desiccators to attain constant weights before being transferred into air-tight plastic bottles. All the samples were sieved with a 200µm sieve before metal analysis.

**2.4 Analysis of Sediment Samples**

**2.4.1 Determination of pH, Organic Matter and Total Metal Content in Sediment**

Sediment pH was determined using a pH meter after shaking 5g of each sample with 5ml water and allowed to stay overnight. The organic matter content was determined by Loss on Ignition (LoI) using 5g each of soil and sediment samples heated at 450°C for I hr [8]. The total metal concentrations were determined using Atomic Absorption Spectrophotometer (AAS) after digestion with Aqua-regia.

**2.4.2. Geochemical partitioning of metals in sediment**

1g each of air dried sediment samples were extracted in accordance with Tessier's extraction scheme [9]. At the end of each extraction step, the samples were centrifuged for 10 minutes and the supernatant decanted, filtered and analyzed for metal content using AAS. Each step was followed by a wash stage in which 8ml of deionized water was added to the residue and centrifuged for 10 minutes to remove any trace of previous extracting solution and the supernatant discarded. The extractions were carried out as follows;

**2.4.2.1 Exchangeable fraction:**

1g each of the air dried sediment samples was leached with 8ml of 1M $MgCl_2$ at pH 7 (adjusted with NaOH) for 1hr with continuous shaking using mechanical shaker. The solution was then filtered using a Whatman filter paper No. 1 into a 100ml volumetric flask. The filtrate was made up to the mark with distilled water.

**2.4.2.2 Carbonate bound fraction**

8ml of NaOAc at pH 5 (adjusted with acetic acid) was added to the residue obtained from exchangeable fraction and leached for 5hrs at room temperature with continuous agitation using mechanical shaker. The leachate was filtered and transferred into a 100ml conical flask and made up to the mark with distilled water.

**2.4.2.3 Iron-Manganese Oxide fraction**

To the residue obtained from the carbonate fraction, 20ml of 0.04M $NH_2OH.HCl$ (Hydroxylamine hydrochloride) in 25% acetic acid (v/v) was added. The sample was placed in a water bath at $90^O$C and leached for 6hrs with occasional shaking. The leacheate was then transferred into a 100ml volumetric flask and made up to the mark with distilled water.

**2.4.2.4 Organic bound fraction**

To the residue obtained from the Iron-Manganese fraction, 3ml of 0.02M nitric acid ($HNO_3$) and 5ml of 30% hydrogen peroxide ($H_2O_2$) at pH 2 (adjusted with $HNO_3$) were added. The mixture was heated in water bath at $85^O$C for 2hrs with occasional shaking. 9ml of 30% $H_2O_2$ at pH 2 was further added and the mixture continuously heated at $85^O$C for 3hrs. Another 9ml of 3.2M ammonium acetate $CH_3COONH_4$ in 20% (v/v) $HNO_3$ was then added and the mixture shaken for 0.5hr at a low temperature of about $25^O$C. The supernatant was filtered and transferred into a 100ml volumetric flask and made up to the mark.

**2.4.2.5 Residual fraction**

The concentration of metals in the residual fraction of the sediment and soil was calculated by subtracting the concentration in the first four fractions from the total content previously determined by the aqua-regia digestion [8].

**2.5 Quality control/Assurance**

Sediment samples were collected with plastic bags to avoid contamination. Samples were kept in polythene bags well covered while transporting them from field to the laboratory to avoid contamination from the external environment. Reagent blanks were used in all to check reagent impurities and other environmental contaminations during analysis [10]. Analytical reagent (AnalaR) grade chemicals and distilled water were used throughout the study. All glassware and plastic containers used were washed with detergent solution followed by 20% (v/v) conc. $HNO_3$ acid and then rinsed with water and finally with distilled water [11]. All the instruments used were calibrated before use. Tools and work surfaces were carefully cleaned for each sample during grinding to avoid cross contamination [10].

**2.6 Statistical analysis**

Tests for significant difference were carried out using the analysis of variance (ANOVA) of the Statistical Package for Social Sciences (SPSS) computer programme. Means were separated using the Tukey test.

## III. RESULTS AND DISCUSSION

**3.1 Sediment Analysis**

**3.1.1 pH, Organic Matter and Total Metal Content in Sediment**

The mean values of pH and OM (%) and total metal content in sediment obtained from the four stations at River Delimi and Lamingo Dam within Jos metropolis are shown in Table 1.

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*Table 1 Mean pH, Organic Matter (OM) and total metal contents (mg/kg) in sediment from the four sampling stations at River Delimi and Lamingo Dam in Jos city.*

| STATION | pH | OM | Cd | Cu | Pb | Zn |
|---|---|---|---|---|---|---|
| **I** | 6.5[c] | 4.5[c] | 1.330(±0.390)[a] | 3.701(±0.767)[a] | 1.840(±1.775)[a] | 3.558(±0.537)[a] |
| **II** | 6.6[c] | 4.6[c] | 1.515(±0.350)[a] | 3.966(±1.844)[a] | 1.673(±1.111)[a] | 4.390( ±0.377)[a] |
| **III** | 6.6[c] | 4.1[c] | 1.301(±0.371)[a] | 1.898(±0.947)[b] | 2.053(±1.886)[a] | 2.680(±0.477)[b] |
| **IV** | 7.3[t] | 4.9[c] | 0.900(±0.314)[b] | 1.983(±0.176)[b] | 1.111(±0.793)[a] | 2.405(±0.645)[b] |
| **USEPA LIMIT** | - | - | 0.68 | 18.70 | 30.20 | 124.00 |

Means followed by same letter within column are not statistically different at 95% level of confidence

Data analysis revealed that there was a significant variation in the mean pH values of sediment samples collected at the study compared to the control site. Thus, a significantly higher value of 7.3 was recorded for sediment samples obtained from station IV compared to statistically similar value of 6.5 for station I and 6.6 each for stations II and III.

The acidic nature of the sediment at the study site is an indication that the metals can easily be desorbed from the sediment into the water column making them more bioavailable to biota. The high pH value (above 7) at the control site means that the metals cannot be easily desorbed from the sediment to the water phase and therefore less bioavailable to biota.

There was no significant variation in the mean OM content in sediments collected from the various sampling stations. The mean values of 4.5%, 4.6%, 4.1% were recorded for samples collected from stations I, II and III respectively (study site), while station IV (control site) had a mean OM value of 4.9%. However, the relatively higher organic matter content in the sediment at the control station means that the metals are less likely to be released into the water column due to formation of metal-organic matter complex. Organic matter in the sediment is known to play an important role in the adsorption and retention of heavy metals.

**3.1.2 Total Metal Contents in Sediment**

Sediment samples collected at stations I, II and III had statistically similar ($p > 0.05$) mean Cd values of 1.330mg/kg, 1.515mg/kg and 1.301mg/kg respectively. These values were significantly higher ($p \leq 0.05$) than 0.900mg/kg recorded in samples collected at station IV.

There was significant variation ($p \leq 0.05$) in the mean Cu concentration in sediment obtained from the various sampling stations. The mean Cu concentrations in sediment obtained from stations I (3.701mg/kg) and II (3.966mg/kg) were statistically similar ($p > 0.05$), but significantly higher ($p \leq 0.05$) than 1.898mg/kg and 1.983mg/kg obtained from stations III and IV respectively.

No significant variation ($p \leq 0.05$) was observed in the mean Pb concentrations in sediment samples collected at the various sampling stations in the study as well as the control sites. The mean values of 1.840mg/kg, 1.673mg/kg and 2.053mg/kg recorded in sediment samples obtained respectively at stations I, II, and III and 1.111mg/kg recorded at station IV were all statistically similar ($p > 0.05$).

The concentrations of all the metals were generally at their maximum in the study site compared to what were obtained in the control site. This corroborates the findings of [12] who collected sediment samples from Yamuna River which passes through Delhi and Agra urban centers and analysed them for concentration and distribution of nine heavy metals and obtained the following results; Cr (157–817 mg/kg), Mn (515–1015 mg/ kg), Fe (28,700–45,300 mg/kg), Co (11.7–28.4 mg/kg), Ni (40–538 mg/kg), Cu (40–1204 mg/kg), Zn (107–1974 mg/kg), Pb (22–856 mg/kg) and Cd (0.50–114.8 mg/kg). The levels of metals were compared with the average shale concentration and showed exceptionally high values for chromium, nickel, copper, zinc, lead and cadmium in the two urban centers. The authors concluded that high levels of these metals were found in sediments obtained from the urban centers because they received domestic and industrial wastes. Another analysis conducted by [13], however reported the detection limit of heavy metals in sediment of two freshwater lakes as 0.02 mg/kg for Cd, 0.36 mg/kg for Cu, 0.48 mg/kg for Pb, and 7.8 mg/kg for Zn which were below the values obtained in this investigation except for Zn mean concentration. The lower values were obtained by the author because the lake is located at a site far away from urban environment and therefore has less anthropogenic influence. A comparison of the mean metal concentrations obtained in this study with [14] threshold effect limits (Cd, 0.68mg/kg; Cr, 52.30mg/kg; Cu, 18.70mg/kg; Pb, 30.20mg/kg and Zn, 124.00mg/kg) in sediment revealed that the concentrations of all the metals under investigation except Cd falls within the recommended threshold limits.

**3.2 Geochemical partitioning of metals in sediment**

**3.2.1 Partitioning of Cadmium in sediment**

In sediment samples obtained at station I, II, and III of the study site, Cd was mostly concentrated in exchangeable phase, while in station IV – the control site the metal was

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mostly found in residual fraction (Figure 1). Mean values of 38.3%, 14%, 7.1%, 15.5% and 25.1% were recovered from exchangeable, carbonate, Fe/Mn oxide, orgranic and residual fractions respectively in sediment samples collected from station I. The percentage recoveries of the metal at station II were; exchangeable (32.4%), carbonate (9.0%), Fe/Mn oxide (9.6%), organic (19.6%) and residual (29.4%). At, station III, the metal behaved in a similar manner with the exchangeable fraction having the highest metal load of 44.7% recovery, The percent recoveries in carbonate, Fe/Mn oxide, organic and residual fractions were 9.1%, 5.6%, 12.2% and 28.4% respectively. On the contrary however, a greater portion of Cd (47.0%) in sediment at station IV (control site) was found associated with the residual and the least (7.7%) with the exchangeable fractions. Partitioning to other fractions were carbonate (13.4%), Fe/Mn (9.9%) and organic (22.0%).

The binding of Cd to the non-residual fractions especially the exchangeable and organic phase is generally reflective of sites with anthropogenic contamination. This corroborates the findings of [15] who opined that Cd is generally a mobile and bioavailable element that tends to preferentially accumulate more in exchangeable fraction. At station IV – the control site however much more (47.0%) Cd was recovered in residual fractions. The Cd at this site is therefore not readily available since in geochemical partitioning, metals bound to residual fraction are notably fixed within the crystal lattice and are usually considered to be of primary mineral phase. A substantial amount (22.0%) of this metal was also found associated with organic phase of the non-residual fractions. This further reduces its bioavailability since metals bound to this fraction are only released under low pH (oxidizing condition) and the sediment pH value (7.3) at this station is relatively high.

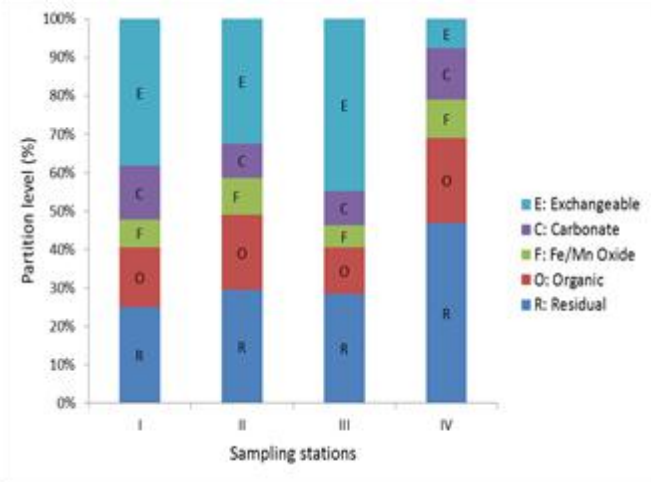


*Fig.1: Partitioning of cadmium into different geochemical fractions of sediment obtained from four sampling stations along River Delimi and Lamingo Dam in Jos metropolis*

**3.2.2 Partitioning of Copper in sediment**

The partitioning of Cu in sediment is as presented in Figure 2. At station I, Cu was mainly concentrated in residual fraction with mean value of 27.8%. This is closely followed by 22.2% recovered from exchangeable fraction. The carbonate bound, Fe/Mn oxide and carbonate fractions had 13.4%, 18.2% and 18.4% mean recoveries respectively. The dominant fraction with respect to the distribution of Cu in sediments obtained from station II is exchangeable with mean value of 37.3%. The lowest value (12.2%) was however recovered in carbonate phase. The mean recovery values of 15.9%, 13.8% and 20.8% were obtained in Fe/Mn oxide, carbonate bound and residual fractions respectively. The metals partitioning in sediment at station III is such that highest mean value of Cu (34.4%) was obtained in residual while the least was recorded in the exchangeable phase with mean value of (13.3%). The carbonate, Fe/Mn oxide and organic fractions had 16.6%, 13.5% and 22.2% mean metal recoveries respectively. The distribution of the metal at station IV was equally dominated by the residual fraction. The mean recovery were exchangeable (10.2%), carbonate (18.3%), Fe/Mn oxide (16.3%), organic (17.2%) and residual (38.0%).

The partitioning of Copper in the sediment samples obtained from the study site revealed a somewhat proportionate distribution of the metal in the various geochemical fractions although the metal tends to accumulate more in non – residual fractions especially the easily exchangeable fraction at stations I and II and organic fraction at station III. At station IV, the residual fraction had 38% of the total metal concentration with the remaining portion distributed among the non-residual fractions especially carbonate, Fe/Mn oxides and organic fractions. The metal in the sediments at stations I, II and III is easily available while at station IV it is only available under specific environmental conditions. For example, under reducing condition, the metal in Fe/Mn oxide can be released. The oxidizing condition however will lead to the release of those portions associated with organic fraction. However since the pH value of sediment at this sampling station is 7.3, the release of Cu will be more in the portion associated with the Fe/Mn oxide fraction.

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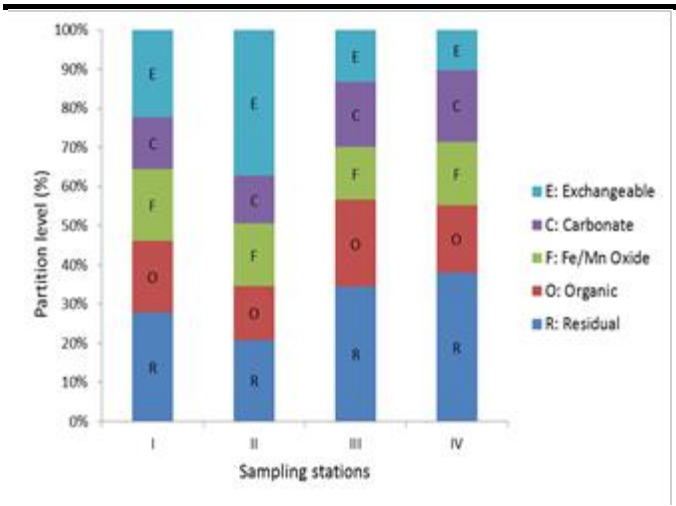


*Fig.2: Partitioning of copper into different geochemical fractions of sediment obtained from four sampling stations along River Delimi and Lamingo Dam in Jos metropolis.*

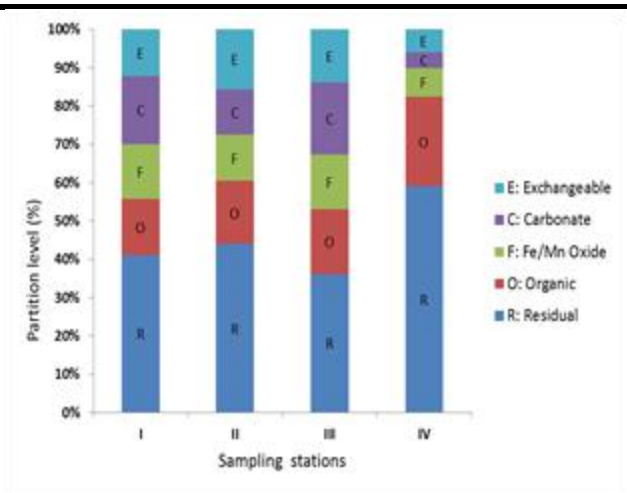


*Fig.3: Partitioning of lead into different geochemical fractions of sediment obtained from four sampling stations along River Delimi and Lamingo Dam in Jos metropolis*

### 3.2.3 Partitioning of Lead in sediment

At station I, residual was the dominant fraction for Pb (mean = 40.9%), while the exchangeable was the least (mean = 12.8%). The carbonate, Fe/Mn oxide and organic fractions had 17.2%, 14.3% and 18.4% mean Pb recoveries respectively (Figure 3).There was a similar higher mean Pb recovery (43.9%) from residual fraction at station II, however the least value (12.0%) was recorded in Fe/Mn oxide fraction. The mean concentrations in exchangeable, carbonate and Organic fractions were 15.7%, 11.9% and 16.5% respectively. Relatively higher mean Pb value (36.1%) was recovered from residual at station III. The next important fractions were carbonate and organic fractions having 18.9% and 17.0% mean metal recoveries respectively. The mean concentrations recovered in the other fraction were exchangeable (13.8%) and Fe/Mn oxide (14.2%). The residual fraction also dominated the distribution of the metal at station IV with mean recovery of 59.1%. The exchangeable, carbonate, Fe/Mn oxide and organic fractions had 6.0%, 4.1%, 7.5% and 23.3% mean values respectively.

The substantial amount recovered in the non – residual phase also confirmed that there has been a lot of anthropogenic interference on the sediment quality. At the control site however, the metal tends to be partitioned predominantly into the residual and organic fractions with the exchangeable accumulating the least amount. The metal at station IV may be considered to be less mobile because of its preference to the residual fraction and its high stability constants for the formation of lead–organic matter complexes.

### 3.2.4 Partitioning of Zinc in sediment

Zn partitioning in sediment samples (collected from both study and control sites) is dominated by residual fraction (Figure 4). At station I, the result showed that residual had the highest mean Zn value of 28%, followed by 23.8% in exchangeable, 18.2% in Fe/Mn oxide, 15.6% in organic and the least 14.4 recovered in carbonate fraction. The portioning at station II revealed a higher mean recovery value of 34.5% in residual, followed by 22.3% in exchangeable, 15.9% in organic and the least values of 13.4% each in carbonate and Fe/Mn oxide. At station III, greater portion (50.7%) of Zn was present in residual while the non-residual fractions exchangeable, carbonate, Fe/Mn oxide and organic fractions had 8.6%, 11.1%, 13.7% and 15.9% mean concentrations respectively. Residual was also the dominant fraction in the distribution of Zn at station IV. The mean metal concentrations in ascending order were exchangeable (7.4%), carbonate (12.6%), organic (12.8%), Fe/Mn oxide (15.2%) and residual (52.0%).

The partitioning of Zn follows almost the same pattern to that of Pb with the non-residual fraction dominating the distribution of the metal, although unlike Pb, substantial amount of Zn was also found in exchangeable fraction of the non – residual phases especially at stations I and II. It is not surprising that there was high accumulation of Zn in exchangeable fraction at stations I and II considering the fact that the two stations had the busiest human activities notably mechanic workshops and other metal works.

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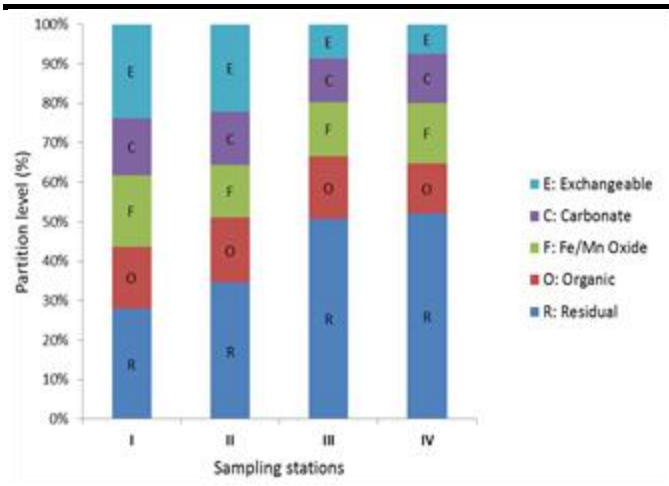


*Fig.4: Partitioning of Zinc into different geochemical fractions of sediment obtained from four sampling stations along River Delimi and Lamingo Dam in Jos metropolis*

## IV. CONCLUSION

The result of this investigation revealed that high concentrations of Cd, Cu, Pb and Zn where recovered in bottom sediment obtained from River Delimi compared to the control site. Partitioning of these heavy metals into different geochemical fractions of sediment also revealed that sediment samples obtained from the River Delimi had more metals associated with non-residual mobile fractions than those from the control sites. This suggests that there are more anthropogenic and potentially available metal species in sediments obtained from River Delimi compared to the control site. This may not be unconnected to the indiscriminate dumping of domestic and industrial wastes into the Delimi River contrary to the situation at Lamingo Dam, the control site where no intentional dumping of such wastes occurs being a protected drinking water reservoir. It is recommended that measures should be put in place by the Plateau State Environmental Protection Board and other relevant government agencies to check the unnecessary dumping of domestic and industrial waste into the Delimi River.

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# Air Quality Changes and Geospatial Dispersion Modeling in the Dry Season in Port Harcourt and its Environs, Niger Delta, Nigeria

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***Abstract—*** *This work is a geospatial analysis of air dispersion for the purpose of establishing the concentration trend of air pollutants within the study area. Pollutants of consideration are $SO_2$, $NO_2$, CO, $H_2S$, $NH_3$, VOCs, $CH_4$, $PM_{2.5}$, $PM_{10}$, TSP, while area of interest are Port Harcourt, Obio/Akpor, Eleme, Oyigbo, Etche and Ikwerre Local Government Areas. This is an approach to identify the hotspots and how they are dispersed to impact on other parts of the region. This is an attempt to predict future pollution trends, but an approach to gain understanding of the general scenarios of air quality status and how they impact on receptor areas some kilometers away from the hotspot. Pollution hotspots are locations where emissions from specific sources such as water or air pollution may expose local populations to elevated health risks and environment degradation. It indicates areas with strong pollution sources and high industrial activities of adverse effect*

***Keywords— Air Quality Changes, Dry Season, Geospatial, IPPC, Hotspots, Modeling.***

## I. INTRODUCTION

Pollution is defined by the European Union 1996 Council Directive on Integrated Pollution Prevention and Control (IPPC) as "the direct or indirect introduction as a result of human activity, of substances, vibrations, heat or noise into the air, water or land which may be harmful to human health or the quality of the environment, result in damage to material property, or impair or interfere with amenities and other legitimate uses of the environment". Inorganic and organic air pollutants cause negative health and environmental effects such as respiratory ailments, premature deaths. Air pollution-related deaths worldwide are estimated to be up to 2 million per annum.

Other environmental consequences of air pollution include acidification of soil and water and loss of plant and animal life. Air quality assessment studies in Nigeria have focused mainly on urban centres where industrial processes, domestic activities and traffic congestion constitute major sources of air pollution.

Most of these studies are independent as there are no systematic measurements of air quality public agencies. Meteorological parameters influence aids to drive the air pollutants from the pollutant hotpots to non-sources areas (Antai *et al.,* 2017, Everitt, 1992 and Esplin, 1995). This is the trend that has put man in alert in his own environment since the air has no boundary or barrier from one man to another.

The aim of this research is to assess changes in existing physical and chemical characteristic of the air quality and to determine the hotspots and assess the level of concentration of air pollutants dispersion trends in the study area. Results of geospatial analysis and generalized additive models revealed that sources of pollutants in the study areas are both localized in the up-land area and the region around the coastal area.

### Description of the Study Area

### Location

Port Harcourt metropolis is located between latitudes $4^0$35' and $5^0$30' North and between longitudes $6^0$54' and $7^0$08' East. It covers an estimated area of 1811.6 square kilometer. Port Harcourt, the capital of Rivers State, was established in 1914 by the British colonial administration under Lord

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Lugard to meet the pressing economic needs of the Europe the city, which lies at the heart of the Niger Delta, is one of the world's richest wetlands and is bounded on the south by the Atlantic Ocean, to the North by Imo and Abia States, to the East by Akwa Ibom State and to the West by Bayelsa and Delta States respectively. The spatial coverage of this study extends through beyond in Port Harcourt and its environs, Port Harcourt, Obio/Akpor, Eleme, Oyibo, Ikwerre and Etche Local Government Areas.

*Table.1: Sampling Points Key, Description of Sampling Points, Coordinate and Frequency of Monitoring*

| Sampling Point Code: | Description of Sampling Points | Coordinates | Frequency of Monitoring/Hourly |
|---|---|---|---|
| SP 1 | Onne Roundabout by FLT and FOT Signboard | N $04^0$ 43'. 207'' E $007^0$ 09'.478'' | Morning, Afternoon and Evening |
| SP 2 | Notore Road by Notore Garden Camp, Onne | N $04^0$ 44'. 147'' E$007^o$ 08'. 526'' | Morning, Afternoon and Evening |
| SP 3 | Onne (Trailer park) Junction by East-West Road | N $04^0$ 45'. 510'' E $007^0$ 09'. 516'' | Morning, Afternoon and Evening |
| SP 4 | Port Harcourt Refinery Junction by East-West Road, Alesa | N $04^0$ 47'. 066'' E $007^0$ 07'. 001'' | Morning, Afternoon and Evening |
| SP 5 | Agbonchia by Zina Motel Junction Eleme | N $04^0$47'. 867'' E $007^0$ 07'. 358'' | Morning, Afternoon and Evening |
| SP 6 | Eleme Petrochemical (Ndorama gate) Aleto, Eleme | N $04^0$ 48'. 744'' E $007^0$ 05'. 842'' | Morning, Afternoon and Evening |
| SP 7 | Sandfilled Roundabout, Akpajo | N $04^0$ 49'. 402'' E $007^0$ 05'. 276'' | Morning, Afternoon and Evening |
| SP 8 | Okoloma Afam by Afam Power Plant | N $04^0$ 51'. 058'' E $007^0$ 15'. 088'' | Morning, Afternoon and Evening |
| SP 9 | Izuoma Asa by Dominican College | N $04^0$ 51'. 458'' E $007^0$ 10'. 717'' | Morning, Afternoon and Evening |
| SP 10 | Oyigbo by Eke Oyigbo Market | N $04^0$ 52'. 561'' E $007^0$ 08'. 889'' | Morning, Afternoon and Evening |
| SP 11 | Oyigbo Junction by Port Harcourt-Aba Road | N $04^0$ 52'. 852'' E $007^0$ 07'. 959'' | Morning, Afternoon and Evening |
| SP 12 | Shell Flow Station/Location Junction, Umuebulu 4, Etche | N $04^0$ 53'. 6107'' E $007^0$ 07'. 302'' | Morning, Afternoon and Evening |
| SP 13 | Shell Gas Plant, Umuebulu 4, Etche | N $04^0$ 53'. 674'' E $007^0$ 07' 129'' | Morning, Afternoon and Evening |
| SP 14 | Igbo Etche Junction-Umasikpo, Igbo Etche | N $04^0$ 56'. 788'' E $007^0$ 04'. 94'' | Morning, Afternoon and Evening |
| SP 15 | Eleme Junction by Oilmill Bus Stop | N $04^0$ 51'. 267'' E $007^0$ 03'. 843'' | Morning, Afternoon and Evening |
| SP 16 | Rumukrushi Bus Stop by Rumukrushi Park | N $04^0$ 50'. 992'' E $007^0$ 03'. 201'' | Morning, Afternoon and Evening |
| SP 17 | Rumukrushi Tank by East-West Road | N $04^0$ 51'. 859'' E $007^0$ 03'. 364'' | Morning, Afternoon and Evening |
| SP18 | Eneka Roundabout, Rumu-olukwu, Eneka | N $04^0$ 53'. 756'' E $007^0$ 02'. 392'' | Morning, Afternoon and Evening |
| SP 19 | Artillery Junction by Okporo Road | N $04^0$ 50'. 612'' E $007^0$ 02'. 298'' | Morning, Afternoon and Evening |
| SP 20 | Rumuobiakani Junction by Oginigba/Old Aba | N$04^0$ 50'. 208'' E $007^0$ 02'. | Morning, Afternoon and |

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| | | | |
|---|---|---|---|
| | Road | 066'' | Evening |
| SP 21 | Rumuomasi in- Between Aba Road Rumuomasi Junction and Old Aba Road | N $04^0$50'. 236'' E $007^0$ 01' 548'' | Morning, Afternoon and Evening |
| SP 22 | Rumuodara Junction by East-West Road | N $04^0$ 51'. 622'' E $007^0$ 01' 776'' | Morning, Afternoon and Evening |
| SP 23 | Eliozu Flyover Junction | N $04^0$ 51'. 570'' E $007^0$ 01'. 307'' | Morning, Afternoon and Evening |
| SP24 | Rukpokwu Roundabout | N $04^0$ 53'. 447'' E $007^0$ 00'. 140'' | Morning, Afternoon and Evening |
| SP 25 | Igwuruta Roundabout by Air Port Road | N $04^0$ 57'. 400'' E $007^0$ 00'. 690'' | Morning, Afternoon and Evening |
| SP 26 | Port Harcourt International Air Port Junction/Roundabout Omagwa | N $04^0$ 58'. 858'' E $006^0$ 56'. 989'' | Morning, Afternoon and Evening |
| SP 27 | Greater Port Harcourt in-Between Air Port and Obirikwere Road by H and H Engineering Ltd | N $04^0$ 57'. 421'' E $006^0$ 56'. 965'' | Morning, Afternoon and Evening |
| SP 28 | Aluu Roundabout, Aluu | N $04^0$ 56'. 019'' E $006^0$ 56'. 547'' | Morning, Afternoon and Evening |
| SP 29 | Choba Junction, By Uniport East-West Road | N $04^0$ 53'. 917'' E $006^0$ 54'. 400'' | Morning, Afternoon and Evening |
| SP 30 | Rumuosi Junction by East-West Road | N $04^0$ 52'. 951'' E $006^0$ 56'. 461'' | Morning, Afternoon and Evening |
| SP 31 | Nkpolu Junction by East-West Road | N $04^0$ 52'. 158'' E $006^0$ 58'. 862'' | Morning, Afternoon and Evening |
| SP 32 | Rumuokoro Junction/Roundabout | N $04^0$ 52'. 612'' E $006^0$ 59'. 855'' | Morning, Afternoon and Evening |
| SP 33 | Wimpy Junction by Ikwere Road | N $04^0$ 49'. 835'' E $006^0$ 58'. 924'' | Morning, Afternoon and Evening |
| SP 34 | Location Junction by NTA and Ada George Road | N $04^0$ 51'. 137'' E $006^0$ 58'. 516'' | Morning, Afternoon and Evening |
| SP 35 | Rumuokuta Junction/ Roundabout | N $04^0$ 50'. 271'' E $006^0$ 59'. 308'' | Morning, Afternoon and Evening |
| SP 36 | Rumuigbo by Obiwali Junction | N $04^0$ 50'. 843'' E $006^0$ 59'. 421'' | Morning, Afternoon and Evening |
| SP 37 | Akar Base, Saipem Gate, Rumuolumeni | N $04^0$ 46'. 550'' E $006^0$ 58'. 013'' | Morning, Afternoon and Evening |
| SP38 | University of Education's Gate, Rumuolumeni | N $04^0$ 48'. 420'' E $006^0$ 56'. 061'' | Morning, Afternoon and Evening |
| SP 39 | Eagle Island Gate by Illoabuchi T Junction | N $04^0$ 47'. 164'' E $006^0$ 58'. 806'' | Morning, Afternoon and Evening |
| SP40 | Agip Junction by Agip Flyover | N $04^0$ 48'. 825'' E $006^0$ 59'. 018'' | Morning, Afternoon and Evening |
| SP41 | Mile 3 by Mile 3 Park | N $04^0$ 48'. 158'' E $006^0$ 59'. 409'' | Morning, Afternoon and Evening |
| SP 42 | Illoabuchi by Ukuoto Street, Mile 2 | N $04^0$ 47'. 451'' E $006^0$ 59'. 279'' | Morning, Afternoon and Evening |
| SP 43 | Ikoku by Eko Bank | N $04^0$ 48'. 043'' E $006^0$ 59'. 698'' | Morning, Afternoon and Evening |
| SP 44 | Waterline Junction by Olu Obansanjo/Port Harcourt Aba Road | N $04^0$ 49'. 016'' E $007^0$ 00'. 562'' | Morning, Afternoon and Evening |

| | | | |
|---|---|---|---|
| SP 45 | Garrison Junction by Ogunabali/Port Harcourt Aba Road | N $04^0$48' 335'' E $007^0$ 00'. 566'' | Morning, Afternoon and Evening |
| SP 46 | Rumuola Junction/Flyover by Port Harcourt Aba Road | N $04^0$ 49'. 945'' E $007^0$ 00'. 315'' | Morning, Afternoon and Evening |
| SP 47 | Woji by Woji Town Hall | N $04^0$ 49'. 820'' E $007^0$ 03'' 018'' | Morning, Afternoon and Evening |
| SP 48 | YKC Junction, Woji | N $04^0$ 49'. 338'' E $007^0$03'. 228'' | Morning, Afternoon and Evening |
| SP 49 | Elelenwo by Woji T-Junction/Health Center | N $04^0$ 49'. 807'' E $007^0$ 04'. 279'' | Morning, Afternoon and Evening |
| SP 50 | Trans-amadi by Slaughter Junction/ Roundabout | N $04^0$ 48'. 760'' E $007^0$ 02' 688'' | Morning, Afternoon and Evening |
| SP 51 | Gbalajam | N $04^0$ 48'. 713'' E $007^0$04'. 208'' | Morning, Afternoon and Evening |
| SP 52 | Mothercat Junction, Trans-amadi | N $04^0$ 48'. 304'' E $007^0$ 01'. 683'' | Morning, Afternoon and Evening |
| SP53 | Trans-amadi Gas Turbine by Total E & P Back Gate | N $04^0$ 49'. 003'' E $007^0$ 01'. 834'' | Morning, Afternoon and Evening |
| SP 54 | Abuloma Jetty Road by Okuru Link Road, Abuloma | N $04^0$ 46'. 930'' E $007^0$ 03'. 277'' | Morning, Afternoon and Evening |
| SP55 | Sansung Roundabout/Junction by Peter Odilli Road | N $04^0$ 47'. 728'' E $007^0$ 02'. 316'' | Morning, Afternoon and Evening |
| SP 56 | Nkpogu Junction, Trans-amadi | N $04^0$ 48' 570'' E $007^0$ 00'. 994'' | Morning, Afternoon and Evening |
| SP 57 | Eastern By Pass Roundabout/Junction | N $04^0$ 47'. 583'' E $007^0$ 00'. 948'' | Morning, Afternoon and Evening |
| SP 58 | Abonnema Jetty by Shell Kidney Island | N $04^0$ 46'. 583'' E $007^0$ 00'. 315'' | Morning, Afternoon and Evening |
| SP 59 | Tombia Extension by Prof Abowie GRA Phase II | N $04^0$ 49'. 509'' E $006^0$ 59'. 454'' | Morning, Afternoon and Evening |
| SP 60 | King Perekule Junction by Evo street GRA Phase II | N $04^0$49'. 171'' E $007^0$ 00'. 070'' | Morning, Afternoon and Evening |
| SP 61 | Mile 1 Opposite Isaac Boro Park | N $04^0$ 47'. 237'' E $007^0$ 00'. 229'' | Morning, Afternoon and Evening |
| SP 62 | Rumuibekwe by Gram Diagnostic Laboratory | N $04^0$ 50'. 375'' E $007^0$ 03'. 003'' | Morning, Afternoon and Evening |
| SP 63 | Stadium Road by Mummy B Junction | N $04^0$ 49'. 615'' E $007^0$ 00'. 930'' | Morning, Afternoon and Evening |
| SP 64 | BMSH Junction Old GRA | N $04^0$ 46'. 817'' E $007^0$ 00'. 905'' | Morning, Afternoon and Eveningv |
| SP 65 | Moscow Road by NNPC/House of Assembly Complex | N $04^0$ 46'. 159'' E $007^0$ 01'. 166'' | Morning, Afternoon and Evening |
| SP66 | Lagos Bus Stop, Town, Port Harcourt | N $04^0$ 45'. 697'' E $007^0$ 01'. 132'' | Morning, Afternoon and Evening |
| SP 67 | UPE Junction, Borokiri, Port Harcourt | N $04^0$ 44'. 930'' E $007^0$ 02'. 489'' | Morning, Afternoon and Evening |

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| | | | |
|---|---|---|---|
| SP 68 | Nembe Water Side, Port Harcourt | N $0^0$ 45'. 500'' E $0007^0$ 01'. 361'' | Morning, Afternoon and Evening |
| SP 69 | Ibeto Cement Gate, Bundu Ama Estate, Port Harcourt | N $04^0$ 44'. 879'' E $007^0$ 00'. 379'' | Morning, Afternoon and Evening |
| SP70 | Makobar Area, Town, Port Harcourt | N $04^0$45'. 509'' E $007^0$ 00'. 533'' | Morning, Afternoon and Evening |
| SP 71 | Agudama Avenue Junction D/Line, Port Harcourt | N $04^0$ 48'. 367'' E $007^0$ 00'. 166'' | Morning, Afternoon and Evening |

## II. METHOOLOGY

A total number of seventy one (71) sampling points (Table 1) were selected in Port Harcourt and its environs using WHO's (2005) guideline for site selection studies for population density, topography, industrial clusters, and heavy traffic.

All the sampling points selected were geo-referenced using GPS model 76Cx Garmin Global positioning system.

Field observations were carried out visually and recorded in the field notebook. Camera was used to take photographs to show evidence of important features and activities that may be the primary sources of the air pollutants.

**Validity/Reliability of Instrument**

All the portable in-situ meters for the field air quality and meteorological parameters including noise level measurement were certified calibrated by the manufacturer prior to mobilization to the field data gathering. Quality assurance and control measures were carried out accurately as per the equipment manufacturer's directive and battery were fully charged.

**Pollutant Mapping**

Concentration levels at each location were mapped out using ArcGIS 10.2 software. The software integrated the spatial air pollutants data from the sampling points within the Port Harcourt and its environs and analyzed them as input variables for graphical presentation to produce curves or contours of air pollutants levels.

## III. RESULTS AND INTERPRETATION

**Distribution of $SO_2$ in Study Area in the Dry Season**

In the dry season, $SO_2$ hotspot is visible within Eleme with the highest concentration within 1.10 to 1.18ppm (Figure 1). It occupies an aerial coverage of about 3,725meters radius. It has a larger influence on Port Harcourt, followed by Obio/Akpor and Oyigbo while its minor influence is in Ikwerre as shown in Figure 1. Its least influence is on Etche area. It is predicted that the hotspot will contribute about 0.035ppm to 0.080ppm to the background concentration of $SO_2$ in parts of Port Harcourt, Obio/Akpor, Oyigbo, Etche and Ikwerre Local Government Areas respectively.

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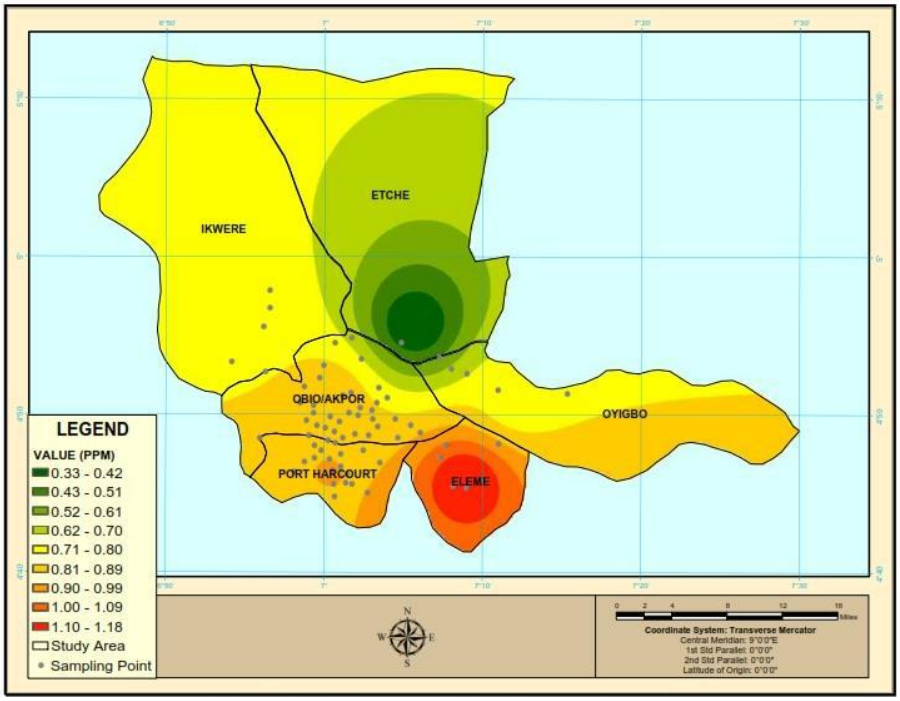


*Fig.1: Distribution in $SO_2$ of the Study Area in the Dry Season*

**Distribution of $NO_2$ in the Study Area in the Dry Season**

In the dry season, $NO_2$ hotspots are visible within Eleme and part of Oyigbo with the highest concentration within 0.82 to 0.88ppm (Figure 2). It occupies elongated area coverage of about 17670meters. It showed moderate influence in Obio/Akpor and Ikwerre It has a larger influence on Oyigbo followed by Etche with the least influence in Port Harcourt area as shown in Figure 2. It is predicted that these hotspots will contribute about 0.05 to 0.070ppm to the background concentration of $NO_2$ in parts of Oyigbo, Etche, Obio/Akpor, Port Harcourt and Ikwerre.

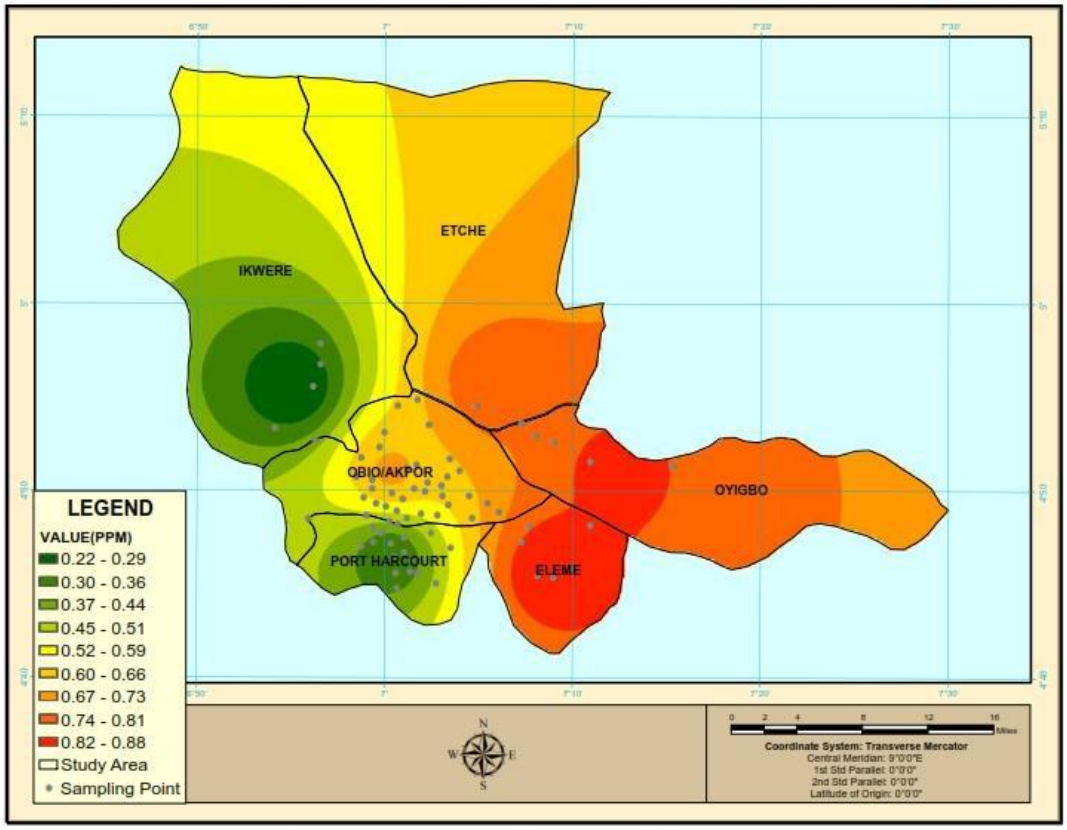

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*Fig.2: Distribution of $NO_2$ in the Study Area in the Dry Season*

**Distribution of $H_2S$ in the Study Area in the Dry Season**

In the dry season, $H_2S$ hotspot is visible within Eleme with the highest concentration within 1.70 to 1.87ppm as shown in Figure 3. It occupies an aerial coverage with a 2670meters radius and has a moderate influence on Oyigbo and its least influence is in Port Harcourt, Obio/Akpor and Etche. It is predicted that this hotspot will contribute additional 0.05 to 0.1ppm to the background concentration of $H_2S$ in parts of Port Harcourt, Oyigbo, Obio/Akpor and Etche.

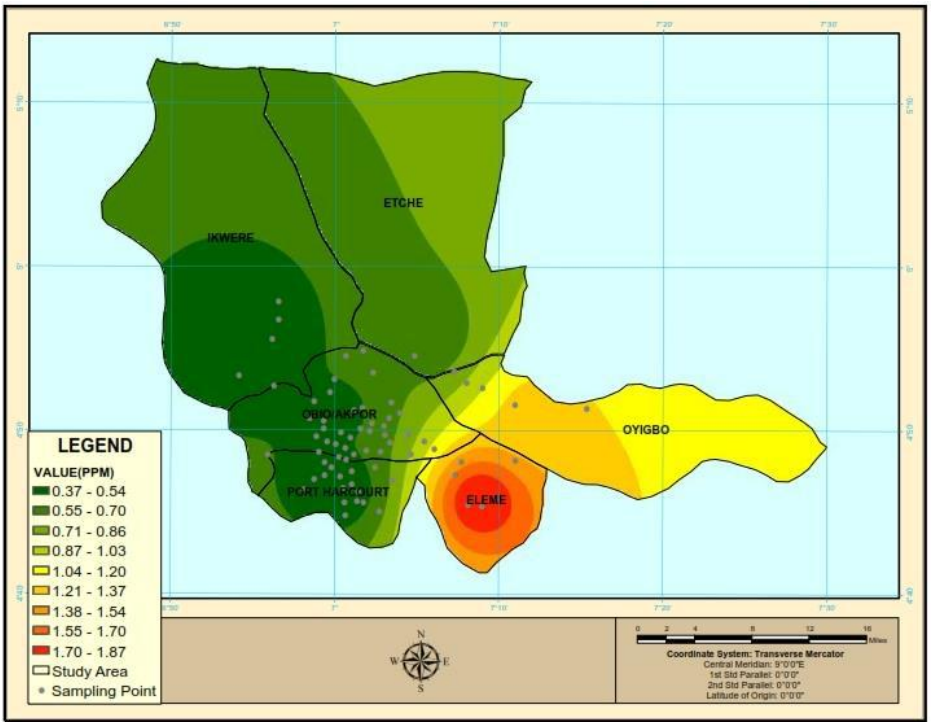


*Fig.3: Distribution of $H_2S$ in the Study Area in the Dry Season*

**Distribution of VOCs in the Study Area in the Dry Season**

In the dry season, VOCs hotspot is visible within Eleme with the highest concentration within 6.26 to 6.82ppm as shown in Figure 4. It occupies an aerial coverage of about 3,850meters radius. It has a larger influence on Obio/Akpor. It has least influence on Port Harcourt, followed by Oyigbo, Etche and Ikwerre LGAs. It is estimated that this hotspot will contribute additional 0.25ppm to 0.41ppm to the background concentration of VOCs.

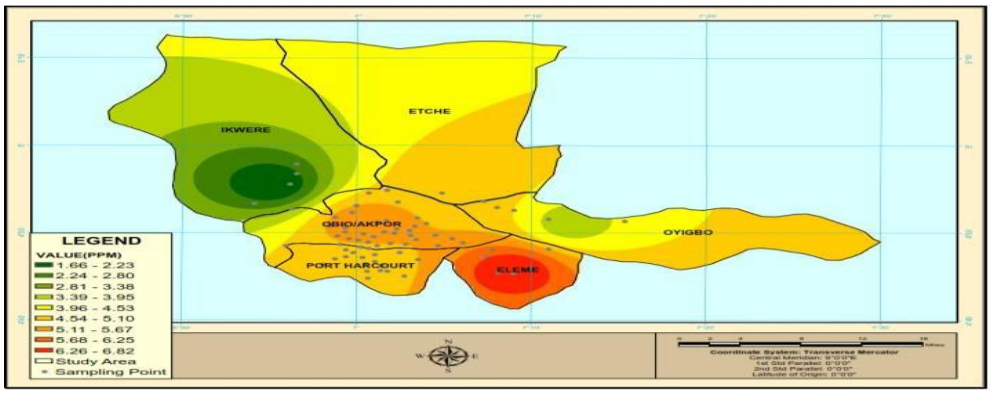


*Fig.4: Distribution of VOCs in the Study Area in the Dry Season*

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**Distribution of CO in the Study Area in the Dry Season**

In the dry season, Carbon Monoxide (CO) hotspot is visible within Eleme with highest concentration within 25.06 to 27.85ppm as shown in Figure 5. It occupies an aerial coverage with a 4350meters radius. It has a minor influence on Obio/Akpor, followed by Oyigbo and Port Harcourt. Its least influence is on Ikwerre and Etche areas. It is predicted that this hotspot contributes additional 1.1 to 2.05ppm to the background concentration of carbon monoxide in parts of Obio/Akpor, followed by Oyigbo and Port Harcourt.

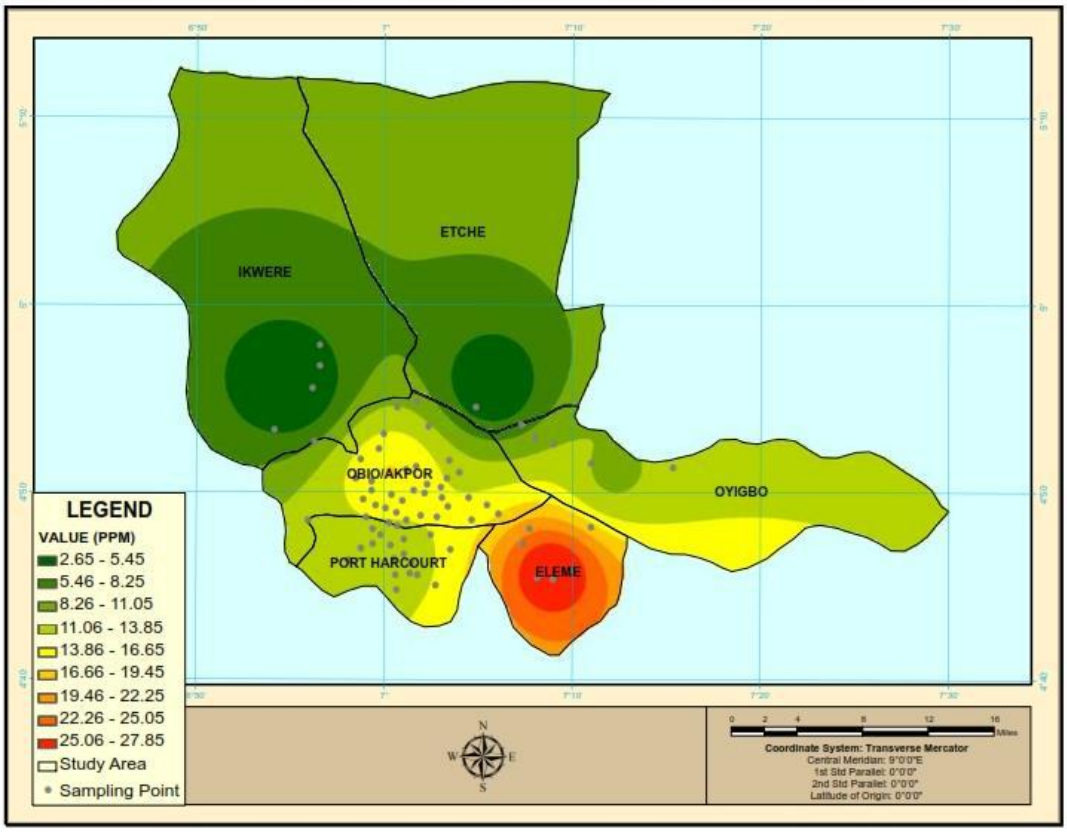


*Fig.5: Distribution of CO in the Study Area in the Dry Season*

**Distribution of $NH_3$ in the Study Area in the Dry Season**

In the dry season, ammonia hotspot is visible within Eleme with highest concentration within 5.32 to 5.98ppm as shown Figure 6. It occupies an aerial coverage of 3500meters radius. It has a very minor influence on Oyigbo, but less on Obio/Akpor and Port Harcourt. Its least influence is on Ikwerre and Etche areas. It is predicted that this hotspot will contribute additional 0.15 to 0.2ppm to the background concentration of ammonia in parts of Oyigbo, Obio/Akpor and Port Harcourt.

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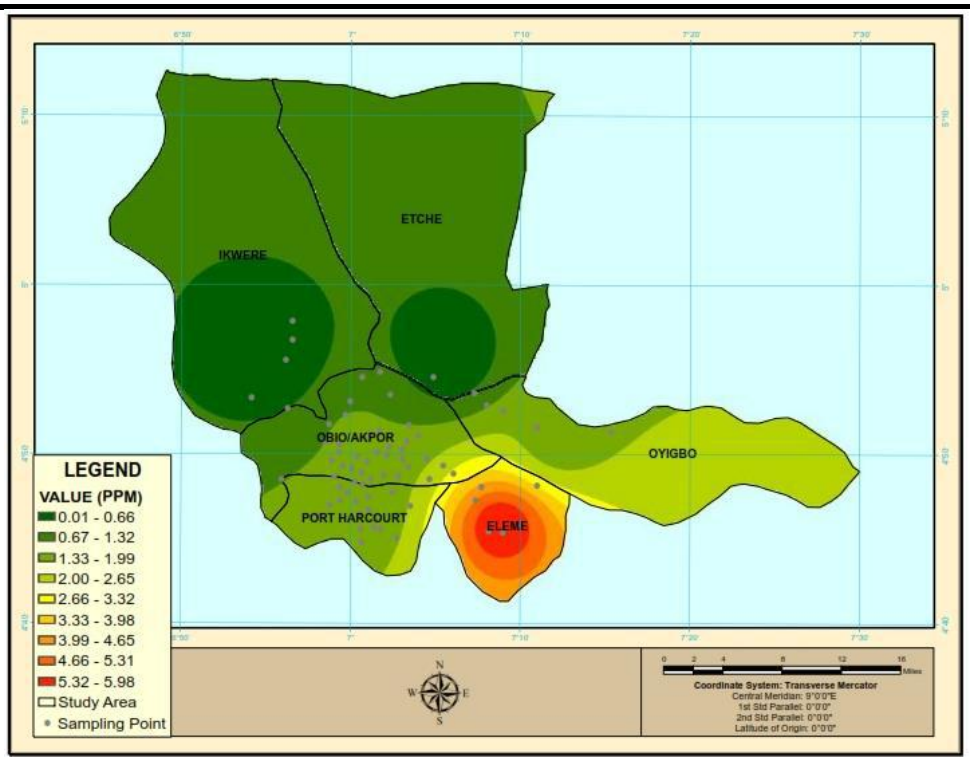


*Fig.6: Distribution of $NH_3$ in the Study Area in the Dry Season*

**Distribution of $CH_4$ in the Study Area in the Dry Season**

In the dry season, methane hotspots are visible within Eleme followed by Obio/Akpor and Port Harcourt with highest concentration within 6.86 to 7.48ppm as shown in Figure 7. It occupies an aerial coverage of 3750meters radius. It has a moderate influence on Oyigbo. It has least influence on Ikwerre and Etche LGAs. It is predicted that these hotspots contribute about 3.0 to 4.0ppm to the background concentration of methane ($CH_4$) in parts of Oyigbo, Ikwerre and Etche.

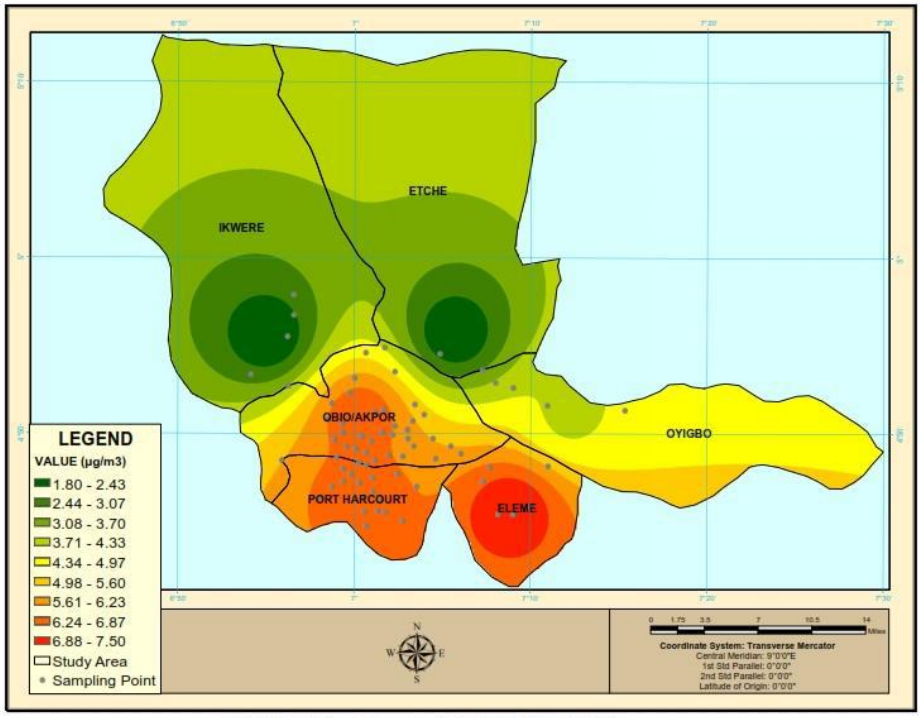


*Fig.7: Distribution of $CH_4$ in the Study Area in the Dry Season*

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**Distribution of TSP in the Study Area in the Dry Season**

In the dry season, TSP hotspots are visible within an extended area of Obio/Akpor and Eleme followed by Port Harcourt with highest concentration within 716.27µg/m$^3$ to 792.81µg/m$^3$ as shown in Figure 8. It occupies elongated aerial coverage of 26,466meters length. It has a larger influence on Ikwerre followed by Oyigbo and showed its moderate influence on Etche area. It is predicted that these hotspots will contribute additional 2.0µg/m$^3$ to 3.4µg/m$^3$ to the background concentration of TSP in parts of Ikwerre, Oyigbo and Etche.

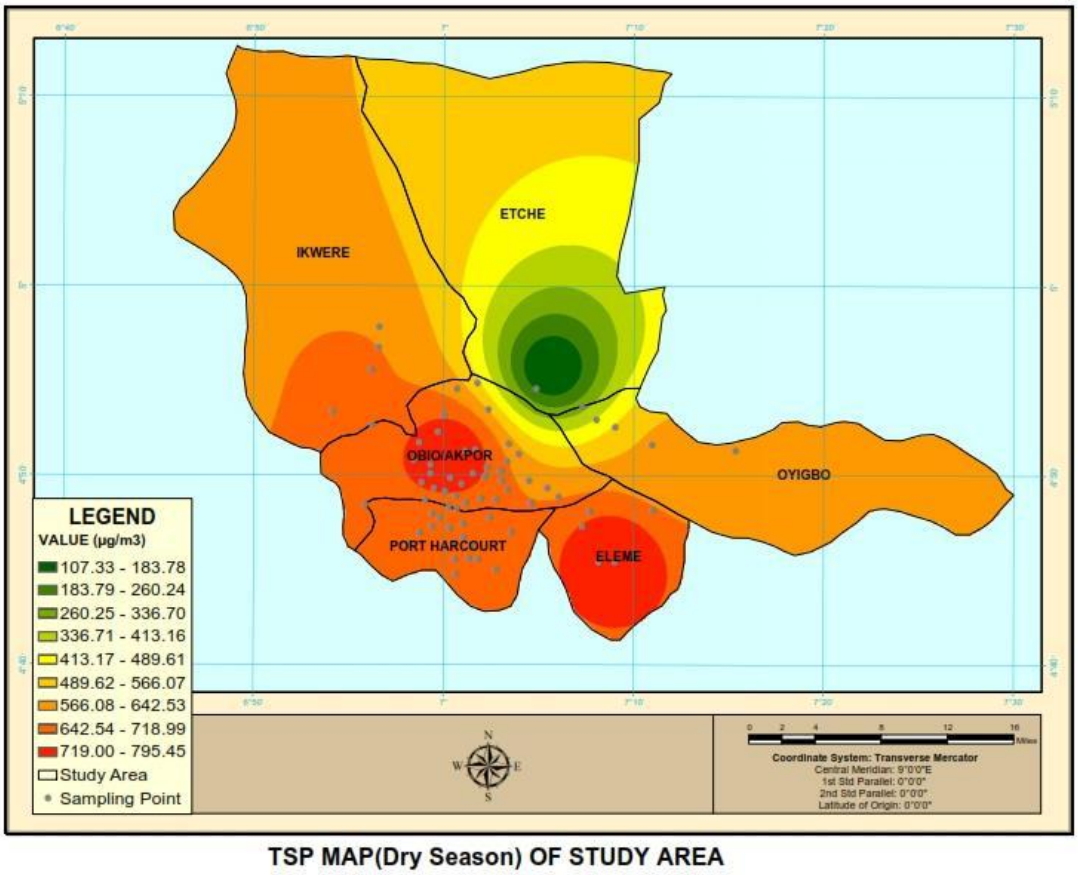


*Fig.8: Distribution of TSP in the Study Area in the Dry Season*

**Distribution of $PM_{10}$ in the Study Area in the Dry Season**

In the dry season, $PM_{10}$ hotspots are visible within an extended area of within Obio/Akpor and Eleme followed by Port Harcourt with highest concentration within 501.39µg/m$^3$ to 554.09µg/m$^3$ as shown in Figure 9. It occupies elongated aerial coverage of 27200meters length. It has a larger influence on Oyigbo but less on Ikwerre and Etche. It is predicted that these hotspots will contribute about 10µg/m$^3$ to 25µg/m$^3$ to the background concentration of $PM_{10}$ in parts of Oyigbo, Ikwerre and Etche.

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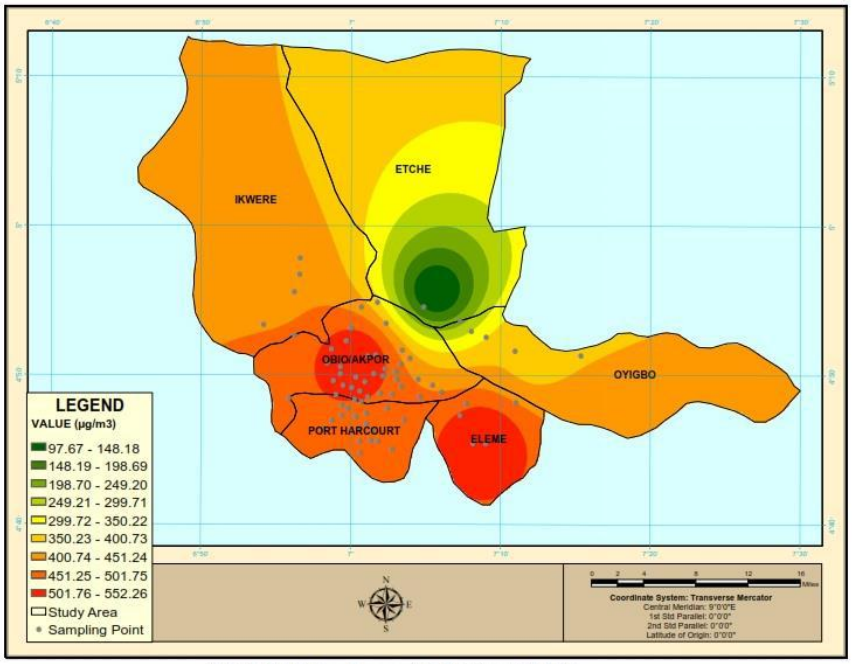


*Fig.9: Distribution of $PM_{10}$ in the Study Area in the Dry Season*

**Distribution of $PM_{2.5}$ in the Study Area in the Dry Season**

In the dry season, $PM_{2.5}$ hotspots are visible within Eleme followed by Port Harcourt and Obio/Akpor with highest concentration within 84.15µg/m$^3$ to 92.19µg/m$^3$ as shown in Figure 10. It occupies an aerial coverage of 4100meters radius. It has a larger influence on Ikwerre and Oyigbo, but least influence on Etche area. It is predicted that these hotspots contribute about 3.5µg/m$^3$ to 5.5µg/m$^3$ to the background concentration of $PM_{2.5}$ in parts of Ikwerre, Oyigbo and Etche,

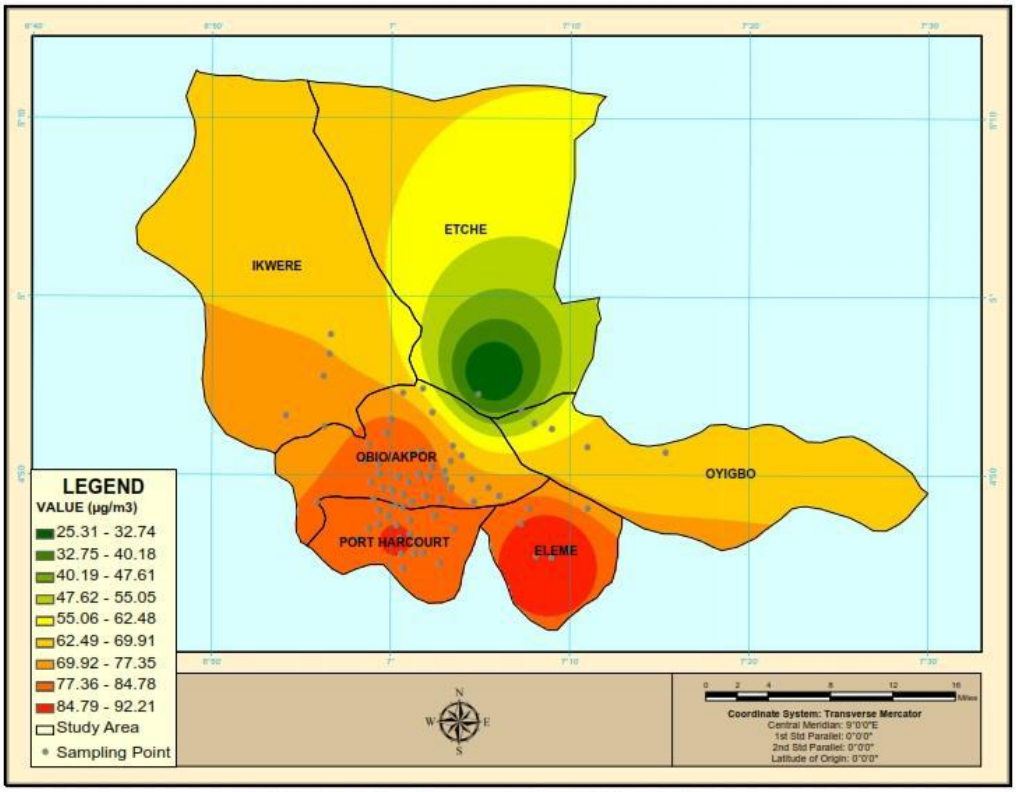


*Fig.10: Distribution of $PM_{2.5}$ in the Study Area in the Dry Season*

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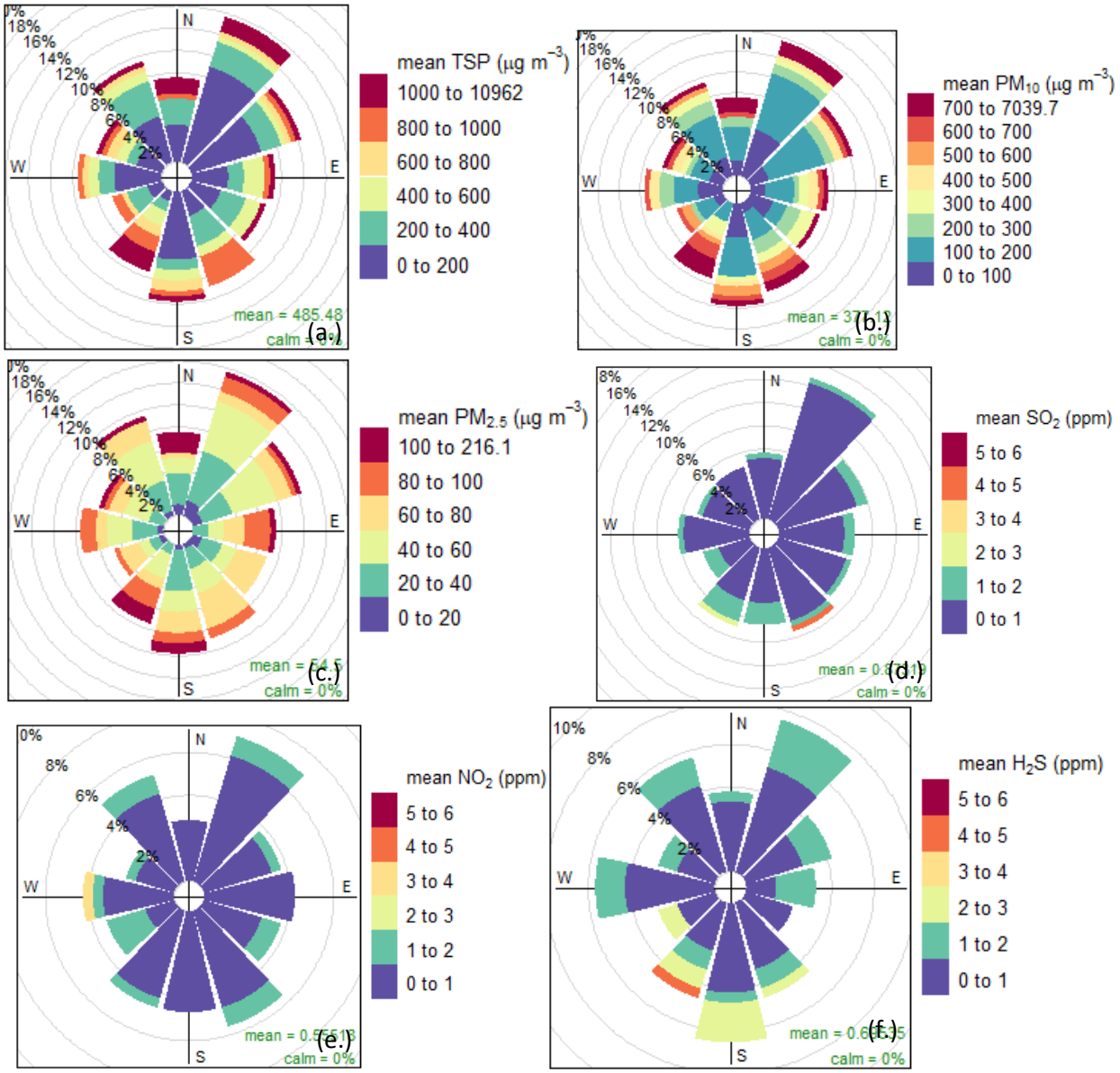
## IV. DISCUSSION

### Evaluation of Pollutants Dispersion Pattern in the Study Area in the Dry Season

The pollutants dispersion patterns in the study area in the dry season were evaluated with the aid of pollution roses and bivariate polar plots of each pollutant with respect to wind speed and wind direction. The dry season results are presented in Figures 11 (a-j) and 12 (a-j). The pollution roses and polar plots were developed using the mean concentration of each pollutant in different wind speed and percentage frequency count of wind direction categories (Munir, 2016). They were simulated with the aid of Generalized Additive Model (GAM) smoothing techniques Carslaw, (2015) that depict pollutant concentrations as a continuous surface.

Pollution roses (Figure 11 (a-j)) showed that pollutant concentrations increase with increased wind speed. Low concentrations of pollutants were obtained at low wind speed and vice-versa. This implies that wind speed has positive influence on the concentration levels of pollutants in the study area.



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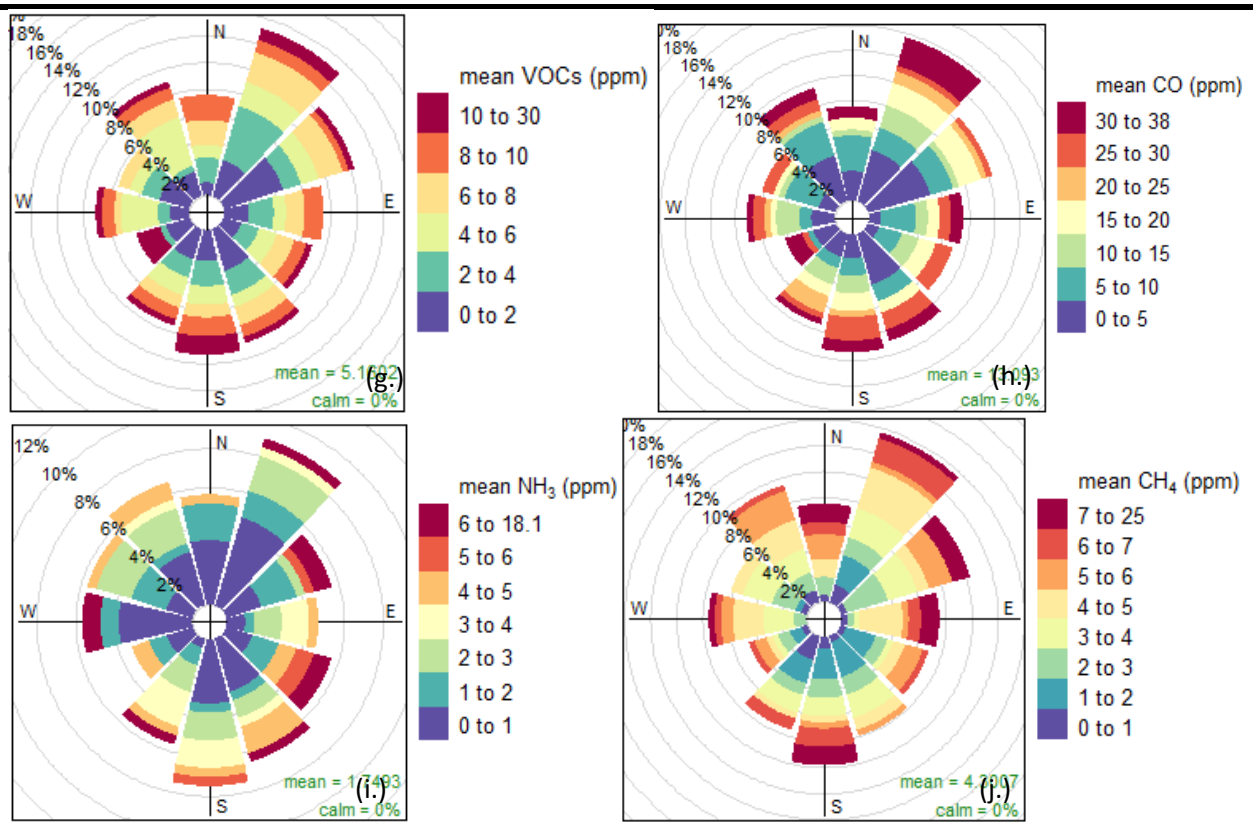


*Fig.11(a-j): Pollution Roses of Pollutants in the Study Area in the Dry Season*

The pollutant polar plots (Figure 12 (a-j)) showed that concentrations of pollutants in the area are associated with wind speed up to 3.5m/s. It is also observed from Figure 12 (a-j) that pollutant concentrations increase with increased wind speed.

Surface polar plots of pollutants concentrations in the study area revealed that high concentrations of $SO_2$, $NO_2$, $NH_3$, $H_2S$ and VOCs are associated with the south-west and south-east directions and are dispersed toward the north-east and north-west directions. This may imply that sources of these pollutants are in the southern part, which is the coastal region of the study area. Industrial activities, especially in Eleme area (refineries, petrochemical company, fertilizer companies, industrial waste management facilities, civil construction, gas flaring, and vehicular movement) and the release of black carbon (black soot) due to illegal refineries in the coastal area may be the sources of these pollutants (Ahove, 2006, Akpan *et al.*, 2014 and Antai *et al.*, (2016)).

Figure 12 (a-j) also indicate that concentrations of CO is associated with south-west, south-east and north-east directions and are dispersed toward the north-west directions. This may imply that sources of this pollutant are both in the southern and northern parts, which are the coastal and up-land areas. Industrial activities, vehicular exhaust emissions, gas flaring and oil and gas exploitation in Eleme, Port Harcourt, Obio/Akpor and Etche areas might be the sources of these pollutant (Bhatia, 2011, Bleta *et al.*, 2017, Efe, 2005 and Emmanuel *et al.*, 2009) .

Similarly, concentrations of Methane ($CH_4$) and Particulate matter (TSP, $PM_{10}$ and $PM_{2.5}$) are associated with both northern and southern directions. This showed that activities in the both the coastal and up-land areas are responsible for the release of these pollutants into the environment. In other words, industrial activities, vehicular exhaust emissions, civil construction, the released of black carbon (black soot) due to illegal refineries in the coastal area, gas flaring and oil and gas exploitation in Eleme, Port Harcourt, Obio/Akpor, Etche and Ikwerre areas may be the sources of $CH_4$ and Particulate matter in the air environment of the study area in the dry season period.

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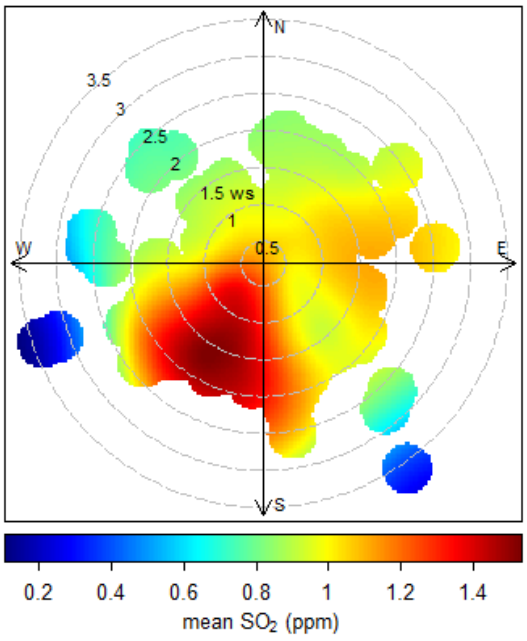


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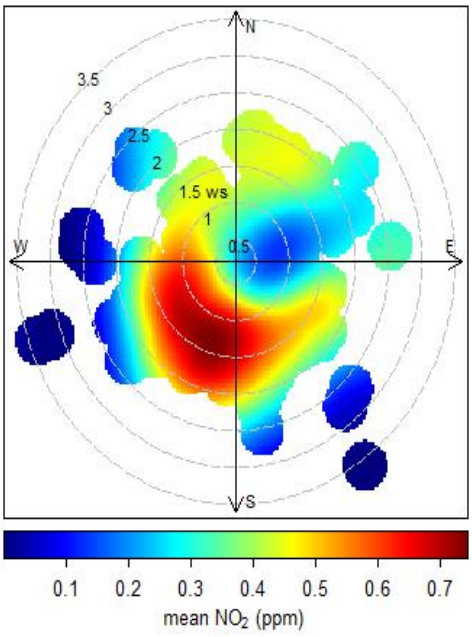


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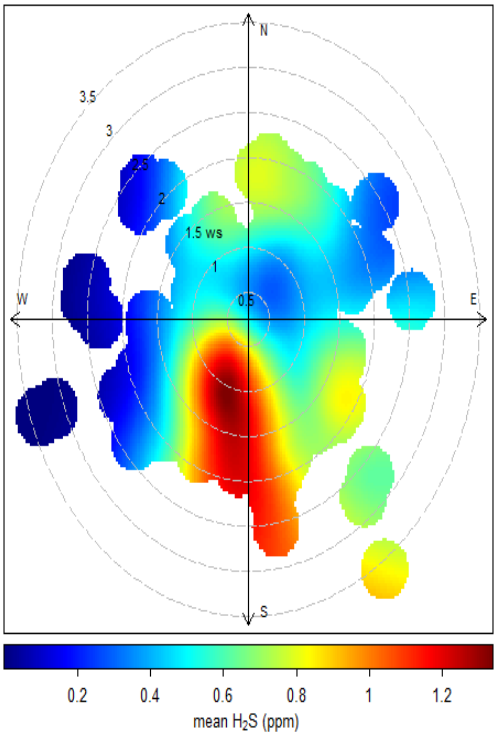


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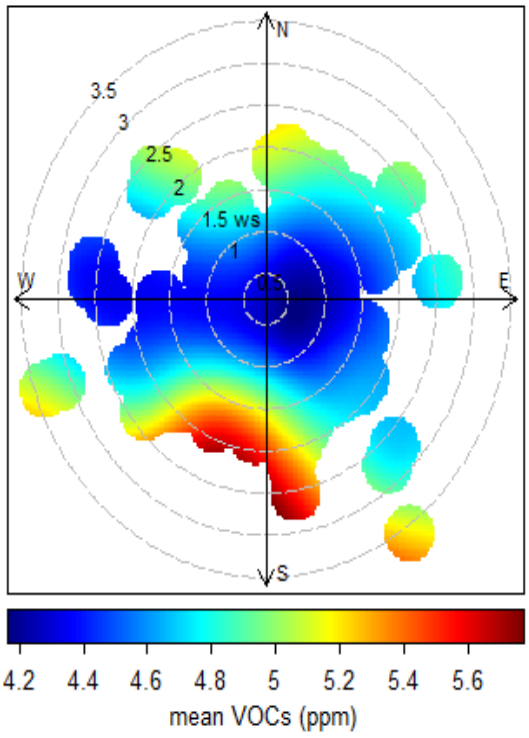


(d.)

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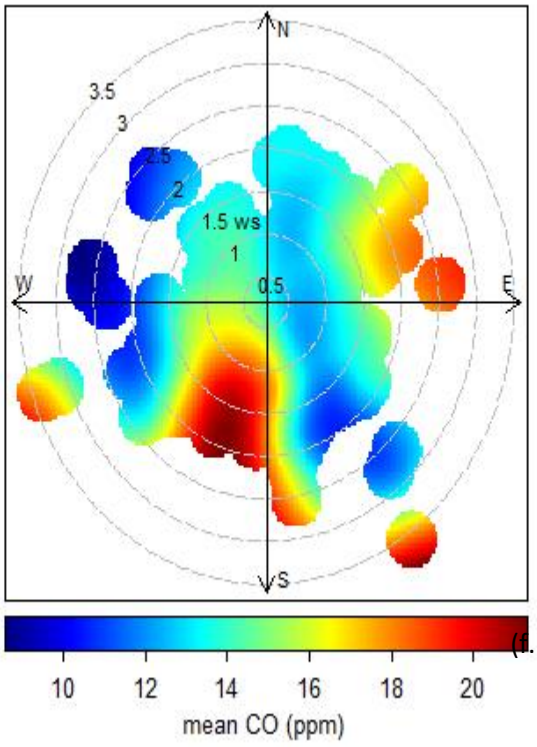
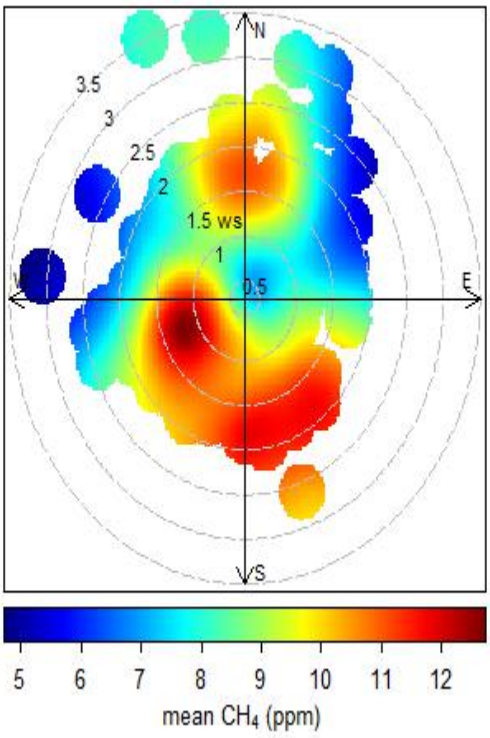
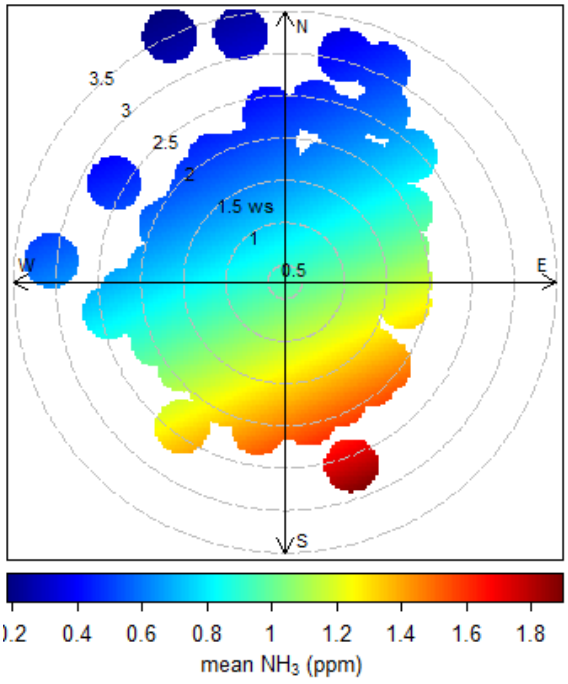


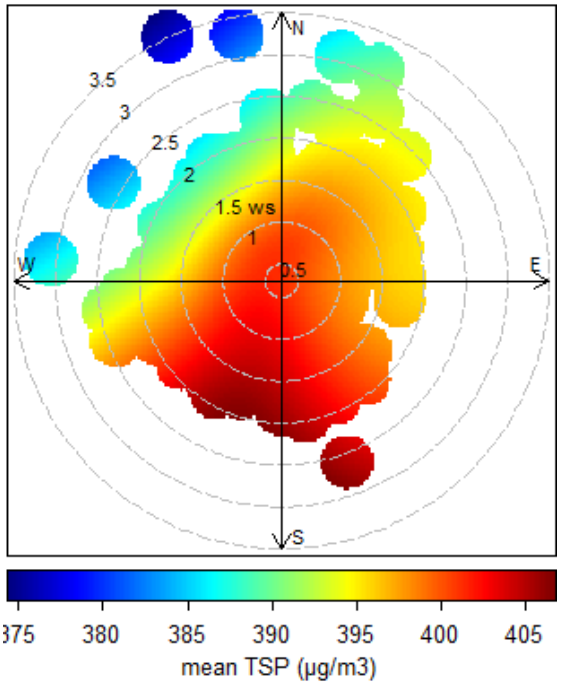

(e.)




(f.)




(g.)




(h.)

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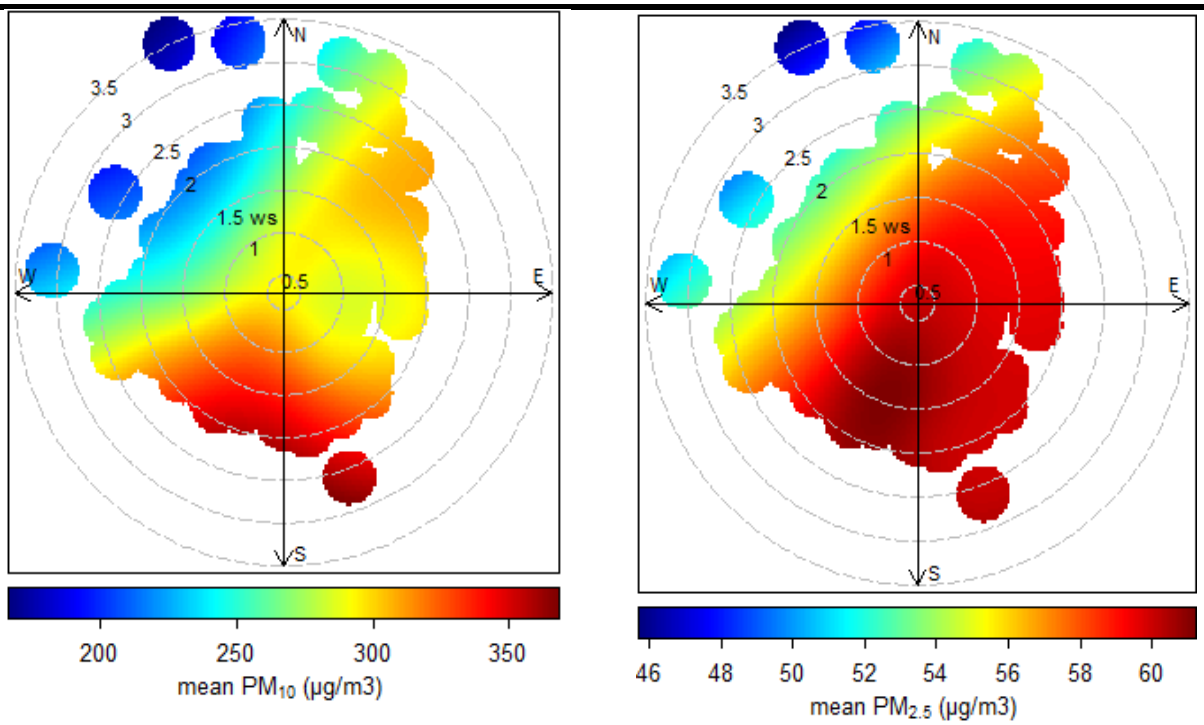
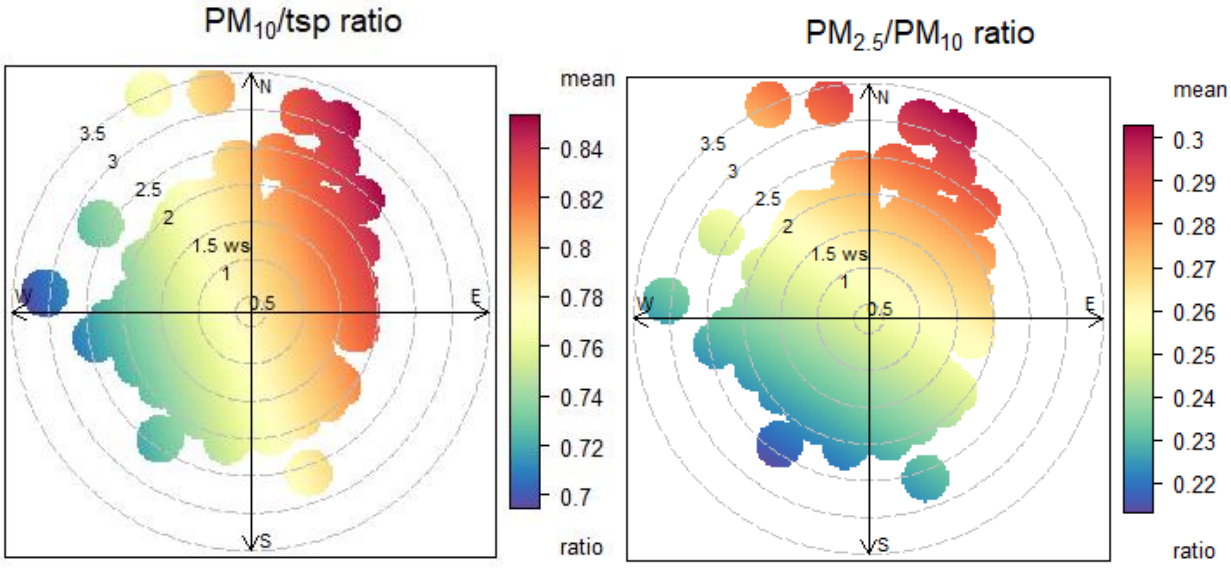



(j.) (i.)

*Fig.12 (a-j): Polar Plots of Pollutants in the Study Area in the Dry Season*

**Determination of Particulate Ratio (TSP: $PM_{10}$:$PM_{2.5}$) in the Dry season**

Particulate mass concentration ratios $PM_{10}$/TSP and $PM_{2.5}/PM_{10}$ in the dry season were determined in the modeling process. Result (Figure 13 (a-b)) indicates that the mean mass concentration ratio of $PM_{10}$/TSP was 0.84, while the mean mass concentration ratio of $PM_{2.5}/PM_{10}$ was 0.30. This reveals that $PM_{10}$ constitutes 84% of the concentration of total suspended particulate (TSP) measured in the study area, out of which 30.0% is composed of $PM_{2.5}$. The $PM_{2.5}/PM_{10}$ ratio obtained in the dry season is below the range (0.5-0.8) found in urban areas of developing countries by World Health Organization (WHO, 2006). This $PM_{2.5}/PM_{10}$ ratio could be considered hazardous to human health. It revealed that black smoke emanating mostly from the combustion of hydrocarbon fossil fuel is concentrated in this fine fraction of particulate.




(a.) (b.)

*Fig.13(a-b): Particulate Ratio in the Study Area in the Dry Season*

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## V. CONCLUSION

There were relatively high concentrations of air pollutants especially dominated in southern area in the dry season. Results of geospatial analysis and generalized additive models revealed that sources of pollutants in the study area are localized in the up-land area and region around the coastal area and dispersed towards the southern and northern parts of the study area in the dry season.

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# Prediction and Modeling of Dry Seasons Air Pollution Changes Using Multiple Linear Regression Model: A Case Study of Port Harcourt and its Environs, Niger Delta, Nigeria.

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***Abstract*—***The influence of meteorological parameters on air pollutants over Port Harcourt and its environs in the dry season was modeled using multiple linear regressions model. Results indicated that meteorological parameters significantly influenced pollutant concentrations; results also showed poor linear relationships between meteorological parameters and pollutant concentrations, and that meteorological parameters are poor predictor variables of concentrations of air pollutants in the area. Pollution roses of pollutants dispersion pattern in the study area showed that pollutant concentrations increase with increased wind speed. Result also showed that wind speed exerts positive influence on the concentration levels of pollutants in the study area. The yearly prediction of air pollutants was also carried out using a ten-year data from previous studies conducted in the study area. The prediction was done using regression analysis and year as the predictor variable to develop a model. The relationship between air pollutants and year was therefore established for the annual prediction of the future pollutant concentrations in the dry seasons for period of the next fifteen years.*

***Keywords—Multiple linear Regressions Model, Air Pollution changes, Meteorological variables concentration.***

## I. INTRODUCTION

Air quality impacts on the environment can therefore be quantified by simulating environmental conditions using analytical tool known as modeling (Okpala *et al.*, 2013).

The simulating of real-life environmental situations can use the systematic method called modeling. Modeling is a tool by which mathematical equations are used to predict the air pollutants future behaviour.

Modeling assists in studying and predicting the impacts of various environmental components and also viewing the environment as a system by representing simplified variation of environmental system mathematically and also prediction, testing and comparison of reasonable alternative situations (Okpala *et al.*, 2013).

The effective and efficient way to understand the interactions of various air pollution scenarios as relate with meteorology, topography and existing air quality characteristics are air pollution models (Okpala *et al.*, 2013).

The relative high concentration of air pollutants in Port Harcourt can be attributed majorly to industrial activities such as oil and gas related activities and vehicular emissions (Antai, 2016). Geographical and meteorological conditions of the study area can also influence some local background concentration of air pollutants since there is a relationship between air pollution and meteorological variables, thus air pollution modeling is the development of a functional relationship between air pollutions concentration and other control variables.

Most of the conventional models have been proved inaccurate (Esplin, 1995). These models depend basically on detailed knowledge of pollutant sources, topography in the surrounding environment (Elangasinghe *et al*., 2014).

Multiple linear regression (MLR) model was developed and applied to predict the variations of air pollutants concentrations with meteorological parameters of the study area. This study highlights how the relationships between

measured air pollutant concentrations and meteorological parameters were modeled using multiple linear regressions and generalized additive model.

## STUDY LOCATION

### Description of the Study Area

### Location

Port Harcourt metropolis is located between latitudes $4^0$35' and $5^0$30' North and between longitudes $6^0$54' and $7^0$08' East. It covers an estimated area of 1811.6 square kilometer and is the capital of Rivers State. Port Harcourt was established in 1914 by the British colonial administration under Lord Lugard to meet the pressing economic needs of the Europe. Port Harcourt which lies at the heart of the Niger Delta, one of the world's richest wetlands, is bounded on the South by the Atlantic Ocean, to the North by Imo and Abia States to the East by Akwa Ibom State and to the West by Bayelsa and Delta State respectively. Some of the well known residential areas in Port Harcourt and its environs include: Port Harcourt, Obio/Akpor, Eleme, Oyigbo, Ikwerre and Etche Local Government Areas (LGAs) (Awosika, 1995).

## II. METHODOLOGY

### METHOD OF DATA ANALYSIS AND MODELING

Mean concentration of air pollutants was computed using equation (1)

$$\bar{X} = \frac{\sum_{i=1}^{n} X_{meas,i}}{N} \quad (1)$$

Standard deviation was computed using equation (2)

$$s = \sqrt{\frac{\sum \left(X_{meas,i} - \bar{X}\right)}{N-1}} \quad (2)$$

Standard error estimate was determined using equation (3)

$$\sigma_X = \frac{s}{\sqrt{N}} \quad (3)$$

where, s is the standards deviation, $X_{meas,i}$ is the measured $i^{th}$ data point, $\bar{X}$ is the mean and N is the total number of data set.

### Coefficient of variation of air pollutants

The coefficient of variation of each parameter was computed using Equation (4)

$$\%CV = \frac{S}{\bar{X}} = \frac{\sqrt{\frac{\sum \left(X_{meas,i} - \bar{X}\right)^2}{N-1}}}{\frac{\sum_{i=1}^{N} X_{meas,i}}{N}} \quad (4)$$

Computation of Exceedance Factor (EF)

A factor known as Exceedance Factor (CPCB, 2006) was used to determine pollutants compliance with national and international standards.

The Exceedance Factor (EF) was calculated using equation (5) as follows:

$$\text{Excedence Factor (EF)} = \left(100 \frac{C_i}{C_{std}}\right) \quad (5)$$

where $C_i$ is the measured concentration of the $i^{th}$ parameter in the ambient air.

$C_{std}$ is the regulatory standard recommended for the $i^{th}$ parameter.

For EF < 100, the parameter is said to be withing permissible limit, and for EF > 100, the parameter is said to exceed permissible limit. The EF for each pollutant was computed based on the Federal Ministry of Environment (FMEnv) stipulated permissible limit as contained in FEPA (1991, 1992) and National Ambient Air Quality Standards (NAAQS).

### Model Development

Multiple linear regression (MLR) models were applied to predict the variations of pollutant concentrations with meteorological parameters. The following steps were applied in the model building process.

i. Data was collected through field measurement.
ii. Data was prepared and analysed using statistical software.
iii. Appropriate variables were selected as input parameters.
iv. Models were built using the variables.
v. Models were tested and validated models and

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vi. Pollutants were predicted using built models.

Multiple linear regression (MLR) modeling approach was employed to model the influence of meteorological variations on air pollutants.

Modeling was based on the following fundamental approaches:

$$\text{outcome}_i = (\text{model}) + \text{Error}_i \quad (6)$$

$$Y_i = (b_o + b_1 X_{i1} + b_2 X_{i2} + \ldots + b_n X_n) + \varepsilon_i \quad (7)$$

$$y_i = \beta_o + \sum_{i=1}^{n} \beta_i x_i + \varepsilon_i \quad (7)$$

Where; $Y_i$ and $y_i$ are model outcomes or outputs,

$X_1, X_2, \ldots\ldots, X_n$ are predictor variables,

$b_0, b_1, b_2, \ldots\ldots, b_n$ are regression coefficients, and

$\varepsilon_i$ is the error factor called residual.

Multiple linear regressions (MLR) modeling technique was employed to predict air pollutants concentration in the study area using wind speed (Ws), wind direction (Wd), temperature (Temp), air pressure (Ap) and relative humidity (Rh) as predictor variables. The multiple linear regressions were performed using Statistical Package for the Social Science (SPSS) software, originally developed by International Business Machines (IBM). Stepwise regression approach was used to determine the relationship between air pollutants and individual meteorological parameter. Stepwise regression of independent parameter was performed using Equations (7) and (8).

$$PM_{pred} = f(X_i)$$
$$PM_{pred} = f(Wsp_i, \text{Wd}_i, \text{Temp}_i, \text{Rh}_i) \quad (8)$$

**Model Validation**

The model performance was evaluated in consonance with guidelines instituted by EPA (2007). Specific analyses was performed to validate the model outputs against measured data. Both quantitative (statistical) and qualitative (visual) methods were adopted. Measured data was paired against predicted values. Various statistical parameters such as mean square error (MSE) , root mean sqaure error (RMSE) were used to validate and determine the quality of the prediction models. In addition, a measure of goodness of fit known as coefficient of determination, R-sqaure ($R^2$) was used to determine the total variability in the dependent variables that is accounted for by the model eqautions.

The mean square error (MSE) was computed as the mean difference between predicted and measured values using Equation (9), while the root mean square error was computed using Equation (10).

$$MSE = \frac{1}{N} \sum_{i=1}^{n} (Y_{pred,i} - X_{meas,i}) \quad (9)$$

$$RMSE = \left[ \frac{1}{N} \sum_{i=1}^{n} (Y_{pred,i} - X_{meas,i})^2 \right]^{\frac{1}{2}} \quad (10)$$

where N is the number of measured data or observations.

Sum of square error (SSE) will be calculated using equation (11)

$$SSE = \sum (X_{meas,i} - \bar{X})^2 \quad (11)$$

The sum of squares of the regression model ($SS_M$) was computed using Equation (12).

$$SS_M = \sum_i (Y_{pred,i} - X_{meas,i})^2 \quad (12)$$

The residual sum of squares (RSS) was computed using Equation (13)

$$RSS = \sum_{i=1}^{n} (\varepsilon_i)^2 = \sum_{i=1}^{n} (y_i - f(x_i))^2 \quad (13)$$

The residual sum of square error is therefore computed as

The residual sum of squares ($SS_R$) was computed using Equation (14).

$$SS_R = \sum_i (Y_{pred,i} - \bar{X})^2 \quad (14)$$

The total sum of squares ($SS_T$) was computed using Equation (15).

$$SS_T = SS_M + SS_R = \sum_i (X_{meas,i} - \bar{X})^2 \quad (15)$$

**Coefficient of determination R-sqaure ($R^2$)**

The coefficient of determination is the proportion of the total sample variability explained by the regression models and indicates how well the models fit the data. The coefficient of determination was computed using Equation (16).

$$R^2 = \frac{\text{Explained variation}}{\text{Total variation}} = \frac{SS_M}{SS_T} = \frac{\sum_i (Y_{pred,i} - \bar{X})^2}{\sum_i (X_{meas,i} - \bar{X})^2} \quad (16)$$

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where $Y_i$ is the predicted concentration of pollutant, $X_{meas,I}$is the individual measured concentration of air pollutant and $\overline{X}$ is the mean concentration of measured pollutant.

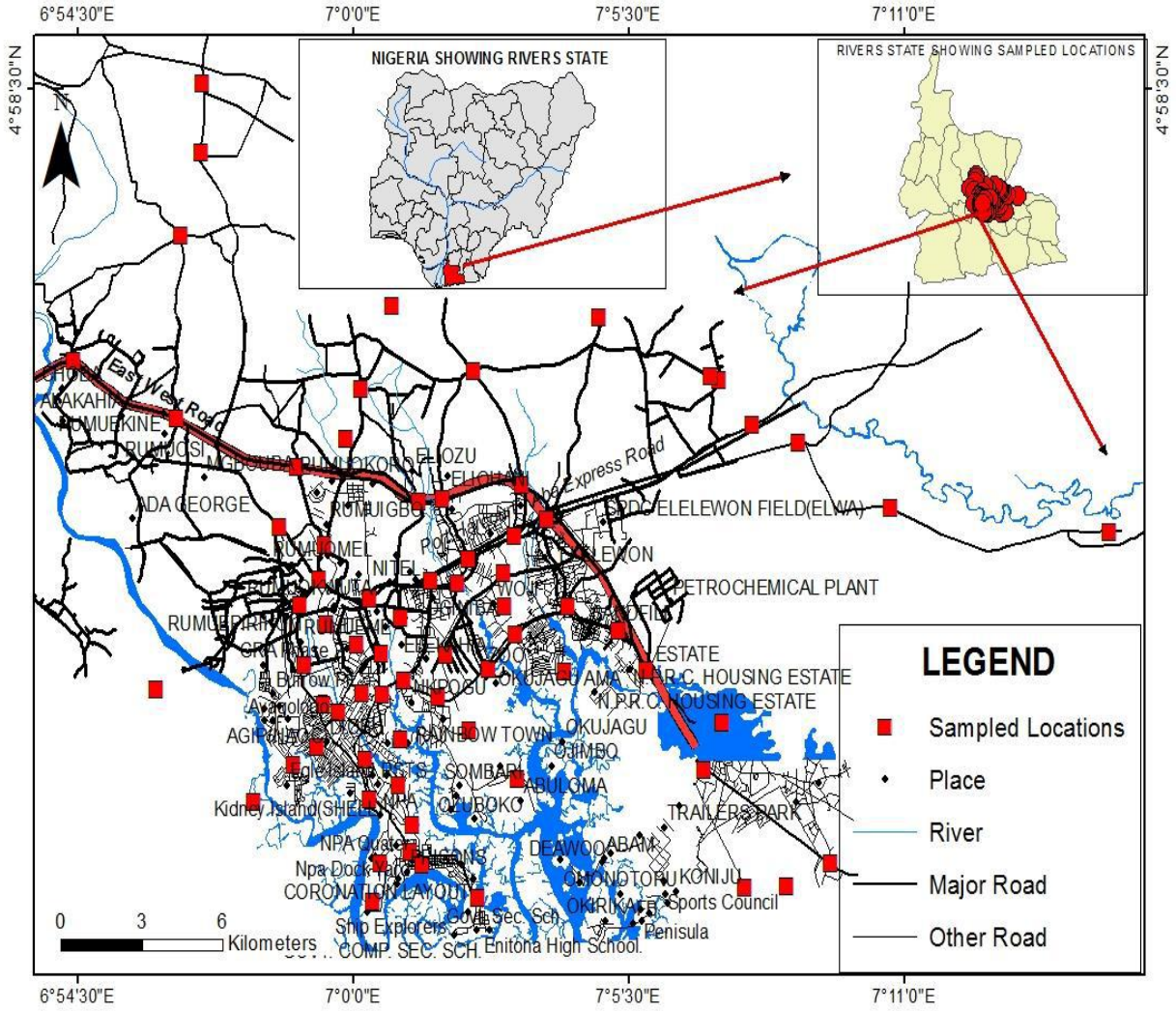


*Fig.1: Port Harcourt and its Environs showing Sampling Points for the Study.*

## III. PRESENTATION OF RESULT

### (i) Variation of Volatile Organic Compounds (VOCs) with Meteorological Parameters in the Dry Season

The results (shown in Figure 2 (a-e)) indicated that VOCs varied significantly with temperature, and positively correlated with wind speed. The stepwise regression linear models (shown in Table 1) show that the linear relationships between VOCs and wind speed, wind direction, relative humidity and air pressure are not significant at 0.05 confidence levels. However, the relationship between ambient temperature and VOCs concentrations is significant at 0.01 confidence level for a 2-tail test with a coefficient of determination ($R^2$) of 0.015). This implies that though VOCs varies significantly with temperature, only a fraction of 1.5% of the variation can be explained. Results (Table 1) further indicated that wind speed, wind direction, relative humidity and air pressure respectively accounted for 1.8%, 0.18%, 0.14% and 0.014% of the variation.

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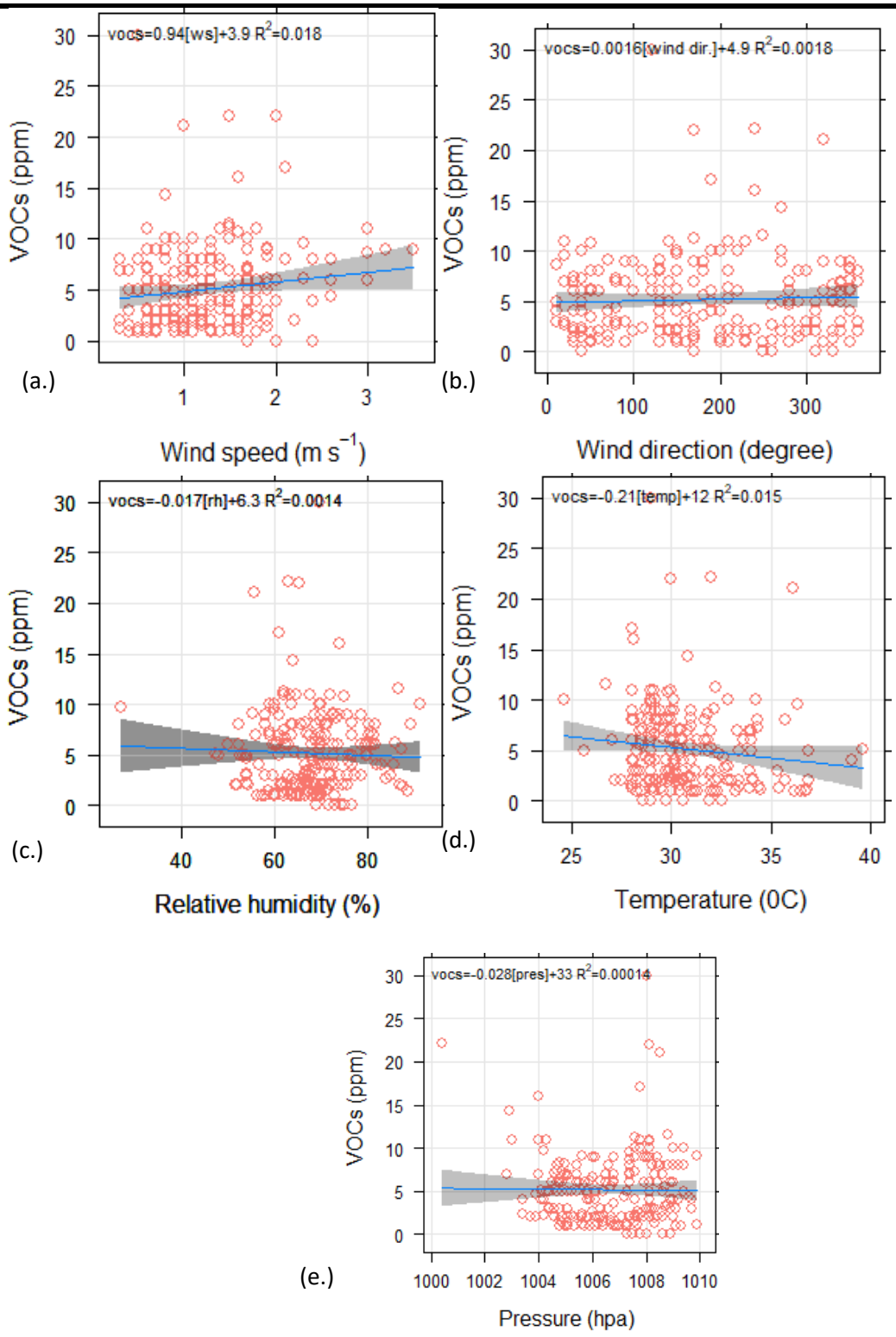


*Fig.2 (a-e): Relationship between Predicted VOCs and Meteorological Parameters in the Dry Season*

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*Table.1: Stepwise Linear Models for Dry Season VOCs*

| Pollutant | Model | $R^2$ | t-statistic | Sig. (2-tailed) |
|---|---|---|---|---|
| VOCs | = 3.9 + 0.94*Wsp | 0.018 | 1.807 | 0.072 |
| | = 4.9 + 0.0016*Wd | 0.0018 | 0.294 | 0.769 |
| | = 6.3 – 0.017*Rh | 0.0014 | -1.692 | 0.092 |
| | = 12 – 0.21*Temp | 0.015 | -2.084 | 0.038* |
| | = 33.0 – 0.028*Pres | 0.00014 | -0.070 | 0.944 |

***** Correlation is significant at the 0.05 level (2-tailed).

A multiple linear regression model for the prediction of VOCs was developed using all the meteorological parameters as predictor variables. The model for the prediction of VOCs concentrations was therefore derived as shown in Equation (17). The derived Equation (17) was used to predict the concentrations of VOCs in the study area in the dry season.

$$VOCs = 28.755 + 0.901*Wsp + 0.001*Wd - 0.063*Rh - 0.279*Temp - 0.012*Pres \quad (17)$$

*Table.2: Analysis of Variance (ANOVA) for Dry Season VOCs Prediction Model*

| Model | SSE (ppm) | df | MSE (ppm) | RMSE (ppm) | F | Sig. |
|---|---|---|---|---|---|---|
| Regression ($SS_M$) | 159.996 | 5 | 31.999 | 5.6568 | 1.857 | 0.103* |
| Residual ($SS_R$) | 3567.538 | 207 | 17.234 | | | |
| Total ($SS_T$) | 3727.534 | 212 | | | | |

*Not significant at the 0.05 level (2-tailed).

The mean square error (MSE) and the root mean square error were computed to be 31.999ppm and 5.6568ppm respectively. The model sum of squares error ($SS_M$), residual sum of squares error ($SS_R$) and total sum of squares error ($SS_T$) were computed to be 159.996ppm, 3567.538ppm and 3727.534ppm respectively as shown in Table 2. The result (Table 2) showed that meteorological parameters significantly (P-value <0.05) influence the concentrations of VOCs in the area. However, the goodness of fit (Figure 3) shows a poor linear relationship between VOCs and meteorological parameters with a coefficient of determination ($R^2$) of 0.043. This implies that meteorological parameters accounted for only 4.3% of the variation of VOCs concentrations in the area. The goodness of fit between predicted and measured concentrations of VOCs is shown in Figure 3, while the predicted values are plotted against measured values as shown in Figure 4.

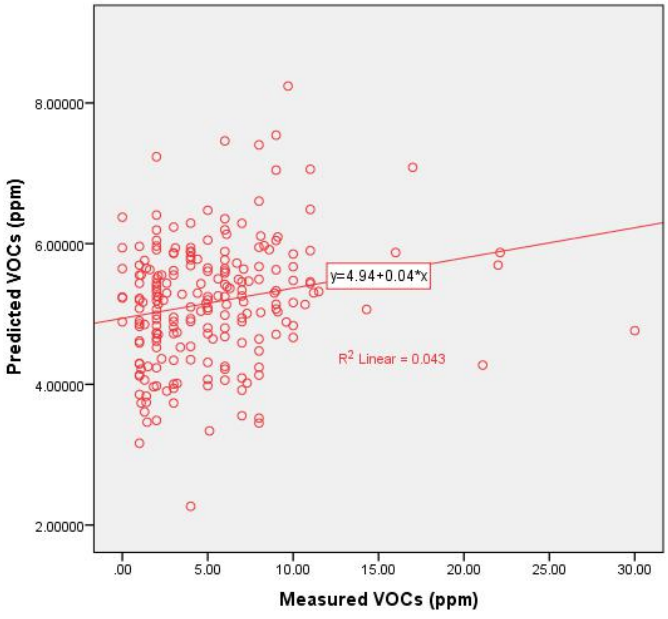


*Fig.3: Relationship between Predicted VOCs and Measured VOCs in the Dry Season*

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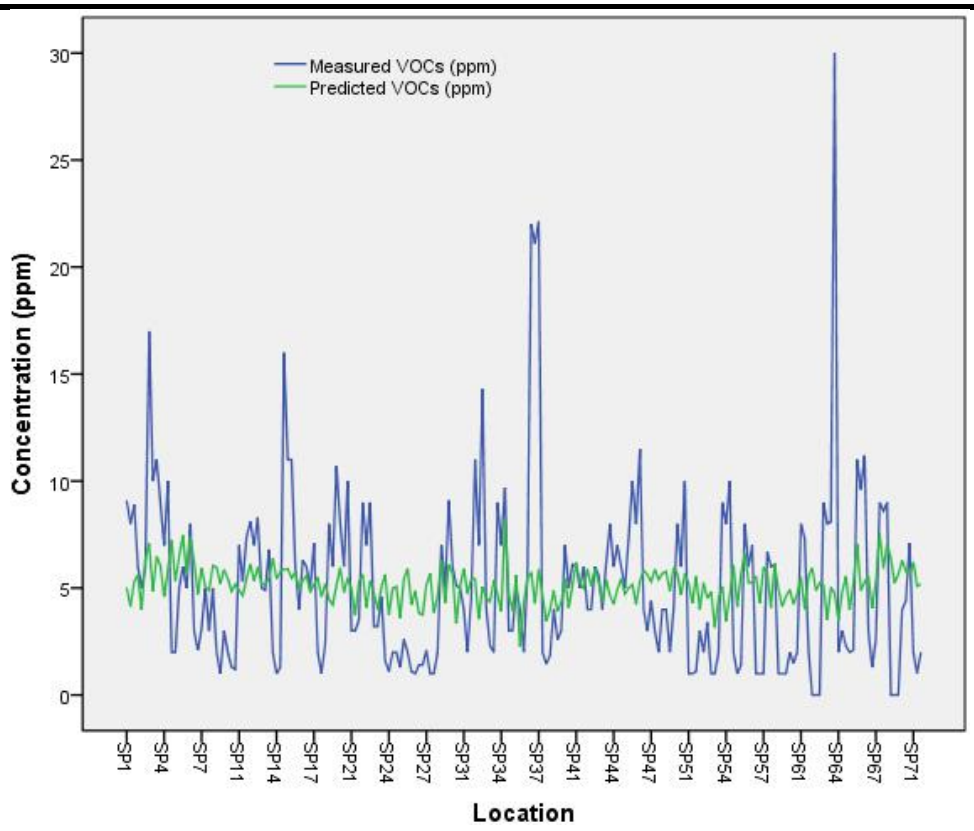
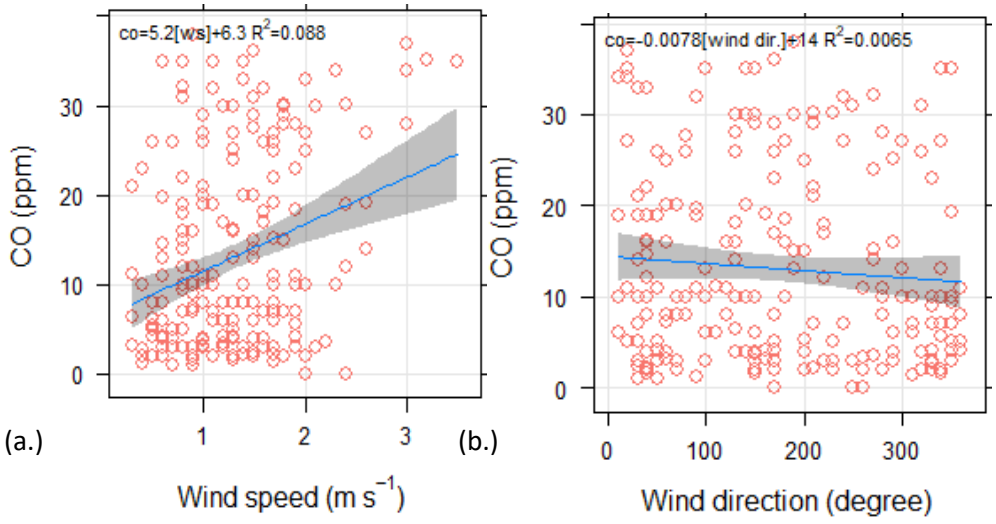



*Fig.4: Predicted VOCs versus Measured VOCs in the Dry Season*

**Variation of Carbon Monoxide (CO) with Meteorological Parameters in the Dry Season**

Results (shown in Figure 5 (a-e)) showed that concentrations of CO correlated significantly with wind speed in a positive manner. The stepwise regression linear models (shown in Table 3) show that the linear relationships between concentrations of CO and wind direction, relative humidity, temperature and air pressure are not significant at 0.05 confidence levels. However, the relationship between wind speed and concentrations of CO is highly significant at 0.01 confidence level for a 2-tail test with a coefficient of determination ($R^2$) of 0.088. This implies that though concentrations of CO vary positively with wind speed, only a fraction of 8.8% of the variation can be explained.



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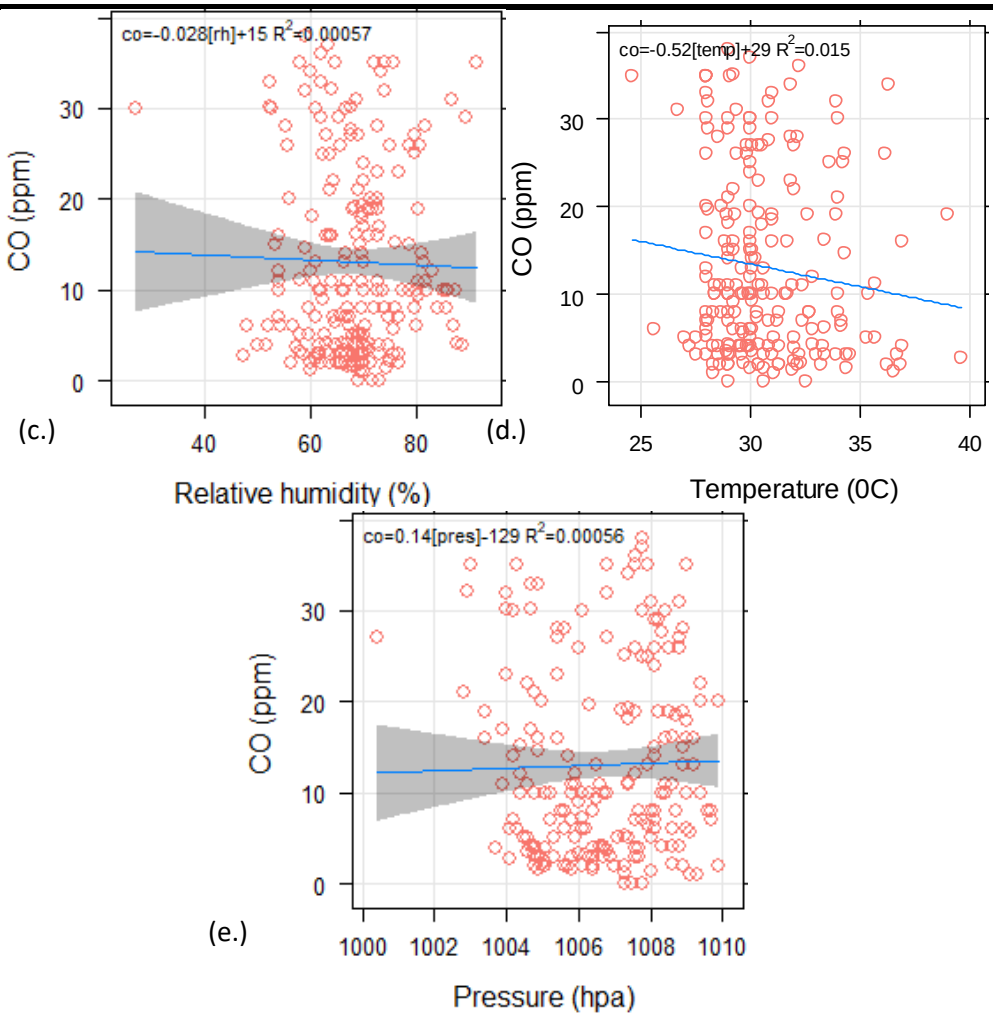


*Fig.5 (a-e): Relationship between Predicted CO and Meteorological Parameters in the Dry Season*

*Table.3: Stepwise Linear Models for Dry Season CO*

| Pollutant | Model | $R^2$ | t-statistic | Sig. (2-tailed) |
|---|---|---|---|---|
| CO | = 6.3 + 5.2*Wsp | 0.088 | 4.612 | 0.000** |
| | = 14 - 0.0078*Wd | 0.0065 | -1.665 | 0.097 |
| | = 15.0 - 0.028*Rh | 0.00057 | -1.921 | 0.056 |
| | = 29.0 - 0.52*Temp | 0.015 | -1.901 | 0.059 |
| | = – 129.0 + 0.14*Pres | 0.00056 | 0.153 | 0.878 |

**Correlation is significant at the 0.05 and 0.01 levels (2-tailed).

A multiple linear regression model for the prediction of CO was developed combining all meteorological parameters as predictor variables. A model for the prediction of CO concentrations was thus derived as shown in Equation (18). The derived Equation (18) was used to predict the concentrations of CO in the study area in the dry season.

$$CO = -24.993 + 5.489*Wsp - 0.011*Wd - 0.171*Rh - 0.608*Temp + 0.063*Pres \quad (18)$$

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*Table.4: Analysis of Variance (ANOVA) for Dry Season CO Prediction Model*

| Model | SSE (ppm) | df | MSE (ppm) | RMSE (ppm) | F | Sig. |
|---|---|---|---|---|---|---|
| Regression ($SS_M$) | 2785.668 | 5 | 557.134 | 23.604 | 5.650 | 0.000* |
| Residual ($SS_R$) | 20413.113 | 207 | 98.614 | | | |
| Total ($SS_T$) | 23198.782 | 212 | | | | |

*Significant at the 0.01 level (2-tailed).

The mean square error (MSE) and the root mean square error were computed to be 557.134ppm and 23.604ppm respectively. The model sum of squares error ($SS_M$), residual sum of squares error ($SS_R$) and total sum of squares error ($SS_T$) were computed to be 2785.668ppm, 20413.113ppmand 23198.782ppm respectively as shown in Table 4. The result (Table 4) showed that meteorological parameters significantly (P-value <0.05) influence the concentrations of CO concentration in the area. However, the goodness of fit (Figure 6) between predicted and measured values showed a poor linear relationship between CO concentrations and meteorological parameters with a coefficient of determination ($R^2$) of 0.120. This implies that meteorological parameters accounted for only 12.0% of the variation of concentrations in the area in the dry season. The goodness of fit between predicted and measured concentrations of CO is shown in Figure 6, while the predicted values are plotted against measured values as shown in Figure 7.

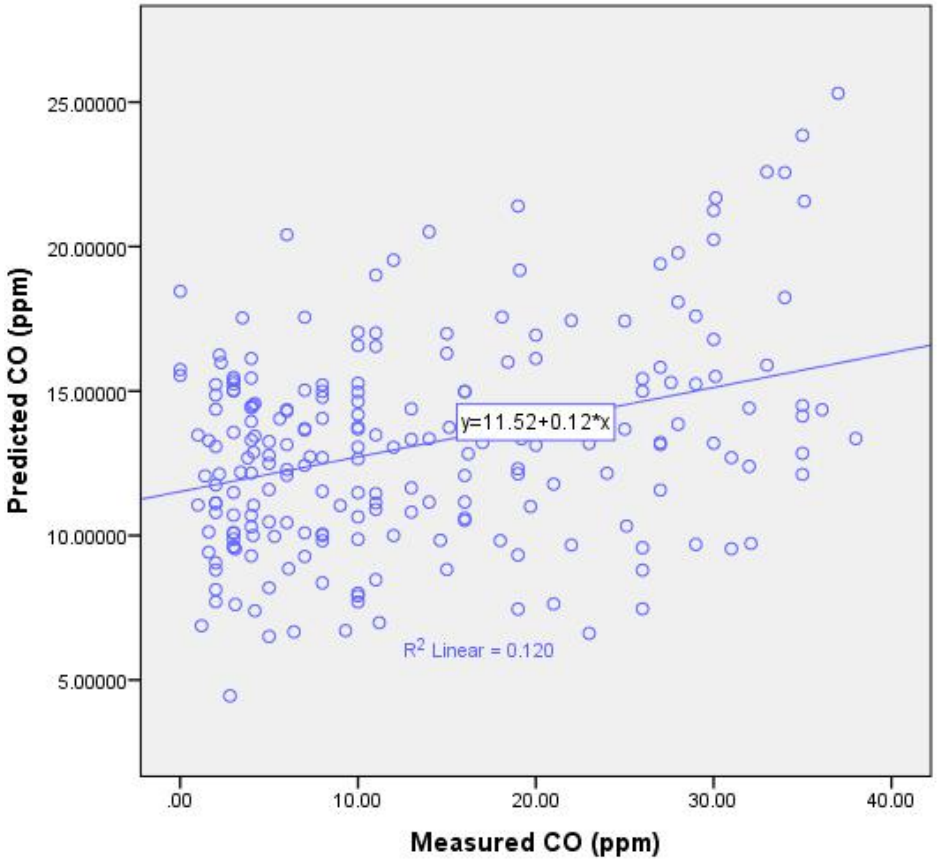


*Fig.6: Relationship between Predicted CO and Measured CO in the Dry Season*

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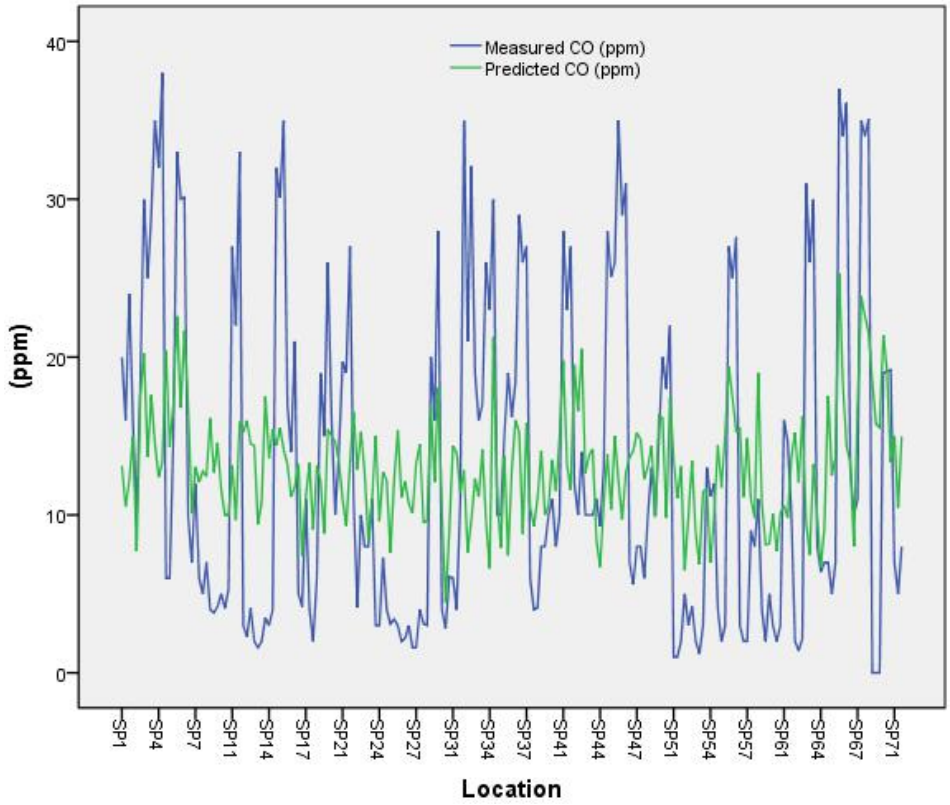
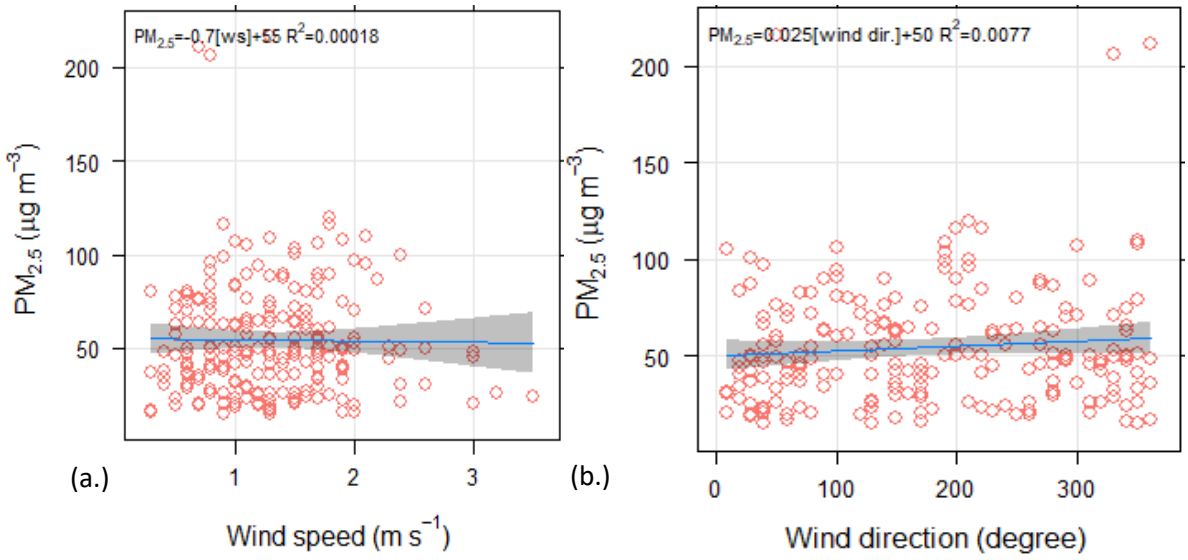



*Fig.7: Predicted CO versus Measured CO in the Dry Season*

**Variation of $PM_{2.5}$ Particulate Matter with Meteorological Parameters in the Dry Season**

The results (shown in Figure 8 (a-e)) indicated that $PM_{2.5}$ varied significantly with relative humidity and temperature and positively increased with wind speed and air pressure. The stepwise regression linear models (shown in Table 5) show that the linear relationships between $PM_{2.5}$ and wind speed, wind direction and air pressure are not significant at 0.05 confidence levels. However, the relationship between relative humidity and concentrations of $PM_{2.5}$ particulate matter is highly significant at 0.01 confidence level for a 2-tail test with a coefficient of determination ($R^2$) of 0.047. This implies that though $PM_{2.5}$ varies significantly with relative humidity, only a fraction of 4.7% of the variation can be explained.



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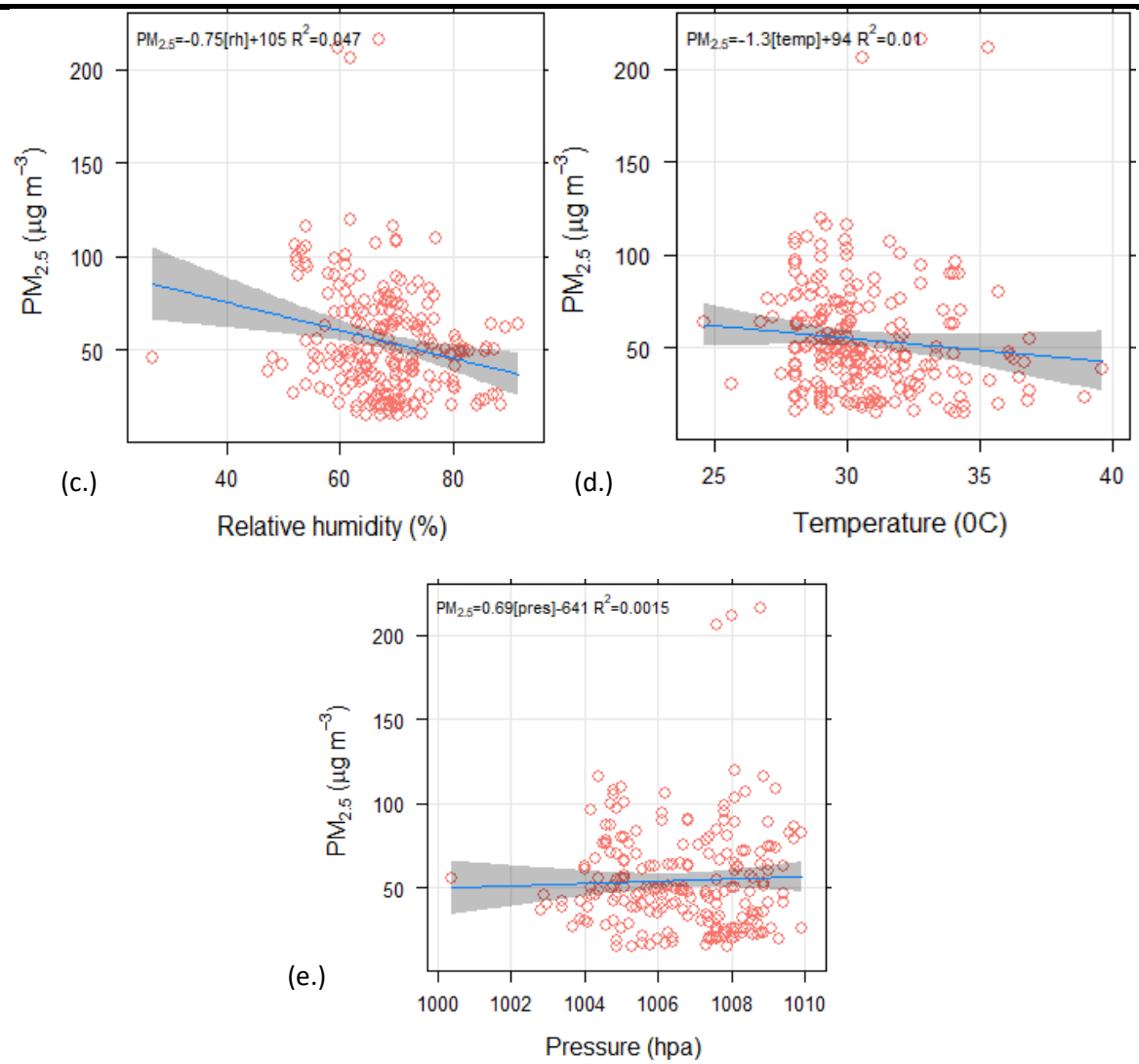


*Fig.8 (a-e): Relationship between Predicted $PM_{2.5}$ and Meteorological Parameters in the Dry Season*

*Table.5: Stepwise Linear Models for $PM_{2.5}$ in the Dry Season*

| Pollutant | Model | $R^2$ | t-statistic | Sig. (2-tailed) |
|---|---|---|---|---|
| $PM_{2.5}$ | = 55 – 0.7*Wsp | 0.00018 | - 0.334 | 0.739 |
| | = 50 + 0.025*Wd | 0.0077 | 1.637 | 0.103 |
| | = 105 – 0.75*Rh | 0.047 | - 4.846 | 0.000* |
| | = 94 – 1.3*Temp | 0.01 | - 3.492 | 0.001* |
| | = - 641 + 0.69*Pres | 0.0015 | 1.835 | 0.068 |

***** Correlation is significant at the 0.05 and 0.01 levels (2-tailed).

A multiple linear regression model for the prediction of $PM_{2.5}$ was developed using a combination of all the meteorological parameters as predictor variables. The following predictive model for concentration of $PM_{2.5}$ particulate was derived as shown in Equation (19).The derived Equation (19) was used to predict the concentrations of $PM_{2.5}$ in the study area in the dry season.

$$PM_{2.5} = -2014.453 - 1.187*Wsp + 0.031*Wd - 1.288*Rh - 3.333*Temp + 2.24*Pres \quad (19)$$

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*Table.6: Analysis of Variance (ANOVA) for Dry Season $PM_{2.5}$ Prediction Model*

| Model | SSE (µg/m$^3$) | df | MSE (µg/m$^3$) | RMSE (µg/m$^3$) | F | Sig. |
|---|---|---|---|---|---|---|
| Regression ($SS_M$) | 25849.946 | 5 | 5169.989 | 71.903 | 5.894 | 0.000* |
| Residual ($SS_R$) | 181565.412 | 207 | 877.128 | | | |
| Total ($SS_T$) | 207415.358 | 212 | | | | |

*Significant at the 0.01 level (2-tailed).

The mean square error (MSE) and the root mean square error were computed to be 5169.989µg/m$^3$and 71.903µg/m$^3$ respectively. The model sum of squares error ($SS_M$), residual sum of squares error ($SS_R$) and total sum of squares error ($SS_T$) were computed to be 25849.946µg/m$^3$, 181565.412µg/m$^3$and 207415.358µg/m$^3$ respectively as shown in Table 6. The result (Table 6) showed that meteorological parameters significantly (P-value <0.05) influence the concentrations of $PM_{2.5}$ in the area. However, the goodness of fit (Figure 9) shows a poor linear relationship between $PM_{2.5}$ and meteorological parameters with a coefficient of determination ($R^2$) of 0.125. This implies that only 12.5% of the variation of $PM_{2.5}$ concentrations can be explained by the meteorological parameters. The goodness of fit between predicted and measured concentrations of $PM_{2.5}$ is shown in Figure 9, while the predicted values are plotted against measured values as shown in Figure 10.

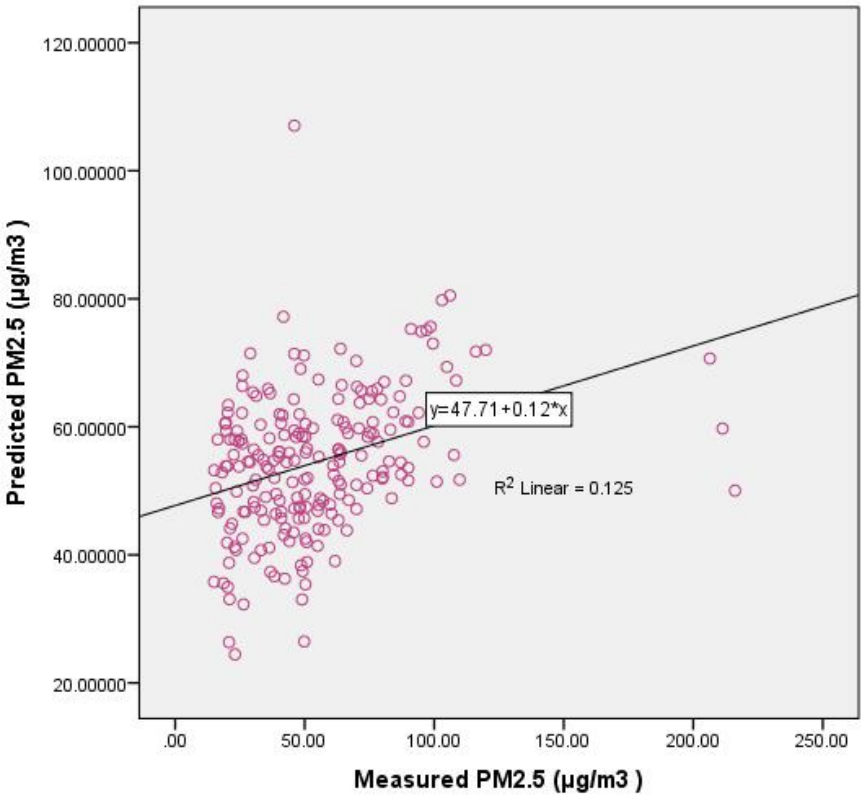


*Fig.9: Relationship between Predicted $PM_{2.5}$ and Measured $PM_{2.5}$ in the Dry Season*

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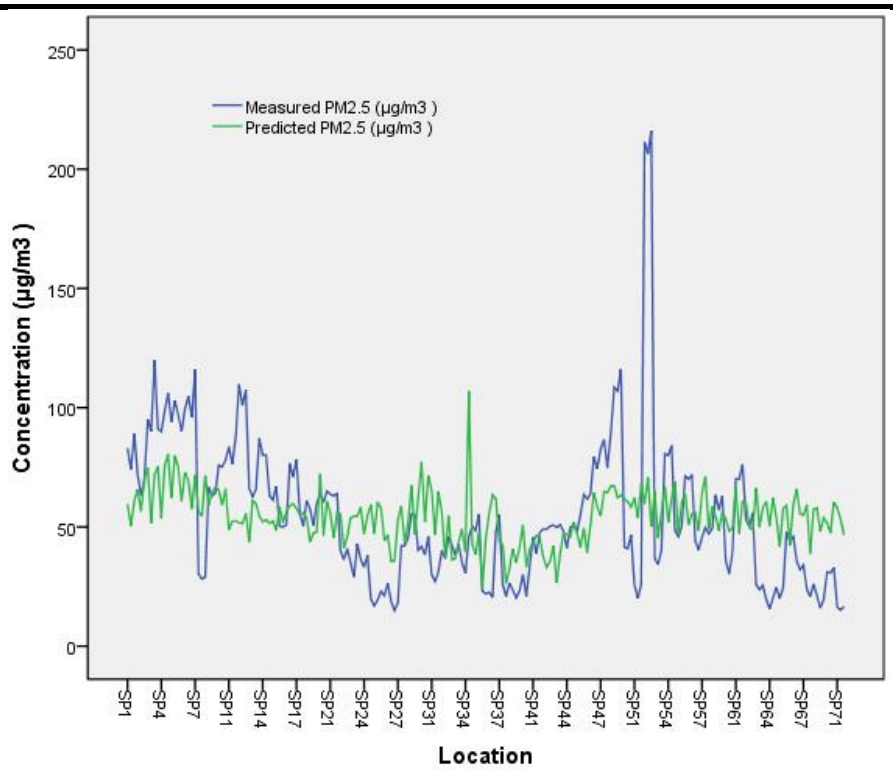


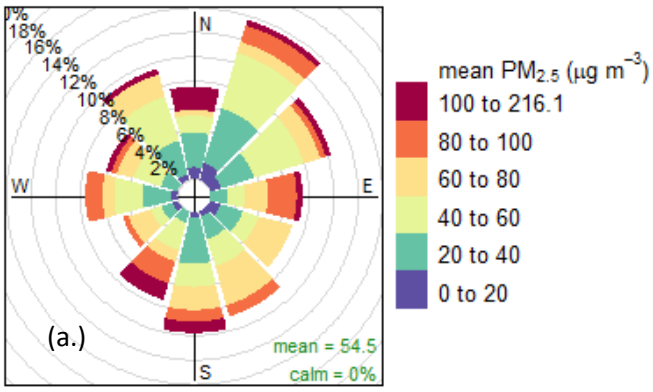

*Fig.10: Predicted $PM_{2.5}$ versus Measured $PM_{2.5}$ in the Dry Season*

## IV. INTERPRETATION AND DISCUSSION MODELING THE RELATIONSHIP BETWEEN AIR POLLUTANTS AND METEOROLOGICAL PARAMETERS IN THE DRY SEASON

### (a) Evaluation of Pollutants Dispersion Pattern in the Study Area in the Dry Season

The pollutants dispersion patterns in the study area in the dry season were evaluated with the aid of pollution roses and bivariate polar plots of each pollutant with respect to wind speed and wind direction. The dry season results are presented in Figures 11 (a-c) and 12 (a-c). The pollution roses and polar plots were developed using the mean concentration of each pollutant in different wind speed and percentage frequency count of wind direction categories (Munir, 2016). They were simulated with the aid of Generalized Additive Model (GAM) smoothing techniques Carslaw, (2015) that depict pollutant concentrations as a continuous surface.

Pollution roses (Figure 11 (a-c)) showed that pollutant concentrations increase with increased wind speed. Low concentrations of pollutants were obtained at low wind speed and vice-versa. This implies that wind speed has positive influence on the concentration levels of pollutants in the study area.



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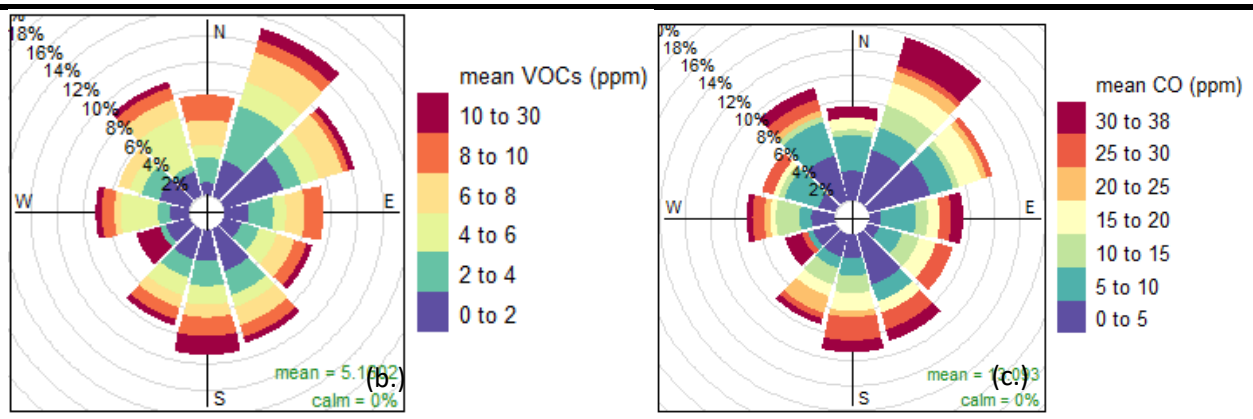


*Fig.11 (a-c): Pollution Roses of Pollutants in the Study Area in the Dry Season*

The pollutant polar plots (Figure 12 (a-c)) showed that concentrations of pollutants in the area are associated with wind speed up to 3.5m/s. It is also observed from Figure 12 (a-c) that pollutant concentrations increase with increased wind speed (Folorunsho *et al*., 1995).

Surface polar plots of pollutants concentrations in the study area revealed that high concentrations of $SO_2$, $NO_2$, $NH_3$, $H_2S$ and VOCs are associated with the south-west and south-east directions and are dispersed toward the north-east and north-west directions (Jimmy *et al.,* 2013). This may imply that sources of these pollutants are in the southern part, which is the coastal region of the study area. Industrial activities, especially in Eleme area (refineries, petrochemical company, fertilizer companies, industrial waste management facilities, civil construction, gas flaring, and vehicular movement) and the released of black carbon (black soot) due to illegal refineries in the coastal area may be the sources of these pollutants (Antai, 2017).

The Figure also indicated that concentrations of CO is associated with south-west, south-east and north-east directions and are dispersed toward the north-west directions. This may imply that sources of this pollutant are both in the southern and northern parts, which are the coastal and up-land areas. Industrial activities, vehicular exhaust emissions, gas flaring and oil and gas exploitation in Eleme, Port Harcourt, Obio/Akpor and Etche areas might be the sources of these pollutant (Antai *et al*., 2016).

Similarly, concentrations of Methane ($CH_4$) and Particulate Matter (TSP, $PM_{10}$ and $PM_{2.5}$) are associated with both northern and southern directions. This showed that activities in the both the coastal and up-land areas are responsible for the release of these pollutants into the environment (Kochubovski *et al*., 2012). In other words, industrial activities, vehicular exhaust emissions, civil construction, the released of black carbon (black soot) due to illegal refineries in the coastal area, gas flaring and oil and gas exploitation in Eleme, Port Harcourt, Obio/Akpor, Etche and Ikwerre areas may be the sources of $CH_4$ and particulate matter in the air environment of the study area in the dry season period (Antai *et al.,* 2017).

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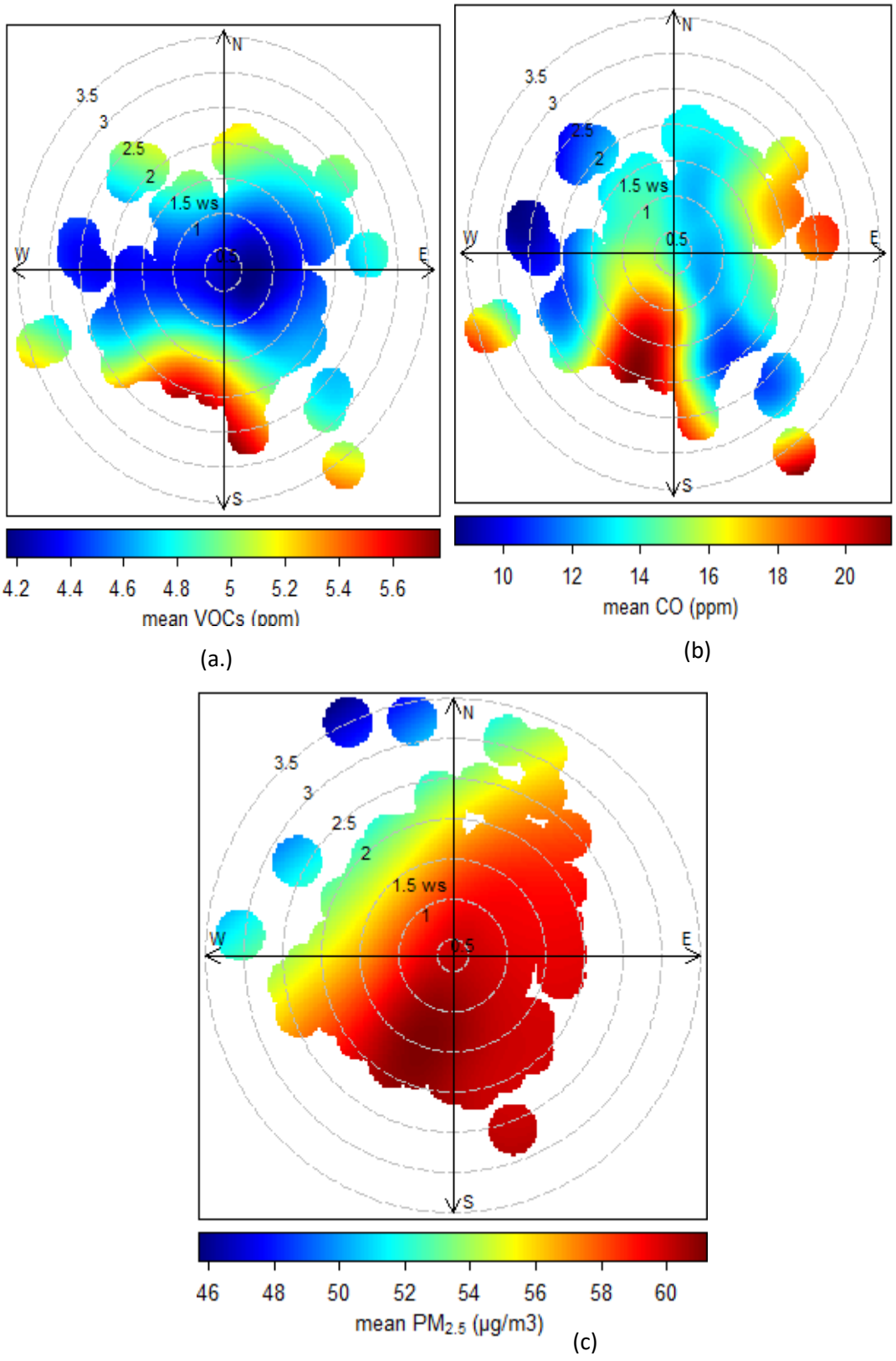


*Fig.12 (a-c): Polar Plots of Pollutants in the Study Area in the Dry Season*

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**Yearly Prediction for 15 Years for Dry Seasons**

Yearly prediction of air pollutants was carried out using a ten year data from previous studies conducted in the study area.

The prediction was done using regression analysis and year as the predictor variable. The relationship between air pollutants and year was therefore established. The annual prediction of pollutant concentrations was made for the dry seasons. The prediction models for each pollutant in the dry season are presented in Equations (20 to 29).The prediction was made for a period of fifteen years (2017 to 2031) and the results of the annual prediction are presented in Table 7 for the dry seasons.

**Dry Season Yearly Prediction**

$$TSP = -66243.8 + 33.07812*Year \quad (20)$$

$$PM_{10} = -5173.13 + 2.603*Year \quad (21)$$

$$PM_{2.5} = -6343.97 + 3.162*Year \quad (22)$$

$$SO_2 = -1118.987 + 0.55645*Year \quad (.23)$$

$$NO_2 = -180.411 + 0.091*Year \quad (24)$$

$$H_2S = -80.7741 + 0.041*Year \quad (25)$$

$$VOCs = -1370.99 + 0.6889*Year \quad (26)$$

$$CO = -716.003 + 0.3594*Year \quad (27)$$

$$NH_3 = -273.036 + 0.13654*Year \quad (28)$$

$$CH_4 = -610.2105 + 0.30321*Year \quad (29)$$

*Table.7: Predicted Yearly Dry Seasons Values for 15 Years*

| Year | TSP | $PM_{10}$ | $PM_{2.5}$ | $SO_2$ | $NO_2$ | $H_2S$ | VOCs | CO | $NH_3$ | $CH_4$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | µg/m$^3$ | | | | | ppm | | | |
| **2017** | 474.77 | 77.12 | 33.78 | 3.37 | 3.14 | 1.92 | 18.52 | 8.91 | 2.37 | 1.36 |
| **2018** | 507.85 | 79.72 | 36.95 | 3.93 | 3.23 | 1.96 | 19.21 | 9.27 | 2.50 | 1.67 |
| **2019** | 540.92 | 82.33 | 40.11 | 4.49 | 3.32 | 2.00 | 19.90 | 9.63 | 2.64 | 1.97 |
| **2020** | 574.00 | 84.93 | 43.27 | 5.04 | 3.41 | 2.05 | 20.59 | 9.98 | 2.77 | 2.27 |
| **2021** | 607.08 | 87.53 | 46.43 | 5.60 | 3.50 | 2.09 | 21.28 | 10.34 | 2.91 | 2.58 |
| **2022** | 640.16 | 90.14 | 49.59 | 6.15 | 3.59 | 2.13 | 21.97 | 10.70 | 3.05 | 2.88 |
| **2023** | 673.24 | 92.74 | 52.76 | 6.71 | 3.68 | 2.17 | 22.65 | 11.06 | 3.18 | 3.18 |
| **2024** | 706.31 | 95.34 | 55.92 | 7.27 | 3.77 | 2.21 | 23.34 | 11.42 | 3.32 | 3.49 |
| **2025** | 739.39 | 97.95 | 59.08 | 7.82 | 3.86 | 2.25 | 24.03 | 11.78 | 3.46 | 3.79 |
| **2026** | 772.47 | 100.55 | 62.24 | 8.38 | 3.95 | 2.29 | 24.72 | 12.14 | 3.59 | 4.09 |
| **2027** | 805.55 | 103.15 | 65.40 | 8.94 | 4.05 | 2.33 | 25.41 | 12.50 | 3.73 | 4.40 |
| **2028** | 838.63 | 105.75 | 68.57 | 9.49 | 4.14 | 2.37 | 26.10 | 12.86 | 3.87 | 4.70 |
| **2029** | 871.71 | 108.36 | 71.73 | 10.05 | 4.23 | 2.41 | 26.79 | 13.22 | 4.00 | 5.00 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **2030** | 904.78 | 110.96 | 74.89 | 10.61 | 4.32 | 2.46 | 27.48 | 13.58 | 4.14 | 5.31 |
| **2031** | 937.86 | 113.56 | 78.05 | 11.16 | 4.41 | 2.50 | 28.17 | 13.94 | 4.28 | 5.61 |

## V. CONCLUSION

The result of multiple linear regressions and generalized additive model in this study revealed that changes in the air pollution of Port Harcourt city and its environs are directly induced and influenced by changes in the meteorological variables in the dry season.

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# Development of Vegetable Seeds Incorporated Cookies: Nutrient Composition, Functional Properties, Mineral Analysis and Sensory Evaluation

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***Abstract*****—** *Jackfruit and Okra are popular fruit crops grown in India. The seeds are the by-product obtained during the processing of these crops. Jackfruit seed possess disposal problem if not handled properly. Scientific data shows that jackfruit and okra seed powder has various health benefits like prevent cancer, lowers the risk of heart disease, improves digestive system, boost immunity in the body, maintains blood glucose levels and helps to increase bone mineral density. The seeds of these crops are particularly a rich source of proteins, starch, minerals and dietary fibres along with phytonutrients. The present study was designed to investigate proximate composition, functional properties and mineral analysis of the best composite flour. Randomization of variables were done along with their nutrient composition. The flour having the highest nutritional value was selected as the best composite flour. Sensory evaluation of the developed food product by incorporating Okra seed flour, Jackfruit seed flour and Wheat flour was also done. After that physical properties of the most acceptable food product were carried out. Results for proximate composition revealed that jackfruit seed flour had an appreciable amount of moisture and protein i.e. 9.08±0.6 and 5.12±0.43 and low amount of fat i.e. 3.6±0.3. While okra seed flour possesses a high amount of moisture and ash i.e. 6.7±0.07 and 6.61±0.4. Estimation of functional properties revealed that water absorption capacity i.e. 1.68±0.051 and oil absorption capacity i.e. 1.81±0.057 of the composite flour was high but the bulk density 0.95±0.02 was very low. Mineral analysis depicted the presence of higher amount of calcium i.e. 3.49±0.02 and iron i.e. 2.71±0.01 but low amount of potassium i.e. 1.46±0.02 in the best composite flour. Sensory evaluation of the product developed (Cookies) was carried out using 9-point hedonic scale with various attributes in four concentrations i.e. 5%, 10%, 15% and 20% and were compared with the standard. Sensory evaluation revealed that up to the level of 5% the developed products were more acceptable than standard. The physical properties with 5% level of incorporation were carried out. Results depicted that oil absorption capacity was high i.e. 4.88±0.02 and good amount in dispersibility i.e. 4.01±0.01 water absorption capacity i.e. 3.74±0.01 but low amount of percent solubility i.e. 1.66±0.01. Thus, the results signify that okra and jackfruits seeds are a good source of various nutrients, functional and mineral properties.*

***Keywords— Jackfruit seed flour, Okra seed flour,By-product, Phytonutrients, Standardization of flour, Functional properties.***

## I. INTRODUCTION

Fruit and vegetable processing has increased considerably during the last 25 years (Indrani*et al*, 1997). This has reflected the increase in demand for pre-processed and packaged food, particularly ready meals. During the period, many modern processes were developed and implemented but disposal of waste was not the major issue it is today. Competitive advantage was often achieved by exploiting the benefits of economies of scale, and strategies consequently involved the centralization of processing activities. This resulted in localized production of large tonnages of waste co-products. These were often disposed of relatively cheaply by landfill, land spreading or selling as animal feed or for its production. The issue of waste in our modern society has become more prominent since it contributes too many of the problems of global environmental sustainability (Claughton*et al*, 1989). Vast quantities of food processing co-product wastes are produced throughout the world.

For most fruits and vegetables, one can estimate the likely waste as approximately 30% or more of processed material or even in some processes; it may be up to 75% (Sathe *et al*, 1982). Nowadays, the high volume of waste produced marks food industry. According to the recent research conducted by FAO, about 1.3 billion tons of food has been wasted worldwide per year, which represents one third of

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the total food industry production (FAO, 2009). The largest amount of loss is verified by fruits and vegetables, representing 0.5 billion tons. In developing countries, fruit and vegetable losses are severe at the agricultural stage but are mainly explained by the processing step, which accounts for 25 % of losses. The growing, processing and preparation of fruit and vegetables result in the production of varying degree of waste material. The waste material may be in the form of leaf, straw and seeds waste during harvesting, processing industry waste and after processing waste (Devraj *et al*, 2008). The waste obtained from fruit processing industry is extremely diverse due to the use of wide variety of fruits and vegetables, the broad range of processes and the multiplicity of the product (William *et al*, 1987).

The by-product seeds represent an important source of sugars, minerals, organic acid, dietary fibre and phenolics, which have a wide range of action, which includes antiviral, antibacterial, cardio-protective and anti-mutagenic activities (Kumar *et al*, 1988). Because of increasing threat of infectious diseases, the need of the hour is to find natural agents with novel mechanism of action. Natural products provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Fruit and vegetable seeds are thrown into the environment as agro waste which can be utilized as a source of anti-microbes (Bremner*et al*, 1996). However, there is currently no major exploitation of these sources, due to the poor understanding of their nutritional and economic value, adding that there is a great opportunity for agribusiness in the area. (Jain *et al*, 2008). Utilization of those by-products as a valuable source of natural food additives appears to be a good alternative toward mitigation of environmental problems and for further exploitation of food additives or supplements having high nutritional value and economically attractive. The transformation of these by products into a "product" with high added value makes it possible for the companies to reduce their cost of treatment, and even to generate additional profits, and thus to improve their competitiveness (Bobbio*et al*, 1978).

The antioxidant capacity of seed is high. Carotenoids are phytochemicals presented in considerable amount in fruit tissue (Rufino *et al*, 2010). Carotenoids play a potentially important role in human health by acting as biological antioxidants, protecting cells and tissues from the damaging effects of free radicals and singlet oxygen and are used as natural colorants in the food industry (Singh *et al*, 2001). The characteristic feature of some tropical exotic fruit by-products like jackfruit seeds is that it has high contents of soluble dietary fibre, which is reported to have more health beneficial effects. The waste generated during the processing of passion fruit mainly consists of peel and seeds.

Despite the high content of bioactive compounds in the skins and seeds of exotic fruits, attention must be paid to antinutritional and toxic factors, like high tannin content in these tissues (Abdalla *et al*, 2007). Tannins are considered nutritionally undesirable because they precipitate proteins, inhibit digestive enzymes and affect the utilization of vitamins and minerals (Awal*et al*, 1991). However, many tannin molecules have been reported to reduce the mutagenicity of a number of compounds and it all depends on the concentration at which it is used or consumed. To avoid these problems, it is recommended that during the preparation of extracts from these by-products, acidic and/or alkaline hydrolysis are recommended in order to inactivate these compounds (Rufino *et al*, 2010).

Fruit production, trade and consumption have increased significantly on the domestic and international markets due to their attractive sensory properties and a growing recognition of its nutritional and therapeutic value (Samadder*et al*, 1990). In many cases the raw fruit is not consumed directly by humans, but first undergoes processing to separate the desired value product from other constituents of the plant tissue. Like tropical crops such as jackfruit, pineapple, papaya and mango are typically valued for their fruit. Processing of these crops typically involves separating the valuable fruit part from by-products such as skin and seeds (Aguilar *et al*, 2010). The mass of by-products obtained as a result of processing tropical exotic crops may approach or even exceed that of the corresponding valuable product, affecting the economics of growing tropical exotic crops (Miljko*et al*, 2002).

The antimicrobial power of seeds and herb extracts has been recognized for centuries and mainly used as natural medicine. However, the trends in using these compounds as food preservatives is increasing now days (Ayalazala*et al*, 2011). In addition, plants produce a wide range of volatile compounds, some of which are important for flavour quality factors in fruits, vegetables, spices, and herbs. Ever since, natural colours from spices and herbs as well as fruits and vegetables have been part of the everyday diet of humans (Soepadmo*et al*, 1992). It is well known that agro industrial by-products are rich in dietary fibres (DF). The DF additive provides economic benefits to the food, cosmetic and pharmaceutical industries (Ajila*et al*, 2010).

Apart from the well-known health effects, dietary fibre shows some functional properties as food additives, such as water-holding capacity, swelling capacity, increasing viscosity or gel formation, which are essential in formulating certain food products (Azad *et al*, 1990). On the other hand, foods are perishable products as a cause of their intrinsic characteristics. Microbial growth, sensorial attribute decay and loss of nutrients are amongst the major causes that compromise the quality and safety of food

produce (Janevska*et al,* 2010). Chemical synthetic additives can reduce food decay but consumers are concerned about chemical residues in the products (Ayala*et al,* 2011). Regarding the food safety issues, one of the major emerging technologies is the application of natural additives. We have to consider that the high content of bioactive compounds present in fruit byproducts can be used as natural food additives. If this approach is realized, it would be feasible to fulfil the requirements of consumers for natural and preserved healthy food. In addition, the full utilization of fruits could lead the industry to a lower-waste agribusiness and increasing industrial profitability.

Several potential uses can be considered for fruit and vegetable by-products, one of the majors can be as food additives (antimicrobials, antioxidants, colorants, flavouringsand thickener agents) (*Arya et al,* 2011). As anti-browning additives fruit and vegetables by-products are sources of a great variety of antioxidants, and their particular properties may be useful in maintaining food quality avoiding enzymatic browning in fruits (Singh *et al,* 2001). The enzymatic browning caused by polyphenol oxidase (PPO) is a major detrimental factor of the quality of fresh-cut fruits and vegetables (Sharman*et al,* 2000). To avoid this problem, several additives have been applied mainly by dipping, spraying or vacuum impregnation. Antioxidants from fruit and vegetables by-products may be grouped in accordance to their mode of action, i.e. as acidulants, reducing and/or chelating agents and enzyme inhibitors. Therefore, their beneficial effects may differ among treated product and matrix applied (Aykroyd *et al,* 1966).Considering that peels and seeds of most exotic fruits are not consumed and rarely approached, the high number of bioactive compounds presented in these non-edible parts could be used for different purposes in the food industry such as enrichment or development of new products.

Okra (*Abelmoschus esculentus Lam.)* is an economically important, tall growing vegetable crop grown in tropical parts of the world. In India, it ranks number one in its consumption but its original home is Sudan and Ethiopia. It is cultivated throughout the tropical and warm temperate regions of the world for its green edible fibrous fruit and pods containing round, white seeds as well as for its ornamental value (Gunasena*et al,* 1966). It is an oligo purpose crop, but it is usually consumed in a variety of ways. Okra plays an important role in the human diet by supplying fats, proteins, carbohydrates, minerals and vitamins. Moreover, its mucilage is suitable for certain medical and industrial applications. The mature okra seeds are a good source of oil and protein. They are rich in unsaturated fatty acids such as linoleic acid, which is essential for human nutrition. Okra seeds are popular due to their high fibre, vitamin-C and folate content (Glacodvin*et al,* 2016). It also contains antioxidant properties. The seeds are known to be rich in high quality protein especially with regards to its content of amino acids relative to other plant protein source. Okra seeds do not only contain nutritionally important bio-compounds but are also sources of other Phyto compounds which at certain critical levels are significant anti-nutritional effects (Jacob *et al,* 2015). The seeds are inexpensive and widely distributed. Theses seeds can be used as an anti-diabetic and is beneficial in chronic or acute eczema.

Jackfruit (*Artocarpusheterophyllus Lam.*) is a popular food crop that is widely grown in Bangladesh and other tropical areas. Jackfruit seeds are light brown to brown, rounded, 2–3 cm (0.8–1.2 in) in length by 1–1.5 cm (0.4–0.6 in) in diameter, and enclosed in a thin, whitish membrane (Dang *et al,* 2014). Up to 500 seeds can be found in each fruit. As jackfruit is highly seasonal and seeds have shorter shelf life, hence go waste during the seasonal glut. Therefore, the seeds can be stored up to a month in cool, humid conditions. They can be eaten in boiled or roasted form. Thus, the jackfruit seeds are not only rich in proteins, starch and dietary fibers but can also be regarded as an abundant yet cheap source of the nutrients. Lectin, a class of glycoproteins found in jackfruit seed, possess an anti-bacterial, anti-fungal and anti-carcinogenic properties (Adekunle *et al,* 2008). Jackfruit contains both water and fat-soluble vitamins along with minerals like vitamin A, vitamin C, thiamine, riboflavin, calcium, potassium, iron, sodium, zinc and niacin among many other nutrients. Jackfruit has a low caloric content. 100 g of jackfruit only contains 95 calories. Jackfruit is a rich source of potassium with 303 mg found in 100 g of jackfruit (Chandrasekharappa*et al,* 1989). Studies show that food rich in potassium helps to lower blood pressure. Jackfruit contains phytonutrients: lignin's, isoflavones and saponins that have health benefits that are wide ranging. These phytonutrients have anticancer, antihypertensive, antiulcer and antiaging properties. The phytonutrients found in jackfruit, therefore, can prevent forming of cancer cells in the body, can lower blood pressure, can fight against stomach ulcers, and can slow down the degeneration of cells that make the skin look young and vitae (Yanty*et al,* 2007). Jackfruit also contains niacin which is necessary for energy metabolism, nerve function, and the synthesis of certain hormones (Rababah*et al,* 2006).

## II. OBJECTIVES

- To estimate Nutrients elements of the samples (okra seed flour, jackfruit seed flour and whole wheat flour)
- To prepare composite flour
- To determine Functional properties and Mineral composition of Best Composite flour

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- To prepare cookies from the selected composite flour and check their Physical properties
- To assess the acceptability of the prepared cookies by Organoleptic Evaluation

## III. METHODOLOGY

Methodologies inquire into potentialities and limitation of some technique or other it is a plan or procedure for carrying out strategies for obtaining valid information.

**Methodology of the study was divided into 5 phases:**

**PHASE I**

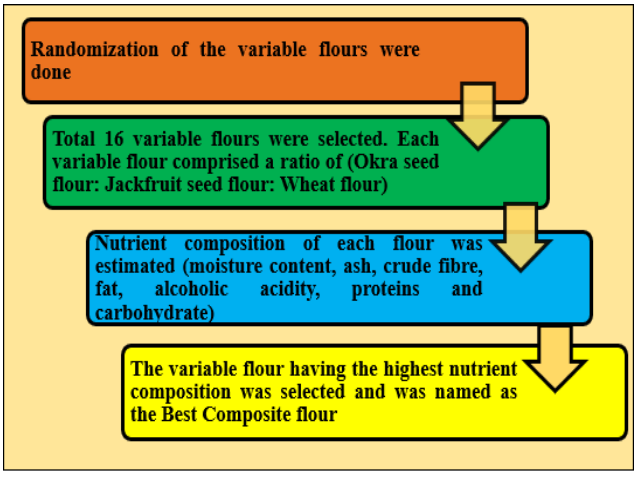
**Analysis of Chemical properties of Okra seed flour, Jackfruit seed flour and Whole wheat flour**

This was the primary phase of our present study. After collecting the seeds and preparing them into flour, the proximate composition of the three flours i.e okra seed flour, jackfruit seed flour and wheat flour were estimated individually. The proximate composition of the flour includes the moisture content (%), fat (%), crude fibre (%), ash (%), protein (%) and carbohydrates (%).

**PHASE II**

**Standardization of Composite flour**

In this, random selection of flour variables were done and were divided into different ratios like Okra seed flour, whole wheat flour and jackfruit seed flour (5:85:10). Total 16 variables were studied and for each variable, the proximate composition was calculated. The results of all 16 variable flours were compared and the best composite flour was selected according to the nutrient composition.




**Phase III**

**Best Flour According To Nutritional Quality**

The third phase of our present study was to find the Functional properties and Mineral analysis of the best composite flour. For the estimation of functional properties, I did the test of Bulk density (%), Oil absorption capacity (%) and Water absorption capacity (%) to know the bulk and consistency of the composite flour. For mineral analysis, the test of Iron (mg/100g), Calcuim (mg/100g), Zinc (mg/100g) and Potassium (mg/100g) were done.

**PHASE IV**

**Preparation of product (Cookies) and estimate Physical properties**

In this phase, product was developed i.e. cookies and sensory evaluation of the product was done. Sensory evaluation of the developed food product was carried out using Triangular test for selection of the panel and for judging the formulated products, 9-point Hedonic rating scale with various attributed was conducted. There was Standard (S) and its four variation were made by incorporating okra seed flour, jackfruit seed flour and wheat flour with the amount 5%, 10%, 15% and 20% assigned as Sample A1, Sample A2, Sample A3 and Sample A4 respectively. After completing the sensory evaluation, the physical properties of the most acceptable product was carried out. In the physical properties, we estimated the swell power (%), foaming capacity (%), protein solubility (%), oil and water absorption capacity (%) of the product (cookies).

**PHASE V**

**Statistical analyis and Report writing**

This was the last phase of our present study. In this phase, we did statistical calculations i.e. estimation of mean, standard deviation and student's t-test. After the statistical analysis, report writing of our present study was done.

**Collection of Okra & Jackfruit Seeds**

The seeds were collected from the local market of Kashmere gate district New Delhi., India during the late rainy season in the month of July-September.

**Preparation of Flour**

Okra and Jackfruit pods were washed, cut into slices and the seeds were extracted, washed, drained and dried at temperature of 60℃ for 4 hours. The dried seeds were milled and sieved through 0.45 mm mesh sieve. The seed flour obtained were then sealed in a cellophane bag and stored thoroughly at room temperature, for further testing and incorporation in food products.

## IV. RESULTS AND DISCUSSION

Oncethe study has been conducted the next step involved the recording of results. Varied data was collected with the help of various methods in order to accomplish the study (Akuboor *et al*, 2003).

The purpose of any report is the dissemination of knowlegde and broadcasting the generation to ensure their widest use. The true and objective recording of the result is of paramount importance. It is the final step of a research endeavor to come up to a logical conclusion. Result of the research, thus constitute the final step, through which answer to the questions are sought.

Commencing with the collection of seeds (*Artocarpus heterophyllum Lam.)* and (*Abelmoschus esculentus Lam*.) and preparation of powder. This project was an attempt to assess the nutriotional, functional and mineral properties of okra seed flour, jackfruit seed flour and wheat flour.

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Further the study includes product development (Cookies) from the seeds powder and assessing their acceptability through 9-point hedonic scale and the most acceptable product to be further tested for the physical properties. At the end, statistical analysis and report writing was done.

The results of the study have been enumerated and disussed under the following heads:

1.1 Proximate composition
1.2 Nutrient composition of all Composite flour
1.3 Functional properties
1.4 Mineral analysis
1.5 Product development
1.6 Sensory evaluation
1.7 Physical parameters

*Table 1.1. Proximate composition of Okra seed flour, Jackfruit seed flour and Wheat flour*

| Proximate Analysis | Okra seed flour | Jackfruit seed flour | Wheat flou |
|---|---|---|---|
| Moisture Content (g/100g) | 7.70±0.07 | 9.08±0.6 | 10.33±0.45 |
| Fat (g/100g) | 3.62±0.3 | 4.63±0.3 | 2.33±0.1 |
| Crude Fibre (g/100g) | 5.64±0.4 | 7.80±0.42 | 3.90±0.1 |
| Ash Content (g/100g) | 6.61±0.4 | 5.34±0.07 | 2.12±0.3 |
| Protein (g/100g) | 5.99±0.4 | 8.12±0.43 | 11.97±0.6 |
| Carbohydrate (g/100g) | 65.01±0.23 | 71.47±0.56 | 77.35±0.51 |

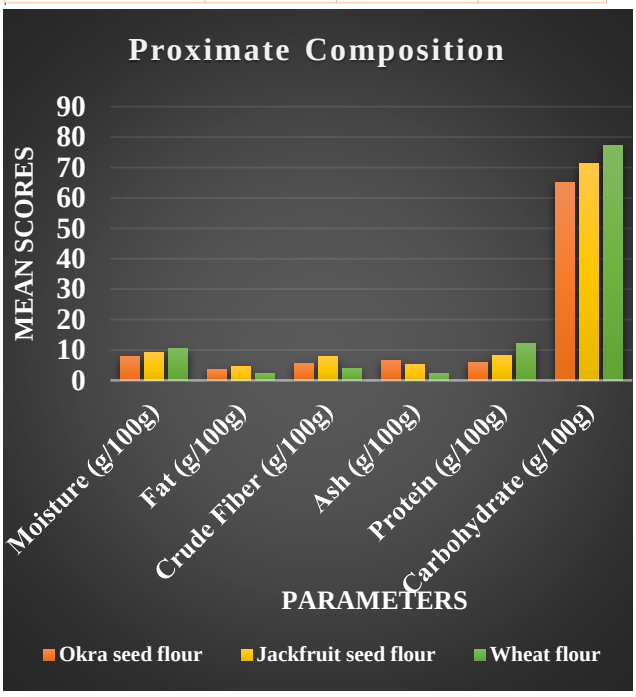


*Fig.1.1: Proximate composition of Okra seed flour, Jackfruit seed flour and Wheat flour*

## DISCUSSION

As reported in the study of (Lakshiminarayana*et al*, 2014), the moisture content of okra seed flour, jackfruit seed flour and wheat flour were 9.2±0.01, 8.64±0.1 and 12.56±0.05. Low moisture content enhances the shelf life of a food product. Fat content was 3.62±0.3. 4.63±0.3 and 4.01±0.3. Crude fiber was 3.01±0.01, 4.60±0.22 and 4.02±0.1. The ash content was 5.33±0.2, 5.1±0.4 and 3.45±0.1. The presence of protein is 6.56±0.2, 5.01±0.1 and 8.12±0.1. Carbohydrate in the flour was 56.51±0.1, 83.07±0.5 and 73.15±0.2. This suggests that the product can be good source of crude fiber and protein. Crude fiber is the residue that remain after a food sample has been subjected to treatment by acid and then by alkali under standard condition.it cleanses the digestive tract, by removing the potential carcinogens from the body and prevents the intake of excess starchy food. Jackfruit has been recognized as an excellent source of fiber, which is an important consideration for people who suffer from elevated cholesterol levels and in helping to cleanse the colon.

*Table No. 1.2 Nutrient composition of all composite flour*

| | Composite Mixed Flour | | | Nutrients | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| S. No | Okra seed flour | Wheat flour | Jackfruit seed flour | Moisture Content (g/100g) | Fat (g/100g) | Crude Fibre (g/100g) | Ash (g/100g) | Alcoholic acidity (g/100g) | Protein (g/100g) | Carbohydrate (g/100g) |
| 1. | 10 | 50 | 40 | 9 | 15.76 | 3.5 | 1.98 | 1.12 | 2.84 | 66.91 |
| 2. | 5 | 85 | 10 | 9 | 15.62 | 2.1 | 4.9 | 1.99 | 3.69 | 64.68 |
| 3. | 7.5 | 52.5 | 40 | 9.5 | 15.54 | 1.9 | 4.95 | 0.25 | 2.41 | 65.71 |
| 4. | 6.25 | 76.25 | 17.5 | 9.5 | 17.19 | 3.3 | 2.94 | 2.24 | 2.27 | 64.81 |
| 5. | 5 | 55 | 40 | 7 | 16.07 | 2.7 | 4.95 | 1.83 | 3.12 | 66.18 |
| 6. | 10 | 50 | 40 | 6.5 | 19.23 | 3.3 | 3.92 | 2.10 | 2.70 | 64.39 |
| 7. | 10 | 60 | 30 | 10 | 17.79 | 1.7 | 3.01 | 0.56 | 2.98 | 64.51 |
| 8. | 5 | 85 | 10 | 10 | 17.75 | 2.0 | 2.46 | 0.47 | 1.85 | 66.91 |
| 9. | 5 | 65 | 35 | 8.5 | 16.78 | 3.2 | 2.94 | 2.32 | 2.98 | 59.71 |
| 10. | 7.5 | 52.5 | 40 | 10 | 17.79 | 2.0 | 2.46 | 1.45 | 1.85 | 66.91 |
| 11. | 10 | 70 | 20 | 10 | 14.53 | 1.7 | 3.01 | 1.68 | 2.98 | 64.53 |
| 12. | 10 | 80 | 10 | 8.5 | 23.77 | 2.8 | 0.98 | 2.22 | 2.56 | 70.62 |
| 13. | 6.25 | 61.25 | 32.5 | 8.5 | 12.65 | 3.1 | 2.94 | 0.88 | 1.99 | 59.71 |
| 14. | 10 | 80 | 10 | 9 | 12.54 | 3.2 | 4.83 | 1.53 | 1.14 | 69.35 |
| 15. | 10 | 65 | 25 | 7 | 16.05 | 2.7 | 4.95 | 2.01 | 3.12 | 66.18 |
| 16. | 5 | 55 | 40 | 8.5 | 14.52 | 2.8 | 0.98 | 1.85 | 2.56 | 70.67 |
| Mean | | | | 8.78125 | 16.4738 | 2.625 | 3.2625 | 1.53125 | 2.62688 | 65.7375 |
| S.D | | | | 1.125 | 2.66679 | 0.62876 | 1.36396 | 0.67754 | 0.60929 | 3.10822 |

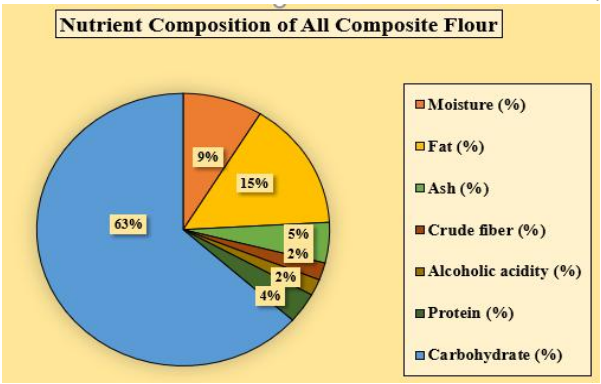


*Fig.1.2: Nutrient composition of the best composite flour*

## DISCUSSION

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**Table 1.2** Represents the nutrient composition of all composite flours.Basically, it was done to find out the best composite flour according to the nutrient composition. Randomization of the variables were done during the on-going process of our study. Total 16 variables were selected having different ratios of okra seed flour, jackfruit seed flour and whole wheat flour. After distributing the variable flours with the respective ratios, the nutrient composition for each variable flour was estimated. Under nutrient composition: moisture content, ash, crude fibre, fat, alcoholic acidity, proteins and carbohydrate test were done. The results were compared and it was seen that "Variable 2" which was a mixture of (Okra seed flour 5% + Wheat flour 85% + Jackfruit seed flour 10%) depicted a high percentage of the nutrients especially fat i.e. 15.62 g/100g, ash i.e. 4.9 g/100g,protein i.e. 3.69 g/100g and carbohydrate i.e. 64.68 g/100gas compared to other variables which depicted a lesser value of nutrients. "Variable 2" was considered as the most suitable blending ratio for the production of cookies from okra seed flour, jackfruit seed flour and wheat flour. Thus "Variable 2" was selected as the Best Composite Flour and further testing were done on this flour.

*Table 1.3. Functional properties of the best composite flour*

| Functional Properties | Mean±SD |
|---|---|
| Bulk Density (g/ml) | 0.95±0.02 |
| Water Absorption Capacity (g/ml) | 1.68±0.051 |
| Oil Absorption Capacity (g/ml) | 1.81±0.057 |

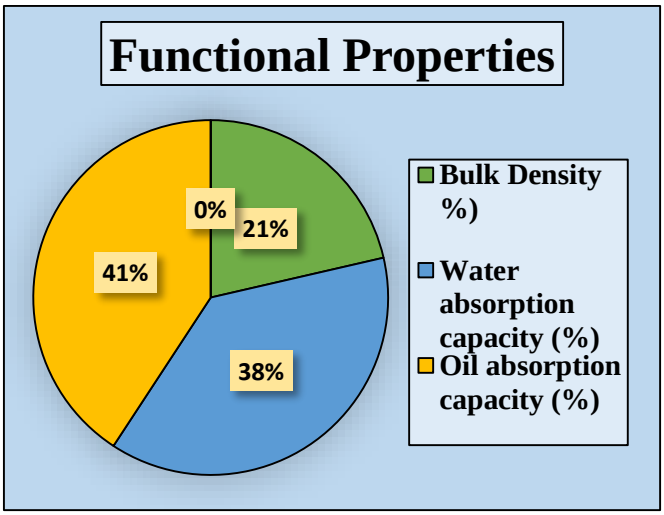


*Fig.1.3: Functional properties of the best composite flour*

**DISCUSSION**

Bulk density gives an indication of the relative volume of packaging material required. As reported by (Niba*et al*, 2001), the bulk density of the flour was 1.24±0.1 g/g which was higher than the results of our present study i.e. 0.95±0.02 g/g. Water absorption capacity is important in bulking and consistency of product as well as in baking applications. The result shows that the water absorption capacity of the composite flour was 1.10±0.1 g/g which was lower than the findings of our study i.e. 1.68±0.051 g/g. Oil absorption capacity is the ability of the flour protein to bind fat by capillary attraction. The oil absorption capacity of the flour was found to be 1.21±0.02g/g whereas the results of our finding shows the oil absorption capacity of the composite flour as shows that the oil absorption capacity of the flour was 1.81±0.057 g/g. An increase in the oil absorption capacity could be attributed to the increase in the protein content of the composite flour. It is also important as oil acts as a flavour retainer and improves mouth feel.

*Table No.1.4 Mineral composition of the best composite flour*

| Mineral Content | Mean±SD |
|---|---|
| Iron (mg/100g) | 2.71±0.01 |
| Zinc (mg/100g) | 1.61±0.02 |
| Calcium (mg/100g) | 3.49±0.02 |
| Potassium (mg/100g) | 1.46±0.02 |

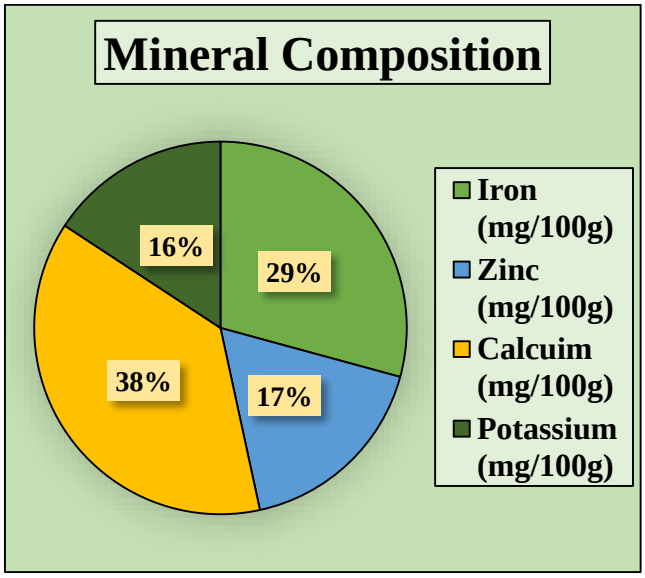


*Figure 1.4 Mineral composition of the best composite flour*

**DISCUSSION**

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As reported by (Abbah *et al*, 2015), the iron present in the composite flour was 1.52±0.07 which was lower than the findings of our present study i.e. 2.71±0.1. Iron has been reported as an essential trace metal and plays numerous biochemical roles in the body, including oxygen binding in hemoglobin and acting as an important cataltic center in enzyme. (Adeyeye *et al*, 1990). Zinc is an essential trace element that helps to stimulate the activity of different enzymes. In the study of (Abbah *et al*, 2015) the zinc content in the flour was 0.95±0.01 which was lower than the findings of our study i.e. 1.61±0.02. Calcuim is a major mineral sustaining strong bones and plays a part in muscles contraction and relaxation, blood clotting, synaptic transmission and absorption of vitamin B12 (Singh *et al*, 2003). The calcuim obtained from the flour was 3.01±0.01. which was similar to the findings of our present study i.e. 3.49±0.02. Potassium is an essential mineral micronutrient which is required for regulating fluid balance and controlling the electrical activity of the heart and muscles.The potassium content of our study was 1.46±0.02 which was lower than the findings of (Adenkule *et al*, 2004) i.e. 2.82±0.07. Calcuim, Iron and Zinc play a central role in the normal regulation of blood pressure. In particular, these elements have important inter-relation in the control arterial resistance.

**Product Development**

In the present study, a product was developed i.e. Cookies with the variations. There was a Standard (S) and its four variation were made by incorporating okra seed flour, jackfruit seed flour and wheat flour with the amount 5%, 10%, 15% and 20% assigned as Sample A1, Sample A2, Sample A3 and Sample A4 respectively.

**Table No. 1.5 Formulation of the best composite flour**

| Sample | Concen-tration | Okra seed flour | Jackfruit seed flour | Whole wheat flour |
|---|---|---|---|---|
| A1 | **5%** | **5%** | **10%** | **85%** |
| A2 | **10%** | **5%** | **10%** | **85%** |
| A3 | **15%** | **5%** | **10%** | **85%** |
| A4 | **20%** | **5%** | **10%** | **85%** |

**Keywords:**

**S:** Standard

**A1:** 5% incorporation of okra seed flour, jackfruit seed flour and whole wheat flour

**A2:** 10% incorporation of okra seed flour, jackfruit seed flour and whole wheat flour

**A3:** 15% incorporation of okra seed flour, jackfruit seed flour and whole wheat flour

**A4:**20% incorporation of okra seed flour, jackfruit seed flour and whole wheat flour

**COOKIES**


**Plate 4.5 (a) Standard**


**Plate 1.5 (b) 5% incorporation of best composite flour**


**Plate 1.5 (c) 10% incorporation of best composite flour**

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**Plate 1.5 (d) 15% incorporation of the best composite flour**


**Plate 1.5 (e) 20% incorporation of the best composite flour**

**Sensory analysis**

Sensory evaluation of the developed food product was carried out using Triangular test for selection of the panel and for judging the formulated products 9-point Hedonic rating scale with various attributed was conducted.

**Hedonic rating test**

Overall acceptability depends upon the concentration or amount of particular component, the nutrition and hidden attributes of a food and its palatability or sensory qualities. The newly developed products were graded by using 9 point hedonic scale, in which a score of 9 stood for 'like extremely' and score of 1 indicated 'dislike extremely'. The data obtained by hedonic scale was averaged by calculating the mean. The mean scores of different attributes were predicted below.

**Table No. 1.6Sensory Evaluation of Cookies**

| ATTRIBUTES | STANDARD | A1 | A2 | A3 | A4 |
|---|---|---|---|---|---|
| COLOR | 8.75±0.44 | 8.55±0.51$^{ns}$ | 8.50±0.60$^{ns}$ | 8.10±0.72* | 7.55±0.60$^{ns}$ |
| APPEARANCE | 8.55±0.51 | 8.45±0.51$^{ns}$ | 8.36±0.77$^{ns}$ | 7.70±0.80$^{ns}$ | 7.35±0.67$^{ns}$ |
| FLAVOR | 8.7±0.47 | 8.45±0.51$^{ns}$ | 8.30±0.66* | 7.90±0.64$^{ns}$ | 7.25±0.64$^{ns}$ |
| TASTE | 8.75±0.44 | 8.40±0.51* | 8.15±0.43$^{ns}$ | 7.70±0.57$^{ns}$ | 7.45±0.6$^{ns}$ |
| OVERALL ACCEPTABILITY | 8.81±0.41 | 8.45±0.6* | 8.40±0.5* | 7.65±0.49$^{ns}$ | 7.25±0.79$^{ns}$ |

***= significant difference**

**ns= no significant difference**

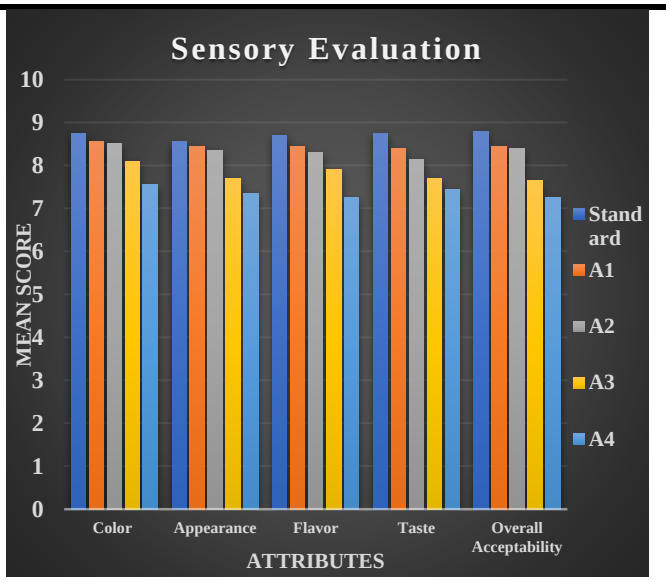


*Fig.1.6: Sensory evaluation of Food product development of (Cookies)*

**DISCUSSION**

As presented in table1.6. the cookies were prepared by the best composite flour which comprised of Okra seed flour, Jackfruit seed flour and Wheat flour in the ration of (5:10:85). The cookies were made by incorporating okra seed flour, jackfruit seed flour and wheat flour in four differenet concentration i.e. 5% (Sample A), 10% (Sample B), 15% (Sample C) and 20% (Sample D) respectively and were compared with the standard. The result states Mean±SD for all attributes (appearance, color, texture, taste and overall acceptability). The mean score secured for taste were ranging from 8.7±0.48 to 8.4±0.5 with the minimum score recorded for the sample D i.e. 7.2±0.6 with 20% level of incorporation. The mean score secured for appearance of cookies ranging from 8.7±0.4 to 8.5±0.5 with the minimum score recorded for the variant A4. Similarly in flavor and color the score ranges from 8.7±0.4 to 8.4±0.5, 8.7±0.4 to 8.5±0.5 with the minimum score recorded for Sample D. For overall acceptability mean score ranging from 8.8±0.4 to 8.4±0.6 with the maximum score recorded for the sample A i.e. 8.4±0.6. Thus it can be conclued that the most acceptable food product is Sample A by the semi trained members.

*Table No. 1.7 Physical properrties of the product developed (Cookies) with 5% incorporation of the best composite flour*

| Physical Parameters | Mean±SD |
|---|---|
| Water absorption capacity (%) | 3.74±0.01 |
| Oil absorption capacity (%) | 4.88±0.02 |
| Foaming capacity (%) | 4.01±0.01 |
| Swell Power (%) | 3.62±0.02 |
| Protein solubility (%) | 1.66±0.01 |

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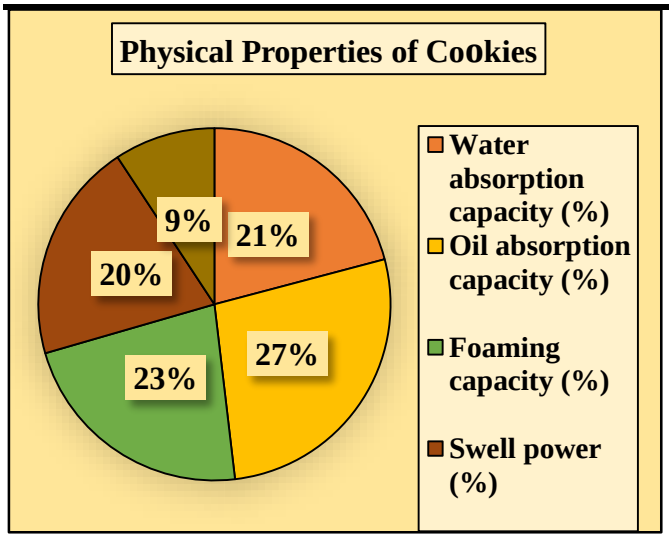


*Fig.1.7: Physical Properties of the product developed (Cookies) with 5% incorporation of the best composite flour*

**DISCUSSION**

Water absorption capacity is important in bulking and consistency of product as well as in baking application as reported by (Niba*et al,* 2001). The water absorption capacity of the flour was 3.74±0.01which was lower than the findings of (Niba*et al,* 2001) i.e. 2.95±0.01.The oil absorption capacity of the flour was high i.e. 4.88±0.02. The oil absorption capacity obtained from the flour was similar to the findings of (Abu *et al,* 2014) i.e..4.53±0.01.The Foaming capacity of the flour was found to be 4.01±0.01 and it was similar to the findings of (Rababah*et al,* 2006) i.e. 3.96±0.02. The composite flour has high surface viscosity property which means that the composite flour increases the hydrophobic nature of the protein matrix. Thus, the flour may have potential use as raw matter in the food industry.Swell power determines the extent to which a flour sample increases in volume when soaked in water in relation to its initial volume. The swelling power of the flour was 3.62±0.02 which was higher than the findings of Okaka and Potter (1977) i.e..4.11±0.01. The solubility of protein is considered as that proportion of nitrogen in a protein product which is in the soluble state under specific conditions. Solubility is the amount of protein in a sample that dissolves into solution. The percent solubility of the flour was 1.66±0.01 while in the study of (Rababah*et al,* 2006) it was 0.96±0.01.

## V. CONCLUSION

The by-product seeds represent an important source of sugars, minerals, organic acid, dietary fibre and phenolics which have a wide range of action. These includes antiviral, antibacterial, cardio-protective and anti-mutagenic activities (Kumar *et al,* 1988). Fruit and vegetable seeds are thrown into the environment as agro waste which can be utilized as a source of anti-microbes (Bremner*et al,* 1996). Utilization of these by-products as a valuable source of natural food additives appears to be a good alternative toward mitigation of environmental problems and for further exploitation of food additives or supplements having high nutritional value and economically attractive. The transformation of these by products into a "product" with high added value makes it possible for the companies to reduce their cost of treatment and even to generate additional profits and thus to improve their competitiveness (Bobbio*et al,* 1978). Considering that seeds of most exotic fruits and vegetables are not consumed and rarely approached, the high number of bioactive compounds presented in these non-edible parts could be used for different purposes in the food industry such as enrichment or development of new products.

In conclusion, okra and jackfruit seeds are nutrient rich food that provides sufficient amount of nutrition needed for normal body function, maintenance and reproduction. The outcome of the study demonstrated that *Artocarpusheterphyllus Lam.*can serve as a good nutritional spring as they are a rich source of fibre, proteins, iron and zinc in comparison to *Abelmoschus esculentus Lam*. The presence of bio active compounds and phyto-nutrients are an assertion in the management of various ailments including wide-ranging cardiovascular and metabolic benefits, which can be readily incorporated into healthy diets. The functional properties present in *Artocarpusheterphyllus Lam* and *Abelmoschus esculentus* shows a vital role in preventing innumerable health disorders related to oxidative stress, diabetes and cancer.

As indicated in the results, the develop product-Cookies at 5% incorporation of the composite flour also showed a low glycaemic index which can be included in the diet for the management of diabetes more effectively. Thus, keeping in view of the above facts the present study was an attempt to estimate the proximate composition, functional properties, mineral properties and physical properties of the jackfruit seed flour, okra seed flour and wheat flour and development of commonly consumed food products by incorporating it.

In coming next generation, the importance of jackfruit seed and okra seed is going to increase because of their effectiveness, easy availability, low cost. Most of the people do not know their nutritional quality, health benefits and how to use and what to use. They are not utilized to its full potential. So, the basic aim of this study is to create data about the jackfruit and okra seed and its health benefits. A range of products will also be developed the popularize this seed among people and to ease its usage. These also lead to the conception of this project and the objectives designed under the study were successfully attained.

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# D-Amino Acid Oxidase Production from Cassava Glucose Syrup by *Trigonopsis variabilis*

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***Abstract*—** *Three alternative carbon sources (molasses, cassava glucose syrup and sorghum fructose syrup) on the production of D-amino acid oxidase (DAAO) from Trigonopsis variabilis (TvDAAO) has been studied.The aim of this study was to screen out the best alternative carbon sources that can be utilized in TvDAAO production, and to study the effect of the additional nitrogen sources. Screening of carbon sources were carried out using glucose, molasses, cassava glucose syrup (CGS) and sorghum fructose syrup (SFS), at the same glucose levels. Analysis of TvDAAO was measured by o-phenylenediamine/horseradish peroxidase coupling assay. The best alternative carbon source was selected for optimization at various concentrations. The enzyme characteristic was done by determining the stability of enzyme to temperature and pH, and the enzyme kinetic parameter was also observed. The screening showed that cassava sugar syrup is the best alternative carbon source. The optimum concentration of cassava glucose syrup is at 10% of glucose levels, which will produce TvDAAO with the activity equal to 166.8861 U/g yeast cell dry weight. The enzyme characteristics stable at 4-10°C and pH 8, with Vmax value was 0.007 µmol/minute and KM was 78 mM. The used of cassava glucose syrup does not require any additional nitrogen source and it is became the advantageous of CGS as an alternative carbon source in terms of efficiency and economical of TvDAAO production.*

***Keywords*— *Cassava Glucose Syrup, D-Amino Acid Oxidase, Trigonopsis variabilis.***

## I. INTRODUCTION

There are two main groups of cephalosporin antibiotics; the first one is penicillin (G or V) derivative, the second is a cephalosporin C (CPC) derivative. The penicillin-derived products are mainly based on 7-Amino-desacetoxy cephalosporanic acid (7-ADCA) and the CPC-derived was 7-aminocephalosporanic acid (7-ACA). Active semi-synthetic cephalosporin are mostly derived from 7-ACA, whereby the 7-ACA issued as a precursor for the synthesis of the active pharmaceutical ingredient (API). CPC can be converted to 7-ACA in two ways, either chemically or an enzymatically [1]. CPC conversion in two enzymatic steps has become industrial standard for 7-ACA production. Two principle enzymatic routes are proposed (Fig.1), one-step hydrolysis of CPC with a CPC acylase (CA) and two-step cleavage with D-amino acid oxidase (DAAO) and glutaryl acylase (GAC) [2]. Hydrolysis of cephalosporin C into 7-ACA cannot be achieved as a one-step process since no enzyme with such type of activity has been discovered so far [3]. Recent study of CPC acylase was reported by Ma et al. [4].

The D-amino acid oxidase (DAAO, EC 1.4.3.3) is a flavoenzyme that can catalyzes the oxidative deamination of d-amino acids to produces α-keto and ammonia. DAAO plays a role in the production of α-keto acid, which is a useful therapeutic agent for the treatment of chronic uremia [5]. The main use of DAAO is in bioconversion of cephalosporin C (CPC) to 7-ACA. DAAO can be found in mammalian organs, mainly in the kidneys. DAAO can also be produced from the following types of microorganism, such as the yeasts Trigonopsis variabilis [6], Rhodotorula gracillis [7] and Candida tropicalis [8], the fungi Neurospora crassa [9]; Rhodosporidium spec. [10], Fusarium solani [11]. Only two enzymes, namely DAAO from Rhodotorula gracilis and Trigonopsis variabilis, have been developed into an industrial biocatalyst. Trigonopsis variabilis DAAO (TvDAAO) has the highest catalytic activity for CPC oxidation that's practical importance was reported[12].

Currently, Indonesia is still importing and spending a lot of money on various types of antibiotic raw materials for the use of local antibacterial industry and research purposes. This is due to the absence of intensive efforts made to produce raw materials of drugs using local resources. The production of antibiotic, such as penicillin and

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cephalosporin industry, can be made by fermentation process. Agro industrial residuals have potentially been used as substrate in fermentation, not only for the production of enzymes but also others secondary metabolites. Cane molasses, an important residue of the sugar industry, have the potential as a cost-effective carbon source that could serve as nutrients for industrial enzyme-producing microorganisms, especially filamentous fungi [13]. Meanwhile, the use of other agro industrial residual, such as cassava glucose syrup and sorghum syrup, is limited or nonexistent. Countries like Indonesia with the abundant of the agro industrial residual have great potential to utilize it maximally.

This study aims to screen out the best alternative carbon sources that can be utilized in TvDAAO production, and to study the effect of the additional nitrogen sources, so that the TvDAAO production process becomes more efficient and economical. This study also to optimize the concentration of the best alternative carbon source.

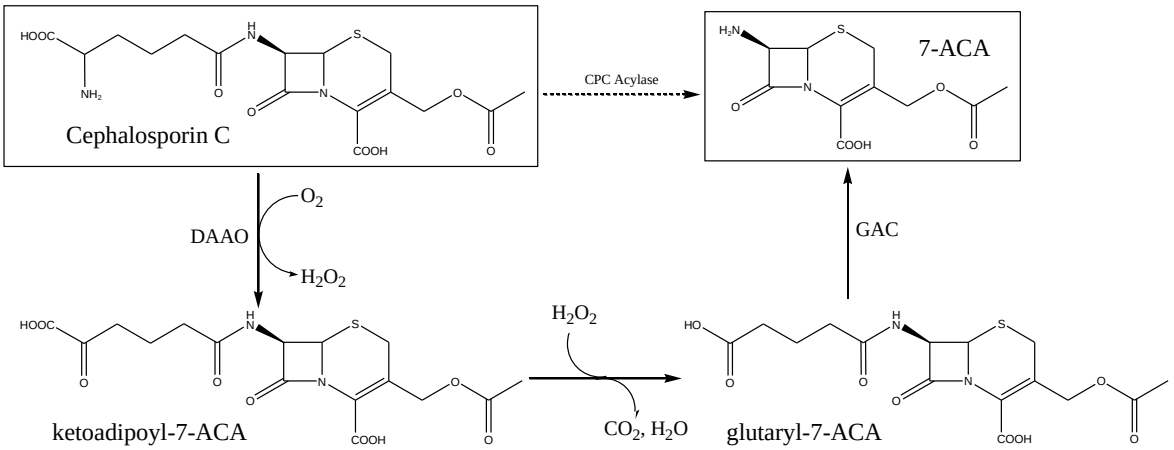


*Fig. 1 : Enzymatic cleavage of CPC into 7-ACA by CPC acylase (upper, dashed line) and by two enzyme using D-Amino acid oxidase (DAAO) and Glutaryl Acylase (GAC) (lower, solid line).*

## II. MATERIALS AND METHODS

### 1. Materials

The microorganism used during the study was yeast, Trigonopsis variabilis was used as source of the enzyme, obtained from Biotechnology Collection Center-BPPT (Serpong, Indonesia). Molasses were obtained from Sragi Sugar Factory (Pekalongan, Central Java). Cassava glucose syrup were obtained from PT. Rejo Madusari (Pati, Central Java). Fructose sorghum syrup was obtained from PT. Sedana Agro (Sleman, Yogyakarta). DL-alanine were purchased from HiMedia. Glucose, K2HPO4, KH2PO4, MgSO4.7H2O, NaCl, CaCl2, MnCl2.4H2O, ZnSO4.7H2O, CuCl2.3 H2O, H3BO3, FeCl3.6 H2O were purchased from Merck (Darmstadt, Germany). The other chemicals, such as thiamin, biotin, o-phenylenediamine, horseradish peroxidase were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). All experiments were carried out in triplicate.

### 2. Culture medium and cultivation conditions

The strain was maintained in yeast malt medium. The cultures were kept at 4°C and subculture at regular intervals of 30 days.

Inoculum were prepared by transferring 1 mL suspension of the organism (OD600 = 1.0 – 1.2) from the slant culture to 50 mL pre culture medium (pH 6) in 250 mL Erlenmeyer flask containing glucose 22 g/L, DL-alanine 4 g/L, K2HPO4 2 g/L, KH2PO4 0.21 g/L, MgSO4.7H2O 0.5 g/L, NaCl 0.1 g/L, CaCl2 0.1 g/L, MnCl2.4H2O 0.105 g/L, ZnSO4.7H2O 0.0231 g/L, CuCl2.3 H2O 0.042 g/L, H3BO3 0.105 g/L, FeCl3.6 H2O 0.0714 g/L, 0.24 mg/L thiamin and 0.02 mg/L biotin. Incubation was carried out in orbital shaker at 30°C and 200 rev/min for 24 h for inoculum development.

Production of TvDAAO was carried out by transferring 8% inoculum (OD600 = 0.7 – 0.8) to 50 mL production media(pH 6) in 250 mL Erlenmeyer flask containing glucose 32 g/L, DL-alanine 6.2 g/L, K2HPO4 2 g/L, KH2PO4 0.21 g/L, MgSO4.7H2O 0.5 g/L, NaCl 0.1 g/L, CaCl2 0.1 g/L, MnCl2.4H2O 0.105 g/L, ZnSO4.7H2O0.0231 g/L, CuCl2.3 H2O 0.042 g/L, H3BO3 0.105 g/L, FeCl3.6 H2O 0.0714 g/L, 0.24 mg/L thiamin and 0.02 mg/L biotin.

Cassava glucose syrup and DL-alanine was sterilized using 0.2 µm filter. The other components were sterilized in the autoclave at 121 °C for 15 min. Incubation was carried out in an orbital shaker at 30°C and 200 rev/min for 24 h for inoculum development.

**3. Fermentation and permeabilization of cell mass**

Initially, the production of TvDAAO was carried out in fermentation at 30°C and 140 rev/min for 72 h. The cells were harvested and neutralized to pH 6.0 using potassium hydroxide, then centrifuged at 10,000 g at 4°C for 10 min to obtain cell mass, and then washing with potassium phosphate buffer (pH 8.0). About 1 mL of the cell suspension were analyzed gravimetrically to obtain the cell mass.

The rest of washed cells were suspended using the same buffer. The suspension cells were permeabilized using 5% toluene-ethanol (1:1) and held for 1 h at 37°C. The permeabilized cells were used for enzyme assay.

**4. Enzyme Assays**

The DAAO activity were measured by o-phenylenediamine/horseradish peroxidase coupling assay[14]. The reaction mixture contained 30 mM D-alanine, 0.03% o-phenylenediamine, 1700 U horseradish peroxidase and DAAO of interest in 100 mM potassium phosphate buffer (pH 8.0). The reaction was monitored by an increase in absorbance at 450 nm for 3 min at 25°C.

One unit of enzyme activity was defined as the enzyme needed to produce 1 micromole of H2O2 per min at 25°C and pH 8.0.

**5. Screening an alternative carbon source and the effect of the additional nitrogen source**

The screening of alternative carbon sources were done by replacing glucose with molasses, CGS and SFS, without or with the addition of a ammonium sulphate (0.5%) as a nitrogen source. The sugar levels of alternative carbon source that being used is equal to glucose concentration (3.2%). Sugar levels in alternative carbon sources were analyzed using dinitro salicylic acid (DNS) method. Measured sugar levels are equivalent to glucose.

**6. Optimization of the best alternative carbon source concentration**

The best alternative carbon source was selected for optimization. The optimization was carried out at various concentrations.

**7. Enzyme characterization**

The enzyme characteristic was done by determining the stability of enzyme to temperature and pH, and the enzyme kinetic parameter was observed.

The temperature stability of TvDAAO was studied in a 0.1-M potassium phosphate buffer, pH 8.0. A series of 0.5 ml plastic test tubes containing 100 µl of the enzyme solution were prepared for each experiment. The tubes were placed to a preheated to the desired temperature water thermostat (temperature control accuracy ± 0.1°C). The test tubes were sampled one by one after fixed time intervals, rapidly cooled for 1-2 min in ice, and the enzyme activity was measured as described above.

The pH stability of TvDAAO was studied in a 0.1-M potassium phosphate buffer, pH 8.0. A series of 0.5 ml plastic test tubes containing 100 µl of the enzyme solution were prepared for each experiment. The pH was adjusted with H3PO4/KOH to the desired pH. The tubes were placed to a preheated at 25°C water thermostat. The test tubes were sampled one by one after fixed time intervals, rapidly cooled for 1-2 min in ice, and the enzyme activity was measured as described above.

The enzyme kinetic parameters were done by determining the maximum reaction rate (Vmax) and Michaelis constant (KM), the concentration of the corresponding D-amino acid was varied from 10 to 300mM. The concentration of the active enzyme was measured as described above. The enzyme kinetics parameters (KM and Vmax) were determined by plotting the relationship graph between 1/V and 1/S, then determined the values of KM and Vmax based on the Lineweaver-Burk curve equation.

## III. RESULTS AND DISCUSSION

**1. Screening an alternative carbon source and the effect of the additional nitrogen source**

The screening of an alternative carbon sources were done with a one-at-a-time strategy. Types of alternative carbon sources used are molasses, cassava glucose syrup and sorghum fructose syrup. The screening was done without or with the additional of a nitrogen source. The additional nitrogen source selected was ammonium sulfate, in which ammonium sulfate was one of the best source of nitrogen for yeast culture [15; 16]. The results of the screening were shown in Table 1.

Table 1 shows the TvDAAO activity resulting from fermentation using various types of carbon sources as nutrients in the production process. From various carbon source, glucose with ammonium sulfate was found to be the most suitable to induce the TvDAAO production. Glucose requires no extra step to enter in glycolytic pathway. However, for better results, the uses of glucose need an additional nitrogen source, where nitrogen plays a role in the formation of amino acids for the production of enzymes. It is made to be less efficient and uneconomical.

It is different when using agro-industry residues as an alternative carbon source. The best alternative carbon source is cassava glucose syrup, with enzyme activity obtained is 73.5079 U/g. Cassava glucose syrup contains glucose as the main sugar [17], which is glucose was the best carbon source for TvDAAO production. The use of cassava glucose syrup as a carbon source can be done without the addition of additional nitrogen sources. This is because the amount of nitrogen required during the fermentation process has been fulfilled from the cassava

sugar syrup itself and from DL-Alanine. Portilho [18] mentioned that the C/N ratio in cassava glucose syrup was above 20. The use of an additional nitrogen source will decrease the resulting TvDAAO, because nitrogen can lead to the formation of ammonium, where excessive ammonium can cause cell death[19]. This is an advantage in the use of cassava glucose syrup as an alternative carbon source of glucose substitutes, because cassava glucose syrup can act as a source of carbon as well as a source of nitrogen, so the production becomes more efficient and economical.

*Table 1. Screening of various types of carbon source, with or without an additional NH4(SO4)2*

| Carbon Source | Enzyme activity (U/g yeast cell dry weight) | |
|---|---|---|
| | without $NH_4(SO_4)_2$ | with $NH_4(SO_4)_2$ |
| Glucose | 52.5286 ± 0.3880 | 100.9528 ± 0.7311 |
| Molasses | 47.9158 ± 0.7447 | 0.3421 ± 0.0126 |
| Cassava glucose syrup | 73.5079 ± 1.4246 | 0.2666 ± 0.0080 |
| Sorghum fructose syrup | 42.9478 ± 0.6666 | 0.5628 ± 0.0336 |

**2. Optimization of cassava glucose syrup concentration**

The productivity of cassava glucose syrup can still be improved by increasing the concentration of cassava glucose syrup (Figure 2). The concentration of cassava glucose syrup is measured as the glucose content contained in the cassava glucose syrup. Figure 2 shows that the highest enzyme activity (166.8861 U/g yeast cell dry weight) was obtained at 10% of glucose levels in cassava glucose syrup. The productivity of the use of cassava glucose syrup in TvDAAO production can still be optimized using a response surface method (RSM), which will be discussed in another article.

**3. Enzyme characterization**

The result of stability test of temperature and pH can be seen in Figure 3 and 4. The enzyme was stable at 4-10°C (Fig. 3). The results obtained are similar to those reported by Kubicek-Pranz & Rohr [20], which state that the TvDAAO enzyme is stable at 4°C. Meanwhile, on the stability test of pH (Fig. 4), it is known that the enzyme is quite stable at pH 8. At pH 5, the TvDAAO enzyme will precipitate because the isolation point of TvDAAO enzyme is at pH 5.1[21].

The enzyme kinetics parameters, including Vmax and KM, are determined by the Lineweaver-Burk curve equation (Fig. 5). The results obtained Vmax value of 0.007 µmol / minute and KM of 78 mM. The value of KM obtained is not significantly different from the value of KM obtained by Szwajcer & Mosbach [22] that is equal to 76 mM.

## IV. CONCLUSION

Cassava sugar syrup was the best alternative carbon source to be utilized in TvDAAO production by fermentation process. Cassava sugar syrup can act as a source of carbon as well as a source of nitrogen, so that the production of TvDAAO become more efficient and economical. The optimum concentration of cassava glucose syrup is at 10% of glucose levels, which produced TvDAAO having activity equal to 166.8861 U/g yeast cell dry weight. The enzyme stable at 4-10°C and pH 8, with Vmax value was 0.007 µmol/minute and KM was 78 mM.

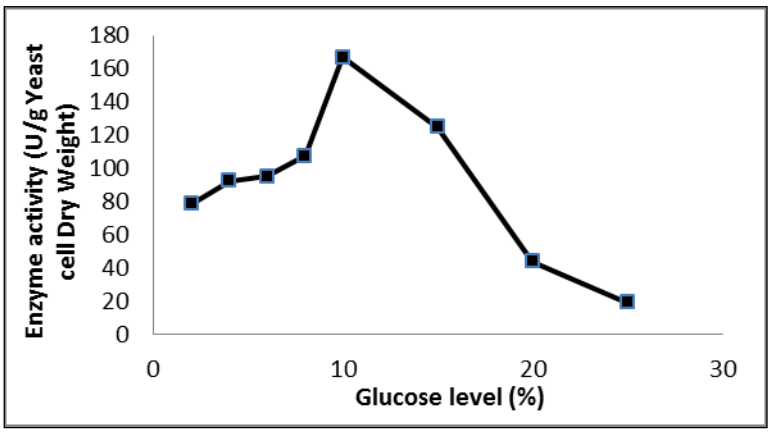


*Fig. 2 : Effect of various concentration of cassava glucose syrup without additional nitrogen source*

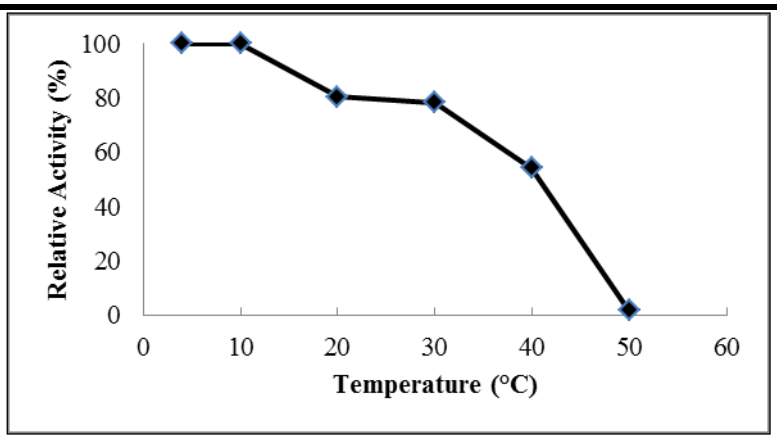


*Fig. 3 : Stability of the enzyme to temperature*

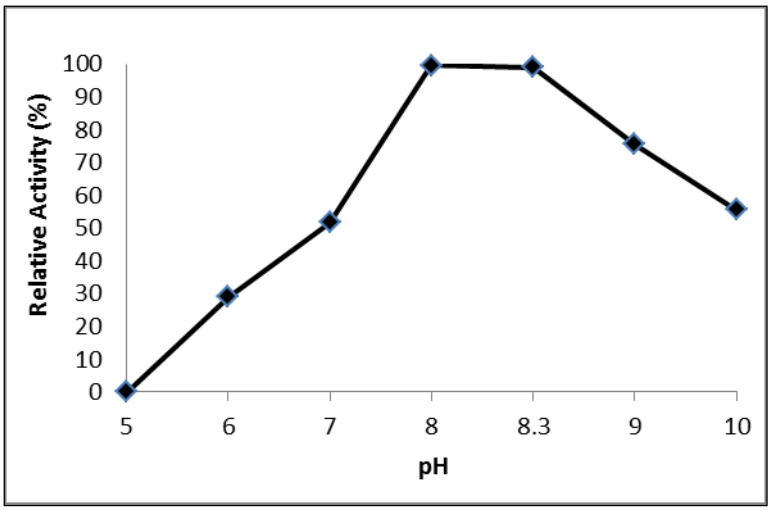


*Fig. 4 : Stability of the enzyme to pH*

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# Parameter: The Area of Microclimate Gradient Diurnal Dynamic for Characterization and Monitoring of Forest Ecosystem and Environment

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***Abstract*—***Microclimate forests are usually described by the parameters: quantity of microclimate differences of interior-exterior, the depth of the effect of edge and gradient. These parameters can characterize ecosystem conditions but their quantities are often inconsistent and thus less valid for monitoring ecosystem and adjacent environmental changes.This paper introduces the concepts, methods, and the results of the application of the parameters: the area of microclimate gradiet diurnal dynamic which the advantage in: (1) characterize ecosystem conditions and their interactions with adjacent environments, (2) categorize transects (in forest ecosystems) based on ecosystem conditions and their interactions with adjacent environments, (3) monitoring the forest ecosystem changes (deforestration, natural damage etc), (4) determine the time of thermal equilibrium between forest and environment.*

***Keywords*— microclimate, parameter, gradient, diurnal dynamic.**

## I. INTRODUCTION

The microclimate variables used by many researchers in describing the microclimate of forest ecosystem are: the intensity of solar radiation penetration, air temperatire, air humidity, wind speed (Hennenberg *et al*, 2008, Davies_Colley *et al*, 2000). Parameters used to express the quantity of forest microclimate are: the maximum difference of interior-exterior, the depth of edge effect, the maximum gradient at the forest boundary. These quantities can not determine the ecosystem capacity in controlling the total daily thermal diffusion between the ecosystem and the environment. Quantity of these parameters, are not consistent for the data measured at the different day, although in the same weather condition.

Mathematical modeling of temporal changes and spatial variation can produce microclimate gradient data at the edge of foret ecosystem. The mathematical modeling of daily microclimate gradient dynamics, yield the functions that describe the thermal interaction between forest and environment. Interesting information obtained from the graph of micaroclimate gradient dynamic functions are: the duration of thermal diffusion from the environment to the ecosystem and vice versa, the time of diffusion transition (marked by gradient value = 0), and the area of intersection between the dynamic gradient curve with the thermal equilibrium line. The area surrounded by gradient dynamic curve of microclimate variables may consist of two or one plane only, depending on the ecosystem condition and its adjacent environment. If the curve forms two planes of gradient dynamic, the one plane represents the thermal diffusion from the environment into the ecosystem while the other plane represent the thermal diffusion in the opposite direction. This area of mocroclimate gradient dynamics is related to the acceptance of solar radiation and thermal energy storage by the interior of the forest through the diffusion process. The thermal diffusion mathematical model that produce a gradient function is developed based on the assumption of steady flow of thermal energy. This article describes the method and examples of application of parameter "the area of diurnal dynamic of microcilamet gradient" in characterizing the interaction of forest ecosystem with the environment. These examples resulted from measurements on several transects, in 2011, 2012, 2014, and 2016.

## II. LITERATURE REVIEW

Microclimate is defined as the climate condition of the localized area as a different zone with surrounding environment (Chen *et al*. 1999; Medellu, 2012). The microclimate variables were studied by experts are the intensity of solar radiation, air temperature and air humidity (Hennenberg *et al*., 2008; Medellu, 2012; de Lima *et al*. 2013). Davies-Colley *et al*. 2000, and Medellu, 2012) also

measure wind speed, soil temperature and humidity for charachterization of ecosystem. The microclimate variables change temporally following the changes of solar radiation intensity (Newmark. 2001; Medellu, 2012), Microclimate variables varies spatially due to local conditions or the earth surface (Medellu, 2012, de Paula *et al*, 2016). Spatial variation of forest microclimate influenced by the structure of the forest such as variation of tree high, the patch and the gap in the forest (Pinto *et al*., 2010; Zulkiflee and Blackburn, 2010; Medellu, 2012). Forest microclimate is significantly influenced by the density of canopy (Renaud *et al*, 2010). The results of research prove that the microclimate variables are very sensitive to change due to variability and the change of forest ecosystems and surrounding environment (Godefroid *et al*., 2006; Berger *et al*. 2008, Gradstein, 2008). Microclimate parameters describe the condition and the changes of microclimete variables quantitatively. Microclimate parameters often used by experts were: the maximum difference of edge – interior, the depth of edge effect, and the maximum edge. The depth of edge effects can indicates a fragmentation or gaps in the forest or changes in the structure of the forest (Harper *et al*. 2005, Medellu *et al*, 2012; Magnago *et al*. 2015). Edge gradient associated with the flow of thermal energy between the environment and forest ecosystems (Heithecker and Halpern, 2007; Medellu, 2013; Chatterjea, 2014). Numerical value of the depth of edge effects and the maximum edge gradient were different for transects located in different forest ecosystems and environment (Medellu, 2013; Chatterjea, 2014; Kolasa, 2014). The results of research for the air temperature and air humidity, shows the matching between parameters for transects in the similar condition of forest ecosystem and environment (Medellu 2012, 2013). The microclimate parameters also become an indication of organic conditions around the edge (Wermelinger *et al*., 2007; Horak and Rebl, 2012; Vodka and Cizek, 2013). The depth of edge effect used to identify the transition zone (Baker *et al*, 2016; Schmidt *et al*, 2017). Laurance *et al*., (2011), Pütz *et al*. (2014), and Chaplin-Kramer *et al*. (2015) used the edge effect parameter to estimate the carbon stocks in the zone. These results proved that the microclimate parameters can be used for characterization of ecologicall condition of the forest ecosystems and interaction with the environment (Renaud *et al*, 2010; Medellu, 2012, 2013).

If the measurement repeated in two consecutive days with the same weather conditions (no rain and wind speed less than 2 km/hour), the value of parameters: maximum difference of edge-interior, the depth of edge effect and the maximum edge gradient of air temperature and air humidity were fluctuated and occured in the different time (Medellu, 2012). The depth of edge effect will show two to four top values in different time during the day (Medellu, 2012). Chen *et al* (1999) found the top value of the depth of edge effects occur four to six times a day. This daily fluctuation was the reason for De Siqueira *et al* (2004) to use the variance of the depth of edge effect data. This result indicates that the microclimate parameters can be used to characterize the interaction between the forest and the adjacent environment (Chen *et al*, 1999; Medellu, 2012, 2013), but less valid if used as a reference for monitoring the changes of the ecosystem and its environment.

In 2012 I publish the parameters "area of microclimate gradient diurnal dynamic" through the dissertation entitled “Mathematical Modeling of Daily Dynamics of Microclimate Gradients in Mangrove Forest. This parameter indicates the change of microclimate variables during one day or one period of sun illumination, to obtain the daily response of forest ecosystem and environment on solar radiation. The reason for the using of this parameter was in line with Godefroid *et al.* (2006) and Laurance *et al*. (2011), who proposed that the effect of microclimate in the transition zone is the cumulative response on solar radiation. Determination of the area of microclimate gradient diurnal dynamic includes the stages of the temporal and spatial modeling, determination of edge gradient, modeling the edge gradient function, determination the area of microclimate gradient diurnal dynamic, and the coefficient of microclimate gradient diurnal dynamic. The area of microclimate gradient diurnal dynamic is the area (abstract) surrounded by the microclimate gradient curve with the line of thermal equilibrium. Thermal equilibrium line is the line in two-dimensional coordinate system i.e. gradient versus time, which the gradient value is zero. Thermal equilibrium line indicates the time of the changes of thermal diffusion direction between forest ecosystems - environment. The area of microclimate gradient diurnal dynamic describe the change of microclimate variables during one day according to the period of sun illmunination, acceptance-storage-reemissin of thermal energy by forest and the environment. This parameter is also influenced by the weather conditions i.e. rainfall and wind speed (Medellu, 2012; 2013). The rainfall and wind speed must be controlled during the measurement to ensure that the value will represent the condition and the changes of forest ecosystem and environment.

## III. METHOD

The parameter: area of microclimate gradient diurnal dynamic was developed through the mathematical modeling the daily gradient changes. The basic concept of modeling is

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the diffusion of thermal energy caused by the sun radiation and the process of absorbtion, thermal emission by medium (soil, water, vegetation etc. The difference in the temperature (and other microclimate variables) causes thermal diffusion horizontally through the border of ecosystem and the environment. The research procedure related to the application of parameter "the Area of Microclimate Gradient Diurnal Dynamic" was as follows:

1.1. The identification and determination of` the transect

The identification and determination of transect is very important to get the diversity of thermal energy flow between forest and environment. Many transect are selected based on the diversity of the ecosystem and environmental conditions, namely: (1) the existence of variations in forest structure (e.g.: patch, the gap, and forest fragmentation), (2) mangrove type and canopy cover, (3) the adjacent environment (sea, asphalt roads, land with or without the vegetation, settlements etc). Transects were taken in the perpendicular direction on edge of forest (forest-environment boundaries).

1.2. Determination of measurement position

For each transect, the position of the measurement using the logarithmic distance, starting from the edge as the zero point. The distance of measurement points to the edge of the forest are: 0 (forest edge), 1 m, 2 m, 4 m, 8 m, 16 m, and 32 m inward the forest (Medellu, 2013). The measurement is also done on the outward positions in 2 m and 4 m from the edge. The distance between positions are smaller near the edge and increase with the increasing of the distance from the edge. This measurement position is more guarantee the validity of the spatial function modeling of microclimate variables. The position of measurement follows the phenomenon of thermal energy absorption by the medium (air, water) which is greater around the edge and decreased with the increasing of the distance from the edge. Theoritically, the physics variables (f) change due to absorption as $f = k_o.e^{-k1x}$, where $k_1$ is the constant of absorption, x is the distance from the edge, and $k_0$ is physics variables value at the edge. The value of f is greater near the edge and gradually desreased by the increasing of the distance from the edge.

1.3. Variables, measurement and tabulation of data**.**

The measurement of variables on each position is done with one hour interval. The measurement was conducted by switch from one position to the next position (moving station). The measurement on each position is done simultaneously for four variables i.e: air temperature, air humidity, the intensity of the light and the water/mud temperature. The measurement of air temperature, air humidity and solar illumination was done on the height of 50 cm above ground, using instrument "four in one", which also measured the wind speed for controlling. The measurement on the position of 50 cm vertically assumed represents the vertical variation of air temperature and humidity (Didham and Ewers, 2014). Wind speed measured for controlling the other microclimate variables. The measurement is only done if the wind speed is less than 2 km/hour that quarantee the free or unforced diffusion. Water or mud temperature measured using the water/land termometer, with the depth variation of 0 - 2 meters. The measurement position is determined using GPS. The measurement position marked for the next measurement. One day measurement on each position produced 24 data. The measurement result data recorded in form as Table-1

*Table.1: The matrix for the recording the data*

Variable: ……Location:…….transect: ……..…..

| Posi tion | Time of measurement | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.00 | 7.00 | .. | t1 | .. | 5.00 | 6.00 |
| - 4 m | | | | | | | |
| - 2 m | | | | | | | |
| 0 | | | | T(0,t1) | | | |
| 1 m | | | | | | | |
| 2 m | | | | | | | |
| 4 m | | | | | | | |
| 8 m | | | | | | | |
| 16 m | | | | | | | |
| 32 m | | | | | | | |

(Source: Medellu, 2012, 2013)

1.4. Analysis: mathematical modeling and the determination of the microclimate parameters

As described in Medellu (2012, 2013), the steps of analysis and mathematical modeling for determination the microclimate parameters are as follows:

1.5. The modeling of temporal function of microclimate variables.

The modeling of temporal function performed for each measurement position (data rows in Table-1). For each position there were twenty-four data. The mathematical modeling of microclimate function using the procedure of Fourier function modeling, according to sinusoidal changes of data as the respons of earth surface on sun illumination. The periodic (Fourier) function for each measurement position was:

$$T(t) = To + \sum_{m=1}^{N/2} a_m \cos \omega_m t + b_m \sin \omega_m t \ldots (1)$$

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where,

$\omega_m = 2\pi m/N$ ……(2a)

$$a_m = \frac{2}{N}\sum_{t=0}^{N-1} f(t)\cos\omega_m t \quad \text{……..(2b)}$$

$$b_m = \frac{2}{N}\sum_{t=0}^{N-1} f(t)\sin\omega_m t \quad \text{……..(2c)}$$

$T_0$ is the mean of microclimate data, m is the harmonic enumerator, and N is the number of pair of data: independent variable (time (t)) – dependent viable (microclimate – T(t)). N/2 is the number of harmonic that is the number of sinusoidal component of Fourier function construted from 24 pairs of data. There are 12 harmonics for variabels: air temperature, air humidity and water temperature, and 6 harmonic for light intensity. The steps of Fourier function modeling are:

1.5.1. Determine the coefficient $a_m$ and $b_m$, using the equation (2b) and (2c).

1.5.2. Determine the coefficient of $c_m^2 = a_m^2 + b_m^2$.

1.5.3. Determine the contributions of diversity: $s_m = (c_m^2/(2.\sigma)).100$

σ is standart of deviation of microclimate data. Through these steps, obtained the value $a_m$, $b_m$, $c_m$ and $s_m$ for m = 1, 2, .....,12.

Based on the value of the contribution of diversity ($s_m$), determined the number of harmonic component needed to construct the Fourier function that is considered valid. The validity of modeling of Fourier function indicates by the vaule of total contributions of diversity. More number of harmonic components, more precision of Fourier function. If the entire hamonic used to construct the function, the total contribution of diversity reached 100 percent. Through these stages can be displayed temporal changes of microclimate variables for each measurement position. Through these stages also obtained the maximum value of microclimate at the day and night, and the maximum difference of edge-interior of forest. The sinusoidal model of the daily changes of air temperature and air humidity also described by Davies-Colley *et al*. (2000), Spittlehouse *et al*. (2004), and Saxena,(2007).

1.6. The synchronization of data.

The synchronization must be done because the data were not measured simultaneously, but switching from one to the next position, along transect. Synchronization is done by measuring the difference of time measurement between the two consecutive positions and then submits into the temporal function to get a new microclimate data. This process of synchronization does not alter the temporal function but gives a new data wich synchron between positions along transect. The synchronized data used for analysis and modeling of spatial function wich describe the microclimate variations along transect.

1.7. Modeling of spatial function.

Modeling of spatial function using the exponential model as presented in equation (3). This hypothetical function contains four unknown constants. These constants can be determine at least using three pairs of data (distance (x) – microclimate T(x)), including the edge data as a reference of position: x = 0.

$T(x) = k_1 + k_2.e^{k3 - k4.x}$ ……(3)

where x is the distance from the reference or the edge of the forest. The constants: $k_1$, $k_2$, $k_3$ and $k_4$ obtained by computer iteration techniques, using the pair of data:. $(0,T_0)$, $(x_1,T_1)$, and $(x_2,T_2)$. Stages of iterations to generate the constants of spatial function are:

$(T_0-T_1)/(T_0-T_2)=[(\exp(k_4.x_2).(\exp k_4.x_1-1)]/$
$[(\exp(k_4.x_1).\exp(k_4.x_2-1)]$

$k_3 = (T_0 - T_1)/(1-1/\exp(k_4.x_1))$

$k_2 = (T_0 - T_1)/(\exp(k_3) - \exp(k_3 - k_4.x_1))$

$k_1 = y_0 - k_2.\exp(k_3)$

The validity of spatial function is indicated by the biased of model data to the measured data. The spatial function can be used to generate the microclimate data on the other position in a range or outside the range of the measurement position. The software outputs through this stage are: (1) the depth of edge effect, obtained using condition: $dT(x)/dx = 0$, (2) edge gradient value $dT(x)/dx$ for x = 0, (3) the maximum value of edge gradient at the day and night. Edge gradient value obtained using the equation:

$G = dT(x)/dx\,\big|_{x=0} = - k_2.k_4.\exp(k_3)$ ……..(4)

Edge gradient value is a function of time. Modeling of edge gradient function produces the function of diurnal dynamics of microclimate gradient.

1.8. Modeling of diurnal dynamics gradient.

Edge gradient data are fluctuates sinusoidally to follow the quantity and the direction of thermal diffusion through the forest edge. The modeling of diurnal dynamics gradient function using the procedure of periodic function modeling as described in point a.

1.9. Determine the area of the diurnal dynamics gradient of microclimate.

The area of microclimate gradient diurnal dynamic is the area restricted by the curve of microclimate gradient dynamic with the thermal equilibrium line. The thermal equilibrium line is the line that has a zero gradient value. Physically, the thermal equilibrium line shows the

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condition where no thermal diffusion between forest and environment.

The measurement for 24 hours can produce two areas of diurnal dynamics gradient, above and below the thermal equilibrium line, depends on the changes of gradient sign (Figure-1).

If the gradient has the negative sign, the area of diurnal dynamics gradient lies below the line of equilibrium, indicates the thermal diffusion from the environment to the forest. If the gradient sign is positive, the area of diurnal dynamic gradient lies above the equilibrium line that indicates the thermal diffusion from forest to environment. The area of diurnal dynamics microclimate gradient (A) determined using the the numerical integral:

$$A = \sum_{i=1}^{n} \left| G_t . \Delta t \right| \ldots\ldots\ldots 5)$$

Where n is the number of elements of area. $G_t$ is the value of the function gradient. $\Delta t$ is the interval of time sampling.

1.10. Location of research

Research was performed on several locations that to show the consistent results according to ecosystem and environment conditions. Research in 2011 taken on 10 transects which the condition as describes in Tabel-2

*Table.2: Research location and transects condition in May 2011*

| Location | Transect number | Edge coordinate* | | Mangrove type and characteristic of ecosystem | Adjacent environment |
|---|---|---|---|---|---|
| | | Latitude | Longitude | | |
| Talengen Bay, District of Sangihe | 1 | 3°35'20.14" | 125°34'10.68" | fringe, homogeny, *Rhizophora*, gap at 36 m from the edge, canopy cover 72 %-85 % | Sea, Talengen Bay |
| | 2 | 3°35'25.17" | 125°34'8.22" | fringe, homogeny, *Rhizopho ra*, canopy cover 75%-85 % | Sea, Talengen Bay |
| | 3 | 3°35'31.70" | 125°33'59.55" | riverine, homogeny, *Rhizophora*, canopy cover: 78% – 88% | River/Talengen Bay |
| Ratatotok Bay, District of South-East Minahasa | 1 | 0°52'9.09" | 124°42'21.52" | hammock, fragmented at 12 m; bruguiera: 0-12m, canopy cover of 90% - 95% | Pavement/shrubs mangrove at 8 m from edge |
| | | | | Domination of Avicenia 12 - > 80 m, canopy cover 55% - 70% | Mangrove (heterogeny) |
| | 2 | 0°51'2195" | 124°42'24.82" | fringe, homogeny, *Rhizopho ra*, canopy cover 75 %-80 % | Coast (shrubs) |
| | 3 | 0°50'59.76" | 124°42'29.69" | Basin. Heterogeny, variation in high and canopy cover (40% - 65%) | Coast.shrub, sea infront |
| | 4 | 0°50'50.53" | 124°42'11.51" | Basin heterogeny, domination of A*vicenia*, canopy cover 35% - 55% | Pavement/schrubs |
| | 5 | 0°51'52.42" | 124°42'2.61" | Scrub heterogeny, domination of A*vicenia*, canopy cover 50% - 60% | Coast/shrub |
| Arakan village, South Minahasa | 1 | 1°22'8.87" | 124°32'49.12" | Fringe, homogeny, *Rhizophora*, canopy cover 75% – 85% | Sea |
| | 2 | 1°21'59.07" | 124°32'55.33" | Basin, heterogeny in mengrope type, high and canopy cover. (55% - 65%) | Coast, shrub & high trees |

* The position of the zero point on transect, located at the edge of the forest. Source: Medellu, 2012.

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## IV. RESULTS AND DISCUSSION

4.1. Daily fluctuation of microcimate variabels

Figure-1 shows the graph of the air temperature gradient changes of transect number-2, located in Talengen Bay, start from 07.00 a.m, date of May 8th 2011 to 07.00 a.m. on May 9th 2011.

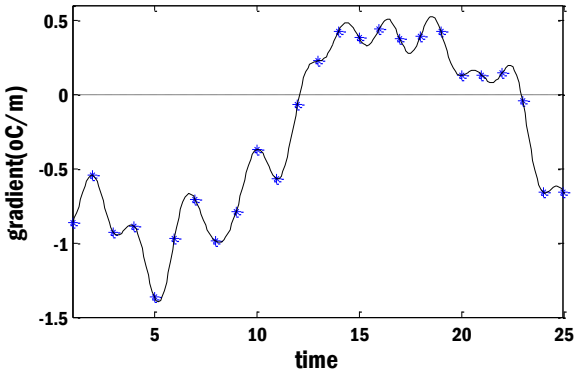


*Fig.1: Graphic of air temperature gradient of transect-2, Talengen Bay*

The horizontal line represents the thermal equilibrium line between the mangrove ecosystem and the adjacent environment. The number 1 on the abscissa was in accordance with 07.00 a.m., while the number 25 associated with 07.00 a.m. of the next day. The gradient dynamics curve that lies below the thermal equilibrium line shows the direction of the thermal diffusion from the environment into the ecosystem. The negative sign of gradient represents the thermal diffusion during the day. At night, the direction of the thermal diffusion from the ecosystem to the environment and the gradient dynamics curve lies above the thermal equilibrium line.

The function of air temperature gradient of transect number-2, located in Talengen Bay is:

Gt(2) = -0.2944 -0.2822*COS((2πt/180)/24) -0.7046* SIN((2πt/180)/24)+ 0.0246*COS((4πt/180)/24)+ 0.0455* SIN((4πt/180)/24) -0.0479* COS((6πt/180)/24) -0.0306* SIN((6πt/180)/24) -0.0440* COS((8πt/180)/24)+ 0.0658* SIN((8πt/180)/24) -0.0937* COS((10πt/180)/24)+0.0001* SIN((10πt/180)/24) -0.0812* COS((12πt/180)/24)+ 0.0189* SIN((12πt/180)/24) +0.0163* COS((14πt/180)/24) -0.0278* SIN((14πt/180)/24)+ 0.0649* COS((16πt/180)/24)+ 0.0734* SIN((16πt/180)/24) -0.0478* COS((18πt/180)/24) -0.0040* SIN((18πt/180)/24)+ 0.0099* COS((20πt/180)/24) -0.0577* SIN((20πt/180)/24)+ 0.0359* COS((22πt/180)/24)+0.0021* SIN((22πt/180)/24)+ 0.0808* COS((24πt/180)/24) -0.0402* SIN((24πt/180)/24)

Graph of gradient dynamics of air humidity, sun light intensity and sea water temperature of the same transect, measured simultaneously with the air temperature, respectively presented in Figure-2, Figure-3, and Figure-4

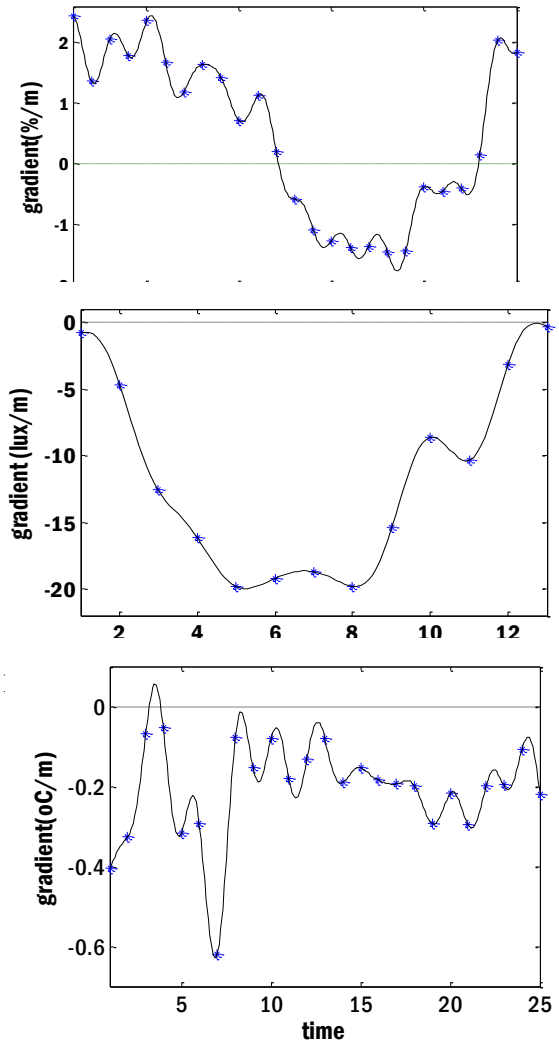


*Fig.4: Graphic of water temperature gradient of transect-2 Talengen Bay*

Figure-3 to show the light intensity gradient at the edge of mangrove forest measured at 06.00 to 18.00, Mayth 9 2011.

At night, the air temperature in mangrove forest was higher than in edge or in the environment, which shows the direction of thermal diffusion was from the forest to the environment (Medellu 2012, Medellu, 2013). This result was accordance with Renaud *et al*. (2010). The

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graph of diurnal dynamic of air temperature gradient generally shows more fluctuate on the day than night. This fluctuation caused by the change of sun elevation, cloud cover, wind speed and its direction. Air humidity gradient graph shows that in the daytime, air humidity in interior of forest higher than the air humidity above the open sea surface. This result in accordance with Renaud *et al.* (2010); Wicklein *et al*., (2012), and Williams-Linera *et al*., (1998) as reported in Schmidt *et al.* (2017). The air above the open sea surface, receive the direct warming by sun shining, and then the humidy decreased faster than the air under the mangrove canopy. At night, the air above the open sea surface shows the slightly higher moisture than the air under the mangrove canopy. The function of the radiation gradient dynamic consists of 6 harmonics part and one part of illumination gradient mean. The water temperature gradient has the negative sign throughout the day (24 hours), shows that the water temperature of open sea surface is higher than under the mangrove canopy. Physically, this condition caused by the higher acceptance and storage of solar energy by the open sea. This result was in line with Hawley (2010) conclusion which to compare the water temperature of open sea with under the closed canopy. Another factor influence the water temperature under the canopy was the flow of fresh water from the land inform of water leaks, through openings or pores of soil.

4.2. Comparison between transect in different ecosystem and adjacent environment

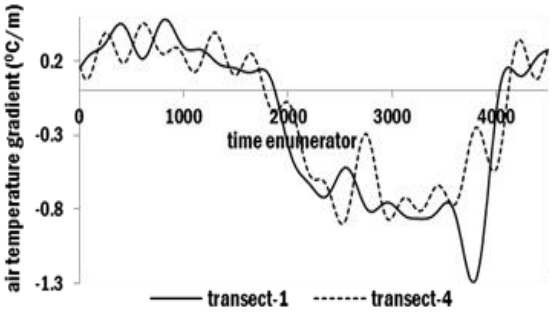


*Fig.5: The graphs of air temperature gradient of transect no 1 and no 4, Location: Ratatotok Bay*

The differences of the area of air temperature gradient diurnal dynamic between the two transects that measured at the same time and on the same weather conditions (no rain), proves that the difference is caused mainly by canopy cover (sea basic data in Table-2). Figure-5 shows the morning thermal eguilibrium reached earlier in transect-4 then in transect-1; on the afternoon the thermal equilibrium reached earlier in the transect-1 than in the transect-4. Physically this is influenced by the process of acceptance and storage of thermal energy by mangrove ecosystem, among others related to canopy density and temperature changes around the mangrove edge.

4.3. Weather influence on the microclimate of mangrove

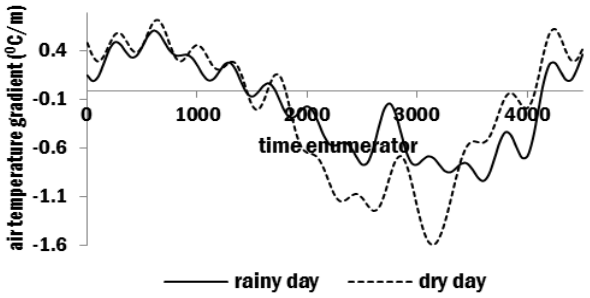


Figure-6. The graph of air temperature gradient in rainy and dry day, Location: Ratatotok Bay

until 19.00 p.m., October 14, 2011. The condition of the mangrove ecosystem and environment along transect 2 on the first and second measurements were the same. The area of air temperature gradient diurnal dynamis in dry day was 9.024$^0$C.hours/m at the day, and 3.064$^0$C.hours/m at night. The area of air temperature gradient diurnal dynamis in rainy day was 7.30$^0$C.hours/m at the day, 2,623$^0$C.hours/m at night. Decreasing the area of air temperature gradient diurnal dynamic due to rainfall was in line with the decreasing of other parameters such as the maximum difference of interior - edge, maximum edge gradient and the depth of edge effects.

4.4. The monitoring the change of forest width and the gap size in mangrove forest

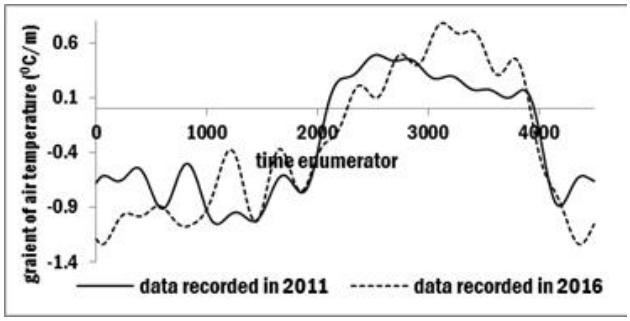


*Fig.7: Graph of air temperature gradient of the transect 1, location Talengen Bay, result of measurement in 2011 and 2016*

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Figure-7 shows two graphics of air temperature gradient dynamic of the transect-1 at Talengen Bay in two different ecosystem conditions. The solid curve related to the first measurement conducted at 07.00 a.m. on May 8, 2011 to 07.00 a.m. on May 9, 2011 In interior of mangrove there is the gap which the width was 30 m, while the distance of the gap edge from the main edge (border of forest with the open sea) was 68 m.

The second measurement has been done at 07.00 a.m. on July 20, 2016 to 07.00 a.m., July 21, 2016. In the second measurement the width of gap decreased to 18 m, while the width of mangrove or the distance of the two edges was increased to 76 m. The green color graph shows the air temperature gradient dynamic constructed by the data of second measurement. Weather conditions for the first and second measurement were the same, by controlling wind speed less than 2 km/hour and no precipitation. The dense of canopy was relatively in the same range, around 72 %-85% in the first and (72 %-86%) in the second measurement. The area of air temperature gradient diurnal dynamic derived from the first measurement data are 9.586$^0$C.hours/m at the day, and 3.034$^0$C.hours/m at night. In the second measurement, the area of air temperature gradient diurnal dynamic was 9.982$^0$C.hours/m at the day, 3.424$^0$C.hours/m at night.

4.5. The grouping of mangrove ecosystem based on the area of air temperature and humidity gradient diurnal dynamic

Figure-8 and Figure-9 shows the map of the transect grouping based on the area of air temperature gradient (absis) and the humidity gradient (ordinate) on night and day. The numbers on the map represent the number of ten transects on Table-1, without distinguish location, for example the transect number 4 was the transect no-1 Location Ratatotok etc. Based on the area of air temperature and humidy gradient data at night and day, the transects grouping was:: (a) group of transects (1,2,3,5,9), and (b) group of transects (6,7,8). Transect-4 and transect-10 was not grouping, each transect stand alone.

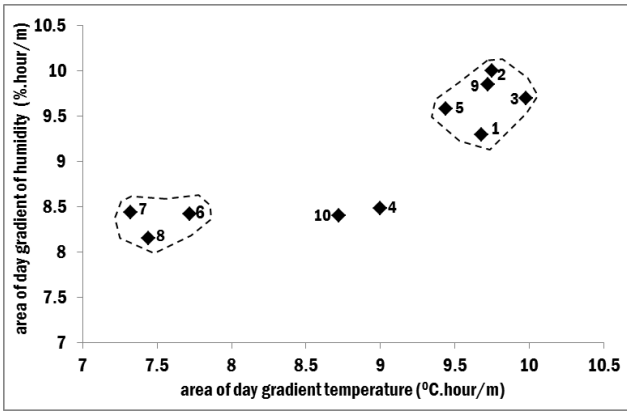


*Fig.8: Transect grouping based on the area of gradient dynamic of temperature versus humidity at the day*

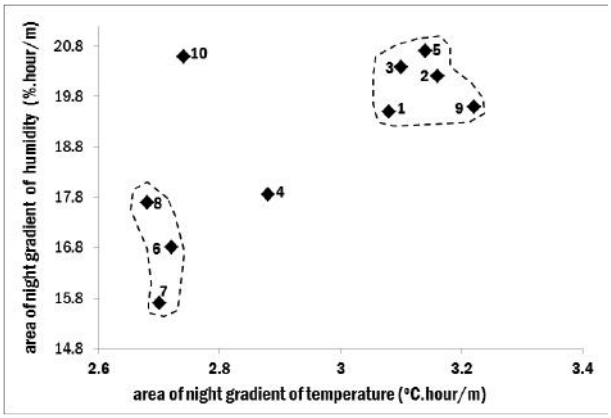


*Fig.9: Transect grouping based on the area of gradient dynamic of temperature versus humidity at night (Medellu, 2013)*

Group of transects (a) have the high value of the area of air temperature and humidity gradient at the day and night. The group (b) represesnts transects with the low value of the area of air temperatre and humidity gradient at the day and night

## V. CONCLUSION

The area of microclimate gradient diurnal dynamic represents the one day accumulative response of forest ecosystem and environment wich more stable than the other parameters: interior-edge differences, the depth of depth edge, maximum edge gradient. Parameter the area of microclimate gradient diurnal dynamic can be used to: (1) characterize, identify and classify or grouping the transects, (2) monitor the changes in forest structure, the change of forest or changes the gap size in the forest, (3) monitor the impact of environment to forest ecosystems. The application of parameter the area of microclimate gradient diurnal dynamic for mangrove ecosystem monitoring, limited to variable: air temperature, air humidity and illumination. For variable of water/mud temperature needed the control of water flow which caused the complex of spatial variation and temporal changes of water temperature. To quarantee the good ouput, the measurement of microclimate conducted in the condition of the wind speed less than 2 km/hour; this to ensure the unforced thermal diffusion.

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# Analysis of the Marketing Margin of Soyabeans in Benue State, Nigeria

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***Abstract—*** *The study examined the marketing margin of soyabeans marketers in Benue State, Nigeria. A sampling frame of 914 registered soyabeans marketers was obtained from soyabeans marketers association in the study area. Stratified random sampling techniques was employed to obtain 278 market actors (producer-marketers, wholesalers and retailers) for the study. Data were collected with the use of a well structured questionnaire and analyzed using descriptive and inferential statistics. The result on market conduct, showed that all the respondents (100 %) agreed that they sold their soyabeans immediately after harvest, they rely on family or personal savings for business finance, they do not advertise their soyabeans for sell and they have not attended any training on soyabeans. The result further showed a marketing margin of ₦88.91, ₦85.78 and ₦85.99 respectively. The major problems faced by soyabeans marketers in the study area include inadequate capital, high transportation cost, lack of access to credit facilities, heavy imposition of taxes or levies and poor storage and warehousing facilities. It was recommended that soyabeans marketers in the study area should form cooperative society to have access to loan from both formal and informal sources for better capital base and higher output. In conclusion soyabeans marketing is a profitable and efficient business, with attractive net return on investment in the study area.*

***Keywords— Analysis; soyabeans marketing; marketing margin; Benue State.***

## I. INTRODUCTION

Soyabeans, a member of the family leguminoceae, subfamily papiplonaceae, and the genus *Glycine max* (L) Merril, is an annual food legume and an important food, livestock feed, oil, milk production and cash crop in the world. It has been the dominant oilseed produced since the 1960s and is used as human food; medicals, and for various industrial purposes (Adedoyin *et al.,* 1998).

One of the major food problems in Nigeria is the gross deficiency in protein intake, both in quantity and quality (Dashiell, 1998). Although, protein in human diet is derived from both plant and animal sources, the declining consumption of animal protein due to its high prices requires alternative sources. Soyabeans provides a cheaper and high protein rich alternative substitute to animal protein. It is an important crop in the world and has been the dominant oilseed since the 1960s (Smith and Huyser, 1987). It is a multipurpose crop and its importance ranges from its use in milk production, oil processing, livestock feeds, medical, industrial and human consumption and more recently, as a source of bio-energy (Adedoyin *et al.,* 1998, Myaka *et al.,* 2005). Soyabeans is the richest source of plant protein known to man (Odusanya, 2002). It is also an important source of income.

According to the United State Department of Agriculture, USDA attaché report (2008) Nigeria domestic production of soyabeans is continuing to trend upwards but still does not meet the rapidly growing demand from the poultry and vegetable oil producers. This deficit cause the price of soyabeans to double within eight months and peak at $1,100 per ton in august 2008.This high price severely impacted poultry and soyabeans meal import are raising. The rapid growth in the poultry sector in the past 5 years which is about 39% per annum has boosted demand for soyabeans meal in Nigeria. However, although domestic production has been unable to satisfy local demand, in addition soybean crushers in the country are also operating below capacity and are unable to satisfy the growing demand for vegetable oil estimated at about 300,000tonnes annually.

The Nigerian National Commission on Agriculture defines agricultural marketing as a process which starts with a decision to produce a saleable farm commodity and it involves all aspects of market structure and systems both functional and institutional, based on pre-harvest and post-harvest operations, assembling, grading, storage, transportation and distribution (Agricultural Marketing Resource Centre, 2007).

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Research development and investment effort have often been focused on primarily on production increases without a well-developed marketing system which leads to all possible gains from the production effort going into the drains of post-harvest losses. Often times, marketers are compelled if not forced to sell their product at a very low price to avoid huge wastage or total loss and this reduces their marketing margins and marketing efficiency.

As important as marketing is, most of the studies on soyabeans have concentrated on production (Adekunle, *et al.* 2003; Abu 2012; Shalma; 2014; Agada 2015. Since proper marketing of soyabeans is made available to all and sundry and even though there have been a few scholarly investigation into soyabeans marketing in Benue State not much has been done on the profitability of soyabeans marketing in Benue State Nigeria. It is against this background that the research was carried out.

**Objectives of the Study**:

i. determine the conduct of soyabeans marketers in Benue State;
ii. examine the marketing margin of soyabeans marketing actors in the study area;
iii. Identify the problems faced by fish marketers in the study area.

**Statement of Hypothesis**

There was no significant difference in the marketing margin of soyabeans marketers in Benue State.

## II. METHODOLOGY

**Area of study**

The area of this study is Benue State of Nigeria. Benue state was created in 1976 and is located in the middle belt region of Nigeria with the capital at Makurdi. Benue state lie approximately between latitudes $6^0 30'$N and $8^0 10'$N of the equator and longitudes $6^0 35'$E and $8^0 10'$E of the Greenwich meridian, [Benue State Agricultural and Rural Development Authority, (BNARDA), 2005].

Benue state is considered as one of the hottest States in Nigeria with an average minimum and maximum temperature of $21^0$C and $38^0$ C respectively. It is in the southern guinea savannah ecological zone, which has a typical climate with the clearly marked seasons of dry season (late October to March) and wet season (April to early October). The State annual rainfall ranges from 1700mm in the southern part to 120mm in the northern ecology of the state.

The important feature of the state is the river in which the state derived its name from. The state share boundaries with five States, Nassarawa to the North, Taraba to the East, Cross-River to the Southeast Enugu to the Southwest and Kogi to the west. The southern part of the state is also bounded with republic of Cameroun.

Benue State has a land mass of about 33, 955km$^2$ with 23 Local Government Areas. Geographically and agriculturally, Benue State is divided into three zones, Zone A (katsina-Ala, Ukum, Ushongo, Vandiekya, Logo Kwande and Konshisha local government areas) Zone B (Gboko, Tarka, Buruku, Gwer East , Gwer West, Guma and Makurdi Local government areas), Zone C (Ado , Agatu, Apa, Otukpo, Ohimini, Okpokwu, Ogbadibo, Obi and Oju local government areas).

The state has a total population of 4,219,244 million people (National Population Commissions 2006). About 80$^{\%}$ of the state population is directly involved in agriculture. It is also called the food basket of nation, because the state produces agricultural products in large quantities. Some farmers in the study area have taken poultry production as their source of livelihood.

**Population and Sampling Procedure.**

The population of the study comprised all soyabeans traders in Benue State, with particular interest on producer marketers, retailers and wholesalers . The sampling frame of 914 registered soyabeans marketers was obtained from soyabeans Marketers Association in Benue State. Stratified random sampling technique was used to select respondents for this study. Three strata were used for this study. This includes producer marketer wholesaler and retailers. Yamene (1967) formula was used to determine the sample size (278) as follows:

$$n = \frac{N}{1+N(e)^2}$$

where:

n = sample size

N = population

e = level of significance (0.05)

Bowley's proportional allocation technique was used to determine the total sample size that was drawn from each of the selected Local Government Areas. The determination of the sample to be drawn from each LGA was done with the aid of Bowley (1926) formula as follows:

$$n_{h=} \frac{n \times N_h}{N}$$

Where:

$n_h$ = Number of unit to be allocated to each stratum

n = Total sample size

$N_h$ = Total number of elements in each stratum

N = Total population of the study

The total number of units drawn from Tarka Local Government Area was 87, 57 and 24 for producer marketers, wholesalers and retailers respectively while that of Konshisha was 59, 38 and 13 for producer marketers

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wholesalers and retailers respectively. The sampling frame is shown in table 1.

*Table.1: Sample Size Determination*

| LGA | Producer marketers | | Wholesalers | | Retailers | |
|---|---|---|---|---|---|---|
| | N | Nh | N | Nh | N | nh |
| TARKA | 287 | 87 | 188 | 57 | 79 | 24 |
| KONSHISHA | 194 | 59 | 124 | 38 | 42 | 13 |

Source: Soyabeans Marketers Association in Benue State, 2016.

**Data collection and Analysis**

Data for this study was collected from primary sources. The primary data was generated by the researcher through the use of trained enumerators, personal interview techniques and a well structured questionnaires which was administered to 278 soyabeans marketers in Benue State. 277 questionnaire were retrieved and used in data analysis. Data collected were analysed using descriptive and inferential statistics. The descriptive statistics were frequency distribution, percentages and mean while the inferential statistics were marketing margin model, Analysis of variance, Duncan multiple range test and one-way ANOVA was used to test the hypothesis.

**Model Specification**

**Marketing Margin Analysis**

Marketing margin$= \frac{\text{selling price}-\text{supply price}}{\text{selling price}}\ x\ 100$

Net Marketing Margin was estimated using the formular:

NMM=GM-TMC

NMM= Net Marketing Margin (Naira)

GM= Gross Margin (Naira)

TMC= Total Marketing Cost (Naira)

1 bag of soyabeans is equivalent to 100kg

**Garrett's ranking technique**

Problem associated with soyabeans marketing was achieved using Garrett ranking technique this was used by Nirmala and Suhasini (2013) as specified below:

$$percent\ position = \frac{100(Rij - 0.50)}{Nij}$$

where,

Rij is the rank given by ith item by jth individual;

Nij is the number of items ranked by the jth individual

## III. RESULTS AND DISCUSSION

**Market Conduct for Soyabeans in Benue State**

The distribution of respondent according to market conduct is shown in Table 2. The result revealed that all the respondent 100 % indicated their soyabeans was sold at lower prices during the harvest season since there is much soyabeans in the market . Most producer marketers sell their soyabeans during the harvesting season to meet pressing domestic needs. This is because most of them are small holder farmers with little financial capacity. This result agrees with Onu and Iliyasu (2008) that high proportions of grain sales by producers take place during harvest period.

Furthermore, the percentage distributions of respondents by advertising showed that 100 % of the respondents in the study area do not advertise their soyabeans to prospective buyers. This is because the market is structured in a way that sellers can take their soyabeans to particular stalls or position in the market where the middlemen either buy and sell or buy for some companies. This result is in agreement with earlier findings by Abah (2015) that 96.95 % of paddy rice marketers in Benue State do not advertise their paddy rice to prospective buyers.

More so, the distribution of respondents by source of business finance showed that majority 67.9 % depends on personal funding, 22.4 % source their money from family and friends while 9.7 % source their money from bank for their business. Therefore, the table further suggested that, majority of the marketers attracting about 67.9 % employed personal savings to finance their soyabeans marketing business. This may have the tendency to restrict their marketing activities and retard the expansion of the enterprise since capital is the main incentive by which marketers expand their marketing business. This result is in consonance with that of Fadipe *et al.* (2015) who found that high proportion (50 %) cocoyam marketers use personal savings as their source of income in sagamu local government area of Ogun State.

Conversely, all the respondent 100 % in the study area have not attended training on soyabeans cultivation or soyabeans marketing. This indicates that there is no research and development practice in soyabeans market in Benue State. This study contradicts earlier study by Abah (2015) that there is free of charge training to rice famers by government extension services and OLAM in Benue State.

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*Table.2: Market Conduct of Soyabeans in Benue State (n=277)*

| Parameter | Frequency | Percentage | (%) |
|---|---|---|---|
| **Price of soyabeans is higher at harvest season** | 0 | | |
| Yes | 277 | 100 | |
| No | | | |
| **Advertisement** | | | |
| Yes | 0 | | |
| No | 277 | 100 | |
| **Financial policy** | | | |
| Personal savings | 188 | 67.9 | |
| Banks | 27 | 9.7 | |
| Family and friends | 62 | 22.4 | |
| Total | 277 | 100.0 | |
| **Attended training** | | | |
| Yes | 0 | | |
| No | 277 | 100 | |

Source: Computed from field survey data, (2017).

**Marketing Margin of Soyabeans Marketers in Benue State**

The marketing margin of soyabeans marketers was presented in Table 2. The result showed that on the average the marketing margin of soyabeans marketers were ₦88.91, ₦85.99 and ₦85.78 per 100kg bag for producer marketers wholesaler and retailers, respectively. In Table 3 the calculated average marketing margins for producer marketers is higher than that of retailers and wholesalers. The estimated margins for wholesalers and retailers were correspondingly low. The low level of the average marketing margin of the marketers is largely attributed to the exploitative activities of the agents. This finding contradicts Jongur and Ahmed (2008) who found that farmers margin was as high as 96.81% and the remaining 31.19% went to the middlemen involved in sorghum marketing in Adamawa central zone.

*Table.3: Marketing Margin of Soyabeans Marketers in Benue State (n=277)*

| Variables | n | Mean | Std. deviation | Minimum | Maximum |
|---|---|---|---|---|---|
| Retailers | 37 | 85.78 | 3.414 | 75.031 | 91.437 |
| Wholesalers | 94 | 85.99 | 4.076 | 68.566 | 92.888 |
| Producer marketers | 146 | 88.91 | 3.739 | 74.174 | 99.22 |

Source: Computed from field survey data, 2017.

**Result of Hypothesis**

**Differences in the Marketing Margin of Soyabeans Marketing Actors in Benue State.**

One-way ANOVA was used to determine the marketing margin of the market participants in Table 3 the result shows that ($F = 21.029$; $P \leq 0.00$). The F statistics was significant at 5% therefore the null hypothesis which state that there is no significance difference in the marketing margin of the marketing actors is rejected and the alternative hypothesis accepted. The Duncan multiple range test indicated that producer marketers had the highest marketing margin (₦88.91) followed by wholesalers (₦85.99), while retailers had the least marketing margin of (₦85.78). The difference in the marketing margin of the respondents is largely attributable to the exploitative activities of middlemen. This finding agrees Achike and Anzaku (2010) that significant difference existed in marketing margins among benniseed traders in Nassarawa State, Nigeria.

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*Table.4: Difference in the Marketing Margin of Soyabeans Marketing Actors in Benue State.*

| Variables | Groups | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Marketing margin | Between Groups | 612.872 | 2 | 306.436 | 21.029* | 0.000 |
| | Within Groups | 3992.680 | 274 | 14.572 | | |
| | Total | 4605.552 | 276 | | | |
| DMRT | Retailer | 85.78 | | | | |
| | Wholesalers | 85.99 | | | | |
| | Producer marketers | 88.91 | | | | |

* denotes statistically significance at 5% level

DMRT: Duncan multiple range test

Source: Computed from field survey data, 2017

**Constraints Associated with Soyabeans Marketing in Benue State**

As contained in Table 5 which indicated the problems associated with soyabeans marketing in the study area, it reveals that, high transportation cost, poor storage/warehousing facilities, lack of access to credit, debt management or bad debt, heavy imposition of taxes or levies lack of insurance against theft and fire, seasonality, robbery, inadequate capital and adulteration were some of the problem identified as militating against soyabeans marketing in the study area.

Among all the problems inadequate capital was ranked first with a mean of 88.46. This was as a result of inadequate sources of finance and the problem of collateral before obtaining loan. Lack of access to credit facilities and High transportation cost was ranked 2nd with a mean of 85.07, this maybe as a result of poor road network linking the rural soyabeans markets to the urban centres.. The 4th ranked problem was heavy imposition of taxies or levies. With a mean of 85.03. Poor storage and warehousing facilities ranked the 5th with a mean of 84.13. The 6th ranked problem was lack of insurance against theft and fire with a mean of 79.83. Robbery /theft ranked 7th with a mean of 30.28. The 8 ranked problems was seasonality with a mean of 24.60 while debt management/bad debt and adulteration with a mean of 16.04 and 10.77 ranked 9 and 10 respectively. Amongst all, the problems inadequate capital, high transportation cost and heavy imposition of taxes and levies ranks the first three major problems. The combine effect of these problems on the marketing system could bring about a distortion in the structure, conduct and performance of the marketing process. Hence, this could lead to the reduction in profit margin of the marketers and consequently, discourage the present and prospective marketers of the commodity in participating in the enterprise in the study area. These findings were in agreement with that of Girei *et al*. (2013) in their study of cowpea marketing that inadequate capital, high cost of transportation, inadequate and poor storage facilities, high taxes were the major problems facing cowpea marketers in North and Yola South Local Government Areas in Adamawa State, Nigeria,

*Table.5: Constraints Associated with Soyabeans Marketers in Benue State (n = 277).*

| Variables | Mean | Rank |
|---|---|---|
| High transportation cost | 85.07 | 2 |
| Poor storage/ warehousing facilities | 84.13 | 5 |
| Lack of access to credit facilities | 85.07 | 2 |
| Debt management or bad debt | 16.04 | 9 |
| Heavy imposition of taxes or levies | 85.03 | 4 |
| Lack of insurance against fire and theft | 79.83 | 6 |
| Seasonality | 24.60 | 8 |
| Robbery/theft | 30.27 | 7 |
| Capital intensive | 88.46 | 1 |
| Adulteration | 10.77 | 10 |

Source: Computed from field survey data, 2017

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## IV. CONCLUSION

The study showed that soyabeans marketing was profitable in Benue State, Nigeria. The profit level was relatively sustainable and can even attract new entrants into business. Hence, it serves as a source of income and employment for the marketers. The major problems of soyabeans marketing in the study area include inadequate capital, high transportation, lack of access to credit facilities heavy imposition of taxes or levies and poor storage and warehousing facilities.

**Recommendation**

I. Efforts should be made to eliminate the constraints to soyabeans marketing in the study area by improving on socioeconomic facilities such as roads, markets stores and related amenities.
II. Government and nongovernmental organization can do their own part by renovating existing bad roads and constructing new ones, especially those that link the points of production to points of consumption.
III. The business can also help in generating jobs and reducing poverty.
IV. Existing association of soyabeans marketers should be strengthen financially by the government through provision of minimal and interest free loans whereas formation of new ones be encouraged by the marketers.

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# Effect of Different Shade Levels on Growth and Yield Performances of Cauliflower.

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***Abstract*****—** *An experiment was conducted during March to July in 2017 (off season) at the Faculty of Agriculture, University of Jaffna, Ariviyal Nagar, Kilinochchi to study the effects of different shade levels on growth and yield performances of cauliflower. Different shade levels such as 25 % (open field), 50 % (single net house) and 75 % (double net house) were used as treatments. The experiment was conducted in completely randomized design (CRD) with four replications. Parametric (growth & yield) analysis were done by using SAS 9.1 package. The influence of environmental variables such as temperature, relative humidity and light intensity were also studied.The result revealed that growing of cauliflower in different shade levels showed great influence on plant growth and yield attributes. There were significant variations in number of leaves, plant height,curd weight, curd diameter and curd circumference of cauliflower under different light intensities. Light intensity in the shade net house was lower than in the open field. The highest vegetative growth and yield were observed in cauliflower which was grown in 50 % shade levels and the lowest yield was in 25 % shade level (open field). It can be concluded that cauliflower can be cultivated in 50 % shade levels successfully to produce quality curd during off season of the dry zone of Sri Lanka.*

***Keywords— Cauliflower, growth, shade levels, yield.***

## I. INTRODUCTION

Agriculture is closely related to the Sri Lanka because of the country's fertility and the different agro climatic zones which are ideal for crop cultivation. The vegetable sub sector is the most important in agricultural sector next to the rice. As with rice, vegetables are grown throughout the country and large numbers of farmers are engaged in this cultivation (Rupasena, 1999). The cool and healthy climatic conditions in the hill country are ideal for temperate vegetable (exotic) crops such as carrot, leek, cabbage, cauliflower, salad leaves, beet, bean, bell pepper and salad cucumber and climatic conditions in the low and mid country are suitable for tropical vegetable crops. Among the exotic vegetables cultivated in Sri Lanka, cauliflower is cultivated in large extent due to its nutritional value and market demand.

Cauliflower (*Brassica Oleracea* Var. botrytis L.) belongs to the family of Brassicaceae originated from Europe and Africa (Ajithkumar*et al*., 2014). Most daily consumed important vegetable of commercial crop in the world. It also has good demand in Sri Lanka. Consuming cauliflower is useful to fight against cancer,boost heart health,rich in vitamins and mineralsboost brain health,detoxification support and digestive benefits. In cauliflower, the edible curd is made up of abortive flowers. The stalk of cauliflower is short, fleshy and closely crowded (Shanmugavelu, 1989). The growers can cultivate a crop in any season under protected environment, as he can provide the temperature, humidity and light, as required by the plant species.

The optimum monthly temperature requirement for cauliflower is 15 to 20 $^{o}$ C with an average maximum of 25 $^{o}$ C and average minimum of 8 $^{o}$ C.Plants require light for optimum growth and development, but the three different aspects of light, quantity, quality and duration, also have a significant influence on growth. A plant under natural conditions receives light from the sun; the amount, quality and duration greatly depend on the season of the year, hour of the day, geographical location and weather. Plants use light as a source of energy for photosynthesis. It is primary metabolites in plants (Kopsell, Kopsell, 2008; Perez-Balibrea*et al*., 2008). The carbohydrates produced during photosynthesis are stored and used by the plant as a food source. Light intensity can affect plant canopy, flowering, leaf size, and colour in both herbaceous (Jeong*et al*. 2009; Vendrame*et al*. 2004) and woody species (Hampson *et al*. 1996).

Sri Lanka is a tropical country and shade nets are used to grow the quality temperate vegetable crops which reduce the crop damages due to heavy rain falls and high solar radiation. In addition, it minimizes the pest and disease damages in many parts of the country. Cultivating the crops under shade nets are needed compulsorily for cultivation of hybrids and some exotic crop varieties because they have are susceptible to extreme external environmental conditions. In net houses,

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the plant growth and productivity can be manipulated by modifying light quantity (Wheeler, 2008). Cauliflower is highly sensitive to pest attack and it is ideal for cool climate. Therefore, growing of this crop under shade condition is ideal in dry zone of Sri Lanka especially during off season to reduce the effect of light intensity and pest incidence to this crop.

There are studies available regarding effect of shade levels on growth and yield performances of cauliflower and other exotic vegetables in the world and few studies are available in Sri Lanka, but none of the study was done in Dry zone of Sri Lanka during off season. To overcome this gap, a research study was carried outwith an objective of evaluating the performance of the cauliflower under different shade levels in Kilinochchi district during off season with the sub objectives of the following,

i. To study the effect of different shade levels on morphological characteristics of cauliflower.

ii. To study the effect of different shade levels on yield of cauliflower.

## II. MATERIALS AND METHODS

A field experiment was carried out at the Faculty of Agriculture, Ariviyal Nagar, Kilinochchiwhich is located at Northern Province of Sri Lankabelongs to the agro-ecological region of Low Country Dry Zone ($DL_3$) to evaluate the effects of different shade levels on the growth and yield performances of cauliflowerduring the period of March to July 2017. Experiment was conducted in Completely Randomized Design (CRD) with four replications. The cauliflower variety Mareet$F_1$ was selected due to its excellent performance in warm conditions.

Treatments were

- $T_1$ – 50 % shade level ( Single net house)
- $T_2$ -75 % shade level (Double net house)
- $T_3$ - 25 % shade level (Open field)

For nursery preparation,Seeds were treated with captan and sowed in a nursery tray with dimensions of 22.5 cm in width and52.5 cm in length consisting of cells dimensions of 6.25 × 6.25× 6.25 cm each. Rooting media was prepared by using top soil, compost and cattle manure at the ratio of 1:1:1 and treated with fungicide captan. Three seeds were planted per cell. Cauliflower seeds germinated 2 days after sowing. Three weeks after germination uniform size cauliflower seedlings were planted in 45 cm height and 22 cm width poly bags filled with same rooting media used in the nursery and as 3 seedlings per bag. After the successful establishment of the seedlings, one vigorous healthy seedling was allowed per bag. Cauliflower bags were arranged according to recommended spacing of 60 cm × 45 cm. After planting watering was done by water can and the surface soil was kept in wet condition, but excess watering was avoided. All other management practices were given as recommendation made by Department of Agriculture.The bag arrangements were shown in the plate 1.


*Plate 1. Cauliflower under different shade levels*

At the time of curd formation, blanching was done in cauliflower. Leaves were tied up with twine to protect the curd from sun burning and browning (Plate 2).

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*Plate 2: Blanching of cauliflower*

Harvesting was done 65 to 80 days after transplanting, when curd reach the proper size, bright white colour and compactness.

**Measurements:**

**1. Weather Parameters**

Light intensity, average temperature and average humidityat each shade levels were measured

**2. Growth Parameters**

Plant height and number of leaves per plant were taken at biweekly interval commencing from 3 weeks after transplanting.

**3. Yield Parameters**

The yield components of cauliflower curd such as weight, diameter and circumference were measured during harvesting time.

**Data Analysis**

The ANOVA was performed by using GLM procedure of the SAS 9.1 computer software package.

## III. RESULTS AND DISCUSSION

The results obtained from this research study were discussed in this chapter.

**1. Light Intensity**

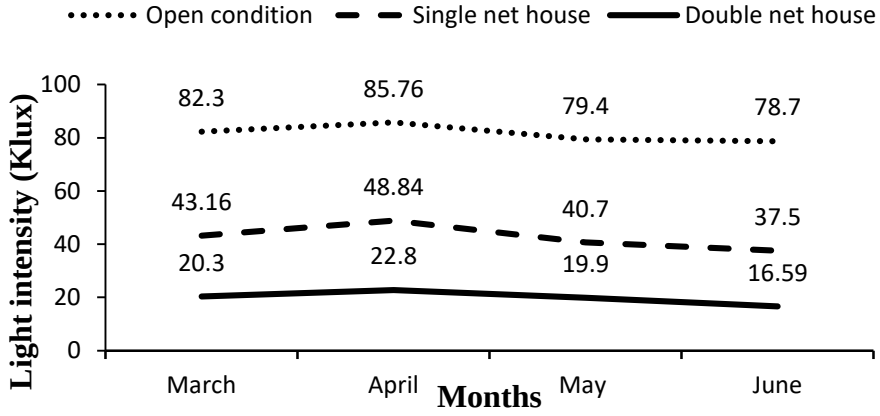


*Fig.1: Mean light intensity (klux) from March to June during Experimental period*

Mean light intensity was higher in open field condition and maximum light intensity was observed during April than other months (Figure 1). Nangare*et al*. (2015) reported that shade nets reduced both light intensity and heat effectively during the daytime while changing the spectrum. He also stated that the significant difference was observed in solar radiation in open condition and inside the shade nets.

**2. Average temperature**

*Table.1: Average temperature from March to June during the Experimental period*

| Treatment | Months | | | |
|---|---|---|---|---|
| | March | April | May | June |
| Open field condition | 32.8 ºC | 37.8 ºC | 35.4 ºC | 34.8 ºC |
| Single net house | 31.4 ºC | 38.8 ºC | 35.7 ºC | 35.8 ºC |
| Double net house | 31.3 ºC | 38.1ºC | 35.2 ºC | 35.1 ºC |

**3. Average relative humidity**

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*Table.2: Average relative humidity during the Experimental period*

| Treatment | Months | | | |
|---|---|---|---|---|
| | March | April | May | June |
| Open condition | 58.1 % | 48.6 % | 50.4 % | 50.0 % |
| Single net house | 59.7 % | 48.1 % | 49.4 % | 49.2 % |
| Double net house | 59.3 % | 47.0 % | 49.8 % | 49.1 % |

There was no much variation in average temperature and relative humidity during the experimental period of March to June among different treatments (Tables 1 and 2).

Nangare*et al.* (2015) stated that there was no significant difference found in average monthly temperature and humidity inside shade net house and open field condition (control).Sajjapongse and Raon (1983) observed the poor head formation, leaf twisting, early bolting and reduced yields when temperate leafy vegetables were grown under hot, high sunlight conditions. Smith *et al.* (1984) also observed that under shading nets, the air temperature was lower than that of the ambient air temperature, depending on the shading intensity. Shade netting not only decreases light quantity but also alters light quality to a varying extent and might also change other environmental conditions.

Growth and yield performance of cauliflower also depends on the season of cultivation. Experimental period was unsuitable season (off season) to cultivate cauliflower. Off season cultivation showed poor growth and yield than proper time of cultivation. Swagatika*et al.* (2006) observed that cauliflower sown in the month of September and grown under shade net recorded the highest values for plant height, number of leaves, girth and curd yield.

**4. Number of Leaves**

Number of leaves is one of the important growth parameter which influenced by genetic and environmental factors. Leaf is the primary source of photosynthesis. Formation of leaves in cauliflower was significantly influenced by different shade levels.

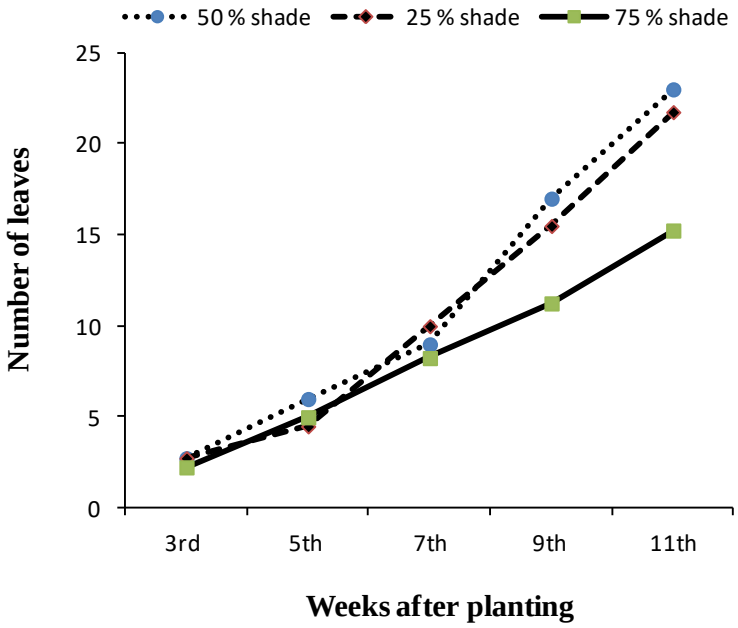


*Fig.2: Number of leaves of cauliflower at biweekly interval*

The leaves growth in cauliflower was significantly differed with different shade levels. The highest number of leaf formation was recorded in 50 % shade level and the lowest number of leaves was recorded in 75 % shade level (Figure 2). This might be due to the favourable effect of 50 % shade net which had increased photosynthetic process in cauliflower due to favourable micro climate.

**5. Plant Height**

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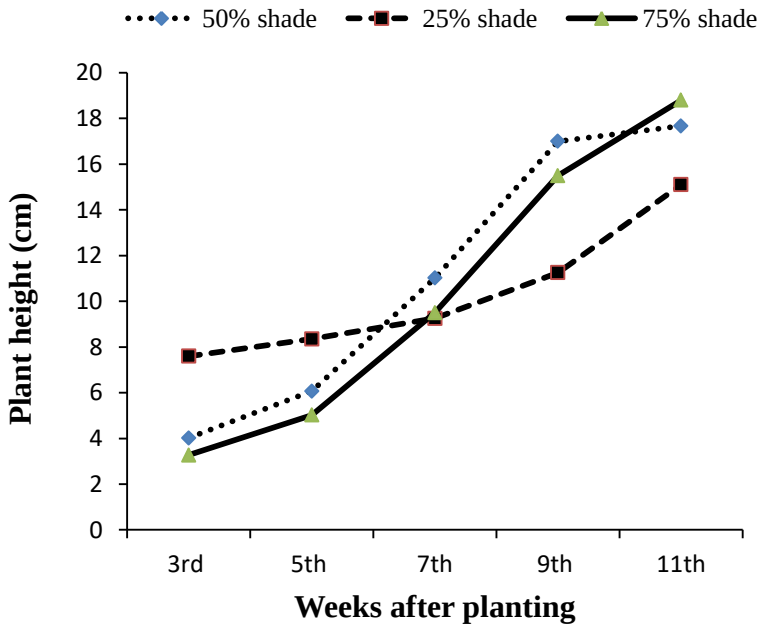


*Fig.3: Average plant height of cauliflower at biweekly interval*

The plant height of cauliflower was significantly influenced under different shade levels throughout the crop growing period. Plant height of cauliflower gradually increased and it was significantly differed among different treatments (Figure 3). The highest height was observed in 75 % shade level that would be due to elongation of internodes to capture more light. Different shade levels could alter the other environmental conditions and develop suitable micro climate inside the shade netswhich may be the reason for differences in plant height and number of leaves under different shade levels. Same results also reported by Swagatika*et al*. (2006), Elad*et al*. (2007), Vethamoni and Natarajan (2008), Haque*et al*. (2009) and Rajasekar*et al*. (2013).

**6. Mean Curd Weight**

The number of days required for curd initiation was influenced due to different shade levels. The curd initiation was found to be earlier in 50 per cent shade level. Late curd initiation was observed in 25 % shade level and curd was not initiated under 75 percent shade level. Mean curd weight of cauliflower was significantly differed. Among treatments, 50 % shade level ($T_1$) gave the highest weight than other treatments. When increasing the shade level, mean head weight was decreased. The highest curd weight of 285 g was obtained in 50 % shade level ($T_1$)which was statistically significant from other two treatments. The lowest curd weight of 160.03 g was obtained from $T_3$ (open field). There was no curd formation in 75 % shade level that could be due to the inadequate light intensity and low photosynthetic activity. Results showed that cauliflower performed well under 50 % shade level. This might be due to the favourable environmental conditions such as light intensity, temperature and relative humidity in 50 % shade levels which had increased photosynthetic process and assimilate accumulation in cauliflower. Similar results were also reported by Swagatika*et al*., (2006) and Vethamoni and Natarajan (2008) in cauliflower.

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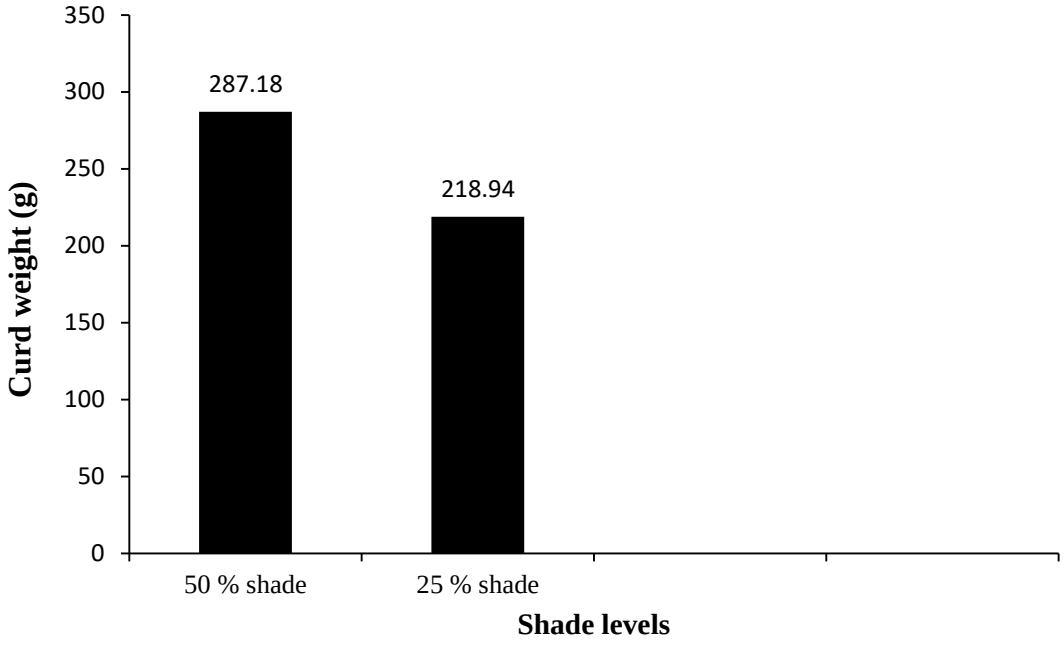


*Fig.4: Mean curd weight of cauliflower*

**7. Mean Curd Diameter**

There was a significant differences was observed in 50 % shade and open field treatments (Figure 5). Maximum curd diameter of 22.53 cm was obtained from 50 % shade level ($T_1$) and minimum curd diameter of 18.53 cm was obtained from 25 % open field condition ($T_3$). This may due to favourable condition in 50 % shade levelthat caused to form compact quality curd. Under high temperature and light effect in open field conditioncurds were perform poorly than 50 % shade level.

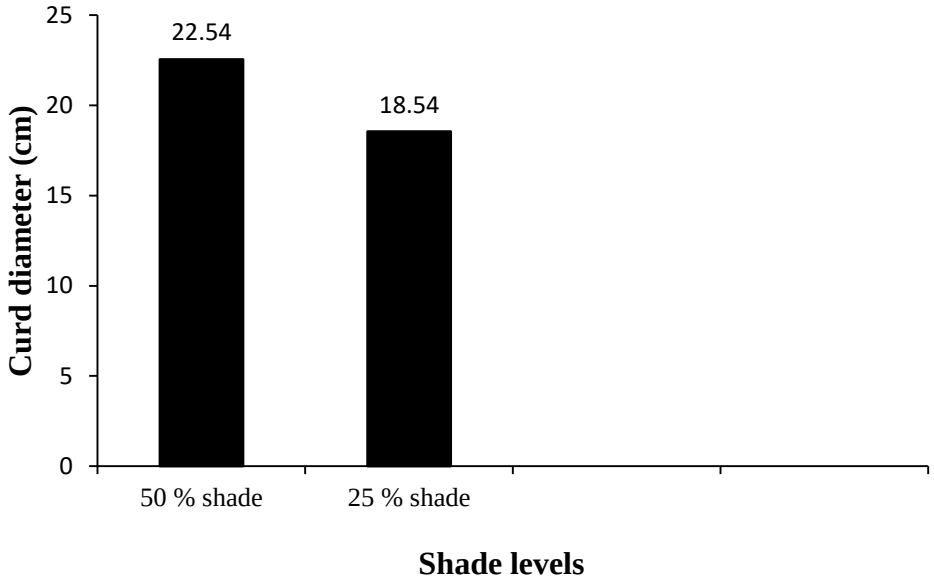


*Fig.5: Mean curd diameter of cauliflower*

**8. Mean Curd Circumference**

Different shade levels showed the significant differences in mean curd circumference of cauliflower (Figure 6). The

maximum curd circumference of 38.88 cm was recorded in 25 % shade levelwith more volume in curds than other treatments. Under lower shade level temperature and light intensity were high it promote more growth of curd. Lower shade level gave more volume curds but curds were loosely arranged and not well compacted.

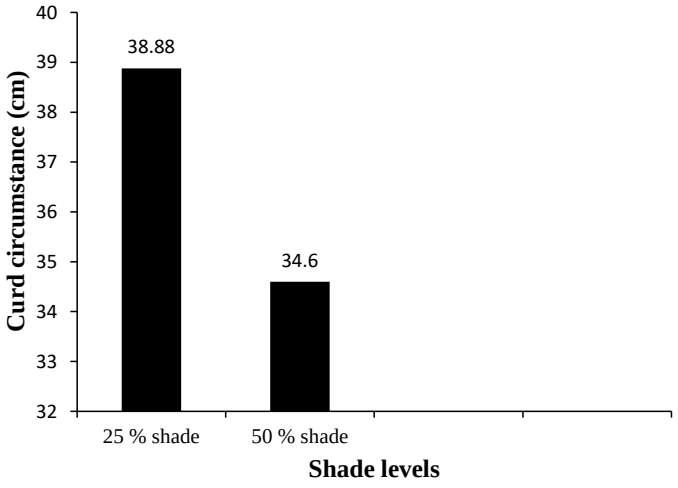


*Fig.6: Mean curd circumference of cauliflower*

## IV. CONCLUSIONS

Cauliflower cultivation in Ariviyal Nagar, Kilinochchi was significantly influenced by the season and weather conditions. Growing of cauliflower under different shade levelsshowed great influenced on its growth and yield performance. Numbers of leaves were high in 50 % shade level (single net) than other shade levels. Average plant height of cauliflower increased with increasing shade levels. The highest plant height was in 75 % shade level than other shade levels.The highest mean curd weight and curd diameter were in 50 % shade level than 25 % shade level (open field) condition. Good quality, compact curds were produced at 50 % shade levels than other shade levels. The highest mean curd circumference was in 25 % shade level (open field) than 50 % shade level.

## V. SUGGESTION

Other levels of shade and naturally available shade scan also be used as shade level and experiment can be repeated for different varieties in both *Maha* and *Yala* seasons to get consistency.

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# Effect of aqueous extracts of neem seeds (*Azadirachta indica*) on the development of Asian Rust of soybean in the Center Region of Cameroon

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***Abstrat*—***A study realized during two consecutive years at Mimetala and Nkometou, permitted to evaluate the antifungal potential of aqueous extract of neem seeds (AENS) on the Asian rust of soybean development. Varieties of soybean used, were a locale, TGX1835-10E and TGX1910-14E. A system of completely randomized blocks was used containing five treatments: $T_0$ (control), $T_1$, $T_2$, $T_3$ (consisting of 25, 50, 100 g/l of extract respectively) and $T_5$ (treated with Plantineb 80wp). The growth and disease parameters, yield were evaluated under the influence of different treatments. During the two campaigns, growth parameters varied significantly and proportionately for different doses of extracts, $T_4$ having the largest values followed by $T_3$at Mimetala. Those parameters were high on locale and TGX1910-14E varieties during the two campaigns. In 2015 as in 2016, TGX1835-10E was the most attacked by the disease in the two sites. Its incidence in the controlwas 100% and 80% in 2015 and 97.5 and 75% in 2016 respectively at Mimetala and Nkometou. TGX 1910-14E was the least attacked with an incidence in the control of 47.5% and 45%, and 5% in $T_3$ and $T_4$respectively in 2015 and 2016. Disease was most severe at Nkometou on local (67%)and least at Mimetala on TGX 1910-14E (22.3%) in the control. The yielding was lightly high in 2016 than 2015 and high values were obtained at Mimetala in $T_4$ and $T_3$. Results suggest that* AENS*is a biocidal substance with an antifungal activity and should be applied at high doses.*

***Keywords*—Antifungal potential, aqueous extract, Glycine max, Asian rust, Azadirachta indica.***

## I. INTRODUCTION

Soybean (Glycine max), an annual plant of the Fabaceae family, is present in more than 80 countries worldwide. Native from East Asia (Qiu & Chang, 2010), it is grown mainly for its highly rich seeds in protein (40%), fat (20.2%) and trace elements (Labat, 2013). The world production of soya increased considerably during the 20th century. FAO currently estimates it at approximately 315 million tons; the main producers are the United States, Brazil and Argentina with about 82%. However this very high productivity results from the increase of cultivated surfaces inducing large deforestation. In South America, cultivated surfaces passed from 18 to more than 40 million hectares (Anonymous, 2016). Today, in Cameroon and many other African countries, soybeans are widely used in various forms of food to curb malnutrition. However, multiple infections substantially reduced the productivity of soybeans. Several fungal diseases affect many varieties of soybean in the field; besides, the Asian rust is nowadays the most widespread disease throughout the world. Two main methods of control are used to remedy these pathologies, namely genetic and chemical control. These individual methods have shortcomings and are constraining to the environment (Ambang *et al*., 2008). Chemical control is the most used and most effective; based primarily on the use of synthetic pesticides it is costly and harmful because of the concentration of residues in food chains (Ngando *et al*., 2006). In addition, improved varieties used in genetic control do not unfortunately totally resist against these diseases. An alternative, the use of environmentally friendly biopesticides is conducive to healthy and sustainable agriculture. In the search of natural pesticides, neem (*Azadirachta indica*) is one of the most widely used plants. This plant of the Meliaceae family is originally from eastern India and the pesticide potential of the extracts has already been demonstrated on several plants. Thus, it has fungicidal (Sharma *et al*., 2003; Mohammad *et al*., 2010; Mboussi *et al*., 2016), insecticide (Awad *et* Shimaila,

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2003; Da Silva *et* Martinez, 2004), and nematicide (Javed *et al*., 2007; Kosma *et al*., 2011) properties.Its effectiveness in the fight against Asian rust of soybean would enhance theprotection of plants in the field and the environment. The main objective of this study is to test the antifungal effects of aqueous extracts of neem seeds on the development of Asian rust of soybean in the farm.

## II. MATERIALS AND METHODS

### 2.1. Materials

The plant material consisted of three varieties of soybean (Local bought in a maket, TGX1835-10E ameliorated at the Institute of Agricultural and Development Research (IADR) of Foumbot and TGX1910-14E ameliorated at IADR of Maroua) and neem seeds collected in the Northern region. Chemical material used was plantineb 80 Wpwhich is at 80% composed of Manebe and was applied at de recommended dose: 5.33g/l (Fig.1). Several other laboratory materials were used.


*Fig.1. Materials (a: neem seeds; b, c, d: respectively the local, TGX1835-10E and TGX1910-14E varieties of soybean, c: chemical fungicide).*

### 2.2.Methods

#### 2.2.1. Preparation of extract and plants treatment

Aqueous extracts was obtained using the method of Kumar (2003) based on maceration. Mature fruits harvested in the northern part of the country were broken to collect the seeds. These were then dried at ambient temperature for 2 to 3 days and crushed. To obtain $C_1$, $C_2$ and $C_3$ concentrations, respectively 250, 500 and 1000 g of neem seeds powder were introduced in 10 L of tap water, kept for about 12 hours and filtered with the muslin tissue. These solutions were directly applied in the field by adding ten (10) g of soap (Total clean) used as wetting. The solution of Plantineb 80 WP was used at the recommended doses, which is 5.33 g/L.Spraying was made weekly for the extract and the chemical fungicide. The aqueous extracts of neem seeds were applied at the dose of 250 L/ha and the fungicide at 2.5 Kg/ha. Their application was done the 4$^{th}$ WAS but the parameters taking was done from the 2$^{nd}$ WAS.

#### 2.2.2. Experimental design

The experimental design used in each site was a system of completely randomized blocks containing five (05) treatments which are $T_0$ (control), $T_1$, $T_2$, $T_3$ (consisting of 25, 50 and 100 g/l of extract respectively) and $T_5$ (5.33 g/l of Plantineb 80 wp) repeated three times.

#### 2.2.3. Evaluation of the growth parameters

The height and the number of leaves are the two main parameters evaluated. The height was evaluated by measuring with a decameter, the distance from the soil area to the apex of the plant. The number of leaves was determined by counting. These parameters were evaluated in the regular manner and by meantime of 2 weeks. The averages were compared between all treatments in relation to control.

#### 2.2.4. Evaluation of the incidence and the severity of disease

The plants identified as ill were counted, then, labeled per plot and per varieties. These plants were then counted per attack degree and the quantification of the disease was made on the unitary plot during the time. The incidence and the severity were evaluated by adopting the formula of Tchoumakov and Zaharova, (1990).The incidence which is the proportion of ill plants in an experimental unit, apart from the attack gravity of each plant was determined using the following formula:

$$I = \frac{Ni}{Nt} x\ 100$$

Where: Ni= number of ill plants Nt= number of plants studied and I= incidence in %

The severity measures the quantity of disease on a plant organ or on an entered plant. It is the attack degree of an organ or the entered plant by the disease. It is expressed by the following formula:

$$S = \frac{\sum AB}{Nm} \times 100$$

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Where: ∑ABis the sum of multiplications of the number of ill plants (A) with the correspondentinfection degree **(**B**)** given in %; and Nm is the total number of ill plants.

The scale used for the infection degree (B) was adopted to those proposed by Wangungu *et al.,* (2011) where 1= 0 % of plant infection; 2= infection covering 1 - 15 % of the plant; 3= infection covering 16 - 40 % of the plant; 4= infection covering 41 - 75 % of the plant; 5= infection covering 76 - 100 % of the plant;

**2.2.5. Determination of the impact of disease and different defensives substances tested on the production**

Pods were harvested and sacked by plant of soybean, and then the sachets were gathered per plot and put in bags initially labeled. After, the cockles were removed and the grains were dried under the sun for 7 days. The yielding was evaluated by heaving with a precision scale the grains of each plant and each plot. The values obtained were estimated at the hectare by adopting the formula of Svecnjak *et al.,* (2006) following:

$$Y = \frac{Wp \times Np}{S} \times 10000$$

With Wp = Weight of dried soybean grains per plant; Np = number of soybean plant per treated elementary plot (which is 160); S = surface of each treated elementary plot (which is 11.75 $m^2$); 10 000 $m^2$= 1hectare.

**2.2.6. Statistical analysis**

Data obtained for all the studied parameters were automated and subjected to an analysis of variance multi varied MANOVA using the software SPSS/IBM version 20.0. Different averageswere compared using the PPDS of Duncan with the probability threshold p= 5 %. Histograms were plotted using Microsoft Office Excel 2010 software.

## III. RESULTS AND DISCUSSION

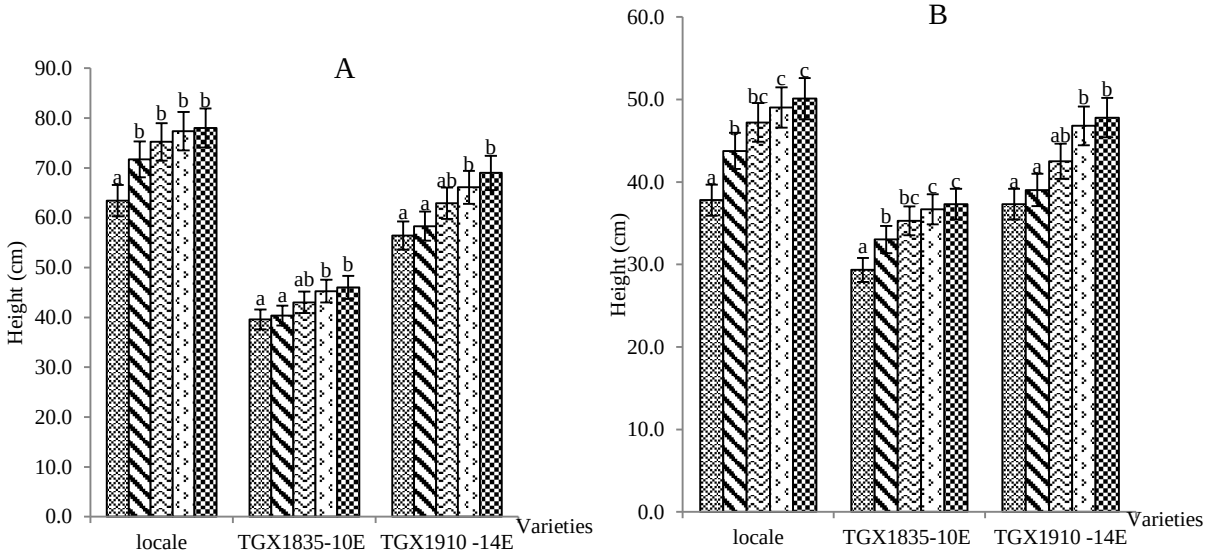
### 3.1. Results

**3.1.1. Effect of Aqueous extract of Neem seeds on the growth parameters**

**3.1.1.1. Variation of the height of plants**

As much in the 1st campaign as in the 2nd, it was noticed that irrespective of the varieties, the size of the plants was smaller at Nkometou than at Mimetala. In addition, the TGX 1835-10E variety was the one with the smallest height, confirming its dwarf variety characteristic. In all the varieties and in the two sites, the height of the plants varied very little between the different treatments during the first 4 weeks after sowing (WAS). From the 6th WAS (2 weeks after the first treatment), there was a variation in size proportional to the different treatments. For TGX 1835-10E, the size remained relatively stable from the 8th WAS in the Mimetala and Nkometou sites. However, local and TGX 1910-14E varieties, saw their size increase even after the 10th WAS. The largest sizes were obtained at Mimetala on the local variety at all doses tested with 63.4; 71.7; 75.2; 77.4 and 78 cm respectively in treatments T0, T1, T2, T3 and T4 at Mimetala against 37.8; 43.8; 47.2; 49 and 50.1 cm at Nkometou 10 SAS in 2015. These values were 56.2; 65.2; 68; 71.6 and 73.2 cm respectively at Mimetala against 35.9; 38.7; 39.8; 43.9 and 45 cm at Nkometou in 2016.

The statistical analysis showed that there was no significant difference between all treatments up to the 4th WAS but that from the 6th WAS there was a significant difference between the T2, T3, T4, ratio T0 the other two, and no significant differences between treatments T3 and T4. Taking into account the size parameter of the plants, the analysis shows that there is a significant difference between all the varieties (Fig.2).



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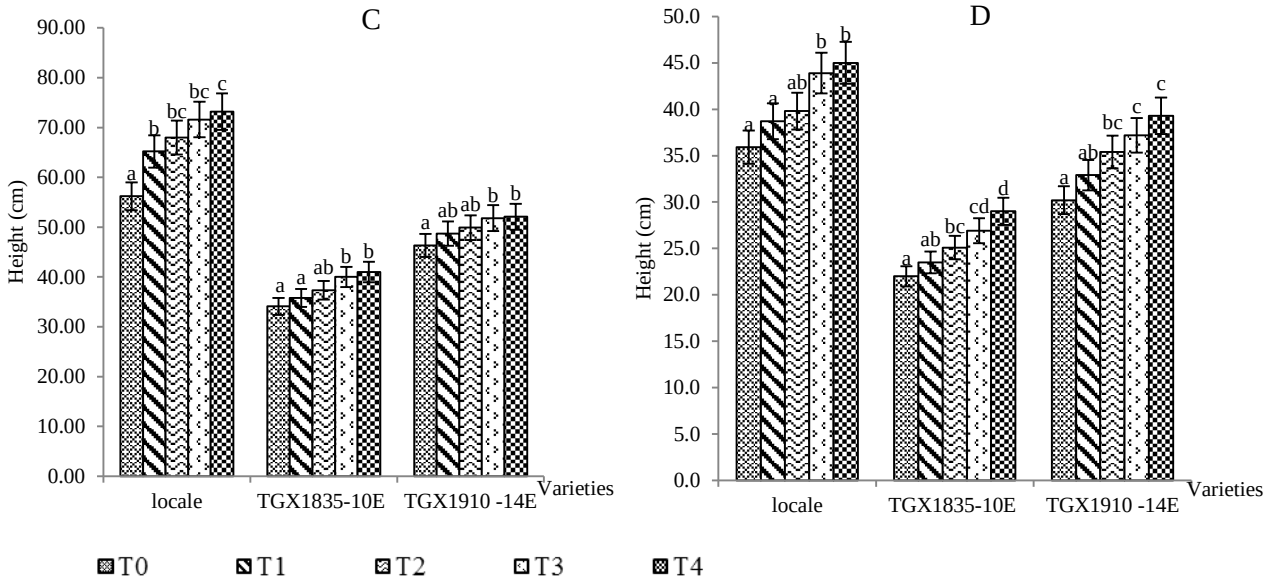
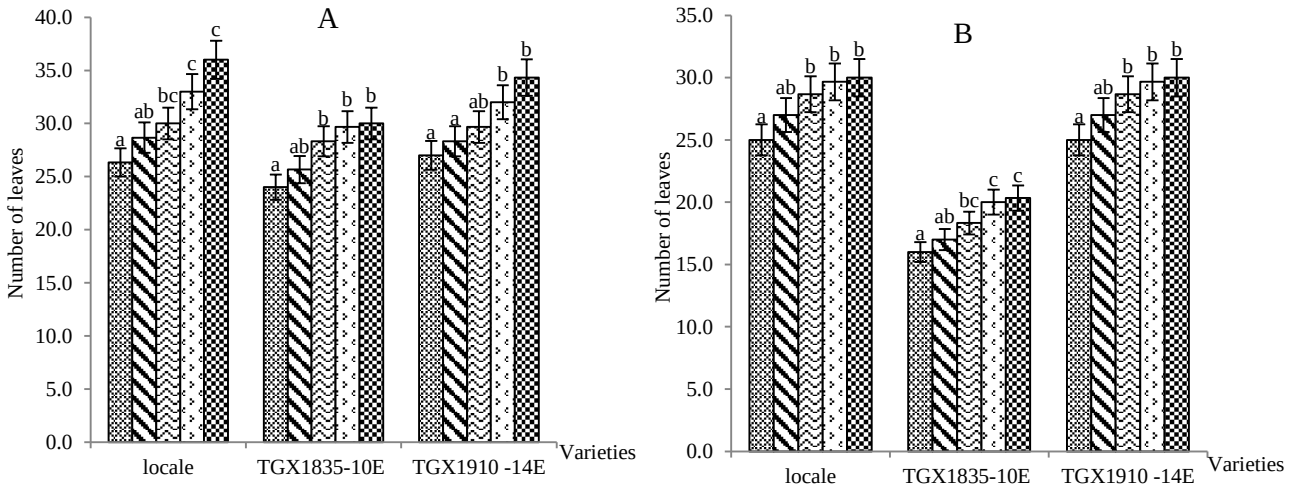



*Fig.2. Plants height of the three varieties 10 WAS in the different treatments during the two campaigns in the sites: A and B (Mimetala and Nkometou in 2015; C and D Mimetala and Nkometou in 2016).*

**3.1.1.2. Variation of the number of plant leaves**

Irrespective of the varieties, the number of leaves of the plants was lower at Nkometou than at Mimetala during the two campaigns. TGX 1835-10E was the variety with fewer leaves. In all the varieties and in the two sites, the number of leaves of the plants varied very little between the different treatments during the first 4 weeks after sowing (WAS). From the 6th WAS (2 weeks after the first treatment), there was a variation in the number of leaves in proportion to the different treatments. For TGX 1835-10E, the number of leaves remained relatively stable from the 8th SAS with an average of 25; 25.7; 28.3; 29.7 and 30, then 16; 17; 18.3; 20 and 20.3 respectively in the $T_0$, $T_1$, $T_2$, $T_3$ and $T_4$ treatments at the Mimetala and Nkometou in the 2015. During the same period in the 2016 season, the average number of leaves for this variety was 15 ; 16; 17.3; 19 and 20, then 13.7; 14.7; 16.7; 18.7 and 19.7 respectively at Mimetala and Nkometou. However, the local and TGX 1910-14E varieties, have seen their number of leaves always increase even after the 10$^{th}$WAS. These two varieties had practically the same number of leaves in both sites and at all doses tested.

The statistical analysis showed that there is no significant difference between all treatments up to the 4th WAS, but that from the 6th WAS there is a significant difference between the $T_2$, $T_3$, $T_4$ treatments, Compared to the other two, and no significant differences between treatments $T_3$ and $T_4$. Taking account of the leaf number of plants, the analysis shows that there is a significant difference between TGX 1835-10E compared to the other two varieties and no significant differences between local varieties and TGX 1910-14E According to duncan test alone of 5% (Fig.3).



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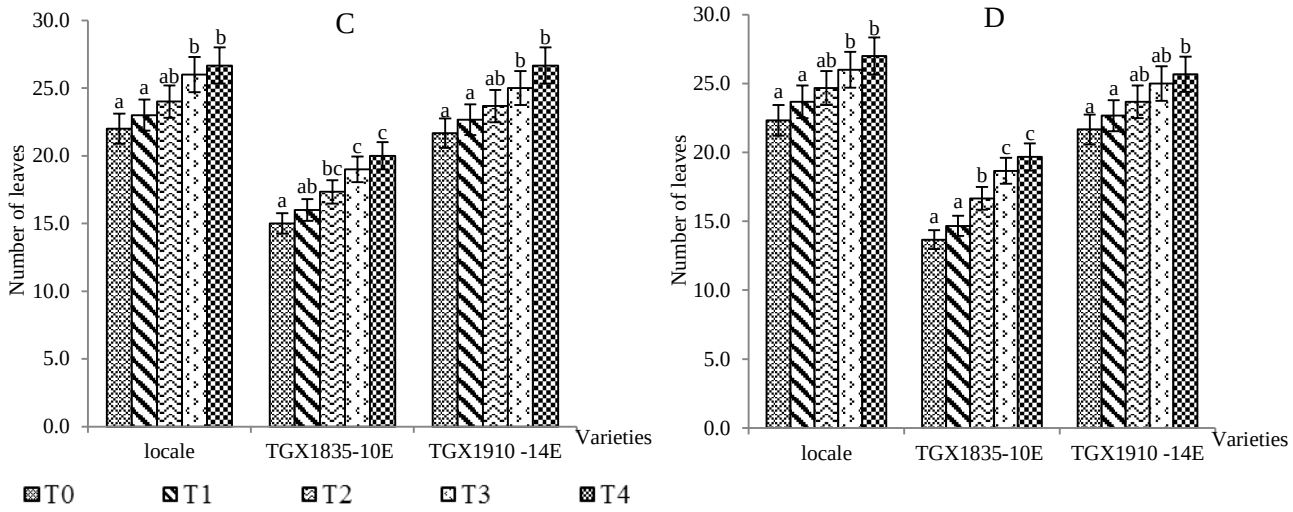
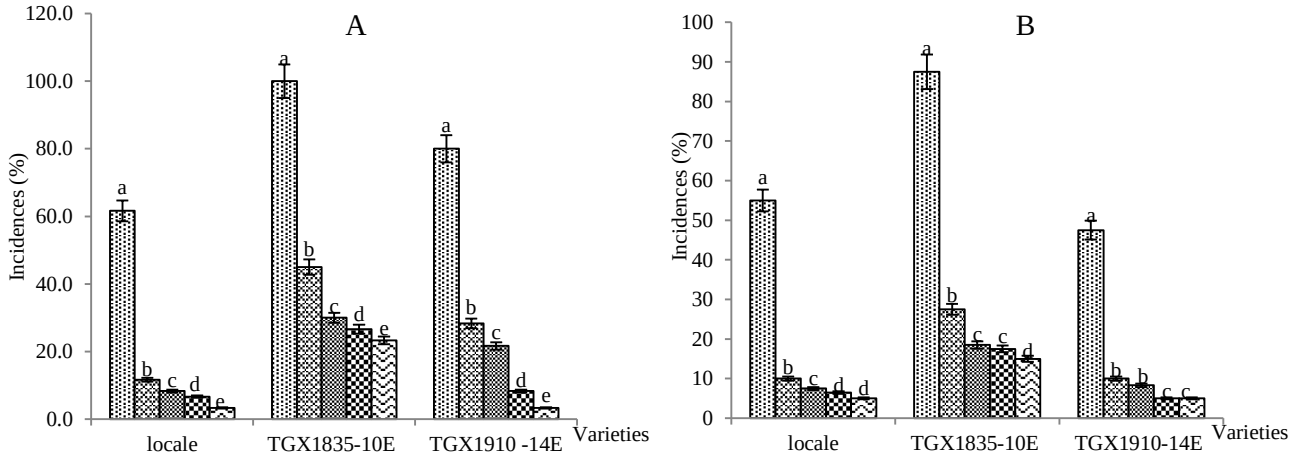



*Fig.3. Number of leaves of the three varieties 10 WAS in the differents treatments during the two campaings in the sites: A and B (Mimetala and Nkometou in 2015; C and D Mimetala and Nkometou in 2016).*

**3.1.2. Effect of Aqueous extract of Neem seeds on the disease parameters**

**3.1.2.1. Evolution of the incidence**

Disease incidence increased over the time in T0 treatment (control), for all varieties and at both sites during the two campaigns. Overall, TGX 1835-10E was the most affected by the disease in both campaigns. Indeed, from the 6th week after sowing (WAS) at Mimetala in 2015, all the plants of this variety were already affected by the disease. That incidence of 100% remained definitively constant in the control. In Nkometou, the incidence of the disease for the same variety reached about 80.0% in the 12th WAS. In 2016 at the same period, that variety presented an incidence of 97.5 and 75% respectively at Mimetala and Nkometou in the control. Between the 4th and 6thWAS, the plants, although receiving treatments, saw the incidence of the disease slightly increase in all treatments. However, from the 6th WAS and irrespective of the varieties and sites, the incidence of the disease decreased over the time depending on the different concentrations of extracts tested. The variety lowest effected were TGX 1910-14E at Nkometou with the values of45% in the control and 5% in the T3 and T4 in 2015 at the 12th WAS. In 2016, at the same period, those values were 47.5 % and 5 % in the same treatments (Fig.4). The statistical analysis showed that there was a significant difference between all the treatments compared to the control and that at the 12th WAS there was no significant difference between the T3 treatments (100 g / l extract) and T4 (Systemic fungicide).



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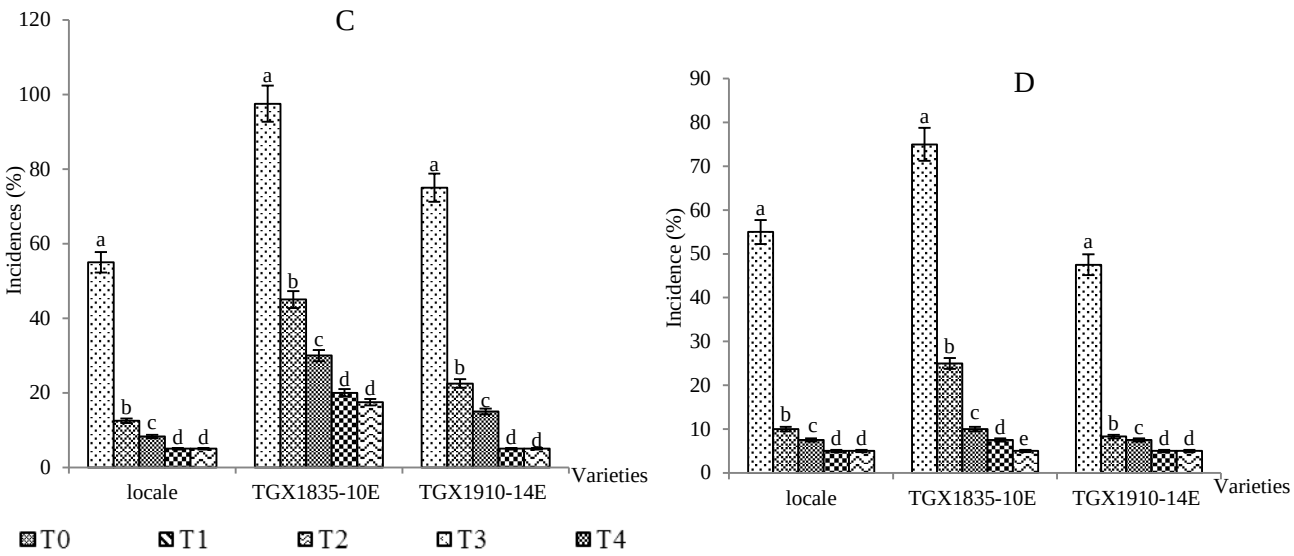
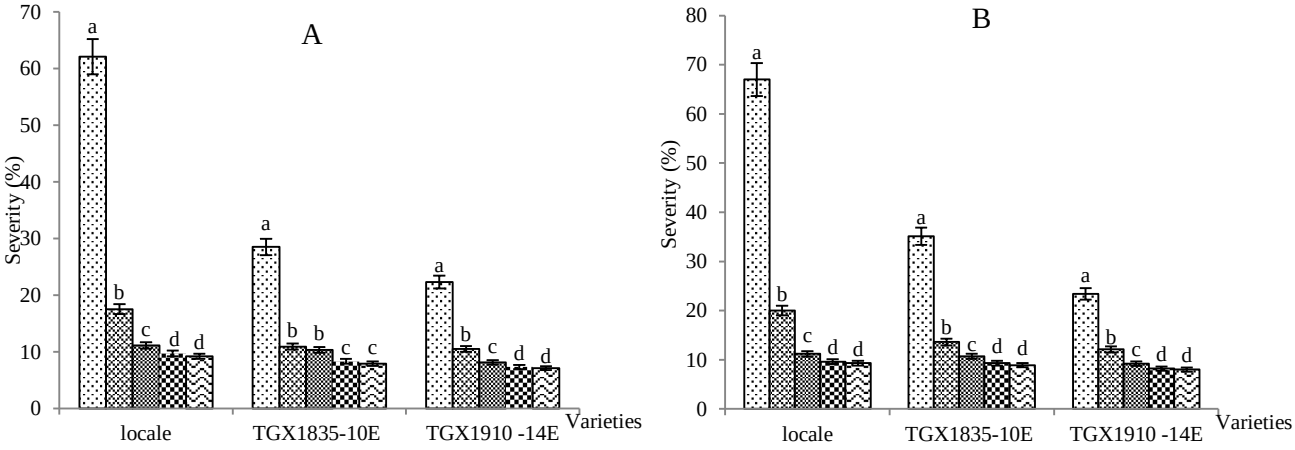



*Fig.4. Variation of the incidence 12 WAS in the differents treatments during the two campaings in the sites: A and B (Mimetala and Nkometou in 2015; C and D Mimetala and Nkometou in 2016).*

**3.1.2.2. Evolution of the severity**

The variation of the severity was practically the same at Nkometou as at Mimetala in 2015 just as in 2016.Indeed,the severity of the disease varied very little between treatments until the 6th week after sowing independently of the varieties and the sites. From the 8th SAS, the severity decreased with the different concentrations of extract tested. The disease was more severe at Nkometou than Mimetala on the local variety in both campaigns. In 2015 at Mimetala, that variety had a severity of approximately 62.1 % in the $T_0$ treatment (control) and 9.7 and 9.2 % respectively in the $T_3$ and$T_4$ treatments at the 12th SAS. At Nkometou, the local variety had a severity of 65 % in the $T_0$ treatment. At the same period in 2016 at Mimetala, the severity was 67 % in the treatment $T_0$, then 9.6 and 9.3 %in the $T_3$ and$T_4$ treatments. At Nkometou, the severity was 77 % in the treatment $T_0$ (control), then 10.1 and 9.7 % in the $T_3$ and$T_4$ treatments. This disease was less severe on the TGX 1910-14E variety with a severity of, and 22.3 % in the control and 7.3 and 7.1 % respectively in the $T_3$ and$T_4$ treatments at Mimetala in 2016. At Nkometou the severity was 23.4 % in the control and 8.2 and 8 % respectively in the $T_3$ and$T_4$. At the same period in 2016, the severity of that variety at Mimetala was 50% in $T_0$ and 7.9% and 7.7% respectively in the T3 and T4 treatments. At Nkometou it was 52.5 % in $T_0$ and 8.3 % and 8 % respectively in the T3 and T4 treatments.

The statistical analysis showed that there was a significant difference between all the treatments compared to the control and that at the 12th WAS, regardless of the varieties, there was no significant difference between the T3 and T4 treatments (Fig.5).



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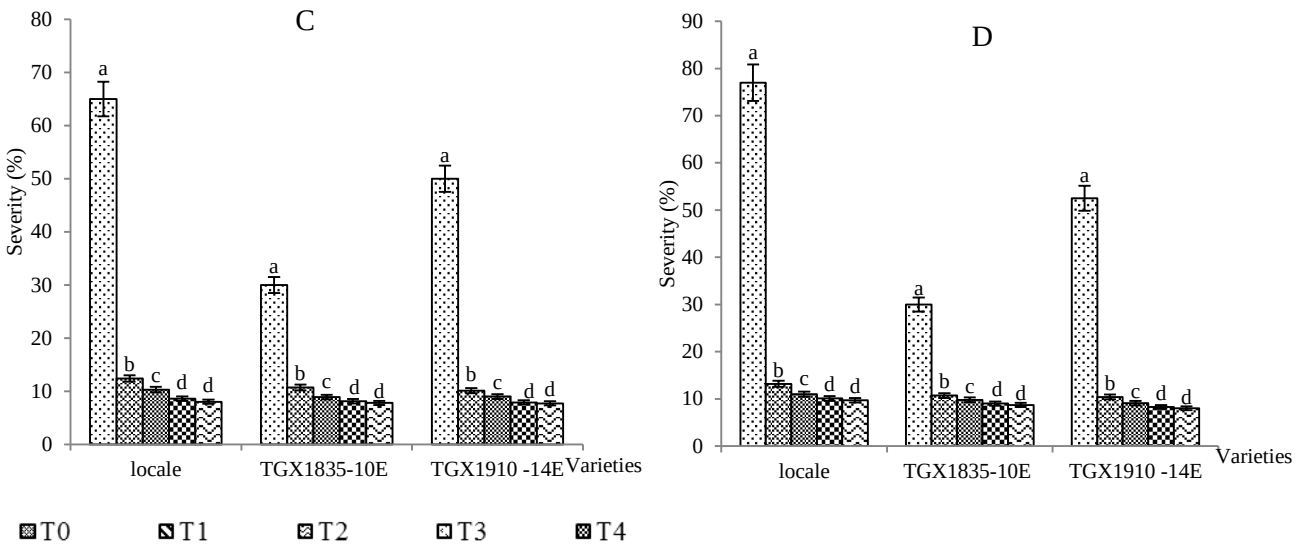
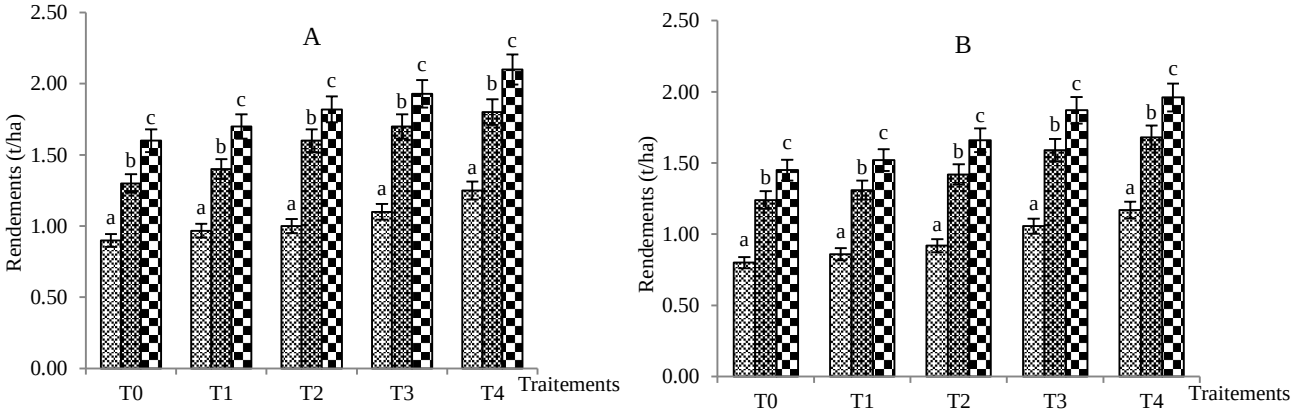



*Fig.4. Variation of the severitiy 12 WAS in the differents treatments during the two campaings in the sites: A and B (Mimetala and Nkometou in 2015; C and D Mimetala and Nkometou in 2016).*

**3.1.2. Effect of Aqueous extract of Neem seeds on the grain yield**

Overall, grain yield was lower at Nkometou than at Mimetala during both campaigns. Independently of the sites, the $T_0$ treatment of all varieties presented the lowest yield certainly induced by the disease. For all varieties and at all sites, yield was proportional to the different treatments. During growth, the local variety had virtually the same behavior as TGX 1910-14E. However, the lowest grain yield was obtained on the local variety with an average of 0.90; 0.97; 1.0; 1.1 and 1.25 t/ha respectively in T0, T1, T2, T3 and T4 treatments at Mimetala; and 0.80; 0.86; 0.92; 1.06 and 1.17 t/ha respectively in the same treatments at Nkometou in 2015. In 2016, that yield was 0.88; 0.95; 0.95; 1.15 and 1.29 t/ha respectively in T0, T1, T2, T3 and T4 treatments at Mimetala; and 0.84; 0.86; 0.92; 1.1 and 1.14 t/ha respectively in the same treatments at Nkometou. The highest yield was obtained on the TGX 1910-14E variety with an average of 1.6; 1.7; 1.8; 1.93 and 2.05 t/ha respectively in T0, T1, T2, T3 and T4 treatments at Mimetala; and 1.45; 1.52; 1.66; 1.87 and 1.96 t/ha respectively in the same treatments at Nkometou in 2015. The TGX 1910-14E variety yield in 2016 was 1.55; 1.6; 1.8; 1.89 and 2.0 t/ha respectively in T0, T1, T2, T3 and T4 treatments at Mimetala; And 1.40; 1.50; 1.6; 1.85 and 1.93 t/ha respectively in the same treatments at Nkometou. Statistical analysis showed that there was a significant difference between all varieties and even between all treatments compared to control $T_0$ for all varieties. However, there is no significant difference between treatments $T_3$ and $T_4$ (Fig.5).



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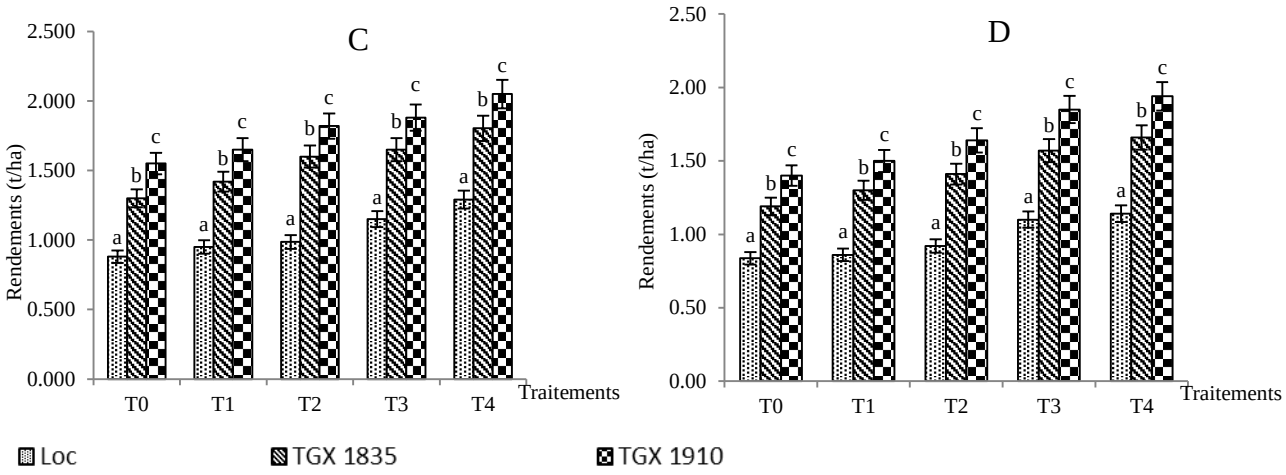


*Fig.5. Variation of the grain yield in the differents treatments during the two campaings: A and B Mimetala and Nkometou in 2015; C and D Mimetala and Nkometou in 2016).*

**3.2. Discussion**

Results showed that treatments with aqueous extract of neem seeds positively influenced the height of soybean. The plots treated with aqueous extracts of neem seeds showed larger height compared to the control. These might account for the presence in the extracts of saponins and polyphenols, which are active compounds stimulating the growth and yield of plants, as demonstrated by Andresen and Cedergreen in 2010. Similar results were observed by Okunlola & Ofuya in 2013 who showed the growth stimulator effect of *Azadirachta indica* and *Piper guineense* on the growth of jute alone and in polyculture. Likewise, Nahakand Sahu in 2014 showed thataqueous extract of neem leaves stimulated significantlythe height growth of eggplant.Moreover, the treatments do not influence significantly the average number of leaves. The variation of the number of leaves was proportional to the different doses of extract tested. These results are similar to those obtained by Sahu *et al*., in 2012 who demonstrated a significant increase of the number of leaves of tomato (*Lycopersicum esculentum*) under the influence of many medicinal plant extracts.

The results of this work showed the positive effect in the threshold of probability of 5 %, of the aqueous extract of neem seeds in the reduction of Asian soybean rust in the field. The disease parameters were reduced on the treated plots compared to the control plots to which disease was highly marked. The incidence and the severity of the soybean rust were significantly curtailed on the treated plots compared to the control plots crammed with this disease. Devaraj *&al*., in 2013 obtained a similar efficacy of neem extracts and the chemical fungicide on the development of Asian soybean rust caused by *Phakopsora pachyrhizi*. Likewise, Pattnaik *et al*., in 2012 obtained similar results when evaluating the effectiveness of aqueous extracts of the *azadirachta indica* leaves while controlling foliar spots of *Lycopersicum esculentum*. In addition, there was no significant difference between $T_3$ (100 g / L of AENS) and $T_4$ (chemical fungicide). Thus, at high doses, AENS may be as effective as the systemic fungicide.

The soybean grains yield increased proportionally to different doses of extracts tested compared to the absolute control. It suggests that the neem extracts stimulate the biomass and the soybean yield. Okunlola and Ofuyaobtained similar results when evaluating the effect of *Azadirachta indica* and *Piper guineensis* on the growth and the jute yield alone and in polyculture.

## IV. CONCLUSION

This study is the evaluation of the antifungal potential of aqueous extracts from neem seeds on the development of Asian soybean rust in the field. It revealed that these extracts have active compounds capable of stimulating the growth and yield. At the high doses, this extract significantly reduced the disease parameters as the systemic fungicide. Besides, at the dose of 100 g/L of aqueous extract of neem seeds, the yield was practically similar to that obtained with the systemic fungicide. This extract could be used as an alternative to synthetic fungicides and thus ensure a long-lasting and sustainable agriculture.

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# Women in Rural Development: An Appraisal of Yam Chips Processors in Saki Area Oyo State, South West Nigeria

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***Abstract—*** *An investigation was carried out to appraise the processing of yam chips in Saki Area of Oyo State, South West, Nigeria. Purposive stratified random samplings of 150 respondents in 8 processing centres using structured questionnaire were adopted. Descriptive statistics (frequency and percentages) were used to analyse the socio-economic variables while chi-square was used to capture the relationship that exists between socio-economic factors and output, and also between year of experience and output. However, the study revealed that 95% of the processors were females with age ranging between 20-60 years. Majority of the processors (76%) were married, while 43% had junior and secondary school education. Eighty-five percent of the processors were Muslims. Using chi-square, all the tested socio-economic variables had no statistical effects on output and socio – economic characteristics of the processors. The study also revealed factors militating against large scale production of yam chips in this area of study. Recommendations such as provision of loan and credit facilities, provision of modern equipment and social amenities were suggested in order to increase production thus assisting in alleviating poverty in this area.*

***Keywords— Yam chips, Processors, Poverty, Rural Development, Dioscorea spp.***

## I. INTRODUCTION

Yam (*Dioscorea spp*) is an important source of carbohydrate for many people of the sub-Saharan region especially in the yam zone of West Africa (Akissoe et al, 2003). Yam belongs to the genus *Dioscorearotundata, D. cayensis, D. alata, D. dumetorum* and *D. esculenta* (Coursey, 1967). The tuber is economically the most important part of the plant. Yam is an impotant food crop especially in the yam zones of West –Africa comprising Cameroon, Nigeria, Benin, Togo, Ghana and Cote D 'Ivoire .This zone produces more than 90% of the total production which is estimated at about 20-25 million tons per year (Sanusi and Salimonu, 2006).Nigeria is the main producer of yam in the word with about 71% of the word output followed by Ghana,Cote d' Ivoire, Benin and Togo (FAO,2002). Yam production in Nigeria has tripled over the past 40 years from 6.7 million tonnes per annum in 1961 to 27 million tonnes per annum in 2001 (FAO, 1999) and 35.017 million tonnes per annum in 2008 (FAO, 2010). These figures account for 68.0% of the world population in 2008 thus making Nigeria the largest producer in the world (FAO, 2010).

Yam production was indigenous to the forest areas of the country (Coursey and Coursey, 1971; Hahn et al, 1987) but in recent times, yam production has shifted to Guinea and even Sudan Savanna zones due to shortage of arable land in the forest areas under increasing population pressure (Manyong and Oyewole, 1997). Yam has some inherent characteristics which make it attractive and it is rich in carbohydrate especially starch and moreover has multiplicity of end use (FAO, 1987). Yam could be eaten as boiled yam or fried in oil. It can also be processed into yam chips. Moreover, yam is also a source of industrial starch, although the quality of starch of some species is said to be comparable to cereal starchy (Osisiogu and Uzo, 1973).The major constraint is that yam is a perishable food item. The tubers cannot be store for more than a few weeks after harvesting. Moulds and bacterial have been implicated in the deterioration of stored yam. A loss of 10-15% in the first three months and losses approaching 50% after six months storage have been reported by Coursey (1967) and Asiedu (1989). However, the processing of yam tubers into staple non-perishable and easily transportable chips offers an alternative storage in fresh form. The stages of yam chips processing involves the peeling of the epidermis of the tubers, slicing the tuber to thickness of about 1cm, parboiling the yam slices (for 30min at $70^0$C) and finally sun drying for about 4-10 days to reduce the moisture content to about 10%. The parboiling of the slices softens the tissue considerable and gives a more palatable production. However, Ezeh 1992

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reported that it is not in all cases that the tubers are parboiled especially in Nigeria.

The microbiological quality of chips is closely related to the rate of drying. Processing occurs within the periods of harmattan, with a very dry wind coming from Sahara during the dry season (Akissoe et al 2006). Yam chips are stabilised with a moisture of about 10 to 13% as against 60-75% in fresh tubers and can be kept for up to a year when stored in water insect proof condition. Yam chips are competitive with respect to other starchy products. The chips are mainly eaten in paste form prepared from the flour obtained by grinding them (Vierner et al, 2010). The flour can be turned into granules or mixed with biscuits as weaning food for babies.

In Nigeria, women play a vital role in providing food and nutrition for their family through their roles as food producers, processors, traders and income earners (Siyanbola & Elegbede, 2008). Although, still using traditional methods which are tedious and often inefficient. The involvement of women in food processing and storage has assisted a lot to reduce spoilage and wastage which usually lead to a reduction in national income and nutrition standards and substantial reduction in the ratio of food supply (Olatoye, 1989). Production of yam chips may act as a catalyst to food production with the rapid pace of urbanization in Nigeria. This can be through the development of sustainable intermediation system (marketing, transportation and processing between urban and rural areas .These systems should be able to guarantee outlets for farmers ,thereby encouraging them to increase production and at the same time available on a permanent basis products that are adapted to the eating habits and budget of urban consumers . The tradition processing method has become popular over the years in the South –Western part of Nigeria.

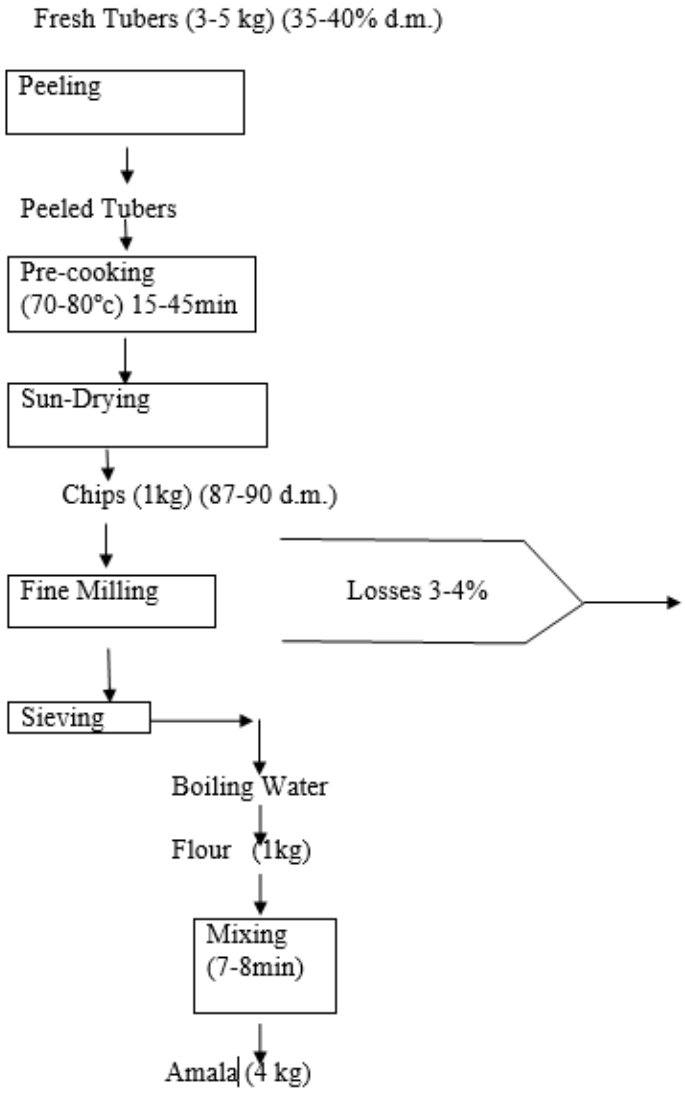


*Fig.2: The processing of yam tubers into chips,flour and "Amala "*

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**Objectives of the Study**

Specific objectives of this study are:

i. To describe the socio-economic characteristics of the yam chips processors in Saki Area of Oyo State, Nigeria.
ii. To appraise the level of yam chips production in Saki Area of Oyo State, Nigeria.
iii. To identify reasons for women involvement in chips processing.
iv. To identify constraints faced by yam chips processors in this area.
v. To examine the relationship between the socio-economic factors and output.
vi. To determine the occupational hazards of yam chips production.
vii. To make recommendations.

## II. METHODOLOGY

**Sampling Procedure and Data Collection**

This study was conducted at eight processing centers at Saki, Area of Oyo State, South- West Nigeria. ( Fig 2.) A trial survey was conducted from October 2009 to February 2010 while the final survey was carried out from October 2010 to February 2011. Saki is situated on Longitude 03º 4E and Latitude 8º75N. It is about 180km North West Ibadan. About 80% of the people of this area are predominantly farmers planting mainly tuber crops such as yams, cassava and cereals. The climate of the study area is tropical climate with a moderate annual rainfall .The raining season lasts for 8-9 months with a short dry season of 3-4 months .The mean annual temperature is about 80For 27C.

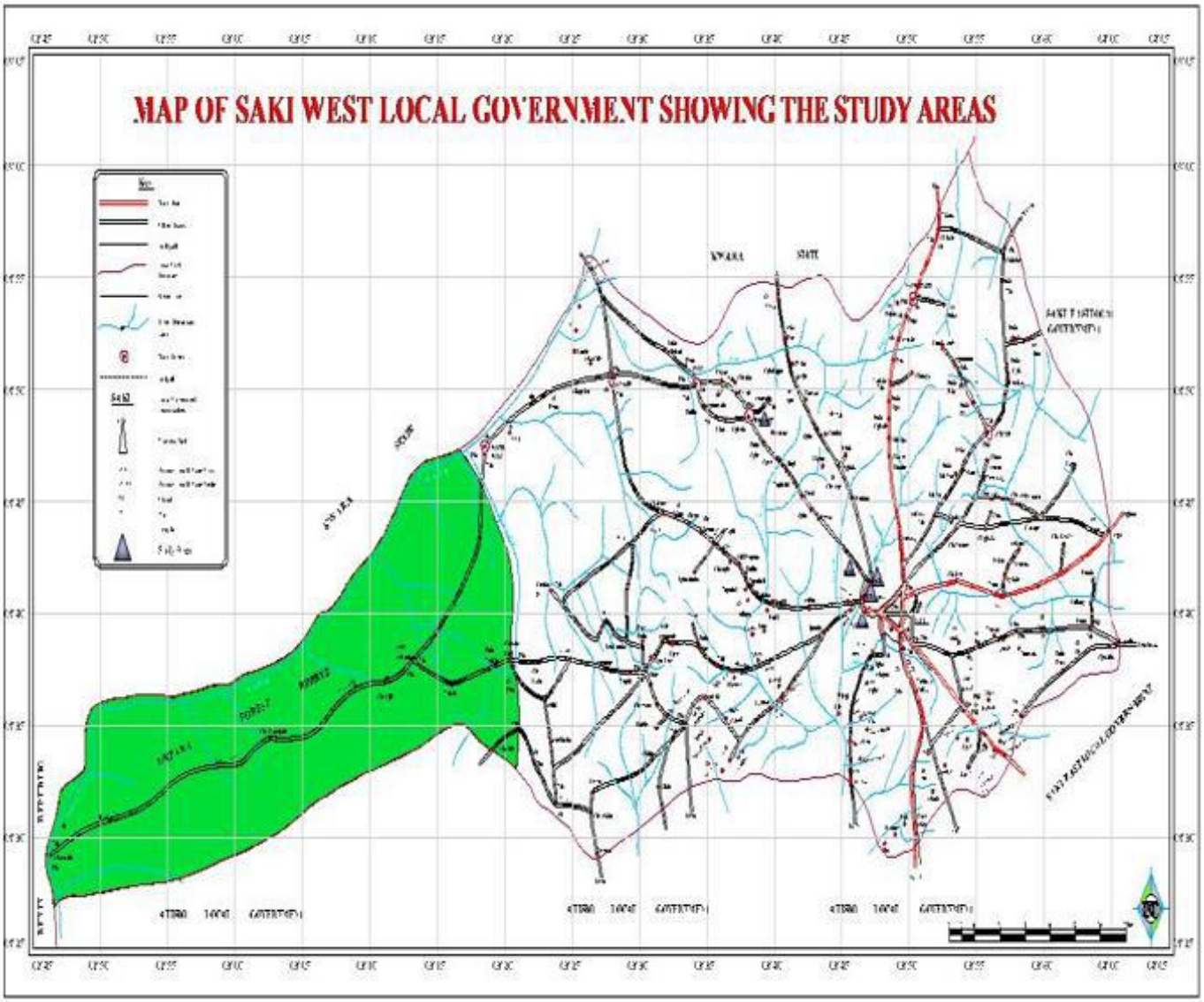


*Fig.2*

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The area experiences North-East trade wind which brings harmattan between November to March being nearer to the Northern belt of the country. There is usually a drop in relative humidity during the period of harmattan. Primary and secondary data were used in the study. Primary data were generated through a set of well structured questionnaire administered on the processors at the eight processing centres. Purposive sampling approach was adopted to collect the data. One hundred and fifty (150) yam chips processors out of an estimated 165 processors were interviewed.

Data collected were age, education religion, occupation, gender, marital status and year of processing experience. Others include processing time, equipment quality and storage observations were also made about processing constraints, possible by products and environmental pollution. Secondary data were sourced from literature and relevant work in this area. Descriptive statistics tools (frequency distribution and percentage) were used to analyse the data collected from the study. Chi-square was used to examine the relationship between socio-economic characteristics of the processors and output.

## III. RESULTS

*Table.1: Distribution of Respondents according to Socio-economic Information*

| **Age** | **Frequency** | **Percentage** |
|---|---|---|
| 20-29 | 35 | 23.33 |
| 30-39 | 40 | 26.67 |
| 40-49 | 46 | 30.67 |
| 50-59 | 20 | 13.33 |
| 59-60 | 09 | 6.00 |
| **Total** | **150** | **100.00** |
| **Sex** | | |
| Male | 08 | 5.33 |
| Female | 142 | 94.67 |
| **Total** | **150** | **100.00** |
| **Marital Status** | | |
| Single | 30 | 20.00 |
| Married | 112 | 75.67 |
| Divorced | 08 | 5.33 |
| **Total** | **150** | **100.00** |
| **Religion** | | |
| Christianity | 15 | 10.00 |
| Islamic | 127 | 84.67 |
| Traditionalist | 08 | 5.33 |
| **Total** | **150** | **100.00** |
| **Educational Level** | | |
| No formal education | 38 | 25.33 |
| Primary Six Leaving Cert. | 45 | 30.00 |
| Junior and Secondary School Education | 64 | 42.66 |
| Tertiary Education (Polytechnic, College of Education and University) | 03 | 2.00 |
| **Total** | **150** | **100.00** |
| **Labour Man/ Days** | | |
| Family | 50 | 33.3 |
| Hired | 100 | 66.7 |
| **Total** | **150** | **100** |
| **Year of Experience** | | |

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| | | |
|---|---|---|
| 1-5 | 45 | 30.0 |
| 6-10 | 60 | 40.0 |
| 11-15 | 28 | 18.67 |
| 16-20 | 12 | 08.0 |
| 21-25 | 5 | 3.33 |
| **Total** | **150** | **100.00** |
| **Output in Bags** | | |
| Below 10 bags | 02 | 1.33 |
| 10-20 bags | 15 | 10.0 |
| 20-30 bags | 28 | 18.67 |
| 30-40 bags | 32 | 21.33 |
| 40-50 bags | 48 | 32.00 |
| Above 50 bags | 25 | 16.67 |
| **Total** | **150** | **100.00** |

**Source: Field survey 2011**

*Table.2: Relationship between Socio-economic Characteristics and Output of the Processors*

| | **Value of level of Significance** | | | |
|---|---|---|---|---|
| Output and Age | 0.534 | 2 | 0.034 | NS |
| Output and Marital Status | 0.098 | 2 | 0.20 | NS |
| Output and Educational Background | 2.897 | 4 | 0.010 | NS |
| Output and Religion | 1.543 | 2 | 0.010 | NS |

*Table.3: Distribution of Respondents according to Production Output*

| **Variable** | **Frequency** | **Percentage** |
|---|---|---|
| High (Above 50) | 25 | 16.67 |
| Medium (31-50) | 80 | 53.00 |
| Low (Below 30) | 45 | 29.00 |

## IV. DISCUSSION

**Socio-economic Factors of the Processors**

**a. Sex of the Processors:** Ninety-fivepercent of the processors were females while the remaining five percent are males who were mostly the children of the processors who do assist in the peeling of the yams and fetching for process. Thus finding supports the earlier findings reported by Sanni (1991), Olatoye (1989), NEST (1991), Siyanbola and Elegbede (2010).

**b. Age of the Processors:** The age of the processors fall within 20 -60 years, however, majority of them fall between 20-49 years which constitute 87% of the total respondents. This implies that most of the respondents are still in their active age. The respondents are at their economically active age and are still in child bearing age. Age is one of the factors affecting decisions and actions made in agriculture, because people thought, behaviours and needs are primarily related to their ages.(Simsek and Karkacur,1996) .

**c. Marital Status:** Majority of the processors were married (seventy -six percent ) which is an indication that most of the respondents have another responsibility outside their income earning occupation such as domestic work and other manual responsibility in line with the observation made by FAO,2009.

**d. Education Background:** Studies have revealed that education influences the adoption of practices in modern Agriculture (Obinne, 1991) The reason been that educated person is more likely to adopt modern practices easily, better innovation s and hence could be a better producer**.** It is expected that the years of education will contribute significantly to decision making of a farmer. The findings support Obinne 1991; Alabi and Aruna 2006 and Ndahitisa 2008 that years of education determiners the quality of skills of farmers, their allocative abilities and how well informed they are to the innovators and technologies around them. Also support the result of Oladipo and Adekunle 2010 that individual s with higher educational attainment are usually being faster adopters of innovation.

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This survey revealed that seventy-five percent of the processors are educated. However, the level of education varied from primary to secondary and tertiary institutions. The percentage of those with no formal education is about 25%. This shows that majority of the processors were educated. This can be accounted for through the free education of Obafemi Awolowo in the Western region coupled with renewed interest of Nigerians in Western education through various awareness campaigns. The high percentage of literates among the processors implies that the processors can easily adopt new techniques of yam chips processing when introduced.

e. **Labour**: Labour is expressed as adult male man - day and it is the summation of family labour and hired labour. Family and hired labour play an important role in agricultural production especially in developing economics where capital is less significant (Meire, 1989). The result showed that majority (63%) of the processors in the study area use more of family labour so as to maximize profit. The implication of this study is that processors will be spending less than hired labour.

f. **Year of Experience:** The highest year of experience was 30 years and the least 2 years.

This implies that more women were involved in the processing in the last 10 years. This may be due to the high unemployment rate in the country presently experience in the country with over 55% of Nigerians of working age unemployed (Financial Standard, June 2009) representing one in five adults.

The knowledge, skill and practices acquired over a certain period that is accrued to farmers in practice. The result majorly falls between 6 and10 years which agrees with the work of Oluwatayo et al 2008 that farmers with more experience would be more efficient, have better knowledge of climatic conditions and market situation and are thus expected to run a more efficient and profitable enterprise.

He also supports findings of Onyebinamci, 2004 that previous experience in farm business management enables farmers to set realistic time and cost targets, allocate, combine and utilize resources efficiently and identify production skills.

g. **Medium of Training of Processors:** Most of the processors had no formal training in yam chips processing. Majority (98%) as indicated during the survey acquire the skill from friends and relatives. Nobody indicated in the questionnaire that they receive any training through media or Agricultural Extension Agents.

h. **Processing Time, Techniques and Packaging:** The period of processing ranges from 1 - 10 days depending on the availability of labour (usually children of processors and hired labours) and intensity of sun. The processing period is shorter (3-4 days) during the peak of harmattan (December to January). This is the peak of yam chips production in this region. The peeled yams are left on the flat rocks or bare ground at the processing sites. Majority of the processors use drums for pre-cooking (85%) while only few use aluminum pots (10%) for economic reason .The use of crude implements is because they lack collaterals to obtain loan since they generate capital for the yam chips production through informal sources (personal savings ,daily contributions and loan from friends and husband).

At the time of the survey, the cost of a drum was #2000.00 while the largest iron pot cost #6000.00. The locally made clay pot costs #800.00. However, only 5% processors indicted that they make use of clay pots which is the cheapest during the survey but break easily and can only take small amount of chips compared to drum and aluminum pots. Some of the processors indicated that they use rust prone drums for pre-cooking and the use of unchanged water throughout the period of processing will obviously contaminate the chips but attendant health risk was not be assessed.

Eighty percent of the processors affirmed that yam tubers do not store well in fresh form and that transportation is costly due to their bulkiness hence processing into chips as reported by Okereke and Nwosu (1981). Dried yam chips are packed into bags of different sizes and kept in store for between 6-7 months. The weight of the bags ranges between 130kg – 150kg. (Average weight is 140kg).

i. **Output:** The production rate is low (majority produce between 31-50 bags) (53%) during the processing period. This low production level of the women can be attributed to their marital status because they have other domestic responsibility therefore sharing their time between such responsibilities.

j. **Useful by Products and Environmental Pollution:** The peels from the yam tubers can be dried and use as feed to livestock. It can also be incorporated into feeds of poultry as a source of carbohydrate as reported by Siyanbola and Amao (2011). The peels form a heap of refuse around the processing centres but do not constitute any environmental pollution.

k. **Occupational Hazards:** The 3 common diseases that affect the processors as indicated by the processors are malaria, catarrh and cough. This may be due to their exposure to mosquitoes and cold at the processing sites.

l. **Processing Constraints:** The constraints highlighted

by the processors are:

- Lack of capital to purchase yam tubers and purchase of stainless drums. They usually generated capital through informal sources such as personal savings, contribution and borrowing from friends.
- Inability to obtain loan from banks because of lack of collaterals especially landed properties.
- Security problem.
- Lack of shelter at the processing site.
- Lack of health facilities for processors and their children.
- Scarcity of labour.
- Lack of water needed for washing and parboiling of peeled yams since the processing period usually fall within the dry season.
- No incentives from government in form of agric loan, tools etc.
- Lack of adequate storage facilities and extension services.

The yam chips processors are women which supports the earlier findings that women are processors of farm produce (Sanni, 1991). The age of the processors falls within 20-60 yrs. This is an indication that most of the processors are within the productive age. The mean number of years of schooling was about 14 years while the highest year of experience was 25years. This implies that most of the processors are literate and with years of experiences which influences decision making in relation to risk aversion.(Asumugha et al (2009). The period of processing observed is from 1-10 days depending on availability of labour and time of processing. During the peak of harmattan period (December - March), it takes a shorter period for the chips to get dry for storage. The use of rust prone drums for the cooking will obviously contaminate the chips but the attendant health risk was not assessed. Majority of the processors (80%) affirmed the yam tubers do not store well in fresh form and that transportation is costly due to their bulkiness. This correlates with reported findings by Charke (1987;Martins (1972) and Ekechhukwe et al (1987).The processors had earlier identified some constraints affecting yam chips processing to be bad road to the processing centers, drudgery of processing , lack of capital for expansion of their activities and environmental conditions

**Conclusions and Recommendations.**

Yam chips processing in the study area are neither subsistence traditional nor village level but it is a well-developed commercial enterprise. Majority of the processors are women with formal education. Yam chips production is relatively simple and calls for no major investment and moreover provides an effective means for producers to boost the value of the crop. The processing of yam tubers into chips will assist in stabilizing market prices thus reducing loss of tubers and provides farmers a safe return with the opportunity to grow more tubers. Women groups should be encouraged and strengthened through the provision of credit facilities extension services, agricultural inputs, processing ,storage and marketing services that will assist to increase production. The entire production and marketing chain offer vast employment opportunities. It also offers prospects for income generation due to the numbers of people involved and the value attached to it. Moreover, the role of women in food production processing and marketing has become more relevant as a way of fighting poverty and ensuring food security.

However, in attaining the Millennium Development Goals (MDGs) of helping those living in poverty and eliminating hunger by 2015 there need for the government to assist the processors by;

- Public enlightenment to educate the processors on ways of improving the quality of their products.
- Provision of stainless drums at subsidized rates.
- Provision of health facilities at the processing centers.
- Giving interest free loan to processors.
- Construction of model processing centers.
- Construction of roads to the processing centers.
- Processors to have access to extension services in order to improve their knowledge of farm management and the need to form cooperative societies.
- Provision of storage facilities.

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# Study of the behavior of cultivars from a world collection of olive (*Olea* spp.) in Morocco

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***Abstract—*** *In Marrakech (Morocco), a world collection of the genetic resources of the olive-tree was established. This collection currently contains 600 cultivars of various origins. However, work of characterization of these cultivars remains very limited. The objective of this study was to emphasize these genetic resources through their agronomic characterization. We studied certain agronomic characters on some of the most productive cultivars during the 4 years of production 2007-2008-2009 and 2010. Fifteen cultivars originating in various countries that show a cumulated average production higher than 20Kg were then selected for studies of behavior namely: strength, floral biology and the content of oil. The study of vigor of all cultivars showed that Alameno de Marchene, Haouzia, Manzanilla de Sevilla, and Sevillano de Jumilla are most vigorous whereas the cultivars Acebuchera and Blanqueta are the least vigorous and could be used for a system of high density. Analysis of floral phenology of the 15 cultivars revealed overlapping between the majorities of them. This result will contribute to the determination of the adequate pollination for each cultivar. The analysis of the index of compatibility showed that four cultivars (Azeitoneira, Koroneiki, Amargoso and Acebuchera) are self-fertilizing. This study proposes a base of knowledge for the valorization of the Moroccan genetic resources of olive.*

***Keywords— Olea europaea spp. vigor, floral biology, pollinic compatibility.***

## I. INTRODUCTION

The olive-tree is one of the most important fruit in the Mediterranean basin (Zohary and Spiegel-Roy, 1975), the domectication and the diversification of the Mediterranean olive tree was verified by (Besnard et al., 2013). The world production was estimated in 2012 at 3.408.500 tons for the olive oil and 2.526.000 tons of olives of table (COI, 2013). In Morocco, the olive tree it's characterized with agronomic and adaptive characteristics likely higher than those of other traditional varieties (use of fruit and oil, vigor, phenotypic plasticity...). The result of clonal selection in INRA, two varieties have been registered in the official catalog (Haouzia and Menara) and are currently massively propagated within the program "Green Morocco" (Khadari et al., 2016). In a parallel to the dominance of the variety Picholine Moroccan, an important diversity of genotypes cultivated locally was potentially recoverable (Khadari et al., 2008; El Bakkali et al., 2013). Until now, the INRA Morocco has not a representative collection of the local genetic diversity of all the traditional agrosystems (Khadari et al., 2008). The only ones of olive trees stemming from prospecting in the regions of Marrakech, Meknes and Taounate are in collection in the INRA (centers of Marrakesh and Meknes) and represent only a part of the existing diversity (Khadari et al., 2008 ; El Bakkali, 2013). In the Word, in spite of high initial varietal diversity, a recent tendency towards the establishment of the modern orchards based on the majority of the productive cultivars leads to the high erosion of this genetic material. Several collections of genetic material of olive-tree were create at the national and regional level to control the genetic resources of olive-tree ex-situ; to required, preserve and use them in certain programs of improvement (El Bakkali et al., 2013; Bartolini, 2008). The first attempt to preserve and characterize the most important cultivars of all the olive-growing countries led to the creation of the World Bank of germoplasm of olive-tree in Cordoue, Spain (OWGB Cordoba). More recently, a second international bank of cultivars of olive-tree was installed with the experimental collection of Tessaoute (Marrakech) in 2003. The world collection of Tassaout was an important genetic reserve of the resources of the olive-tree in Morocco. The characterization of these resources constitutes a paramount study in any program of improvement and conservation. Recently, International Olive Oil Council (IOC; Caballero et al., 2006) proposed a system of classification to standardize the descriptors of olive-tree in World (Ganino et al., 2006). The list constitute an indentity card containing of the data passport of the cultivar (origin, zone of origin and distribution), of each the morphological characters, and the agronomic and gustatory criteria. The objectif of this present study was the characterization and

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the conservation of the genetic resources of olive-tree present in world collection of olive tree in Tassaout, Morocco, in order to have a data bank on the most powerful cultivars in the objective to use them in a program of genetic improvement.

## II. MATERIAL AND METHODS

15 cultivars were studied among the 600 cultivars present in the world collection of the olive-tree; these cultivars were selected on the basis of their production cumulated during the first 4 years of production (Table 1). Each cultivar was represented by four trees of olive the moste productive. Those are laid out with a spacing of 7m X 4m, a density of plantation of 357 feet per hectare. The olive tree was irrigated.

Evaluation of vigor

The vigor was studied on the four trees of each selected cultivar. It was expressed by the following parameters:

- Section of the trunk (ST) in $cm^2$ ST= P2/4π

P= diameter of trunk

- Volume of foliation (VF) in $m^3$ VF= 2/3 π r2 H= 2/3 π D2/4H= 0.5236*(D)2*H D= average diameter = (D1+D2)/2

r= a for circular orbits

H= tree height

- Productive Area (SF) in $m^2$ SF= 2 π r H= π. D.H

D= average diameter = (D1+D2)/2

r= a for circular orbits

H= tree height

Phenological stages

Floral phenology consists in studying the stage of the race of the phenomenon of flowering (beginning flowering, full flowering and fine flowering), according to the Colbrant (1974).

Floral biology

The following parameters were mesured: the floral phenology, rate of flowering, morphology of the floral bunch, rate of fruit set and fruiting rate.

In the second time, we calculated the class of self compatibility which is the ratio of the number of fruits per inflorescence in self pollination by the number of fruits obtained in free pollination (Zapata and Arroyo, 1978). The content of oil of each cultivar was estimated by the method NMR (Nuclear Magnetic Resonance at base Resolution).

The analysis of the variance was carried out by software SPSS ® version 20.0. Before the analysis, the results which are in the form of percentage were standardized by the angular transformation using the following formula Y= 2 x Arcsin$\sqrt{x/n}$ is defined as the inverse sine function of x when -1≤x≤1; where x and n are used to determine the rates

## III. RESULT AND DISCUSSIONS

Tree vigor

The parameters of vigor were calculated for each cultivar, the results are presented in table 2. The average section of the trunk varies from 38 to 110 $cm^2$ with a median value of 80 $cm^2$ (table 2). The analysis of the variance shows that the section of the trunk of the trees varies very highly significant between the studied cultivars (P< 0,001). The multiple comparisons of the averages of the section of the trunk according to the method of Newman-Keuls classify these 15 cultivars in 4 homogeneous groups. It is noticed that the group having the section of the weakest trunk was formed by the cultivar Sebatera with 38 $cm^2$, whereas the cultivar Alameno de Montilla it represents with only the group having the higher section with 110 $cm^2$ (table 2).

The height of the trees varies between 2 m and 3,7 m with a median value of 3 m (table 2). The analysis of the variance showed that this character fluctuates very highly significant between these cultivars (P< 0,001). The Newman-Keuls test gathers these 15 cultivars in seven homogeneous groups. The cultivar espangole Alameno de Montilla was differentiate by the highest value (3,7 m) followed by the cultivar Haouzia also presents high value, this result indicates this national cultivar was strongly vigorous and confirms the result obtained by other work (Boulouha, 2006; Hadiddou et al., 2013).

The area of foliation varies from 6 to 19 $m^2$ with a median value of 15 $m^2$. The analysis of the variance showed the existence of a difference very highly significant between the cultivars for this character (P< 0,001). The test of Newman-Keuls made it possible to distinguish 7 overlapping homogeneous groups. The cultivar Alameno de Montilla has larger area of foliation (19 $m^2$) and does not differ significantly with the cultivars Galega vulgar, Sevillano de Jumilla and Manzanilla of sevilla which present a value of 17 $m^2$. The cultivar Blanqueta has a weak area of foliation (6 $m^2$).

The volume of foliation of the 17 cultivars varies from 2 to 9 $m^3$ with an average volume of 5 $m^3$ (table 1). The analysis of the variance revealed a highly significant difference between the cultivars tested (P<0,001). The Newman-Keuls test revealed two homogeneous groups including three. The group of weak foliation was formed by the majority of them. The highest values of foliation are noted at the cultivars Manzanilla de Sevilla and Haouzia with 9 $m^3$ (table 2).

The results of the vigor of these 15 cultivars under the conditions of the area of Haouz, release a great variability between the cultivars tested for the four parameters studied, with significant area of foliation, the section of the trunk, the total height and the volume of foliation of the tree. This variation observed at the seventh year of

plantation was allotted mainly to the effect of the cultivar. Indeed, work of Del Rio et al. (2006) confirmed the cultivar has responsibility for the total variation observed for the volume of foliation, the productive area and the section of the trunk of the trees with 79%, 82% and 73% respectively. The pedoclimatic conditions apply an effect on the productive expression of the cultivars and the characteristics of the product. It is very rare to see a cultivar expressing the same productive performances in environments different from its origin. In the same way, other factors can affect the development of olive-tree since the moment of its plantation, in particular the medium and in particular the quality of the ground (Gálvez et al., 2004; Hadiddou et al., 2013) and the understock (Loussert and Brousse, 1978). These results relative to these four indices for these cultivars show that the cultivars Alameno de Marchene, Haouzia, Manzanilla de Sevilla, and Sevillano de Jumilla are most vigorous. For the cultivar Haouzia this result was already confirmed by other studies (Boulouha, 2006). However, the cultivars Manzanilla de Sevilla and Blanqueta are not very vigorous and adapt easily to the culture in intensive plantations in their countries of origins (IOC, 2007). The cultivar Koroneiki has an average vigor, which confirms the result of the study of Lavee (2002). Consequently, this cultivar was largely used in super intensive beside Arbequina and Arbosana (Vossen, 2007).

The floral biology of the 15 cultivars of olive-tree studied

Floral phenology

Figure 1 shows the chronology of phenological steps (F), (F1) and (G), at the 15 cultivars studied during the period 2010-2011. The majority of these cultivars show overlappings of the times of flowering. This makes it possible to classify these cultivars in three types:

- Cultivars with early flowering: Koroneiki, Blanqueta, Sevillano de Jumilla, Alameno de Marchene, Negrinha, Haouzia, Azeitonera, Amargoso and Changlot Real.
- Cultivars of season: Galega vulgar, Sebatera, Alameno de Montilla, Fulla of salce, and Manzanilla de Sevilla.
- Cultivar with late flowering: Acebuchera

The time of flowering was variable from one cultivar to another. Indeed, the study of the distribution of the times of flowering shows that the cultivars Koroneiki and Sevillano de Jumilla the duration of flowering was extended (30 days). As for the cultivars Sebatera, Alameno de Mentilla, Azeitoneira and Acebuchera it was shorter (14 days). The koronieki cultivar starts production early in its country of origin (IOC, 2007). The cultivar Galega vulgar start production early in its country of origin and its time of flowering was middling. But the cultivar Manzanilla de Sevilla early starts production its time of flowering is middling (IOC, 2007). The cultivar Changlot Real stare at one time of average flowering whereas the Acebuchera cultivar shows one time of tradive flowering in Spain (IOC, 2007). A comparative study of the times of flowering during two crop years at 5 cultivars (Arbequine, Picholine Languedoc, Picholine Marocaine, Manzanille and Sourani) showed the fluctuations of the dates of flowering from one year to another. This is especially related to the temperatures of Mars and April (Griggs et al., 1975; Nait Taheen, 1993). Several other authors showed that the low temperatures stimulate the formation of the inflorescences (Hartmann et Whisler 1975) whereas the high temperature inhibits their appearance (Ouksili, 1983). The nutritional conditions, in particular the nitrogen, potassium and phosphorus support flowering (Tsikalas and Parchaladis, 1980). However, their availability for the metabolism of the tree depends closely on the hydrous food.

Rate of flowering

Floral biology is an aspect important to study because the processes of floral induction, flowering and fructification are determining in the realization of the production. Although the introduced cultivars were characterized in their countries of origin, the environmental conditions of the collection can have an important influence on their behaviors. The results obtained for the 15 studied cultivars are represented in figure 2. The rate of flowering varies from 11% at the cultivar Acebuchera with 98% noted at the cultivar Sevillano de Jumilla.

The analysis of the variance show the differences very highly significant between the cultivars ($P<0.001$). The multiple comparisons of the averages according to the method of Newmann and Keuls release seven homogeneous groups. The study of the rate of flowering at different the cultivars showed a variation of this parameter at these different cultivars. In the same way, the studies showed the rate of flowering varies from one year to another for same the cultivar, and from the significant differences were obtained during two years of studies for same the cultivars. These differences are generally related to the variations of the climatic conditions and physiological of the old tree to another. For example, at the cultivar Haouzia, the rate of flowering (62%) confirms the stability of this cultivar as the same result was got by Nait Taheen (1993) studied with the collection Menara of Marrakech. The work completed in 1993 by Nait Taheen, showed there exists a positive and significant correlation between the rates of flowering and the extent of the winter cold. The same observations were reported by Hartmann and Prolingis (1975).

Morphology of the floral bunch

Median number of flowers per inflorescence

The results obtained for this parameter are presented in figure 3. It is noted that the number of flowers per inflorescence varies between 9 at the cultivar Changlot Real and 30 at the cultivar Sevillano de Jumilla. The

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number of flowers per inflorescence varies significantly according to the cultivar ($p < 0.001$). The multiple comparison of average shows the existence of ten homogeneous groups which overlap between them. The first group of cultivars with median number of flower per the weakest bunch (less than 12) it was the cultivar Changlot Real. As for the group having the median number of flower per the highest bunch (30) was formed by the cultivar Sevillano de Jumilla.

Average number of hermaphrodite flowers per inflorescence

The majority of the cultivars have a rate of hermaphrodite flowers superior to 50% (figure 4). For the cultivar Sebatera and Haouzia the rate of hermaphrodite flowers was 16 and 18% respectively. While the two cultivars Amargoso and Changlot Real have a very high rate of 95 and 93% respectively. The analysis of the variance shows the existence of a difference very highly significant between the cultivars ($p < 0.001$). The multiple comparison of average made it possible to groups the cultivars in 4 homogeneous groups.

The median number of flower per inflorescence was variable at different cultivars studied. However, for the studies realized on others cultivars showed that there are no significant differences between the cultivars compared to the number of flower per inflorescence. This parameter was regarded for a cultivar given as a stable character (Moundi, 1974). However, our results are in agreement with several authors on others cultivars (Lavee, 1996; Lavee et al., 2002).

Many studies showed that the median number of flowers hermaphrodites varies according to the years of production. This variation was related to the action of the minimal temperatures reigning for the period separating the stage from debourrement from the buds of the stage beginning from flowering, lasting which occur the differentiation and the complete development of the flowers (Spiegel-Roy, 1965; Badr and Hartmann, 1971). This character can be also influenced by the lack of water especially during the period separating floral differentiation until the complete evolution from the flowers (Hartmann and Panestos, 1961).

Rate of fruit set

A counting of the inflorescences of each fruit-bearing branch was carried out. At the time of the fall of the petals, the sachets are removed and with the fruit set, the number of tied fruits was given. The results of the rates of fruit set obtained for each mode of pollination are illustrated by figure 5. The rates of fruit set, in the event of self pollination, are understood between 1% and 99% fruits tied by bunch. Whereas in the event of free pollination, these rates are understood between 42% and 100% fruits tied by bunches. By comparison the rates of fruit set obtained in free pollination; the rates of fruit set in self pollination appear weak at the cultivars Sebatera (1%), Manzanilla de Sevilla (3%), and Haouzia (8%). The other cultivars such as Alameno de Marchene, Galega vulgar, Koroneiki and Amargoso have rates of higher fruit set in free mode of pollination, whereas the cultivars Sevillano de Jumilla and Changlot Real show rates of fruit set in self pollination similar to those of free pollination (figure. 5).

The analysis of the variance show a highly significant differences between the cultivars and the mode in pollination on the fruit set with respectively (P=0,004) and (P=0,006) for the first and the second factor. Thus, the multiple analyses of the averages reveal 5 homogeneous groups. The first group was formed by cultivars for which average of fruit set was very different between the self pollination and free pollination; it acts of the cultivars of the (A) group: Sabatera. The 2nd group (c) contains cultivars which have similar average rates of fruit set in free pollination that in self pollination, they are the cultivars Sevillano of jumilla and Changlot Real. Between the two groups (a and c) was cultivars whose variation of the average rate of fruit set was at least weak between the two modes of pollination, it acts of the group (ab) with cultivars Alameno de Marchene, Galega vulgar, Haouzia, Negrinha and Manzanilla de Sevilla. Whereas the group (abc) with the cultivars Azeitoneira, Amargoso, Acebuchera, Blanqueta, Fulla of salce and Alameno de Montilla.

Fruiting rate

The fruiting rate obtained was between 0 and 92% in self pollination and of 5% and 98% in free pollination. These results represent an increase in fruiting rate into free mode of pollination compared to the self pollination (figure 6). It should be noted also that in self pollination, the cultivars Koroneiki, Alameno de Marchene and Sevillano de Jumilla are characterized by a high fruiting rate (92%, 44% and 40% respectively).

A great variability was observed between the cultivars and the two modes pollination (self pollination and free pollination) for the fruiting rate with (P=0,002) as well for the first and the second factor. The multiple analysis of average revealed 3 homogeneous groups. The group of the cultivars with the similar middling fruiting rate in self pollination and in free pollination, it was about the (A) group with cultivars Acebuchera, Amargoso and Azeitoneira and Koroneiki. The 2nd group (c) contains cultivars with a middling fruiting rate very different between the two modes from pollination; it acts of only one cultivar Sebatera. The other group (b) contains cultivars whose variation of the middling fruiting rate was at least weak between the two modes of pollination for each cultivar.

The majority of the cultivars showed a positive response with free pollination. This positive response appears by an increase very highly significant in rate of fruit set and fruiting rate. The lower level for the fruiting rate, met primarily in the event of self pollination, could be due on a level lower of fecondation and a rejection of its pollen, and in the adverse conditions create inside the isolation. This explains why we met small fruits probably parthenocarpic which develop in great quantity under the conditions of poor pollination and especially when the temperature exceeds 30° C (Lavee, 1996).

These results reveal the cultivars which gave the highest rates of fruit set also generated important rates of fall. Thus, more one tree was charged out of fruit, more the fall of the fruits was important. This phenomenon of nutritive competition between fruits was reported by several authors (Suarez et al.; 1984; Rallo et al., 1981; Rallo and Fernandez-Escobar, 1985; Cuevas and Rallo, 1990). In this case the tree tends to regularize its physiological balance in order to obtain fruits of good gauge. The climate was also another factor to be considered, insofar as the high temperatures advance the fall of the fruits at many cultivars of olive-tree (Rallo et al., 1990). These climatic factors are in our case accentuated by the artificial conditions creating by the conditions of paper sachets.

Self compatibility index

The calculation of the self compatibility index at the 15 cultivars studied makes it possible to classify them in 3 groups:

- Strongly self-compatible cultivars: Azeitoneira, Koroneiki, Amargoso and Acebuchera.
- Partially self-compatible cultivars: Sevillano de Jumilla, and Allameno de Marchene, Galera vulgar,
- Strongly self-incompatible cultivars: Sebatera, Manzanilla of sevilla, Changlot Real, Blanqueta, Haouzia, Alameno de Montilla, Fulla de Salce and Neghinha.

The Studies of behavior of certain cultivars showed the cultivar Picholine Languedoc was highly self-compatible, the cultivar Sourani presents a satisfactory self-fertility. However, the cultivars Picholine Marocain, Manzanille and Arbequine can be regarded as partially self-compatible.

In recent study by Breton et al. (2014; 2016) showed that in the olive tree, the system of reproduction, completely particular compared with the other fruit species, leads at present, most of the orchards to be in money chronic production due to the lack of pollinators.

Other studies showed the same cultivars behave differently with the test of compatibility. Thus, the cultivars considered in this study as self-compatible, compatible or self-incompatible in their countries of origin. The study made by Aoubid in 2009 showed that the cultivar Amellau was self-compatible whereas the same cultivar was noted self-incompatible according to Moutier et al. (2004). The same studies shown as the cultivars Italians Rosino, Santa Caterina and Rossello which are considered, in their country like self-incompatible cultivars (Cimato et al., 1993; Cimato et al., 2001), are self-compatible in the world collection of Tassaout (Aoubid, 2009). The cultivars such as Sassarese, Morchiaio and Gremignolo di Bolgheri kept their degree of compatibility the same one as that obtained by the other authors (Androlakis and Loupassaki, 1990; Cimato et al., 1993; Lavee, 1996; Cimato et al., 2001; Lavee et al., 2002). The cultivar Galega vulgar was slef compatible in its country of origin (IOC, 2007) whereas our study showed the cultivar was partially auto compatible. The cultivar Manzanilla de Sevilla was cultivated without pollinating in Spain (IOC, 2007), pollination seems better in crossed pollination, in the other countries, the use of pollinating was essential this in conformity with our results.

The content of oil

The content of oil of olives of the cultivars was one of the most important parameters. We determined the content of oil of the 15 most productive cultivars during the 4 years of production. The results are indicated in figure 7. All the cultivars present a content of higher oil or equalizes to 40%. The cultivar Koroneiki presents the content of the highest oil (56% MS) whereas the cultivar Manzanilla de Sevilla presents the lowest value (40% MS).

The Koronéiki cultivar was the principal cultivar of oil of Greece according to the IOC (Catalogue of the cultivars), its output oils some in its country of origin very high, and it was very appreciated by its acid content oleic very high. This result shows this cultivar always keeps these large values for the production of olive oil under the Moroccan environmental conditions. The cultivar Galega vulgar was very appreciates by its content of oil in its country of origin, it is used primarily for the oil extraction (IOC, 2007). The cultivar Manzanilla de Sevilla shows a content of stable average oil of good quality in its country of origin, this result similar that which obtained whereas the cultivar Changlot Real shows a high content of oil quality in Spain (IOC, 2007). The Blanqueta cultivar presents a content of high oil soft and fruity of low stability was good quality in its country (IOC, 2007). A recent study in Morocco showed that the contents in oleic acid for Haouzia (76,1 %), Dahbia (75,3 %) and Menara (75,2 %) were higher than that of Arbéquine (66,2 %) (Mahhou et al., 2014).

## IV. CONCLUSION

This works could prove the perfermence of the same cultivars of the world collection of olive tree in Marrakech (Morocco), it acts on the cultivars with same

characteristics; this enables us to think this material deserves being proposed to propagate by the agricultures in the region. This cultivars contrains same important characteristics and can be utilized in the program of genetic improvement. In perspective, these cultivars should multiply in other areas of Morocco, in order to know the differentiation of this cultivars beter the various zones.

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*Table.1: The liste of the cultivars selected*

| Cultivar | Origin | Cumulated average production (2007 to 2010) in Kg |
|---|---|---|
| Blanqueta | Spain | 36 |
| Fulla de salce | Spain | 34 |
| Amargoso | Spain | 32 |
| Acebuchera | Spain | 29 |
| Haouzia | Morocco | 29 |
| Changlot Real | Spain | 27 |
| Negrinha | Portugal | 26 |
| Alameno de Marchene | Spain | 26 |
| Azeitonera, Azeitira | Portugal | 23 |
| Galega vulgar | Portugal | 23 |
| Sebatera | Spain | 22 |
| Sevillano de Jumilla | Spain | 21 |
| Koroneiki | Greece | 21 |
| Alameno de Montilla | Spain | 21 |
| Manzanilla de Sevilla | Spain | 21 |

*Table.2: The vigor of 15 cultivars selected*

| Cultivar | Average of the section of the Trunk ($cm^2$) | Average total height (cm) | Average of the areaof foliation en $m^2$ | Average of volume of foliation en $m^3$ |
|---|---|---|---|---|
| Acebuchera | $67^{ab} \pm 13$ | $235^{ab} \pm 12$ | $9^{ab} \pm 1.8$ | $3^{a} \pm 0.8$ |
| Alameno de Marchene | $80^{bc} \pm 6$ | $369^{e} \pm 13$ | $19^{ef} \pm 1$ | $8^{ab} \pm 1$ |
| Alameno de Montilla | $110^{c} \pm 14$ | $344^{de} \pm 21$ | $17^{def} \pm 3$ | $6^{a} \pm 1$ |
| Amargoso | $70^{ab} \pm 15$ | $243^{ab} \pm 15$ | $12^{bc} \pm 1.6$ | $5^{a} \pm 0.6$ |
| Azeitonera | $66^{ab} \pm 10$ | $308^{cd} \pm 14$ | $16^{cde} \pm 1.7$ | $5^{a} \pm 0.9$ |
| Blanqueta | $53^{ab} \pm 10$ | $200^{a} \pm 16$ | $6^{a} \pm 0.7$ | $2^{a} \pm 0.3$ |
| Changlot Real | $68^{ab} \pm 15$ | $250^{b} \pm 14$ | $12^{bc} \pm 1.9$ | $5^{a} \pm 1.04$ |
| Fulla de Salce | $56^{ab} \pm 8$ | $243^{ab} \pm 9$ | $12^{bc} \pm 1$ | $5^{a} \pm 0.6$ |
| Galega vulgar | $62^{ab} \pm 5$ | $341^{de} \pm 21$ | $18^{ef} \pm 1$ | $7^{a} \pm 1$ |
| Haouzia | $88b^{c} \pm 16$ | $351^{de} \pm 13$ | $21^{ef} \pm 0.8$ | $9^{ab} \pm 0.9$ |
| Koroneiki | $62^{ab} \pm 5$ | $261^{bc} \pm 18$ | $13b^{cd} \pm 1.8$ | $5^{a} \pm 0.8$ |
| Manzanilla de Sevilla | $83^{bc} \pm 18$ | $350^{de} \pm 6$ | $18^{ef} \pm 2$ | $9^{ab} \pm 1$ |
| Negrinha | $66^{ab} \pm 12$ | $329^{de} \pm 17$ | $18^{def} \pm 2.4$ | $6^{a} \pm 1.3$ |
| Sebatera | $38^{a} \pm 16$ | $332^{de} \pm 18$ | $13^{bcd} \pm 2$ | $5^{a} \pm 1$ |
| Sevillano de Jumilla | $80^{bc} \pm 15$ | $348^{de} \pm 21$ | $18^{ef} \pm 1$ | $8^{a} \pm 1$ |
| Valeur moyenne | $80 \pm 3.9$ | $297 \pm 4$ | $15 \pm 0.1$ | $5 \pm 0.1$ |
| CV (%) | 4.8 | 134 | 0.66 | 2 |

*The cultivars which present the same letters do not differ significantly (P>0.05). Test Newmann-Keuls*

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Date of appearance, lasted in phenologic days and spreading out of the three stages Beginning of flowering (F), Full flowering (F1) and Fine flowering (G) at the 15 cultivars studied for the partner 2010-2011

F= Beginning of flowering
F1= Full flowering
G= Fine flowering

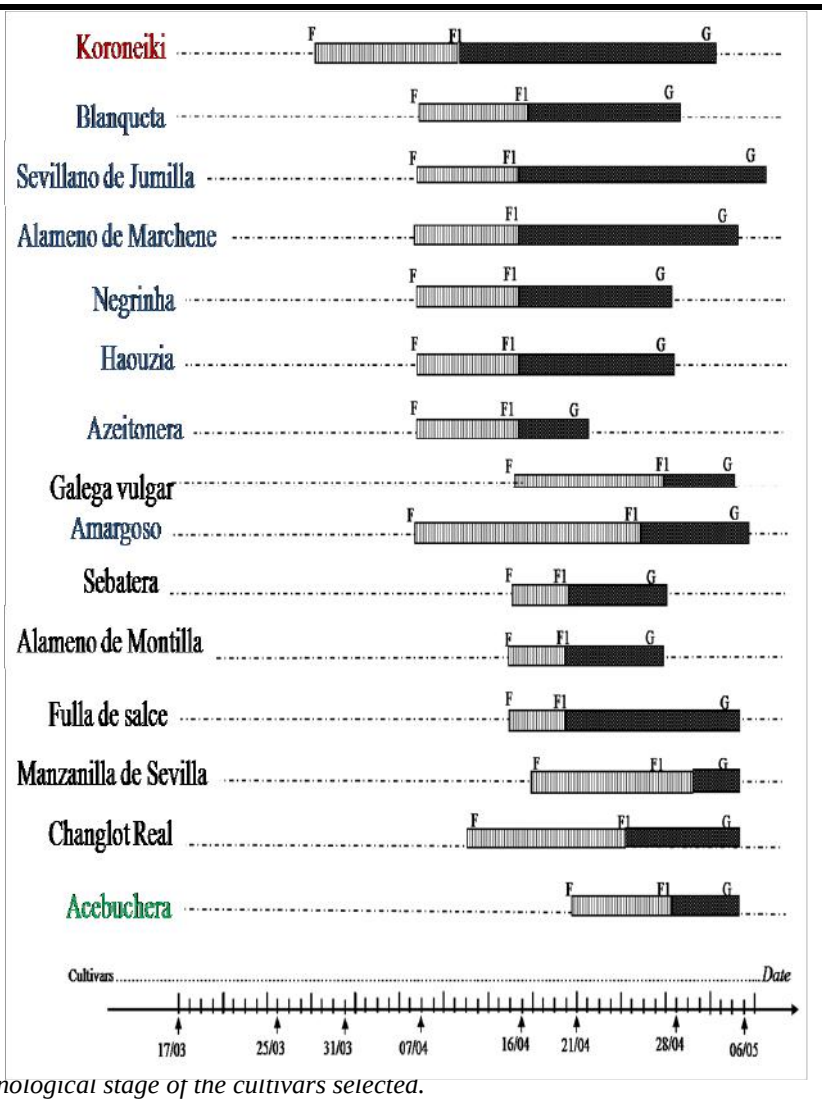


*Fig. 1: Phonological stage of the cultivars selected.*

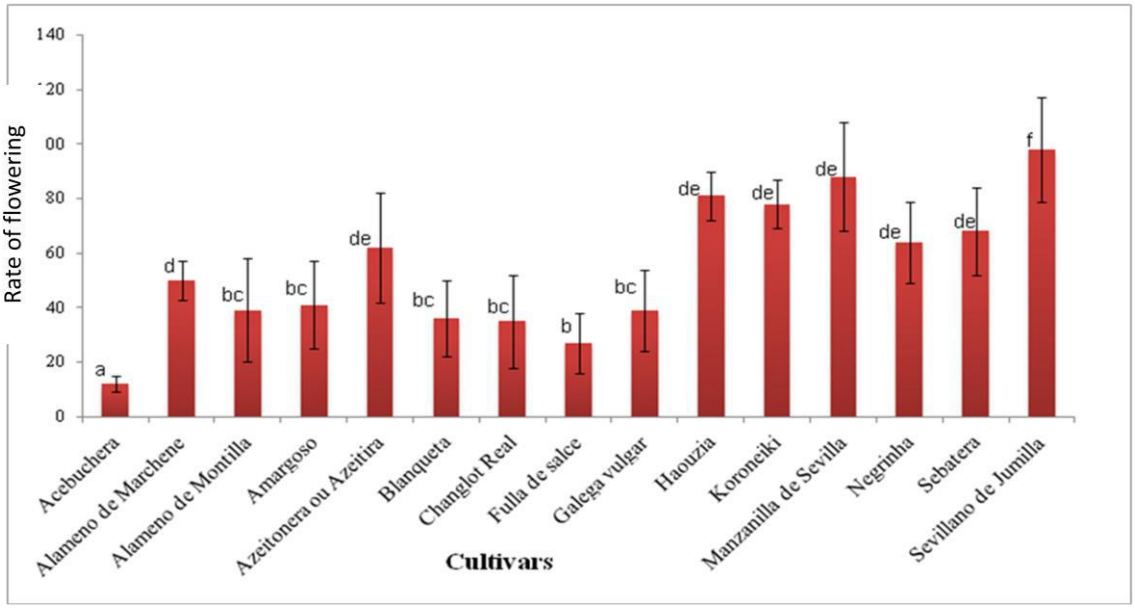


*Fig. 2: Rate of flowering for the cultivars selected.*
*The cultivars which present the same letters do not differ significantly (P>0.05). Test Newmann-Keuls*

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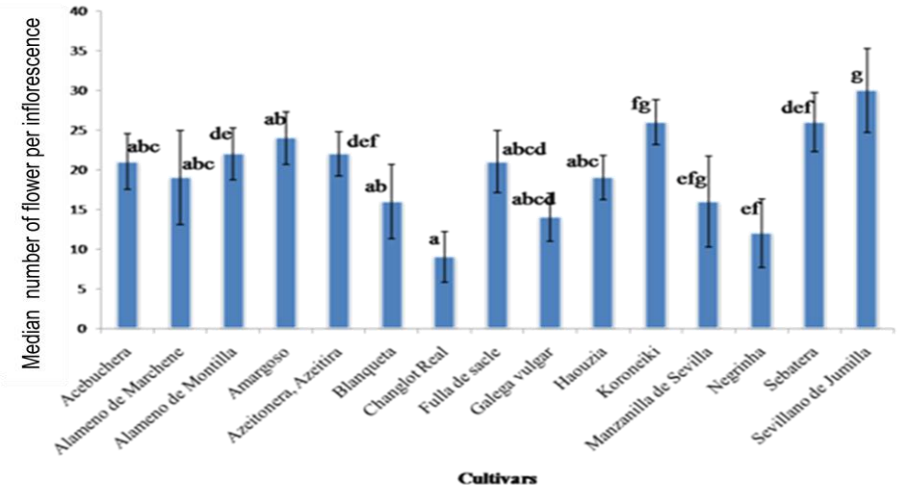


*Fig.3: Median number of flower per inflorescence for the cultivars selected.*
*The cultivars which present the same letters do not differ significantly (P>0.05). Test Newmann-Keuls*

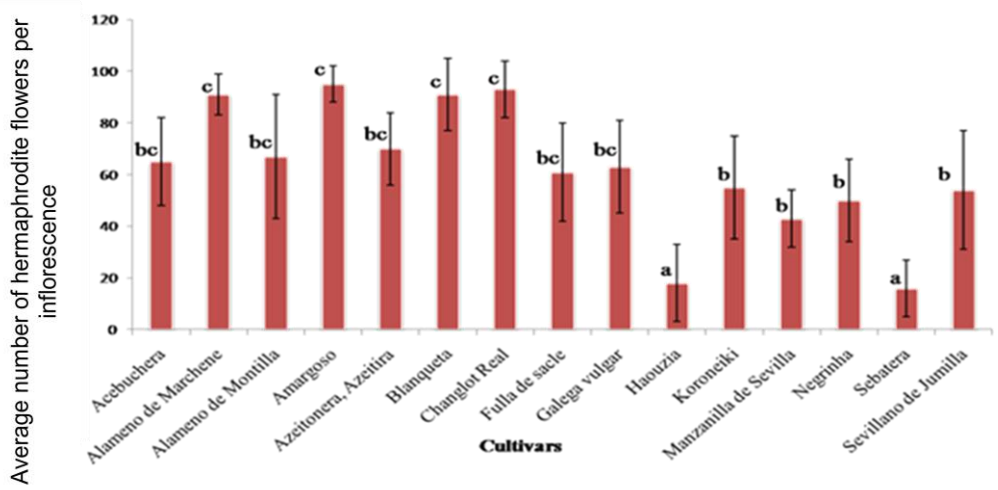


*Fig.4: Median number of hermaphrodite's flowers perinflorescence for the cultivars selected*
*The cultivars which present the same letters do not differ significantly (P>0.05). Test Newmann-Keuls*

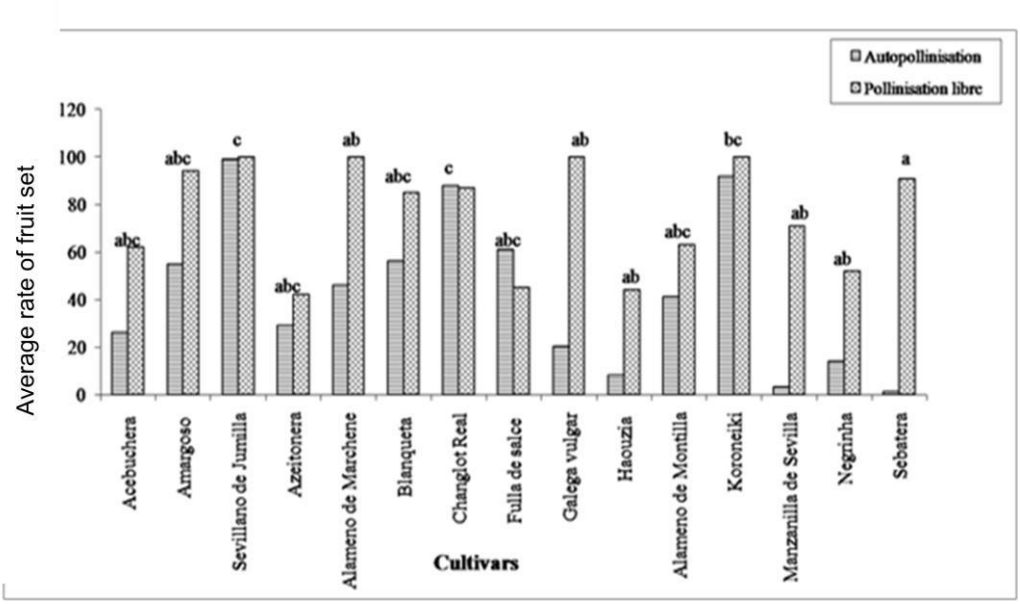


*Fig. 5: Average rate of fruit set for the cultivars selected*
*The cultivars which present the same letters do not differ significantly (P>0.05). Test Newmann-Keuls*

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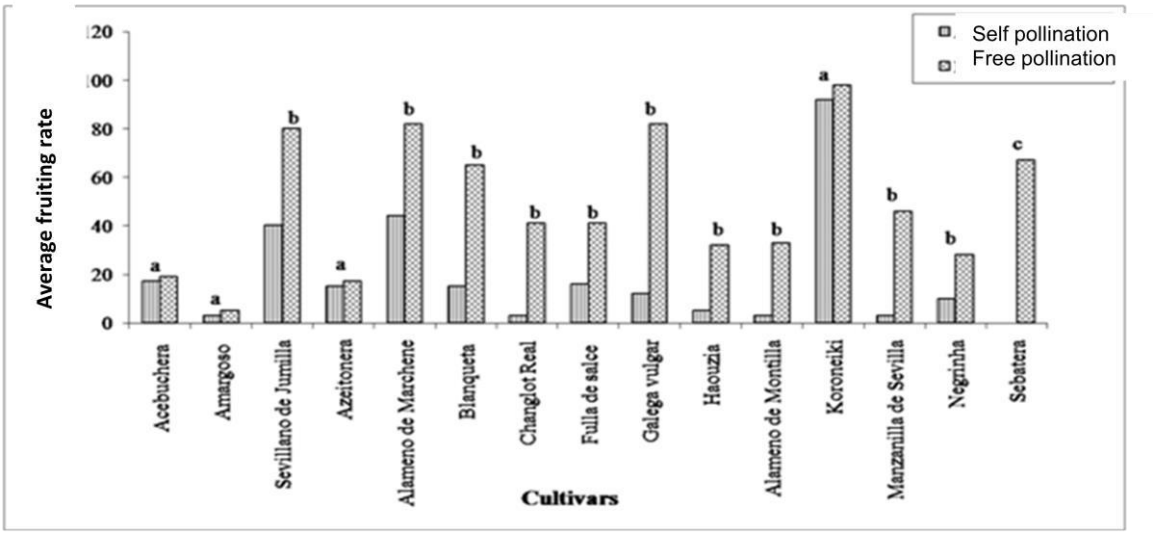


*Fig. 6: Average fruiting rate obtained at the 15 studied cultivars.*
*The cultivars which present the same letters do not differ significantly (P>0.05). Test Newmann-Keuls*

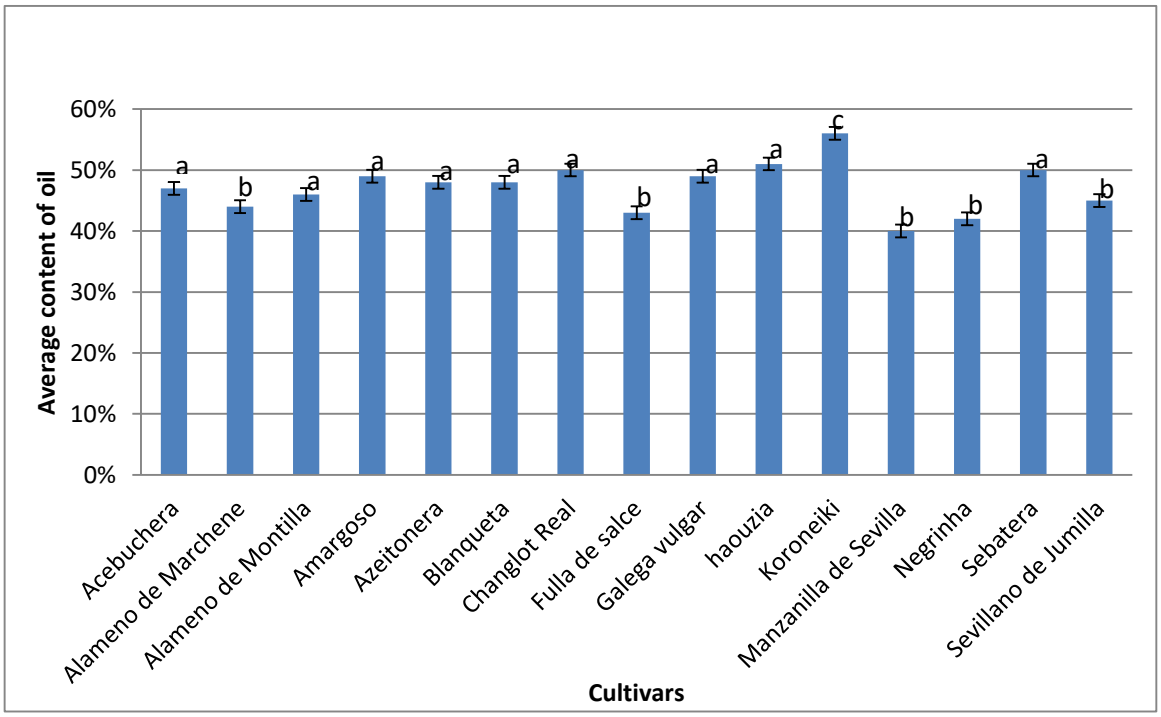


*Fig. 7: Content of oil for the cultivars selected*
*The cultivars which present the same letters do not differ significantly (P>0.05). Test Newmann-Keuls*

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# Effect of Rates of Single Superphosphate added to Poultry Manure on Popcorn (*Zea mays everta*) Production in Jos, Plateau State.

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***Abstract—*** *Field studies were conducted at the Teaching and Research Farm of Federal College of Land Resources Technology, Kuru-Jos in 2013 and 2014 cropping seasons. The effect of rates of single superphosphate fertilizer to be added to poultry manure for Popcorn Zea mays everta Production in Jos was investigated. The experiment was laid out in a Randomized Complete Block Design with four replicates. Five treatments were used: 20 t ha$^{-1}$ Poultry manure (PM) + O kg $P_2O_5$ ha$^{-1}$ (control), 20 t ha$^{-1}$ PM + 20 kg $P_2O_5$ ha$^{-1}$, 20 t ha$^{-1}$ PM + 40 kg $P_2O_5$ ha$^{-1}$, 20 t ha$^{-1}$ PM + 60 kg $P_2O_5$ ha$^{-1}$ and 20 t ha$^{-1}$ + 80 kg $P_2O_5$ ha$^{-1}$. The results revealed that the grain yield of Popcorn were significantly ($P<0.05$) affected by the treatments in both seasons. The application of 20 t ha$^{-1}$ PM + 80 kg $P_2O_5$ ha$^{-1}$ gave the highest grain yield in both seasons, also the soil properties at the end of the experiments improved in terms of organic matter, total nitrogen and exchangeable cations. Hence 20 t ha$^{-1}$ PM + 80 kg $P_2O_5$ ha$^{-1}$ is recommended for Popcorn production in Kuru and its environs.*

***Keywords— Single Superphosphate, Poultry manure, Popcorn.***

## I. INTRODUCTION

Popcorn (*Zea may everta*) just as the ordinary maize is an important crop that has world significance as it is a good source of food not only for humans but also for livestock (Akintola, 1997). In many regions, it is consumed as a vegetable although it is a grain crop. In Nigeria it is mainly consumed as a snack. The grains are rich in vitamins A, C and E, carbohydrates and essential minerals and contain 9 % protein. They also rich in dietary fibres which are food source of energy (IITA, 2007),

Popcorn being a cereal is a very high nutrient demanding crop, requiring adequate nutrient for maximum performance. Among the several other factors which cause a declined in corn yield is soil degradation from intensive alteration are continuous application of high rates of fertilizers which may cause nutrient imbalance and limit the uptake of other essential nutrients, thus limiting the crop performance (Obi, 1991).

Phosphorus is an essential constituents of numerous substance involved in biochemical reactions including photosynthesis and respirations. It is a major component of adenosine diphosphate (ADP) and adenosine triphosphate (ATP). These are used to supply energy for many biochemical reactions in plants and animals. Phosphorus levels in soil can be used as a guide to indicate whether phosphorus fertilizer is required for plant growth (Moody and Balland, 1999).

The objective of this study is therefore to establish the correct rate of SSP to be added to poultry manure for popcorn production in Jos.

## II. MATERIALS AND METHODS

Field trials were conducted at the Teaching and Research Farm of Federal College of Land Resources Technology, Kuru in 2013 and 2014 cropping seasons. The site which falls between Longitude $8^0 5'$ - $9^0 5'$E and Latitude $9^0 5'$- $10^0$ N, at an elevation of 1400 m above sea level. The effect of rates of single superphosphate (SSP) fertilizer to be added to poultry manure for Popcorn (*Zea mays everta*) production was investigated. The experiments were laid out in a Randomised Complete Block Design (RCDD), with four replications. Five treatments were used: 20 t ha$^{-1}$ Poultry manure (PM) + O kg $P_2O_5$ ha$^{-1}$ (control), 20 t ha$^{-1}$ PM + 20 kg ha$^{-1}$$P_2O_5$ , 20 t ha$^{-1}$ PM + 40 kg ha$^{-1}$$P_2O_5$, 20 t ha$^{-1}$ PM + 60 kg ha$^{-1}$$P_2O_5$ and 20 t ha$^{-1}$ + 80 kg ha$^{-1}$ $P_2O_5$ were used with each plot size measuring 3 m x 3 m (9 m$^2$). The yield parameters considered were number of cobs and grain weight, the data collected was subjected to Analysis of Variance (ANOVA) and means were separated using Fishers Least Significant Difference (FLSD).

Soil samples were collected from the plough layer (0 – 15 cm) at the beginning of the experiments and after harvest of the popcorn. The samples were analyzed for, pH, OM, total N, available P, exchangeable cations (Mg, Ca, K and Na) and cations exchange capacity (CEC).

## III. RESULTS AND DISCUSSION

The chemical properties of the soil at the experimental site before application of treatments and after harvest of popcorn for the two cropping seasons are shown in Tables 1 and 2. The pH values in both seasons were below the slightly acidic range (5.5 – 6.5) considered option for normal growth of most crops (Kamprath, 1970; Bruce and Rayment, 1982). This low pH may likely be the cause of the generally low nutrient status of the experimental site in the both seasons (Brady, 1984; McKenzie *et al*., 2004).

The soil analysis results also revealed that organic matter (OM), trial N, available P seasons and CEC slightly improved in all the plots in both seasons at the end of the experiments. This could be as a result of the organic material introduced into the soil in form of poultry manure which also serves as a source of nutrients and accumulation humus.

The number of cobs and grain yield were significantly ($P<0.05$) affected by the application of treatments in both seasons (Table 3). In 2013, the highest numbers of cobs (38 cobs) were obtained with 20 t $ha^{-1}$PM + 60 kg $ha^{-1}$ $P_2O_5$ and 20 t $ha^{-1}$ PM + 60 kg $ha^{-1}$ $P_2O_5$ while the least was 20 t $ha^{-1}$ PM + 0 kg $ha^{-1}$ $P_2O_5$ (15 cobs). In terms of grain yield 20 t $ha^{-1}$ PM + 80 kg $ha^{-1}$ $P_2O_5$ gave the highest yield of 1.94 tons $ha^{-1}$ followed by 20 tons $ha^{-1}$PM + 60 kg $ha^{-1}$ $P_2O_5$. In 2014, a similar trend was observed, with the highest number of cobs obtained with 20 t $ha^{-1}$ PM + 80 kg $ha^{-1}$ $P_2O_5$ (36 cobs) followed by 20 t $ha^{-1}$ PM + 60 kg $ha^{-1}$ $P_2O_5$ (35 cobs). Grain yield of 1.5 tons $ha^{-1}$ was obtained with 20 tons $ha^{-1}$PM + 80 kg $ha^{-1}$ $P_2O_5$ followed by 20 tons $ha^{-1}$PM + 60 kg $ha^{-1}$ $P_2O_5$ (1.41 tons $ha^{-1}$).

## IV. CONCLUSION

The study revealed that popcorn responded well to different rates of single superphosphate (SSP) added to 20 tons $ha^{-1}$ poultry manure. The soil properties at the end of the experiments improved in terms of O.M, total N, available P and CEC. 20 tons $ha^{-1}$ PM + 80 kg $ha^{-1}$ $P_2O_5$ is recommended for optimum popcorn production in Kuru – Jos.

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*Table.1: Chemical Properties of Soil Before and After Treatment in 2013*

| **Properties** | **Before planting** | ***$T_1$** | **$T_2$** | **$T_3$** | **$T_4$** | **$T_5$** |
|---|---|---|---|---|---|---|
| pH $H_2O$ | 5.20 | 5.40 | 5.40 | 5.30 | 5.40 | 5.40 |
| O.M (%) | 0.80 | 0.87 | 0.87 | 0.90 | 0.88 | 0.90 |
| Total N (%) | 0.70 | 0.80 | 0.09 | 0.09 | 0.10 | 0.15 |
| Available P (ppm) | 15.00 | 15.00 | 15.20 | 16.20 | 16.20 | 18.00 |
| Exch. Mg (cmol $kg^{-1}$) | 0.60 | 0.67 | 0.80 | 0.78 | 0.78 | 0.80 |
| Exch. Ca (cmol $kg^{-1}$) | 2.84 | 2.90 | 3.0 | 3.01 | 3.01 | 3.00 |
| Exch. Na (cmol $kg^{-1}$) | 0.22 | 0.26 | 0.23 | 0.24 | 0.24 | 0.24 |
| Exch. K (cmol $kg^{-1}$) | 0.24 | 0.23 | 0.30 | 0.32 | 0.32 | 0.30 |
| CEC (cmol $kg^{-1}$) | 10.20 | 12.20 | 12.80 | 14.20 | 14.20 | 15.20 |

*$T_1$ = 20 tons $ha^{-1}$ PM + 0 kg $ha^{-1}$ $P_2O_5$, $T_2$ = 20 tons $ha^{-1}$ PM + 20 kg $ha^{-1}$ $P_2O_5$, $T_3$ = 20 tons $ha^{-1}$ PM + 40 kg $ha^{-1}$ $P_2O_5$, $T_4$ = 20 tons $ha^{-1}$ PM + 60 kg $ha^{-1}$ $P_2O_5$, and $T_5$ = 20 tons $ha^{-1}$ PM + 80 kg $ha^{-1}$ $P_2O_5$.

*Table.2: Chemical Properties of Soil Before and After Treatment in 2014*

| **Properties** | **Before planting** | ***$T_1$** | **$T_2$** | **$T_3$** | **$T_4$** | **$T_5$** |
|---|---|---|---|---|---|---|
| pH $H_2O$ | 5.30 | 5.40 | 5.50 | 5.50 | 5.50 | 5.50 |
| O.M (%) | 0.83 | 0.90 | 0.92 | 0.90 | 0.91 | 0.99 |
| Total N (%) | 0.09 | 0.10 | 0.20 | 0.22 | 0.30 | 0.35 |
| Available P (ppm) | 15.20 | 15.00 | 15.60 | 16.00 | 17.20 | 17.00 |
| Exch. Mg (cmol $kg^{-1}$) | 0.99 | 0.98 | 0.99 | 1.00 | 1.20 | 1.20 |
| Exch. Ca (cmol $kg^{-1}$) | 3.00 | 2.90 | 3.00 | 3.00 | 3.50 | 4.00 |
| Exch. Na (cmol $kg^{-1}$) | 0.23 | 0.20 | 0.20 | 0.22 | 0.21 | 0.22 |
| Exch. K (cmol $kg^{-1}$) | 0.38 | 0.35 | 0.38 | 0.38 | 0.40 | 0.40 |
| CEC (cmol $kg^{-1}$) | 15.00 | 15.20 | 15.00 | 16.00 | 15.80 | 16.00 |

*$T_1$ = 20 tons $ha^{-1}$ PM + 0 kg $ha^{-1}$ $P_2O_5$, $T_2$ = 20 tons $ha^{-1}$ PM + 20 kg $ha^{-1}$ $P_2O_5$, $T_3$ = 20 tons $ha^{-1}$ PM + 40 kg $ha^{-1}$ $P_2O_5$, $T_4$ = 20 tons $ha^{-1}$ PM + 60 kg $ha^{-1}$ $P_2O_5$, and $T_5$ = 20 tons $ha^{-1}$ PM + 80 kg $ha^{-1}$ $P_2O_5$.

*Table.3: Effects of Treatment on the Number of Cobs and Grain Yield of Popcorn in 2013 and 2014 Cropping Season*

| | **Number of cobs** | | **Grain Yield (tons $ha^{-1}$)** | |
|---|---|---|---|---|
| **Treatments** | 2013 | 2014 | 2013 | 2014 |
| 20 tons $ha^{-1}$ PM + 0 kg $ha^{-1}$ $P_2O_5$ | 15 | 10 | 0.14 | 0.05 |
| 20 tons $ha^{-1}$ PM + 20 kg $ha^{-1}$ $P_2O_5$ | 25 | 21 | 0.80 | 0.70 |
| 20 tons $ha^{-1}$ PM + 40 kg $ha^{-1}$ $P_2O_5$ | 36 | 34 | 1.20 | 1.01 |
| 20 tons $ha^{-1}$ PM + 60 kg $ha^{-1}$ $P_2O_5$ | 38 | 35 | 1.70 | 1.41 |
| 20 tons $ha^{-1}$ PM + 80 kg $ha^{-1}$ $P_2O_5$ | 38 | 36 | 1.94 | 1.50 |
| **FLSD ($p \leq 0.05$)** | 12 | 9.0 | 0.39 | 0.22 |

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# Effect of egg sizes on egg qualities, hatchability and initial weight of the hatched-chicks

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***Abstract—*** *The study evaluates the relationship of egg weights with other egg qualities, hatchability and hatchling weights. One thousand eight hundred eggs from marshal broiler chicken were categorized into small (50 – 59 g), medium (60 – 69 g) and large (≥70 g) of 600 eggs per group in a completely randomized design. Hundred eggs from each group were subjected to quality analysis and the remaining incubated for hatchlings production. The egg length of of large group was the highest (4.71 cm) and lowest in small (4.22 cm). The relationship revealed 32.86% yolk in small size, while 33.71 and 30.99% was observed for medium and large respectively. Also, albumen ranged from 50.85% in small to 55.38% in large. Hatchability result revealed 74.50% for small egg and reduced as sizes increased. Hatchling weight was highest in large (49.19 g) and lowest in small (38.79 g). The regression equations showed that albumen weight and egg size were best predictors of chick's weight through their $R^2$ values (0.68 and 0.57 respectively) compared to yolk weight with $R^2$ of 0.28. It was therefore be inferred that breeder eggs size could affect egg quality parameters and chick's weight.*

***Keyword— Egg weights, hatchability, Egg quality, chick weight.***

## I. INTRODUCTION

The distributions of domestic chicken are on the increase in rural and urban areas of the world and in particular Africa and Asia where they contribute significantly to economic developments of the households. They are nutritionally, economically, and culturally very important to livelihoods of rural households (King'ori *et al*., 2003; Mtileni *et al*., 2010). However, productivity of these chickens is generally low and mortality is high, which suggest that appropriate genetic, nutritional and management interventions are needed to realize their optimal production potential (Okitoi *et al*., 2006; Mbajiorgu *et al*., 2011). Studies on improving productivity of broiler chickens through genetic and nutritional strategies have been well documented (Mbajiorgu *et al*., 2011; Adesola *et al*., 2012). Aside, other relevant factors that could affect the economic development of broiler chicken are needed to be examined. Such factors include the quality of day-old chick and the egg that produced the hatched chick.

The influence of egg weight on hatchability, chick weight and subsequent growth rate and mortality of these chicks is of practical interest to chicken farmers. However, there are ample evidences that egg weight affects subsequent productivity of laying hens and broiler chickens (Alders and Spradbrow, 2001; Swatson *et al*., 2001; King'ori *et al.*, 2003; Rashid *et al*., 2005). Alabi *et al*. (2012a) reported that hatchability and chick weight were higher in larger broiler chicken eggs but the influence of egg weight on productivity of the progenies was not determined. Alabi *et al*. (2012b) then reported that hatchability and post-hatch performance of Potchefstroom Koekoek chickens were higher in larger eggs than in smaller ones. Williams (1994) studied the relationship between egg size and offspring quality in birds and reported that egg size typically affects hatchling mass more strongly than it affects hatching size in birds because the main effect of egg size lies in the mass of the residual yolk sac that the chick retains at hatching. Also, egg weight and chick weight at hatching have been shown to be positively related (Khurshid *et al.*, 2003). The embryo size before and at hatching can be altered by the weight of the egg and the incubation environment (Wilson, 1991). It could therefore be suggested that the potential of the broiler chicken depends, in part, on egg quality, an important parameter for embryogenesis as well as for day-old chick quality and growth. In hatchery, the quality of a day-old chick is usually based on qualitative aspects, such as abnormalities and contamination. This study thus examines the effects of egg sizes on other egg quality parameters, hatchability and the weight of the resulting hatched chicks.

## II. MATERIALS AND METHODS

### Experimental site

The egg quality parameters evaluation was carried out at Animal Production and Health laboratory of The Federal University of Technology, Akure, Nigeria. While the incubation and hatching of fertile eggs were done at a reputable hatchery in Ilara-Mokin, Nigeria. These are located

in the rainforest zone, South West, Nigeria with about 1200-1500mm of average rainfall per annum (Nigerian Meteorological Agency, 2014).

**Hatchable Eggs Sourcing**

One thousand eight hundred marshal broiler chicken eggs were sourced from Oluade breeder farm in Ilara-Mokin, Ondo State, Nigeria. The eggs were cleaned and grouped based on sizes into small (50-59g), medium (60-69g) and large (≥70g). After grouping, the eggs were stored in the cold room to arrest embryonic development prior to incubation.

**Egg Quality Parameters Assessment and Hatching of Eggs**

A total number of three hundred (300) eggs selected from the three sizes (i.e. small, medium and large) with 100 eggs per group were subjected to quality analysis in the laboratory. The eggs parameters that were assessed included; shape index, shell thickness, albumen index, yolk index, yolk weight, albumen weight, yolk height, albumen height and Haugh unit. Some of these parameters were calculated using the following formulae and methods summarized by Yoruk *et al.* (2004).

Shape index (%) = (egg width, cm/egg length, cm) × 100;

Shell thickness (mm × $10^{-2}$) was determined in 3 different parts with a micrometer screw gauge;

Albumen index (%) = (albumen height, mm/average of albumen length, mm and albumen width, mm) × 100;

Yolk index (%) = (yolk height, mm/yolk diameter, mm) × 100;

Haugh unit = 100 × log (AH + 7.57 − 1.7 × $EW^{0.37}$), where AH = albumen height (mm) and EW= egg weight (g). Before the egg quality parameters were determined, the eggs were stored for 24 hours at a room temperature.

The hatching of eggs was done based on the procedure provided by the hatchery.

**Determination of hatchability of egg and initial weight of hatched chicks**

One thousand five hundred eggs of 500 eggs per group were set in the setter according to their groups (small, medium and large). The eggs spent a total number of 21 days in the incubator, after which the chicks were brought out. Counting was done based on the ranges set in the incubator. Percentage hatchability was also determined using the formula;

$$\% \text{ hatchability} = \frac{total\ number\ of\ hatched\ chicks}{total\ number\ of\ fertile\ eggs\ incubated} \times 100$$

Thereafter, the initial weight of each chick was determined using a digital sensitive scale (Wiggen Hauser, WH 200-4, Germany).

**Experimental Design and Data Analysis**

The design of the experiment was a Completely Randomized Design (CRD) with one thousand eight hundred eggs of 600 eggs per group, out of which 100 eggs per group were used for egg quality assessment and the remaining for hatching. All data collected were analyzed by subjecting them to One Way Analysis of Variance using SPSS version 16 package. Where significant differences were found, Duncan multiple Range Test of the same package was used to separate the means. The regression equations were fitted using the mean values of the weight range.

## III. RESULTS

**Quality characteristics of different egg sizes**

The result of the quality characteristics of the different egg sizes of broiler chicken presented in Table 1 showed that there were relationships between the egg sizes and some egg qualities as significant ($P < 0.05$) differences were observed in the egg length, egg width, yolk weight, yolk length, yolk height, albumen weight and albumen length. A significant ($P < 0.05$) difference noticed in the egg length as a result of the egg sizes revealed that egg categorized as large ( ≥ 70) had the highest egg length (4.71cm) followed by those categorized as medium (4.49 cm) and lowest in those categorized as small (4.22cm). The same trend was also noticed in the egg width, albumen weight and albumen length (large > medium > small). The yolk weight and length recorded for the large group though not statistically ($P > 0.05$) different from those in medium group were higher than those recorded for the small group. However, the yolk height of eggs categorized as medium was significantly ($P < 0.05$) higher than those categorized as small. The shell thickness shows no significant variation with respect to egg sizes. The albumen index decreased as the weight of the eggs increased. The same trend was noticed in the haught unit of the eggs though not significantly ($P > 0.05$) affected.

**Correlation of egg sizes with egg yolk and albumen**

Correlation of egg sizes with egg yolk and albumen revealed that the coefficients for egg size with egg yolk and albumen were positive in all the sizes and were highly significant (Table 2). The small categories had significantly ($P < 0.01$) higher egg/yolk weight correlation (0.88) than other groups and lowered as the egg sizes increased. However, the egg/albumen weight correlation was highest in the medium group (0.85) followed by the small group (0.79) and the large group (0.62).

**Effect of egg sizes on hatchability and the Initial weight of day-old chicks**

The result of the egg sizes in relation to hatchability and day-old chick weight revealed that there was a significant ($P < 0.05$) difference in the hatchability of the egg due to sizes (Table 3). The eggs categorized as small had the highest percentage of hatchability (74.50%), followed by the medium (72.50%), and the lowest in the eggs categorized as large

(69.49%). However, the initial weight of the hatched chicks was highest in large size eggs with mean value of 49.19g, followed by the medium (42.72g) and the lowest value was recorded in the small size egg (38.79g).

**Effects of egg sizes on yolk, albumen and chick weight**

The relationships between egg size and yolk weight reveals that 32.86% of the small size eggs were made up the yolk, while 33.71 and 30.99 % made up the medium and large groups respectively (Table 4). The contribution of albumen to the entire egg ranged from 50.85% in small size to 55.38% in the large group. In addition, the ratio of egg to chick indicated that about 70% of egg in the small group translates to chicks, while it was 64 and 68% for medium and large groups respectively. Also, the small sized group had the lowest egg to chick weight loss (16.50 g), while the large had the highest but this was not significantly ($P>0.05$) different from those in the medium. However, the % reduction in weight from egg to chick reveals that medium sized eggs had the highest reduction with 35.99% followed by the large (31.28%) with the small group having the lowest (29.79%) (Table 4).

**Regression equations derived from parameters of the relationship between the day-old chick weight and other egg qualities**

Table 5 shows the regression equations, estimates and coefficients of determination of day-old chick weight measurements in relation to egg sizes, albumen and yolk weights. The relationship between the day-old chick weight and egg sizes as indicated by the coefficient of determination ($R^2$) had a positive value for regression coefficient (0.57). Also, the relationship between the day-old chick weight and albumen weight shows a positive coefficient of determination (0.68) which is more than that of the yolk weight (0.29).

## IV. DISCUSSION

The physical characteristics of the egg have been shown to play an important role in the embryonic development process and a successful hatching (Narushin and Romanov, 2002). In addition, the most influential physical egg characteristics include egg weight, shell thickness and porosity and shape index. The significant differences observed in the egg weight, egg length, egg width, yolk weight, yolk length, yolk height, albumen weight and albumen length further confirmed that these parameters are function of egg size. The coefficient of correlation of egg sizes with yolk and albumen weights in this study were generally high and positive, suggesting that both the yolk and albumen weight highly significantly contributed to the weight of the egg. The relationship between egg size and yolk weight revealed that 32.86% of the small sized egg was contributed by the yolk, while it was 33.71 and 30.99% for medium and large groups respectively. However, the contribution of albumen to the entire egg ranged from 50.85% in small size to 55.38% in the large group. This was contrary to the report of North and Bell (1990) that as egg size increases, yolk size increases more than the quantity of albumen. The present study therefore suggests that the albumen weight contributed more to egg weight than yolk weight having contributed about 53% to the entire egg weight.

The reproductive efficiency of broiler breeders decreases with age, which is related to the internal egg composition or ratio, too large egg weight, poor shell quality leading to increased early and late embryo mortality (North and Bell, 1990; Benette, 1992; Vieira and Moran, 1998; Leeson and Summers, 2000; Tona *et al*., 2004; Joseph and Moran, 2005a; Elibol and Brake, 2008) and albumen quality deterioration (Lapao *et al*., 1999; Tona *et al*., 2004) and increase in yolk cholesterol content (Dikmen and Sahan, 2007).

The yolk index though not according to a particular pattern (ranged: 0.35- 0.37) in this study was lower than 0.50 reported for Fulani-ecotype chicken by Fayeye *et al.* (2005) but the haugh unit (ranged: 75-77) was similar and sometime higher than 75.50 % for Fulani-ecotype chicken by the same authors. These further confirmed that the range of eggs used in this study could be desirable since these two indices were regarded as the best indicators of internal egg quality (Isikwenu *et al.*, 1999), and the higher the yolk index (Ayorinde 1987) and haugh unit the more desirable the egg quality. With reference to Ihekoronye and Ngoddy (1985) high quality egg generally have haugh unit of 70 and above.

The result of this study also showed that weight of broiler chicken eggs had a significant effect on egg hatchability. Heavier eggs (≥70g) had lower hatchability percentage (69.49%) while the small group (50-59g) had the highest (74.50%). This is in agreement with the findings of Constantini and Panella (2001) who reported that eggs with heavier weight have lower hatchability. This could be due to difference in egg weight, egg components like the yolk and albumen percentage, yolk: albumen ratio and incubation time (Suarez *et al*., 1997; Joseph and Moran, 2005b). Yannakopoulos and Tserveni-Gousi (1999) also reported similar results for Japanese chicken. In the result of the latter, it was noted that eggs with heavier weight which were incubated have the lowest hatchability. However, Gonzalez *et al.* (1999) and Abiola *et al.* (2008) found contrary results. These authors found that broiler chicken eggs of medium weight (60-69 g) are more suitable for setting in order to obtain higher hatchability. Farooq *et al.* (2001) and Narkhede *et al.* (1981) found negative correlations between egg weight and hatchability in crossbred chickens as heavier eggs

resulted in lower hatchability. These observations were also similar to those made by Deeming (1995) and De Witt and Schwalbach (2004), who found that hatchability of eggs of ostrich, New Hampshire and Rhode Island Red breeds decreased with increasing egg weight. On the contrary, large sized eggs of indigenous Venda chickens had been reported to have higher hatchability than medium and small sized eggs (Mbajiorgu *et al.*, 2011).

The effect of egg weight on chick weight was significantly higher in heavier egg sized group with the medium group having higher chick weights of about 10.13 % over the small group, while chick's weight from the large group was 15.15 and 26.81 % over the medium and small groups respectively. This was positively and highly correlated with equation $Y= 12.48 + 0.49X_1$, $R^2 = 0.57$. This finding could be due to the fact that heavier eggs contain more nutrients than small eggs (Williams, 1994), which resulted in developing embryos from heavier eggs having more nutrients for their growth requirements. This finding is in agreement with the finding of Vieira *et al* (2005) who found higher chick weight in large size egg in comparison with small ones in 40 weeks old Ross-38 breeders. However, contrary results were found by Asuquo and Okon (1993) which indicated that egg size within the intermediate weight range of 45 to 56 g hatched heavier chicks than small or large eggs. Ng'ambi *et al* (2013) also found similar observation that egg weight was positively correlated with chick hatch-weight in venda chicken. The increase in chick's weight with increasing egg weight was adjudged to the fact that heavier egg contains more nutrient than small egg (Willams, 1994). However, chicks from larger eggs have been shown to have more yolk attachment at hatching (Hassan *et al.*, 2005; Wolanski *et al.*, 2006). Hence heavier chicks tend to present higher body development and smaller yolk sacs due to higher development during incubation, or less developed bodies and larger yolk sacs, allowing them to survive longer before exogenous feed is provided (Skewes *et al.*, 1988). It was therefore concluded that chicks tend to depend on this yolk during the first few hours after hatching (Deeming, 1995). The yolk attachment is utilized by the chicks after hatching and the potential performance of day-old chicks may depend on the quality and quantity of this yolk. In the current study, the ratio of egg to chick indicated that about 70% of egg in the small group translates to chicks, while it was 64 and 68% for medium and large groups respectively. From the result, it was observed that the small sized group had the lowest egg to chick weight loss (16.50 g), while the large had the highest but similar to those in the medium. These results were consistent with those reported by Guill and Washburn (1973) for broiler chickens. Chick/egg weight ratio in the current study was independent of maternal age, as found by Morris *et al.* (1968), who observed that this ratio remained virtually constant over the full range of egg weight.

The regression equations estimates and coefficients of determination ($R^2$) of day-old chick weight measurements in relation to egg sizes ($Y= 12.48 + 0.49X_1$), albumen weight ($Y=17.95 + 0.77X_3$) and yolk weight ($Y= 20.62 + 1.12X_2$) showed that albumen weight and egg sizes could be used for predicting chick weight in Arbor acre breed of broiler with $R^2$ of 0.68 and 0.57 respectively, while the yolk weight may not give a stable prediction for the day-old chick weight having $R^2$ of 0.28. By implication also, the egg size and albumen weight account for about 57 and 68 % respectively, of the variation in chick weight while it was just 28 % of yolk. Thus, for every unit increase in egg weight and albumen weight, there would be a corresponding increase of 0.49 and 0.77 respectively in chick weight, while other factors aside egg weight and albumen weight require 12.48 and 17.95, respectively.

## V. CONCLUSION

It could therefore be concluded that there were strong relationships between the egg sizes and some egg qualities in Arbor acre broiler chicken. Egg categorized as large ( $\geq 70$) had the highest egg length, egg width, yolk weight, yolk length, yolk height, albumen weight and albumen length followed by those in medium size. Egg categorized as small had higher egg/yolk weight correlation than other groups and lowered as the egg sizes increased. Also, eggs categorized as small had the highest percentage of hatchability and decreased as the egg sizes increased. However, the initial weight of the hatched chicks was highest in large eggs.

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*Table.1: Quality characteristics of different egg sizes*

| PARAMETERS | SMALL 50 – 59 g | MEDIUM 60 – 69 g | LARGE ≥ 70 g | ±SEM | P-value |
|---|---|---|---|---|---|
| Egg weight (g) | 54.99[c] | 65.82[b] | 72.19[a] | 0.99 | 0.001 |
| Egg length (cm) | 4.22[c] | 4.49[b] | 4.71[a] | 0.04 | 0.015 |
| Egg width (cm) | 2.65[c] | 2.99[b] | 3.12[a] | 0.03 | 0.01 |
| Yolk weight (g) | 18.11[b] | 22.17[a] | 22.34[a] | 0.31 | 0.01 |
| Yolk length (cm) | 4.51[b] | 4.76[a] | 4.77[a] | 0.03 | 0.02 |
| Yolk height (mm) | 16.10[b] | 17.89[a] | 16.68[b] | 0.25 | 0.02 |
| Yolk index (%) | 36.42 | 37.65 | 35.10 | 0.62 | 0.18 |
| Albumen weight (g) | 27.94[c] | 33.69[b] | 40.00[a] | 0.70 | 0.03 |
| Albumen length (cm) | 9.07[c] | 9.76[b] | 10.52[a] | 0.12 | 0.02 |
| Albumen height (mm) | 6.10 | 6.47 | 6.16 | 0.18 | 0.26 |
| Albumen index (%) | 6.85[a] | 6.66[a] | 5.89[b] | 0.22 | 0.014 |
| Shell thickness (mm) | 0.33 | 0.33 | 0.35 | 0.05 | 0.28 |
| Shape index (%) | 63.42 | 66.60 | 66.62 | 0.79 | 0.16 |
| Haugh unit (%) | 77.48 | 76.46 | 75.28 | 1.38 | 0.31 |

[a.b.c], : Means with different superscripts within a column are significantly different(P<0.05)

*Table.2: Correlation of egg sizes with egg yolk and egg albumen weights*

| Egg Sizes | Correlation Coefficient | |
|---|---|---|
| | Yolk | Albumen |
| Small | 0.88** | 0.79** |
| Medium | 0.79** | 0.85** |
| Large | 0.69** | 0.62** |

*Table.3: Effects of egg sizes on hatchability and the initial weight of hatched chicks*

| TREATMENT | HATCHABILITY (%) | WEIGHT OF DAY OLD CHICKS (g) |
|---|---|---|
| SMALL | 74.50[a] | 38.79[c] |
| MEDIUM | 72.50[b] | 42.72[b] |
| LARGE | 69.49[c] | 49.19[a] |
| ±SEM | 0.78 | 1.54 |
| P-value | 0.03 | 0.001 |

[a.b.c] : Means with different superscripts within a column are significantly different (P<0.05)

*Table.4: Effects of egg sizes on yolk, albumen and chick weight*

| Parameters | Small | Medium | Large | ±SEM | P-value |
|---|---|---|---|---|---|
| Average Egg Weight (g) | 54.99 [c] | 65.82 [b] | 72.19 [a] | 1.00 | 0.001 |
| Average Chick Weight (g) | 38.79 [c] | 42.72 [b] | 49.19 [a] | 1.54 | 0.001 |

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| Parameters | Small | Medium | Large | ±SEM | P-value |
|---|---|---|---|---|---|
| Yolk Weight (g) | 18.11[b] | 22.17[a] | 22.34[a] | 0.31 | 0.01 |
| Albumen Weight (g) | 27.94[c] | 33.69[b] | 40.00[a] | 0.70 | 0.03 |
| % Yolk Weight | 32.86 [a] | 33.71 [a] | 30.99 [b] | 0.25 | 0.01 |
| % Albumen Weight | 50.85 [b] | 51.08 [b] | 55.38 [a] | 0.38 | 0.03 |
| % Egg/Chick Weight | 70.21 [a] | 64.01 [c] | 68.72 [b] | 0.71 | 0.014 |
| Egg/Chick Weight Loss (g) | 16.50 [a] | 22.49 [b] | 22.61 [b] | 0.71 | 0.02 |
| % Egg/Chick Weight Reduction | 29.79 [a] | 35.99 [b] | 31.28 [a] | 0.71 | 0.02 |

[a.b.c] : Means with different superscripts within a row are significantly ($P < 0.05$)different

*Table.5: Regression equations derived from parameters of the relationship between the day-old chick weight and other egg qualities*

| Parameters | Regression equation | $R^2$ | P value |
|---|---|---|---|
| Day-old Chick Weight vs Egg size | $Y= 12.48 + 0.49X_1$ | 0.57 | 0.001 |
| Day-old Chick Weight vs Yolk Weight | $Y= 20.62 + 1.12X_2$ | 0.29 | 0.001 |
| Day-old Chick Weight vs Albumen Weight | $Y= 17.95 + 0.77X_3$ | 0.68 | 0.001 |

Where Y= Day-old Chick Weight, $X_1$= Egg size, $X_2$= Yolk Weight and $X_3$= Albumen Weight

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# Impact of Exchange Rate Deregulation on Manufacturing Sector Performance in Nigeria

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***Abstract—*** *The study examined the impact of exchange rate deregulation on manufacturing output performance in Nigeria over the period 1980 to 2016. The normalized co-integration technique was used to test for long-run relationship between exchange rate and manufacturing output while the granger causality test was used to ascertain the direction of causality between them. Also, the error correction mechanism (ECM) was used to calculate the speed of adjustment of the model to short-run disequilibrium condition. The empirical findings revealed that exchange rate has non-significant positive long-run effect on manufacturing industry output. However, unidirectional causal impact of exchange rate on manufacturing output was established using the pairwise granger causality test. Based on the above result, it is recommended that in discharging the mandate of exchange rate management, the monetary authorities should aim at stabilizing exchange rate through the use of appropriate monetary policy tools as well as support export diversification programmes in order to enhance foreign exchange inflow.*

***Keywords— Exchange rate, Manufacturing output, Exchange rate management, Monetary policy.***

## I. INTRODUCTION

In modern economies, the manufacturing sector is generally regarded as capable of accelerating the growth and development process. One major reason for this is the nature of activities in the sector which is believed to involve significant linkages across other sectors in terms of contribution to and from these sectors (Okigbo, 1993; Opaluwa, Umeh, and Ameh, 2010). However, the manufacturing sector in Nigeria is still under-developed, with very low level of capacity utilization and contribution to aggregate output in spite of the fact that it is considered the fastest growing sector in Nigeria since 1973/1974 (Ojo, 1990; Obadan, 1994). Low level of development in the sector has often been attributed to increasingly dependence on the external sector for import of essential manufacturing inputs (Okigbo, 1993). Inability to source foreign exchange at affordable rates can impair the capacity to import, thereby impacting negatively on manufacturing performance.

The structural adjustment programme (SAP) which was adopted in 1986 to restructure the Nigerian economy led to an increase in agricultural output but also had negative effect on the manufacturing sector (International Labour Organization, 1996). SAP entailed the deregulation of prices (including exchange rate) which led to unstable and rising trends in the general price level. This unintended consequence of SAP led to de-industrialization and rising unemployment in the economy. It should be noted that after 28 years of exchange rate deregulation as entrenched in SAP, the industrialization process in Nigeria is still very slow while unemployment is on the increase. Iyoha (2003) noted that the decline in manufacturing contribution to GNP showed that SAP, indeed, impacted adversely on the operations of the manufacturing sector in Nigeria. The relative share of industrial output in GDP achieved a high level of 45.57 percent in 1980 and a low level of 26 percent in 1986. With the adoption of SAP, the manufacturing sector's relative share of national output declined even further, reaching a low level of 5.2 percent in 1989.Manufacturing capacity utilisation fell from about 73.3 percent in 1981 to 38.3 percent in 1985. This translates to a decline of about 45 percent. It further reduced from 38.1 per cent in 1992 to an all-time low of about 29.29 percent in 1995 and has not exceeded an annual average of 57 percent up to 2010 (CBN, 2015; Achugamonu, 2017)

For an open economy that depends on importation to support domestic production, exchange rate plays a critical role in its ability to attain optimal production capacity. Thus, exchange rate fluctuations/uncertainty which attended the introduction of exchange rate deregulation had serious implications for

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the macroeconomic stability of the country. For example, an over-valued exchange rate hurts the performance of export industries thereby reducing foreign exchange inflow, leading to unsustainable balance-of-payments deficits. On the other hand, excessive devaluation of the domestic currency or depreciation of the exchange rate increases the cost of imported production inputs thereby fuelling inflationary pressures. The Nigerian manufacturing sector imports most of its industrial inputs thereby raising the cost of production. This discourages investment in the sector and in the process retards manufacturing sector output growth.

Based on the above background, this study examined the performance of the manufacturing sector in the post exchange rate deregulation period in order to ascertain the extent to which deregulation has affected the contribution of the manufacturing sector to the economy. Estimation techniques of the Johansen normalized co-integration and Granger causality were employed in the study. The error correction mechanism (ECM) was used to determine the speed of adjustment of the model in the case of a disequilibrium in the system.

## II. INSIGHTS FROM LITERATURE.

Preservation of the value of the domestic currency, maintenance of favourable external reserve position and attainment of both internal and external balance, among others, are major objectives of exchange rate management in Nigeria. Exchange rate policy is an essential component of macroeconomic management in Nigeria because the dynamics of exchange rate have significant implications for a country's balance of payment position, income distribution and growth (Oyejide and Ogun, 1985). It is often argued that the behaviour of exchange rate determines the behaviour of several other macroeconomic variables (Oaikhenan and Edo, 2002). Exchange rate movements, for instance, affect other indicators of a nation's economic health like interest rate, inflation rate, unemployment rate, term of trade (Douglas and Jike,2005). Hence, exchange rate which is a measure of the strength of a currency relative to another currency or a group of currencies is both an instrument of macroeconomic management and an indicator of macroeconomic performance.

Abdullahi (1981) and Ammani (2011) observed that after many years of independence, Nigeria could nether produce sufficient consumer goods for its rapidly increasing population nor provide for the raw material needs of agro-based industries like oil mills, textile and paper mills, the furniture industry etc. let alone producing for export. Indeed, many of the agro-based industries are either closing shop or are operating at sub-optimal levels due to inability to import part or all of the raw materials required to support their operations. In addition, other performance assessment indicators suggest that the country was in need of a structural reform and it was against this background that the structural adjustment programme (SAP) was introduced in 1986to address perceived structural imbalance arising from over-dependence on both consumer and industrial goods imports.

Ahmed and Lipton (1997) opined that structural adjustment refers to a set of measures designed to fast-track or accelerate the process of economic development through correction of structural imbalance in an economy. The World Bank and IMF often emphasize such measures as conditions for financial support. These reforms aim at eliminating distortions such as currency overvaluation, high fiscal deficits, trade restrictions and inefficiencies in public service delivery which impair efficiency in allocation of economic resources. The structural adjustment programme (SAP) derived from the Washington consensus or agreement was adopted in the mid-1980s to restructure and redirect the Nigerian economy, eliminate price distortions and diversify its productive base. This was a follow-up to earlier failed attempts to lift the country out of the adverse macroeconomic condition that confronted it in the early part of that decade. Exchange rate deregulation was a major policy instrument of the structural adjustment programme.

However, exchange rate deregulation had unintended consequences on the Nigerian economy thereby bringing to question whether it was indeed a suitable option for Nigeria at the time it was introduced (Ude, 1996).The consequences, according to Osisioma (2004), include a general hike in prices of most finished goods, low aggregate demand for manufactured goods, accumulation of inventories of unsold finished products, and production cut-backs. Uche (2000) attributed the failure of the deregulated regime the promote economic stability and jump-start the growth process of non-oil sectors, like manufacturing, largely, to lack of fiscal discipline and deficit budgeting. Also, Oyejide (1985) and Umubanmwen (1993) emphasized the adverse consequences of the Bretton Woods system which induces variability in the exchange rate and which also reduces the ability to import on the country that adopts Washington Consensus. Drawing from the above scenario, Anyanwu, Oyefusi, Oaikhenan, and Dimowo (1997) argued that currency devaluation, occasioned by exchange rate deregulation has not significantly affected economic performance positively in Nigeria. An assessment of the competitiveness of the real exchange rate constitutes a major component of a country's macroeconomic performance. Some developing nations are believed to have adopted currency devaluation as a policy option for boosting domestic export (Haddad and Pacavo,

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2010). According to Sanger and Wines (2010), China effectively used this strategy to drive domestic production and enhance its export competitiveness.

Over the years scholars have examined the link between exchange rate and economic performance in both developed and developing economies but not many have focused on sectoral impact of exchange rate, particularly in a developing economy like Nigeria. Also, evidence from some of these studies have not been consistent. For instance, while studies by Enekwe, Ordu and Nwoha (2013), Adedokun (2012), Modebe, Okoye and Ahmed (2017), Okonkwo (2012), Okoye, Okorie and Nwakoby (2017) presented evidence of significant positive impact of exchange rate on manufacturing performance, others by Ayinde (2014), Maduabuchi and Ajudua (2014), Yaqub (2010), Arize, Osang and Slottje (2000) showed negative impact of exchange rate on the performance of the sector. However, studies by Opaluwa, Umeh and Ameh (2010), Lawal (2016), Akpan and Atan (2012) and Okoye, Nwakoby, Modebe and Okorie (2016) did not produce evidence that exchange rate has significant impact on manufacturing performance. Studies by Rodriguez and Diaz (1995), Rogers and Wang (1995),Berman, Martin and Mayer (2012) offer greater insight for a deeper understanding of the nexus between exchange rate and manufacturingoutput. These studies specifically showed the exchange rate depreciation is an impediment to manufacturing sector performance. A similar study by Ehinomen and Oladipo (2012) aligned with the outcome of the above studies. It showed that exchange rate appreciation supports manufacturing output growth. This result however contradicts Branson and Love (1988) which reported negative impact of exchange rate appreciation on manufacturing performance. In terms of causality, Okoye and Nwakoby (2015) established causal link from manufacturing capacity utilization to exchange rate, an indication that manufacturing operations in Nigeria affect exchange rate movements.

## III. SCOPE AND METHODOLOGY

The study covered the period 1980-2016. Quantitative technique of data analysis was adopted in investigating the relationship between the dependent variable (manufacturing output) and the independent variables (exchange rate, inflation rate, monetary policy rate, broad money supply, foreign direct investment, and market capitalization). Data for the study were obtained from secondary sources, specifically from CBN statistical bulletins (2016) and World Bank (2018).

### 3.1: Model Specification

The model for the is specified in the implicit form as follows:

IND = *f* (EXRT, INFL, MPR, M2, FDI, MCAP) - - - (i)

Where:

IND = Manufacturing Industry output

INFL = Inflation

MPR = Monetary policy interest rate

EXRT = Exchange rate

FDI = Foreign direct investment

M2 = Broad money supply

MCAP = market capitalisation.

The above model can be re-specified explicitly as:

$IND = EXRT^{\alpha 1}. e^{\alpha 2\, INFL}. e^{\alpha 3\, MPR}. FDI^{\alpha 4}. M2^{\alpha 5}. MCAP^{\alpha 6} e^{-}$ (ii)

The above model indicates thatmanufacturing industry output is a function of exchange rate, inflation rate, monetary policy rate, foreign direct investment (net inflows percentage of GDP), financial deepening and market capitalization. Inflation rate and monetary policy rate (interest rate) are exponential due to an intention to take the double log of the model for linearization purpose of which inflation and monetary policy are already smoothened.The variables are logged to ensure comparability of the variables on the same scale.

This, taking the log of the variables in order to ensure linearity in the equation, we have:

$LIND_t = \alpha_0 + \alpha_1 LEXRT_t + \alpha_2 INFL_t + \alpha_3 MPR_t + \alpha_4 LFDI_t + \alpha_5 LM2_t + \alpha_6 LMCAP_t + u_t$ ……. (iii)

All the variables are as previously defined above.

From theory, the *apriori* expectation of the relationship between the independent variables and industrial output are as follows: exchange rate, inflation rate, and monetary policy rate are expected to have a negative and statistically significant relationship with industrial output such that an increase in exchange rate, inflation rate and monetary policy rate, will lead to a reduction in manufacturing industry output; market capitalisation, foreign direct investment and financial deepening are expected to have a positive and statistically significant relationship with manufacturing output.

### 3.2: Technique of Estimation.

The Augmented Dickey Fuller (ADF) unit root test was conducted to test for stationary trend in the series because research has shown that time series data are often non-stationary and could produce spurious estimates (Granger, 1996; Popola, Ejemeyovwi, Alege, Adu, and Onabote, 2017). The null hypothesis of non-stationary trend is rejected if the AD Fteststatistic,at 5 per cent, is greater than or equal to the Mac Kinnon critical value, otherwise it is accepted (Popoola

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*et al*, 2017).The Johansen co-integration technique was used to establish evidence of long-run relationship among the variables. Evidence of co-integrating relationship is established if the trace statistic and or the Max Eigen-value statistic is equal to or greater than the critical value at 5 per cent.

The error correction (ECM) mechanism was used to ascertain short-run adjustment dynamics of the model. The ECM coefficient shows how quickly variables respond to short-run disequilibrium, should there be a disturbance to the model. The error correction technique corrects for short-run disequilibrium by restoring or tying the value of the dependent variable to its long-run equilibrium. The Johansen normalized co-integration was conducted to determine the long-run effect of exchange rate on manufacturing output.

## IV. RESULTS AND DISCUSSION

The results of the various tests are presented and discussed in this section as follows:

### 4.1: Results of ADF unit root test

The result of the unit root test is shown in table 1 below:

*Table.1: Unit root result*

| Variable | ADF t statistic value | Critical Value at (5 percent) | Order of Integration | Remarks |
|---|---|---|---|---|
| LIND | -5.1585 | -2.9706 | I(1) | Stationary |
| LEXRT | -5.0223 | -2.9511 | I(1) | Stationary |
| LFDI | -11.1674 | -2.9511 | I(1) | Stationary |
| INFL | -5.4164 | -2.9511 | I(1) | Stationary |
| LM2 | -3.5699 | -2.9540 | I(1) | Stationary |
| MPR | -3.9245 | -2.9798 | I(1) | Stationary |
| DLMCAP | -4.0969 | -2.9511 | I(1) | Stationary |

Source: Authors' Computation, 2018

Based on the ADF unit root test statistics, it was found that all the variables are non-stationary at level. However, stationary trend was achieved after taking the first difference at 5 per cent significance level. Given the stationary trend of all variables at their first difference (I(1)), investigation of the long run relationship using the Johansen co-integration method was conducted. The results of the Johansen co-integration trace and max eigen value results are shown in tables2 and 3 below:

### 4.2: Co-integration Test

The result of the co-integration test is presented below:

*Table.2: Johansen co-integration test result (Trace test)*

Unrestricted Co-integration Rank Test (Trace)

| Hypothesized No. of CE(s) | Eigenvalue | Trace Statistic | 0.05 Critical Value | Prob.** |
|---|---|---|---|---|
| None * | 0.811278 | 172.7168 | 134.6780 | 0.0000 |
| At most 1 * | 0.697398 | 116.0225 | 103.8473 | 0.0061 |
| At most 2 | 0.493354 | 75.38096 | 76.97277 | 0.0657 |
| At most 3 | 0.481034 | 52.26293 | 54.07904 | 0.0720 |
| At most 4 | 0.328736 | 29.96174 | 35.19275 | 0.1644 |
| At most 5 | 0.226530 | 16.40957 | 20.26184 | 0.1561 |
| At most 6 | 0.202095 | 7.676041 | 9.164546 | 0.0951 |

Trace test indicates 2 cointegrating eqn(s) at the 0.05 level

* denotes rejection of the hypothesis at the 0.05 level

**MacKinnon-Haug-Michelis (1999) p-values

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Table 2 shows that the trace statistic (172.72) is greater than 5% critical value (134.67) for the first equation and the same applies for the following equation. Hence, the null hypothesis of no co-integrating equation is rejected and the alternate hypothesis of co-integrating equations is accepted.

*Table.3: Johansen co-integration test result (Max Eigen test)*

Unrestricted Cointegration Rank Test (Maximum Eigenvalue)

| Hypothesized No. of CE(s) | Eigenvalue | Max-Eigen Statistic | 0.05 Critical Value | Prob.** |
|---|---|---|---|---|
| None * | 0.811278 | 56.69434 | 47.07897 | 0.0035 |
| At most 1 | 0.697398 | 40.64150 | 40.95680 | 0.0542 |
| At most 2 | 0.493354 | 23.11803 | 34.80587 | 0.5891 |
| At most 3 | 0.481034 | 22.30119 | 28.58808 | 0.2574 |
| At most 4 | 0.328736 | 13.55217 | 22.29962 | 0.5037 |
| At most 5 | 0.226530 | 8.733526 | 15.89210 | 0.4629 |
| At most 6 | 0.202095 | 7.676041 | 9.164546 | 0.0951 |

Max-eigenvalue test indicates 1 cointegrating eqn(s) at the 0.05 level

* denotes rejection of the hypothesis at the 0.05 level

**MacKinnon-Haug-Michelis (1999) p-values

Table 3 complements the result shown in table 2. Here, the Max-Eigen statistic (56.69) is greater than 5% critical value (47.07) for the first co-integrating equation. Though the other equations show the absence of or no co-integration, this is sufficient evidence of co-integration. Hence, a rejection of the null hypothesis of no co-integrating equations and acceptance of the alternate hypothesis of presence of co-integration.

**4.3: Long-run Estimation**

Evidence of long-run response of manufacturing to changes in the explanatory variables is presented in table 4'

*Table.4: Normalized co-integrating coefficients*

| Normalized co-integrating coefficients (standard error in parentheses) | | | | | | |
|---|---|---|---|---|---|---|
| LIND | LEXRT | INFL | LMCAP | LFDI | MPR | LM2 |
| 1.000000 | 0.115737 | 0.012056 | 0.522986 | -0.670851 | -0.035878 | -0.508188 |
| | (0.08951) | (0.00104) | (0.06783) | (0.09817) | (0.00684) | (0.08379) |
| | 1.2930 | 11.5923 | 7.7102 | 6.8332 | 5.2453 | 6.0650 |

Source: Authors' Computation, 2018

The long-run model estimation based on Johansen normalized co-integration test (table 4) shows non-significant positive effect of exchange rate (t=1.2930) on manufacturing output at 5 percent level of significance. Exchange rate coefficient of 0.115737 implies that 1 percent increase in exchange rate will induce a less than proportionate increase in manufacturing output. Though this result is not consistent with *apriori* expectation, it explains the extent to which the nation's manufacturing sector depends on foreign imports for the sustenance and expansion if its operations.

The t-statistic for inflation rate (11.5923) and the coefficient (0.012056) indicate statistically significant positive effect of inflation rate on manufacturing output. Specifically, 1 percent increase in capital will induce a less than proportionate percent increase in manufacturing output. This result also does not *apriori* expectation but it is an indication low productive capacity of the sector. The estimates for market capitalization (t=7.7102 and α=0.522986) show significant positive effect on manufacturing output, an indication that an increase in market capitalization enhances the capacity of the market to support manufacturing operations thereby raising the output of the sector. This is in agreement with *a priori* theoretical expectation.

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For foreign direct investment, the t-statistic (6.8332) and α coefficient (-0.670851) indicate significant negative effect of foreign direct investment on manufacturing output. This outcome implies the foreign direct investment inflow leads to reduction in the output of the manufacturing sector. It is however not in agreement with theory.

The result further shows that monetary policy rate (proxied as interest rate) has significant negative effect on the output of the manufacturing sector such that if interest rate is raised by 1 per cent, there is a decline in manufacturing output by about 0.05 per cent. This outcome indicates that manufacturers react to high interest rates by borrowing less, thereby not being able to produce more or even maintain existing production level.

Finally, the result financial deepening (proxied as M2) shows statistically negative effect of broad money supply on manufacturing output performance. The coefficient of -0.035878 indicates that 1 per cent increase in money supply reduces manufacturing output by about 0.04 per cent. This does not agree with *a priori* expectation but it suggests diversion of monetary aggregates away from manufacturing, possibly to sectors that offer high and fast returns.

**4.4: Granger Causality Test**

The granger causality was conducted to determine how changes in one variable affect the behaviour of the other variable. The results are presented in tables 5 and 6.

*Table.5: Granger Causality Result 1*

| **Pairwise Granger Causality Tests** | | | |
|---|---|---|---|
| **Lags: 1** | | | |
| Null Hypothesis: | **Obs** | **F-Statistic** | **Prob.** |
| LEXRT does not Granger Cause LIND | 35 | 13.8001 | 0.0008 |
| LIND does not Granger Cause LEXRT | | 0.05905 | 0.8096 |

*Table.6: Granger causality Result 2*

| **Pairwise Granger Causality Tests** | | | |
|---|---|---|---|
| **Lags: 2** | | | |
| Null Hypothesis: | **Obs** | **F-Statistic** | **Prob.** |
| LEXRT does not Granger Cause LIND | 34 | 5.00701 | 0.0136 |
| LIND does not Granger Cause LEXRT | | 0.04064 | 0.9602 |

Source: Authors' Computation, 2018

To ensure consistency in the result, the causal relationship between exchange rate and manufacturing output was examined using the pairwise granger causality method. The results of the analysis at lag one and two show that a significant unidirectional relationship exists between exchange rate and manufacturing output in Nigeria with causality running from exchange rate to manufacturing output. This implies that exchange rate significantly affects manufacturing sector output at 5 percent significance level.

Based on the result, the alternative hypothesis is accepted for the two results since the p-value of the f-statistics at lag 1 and lag 2 show (0.0008 and 0.013 respectively) are significant at 5% level of significance (> 0.05).

**4.5: Error Correction Mechanism**

To check for the ability of the model to adjust to short-run disequilibrium, the error correction mechanism model (ECM) was employed and the result is as presented in table7.

*Table.7: Short-Run Model– ECM Result*

| **Error Correction:** | **D(LIND)** | **D(LEXRC)** | **D(INF1)** | **D(LMCAP)** | **D(LFDIC)** |
|---|---|---|---|---|---|
| **CointEq1** | -0.415817 | -0.996682 | 21.64445 | 0.682066 | 1.407051 |
| | (0.13793) | (0.62770) | (68.8734) | (0.60530) | (0.93367) |
| | [-3.01478] | [-1.58783] | [ 0.31426] | [ 1.12682] | [ 1.50702] |

Source: Authors' Computation, 2018

From the result, ECM is negative (-0.42). The speed of adjustment to equilibrium in its current period is about 42 per cent This implies that about 42 per cent of the disequilibrium in the RGDP is offset by the short-run adjustment in each

period. The coefficient of adjustment of the ECM is correctly signed i.e. negative. It lies between the theoretical expectations (from -1 to 0). The negative sign indicates convergence in the long-run. Thus, the model will rightly act to correct any deviation of the dependent variable from its long-run equilibrium value.

## V. CONCLUSION AND RECOMMENDATIONS

The study examined the impact of exchange rate deregulation effects on manufacturing industry output in Nigeria. The unit root test revealed that all the variables attained stationary trend at first difference. The normalized Johansen co-integration technique was used to ascertain evidence of long-run relationship between the explanatory variables and manufacturing industry output. The empirical findings revealed that exchange rate has non-significant positive long-run effect on manufacturing industry output. However, unidirectional causal impact of exchange rate on manufacturing output was established using the pairwise granger causality test.

Based on the above findings, the study concludes that exchange rate deregulation policy has significant effect on the performance of the Nigerian manufacturing sector. Given that exchange rate has a significant relationship with manufacturing industry output, it is recommended that in discharging the mandate of exchange rate management, the monetary authorities should aim at stabilizing exchange rate through the use of appropriate monetary policy tools as well as support export diversification programmes in order to enhance foreign exchange inflow.

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# Effect of Abattoir Activities on the Ground Waters around Bodija and Akinyele Abattoirs in Oyo State

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***Abstract*****—** *This study was carried out to examine the effect of abattoir activities on ground waters around Bodija and Akinyele abattoirs in Oyo state. The work was premised on the fact that untreated wastes from the abattoir are discharged directly into open drainage which flows into a nearby stream. Sixty structural questionnaires were administered and retrievedin the study areas with thirty used in each of the two abattoirs. The survey shows that 100% of the abattoir operators in both abattoirs disposed wastes manually using spade, 90% sweep and wash the wastes into open drainages as 90% do treat their wastes before disposal at the dumping site. Physical, chemical and microbiological analysis of water samples from the well around the two abattoirs revealed no significant different in the two abattoirs. Turbidity, Total dissolved solid (TDS), and total suspended solid (TSS) were significantly higher in Akinyele abattoir than Bodija abattoir. Total coliform count (TCC) was 6.3x105 in the well around Bodija abattoir and was not significantly lower than that around Akinyele abattoir which was 7.6x105. Although Total aerobic count (2.1x106) was higher in the wells around Bodija than those around Akinyele (1.7x106) the result clearly shows that both total aerobic count and total coliform count are beyond the maximum permissible limits from bodies in charge of Health and Environment. Biochemical oxygen demand (BOD) in Bodija (5.06) was also significantly higher than that of Akinyele (2.95). This result shows that more pollutants are present in the wells around Bodija abattoir. The high microbial load and its health implications confirm the need to enforce treatment of abattoir wastes before dumping into the environment and provision of portable water for the abattoir operators and the dwellers around the abattoirs.*

***Keywords*****— Abattoir; Wastes; Water quality, Pollution.**

## I. INTRODUCTION

The importance of water to human and other biological systems cannot be over emphasized as water shortage or its pollution can cause severe decrease in productivity and deaths of living species. [1] observed that water quality degradation interferes with vital and legitimate water quality uses at any scale. Pollution of water resources reduces the availability of clean and safe drinking water to most of the world's population. [2] reported that in developing countries an estimated 80% of all diseases and over one third of deaths are caused by consuming contaminated water.

Waste generated by abattoirs include liquids and solid waste, made up of paunch content, bones,horns, and faecal components, slurry of suspended solids, fat, blood and soluble materials [3]. These wastes tend to be worrisome due to the high content of putresible organic matter, which can lead to the depletion of oxygen and an impairment or disruption of water eco-functionality and a preponderance of disease-causing organisms.

[3] Identified improper management and supervision of abattoir activities as a major source of risk to public health in South Western Nigeria as abattoir wastes contain several pathogenic species. There is no special waste disposal system or treatment. Dung is piled up and waste water containing blood and dung are discharged into a nearby stream without treatment. These result into pollution of surface and underground water especially of the abattoir and residents in the abattoir vicinity.

While the slaughtering of animals results in significant meat supplies, a good source of protein and production of useful by-products such as leather, skin and bones, the processing activities involved sometimes result in environmental pollution and other health hazards that may threaten animal and human health. In most developing countries, location and operation of abattoirs are generally unregulated they are usually located near water bodies where access to water for processing is guaranteed.

There is also the major challenge of handling animal by-products, waste products and effluents from processing activities at the abattoir. The problem of unhygienic nature and practices in abattoirs in Nigeria could also to a large extent affect the surrounding ecosystem. It has been implicated with pollution of the soil, surface and ground

water [4] and [5]. In many developing nations like Nigeria, many abattoirs dispose off their waste directly into streams or rivers and also use water from the same source to wash [6].

The need to avoid ground water pollution and the associated human health risks in meat slaughtering operations is of paramount importance in our society makes this study of great importance. This study examined the socio-economic characteristics of abattoir operators in Bodija and Akinyele slaughtering houses, identified the various waste management practices in the selected abattoirs, chemical and microbiological properties of utility waters around the slaughtering houses, identified the pollutants present in the utility water around the study areas.

## II. RESEARCH METHODOLOGY

The study was carried out in Ibadan, the capital city of Oyo State, Nigeria. It is located on geographic grid reference longitude 3° 5E, latitude 7° 20N with a population of over 3 million people [7] and having Federal, State and Local Government participation in meat processing hygiene and inspection. Two major abattoirs within Ibadan were purposively selected for this study, which were Bodija and Akinyele Abattoirs.

The primary data for the study was obtained using a well-structured questionnaire which was designed for the abattoir users to obtain information on ownership, year of establishment, available facilities in the abattoir, average number of animals killed per day, operation and activities, waste disposal methods employed, and other abattoir management issues.

Study population of this study consists of the abattoir operators in the study areas while thirty questionnaires were administered in each of the abattoirs to abattoir operators so as to assess their ethical behaviours. The investigator collected the questionnaires on the spot to ensure that all questionnaires were properly filled and collected emblock.

The second study was conducted where well water samples located within 0-250m radius along each of the two abattoir premises were collected and analysed for physical and chemical properties which included Temperature, Turbidity, pH, Dissolved oxygen (D.O), Total suspended solid (T.S.S), and Biochemical Oxygen Demand (B.O.D), also the levels of the following metals in the water samples was determined: copper, iron, zinc and lead. In addition to this, total microbial count and identification was done.

A total of six well water samples were used at Bodija abattoir while three well samples were used at Akinyele slaughter slab. In Akinyele, there were only three wells within the range of study. Well water samples were collected in 500ml PVC plastic containers previously cleaned by washing in non-ionic detergent, rinsed with tap water and later soaked in 10% $HNO_3$ for 24 hours and finally rinsed with deionized water prior to usage. For Dissolved Oxygen (DO) and Biochemical Oxygen Demand (BOD]- testing, samples were collected in 150 ml bottles. During sampling, sample bottles were rinsed with sampled water three times and then filled to the brim. To ensure that changes in sample properties did not occur while in transit to the laboratory, the bottles were placed in a cooler box, and appropriate preservation methods were applied.

The samples were labelled and transported to the laboratory. Samples were collected two times a week (Wednesdays and Fridays) for a period of three weeks. Parameters like temperature and $P^H$ were done on the spot of sample collection. Temperature was measured with the aid of mercury in bulb thermometer while the $P^H$ was measured with a $P^H$ meter. Physico-chemical parameters such as biochemical oxygen demand (BOD), dissolved oxygen (DO), total suspended solids (TSS), were used to determine the water quality and pollution effects from abattoir wastes. All chemical tests were done based on standard methods- [8]. Data collected through the survey were analysed using descriptive analysis.

## III. RESULTS AND DISCUSSION

*Table.1: Demographic status of abattoir operators*

| Location<br>Variable | Bodija n=30<br>Frequency/ % | Akinyele n=30<br>Frequency/ % | Total n=60<br>Frequency/ % |
|---|---|---|---|
| Age (yrs) | | | |
| 20-40 | 25(83.30) | 20(66.70) | 45(75.00) |
| 41-60 | 4(13.30) | 10(33.30) | 14(23.33) |
| Above 60 | 1(3.30) | 0.00(0.00) | 1(1.67) |
| Gender | | | |
| Male | 29(96.70) | 29(96.70) | 58(96.67) |
| Female | 1(3.30) | 1(3.30) | 2(3.33) |
| Marital status | | | |
| Single | 1(3.30) | 0.00(0.00) | 1(1.67) |

| | | | |
|---|---|---|---|
| Married | 29(96.70) | 30(100.00) | 59(98.33) |
| Religion | | | |
| Christian | 12(40.00) | 0.00(0.00) | 12(20.00) |
| Islam | 18(60.00) | 30(100.00) | 48(80.00) |
| Household size | | | |
| 1-5 | 14(46.70) | 12(40.00) | 26(43.33) |
| 6-10 | 13(43.30) | 14(46.70) | 27(45.00) |
| 11-15 | 3(10.00) | 4(13.30) | 7(11.67) |
| Educational level | | | |
| No formal education | 3(10.00) | 13(43.30) | 16(26.67) |
| Primary education | 13(43.30) | 17(56.60) | 30(50.00) |
| Secondary education | 12(40.00) | 0.00(0.00) | 12(20.00) |
| Adult education | 2(6.70) | 0.00(0.00) | 2(3.33) |

Percentage in parenthesis.

Table 1 above revealed that 75% of the operators were between the ages of 20-40 years. This result clearly contradicted the general (non-documented) belief that abattoir operators and meat sellers are majorly elderly people. Also 96.62% were males while the remaining 3.33% were females. This was close to the report of [9] who reported 100% abattoir workers to be male. This result shows that this job is dominated by males, this might not be unconnected with the nature of the job and the general belief that the trade is for men. Result also shows that 50% of the respondents with all of them 40% from Bodija abattoir.

*Table.2: Number of cattle slaughtered per day*

| Number of animals | Bodija n=30 | Akinyele n=30 | Total n=60 |
|---|---|---|---|
| 60-120 | 2(6.70) | 30(100.00) | 32(56.67) |
| 121-180 | 2(6.70) | 0.00(0.00) | 2(2.30) |
| 181-240 | 10(33.30) | 0.00(0.00) | 10(16.67) |
| Above 240 | 16(53.30) | 0.00(0.00) | 16(27.78) |
| Total | 30(100.00) | 30(100.00) | 60(100.00) |

Percentage in parenthesis.

Result from table 2 above shows that 6.7% of the respondents submitted that an average of 61-120 cattle are slaughtered per day in Bodija market while 6.7% also agreed that the number of cows slaughtered per day is between 121-180 about 33.3% affirmed that 181-240 were usually slaughtered however 53.3% agreed that more than 240 cattle are slaughtered in Bodija market per day. This result is in line with the findings of [10] that reported about 350 cattle per day from their personal observation. However, in Akinyele cattle market, all the respondents (100%) agree that a range 61- 120 cattle are slaughtered per day at the market. This shows that more cattle are slaughtered at Bodija than Akinyele. This result may be subjective as most of the traders usually have fear of disclosing the true picture of their performance for the fear of taxation. This enormous number of cattle being slaughtered daily implies that much waste and waste water are being released into the neighbouring environment and may be hazardous to the environment.

*Table.3: Available facilities in the two abattoirs*

| Location | Bodija n=30 | | Akinyele n=30 | | Total n=60 | |
|---|---|---|---|---|---|---|
| Variables | Frequency/(%) | | Frequency/(%) | | Frequency/(%) | |
| | Yes | No | Yes | No | Yes | No |
| Water closet | 22(73.30) | 8(26.70) | 8(26.70) | 22(73.30) | 30(50.00) | 30(50.00) |
| Incinerators | 4(13.30) | 26(86.70) | 5(16.7) | 25(83.30) | 9(15.00) | 51(85.00) |
| Refuse disposal bay | 20(66.10) | 10(33.30) | 4(13.30) | 26(86.70) | 24(40.00) | 36(60.00) |
| Lairage | 25(83.30) | 5(16.7) | 25(83.30) | 5(16.7) | 50(83.30) | 10(16.70) |
| Proper drainage | 13(43.30) | 17(56.70) | 7(23.30) | 23(76.7) | 20(33.30) | 40(66.70) |
| Sick bay | 5(16.7) | 25(83.30) | 19(63.30) | 11(36.70) | 24(40.00) | 36(60.00) |
| Slaughter unit | 29(96.70) | 1(3.30) | 29(96.70) | 1(3.30) | 58(96.70) | 3(3.30) |
| Dressing unit | 15(50.00) | 15(50.00) | 17(56.70) | 13(43.30) | 32(53.30) | 28(46.70) |

Percentage in parenthesis

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Table 3 above showed that higher proportion (60%, 85%, 66.7% and 60%) of the respondents submitted that there is no refuse disposal bay, incinerator, proper drainage and sick bay respectively in their abattoirs. This explored the different facilities available in the two abattoirs and ascertained that there has not been any improvement on the findings of [11] who reported that the state of some abattoirs in Nigeria is such that encourages unsanitary practices as they are usually without modern waste disposal facilities. This condition will present the abattoir operation as a threat to the society despite the service they render.

The table however revealed that 50% of the total respondents with 73.3% from Bodija affirmed the availability of water closet toilet in the abattoir for their use but from Akinyele, 73.3% disagreed with this. This shows that not all abattoirs have toilet facilities and from personal observation, they only have pit latrines in Akinyele and it is located close to the abattoir. 66 .1% of the respondents from Bodija abattoir confirmed that they have a refuse disposal unit, and 86.7% from Akinyele disagreed. This also shows that while Bodija has this facility, Akinyele abattoir did not. Incinerator is not available in both abattoirs with 86.7% and 83.3% of the respondents from the two abattoirs not agreeing with this respectively. Lairage is present in both markets as 83.3% of the respondents from each of the abattoirs affirmed it.

The table also shows that what is available in most of our abattoirs cannot be referred to as proper drainage system as 66.7% of the total respondents support this fact. Also, majority of the respondents in Bodija abattoir (83.3%) agreed that they do not have a sick bay for their animals while 63.3% from Akinyele said they have it thus giving an average of 40% affirming it and 60% answered in the negative. The implication of this is that majority of the abattoir do not have this facility. Ante mortem inspection unit which is very important for the inspection of animal due for slaughtering is not available in most of our abattoirs as 60% confirmed this while 40% disagreed. This result is in line with previous studies [11] and [12].

*Table.4: Type of waste generated in the abattoir*

| Waste | Bodija n=30 | | Akinyele n=30 | | Total n=60 | |
|---|---|---|---|---|---|---|
| | Yes | No | Yes | No | Yes | No |
| Fat | 25(83.30) | 5(16.70) | 13(43.30) | 17(56.70) | 38(63.30) | 22(36.70) |
| Blood | 23(76.70) | 7(23.30) | 21(70.00) | 9(30.00) | 44(73.30) | 16(26.70) |
| Bone | 23(76.70) | 7(23.30) | 23(76.70) | 7(23.30) | 46(76.70) | 14(23.30) |
| Hoof and horns | 28(93.30) | 2(6.70) | 24(80.00) | 6(20.00) | 52(86.70) | 8(13.30) |
| Faecal material | 25(83.30) | 5(16.70) | 19(63.30) | 11(36.70) | 44(73.30) | 16(26.70) |
| Rumen and gut content | 25(83.30) | 5(16.70) | 24(80.00) | 6(20.00) | 49(81.70) | 11(18.30) |
| Foetus | 22(73.30) | 8(26.70) | 21(70.00) | 9(30.00) | 43(71.70) | 17(28.30) |
| Wastewater | 29(96.70) | 1(3.30) | 23(76.70) | 7(23.30) | 45(75.00) | 15(25.00) |
| Slurry liquids | 28(93.30) | 2(6.70) | 24(80.00) | 6(20.00) | 52(86.70) | 14(23.30) |

Percentage in parenthesis.

The result in table 4 above shows wastes generated in the abattoirs with the answer 'yes' having the majority. High percentage (> 70%) of the respondents agreed that fat (63.3%), blood (73.3%), bone (76.7%), hoof and horn (86.7%), faecal material (73.3%), rumen contents (81.7%), foetus (71.7%), wastewater (75%) and slurry liquid (86.6%) are parts of the wastes produced in the study areas. This result is in agreement with the findings of [13] and [3] who identified all the products mentioned above as waste generated in various abattoirs across the country. It is important to know that where any of these waste products are poorly managed they constitute great threat to ground water in the immediate environment.

*Table.5: Method of abattoir waste removal*

| Method of waste removal | Bodija n=30 Frequency (%) | | Akinyele n=30 Frequency (%) | | Total n=60 Frequency (%) | |
|---|---|---|---|---|---|---|
| | Yes | No | Yes | No | Yes | No |
| Manual scraping with spade | 30(100) | 0(0.00) | 30(100) | 0(0.00) | 60(100) | 0(0.00) |
| Sweeping and washing into open drainage | 26(86.0) | 4(13.3) | 28(93.3) | 2(6.7) | 54(90) | 6(10.0) |
| Mechanical scraping | 2(6.7) | 28(93.3) | 1(3.3) | 29(96.7) | 3(5.0) | 57(95.0) |
| Hydraulic flushing | 2(6.7) | 28(93.3) | 2(6.7) | 28(93.3) | 4(6.67) | 56(93.33) |

Percentage in parenthesis.

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Table 5 shows that all respondents (100%) agreed that they usually employ manual form of waste removal by scraping with spade, and that they (90%) usually sweep and wash the waste into open drainage (table 5). This is in line with the findings of [6] who reported that animal blood is released untreated into the flowing stream while the consumable parts of the slaughtered animals are washed directly into the flowing water in many developing nations. Result further shows that majority of the respondents (95%) agreed that they do not use mechanical scraping and 93.3% confirmed not using hydraulic flushing. This result thus shows that our abattoir operators are yet to adopt modern method of removing abattoir waste.

*Table.6: Method of treating abattoir waste*

| Waste treatment methods | Bodija n=30 Frequency (%) | Akinyele n=30 Frequency (%) | Total n=60 Frequency (%) |
|---|---|---|---|
| No treatment | 28(93.7) | 26(86.6) | 54(90.0) |
| Chemical treatment | 0(0.0) | 4(13.3) | 4(6.6) |
| Burning | 1(3.3) | 0(0.0) | 1(1.7) |
| Chemical treatment and burning | 1(3.3) | 0(0.0) | 1(1.7) |

Percentage in parenthesis.

Since majority of the respondents (90%) agreed that they do not treat their wastes (table 6), it implies that most abattoirs in this country do not treat their waste in anyway before disposing it off. This result is in agreement with the findings of [14] who reported that there is no special waste disposal system or treatment in our abattoirs. Dung is piled up and waste water containing blood and dung are discharged into a nearby stream without treatment. This results into pollution of surface and underground water especially of the abattoir and residential area around the abattoir vicinity. Bones and hooves collected in the abattoir are burnt at the abattoir site causing smoke and air pollution in the environment.

*Table.7: Disposal of wastes*

| Disposal methods | Bodija n=30 Frequency (%) | Akinyele n=30 Frequency (%) | Total n=60 Frequency (%) |
|---|---|---|---|
| Disposal in the nearby river | 7(23.3) | 23(76.7) | 30(50.0) |
| Burning | 3(10.0) | 3(10.0) | 6(10.0) |
| Disposal at the dump site | 20(66.7) | 4(13.3) | 24(40.0) |
| Total | 30(100.0) | 30(100.0) | 60(100.0) |

Percentage in parenthesis.

Table 7 shows that majority of the respondents from Bodija (66%) usually dispose abattoir waste at the dumpsite while at Akinyele abattoir, majority of the respondents (76.7%) usually dump the waste into nearby river. The implication of this result is that disposal in the nearby river and disposal in the dumpsite are the two major ways of disposing abattoir waste in Ibadan. This probably account for pollution of air, land and water in abattoir vicinity as reported by [14] that there is no special waste disposal system or treatment. Dung is piled up and waste water containing blood and dung are discharged into a nearby stream without treatment. This results into pollution of surface and underground water especially of the abattoir and residents in the abattoir vicinity. This result is also in line with those of [15], [6] and [4]. These methods of waste disposal are dangerous for the quality of both ground and surface water in the abattoir environment.

*Table.8: Perception of both Bodija and Akinyele respondents on waste disposal methods*

| Items | SA | A | U | D | SD |
|---|---|---|---|---|---|
| My waste disposal method constitutes a threat to the environment | 13(21.7) | 34(56.7) | 5(8.5) | 7(11.7) | 1(1.7) |
| My waste disposal method is a source of pollution to a nearby well water | 7(11.7) | 5(8.5) | 10(16.7) | 29(48.3) | 9(15) |
| My waste disposal method is a source of pollution to play grounds in the neighbourhood | 10(16.7) | 5(8.5) | 14(23.3) | 28(46.7) | 3(5.0) |
| My waste disposal method constitutes a barrier to | 8(13.3) | 7(11.7) | 8(13.3) | 30(50.0) | 7(11.7) |

| | | | | | |
|---|---|---|---|---|---|
| the free flow of water in nearby stream | | | | | |
| My waste disposal method can lead to outbreak of disease in the neighbourhood | 15(25) | 10(16.7) | 6(10) | 18(30) | 11(18.4) |

Percentage in parenthesis

KEY: SA- Strongly agree A-Agree U-Undecided
D- Disagree SD-Strongly disagree

Table 8 shows that 56.7% of total respondents agreed that their waste disposal methods constitute a threat to the environment while 50% of the total respondents disagreed that the way of disposing waste in their abattoirs can constitute a barrier to free flow of water. In addition, 50% of the respondents disagreed that their unhealthy way of disposing abattoir waste can lead to outbreak of disease in the neighbourhood. This is in agreement with the discovery of [16] who studied environmental impact of abattoirs on water bodies in Kigali city. When this result is closely examined, it can be seen that majority of the respondents that disagreed are from Akinyele abattoirs since they earlier agreed that their waste disposal method might constitute a threat to the environment; their latter disagreement might not be unconnected with the fact that most of them are not as educated as their counterparts from Bodija, as such may not fully appreciate the consequence of improper waste disposal habits on the immediate environment.

*Table.9: Constraint to waste utilisation*

| Constraint | Bodija n=30 Frequency (%) | | Akinyele n=30 Frequency (%) | | Total n=60 Frequency (%) | |
|---|---|---|---|---|---|---|
| | Yes | No | Yes | No | Yes | No |
| Lack of utilization skill | 18(60) | 12(40) | 26(86.7) | 4(13.3) | 44(73.3) | 16(26.7) |
| Irritation and labour scarcity | 20(66.7) | 10(33.3) | 26(86.7) | 4(13.3) | 46(76.6) | 14(23.3) |
| Lack of vehicle and transportation cost | 18(60) | 12(40) | 22(73.3) | 8(26.7) | 40(66.7) | 20(33.3) |
| Difficulty to burn during rainy season | 18(60) | 12(40) | 17(56.7) | 13(43.3) | 35(58.3) | 25(41.7) |
| High cost of pit and chemical | 25(83.3) | 5(16.7) | 9(30) | 21(70) | 34(56.6) | 26(43.3) |

Percentage in parenthesis

Table 9 shows that 73.3% of the total respondents identified lack of knowledge and skill required as a constraint to waste utilization but 76.6% said irritation and labour scarcity are part of the constraint responsible for their inability to utilize waste. Lack of vehicle to transport the waste and transportation cost was identified by 66.7%, while 58.3% identified difficulty to burn the waste during rainy season as major constraints. Meanwhile, 56.6% identified high cost of pit and chemicals as constraints. The implication of this result is that inability to utilize waste is the reason abattoir waste is poorly managed in this part of the world.

*Table.10: Effects of abattoir operations on the physical, chemical and microbiological properties of well water samples in Bodija and Akinyele abattoirs.*

| Values and constituents | Bodija abattoir | Akinyele abattoir | **Maximum permissible limits |
|---|---|---|---|
| $P^H$ range | $6.78\pm 0.01^a$ | $6.54\pm0.01^b$ | 6.5 - 8.5 |
| Temp ($^0$C) | $27.5 \pm 0.81$ | $27.8 \pm 0.81$ | 40 |
| TDS (mg/l) | $571.14\pm6.01^a$ | $417.28\pm6.01^b$ | 500 |
| TSS(mg/l) | $0.86 \pm0.01^a$ | $0.41 \pm0.01^b$ | NG |
| Turbidity(mg/l) | $4.45\pm0.07^a$ | $4.95\pm 0.07^a$ | 5 |
| D O (mg/l) | $5.31\pm 0.01^a$ | $4.80\pm 0.01^a$ | 5 |
| BOD (mg/l) | $5.06 \pm 0.16^a$ | $2.95 \pm 0.16^b$ | NG |
| Cu (ppm) | $0.00 \pm 0^a$ | $0.00\pm 0^a$ | 1 |
| Fe (ppm) | $0.00\pm 0.01^a$ | $0.05\pm 0.01^a$ | 0.3 |

| | | | |
|---|---|---|---|
| Pb (ppm) | $0.00 \pm 0^a$ | $0.00 \pm 0^a$ | 0.01 |
| Zn (ppm) | $0.03\pm 0.03^a$ | $0.16\pm 0.03^a$ | 3 |
| Total aerobic count(cfu/ml) | $2.1 \times 10^6 \pm 0.05^a$ | $1.7\times 10^6 \pm 0.05^a$ | < 0.01 |
| Total coliform count(cfu/ml) | $6.3 \times 10^5 \pm 0.18^a$ | $7.6 \times 10^5 \pm 0.18^a$ | 0 |

Note: All values are mean± standard error of mean

Mean with the same superscript on the same row are not significantly different.

* * FEPA, (1991)., [21], [19] NG = No guideline.

Table 10 shows that the temperature of the samples collected ranges between $27.5^0$C and $27.8^0$C with the pH values of between 6.54 and 6.78 both of which fall within the FEPA acceptable limit. These values compare well with the past results of [17] and [10], which were 7.0 - 8.3, and 6.92-8.18, respectively. This implies that the pollution level of this study is relatively lower compared with their study locations. Total dissolved solids from Bodija market is higher 571.14 than the standard value which is 500 ± 6.10 (NIS value) and is higher than the permissible limit (500), while that of Akinyele is lower / below the permissible limit (417.28). Turbidity of well water samples in the two location was below the maximum permissible level of 5, with Bodija having 4.45± 0.07 and Akinyele having 4.95± 0.07, but generally from this result, the well samples from Akinyele can be said to be more turbid than that of Bodija, therefore, processing water samples from Akinyele can be more expensive than those from Bodija abattoir because turbidity has been linked with process control in treating water, and high turbidity according to [18] can indicate problems with treatment process especially, coagulation, sedimentation and filtration. Table 10 further shows that the dissolved oxygen (D.O) contents which determines the amount oxygen available for aquatic life was 5.31 in Bodija and 4.8 at Akinyele. Total suspended solids (TSS) were 0.86 and 0.41 at Bodija and Akinyele respectively and they were significantly different from each other while biochemical oxygen demand (BOD) in Bodija (5.06) was also significantly higher than that of Akinyele (2.95). This result shows that more pollutants are present in the wells around Bodija abattoir.

The result above shows that when the values from both abattoirs are compared with that of [19] the level of the following heavy metals – copper (Cu), zinc (Zn), iron (Fe) and lead (Pb) in the wells around the two abattoirs is well below the maximum permissible limit. The result is in line with the work of [17] where they reported that all the aforementioned metals fall within the normal range recommended by [20], [21].

The result clearly shows that both total aerobic count and total coliform count are beyond the maximum permissible limits from bodies in charge of Health and Environment. The well water samples were found to be heavily polluted with microorganisms. The presence of bacteria and coliform should pose a great concern because the presence of coliform indicate recent feacal contamination and the well water samples in question are not only used to wash meat, they act as drinking water to residents especially Akinyele residents. The World Health Organisation [20] recommends zero values for total coliform count.

*Table.11: Effect of abattoir operation on a particular day of the week on the utility water of the residents*

| Values and constituents | Wednesdays | Fridays |
|---|---|---|
| $P^H$ range | $6.48\pm 0.02^a$ | $6.52\pm 0.02^a$ |
| Temp($^0$C) | $27.52\pm 0.8^a$ | $27.70\pm 0.8^a$ |
| TDS (mg/l) | $613.44\pm 12.34^a$ | $527.89\pm 12.34^a$ |
| TSS(mg/l) | $0.67\pm 0.02^a$ | $0.61\pm 0.02^a$ |
| Turbidity(mg/l) | $5.01\pm 0.10^a$ | $4.57\pm 0.10^a$ |
| DO(mg/l) | $6.30 \pm 0.14^a$ | $4.25 \pm 0.14^b$ |
| BOD(mg/l) | $7.05\pm 0.25^a$ | $2.30\pm 0.25^b$ |
| Cu(ppm) | $0.00\pm 0^a$ | $0.00\pm 0^a$ |
| Fe(ppm) | $0.031\pm 0.01^a$ | $0.016\pm 0.01^a$ |
| Zn(ppm) | $0.86\pm 0.02^a$ | $0.83\pm 0.02^a$ |
| Pb(ppm) | $0.00\pm 0^a$ | $0.00\pm 0^a$ |
| Total aerobic count(cfu/ml) | $2.8 \times 10^{6a}$ | $1.4 \times 10^{6b}$ |
| Total coliform count(cfu/ml) | $1.1 \times 10^{6a}$ | $3.7 \times 10^{4b}$ |

Mean with the same superscript on the same row are not significantly different.

The days that were purposely considered were Wednesdays (being a midweek) and Fridays (a time of weekend activities is expected to pick up).

Note: All values are mean± standard error of mean

The result from table 12 shows that the values of turbidity, $P^H$, temperature, TSS and TDS on Wednesdays were not significantly different from the values obtained on Fridays while the values of dissolved oxygen (DO) and biochemical oxygen demand (BOD) were significantly different from each other on both days. The implication of this result is that the dissolved oxygen content and biochemical oxygen demand (BOD) which both have to do with the quality of the water have higher values on Wednesday as compared with Friday. Since BOD indicates the amount of putrescible organic matter present in water, it implies that the level of pollution on Wednesdays is higher than that of Fridays. This might make the cost of treating such water to be higher. Also, it may also mean and implies that less oxygen is available for aquatic life in the water on Wednesdays as compared with Fridays for the number of wells sampled. This is in line with the report of [16] who reported Lower DO usually after the effluent is discharged into the water.

## IV. CONCLUSION AND RECOMMENDATIONS

Though the water quality was generally still above recommended standards, it is however under threat if the present habit of discharging untreated abattoir wastes continues. Residents living in abattoir vicinity may in no distant time begin to experience severe consequences of pollutants from abattoir activities located in their neighborhood. In view of the findings of this work, and in addition to the fact that the abattoir is located in the heart of the town, and also, in view of the fact that the discharge of untreated abattoir wastes may continue unabated and to ensure that health of the dwellers around the abattoir is guaranteed the following recommendations are hereby made:

(i) The management body of the abattoir should see to enforcement of adequate environmental protection in the surroundings of the abattoir through effective management of abattoir wastes.

(ii) Immediate steps should be taken to put in place machinery that will enable treatment of the abattoir wastes before they are disposed.

(iii) Public awareness and enlightenment on possible effect of pollution from abattoir wastes should be made on regular basis by relevant agencies.

*(iv)* Portable water should be regularly provided for the abattoir operators and the dwellers around the abattoirs.

(v) Efforts should be made to commence activities towards the relocation of the abattoir to an area away from residential areas.

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# Integración De La Biodiversidad En La Reducción De La Contaminación En Agua

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***Resumen—*** *Una de las principales causas de la contaminación de los diferentes cuerpos de agua es la cantidad de nutrientes y materia orgánica (MO) que son vertidos como resultado de las diferentes actividades antrópicas; el excesivo enriquecimiento del agua, principalmente con Nitrógeno (N) y Fosforo (P), genera un deterioro del recurso hídrico y en general de los ecosistemas acuáticos debido a la afectación de la calidad fisicoquímica y microbiológica del agua. En esta investigación se presenta los resultados de la evaluación de dos especies de plantas emergentes Scirpus californicus y Typha angustifolia nativas de la zona de estudio utilizadas para la remoción de materia orgánica en sistemas de humedales artificiales. Se obtuvo un porcentaje de remoción de materia orgánica de 86,52 y 86,3% respectivamente en términos de los parámetros DBO y DQO.*
***Palabras claves—fitotecnologías, plantas vegetales emergentes, adsorción, sedimentación, metabolismo bacterial.***

# Integration of Biodiversity in Reducing Pollution in Water

***Abstract—*** *One of the main causes of pollution of the different bodies of water is the amount of nutrients and organic matter (OM) which are discharged as a result of various human activities; excessive enrichment of water, primarily nitrogen (N) and phosphorus (P), generates a deterioration of water resources and aquatic ecosystems generally due to the involvement of the physico-chemical and microbiological quality of water. In this research the results of the evaluation of two species of emergent plants native Scirpus californicus and Typha angustifolia of the study area used for the removal of organic matter in artificial wetlands systems is presented.*
***Keywords— phytotechnologies, emerging vegetable plants, adsorption, sedimentation, bacterial metabolism.***

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## I. INTRODUCCIÓN

Se plantea la hipótesis que la integración de la biodiversidad en la gestión de sistemas de saneamiento ambiental proporciona soluciones viables para afrontar los desafíos socioecológicos como la contaminación antropogénica, el cambio climático y la construcción de comunidades sostenibles.

Los ecosistemas naturales han llamado la atención en las últimas décadas por su efecto significativo en el desarrollo sustentable de la humanidad. El aprovechamiento y aplicación de la biodiversidad en la recuperación de los ecosistemas degradados constituye una estrategia factible aún por explorar. Por tanto, es necesario evaluar nuevas alternativas que involucren especies y procesos que aseguren la capacidad de resiliencia de los ecosistemas.

Los sistemas socioecológicos desde sus funciones y procesos, incluyen los *servicios ecosistémicos* (SE) y el *capital natural (CN)*. El primero ha sido abordado de forma creciente en las últimas décadas (p. ej. Costanza et al, 1997; Clewell 2000; MA 2005, Aronson *et al.* 2006). Los SE hacen referencia a un amplio rango de condiciones y procesos a través de los cuales los ecosistemas naturales y, las especies que los conforman, ayudan a subsistir y satisfacer la vida humana. Estos mantienen la biodiversidad y la producción de bienes de los ecosistemas (Daily 1997).

El CN representa –en términos económicos- las reservas, ganancias e intereses generados a partir de los bienes naturales, es decir los flujos de bienes y servicios de los cuales dependen las sociedades y economías para su supervivencia (Aronson *et al.* 2007 en Prado-Castillo, 2013.

Cada vez existen más evidencias de cómo la degradación del capital natural y la pérdida de servicios de los ecosistemas es una de las mayores barreras para el logro del conjunto de los Objetivos del Milenio-ODM (www.un.org/millenniumgoals/). El ODM 7 "*garantiza la sostenibilidad ambiental*", más allá de constituir una preocupación sectorial, debe convertirse en un objetivo transversal y básico para el logro de los demás ODM. En este sentido, es imprescindible reconocer el papel fundamental de la conservación y la restauración del capital natural como una verdadera herramienta de desarrollo y lucha contra la pobreza (Martín-López *et al.* 2013).

La *Restauración del Capital Natural* (RCN) recoge una serie de conceptos y herramientas que pretenden dicha integración al relacionar directamente el incremento, la inversión o la recuperación de las reservas de CN con la finalidad de promover el bienestar humano y la conservación de los ecosistemas a largo plazo (Clewell y Aronson, 2006, 2007; Aronson *et al.* 2006, 2007).

La RCN está definida según la Alianza RNC (ver www.rncalliance.org) como "cualquier actividad que favorezca la recuperación de capital natural para mejorar el abastecimiento de los bienes y servicios naturales de los cuales dependen la sobrevivencia y bienestar de la sociedad". De esta manera, el diseño de proyectos de RNC implica el buen funcionamiento de los ecosistemas, la conservación de la biodiversidad, los múltiples SE, la sostenibilidad, y los beneficios sociales (Aronson *et al.* 2007).

El déficit en las políticas de salud pública asociado al precario manejo de residuos, gestión del agua potable y aguas residuales en comunidades rurales, constituyen una oportunidad para realizar proyectos de RNC.

El uso de tecnologías apropiadas de pequeña escala, descentralizadas, basadas en recursos locales, de operatividad y mantenimiento sencillo, y que utilizan fuentes naturales de energía, no contaminantes, permitiría de acuerdo con diversos autores (p. ej. Díaz y Masera 1998; Aguilar, 1994) potenciar las capacidades productivas así como un mayor grado de bienestar y autonomía.

La presencia de material orgánico en cuerpos de agua como producto de la actividad humana disminuye la capacidad resiliente de los ecosistemas.

La investigación contempla evaluación de la capacidad de remoción de materia orgánica de las especies *Scirpus Californicus* y *Typha Angustifolia* en un humedal artificial de tipo horizontal y flujo subsuperficial a escala piloto, que permita determinar la viabilidad ecológica, social y económicaen la incorporación de especies nativas en sistemas de tratamiento de aguas residuales.

## II. MATERIALES Y MÉTODOS

El método utilizado en la investigación se desarrolló con la siguiente secuencia:

i). Identificación de las especies nativas emergentes del área de estudio con la ayuda de las comunidades. ii). Definición de los factores de diseño del sistema biológico. iii). Puesta en marcha de la unidad experimental. iv). Descripción morfológica de las especies en el sistema, para la determinación de la capacidad adaptativa de las especies. v). Análisis experimental de los índices de efectividad de las especies emergentes nativas utilizadas en el estudio. vi). Determinación de los índices de remoción de materia orgánica.

Diseño experimental

Se trabajó sobre un diseño factorial que tuvo como variable respuesta la concentración de materia orgánica (DQO y DBO5) en el efluente; y como factores, el tipo de patrón biológico (tres niveles: sistema con patrón biológico 1. *Scirpus Californicus*, sistema con patrón biológico 2. *Typha*

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*Angustifolia* y sistema control blanco) y el tiempo de crecimiento de la planta (observación durante 3 meses de crecimiento). Además, se determinaron para el proceso adaptativo el pH, la conductividad y la temperatura con el propósito de proporcionar condiciones óptimas para desarrollo de las plántulas en el sistema.

Siembra de plántulas y toma de muestras

Se recolectaron en su estado natural 50 plántulas jóvenes de las especies utilizadas en el estudio. Fueron seleccionadas 10 plántulas con características morfológicas similares para ser distribuidas en el sistema con patrón biológico 1. *Scirpus Californicus*, sistema con patrón biológico 2. *Typha Angustifolia* y sistema control blanco. Cada una de las plántulas fue sembrada en el lecho filtrante del sistema (humedal artificial de flujo subsuperficial).

Posteriormente se midieron las variables de respuesta según el siguiente plan de muestreo día cero (0), dos (2), cuatro (4), ocho (8), dieciséis (16), y treinta (30), y se realizó el control diario de los parámetros conductividad, pH y temperatura por 30 días efectivos.

Análisis del laboratorio para el agua tratada.

Las muestras de agua fueron homogenizadas y caracterizadas. La evaluación contempló el análisis pre-test y post-test para determinar y verificar cuales de los sistemas obtuvo mayor remoción de materia orgánica. Todos los análisis realizados se desarrollaron siguiendo los protocolos establecidos por el Standard Methods for the Examination of Wáter and Wastewater, 20 edition, 1998, (Métodos Estándar para la Examinación de Aguas y Aguas residuales institucionales, Edición 20, 1998) de la APHA. AWWA, WEF).

Análisis de datos.

Los datos de concentración de materia orgánica (DQO y DBO5) se presentan como media +/- error estándar. Un análisis univariado fue realizado a las variables cuantitativas de los tratamientos aplicados y a su respectivo control blanco. Se determinó el análisis de medidas de tendencia central con la media, mediana, error estándar y de posición con el rango intercuartílico.

Un análisis de varianza (ANOVA) no paramétrica fue usado para estudiar la dispersión de los datos. La prueba Kruskal - wallis para dos o más factores, se empleó para medir el efecto individual y la prueba U mann - Whilthey para medir el efecto conjunto entre los tratamientos utilizando como variable respuesta la concentración de materia orgánica (DQO y $DBO_5$) en el efluente

El análisis estadístico se realizó utilizando el software Excel y PAST. .

## III. DISCUSIÓN DE RESULTADOS

En la Figura 1 se muestran los niveles de remoción de materia orgánica en términos de la Demanda Biológica de Oxígeno ($DBO_5$). Los sistemas de tratamiento con patrón biológico Typha Angustifolia y Scirpus Californicus alcanzaron puntos de quiebre máximos de 86,3% y 86,52% respectivamente. Entre tanto, el sistema de tratamiento control obtuvo un porcentaje de remoción de 50,42%. Las tendencias evidencian un crecimiento significativo en función del tiempo de análisis, por lo que destaca la estabilidad que adquiere el sistema con el paso del tiempo. Además, se observa la poca diferencia en porcentaje de remoción que se obtuvo en la evaluación de los tratamientos con patrón biológico en función de la DBO5. , mientras que el testigo se mantuvo con índices de remoción interesantes pues evidencia que el proceso de filtración y sedimentación del fluido por el paso del sistema proporciona condiciones de depuración que hacen funcional el sistema. Ver figura 1.

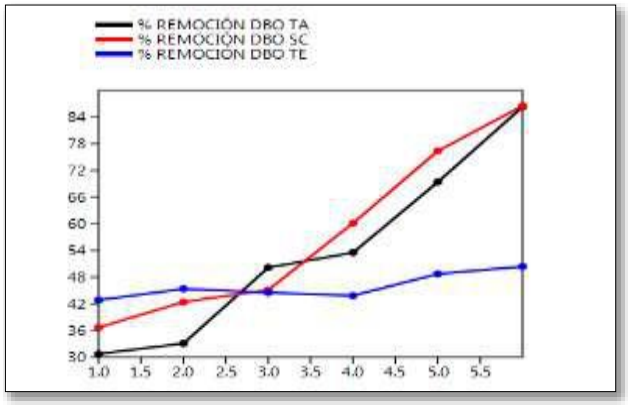


*Figura 1. Índices máximos en porcentajes de remoción de la DBO5 en el sistema*

Fuente: Quintero, Meneses, Devia & Prado. 2016.

Los datos agrupados de la DBO5 se pueden observar en la figura 2, que contiene la dispersión de los datos, medianas y percentiles. La Figura 2 muestra el comportamiento de remoción normal estándar de materia orgánica, observando la similitud de los valores debido al comportamiento y tendencias de remoción afín.

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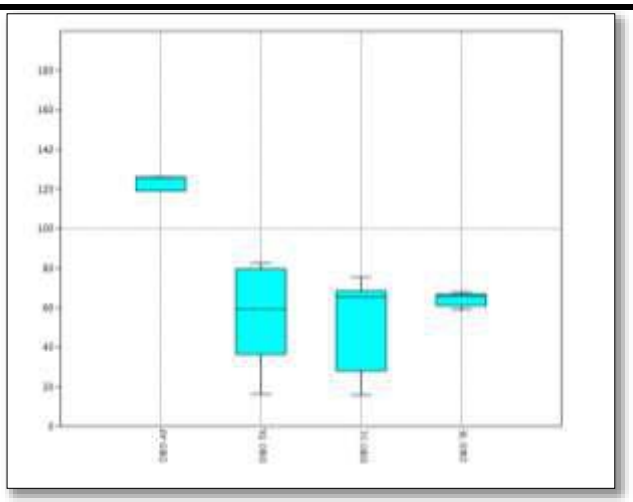

*Figura 2. Análisis comparativo y descriptivo del comportamiento de remoción de la DBO5*
Fuente: Quintero, Meneses, Devia & Prado. 2016.

La Demanda Química de Oxigeno (DQO), el segundo parámetro de respuesta del sistema presentó porcentajes de remoción que indican los niveles destacables de eficiencia de los tratamientos. La curva de remoción fluctuó de la siguiente manera, el punto máximo de remoción del tratamiento con patrón Biológico *Typha Angustifolia* fue 88,21%, para el tratamiento con patrón Biológico *Scirpus Californicus* fue de 89,78% y para el tratamiento control o blanco de 33,48%, como se puede evidenciar en la figura 3.

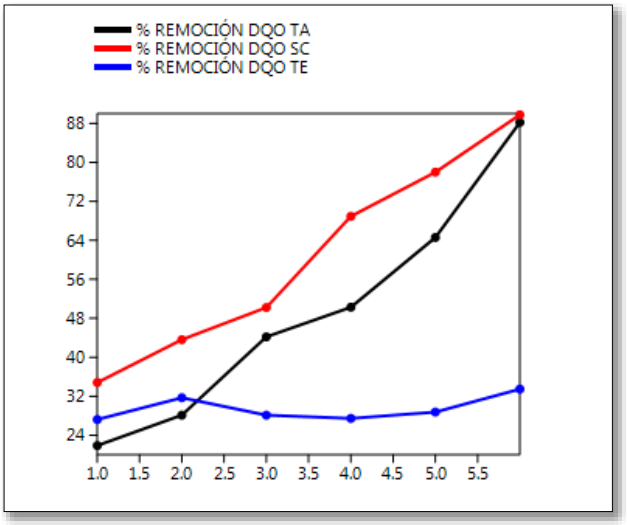


*Figura 3. Índices máximos en porcentajes de remoción de la DQO en el sistema.*
Fuente: Quintero, Meneses, Devia & Prado. 2016.

Es importante destacar las tendencias de remoción, en el patrón biológico *Typha Angustifolia*, a pesar que siempre tuvo porcentajes de remoción menores la curva se mantuvo creciendo en función del tiempo de análisis; con el patrón *Scirpus californicus* se evidencia que los niveles de remoción mantuvieron óptimos en relación a los tratamientos evaluados, pero los puntos máximos en el último día analizado fueron similares.

Los datos agrupados de la DQO se pueden observar en la figura 4, que contiene la dispersión de los datos, medianas y percentiles; permitiendo analizar el comportamiento de remoción normal estándar de materia orgánica; es importante destacar los valores de medianas de los patrones biológicos, ya que aunque presentan similitud, la tendencia establece valores favorables hacia el patrón *Scirpus Californicus*.

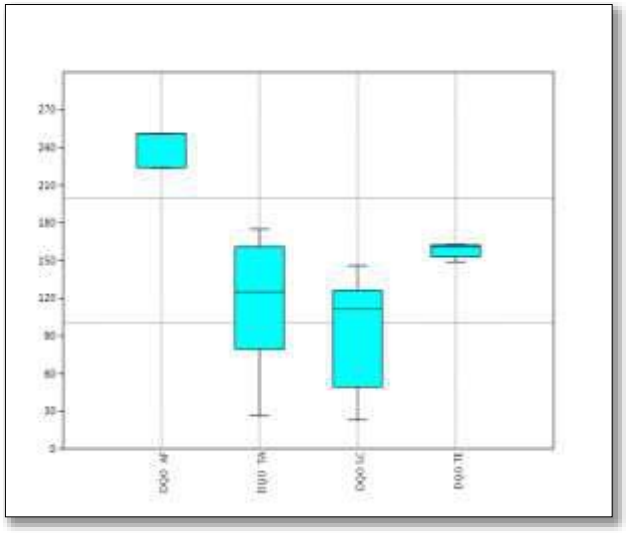

*Figura 4. Análisis comparativo y descriptivo del comportamiento de remoción de la DQO.*
Fuente: Quintero, Meneses, Devia & Prado. 2016.

En el cuadro 1 se presenta el resultado de test de normalidad de los datos DBO y DQO utilizando los softwares estadístico PAST y EXCEL. Se obtuvo un comportamiento anormal, lo que conllevó a un tratamiento estadístico con variables no paramétricas. La prueba de normalidad aplicada fue NORMALITY TESTS. En el cuadro 1 y 2 se presentan los datos de significancia estadística.

*Cuadro 1. Test de normalidad DBO*

| NORMALITY TESTS | | | | |
|---|---|---|---|---|
| | DBO mgO2/l AF | DBO mgO2/l TA | DBO mgO2/l SC | DBO mgO2/l TE |
| N | 6 | 6 | 6 | 6 |
| Shapiro-Wilk W | 0,6827 | 0,9393 | 0,9115 | 0,9036 |
| p(normal) | 0,004039 | 0,6538 | 0,4465 | 0,3959 |

**p< 0.05 Ho.** Los datos se ajustan a una distribución Normal estándar

**p> 0.05 Ha.** Los datos no se ajustan a una distribución Normal estándar

Fuente: Quintero, Meneses, Devia & Prado. 2016.

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*Cuadro 2. Test de normalidad DQO*

| NORMALITY TESTS | | | | |
|---|---|---|---|---|
| | DQO mgO2/l AF | DQO mgO2/l TA | DQO mgO2/l SC | DQO mgO2/l TE |
| **N** | 6 | 6 | 6 | 6 |
| **Shapiro-Wilk W** | 0,6827 | 0,9594 | 0,9525 | 0,8541 |
| **p(normal)** | 0,004039 | 0,8149 | 0,7606 | 0,1697 |
| **p< 0.05 Ho.** Los datos se ajustan a una distribución Normal estándar | | | | |
| **p> 0.05 Ha.** Los datos no se ajustan a una distribución Normal estándar | | | | |

Fuente: Quintero, Meneses, Devia & Prado. 2016.

Obtenido el comportamiento de los datos, en este caso como variables No Paramétricas, se aplicaron las puebras estadísticas de U de Mann-Whitney (Fligner-Kileen test for equal coefficients of variation); para la DBO5. Ver cuadro 3, 4 y 5.

*Cuadro 3. Prueba de Mann whitney para DBO (Typha Angustifolia), en el agua residual comparando el Afluente vs Efluente.*

| Fligner-Kileen test for equal coefficients of variation | | | |
|---|---|---|---|
| **DBO mgO2/l AF** | **DBO mgO2/l TA** | | |
| N: | 6 | N: | 6 |
| CV: | 3,1298 | CV: | 46,224 |
| 95% conf.: | (3,1298 6,2597) | 95% conf: | (19,753 75,937) |
| T : | 7,2285 | p< 0.05 Ha. La relación de datos Afluente/efluente presenta diferencias significativas. | |
| Expected T : | 4,0025 | | |
| z : | 2,2349 | | |
| p (one-tailed): | 0,012713 | p> 0.05 Ho. La relación de datos Afluente/efluente no presenta diferencias significativas. | |
| p (two-tailed): | 0,025427 | | |

Fuente: Quintero, Meneses, Devia & Prado. 2016.

*Cuadro 4. Prueba de Mann whitney para DBO (Scirpus Californicus), en el agua residual comparando el Afluente vs Efluente*

| Fligner-Kileen test for equal coefficients of variation | | | |
|---|---|---|---|
| **DBO mgO2/l AF** | **DBO mgO2/l SC** | | |
| N: | 6 | N: | 6 |
| CV: | 3,1298 | CV: | 47,698 |
| 95% conf.: | (3,1298 6,2597) | 95% conf.: | (24,803 80,966) |
| T : | 7,2285 | p< 0.05 Ha. La relación de datos Afluente/efluente presenta diferencias significativas. | |
| Expected T : | 4,0025 | | |
| z : | 2,2349 | | |
| p (one-tailed): | 0,012713 | p> 0.05 Ho. La relación de datos Afluente/efluente no presenta diferencias significativas. | |
| p (two-tailed): | 0,025427 | | |

Fuente: Quintero, Meneses, Devia & Prado. 2016.

*Cuadro 5. Prueba de Mann whitney para DBO (control), en el agua residual comparando el Afluente vs Efluente*

| Fligner-Kileen test for equal coefficients of variation | | | |
|---|---|---|---|
| **DBO mgO2/l AF** | **DBO mgO2/l TE** | | |
| N: | 6 | N: | 6 |
| CV: | 3,1298 | CV: | 5,4897 |
| 95% conf.: | (3,1298 6,2597) | 95% conf.: | (4,1787 9,6273) |
| T : | 5,9098 | p< 0.05 Ha. La relación de datos Afluente/efluente presenta diferencias significativas. | |
| Expected T : | 4,0025 | | |
| z : | 1,3424 | | |
| p (one-tailed): | 0,08974 | p> 0.05 Ho. La relación de datos Afluente/efluente no presenta diferencias significativas. | |
| p (two-tailed): | 0,17948 | | |

Fuente: Quintero, Meneses, Devia & Prado. 2016.

Para el caso de DQO, se aplicó la misma prueba estadística y los resultados fueron similares, en todos los casos el análisis estadístico arrojó que hay diferencias significativas en las concentraciones que entran al sistema en relación con las que salen. Ver cuadros 6, 7 y 8.

*Cuadro 6. Prueba de Mann whitney para DQO (Typha Angustifolia), en el agua residual comparando el Afluente vs Efluente.*

| Fligner-Kileen test for equal coefficients of variation | | | |
|---|---|---|---|
| **DQO mgO2/l AF** | **DQO mgO2/l TA** | | |
| N: | 6 | N: | 6 |
| CV: | 6,2267 | CV: | 48,35 |
| 95% conf.: | (6,2267 12,453) | 95% conf.: | (17,092 81) |
| T : | 7,2285 | p< 0.05 Ha. La relación de datos Afluente/efluente presenta diferencias significativas. | |
| Expected T : | 4,0025 | | |
| z : | 2,2253 | | |
| p (one-tailed): | 0,013031 | p> 0.05 Ho. La relación de datos Afluente/efluente no presenta diferencias significativas. | |
| p (two-tailed): | 0,026061 | | |

Fuente: Quintero, Meneses, Devia & Prado. 2016.

*Cuadro 7. Prueba de Mann whitney para DQO (Scirpus Californicus), en el agua residual comparando el Afluente vs Efluente.*

| Fligner-Kileen test for equal coefficients of variation | | | |
|---|---|---|---|
| **DQO mgO2/l AF** | **DQO mgO2/l SC** | | |
| N: | 6 | N: | 6 |
| CV: | 6,2267 | CV: | 54,643 |
| 95% conf.: | (6,2267 12,453) | 95% conf.: | (27,7 87,001) |
| T : | 7,2285 | p< 0.05 Ha. La relación de datos Afluente/efluente presenta diferencias significativas. | |
| Expected T : | 4,0025 | | |
| z : | 2,2253 | | |
| p (one-tailed): | 0,013031 | p> 0.05 Ho. La relación de datos Afluente/efluente no presenta diferencias significativas. | |
| p (two-tailed): | 0,026061 | | |

Fuente: Quintero, Meneses, Devia & Prado. 2016.

*Cuadro 8. Prueba de Mann whitney para DQO (control), en el agua residual comparando el Afluente vs Efluente.*

| Fligner-Kileen test for equal coefficients of variation | | | |
|---|---|---|---|
| **DQO mgO2/l AF** | **DQO mgO2/l TE** | | |
| N: | 6 | N: | 6 |
| CV: | 6,2267 | CV: | 3,6112 |
| 95% conf.: | (6,2267 12,453) | 95% conf.: | (2,7699 6,4646) |
| T : | 3,2692 | p< 0.05 Ha. La relación de datos Afluente/efluente presenta diferencias significativas. | |
| Expected T : | 4,0025 | | |
| z : | 0,51469 | | |
| p (one-tailed): | 0,30339 | p> 0.05 Ho. La relación de datos Afluente/efluente no presenta diferencias significativas. | |
| p (two-tailed): | 0,60677 | | |

Fuente: Quintero, Meneses, Devia & Prado. 2016.

En el cuadro 9 se muestra el resultado de la Prueba de Kruskal-Wallis aplicada al universo de datos y variables que intervienen en el proceso de identificación del tratamiento (especie) más eficiente. En éste se observa que para el caso de la DBO, los datos no presentan diferencias significativas. Esto debido a la tendencia creciente en porcentajes de remoción y estabilidad de los datos obtenidos en el periodo de análisis, es decir; los rangos de crecimiento de cada

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tratamiento fueron similares por lo que los sistemas presentaron comportamientos estables en cuanto a niveles de aumento de porcentajes de remoción.

*Cuadro 9. Prueba de Kruskal-Wallis para DBO en los sistema piloto*

| | DBO mgO2/l TA | DBO mgO2/l SC | DBO mgO2/l TE |
|---|---|---|---|
| DBO mgO2/l TA | | 1 | 1 |
| DBO mgO2/l SC | 1 | | 1 |
| DBO mgO2/l TE | 1 | 1 | |
| **p< 0.05 Ha**. La relación de datos Afluente/efluente presenta diferencias significativas. | | | |
| **p> 0.05 Ho.** La relación de datos Afluente/efluente no presenta diferencias significativas. | | | |

Fuente: Quintero, Meneses, Devia & Prado. 2016.

Para el caso de la DBO, los datos presentaron diferencias significativas en la comparación del tratamiento con patrón vegetal *Scirpus Californicus* en relación con el tratamiento Testigo, lo que destaca que los niveles de remoción tienen diferencias relevantes que permiten diferir sobre la eficiencia del sistema con patrón vegetal frente al tratamiento testigo; situación que no se presenta con el tratamiento con patrón vegetal *Typha Angustifolia* en donde estadísticamente no se presentan diferencias significativas con ninguno de los demás tratamientos.

*Cuadro 10. Prueba de Kruskal-Wallis para la DQO en los sistema piloto*

| | DQO mgO2/l TA | DQO mgO2/l SC | DQO mgO2/l TE |
|---|---|---|---|
| DQO mgO2/l TA | | 1 | 0,5982 |
| DQO mgO2/l SC | 1 | | 0,01522 |
| DQO mgO2/l TE | 0,5982 | 0,01522 | |
| **p< 0.05 Ha**. La relación de datos Afluente/efluente presenta diferencias significativas. | | | |
| **p> 0.05 Ho.** La relación de datos Afluente/efluente no presenta diferencias significativas. | | | |

Fuente: Quintero, Meneses, Devia & Prado. 2016.

## IV. CONCLUSIÓN

La evaluación de las especies vegetales *Typha Angustifolia* y *Scirpus Californicus* en función de la remoción de materia orgánica medida a través de las variables de respuesta (DBO5 y) DQO), presentó niveles de remoción destacables, generando una curva de remoción con punto de quiebre máximo de 86,30% para el tratamiento con patrón Biológico *Typha Angustifolia*, 86,52% para el tratamiento con patrón Biológico *Scirpus Californicus* y 50,42% para el tratamiento de control y para la *Typha Angustifolia* fue 88,21%, para el tratamiento con patrón Biológico *Scirpus Californicus* fue de 89,78% y para el tratamiento control de 33,48% respectivamente.

Al realizar el análisis estadístico de la data obtenida se concluye que entre los sistemas de tratamiento que poseían patrón vegetal no hubo diferencias significativas; es decir, los porcentajes y rangos de remoción fueron similares en los dos patrones biológicos utilizados en la investigación. Por lo tanto el punto de análisis va a diferir con respecto a los puntos máximos y mínimos de remoción de materia orgánica en cada sistema.

Los resultado obtenidos en el sistema piloto de humedales artificiales con patrones vegetales *Scirpus Californicus* y *Typha Angustifolia*, destacan esta técnica de reducción de la contaminación orgánica en aguas como una alternativa eficiente a nivel tecnológico, económico, ecológico y social que contribuye al uso, manejo y conservación de la biodiversidad del territorio.

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# Characterization and Suitability Evaluation of Soils of a Toposequence at University of Agriculture Makurdi Teaching and Research Farm for the Production of Rice (*Oryza sativa*) in Makurdi, Benue State.

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***Abstract*—** *A toposequence at University of Agriculture Makurdi Teaching and Research Farm, Benue state was detail surveyed to characterize, classify and assess the suitability of the soils for sustainable rice production. Critical land and soil requirements for rice production were related with data obtained from both field and laboratory studies. The results showed that the soils had sandy loam to clay textures; weak fine crumb to strong coarse subangular blocky structure and friable to very firm consistency. All pedons except 1 had redoximorphic properties. Soil reaction ranged from slightly (6.0) to moderately acid (5.0), organic carbon (0.18-.55%). Total nitrogen and phosphorus were inadequate with low exchangeable cations and micro-nutrients. Pedon 1 was classified as Lithic Ustropept (Plinthic Cambisol (Eutric, Rhodic)) while 11 and 111 were keyed into Typic Plinthudalfs (Eutric, Plinthosols (Clayic, Greyic)). Land characteristics (mean annual rainfall, temperature, relative humidity, topography, coarse fragments and base saturation) were not major limitations for rice production however, there was no highly (S1) or moderately suitable (S2) land for rice cultivation. Productivity index (IPc) ranged between 3.10 and 10.08, and were thus currently not suitable for both upland and lowland rice cultivation by assessments of the two models. Linear model of IPp (17.55-21.06) for upland rice and (11.36) in pedon 1 for lowland rice, showed the soils were currently not suitable for rice cultivation but pedons 11 (29.25) and 111 (37.05) were marginally suitable for lowland rice cultivation. The square root model index of productivity had IPp of 21.77 in pedon 1, 26.12 in 11 and 28.37 in pedon 111. Thus, pedon 1 was currently not suitable; pedons 11 and 111 were marginally suitable for upland rice. Pedon 1 IPp (11.91) was currently not suitable whereas pedons 11 (36.79) and pedon 111 IPp (31.820) were marginally suitable for lowland rice cultivation. The soils' major limitations were the low levels of macro and micronutrients. Management techniques including continuous organic matter incorporation and mineral fertilizers application to the land will adjust the soils structure and boast their fertility level.*

***Keywords*— Characterization, Suitability, Toposequence, rice, productivity.**

## I. INTRODUCTION

Nigeria is the largest producer of rice in the West African sub region. This cereal crop constitutes a major source of calories not only for urban but the rural population with growth demand at 5% annually. Unfortunately this demand has never been met by local production leading to huge rice importation with the balance of payment of over $4 billion between 1991 and 1999 (Akande, 2008). The shortfall which may be due to low yielding rice varieties or low fertility levels of the soils among others calls for urgent need to boast local production. However, one of the problems confronting agricultural productivity in developing countries, Nigeria inclusive, is the ineffective and unplanned use of agricultural land. It is therefore imperative to apply land according to its potential suitability.

To date, the FAO guideline on the land evaluation system is widely accepted for soil evaluation. The system is based primarily on an integration of land qualities as related to individual crop requirements. The similar system developed by Sys *et al* (1993) reports the crop requirements based on the experiments/experience for the land in the tropics.

According to Fasina *et al*., (2007), the primary and most effective land conservation method is appropriate allocation of land to uses for which they are most suitable. Many studies related to various aspect of land suitability for crop cultivation have been conducted on the basis of

FAO framework in different parts of Nigeria (Ajiboye *et al*., 2011; Hassan *et al*., (2002; Fasina *et al*., 2007). Elsewhere, there are records of researches conducted using the FAO framework for land evaluation (Sys *et al*., 1993; Storie, 1933; Van Lanen *et al*., 1992).

Although the multiplicative parametric approach may have been criticized as failing in considering the relative importance of relative stable soil properties capable of dominating crop performance in the determination of suitability classes, the proposed Fuzzy techniques also has some limitations that make its use practically difficult. The matching procedure in land evaluation could be by use of limiting conditions, arithmetic procedures, or modeling. It is apparent that some of these methodologies are subject to human bias. It is pertinent to emphasize that while value judgment is inevitable in any land evaluation exercise; there is nevertheless the need to explore strategies that are intuitively superior and take into cognizance the relative importance of differentiating characteristics to crop performance.

The following soil parameters; cation exchange capacity; soil organic matter content expressed by the organic carbon content, soil depth and stoniness are amongst the main factors that influence crop adaptability to a given land area. Some conservative farming practices could as well accelerate soil chemical and physical degradation and create some of the unfavourable soils.

## II. MATERIALS AND METHODS

**The study area**

The study area lies between Latitude 07º 44.693 and 7º 45.587N and Longitude 008º 37.437E and 008º 37.483E covering an estimated area of 9 hectares. Topography is gently undulating with dominant slopes of between 0 and 5%. Elevation varies between 99 and 123 m above mean surface level. The study sites have tropical subhumid climate characterized by high humidity (> 70%). Annual rainfall ranges between 1220 and 1500mm with annual maximum mean temperature ranging from 29.5 - 33º C. The original semi-deciduous vegetation has been drastically disturbed by farming and timber logging resulting into secondary vegetation succession like bush-regrowth; thick derived savannah has taken over the place. The type of land use is majorly arable cultivation in small holdings; major crops include maize, rice, sorghum, yam, cassava, vegetable, oil palm with fruit orchards scattered over the area. The study area has fine Awe and Makurdi sandstones (upland) of cretaceous sediment while lowland has alluvium-shale intercalation both, underlain by undifferentiated basement complex materials (Offodile, 2014).

**Field work**

An area of 9 hectares was chosen to represent the farming community. The major soil types were identified following the rigid grid soil survey method. Three profile pits were sunk and morphological characterization using the pattern outlined in the soil survey manual (Soil Survey Staff, 2010; Gutherie and Witty, 1982) was carried out. Soil samples were collected from identified profile horizons for laboratory analysis. Based on the morphological characteristics, the landscape segments were classified into two mapping units.

**Laboratory analysis**

Soil samples were air-dried, crushed and passed through a-2mm sieve and analyzed using standard procedure. Soil particle size was determined by hydrometer method (Bouyoucos, 1962) with sodium hexameta-phosphate as the dispersing agent. Soil pH was determined by pH meter in water using a 1:1 soil/water ratio. Total Nitrogen was determined by Microkjeldah method. Organic carbon was determined by Walkley and Black dichromate wet oxidation method (Allison, 1965). Available phosphorus was determined by the ammonium molebdate blue method (Bray and Kurtz, 1945). Exchangeable cations (Ca, Mg, Na and K) were extracted with 1N NH4OAC**.** PH 7.0 (ammonium acetate), K and Na were determined with flame emission photometer while Ca and Mg were determined with atomic absorption spectrophotometer. Exchangeable acidity was extracted wit h 1N KCl (Maclean, 1965). CEC was determined by leaching the soil with 1N salt solution buffered at a given pH which was slightly higher than 7. Effective cation exchange capacity (ECEC) was determined by summation of the exchangeable cations and the exchangeable acidity. Base saturation was calculated as sum of total exchangeable bases (TEB) divided by the CEC x 100.

**Soil Classification and Land Evaluation Procedure**

The Soils were classified according to the USDA Soil Taxonomy (Soil Survey Staff, 2010) and the World Reference Base (2006) classification systems.

The land evaluation was done using the parametric linear models (Storie, 1933) and the square root models (Ogunkule, 1993; Uddoh, 2008; Ajiboye, 2011) of the FAO (1976) framework. Pedons were placed in suitability classes by matching their characteristics/qualities (Table 2) with the established requirement for rice production (Table 1) following the ratings of the characteristics. The most limiting characteristic(s) in a group determine the performance of the pedon, hence the final (aggregate) suitability class.

The groups of land qualities considered for evaluation were climate (c), topography (t), drainage characteristics (w), soil physical characteristics (s) and soil chemical fertility (f). Soil fertility was assessed using soil reaction, macro and micro-nutrients levels. In computing the potential suitability for rice production, the fertility factors that can be amended by fertilizer applications and management practices (level of available macronutrients,

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N, P and K and organic matter content of the soil) were excluded. However, the soil CEC, percent base saturation and pH were considered.

The current suitability was computed linearly using index of current (actual) productivity (IPc) of Storie (1933):

IPc = A X B/100 X C/100… X F/100 ….. (i)

Where,

IPc is index of current actual productivity; A is the overall least rating characteristic and B, C … F is the least rating characteristic for each land group quality.

The potential suitability IPp was similarly computed using the square root model as;

IPp = A √(B/100 X C/100 X … F/100)

Where √ is square root, A is the overall least rating characteristic and B, C … F are the least rating characteristic for each land group quality.

## III. RESULTS AND DISCUSSION

### Physical properties

Table 2 presents the physical and morphological properties of the soils of the toposequence. Pedons 11 and 111 were deep and considered highly suitable while pedon 1 was shallow and moderately suitable for rice cultivation. However all the pedons had plinthite from the B horizon through to the subsoils. The redoximophic conditions of pedons 1 and 11 as indicated by the presence of few to common, fine to coarse, faint to distinct mottles occurring from 20 – 100 cm) may be attributed to plinthite. These soils may not however, have been under permanent water saturation for a period longer than some few months as indicated by the soil colour which ranged from dark reddish brown (5YR3/4) through dull orange (5YR7/4) to grayish yellowish brown(10YR5/2). This condition is not considered as a limitation for rice cultivation.

The soil texture ranged from sandy loam to clay. Upland requires loamy soil while lowland rice requires loamy clay to sandy loamy clay for optimal performance. Thus, the toposequence presents soils with slight limitation to rice yield and were rated 65%. The soil structures ranged between weak fine crumbs to coarse sub angular blocky which are appropriate for upland and lowland rice production respectively (Sys, 1993). The structures were highly suitable for upland and moderately suitable for lowland rice production.

### Chemical properties

The soil chemical properties that could affect soil suitability for the cultivation of rice include acidity, salinity and fertility. The pH of the soil measured in water ranged from 5.48 to 5.95, indicating a moderate acid reaction (James, 2010). This may not pose serious problem for phosphorus uptake and limit micronutrients (Fe, Zn, Cu and Mn) availability which form metallic cations that precipitate into low solubility compounds at high pH levels. Total exchangeable acidity (EA) ranged between 0.06 to 1.07cmolkg$^{-1}$, an indication that exchangeable aluminum was still below toxic level.

The CEC of the soils were very low (<16 cmolkg$^{-1}$) and ranged from 4.45 to 6.12 cmolkg$^{-1}$. The relatively low values of cation exchange capacity (CEC) could be attributed to the low clay and organic matter contents, probably dominated by Kaolinite (Adesemuyi, 2014). The average values of CEC both at the surface and subsurface horizons increased downward the toposequence with the lower slope having the highest average values. Thus, with the relatively high rainfall intensity within the area, fertilizer application must be in several splits, though with increase cost of production, to avoid leaching. The low CEC values of these soils present a moderate limitation to rice cultivation. The soils have medium to high levels of exchangeable potassium, calcium and magnesium with very low levels of sodium, organic carbon, nitrogen and phosphorus (Bray 1). Most of the macronutrients (sodium, organic carbon, nitrogen and phosphorus) and the micronutrient, iron were lower than the critical requirements for rice cultivation. Thus, the greatest limitation to rice production is related to soil fertility status. This result is in agreement with the findings of Ajiboye *et al.*, (2011) and Adesanwo (2002) who evaluated of soils in parts of Ogun state, Nigeria for rice production.

The result showed that iron deficiency rather than the expected toxicity in Nigeria was the limitation of these soils apart from sodium, organic carbon, nitrogen and phosphorus.

### Other qualities

With the mean annual temperature of 33$^{o}$C, total annual rainfall and distribution of >1200mm, solar radiation 13mjcm$^{-2}$d$^{-1}$ and average relative humidity at cropping season of 71 %, the climate of the surveyed area is quite favourable for rice cultivation by Sys (1993). The topography of the toposequence with slope between 0 to 5% is considered adequate. The entire toposequence is well drained except during the rains when the middle and lower slope become saturated after heavy down pours and therefore, considered most suitable for lowland rice cultivation.

### Soil Classification

UAM1pedon possesed neither an ochric epipedon, a petrocalcic horizon nor duripan, but had base saturation of above 50 per cent throughout its 0 cm to 50 cm profile depth. It also displayed an irregular clay distribution with a weak B-horizon (Cambic horizon), formed under typical tropical climatic conditions with heavy rainfall and somewhat extreme temperatures with ustic soil moisture regime. It possessed one or more horizon within 100cm of the mineral soil surface in which plinthic material forms a continuous phase or constitutes one half or more of the

volume. The soil pedons therefore qualified at the subgroup level as Lithic Ustropept (Plinthic Cambisol (Eutric, Rhodic)).

Pedons UAM11and 111 have argillic horizons as evidence by the presence of clay cutans. They also possessed base saturation of more than 70 per cent (by $NH_4OAc$ at pH 7.0) throughout the entire profile depths while Udic soil moisture regime has been inferred for the soils, The soils are dark brown to grey but not dark red or dull red; they possessed no petrocalcic horizon within 1.5m but gradual and clear smooth but not abrupt upper boundaries of argillic horizons. They had no nitric horizon or duripan but plinthic materials and are provisionally classified into Typic Plinthudalfs (Lixic Plinthosols (Eutric, Clayic))

**Evaluation Soils for rice cultivation**

Suitability ratings of the pedons characteristics (Table 4 ) were obtained by comparing their values (Tables 2 and 3) with the land requirements for upland and lowland rice (Table 1 ) using the ratings for the limited characteristics in Table 1. Aggregate suitability ratings (potential and actual) were computed using the linear and square root parametric models.

Most of the macronutrients (sodium, organic carbon, nitrogen and phosphorus) and iron were lower than the critical requirements for rice cultivation except exchangeable potassium contents that ranged 0.19 to .0.36 $cmolkg^{-1}$. All pedons had index of current productivity (IPc) of less than 12.5 and were classified as permanently not suitable (N2) for both upland and lowland rice cultivation according to linear and square root models assessments (Tables 4). The major limiting factors were the low levels of available macronutrients and iron. The evaluation of the potential suitability of the soils without considering the levels of organic carbon, macro- and micronutrients regarded as temporary limitations using linear model indicated that all pedons had index of potential productivity (IPp) of less than 25.0 and are currently not suitable for upland and 11.36 in pedon 1 but 29.25 – 37.05 in pedons 11 and 111 for lowland rice, therefore pedon 1 is permanently not suitable (N2) while pedons 11 and 111 were marginally suitable for it cultivation.

Under the square root model of assessment, pedons 1 and 11 (IPp > 25) were marginally suitable (S3) while pedon 111 (IPp <25) was currently not suitable (N1) for upland rice. Pedons 11 and 111 (IPp .25.0) were marginally suitable (S3) and pedon 1 (IPp <12.5) was permanently not suitable (N2) for lowland rice cultivation (Table 4). The major limitations of the soils for up and lowland rice cultivation were the low levels of macro and micronutrient (Fe).

These deficiencies of the macronutrients (OC and available phosphorus) must be remedied if optimal rice production is to be achieved in the toposequence and indeed Nigeria is to be achieved. Therefore, there is need for fertilizer application strategies beyond mineral fertilizer application while fertility management techniques should be in tune with the diverse farming systems and must include crop rotation, plant residue recycling and organic agriculture as well as rapid grain legume fallowing (mucuna).

Rice is sensitive to micronutrients with iron as most limiting micronutrient limiting rice growth and yield by this study. Generally, Zn, Fe and Mn are most common on neutral and calcareous soil, intensively cropped soils, paddy and poorly drained soils (Ajiboe et al., 2011). Fertilizer recommendation for rice cultivation in many African countries often neglects the importance of these nutrients in achieving good yield. According to Ajiboy *et al.,* (2011), Africa Rice Centre Cotonu Benin accepted the possibility of iron and zinc deficiencies occurring between 1-2 and 3-4 weeks after seedling emergence respectively. The Centre recommended the application of foliar spray of ferrous sulphate or zinc sulphate only as corrective measure. Unfortunately, the current research underscored the need to assess the micronutrient status of the major rice growing area if the country will realize increase per hectare output of rice needed to achieve self sufficiency in rice production. Despite apparent deficiency of N and P in these soils, the present system recommendation (FDPP, 1989) would have corrected these deficiencies without increase in rice yield due to the neglected micronutrient deficiencies. To avoid over application of these micronutrients that may lead to toxicity, the use of organic and green manures have been suggested in India (Ajiboye, *et al*., 2011).

This study opined however that, a suitable combination of organic and inorganic fertilizer at appropriate rates after laboratory and field studies will be of tremendous importance in solving the problem of low fertility soils for rice production in Nigeria.

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*Table.1: Land Requirements for Suitability Classes for Upland and Lowland Rice Cultivation*

| **Land Qualities** | **Rate** | **95-100** | **70-94** | **55-69** | **40-54** | **20-39** | **0.00-19** |
|---|---|---|---|---|---|---|---|
| | **Class** | **$S1_1$** | **$S1_2$** | **S2** | **S3** | **N1** | **N2** |
| **Climate** | **c** | | | | | | |
| Mean Annual Rainfall | mm | >1000 | 900-1000 | 800 – 900 | 600 – 800 | 500 – 600 | < 500 |
| Mean Annual Max. Temp. | °c | >25 | 22-25 | 20-22 | 18-20 | 16 – 18 | < 16 |
| Relative Humidity | % | >75 | 70-75 | 65-70 | 60 – 65 | < 60 | - |
| **Topography** | **t** | | | | | | |
| Slope | % | < 2 | 3-4 | 5–6 | 7-8 | 9 – 10 | > 10 |
| **Drainage** | **w** | | | | | | |
| Wetness | | WD (ID)* | MWD (ID)* | MD | ID (WD)* | PD (WD)* | PD (WD)* |
| Flooding | | F0 | F0 | F1 | F1 | F2 | F3 |
| **Soil Physical Properties** | **s** | | | | | | |
| Texure | | L (LC)* | Lfs (SLC)* | LS (SL)* | S | S | S |

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| Structure | | Cr (SAB)* | Cr (SAB)* | SAB (Cr)* | SAB (Cr)* | Col (Cr)* | Col (Cr)* |
|---|---|---|---|---|---|---|---|
| Coarse Fragments (0-50cm) | % | <3 | 3-5 | 5 – 10 | 10 – 15 | >15 | - |
| Soil Depth | s | >75 | 65 – 70 | 50 – 65 | 35 – 50 | 30 – 35 | <30 |
| **Soil Fertiliy** | **f** | | | | | | |
| pH | water | 5.5 – 6.5 | 5.0-5.5 | 4.5 – 5.0 | 4.0 – 4.5 | <4.0 | |
| CEC | ($cmolkg^{-1}$ clay) | > 16.0 | 12 – 16.0 | 8 – 12.0 | 5.0- 8.0 | <5.0 | - |
| Base Saturation | % | > 80 | 70 – 80 | 50 – 70 | 40 – 50 | 25 – 35 | <25 |
| **Macro-nutrients** | | | | | | | |
| Nitrogen | % | > 2.0 | 1.5 – 2.0 | 1.0 – 1.5 | 0.5 – 1.0 | <0.5 | |
| Avail. P | $mgkg^{-1}$ | > 20 | 15 – 20 | 8 – 15 | 5 – 8 | 3 – 5 | <3 |
| Exractable K | $cmolkg^{-1}$ | > 0.50 | 0.3 – 0.5 | 0.20 – 0.30 | 0.10 – 0.20 | <0.1 | |
| **Micro-nutrients** | **0.5NHCl** | | | | | | |
| Iron | $mgkg^{-1}$ | >4.5 | 3.5 – 4.4 | 2.5 – 3.5 | 1.5 – 2.5 | 1.0 – 1.5 | <1.0 |
| Zinc | ‘’ | 2.0 – 2.5 | 1.5 – 2.0 | 1.0 – 1.5 | 0.8 – 1.0 | 0.6 – 0.8 | <0.6 |
| Mn | ‘’ | 1.5 – 1.7 | 1.0 – 1.5 | 0.8 – 1.0 | 0.6 – 0.8 | 0.5 – 0.6 | <0.5 |

Source: Sys *et al.*, (1993); Ajiboye *et al.*, (2011)

Key: * = Ratings for lowland rice production: SAB – Subangular blocky, Col. – Columnar, Cr – Crumb; WD – Well drained, MWD – Moderately well drained, ID – Imperfectly drained, PD – Poorly drained; L – Loam, SL – Sandy loam, LS – Loamy sand, Lfs – Loamy fine sand, SCL – Sandy clay loam and C – Clay; F0 – Rarely Flooded, F1 – Flooding Expected, F2 – Irregularly Flooded and F3 – Regularly Flooded; C - Clay, CL – Clay Loam, LS – Loamy Sand, SL – Sandy Loam, LCS- Loamy Clay Sand, CS–ClayS and, S–Sand.

*Table.2: Morphological / Physical Properties of Soils of a Toposequence at University of Agriculture Makurdi Teaching and Research Farm.*

| Pedon | | | Gravel | Sand | Silt | Clay | Colour | | Texture* | Structure‡ | Consistency+ | Concretions** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Slope** | **Horizon** | **Depth(cm)** | **%** | | | | **Matrix** | **Mottles¥** | **Class** | | **Moist** | |
| | **1** | | | | | | | | | | | |
| **Up** | **Ap** | 0-21 | 12.9 | 71.7 | 17.8 | 10.5 | 2.5YR 3/2 | - | SL | 1f-ccr | Vfr | Fe- Mn f, r |
| | **A** | 21.31 | 13.4 | 81.4 | 10.5 | 8.1 | 10YR4/4 | - | SL | 2f-csbk | F | Fe- Mn, c, f |
| | **Bw** | 31-50 | 21.6 | 77.4 | 9.2 | 13.0 | 5YR3/3 | - | SL | 3f-csbk | F | Fe-Mn, m; Qtz stones, f; |
| | | M | 16.0 | 76.9 | 12.5 | 10.5 | | | | | | |
| | **11** | | | | | | | | | | | |
| **Mid** | **Ap** | 0-20 | 9.3 | 65.7 | 21.1 | 13.2 | 5YR3/3 | - | SL | 1f-mcr | Vfr | - |
| | **AB** | 20-38 | 11.4 | 61.6 | 19.6 | 18.5 | 5YR6/6 | 1mft 10R4/2 | SL | 1f-csbk | Fr | Fe- Mn, c, r |
| | **$Bvt_1$** | 38-64 | 14.7 | 55.7 | 16.5 | 27.8 | 5YR8/3 | 2md 10R5/6 | SCL | 2f-cgr | F | Fe- Mn,, m; Qtz stones, f |

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| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **$Bvt_2$** | 64-100 | 19.0 | 45.9 | 17.8 | 36.34 | 5YR7/4 | 2md 10YR 7/8 | SC | 3csbk | F | Fe- Mn, c; Qtz stones, f |
| | | M | 13.6 | 57.3 | 18.7 | 24.0 | | | | | | |
| | **111** | | | | | | | | | | | |
| **Lower** | **Ap** | 0-20 | 6.4 | 65.9 | 19.1 | 15.0 | 5YR3/4 | - | SL | 1f-mcr | Fr | Fe- Mn, f, r |
| | **AB** | 20-29 | 8.8 | 61.9 | 17.9 | 20.2 | 7.5YR 5/4 | 2fft; 10YR 7/8 | SCL | 2f-mcr | F | Fe- Mn, c, f |
| | **$Bvt_1$** | 29-39 | 20.3 | 54.7 | 15.6 | 29.7 | 10YR5/3 | 2md; 10YR 7/8 | SCL | 2f—csbk | F | Fe- Mn,, m; Qtz stones, f; |
| | **$Bvt_2$** | 39-95 | 11.6 | 42.4 | 14.0 | 43.6 | 10YR5/2 | 3md; 10YR 7/8 | C | 3f-csbk | Vf | Fe- Qtz gravels, |
| | | M | 11.8 | 56.2 | 16.7 | 27.1 | | | | | | |

**Key:** * – C = Clay, SC = Sandy Clay, SCL = Sandy Clay Loam and SL = Sandy Loam , + -– 1 = f = friable, fr = firm, vf = very friable and vfr = very firm , ** – Fe = Iron, Mn = Manganese, c = common, f = few, m = many, r = round, Qtz = quartz , ‡ – 1 = weak, 2 = moderate, 3 = strong; f = fine, m = medium, c = coarse; cr = crumb, gr = granular and sbk = subangular blocky, ¥ – 1 = few, 2 = common, 3 = many; f = fine, m = medium, c = coarse; ft = faint, d = distinct, p = prominent.

*Table.3: Chemical Characteristics of Pedons at the Toposequence of University of Agriculture Makurdi Teaching and Research Farm, Makurdi.*

| **Pedon** | | | **pH** | **OC** | **TN** | **Av. P** | **$Ca^{2+}$** | **$Mg^{2+}$** | **$K^{2+}$** | **$Na^{+}$** | **CEC** | **TEA** | **BS** | **Zn** | **Mn** | **Fe** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Slope** | **Horizon** | **Depth (cm)** | **$H_2O$** | **%** | | **mg $kg^{-1}$** | **cmol$kg^{-1}$** | | | | | | **%** | **mg$kg^{-1}$** | | |
| **Up** | **1** | | | | | | | | | | | | | | | |
| | **Ap** | 0-21 | 6.16 | 0.26 | 0.029 | 1.55 | 2.09 | 0.97 | 0.23 | 0.08 | 4.45 | 0.00 | 75.96 | 2.72 | 1.60 | 0.99 |
| | **AB** | 21-31 | 6.13 | 0.26 | 0.032 | 0.95 | 1.87 | 1.37 | 0.17 | 0.08 | 4.54 | 0.00 | 75.55 | 3.39 | 0.93 | 0.74 |
| | **Bw** | 31-50 | 5.56 | 0.18 | 0.035 | 0.72 | 2.36 | 2.49 | 0.21 | 0.08 | 6.02 | 0.17 | 85.38 | 3.44 | 0.44 | 0.64 |
| | **M** | | 5.95 | 0.23 | 0.029 | 1.07 | 2.11 | 1.61 | 0.20 | 0.08 | 5.31 | 0.06 | 75.33 | 3.18 | 0.99 | 0.79 |
| **Mid** | **11** | | | | | | | | | | | | | | | |
| | **Ap** | 0 – 20 | 6.08 | 0.55 | 0.040 | 1.62 | 3.21 | 1.01 | 0.15 | 0.08 | 5.44 | 1.01 | 81.80 | 3.17 | 1.87 | 1.62 |
| | **AB** | 20-38 | 5.73 | 0.43 | 0.033 | 1.45 | 3.28 | 2.14 | 0.21 | 0.09 | 6.51 | 2.18 | 87.86 | 3.32 | 1.19 | 1.18 |
| | **$Bvt_1$** | 38-64 | 5.54 | 0.39 | 0.031 | 0.90 | 2.14 | 2.06 | 0.22 | 0.08 | 5.86 | 0.76 | 76.79 | 3.90 | 0.98 | 1.11 |
| | **$Bvt_2$** | 64-100 | 5.41 | 0.23 | 0.027 | 1.29 | 2.11 | 1.49 | 0.19 | 0.08 | 6.66 | 0.31 | 58.11 | 3.55 | 0.88 | 1.03 |
| | **M** | | 5.69 | 0.40 | 0.033 | 1.32 | 2.69 | 1.52 | 0.19 | 0.08 | 6.12 | 1.07 | 75.94 | 3.48 | 1.23 | 1.23 |
| **Lower** | **111** | | | | | | | | | | | | | | | |
| | **Ap** | 0 – 20 | 5.60 | 0.45 | 0.041 | 1.60 | 4.10 | 2.20 | 0.31 | 0.12 | 7.84 | 1.00 | 85.84 | 2.97 | 2.07 | 1.62 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **AB** | 20-29 | 5.45 | 0.45 | 0.043 | 1.48 | 4.80 | 2.60 | 0.42 | 0.10 | 8.44 | 0.40 | 93.84 | 3.52 | 1.09 | 1.26 |
| **$Bvt_1$** | 29-39 | 5.56 | 0.39 | 0.028 | 0.89 | 3.83 | 2.60 | 0.37 | 0.11 | 7.80 | 0.80 | 88.59 | 3.99 | 1.08 | 1.31 |
| **$Bvt_2$** | **39-95** | 5.54 | 0.28 | 0.027 | 1.32 | 3.11 | 1.81 | 0.35 | 0.11 | 6.46 | 0.67 | 83.28 | 3.70 | 0.68 | 1.23 |
| | **M** | 5.54 | 0.39 | 0.035 | 1.32 | 3.96 | 2.30 | 0.36 | 0.11 | 7.64 | 1.00 | 87.89 | 3.52 | 1.23 | 1.36 |

**Key:** TN – Total Nitrogen and Av. P = Available Phosphorus

*Table.4: Suitability Ratings of Pedons Characteristics for Upland and Lowland Rice Cultivation at the Toposequence of University of Agriculture Makurdi Teaching and Research Farm, Makurdi.*

| **Land/Soil Xtics.** | **Unit** | **Upland Rice** | | | **Lowland Rice** | | |
|---|---|---|---|---|---|---|---|
| **Pedon** | | **1** | **11** | **111** | **1** | **11** | **111** |
| **Climate** | **c** | | | | | | |
| **Mean Annual Rainfall** | **mm** | 100 = S1 | 100 = S1 | 100 = S1 | 100 = S1 | 100 = S1 | 100 = S1 |
| **Mean Annual Max. Temp.** | **°c** | 100 = S1 | 100 = S1 | 100 = S1 | 100 = S1 | 100 = S1 | 100 = S1 |
| **Relative Humidity** | **%** | 100 = S1 | 100 = S1 | 100 = S1 | 100 = S1 | 100 = S1 | 100 = S1 |
| **Topography** | **t** | | | | | | |
| **Slope** | **%** | 85 = $S1_2$ | 100 = S1 | 100 = S1 | 85 = $S1_2$ | 100 = S1 | 100 = S1 |
| **Drainage** | **w** | | | | | | |
| **Wetness** | | 100 = S1 | 54 = S3 | 54 = S3 | 54 = S3 | 95 = S1 | 95 = S1 |
| **Flooding** | | 100 = S1 | 100 = S1 | 90 = $S1_2$ | 100 = S1 | 100 = S1 | 90 = $S1_2$ |
| **Soil Phy, Prop.** | **s** | | | | | | |
| **Texure** | **class** | 65 = S2 | 65 = S2 | 85 = $S1_2$ | 65 = S2 | 85 = $S1_2$ | 85 = $S1_2$ |
| **Structure** | | 100 = S1 | 80 = $S1_2$ | 80 = $S1_2$ | 65 = S2 | 65 = S2 | 65 = S2 |
| **Coarse Frag. (0-50cm)** | **%** | 45 = S3 | 65 = S2 | 65 = S2 | 45 = S3 | 65 = S2 | 65 = S2 |
| **Soil Fertiliy** | **f** | | | | | | |
| **CEC** | **$cmolkg^{-1}$** | 55 = S2 | 60 = S2 | 50 = S3 | 55 = S2 | 60 = S2 | 50 = S3 |
| **Base Saturation** | **%** | 80 = $S1_2$ | 80 = $S1_2$ | 80 = $S1_2$ | 80 = $S1_2$ | 80 = $S1_2$ | 80 = $S1_2$ |
| **pH** | **$H_2O$** | 100 = S1 | 100 = S1 | 100 = S1 | 100 = S1 | 100 = S1 | 100 = S1 |
| **Avail. P** | **$mgkg^{-1}$** | 15 = N2 | 20 = N2 | 20 = N2 | 15 = N2 | 20 = N2 | 20 = N2 |
| **Macro-nuts** | | | | | | | |
| **Nitrogen** | **%** | 20 = N1 | 30 = N1 | 30 = N1 | 25 = N1 | 30 = N1 | 30 = N1 |
| **Exractable K** | **$cmolkg^{-1}$** | 55 = S2 | 55 = S2 | 80 = $S1_2$ | 55 = S2 | 55 = S2 | 80 = $S1_2$ |
| **Micro-nuts** | **0.5NHCl** | | | | | | |
| **Iron** | | 15 = N2 | 45 = S3 | 45 = S3 | 15 = N2 | 45 = S3 | 45 = S3 |
| **Zinc** | **$mgkg^{-1}$** | 100 = S1 | 100 = S1 | 100 = S1 | 100 = S1 | 100 = S1 | 100 = S1 |
| **Manganese** | | 65 = S2 | 80 = $S1_2$ | 80 = $S1_2$ | 65 = S2 | 80 = $S1_2$ | 80 = $S1_2$ |
| **Actual Suitability*** | **IPc** | 5.74 = | 5.26 = | 5.26 = | 3.10 = | 9.26 = | 8.78 = |
| **Potential Suitability*** | **IPp** | 21.04 = | 21.06 = | 17.55 = | 11.36 = | 37.05 = | 29.25 = |
| **Actual Suitability!** | **IPc** | 8.55 = | 6.53 = | 6.53 = | 4.07 = | 10.08 = | 9.55 = |
| **Potential Suitability!** | **IPp** | 28.37 = | 26.12 = | 21,77 = | 11.91 = | 36;79 = | 31.82 = |

Key: ***** - Suitability by Linear Model; **!** - Suitability by Square Root Model; Land/Soil Xtics – Land and Soil Characteristics; Coarse Frag. - Coarse Fragments, **Soil Phy. Prop. - Soil Physical Properties, Macro-nuts - Macro-nutrients and Micro-nuts** - Micro-nutrients, IPp - index of potential productivity, IPc - index of current productivity

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*Table.5: Qualitative Land Suitability Classes for the Different Land Indices*

| Symbol | Defination | Land Index |
|---|---|---|
| **S1** | Highly suitable | 75 – 100 |
| **S2** | Moderately suitable | 50 – 75 |
| **S3** | Marginally suitable | 25 – 50 |
| **N1** | Currently not suitable | 12.5 – 25.0 |
| **N1** | Permanently not suitable | 0.00 – 12.50 |

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# Application of Mutagenic Radiation and Research the Optimal doses of Induction of bud break and Vegetative Growth in the Grapevine (*Vitis vinifera (L.*)

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***Abstract—*** *The demand of grape in Morocco is fulfilled through import from foreign countries. The fruits of local cultivars of grapes offer a low yield. Development of seedless grape varieties having increased sweetness, higher yield with better nutritional quality is necessary to reduce the import dependency. The present research activities are the part of a grape improvement project. A pot experiment was conducted at the National Institute of Agronomic Research (INRA), Center Tangier (Morocco), during February to November 2017 to determine the suitable gamma irradiation doses on growth, leaf area and content of chlorophyll of grape seedling. One hundred vegetative bud cutting and three doses of gamma irradiation 20, 30, and 40 Gy were used as treatment. Different irradiation doses and vegetative cutting showed significant variations in respect of plant growth characters, leaf area and Chlorophyll Content. Higher doses of gamma irradiation had showed detrimental effect on grape saplings. Generally, increased in irradiation doses showed decreased and detrimental effects on most of the parameters under study. Maximum numbers of growing bud, leaf area and content of chlorophyll were found at 20 Gy irradiation dose. All parameters showed best results in 20 Gy with bud cutting initiation.*

***Keywords— Grape sapling, Gamma irradiation, Morphological Parameters, Leaf Area, Chlorophyll Content.***

## I. INTRODUCTION

Grape (*vitis vinifera L.*) belongs to the family Vitacae distributed widely all over the world (Olmo et al 1976) and originated in West Asia (Wang et al. 1999). Grape occupies the first position among the fruits in the world in terms of area and production. The world area occupied by grape is 7.63 million hectares producing 64.29 million tons per annum (FAO, 2001). But, in Morocco, the sector of vine of table is based primarily on the introduction of foreign varieties. Despite the fact that the traditional and local cultivars of grapes remain very appreciated by the consumers (Sbaghi, 2008), they do not express all their production potential and therefore offer a low yield. Grapevines may be propagated from seeds, cutting, layers, or grafts. Normally new vines grown from seeds differ markedly from the parent vigne and from each other. However, propagation, by cutting, layers, buds, or grafts, in contrast, produce vines identical with the parents in all varietal characteristics (Gulcan and Llter, 1975). Spontaneous somatic mutation has played a considerable role in improvement of vegetative propagated plants and many of the varieties under cultivation are of this origin, therefore the rate of spontaneous mutations is too low to be efficient means (Donini, 1993).

Mutant has improved cultivar with wide application. In grapevine several mutants, are now growing in preference to the original cultivar. There is great clonal variability among grapevine varieties and this is widely used by plant breeders to develop new varieties (Botta and Me, 1989; Alleweldt et al, 1990; Çoban, 1998). However, chemical and physical mutagenes were also used to increase the variability (Rathjen and Robinson, 1992). To determine the radio sensitivity of grapevine species, cultivars and clones are essential for assessing repair, recovery capacity of the vine from radiation injuries, to measure the influencing factors of these and the radio protective agents (Milosavljeviç and Mijajiloviç, 1965 ; Da Silva and Doazon, 1995). For the radio sensitivity determination the dose-effects in relation to the survival, seedling and plant heights, root shoot growth are measured most commonly (Shin et al., 1998; Hajdu et la, 1994; Körösi et al, 1995).

In order to evaluate gamma-ray (60Co) irradiation as a possible aid to increase the clonal variability, varietal responses to gamma-ray irradiation and cutting responses of each variety were studied. The aim of present study is to determine the radio sensibility of some cutting of the same variety of grapevine.

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## II. MATERIALS AND METHODS

The experiment was conducted out at the National Institute of Agronomic Research tangier (INRA), Morocco. Grapevine Doukkali was used as experimental crop in the study. There was total four lots of 100 bud cutting were irradiated separately by one of the following doses of radiation: 20 Grays (G1), 30 Grays (G2) and 40 Grays (G3). The control cuttings were untreated (G0). All cutting were sown under a greenhouse in plastic bags. The growth of cuttings was counted every 2 weeks during nine months since the date of planting. After irradiation by gamma radiation source, cutting was planted in each pot. Necessary data were recorded on the growth, morphological and biochemical parameters. Total leaf area of the plant was measured with auto matic electronic leaf area meter (model LI-3000, USA) and with imageJ software. Chlorophyll content of leaf was estimated by manual SPAD-502 Plus. The statistical analysis of variance was carried out by software SPSS ® version 20.0. The mean difference of the studies parameters among the treatments was adjusted by Ducan's Multiple Range Test (Gomez et al, 1984).

## III. RESULTS AND DISCUSSION

1. Growth characteristics

The cumulative percentage of bud (CPB) followed a logarithmic progression for both control and irradiated buds (Table 1). The control showed a CPB (96%) higher than the irradiated buds. However, the irradiated buds showed decreasing CPB based on increasing doses of radiation. The lower CPB were noted for the high radiation doses; 2 and 3% for the radiation 40 Grays, 17% for the dose of 30 Grays and 55% for the dose of 20 Grays applied on buds on grapevine.

The correlation between radiation dose and cumulative percent of bud was r= -0.99***. The equation of related regression based on radiation dose is Y=79-2.07X (Fig. 5). Based on the minimum level of 50% of cuttings surviving compared to control, lead us to locate the optima radiation dose between 15 and 20 Grays for Grapevine cuttings. The yield of cuttings surviving of these treatments was satisfactory since the rate of survival was ranged from 17 to 55%.

2. Leaf area plant

A highly significant variation in leaf area plant was observed due to the influence gamma irradiation. The maximum leaf area plant (123.7 $cm^2$) was observed in G1 and the minimum leaf area (74.8 $cm^2$) was observed in G3 (Table 2). The leaf area plant increased in low doses of irradiation. The similar results were also obtained by others (Charbaji et al. 1999; Islam et al, 2015). The maximum and the minimum leaf area plant were found in G2 and G3 respectively was statically different from others. But the leaf area plant found in the G1 was not statically different from the control.

3. Chlorophyll Content

A highly significant variation in chlorophyll content found due to different gamma irradiation doses (Table 3). At the 16/05/2017, the maximum content chlorophyll (22.8 $mg.g^{-1}$) was observed in G1 while the minimum (7.4 $mg.g^{-1}$) was observed in G3 (Table 3). At the 16/06/2017, the maximum content of chlorophyll (26.03 $mg.g^{-1}$) was found in the G1 and the minimum in the G3. But at the 04/07/2017, the maximum content of chlorophyll (32.41 $mg.g^{-1}$) was found in the G2 and the minimum (24.5 $mg.g^{-1}$) was found in the G3. The effect gamma irradiation doses and different vegetative bud stages showed variation in content of chlorophyll. Increase in irradiation doses decreased the amount of chlorophyll content also agreed by others (Lima et al, 1995; Islam et al, 2015).

Conclusion

The irradiation by gamma radiation of buds is a classical method of varietal creation by induced mutagenesis. This method aims to improve or diversify, for one or a few characters, cultivars, which already have a good agronomic value. In this study, it was evident from the result that higher dose had detrimental effect on the plant morphological and biochemical parameters. Among the irradiation doses, 20Gy showed better morphological parameters in M1 generation, however, it is difficult to say at this stage which dose and stage will show maximum mutability. In M2 generation, the expression of mutagenicity will be observed. So, the research works done will push a step forward for further observation and selection of most desirable mutant in M2 and the following generations.

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*Table.1: Cumulative rate of growing of grapevine and irradiated with increased doses (20-30-40 Grays)*

| Gamma irradiation | dates | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 28/02 | 15/03 | 28/03 | 13/04 | 25/04 | 08/05 | 23/05 | 06/06 | 21/06 | 04/07 | 18/07 | 02/08 | 15/08 | 29/08 | 12/09 | 26/09 | 24/10 | 31/10 | 08/11 |
| G0 | 0 | 25 | 36,67 | 61,67 | 86,67 | 93,33 | 96,67 | 96,67 | 96,67 | 96,67 | 96,67 | 96,67 | 96,67 | 96,67 | 96,67 | 96,67 | 96,67 | 96,67 | 96,67 |
| G1 | 0 | 0 | 10 | 31 | 33 | 34 | 39 | 42 | 42 | 44 | 45 | 50 | 55 | 55 | 55 | 55 | 55 | 55 | 55 |
| G2 | 0 | 0 | 0 | 1 | 2 | 4 | 6 | 11 | 16 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 |
| G3 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

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*Table.2: Effect of gamma irradiation on leaf area plant of grape sapling*

| Gamma Irradiation | Leaf area ($cm^2$) |
|---|---|
| G0 | 109,6 ab |
| G1 | 103,7 ab |
| G2 | 98,7 a |
| G3 | 74,8 b |
| *p (α = 0,05)* | *0,028* |

**the means do not differ significantly at 5% level according to Duncan test.*

*Tableau 3: Effect of gamma irradiation on the content of chlorophyll of grape sapling*

| | Chlorophyll of leaf (mg.g-1) | | |
|---|---|---|---|
| Gamma irradiation | 16/05 | 16/06 | 04/07 |
| G0 | 21,2 b | 22,5 b | 25,2 a |
| G1 | 22,8 b | 26,0 b | 28,3 b |
| G2 | 14,7 ab | 24,2 b | 32,4 c |
| G3 | 7,4 a | 16,6 a | 24,5 ab |
| *p (α = 0,05)* | *0,00* | *0,00* | *0,00* |

**The means do not differ significantly at 5% level according to Duncan test.*

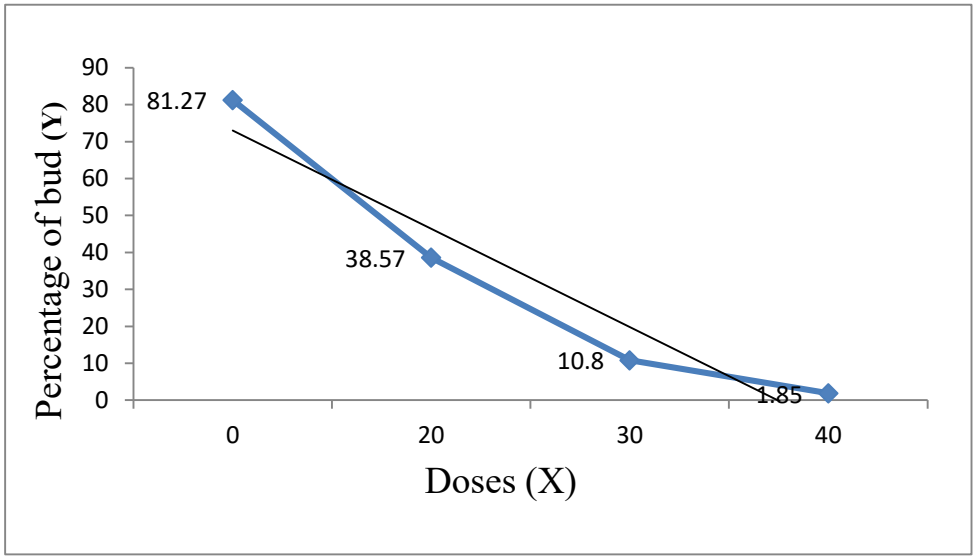


*Fig.2: Average of the percentage of growing of the cuttings of the grapevine*

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# Mass Production of Entomopathogenic Nematodes- A Review

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***Abstract*—***Utilization of entomopathogenic nematodes (EPNs) is an ecofriendly method of crop protection. EPNs can be easily mass produced. Production approaches are either in vivo or in vitro methods (solid and liquid). Most nematodes intended for commercial application are produced in solid or liquid fermentation technology. However, for laboratory research and small greenhouse or field trials, in vivo production of entomopathogenic nematodes is the common method of propagation. Mass production of EPNs is influenced by the amount of progeny required, time, resources, the costs of production, as well as the level of expertise available. The differences in nematode life cycle and bacterial symbiosis play major role in final nematode yields. This review describes the general biology of EPNs and gives an overview of studies to date on EPNs mass production.*

***Keywords*— *Entomopathogenic nematodes, bacterial symbiosis, biocontrol agent, in vivo mass production, in vitro mass production.***

## I. INTRODUCTION

Entomopathogenic nematodes (EPNs) are widely used as biocontrol agent against economically important insect pests in different farming systems, viz. fruit orchards, vegetable garden, turf grass, nurseries and greenhouses which provide environmentally safe and sustainable crop protection. EPNs can be considered good candidates for commercialization as biological control agents as they can rapidly kill the insect host; have a broad pest host range; have active searching behavior; they can be mass produced; have potential for application in integrated pest management programs; and are considered safe for vertebrates and most non-target invertebrates, therefore minimizing the registration requirements (Lacey and Georgis 2012, Lacey et al. 2015) .The use of EPNs for biocontrol involved a step-by-step scientific and technical development. Mass production of the nematodes played a key role in the commercially development of insect pests control. Steiner (1923) identified the species *Aplectana kraussei* for the first time. Later, Glaser and Fox (1930) identified a nematode infecting grubs of the Japanese beetle (*Popillia japonica*) at the Tavistock Golf Course near Haddonfield, New Jersey, USA. This nematode was described by Steiner as *Neoaplectana* (=*Steinernema*) *glaseri* (Rhabditida: Steinernematidae) from Belgium as a natural pathogen of *Hoplia philanthus* (Coleoptera: Scarabaeidae) (Steiner1929). A new species of entomopathogenic nematode, *Heterorhabditis bacteriophora,* was described by Poinar in 1975, as a new species as well as a member of new genus, and family (Heterorhabditidae) of Rhabditida. Currently, over 118 species of *Steinernema* and 20 species of *Heterorhabditis* have been described from different habitats all over the world (Hunt and Sergei,2016). Besides these, other nematode species, *Oscheius* (=Heterorhabditoides) species have been shown to use pathogenic bacteria to parasitize insect hosts. *O. chongmingensis* , *O. carolinensis*, *O.rugaoensis* and *Caenorhabditis briggsae* have been identified as potential insect pathogens (Nguyen and Hunt 2007,Zhang et al.2008,Ye et al.2010,Dillman et al.2012,Zhang et al. 2012).

## II. BIOLOGY OF ENTOMOPATHOGENIC NEMATODES

The life cycle of EPNs is characterized by an egg stage, four juvenile stages, and an adult stage. Only the third juvenile stage is the infective juvenile that is free-living in the soil, non-feeding, encased in a double cuticle with closed mouth and anus and capable of surviving for several weeks in the soil, before infecting a new host individual. Therefore, the only stage used in biological control is the third instar infective juvenile. The infective juveniles actively penetrate through the midgut wall or tracheae into the insect body cavity (hemocoel) containing insect haemolymph. EPNs have a mutualistic partnership with Gram-negative Gamma-Proteobacteria in the family Enterobacteriaceae. *Xenorhabdus* bacteria are associated with steinernematids nematodes while *Photorhabdus* are symbionts of heterorhabditids. *Xenorhabdus* occurs naturally in a special intestinal vesicle of *Steinernema* IJs (Bird and Akhurst1983) while *Photorhabdus* is distributed in the foregut and midgut of *Heterorhabditis* IJs(Boemare et al.1996) .An IJ carries

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between 0 and 2000 cells of its symbiont bacterium in the anterior part of the intestine(Spiridonov et al.1991,Endo and Nickle 1994,Forst and Clarke, 2002). *O. chongmingensis* and *O. carolinensis*, and *Caenorhabditis briggsae* have been found to associate with insect pathogenic bacteria of the genus *Serratia*, while *O. carolinensis* may have additional associates (Torres-Barragan et al. 2011). *O. chongmingensis* and *C. briggsae* require their bacterial partners to cause host death, to grow and reproduce within killed insects, and emerging dauer juveniles are associated with the vectored pathogen (Ye et al.2010) .The nematode provides protected shelter for the symbiotic bacteria and carries the bacteria into the host. Nematode and bacteria overcome the insect immune system and the host insect is killed within 48 hours post infection (Adams and Nguyen,2002) .The bacteria break down the host tissues, and provide food sources for the nematode, which feeds and multiplies on bacterial cells and degrading host tissues. During the process, the bacteria themselves provide a protected niche by producing antibiotics that suppress the competition from other microorganisms(Kondo and Ishibashi, 1986). Due to the different symbiotic bacteria associated with EPN, heterorhabditid nematodes turn the host cadaver red, purple, orange, yellow, brown or sometimes green, whereas steinernematid nematodes turn the insect cadaver tan, ochre, gray or dark gray. $J_4$ stage nematodes develop into egg laying female or male adults in the insect cadaver and hereby run through four juvenile stages ($J_1$ - $J_4$) and the adult stage has up to three generations(Kaya and Gaugler,1993).After reproduction and depletion of all nutrients, a high nematode population density triggers the nematode development into IJs again. In the case of *Steinernema*, IJs become colonized by bacteria *via* one or two founder bacterial cells. The life cycle of Heterorhabditid is similar to that of Steinernematids except for the fact that the IJs always develop into self-reproducing hermaphrodites(Poinar, 1990). Strauch *et al.* (2000) observed that offspring of the first generation hermaphrodites can either develop into amphimictic adults or into automictic hermaphrodite, both can occur simultaneously.The development into amphimictic adults is induced by favourable nutritional conditions, whereas the development of hermaphrodites is induced by low concentrations of nutrient. The lifecycle is completed in a few days and thousands of new IJs emerge, searching for new hosts. The cycle from entry of IJs into a host until emergence of new IJs is dependent on temperature and varies for different species and strains. Generally, life-cycle of EPNs (infective juvenile penetration to infective juvenile emergence) is completed within 12-15 days. The optimum temperature for growth and reproduction of nematodes is between $25^0$ C and $30^0$ C.

## III. MASS PRODUCTION OF ENTOMOPATHOGENIC NEMATODES

The most important requirement for successful and economically reasonable usage of EPNs in crop protection is their production on large scale at competitive cost within a short time (Ehlers,2001). Entomopathogenic nematodes can be easily cultured either *in vivo* or *in vitro* in the laboratory. Mass production of entomopathogenic nematodes has evolved from the first large scale *in vitro* solid media production by Glaser (1940),to the *in vivo* production by Dutky *et al*. (1964) to the three dimensional solid media *in vitro* process by Bedding(1981,1984) and to the *in vitro* liquid fermentation production method by Friedman(1990).

### *3.1 IN VIVO* MASS CULTURE

*In vivo* production is a simple process of culturing EPNs in live insect hosts (Table.1). *In vivo* nematode production is based on the White trap method; the method involves the natural migration of IJs away from the infected host cadaver into a surrounding water layer, from where it can be harvested. This method was devised, reconstructed and later on modified by several workers(White,1927,Dutky et al.1964, Poinar,1979, Woodring and Kaya, 1988,Abdel-Razek and Abd-elgawad,2007, Lindegren et al.1993). Gaugler *et al*.(2002) developed LOTEK system which does not rely on nematode migration to a reservoir. The system consists of perforated trays to secure insects, harvesters with misting nozzles that rinse IJs through the holding trays into a central bulk storage tank and use of a continuous deflection separator for washing and concentrating IJs. The hosts used *in vivo* methods must be susceptible, have high multiplication potential, and reared easily using cheap materials. The choice of host species and nematode for *in vivo* production should depend on nematode yield per cost of insect and the suitability of the nematode for the pest target (Chen et al. 2004,Blinova andIvanova,1987, Costa et al. 2007).The most common insect host used for *in vivo* production is the last instar of the greater wax moth *Galleria melonella* (L.) (Lepidoptera: Pyralidae). *G. melonella* occurs naturally in bee hives and is reared using artificial diets made of cereals, wax, yeast and glycerol. Production of cocoons and the extreme fragility of nematode infected larvae (*G. melonella*) are some of the drawback. The silkworm (*Bombyx mori*) is a Lepidopteran insect that feeds on mulberry leaves and twigs and is highly susceptible to entomopathogenic nematodes. The yellow mealworm, *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae), is an alternative host for *in vivo* nematode

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production. The structural integrity of nematode infected mealworm cadavers has enabled the development of mechanized methods for packing, thereby reducing labor costs. Nematode yield in general is proportional to host size(Flanders et al.1996, Kaya.and Stock, 1997).Maximum number of IJs per larva (*Steinernema* sp. and *Heterorhabditis* sp.) is found in the large sized *Galleria mellonella* larvae (20-22 mm). However, the production of *Heterorhabditis* sp. per unit body weight is always greater than that of *Steinernema* sp. in *Galleria* larvae (Raj Kumar et al.2003). *In vivo* production yields are also dependent on nematode doses (Boff et al.2000).Inoculation method, nematode concentration and host density also effect *in vivo* production of *S. carpocapsae* and *H. bacteriophora* in *G. mellonella* and *Tenebrio molitor* (Shapiro-Ilan and Gaugler,2002,Shapiro-Ilan et al.2012). *In vivo* can be accomplished by pipetting or spraying nematodes onto a substrate, immersion of insects in a nematode suspension, or applying the nematodes to the insect's food. It was observed that host immersion was about 4 times more efficient than pipetting inoculum on to the hosts (Shapiro-Ilan and Gaugler, 2002).Environmental factors including temperature, aeration, and moisture can affect yield (Shapiro-Ilan et al.2012, Grewal et al.1994,Dolinski et al.2007).In general nematode yield is proportional to insect host size (Ehlers and Shapiro Ilan,2005).Other factors affecting yield are inoculum and temperature. The efficiency of *in vivo* culture production also relies on the quality of media, i.e., insect hosts. For example, in production operations that produce their own insect hosts for nematode culturing, a host diet that is improved for insect production translates into improved efficiency in the overall process (Morales-Ramos et al. 2011). Additionally, in a tri-trophic interaction, the nutritional quality of insect host's diet can also impact the quality and fitness of entomopathogenic nematodes that are reared on those insects. The nematode's role in community dynamics will be affected as host diet effects impact entomopathogenic nematode ecology thereby fitness is impacted by differential nutrition (Shapiro-Ilan,2008). Best yields are achieved with intermediate inoculum dosage because higher doses create lower yield due to EPN competition for nutrients(Shapiro-Ilan et al.2002).Optimum production temperatures lie between 18°C and 28°C for different species(Burman and Pye,1980, Hazir et al,2001,Karagoz et al. 2009,Morton and Gracía-del-Pino, 2009).It is also crucial to maintain adequate aeration and humidity throughout the production process(Shapiro-Ilan and Gaugler 2002). Advances in mechanization and production geared toward application of nematodes through infected host cadavers can improve efficiency and economy of scale(Shapiro-Ilan et al.2016).

*Table.1: Nematode mass production in in vivo method.*

| Nematode species | Host | References |
|---|---|---|
| *Neoaplectana carpocapsae* (DD-136 strain), *Steinernema glaseri,S. carpocapsae,S. feltiae, S. masoodi, S. seemae, S. thermophilum,S.*sp. | *Galleria mellonella* | (White, 1927, Dutky,1964,Poinar, 1979, Blinova and Ivanova, 1987, Woodring and Kaya 1988, Lindegren et al. 1993, Gaugler et al.2002, Chen et al. 2004, Abdel-Razek and Abd-elgawad,2007, Costa et al. 2007). |
| *Heterorhabditis bacteriophora, H. indica, Heterorhabditis* sp. | *G. mellonella* | (Poinar, 1979, Woodring and Kaya, 1988, Lindegren et al.1993, Flanders et al.1996, Kaya and Stock, 1997, Boff et al.2000,Raj Kumar et al.2003) |
| *H. bacteriophora* | *Corcyra cephalonica* | (Shapiro-Ilan and Gaugler 2002, Raj Kumar et al.2003) |
| *Steinernema* sp.,*S.glaseri,S. feltiae, S. thermophilum,S. carpocapsae,S.masoodi, S. seemae* | *C.cephalonica* | (Blinova and Ivanova, 1987, Karunakar et al. 1999, Ganguly and Singh,2000, Singh and Gupta 2006, Khan et al. 2007, Ali et al.2008, Shapiro-Ilan et al. 2012). |
| *N.carpocapsae* | *Diatraea saccharalis* | (Folegatti et al.1988) |
| *S. feltiae* | *G.mellonella, Achroia grisella* | (Saenz and Luque, 2000) |
| *H. bacteriophora ,S. carpocapsae,S. glaseri , Heterorhabditis* sp. | *G.mellonella, Achroia grisella Bombyx mori* | (Saenz and Luque, 2000, Zaki et al. 2000, Prabhuraj et al. 2003) |

| | | |
|---|---|---|
| *H.indica*,S. *glaseri* | *Chilo sacchariphagus indicas* | (Karunakar et al. 1999) |
| *H. bacteriophora* | *Tenebrio molitor* | (Shapiro-Ilan et al.2002) |
| *S. masoodi,S. seemae, S. carpocapsae,S. glaseri S. thermophilum,H. indica* | *H. armigera* | (Subramanian,2003 , Ali et al.2008, Rishi and Prasad 2012). |
| *S. carpocapsae* | *S. litura* | (Ali et al.2008, Gupta et al.2008) |
| *H. indica* | *P. xylostella* | (Rishi and Prasad, 2012) |
| *S.*sp., *H. bacteriophora* | *Odontotermes obesus* | (Devi et al.2018) |
| *S. carpocapsae, H. bacteriophora* | *Capnodis tenebrionis* | (Morton and Gracia-del-Pino, 2009). |

### *3.2 IN VITRO* MASS CULTURE

*In vitro* culturing of EPNs is based on introducing nematodes to a pure culture of their symbiotic bacteria in a nutritive, non-living medium. Such media must use sterile ingredients to avoid unwanted bacterial contamination, retain the nematode's specific symbiotic bacterium and provide all the necessary nutrients. The medium is sterilized, and then inoculated with bacteria, followed by the nematodes. Nematodes are then harvested within 2-5 weeks in water. *In vitro* mass production of *Steinernema glaseri* was attempted for the first time in USA for prevention of *Popillia japonica* (Glaser,1932,McCoy and Glaser,1936).The presence of symbiotic bacteria was discovered from DJ (dauer juvenile) of *Steinernema felitiae* (McCoy and Glaser, 1936). Later on *Xenorhabdus nematophilus*, the symbiotic bacteria was isolated and identified from *S. carpocapsae* (Poinar and Thomas, 1966).House *et al.*(1965) devised a dog food based medium to produce the DD-136 strain of *Neoaplectana carpocapsae* on a commercial scale. Hara *et al.*(1981) who stressed on monoxenicity, produced 125 million nematodes / week from 100 dog food agar Petri dishes at a cost of $ 0.28 per million. Bedding (1976) developed methods for production of *Neoaplectana* spp. Bedding (1981) soaked shredded plastic foam in pig's kidney-beef fat homogenate (animal protein and lipid based medium). Several species of neoaplectanid and heterorhabditid nematodes were reared successfully with this method with an average yield of $6\times10^5$-$10\times10^5$ infective juvenile (*N. carpocapsae*) per gram of medium, at a cost of less than $ 0.02 per million. As an improvement to the previous method, Bedding [27] coated shredded polyether polyurethane sponge with a homogenate of chicken offal (for steinernematids) or chicken offal and 10 per cent beef fats (for heterorhabditds), sterilizing the medium in large autoclavable bags and adding the appropriate bacterium and nematode and was able to produce about 50,000 million IJs of *N. bibionis* in a week. In Pakistan, *S. pakistanense, S. asiaticum, S. feltiae* and *H. indica* were mass produced using chicken offal media(Tabassum and Shahina, 2004).Entomopathogenic nematodes were reproduced in solid culture method as 47,000 DJ/ml (Buecher and Popiel,1989).Solid culture method is economically feasible up to a production level of approximately $10\times10^{12}$ nematodes/month(Friedman et al.1989,Ramakuwela et al. 2016).Liquid culture for entomopathogenic nematodes was attempted for the first time by Stoll in 1952 .He cultured them in the shaker by using liver extracts yielding approximately 400 DJ/ml at $21^0$C-$25^0$C and pH of 6.0-6.5, and he had an important observation that, reproduction was more in the dark. Buecher and Hansen (1971) examined the effects of quantity of air flow and shear stress on the growth of entomopathogenic nematodes after the air was supplied to the liquid culture media. Pace *et al*. (1986) attached the flat–blade impeller to the 10 L Bioreactor and then inoculated *Xenorhabdus nematophilus*. After incubation for 24 hours, they reinoculated *Steinernema carpocapsae at* 2,000 DJ/ml and incubated for 10 days while oxygen saturation of 20% was maintained at 23 -28˚C, 180 rpm. *S. feltiae* strain 42 was reared in liquid culture along with its bacterial symbiont, *X. nematophilus.* First-stage juveniles developed into reproducing adults in a maintenance salts medium containing resuspended *Xenorhabdus* cells and the yeast *Kluyveromyces marxianus* or cholesterol. Friedman *et al.* (1989) observed that costs of production decrease rapidly up to a capacity of approximately $50\times10^{12}$ infective juveniles/month in liquid fermentation technique. Using this method *S.carpocapsae*, *S.riobrave*, *S.scapterisci*, *S.feltiae*,

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*S.kushidai* and *S.glaseri* have been produced at 80,000 L scale and *H.bacteriophora*, *H.indica* and *H.megidis* have been produced at 300-2000L level with yield capacity as high as 250,000 IJs /ml (depending on the nematode species). An improved method has been developed by Lunau *et al*.(1993) where axenic nematode eggs are placed on a pure culture of the symbiont. Culture times vary depending on media and species, and may be as long as three weeks though many species can reach maximum IJ production in two weeks or less(Ehlers et al.2000).Large scale production was further advanced through several measures including using bags with gas permeable Tyvac ® strips for ventilation, automated mixing and autoclaving, simultaneous inoculation of nematodes and bacteria, sterile room technology, and automated harvest through centrifugal sifters(Gaugler and Han ,2002,Neves et al. 2001,Wang et al.2007).Once the culture is completed, nematodes can be harvested from media via centrifugation (Surrey and Davies,1996). Media containing materials of plant origin generally were reported to have low productivity than those of animal origin (Abe, 1987, Wouts, 1981, Ehlers, et al.1998,Vyas et al.1999, Shapiro-Ilan and McCoy 2000,Vyas et al.2001,Hussaini et al.2000,2002,2007,Kaya et al. 2006,Prabhu et al.2006, Umamaheswari et al.2008, Somwong and Petcharat,2012, Upadhyay et al. 2013,Sunanda and Siddiqui,2013, Shapiro-Ilan and Xuehong, 2014,Ferreira and Malan,2014, Banu and Meena,2015,Yadav et al.2015).

## IV. STRATEGIES FOR MASS CULTURE

Although these nematodes are easily produced *in vivo* or *in vitro* on various complex semisolid organic media, the cost of mass production using these methods is a major constraint on nematode commercialization. A large scale liquid culture system would constitute a more cost-effective approach. EPN production with *in vitro* solid technology gives rise to higher nematode yields per gram of solid media than *in vivo* technologies. However, costs associated with solid media technologies are much higher than *in vivo* technologies. The high production cost is mainly associated with labour, materials and storage area, while large scale commercial farms' nematode needs can be met by the capital investment mass propagation methods using fermentation chambers [101-103]. Although mass production in submerged culture offers cost-efficiency, capital and technical expertise is still required. Understanding the biology of both the nematodes and bacterial partner is important for mass production. Phase shifting of the bacterial symbiont, time and concentration of the nematode inoculums, low percentages of nematode copulation, and fermentation parameters (oxygen concentration, pH, temperature, agitation, etc.) are some of the other factors which create problem in mass production (Ferreira and Malan,2014 ,Kaya et al.2006,Ehlers,2001,Gil et al.2002,Ehlers,1994,Ehlers et al. 1992,Zervos et al. 1991).The quality of infective juveniles depends on method of production and media composition. Recovery can also be affected by nutritional factors, aeration, $CO_2$, lipid content, and temperature (El-Sadawy, 2011).Diets rich in lipids, glucose and yeast extract content increased juvenile yields in *in vitro* production (Han et al.1992,Kooliyottil et al.2013,Chavarría-Hernández et al. 2010).Nematode virulence is correlated with the percentage of dauer juveniles retaining *Xenorhabdus* and the number of bacteria per dauer juvenile. *Xenorhabdus* subspecies vary in their virulence for a given host. Virulence of *S. glaseri* was restored by culturing these nematodes on *X. nematophilus* subsp, *poinari*. Nematodes with small juveniles were more productive than large nematodes. Nematode yield is inversely proportional to the size of the species. Higher yields of *H. indica* whose juveniles are small in size but *S. yirgalemense* is a large nematode and yet the highest yielding nematode species in *G mellonella* .Maximum average yields reported include 300,000 and 320,000 IJs per ml for *H. bacteriophora* and *S. carpocapsae* respectively, 138,000 per ml for *H. megidis* ,71,470 IJs per ml for *S. feltiae* and 450,000 IJs per ml for *H. indica*. Trait deterioration is a major concern to industrial producers of entomopathogenic nematodes(Bilgrami,2006).Trait changes as a result of continuous subculturing in *S. carpocapsae* and *H. bacteriophora*. These investigators studied trait stability of *P. luminescens* and *X. nematophila* after serial *in vitro* subculturing and demonstrated that phase variation (Phase I to Phase II) in *P. luminescens* and *X. nematophila* strains occurred within ten subculturing cycles. Furthermore, phenotypic variation was controlled in *X. nematophila* strains by selection of primary variants; however, trait change was not detected after prolonged culturing. When phenotypic variation in *P. luminescens* was controlled, changes in the primary variant like cellular morphology and prevalence of inclusion bodies with different sizes were observed((Inman et al.2012,Inman and Holmes,2012).Inman and Holmes (2012) have described the role of trehalose, a non-reducing sugar found in abundance within insect hemolymph that seems to aid in maintenance of Phase I variant of *P. luminescens* over extended periods of time. Minimization of serial passages, introduction of fresh genetic material, improved cryopreservation methods

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of stock cultures (Bai et al. 2004) or creation of homozygous inbred lines are the probable precautions against strain deterioration (Bai et al. 2005, Chaston, 2011).The quality of nematodes produced *in vitro* solid culture is similar to that produced *in vivo* (Dunphy and Webster,1989, Glaser et al.1940,Han et al.1997).High quality of EPNs can be produced using liquid culture provided good media as well suitable environmental conditions in the bioreactor (Johnigk et al.2004,Hirao and Ehlers,2010,Indriyanti and Muharromah,2016).

## V. ECONOMIC VIABILITY

Low-cost mass production of entomopathogenic nematodes (EPNs) is an important prerequisite towards their successful commercialization. During the past few years, a distinct cottage industry has emerged that produces entomopathogenic nematodes mostly *in vivo* for the home lawn and garden markets. Small scale farmers will benefit using cheap materials and those from their farms. However, commercial scale production is impracticable due to high production costs, lacks economies of scale and low nematode yields per gram of insect biomass. The advantage of *in vitro* solid media method are that capital costs are low, limited expertise is required and the logistics of production are flexible. This technology has the lowest mass production costs and is the method of choice for larger companies with multiple products in industrialized countries. Nematodes have been commercially developed by several companies in large liquid fermentation tanks which range from 50,000 up to 100,000 L fermenter(de la Torre,2003, Dillon et al.2012) in North America, Europe, Australia and Asia for the control of a vast array of pests, ranging from pests occurring in greenhouses to those occurring on golf-course turfs. In 1982,the first company which commercialized the liquid culture methods for entomopathogenic nematodes was Biosys (Palo Alto, California).They made mass production of *S. carpocapsae* in large scale of 80,000 L and their commercial products 'Biosafe' and 'BioVector' were used against lawn and garden pests. In 1983, Biotechnology Australia, produced nematodes on particles of sponge impregnated with an artificial diet and the product 'Otinem' was utilized against black vine weevils in Australia and Europe. Currently, E-Nema GmbH and Microbio Ltd. are doing mass production in Europe. Becker Underwood (formerly Micro Bio Ltd.) is owned by a USA company but operates out of Little hampton, United Kingdom, e-nema is based in Germany, and Koppert has its home in The Netherlands In addition, there are smaller producers like Andermatt Biocontrol based in Switzerland, bionema in Sweden and Owiplant in Poland, which produce nematodes using an improved solid-state Bedding system. In Korea, The Sesil, a company has started in vivo nematode production using the greater wax moth, *Galleria mellonella* (L.), larvae. The company produces 200 packs of *S. carpocapsae* Pocheon strain and 380 packs of an unidentified Korean isolate of *Heterorhabditis* sp. a day. The nematodes are sold for use against caterpillars on vegetables, fungus gnats on mushrooms and other insect pests of greenhouse plants. In Korea, WooGene B and G is currently producing the mass culture of entomopathogenic nematodes. A Chinese company Guangzhou Greenfine Biotechnology uses a solid culture method to produce several entomopathogenic nematode species both for Chinese and international markets.

## VI. FUTURE PROSPECTS

Entomopathogenic nematodes have emerged as important biological control agents against soil-dwelling as well as plant-boring insects. The role of nematodes in controlling insect pests will be enhanced by continued research and improved quality control. Recent advances in mass-production and formulation technology, and the discovery of numerous isolates/strains, together with the desirability of reducing pesticide usage, has resulted in a surge of scientific and commercial interest in these insect-killing nematodes. This has culminated in the commercial availability of many nematode products for use in several medium and high-value markets. Each approach has its advantages and disadvantages relative to production cost, technical know-how required, economy of scale, and product quality(Grewal et al.2005)and each approach can be improved further.

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# Rooting development of *Sansevieria trifasciata* (Mother-In-Law Tongue) as influenced by different propagation substrates

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***Abstract*— *Substrates are materials, other than soils in situ, in which plants are grown, and it is often used synonymously with rooting medium. An experiment was conducted at the Crop Type Museum of the Department of Crop, Soil, and Pest Management, the Federal University of Technology, Akure, to determine the rooting development of Sansevieria trifasciata as influenced by different propagation substrates. Results showed that the performance of the Sansevieria trifasciata planted using sand as substrate enhanced growth and root development of Sansevieria trifasciata compare to other substrates used (topsoil, sawdust, and rice hull). The treatment combination of all the substrates Topsoil + Sawdust + Rice hull + Sand performed better than other treatment combinations and sole. This study provided the empirical evidence that substrate combination influenced root development of Sanseveria spp.***

***Keywords*— *Rooting media, Sanseveria spp, Ornamental, Substrate, Growth.***

## I. INTRODUCTION

*Sansevieria spp.* is a perennial herb found in dry tropical and subtropical parts of the world (Randall, 2012).It is considered a "noxious weed" and of great economic importance as an ornamental plant, the source of fibre and as a medicine for curing different ailments. In Nigeria, theleaves and roots of *Sansevieria spp.*are used intraditional medicine for the treatment of asthma,abdominal pains, colic, diarrhea, eczema, gonorrhea,hemorrhoids, hypertension, menorrhagia, piles,sexual weakness, snakebites and wounds of the foot (Osabohien and Egboh, 2008; Ikewuchi *et al.*, 2010).

The term 'growing medium' is amongst others used to describe the material used in a container to grow a plant. The terms 'substrate' (Schroeder and Sell, 2009; Vaughn et al., 2011) and 'rooting medium' (Blok and Verhagen, 2009) are also used as synonyms. In some text, the term 'compost' is often used in place of growing medium. Nair et al. (2011) describe compost as a product obtained as a result of composting operation. For example, a compost heap at the bottom of the garden. However, composted materials have routinely been used as a growing medium or components of growing media (Schroeder and Sell, 2009; Nair et al., 2011). Substrates are materials, other than soils in situ, which support the growth and development of plants. Substrates can either be of organic origin, e.g., tree bark, poultry feathers, peat, compost, or made up of inorganic materials such as vermiculite, mineral wool, and clay (Vaughn *et al.*, 2011;Okunlola, 2016). According to Nair *et al.* (2011), substrates may also contain both organic and inorganic materials such as peat and perlite; coir and clay, peat and compost.Growing media play three significant roles in the plant; it provides aeration and water, allows for maximum root growth and physically supports the plant (Okunlola, 2016). In the last decade, many authors have researched the effects of growing media on the yield of vegetables, and these studies have established the significance of growing media to plant root growth and development.The inorganic media enhance plant growth and development compared to organic ones (Böhme et al., 2001; Okunlola and Oyedokun, 2016). Results from Tzortzakis and Economakis (2008) contradict previous findings. The authors found that plants grew faster in organic media compared to inorganic media. For yield enhancement, several authors have recommended growing vegetables in inorganic media (Rockwool, sand) rather than organic media (Böhme et al., 2001; 2008; Ikeda et al., 2001; Kobryń, 2002). Addition of inorganic substances to organic substances produces higher yield probably owing to increasing water-holding capacity and aeration by organic substances, which demonstrates that inorganic substances could partially replace organic substances (Gao et al., 2010).

However, there is limited information on the effects of the growing medium on root development and growth of ornamental plants such as *Sansevieria spp*. Therefore, the current study aims to examine the influence of different

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propagation substrates on rooting development of *Sansevieria spp*.

## II. MATERIALS AND METHODS

A Completely Randomized Design (CRD) experiment was conductedat the Crop Type museum of the Department of Crop, Soil, and Pest Management, Federal University of Technology, Akure, Ondo State ($7^0$16'N, $5^0$12'E) located in the rainforest zone Southwest Nigeria.

**Procurement and preparation of planting material**

Healthy leaves of *Sansevieria* plant were obtained from the LUCADO horticultural garden, Akure, Ondo State. Selection of the leaves were based on the thickness and colour of the plant; the thick flesh and good colored leaves were selected. The leaves were cut near the base using a pair of scissors,and it was cut at a 45$^o$ angle (approximately 2.5 cm above the top of the soil). Each end of the leaves was marked for easy identification of parts that will develop into the shoot and root when planting. The *Sansevieria* cuttings were stored in a warm, dry area with proper ventilation for a week. Substrateswere filled in polythene pots, watered and the water was allowed to drain for 10 minutes before potting the cuttings. Watering was done at 2 days interval and emerging weeds were hand-pulled from the pots. Root production was checked by gently digging around the base of each cutting with the tip of a pencil.

**Substratesused**

i. Topsoil,
ii. Sawdust,
iii. Rice hull,
iv. Sand,
v. Combination of above substrates (Topsoil + Sawdust + Rice hull + Sand)

**Substrates Analysis**

Before planting, all substrates were analyzed to determine their physiochemical properties.The physiochemical analysis carried out include; Particle Size Analysis, Soil pH, Organic Matter, Potassium (K), available Phosphorus, Calcium ($Ca^{2+}$) and Magnesium ($Mg^{2+}$), Nitrogen,

**Data collection and techniques**

Data were collected weekly on plant height and number of fresh leavesfrom 9– 14 weeks after shoot emergence. At the end of the experiment, the root length, root weight, shoot length and shoot weight data were measured and recorded. Data were analyzed using SPSS version 17.0 and mean separation was done using Duncan's New Multiple Range Test (DMRT) at $P<0.05$.

## III. RESULT

The result presented in Table 2 showed the effect of different substrates on days to shoot emergence of *Sansevieria trifasciata* (Dwarf). Significant differences ($p<0.05$) were recorded on days to shoot emergence of *Sansevieria trifasciata*.The *Sansevieria* cuttings planted in the Sand substrates and substrate combination of Topsoil + Sawdust + Rice hull + Sand emerged 49 days after planting, followed by Sansevieria cuttings planted in the Topsoil growing media (50 days after planting DAP). The *Sansevieria* cuttings planted in the sawdust substrates emerged last at 53 DAP.

Results from Table 3 showed the effect of different substrates on the plant height for *Sansevieriatrifasciata*. A significant difference ($P<0.05$) was recorded in the plant height of *Sansevieriatrifasciata* across the weeks throughout the durationof the experiment (9-14 WAP). However, the treatment that had all the substrate combination (Topsoil + Sawdust + Rice hull + Sand) had the tallest plant followed by Sand and Topsoil. Rice hull substrates had the shortest mean value for most of the parameters considered during the experiment (plant height, number of leaves, Root length, Shoot length, and Shoot weight).

Table 4 showed the response effects of different substrates on leaf number of *Sansevieriatrifasciata* (Dwarf). The result showed significant differences ($P<0.05$) in the number of leaves across the weeks throughout the experiment, although most of the substrates were not significantly different from one another. *Sansevieriatrifasciata*planted Topsoil + Sawdust + Rice hull + Sandwere not significantly different from the one planted in the Sand. The least number of leaveswas recordedinRice husk.

The results in Table5showed the effect of different substrates on the root length, root weight, shoot length and shoot weight for *Sansevieriatrifasciata* (Dwarf). There were significant differences ($P<0.05$) in root length, root weight, shoot length and shoot weight. However, it was observed that the mean value of root length, root weight, shoot length and shoot weight were highest in the treatment combination (Topsoil + Sawdust + Rice hull + Sand) followed by Sand and Topsoil. The rice hull had the least mean value.

## IV. DISCUSSION

This research finding clearly showed that substrates play an essential role in the propagation of plants and root development. Results from the study revealed that there were significant differences among the treatments throughout the duration of the experiment. It was observed that sand performed better than other substrates(except the combination)for the plant height, number of leaves, shoot

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length, root weight, root length and shoot weight. The substrate combination (Topsoil + Sawdust + Rice hull + Sand) enhanced the growth and development of Sansevieria trifasciata. The addition of sand to the substrate combination contributed immensely to it's performance, owing to the ability of sand to retain the right amount of moisture for the plant. This study has also revealed the importance of growing media in root development as earlier highlighted by several authors. Ikeda et al. (2001) stated that inorganic substrates enhanced growth and development of vegetables. Tzortzakis and Economakis (2008) found that plants grew faster in organic media compared to inorganic media. The study further confirmed that inorganic substrates (sand) performed better than the other substrates except for the combination of Topsoil + Sawdust + Rice hull + Sand. The sand substrate had Sansevieria trifasciata with the highest root length, root weight, shoot length and shoot weight mean value. The excellent performance of substrate combination (Topsoil + Sawdust + Rice hull + Sand) may be due to the presence of sand in the media combination. This result conforms with the finding of the following authors Böhme et al., (2001; 2008); Ikeda et al., (2001); Kobryń, (2002); Okunlola and Oyedokun, 2016. The findings from the authors explained that for yield enhancement in vegetables it is better to grow it in inorganic media such as Rockwool or sand. However, the treatment combination of Topsoil + Sawdust + Rice hull + Sand is a typical example of organic and inorganic substrates added together which performed better. than other substrates; the properties of individual substrates may be responsible for this. The organic substrates will help increase water holding capacity and improve aeration while the inorganic substrates will provide required nutrients necessary for plant development. Findings from Gao et al. (2010) also confirmed this result. Stating that the addition of inorganic substances to organic substances produce higher yield probably owing to increased water-holding capacity and aeration by organic substances (Gao et al., 2010).

## V. CONCLUSION

The study provide empirical evidence that the performance of the Sanseveria species planted with sand had better results than those planted with topsoil, sawdust, and rice hull. Sansevieria spp planted with sand had the best performance . However, a combination of organic and inorganic growing media will be recommended owing to its ability to enhance rooting development and vegetative growth. Further research is expected to be conducted to also examine the response of other Sansevieria species to different growing media or substrate.

**List of Tables**

*Table.1: Physiochemical properties of different substrates*

| | Topsoil | Sand | Rice Husk | Sawdust |
|---|---|---|---|---|
| | | **Substrates** | | |
| **Particle size analysis (%)** | | | | |
| Clay | 18.67 | 12.67 | 0 | 0 |
| Silt | 39.67 | 5.67 | 0 | 0 |
| Sand | 41.66 | 81.67 | 0 | 0 |
| | | | | |
| Soil pH | 5.98 | 5.96 | 5.33 | 5.3 |
| Nitrogen (%) | 0.33 | 0.11 | 3.52 | 3.67 |
| Phosphorus (mg $kg^{-1}$) | 5.07 | 3.13 | 34.14 | 17.48 |
| Organic Matter | 3.19 | 0.42 | 34.67 | 32.33 |
| | | | | |
| **Exchangeable cation (cmol $kg^{-1}$)** | | | | |
| Potassium | 0.80 | 0.61 | 2.78 | 2.73 |
| Calcium | 1.14 | 0.45 | 13.81 | 16.99 |
| Magnesium | 3.00 | 2.97 | 12.93 | 13.63 |

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*Table.2: Effect of different substrates on days to shoot emergence of Sansevieriatrifasciata (Dwarf)*

| Substrates | Shoot emergence (days) |
|---|---|
| Topsoil | 50ab |
| Sawdust | 53a |
| Rice hull | 52ab |
| Sand | 49b |
| Topsoil + Sawdust + Rice hull + Sand | 49b |

Means with the same letter in the same column are not significantly different from one another

*Table.3: Effect of different substrates on plant height of Sansevieriatrifasciata (Dwarf)*

| Substrates | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|
| | Weeks after planting | | | | | |
| Topsoil | 1.60b | 2.23b | 3.20b | 4.17b | 4.50b | 6.30c |
| Sawdust | 0.87c | 1.77c | 2.43c | 3.43c | 4.20b | 5.33d |
| Rice hull | 0.57d | 1.40c | 2.07c | 2.93d | 3.70b | 4.67e |
| Sand | 2.00a | 3.50a | 4.27a | 5.63a | 7.13a | 10.03a |
| Topsoil + Sawdust + Rice hull + Sand | 2.07a | 3.67a | 4.43a | 5.43a | 6.50a | 7.97b |

Means with the same letter in the same column are not significantly different from one another

*Table.4: Effect of different substrates on leaf number of Sansevieriatrifasciata (Dwarf)*

| Substrates | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|
| | Weeks after planting | | | | | |
| Topsoil | 1.00a | 2.00b | 2.67c | 4.00b | 4.00c | 6.00b |
| Sawdust | 0.00b | 1.33c | 2.00c | 3.00c | 4.00c | 4.67c |
| Rice hull | 0.00b | 1.00c | 2.00c | 3.00c | 3.00d | 4.00c |
| Sand | 1.67a | 3.00a | 3.33b | 4.33b | 5.67b | 8.33a |
| Topsoil + Sawdust + Rice hull + Sand | 1.67a | 3.00a | 4.00a | 6.00a | 8.00a | 8.67a |

Means with the same letter in the same column are not significantly different from one another

*Table.5: Effect of different substrates on growth parameters of Sansevieriatrifasciata (Dwarf)*

| Treatments | Root length (cm/plant) | Root weight (g/plant) | Shoot length (cm/plant) | Shoot weight (g/plant) |
|---|---|---|---|---|
| Topsoil | 9.23c | 1.28b | 7.12c | 15.68b |
| Sawdust | 8.13d | 0.73c | 6.30d | 10.00c |
| Rice hull | 7.83d | 0.74c | 6.23d | 10.33c |
| Sand | 10.3b | 1.32b | 9.37b | 18.67a |
| Topsoil + Sawdust + Rice hull + Sand | 11.23a | 1.62a | 10.43a | 18.00ab |

Means with the same letter in the same column are not significantly different from one another

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# Bioavailability of Some Heavy Metals in Selected Dumpsites in Ozoro, South-South, Nigeria

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***Abstract—*** *This study investigated the concentrations and distribution patterns of some heavy metals in soil around some waste dumpsites in Ozoro, South-South, Nigeria. Two different dumpsites located at Kwale road and Owhelogbo roads in Ozoro were used for this study. Soil samples were collected at 0 – 15cm depth. A total of two composite samples were collected for this study. The soil samples were air dried, sieved and digested. Sequential extraction was used to separate metals into their various geochemical fractions. Atomic absorption spectrophotometer (Bulk 200 model) was used to determine the metal concentrations in the various geochemical fractions. Physicochemical characteristics like pH and electrical conductivity were determined. pH result ranged from 9.6 – 10.3. The pH values were basic in both sampling sites. The conductivity result for both sites was 81.0 µS/cm. The metal concentrations ranged from 0.29 – 0.53 mg/kg for Iron; 0.22 – 0.47 mg/kg for Copper; 0.16 – 0.21 mg/kg for Cadmium; 0.28 – 0.29 mg/kg for Zinc and 0.24 – 0.27 mg/kg for Manganese. The concentration of metals in both sites is in the order Fe>Cu>Pb>Zn>Mn>Cd for Kwale road and Pb>Fe>Zn>Mn>Cu>Cd for Owhelogbo road. Bioavailability results showed zinc, copper, cadmium, manganese, and lead more associated with the exchangeable fraction for Kwale road while zinc, copper, cadmium, lead and iron were associated more to the carbonate bound fraction for Owhelogbo road. However, lead and zinc associated more with both the carbonate bound and the exchangeable fractions for both sampling sites. There were significant variations in metal concentration from both sites. The mobility factors of metals in the soil profile follows in the order: Cd>Mn>Cu>Pb>Zn>Fe.*

***Keywords— Bioavailability, fraction, heavy metals, pollution, sampling, sites.***

## I. INTRODUCTION

Ozoro is a fast developing town in South-South, Nigeria with a corresponding increase in population leading to increased waste generation. As a result of increased activities occasioned by population growth, dumpsites are proliferated all over the town. Wastes are often deposited at open dumpsites and poor management of these sites could create a number of adverse environmental impacts (Ekwulumgbo et al., 2013). Risks associated with these waste are categorized into three: biological, chemical and physical (Wuana et al., 2012).

Heavy metals are chemical elements mostly with density greater than 4g/cm$^3$ found in all kinds of soils, rocks and water in fresh water ecosystem (Adelekan and Abegunde, 2011).Elements of interest exist in several different forms and are associated with a range of components in the soil which influence their reactivity and hence their mobility and bioavailability (Osakwe and Egharevba, 2008; Yobouet et al., 2010). Information about the physicochemical forms of the element is required for understanding their environmental behaviour (mobility pathways, bioavailability) (Osakwe and Egharevba, 2008). The levels of heavy metals in the environment have been seriously increased during the last decades due to human activities (Binet al., 2001). The presence of heavy metals in the environment is of great ecological significance owing to their toxicity at certain concentrations, translocations through the food chains and non-biodegradability which is responsible for accumulation in the biosphere. The ecological effects of heavy metals in soil are closely related to the distribution of the species in the solid and liquid phases of the soil (Tokalioglu et al., 2003). Depending on their origin, trace elements exist in different mineral forms and chemical compounds and in different combinations with mineral and organic components of soil and sediments which may vary according to various conditions (Tokalioglu et al., 2003). The behaviour and biological impact of heavy metal pollutants in aquatic system is governed by factors such as adsorption, desorption, sedimentation, re-suspension, filtration, complexation, precipitation, solubilisation, biological uptake and excretion (Abehet al., 2007). Comprehensive knowledge of the interaction between the trace elements and the soil matrix is required to judge the environmental impact. The behaviour of the elements in the environment such as bioavailability, toxicity and distribution cannot be reliably predicted on the basis of their total concentration (Tokalioglu et al., 2003; Iwegbue, 2007). The knowledge of both the total concentration and chemical speciation is necessary to characterize the

behaviour of heavy metals in soil (Osakwe and Egharevba, 2008). Chemical speciation is of interest in environmental analysis because the behaviour of trace elements in natural system depends on the forms as well as the amounts which are present (Kot and Namiesni, 2000; Yobout et al., 2010). Because the toxicity of heavy metals is related to their existing species, the speciation of heavy metals is attracting more attention (Omuku et al., 2009). Speciation of metal contaminated soils is important in developing viable and cost effective remediation strategies and in predicting mobility and bioavailability of the metals (Spark, 2003). Water soluble and exchangeable forms are considered readily mobile while metals incorporated into crystalline lattices of clay appears relatively inactive (Kabala and Singh, 2001). Metals precipitated as carbonates occluded in iron, manganese, and aluminium oxides or complexed with organic matter could be considered relatively active or firmly bound depending upon actual combination of physical and chemical properties of the soil (Omuku et al., 2009). Soil texture (clay), pH, organic matter and Fe-Mn oxides have been found to be the most important soil properties and components influencing biological uptake of heavy metals (Oviasogie and Agbimien, 2003; Kabala and Singh, 2001). The process of mobility (distribution), transport and partitioning of trace metals is controlled by physical and biological characteristics of that system (Hlavay et al., 2004). In soil environment, metals occur in both the solid phase and aqueous phase (Uwumarongie et al., 2008). In solution, metals can exist as free ions or as various complexes associated with organic (e.g. carboxyl and phenolic) or inorganic (e.g. $OH^-$, $SO_4^{2-}$, $CO_3^{2-}$) ligands. While in the solid phase it can be retained in organic or inorganic soil components by various sorption mechanism e.g. ion exchange or surface complexation. It can also exist as minerals or be co-precipitated with other minerals e.g. carbonates (Bernhard and Neff, 2001). The process of oxidation, reduction, adsorption, precipitation and desorption is determined by the concentration of ions in the soil. The extent to which the reactions occur is determined by the composition of the soil especially the amount and type of clay minerals, hydrous oxides and organic matter, soil pH, redox status and the nature of the contaminants (Uwumarongie et al., 2008).

The pressing demand to know the various forms, in which metal exist in nature so as to ascertain their toxicity potential and mobility pattern cannot be overemphasized. Sequential extraction method is used to assess the distribution and mobility of heavy metals in soil (Ireneet al., 1998). Tessier et al., (1979) has been the most widely used method of sequential extraction. This present study is focused on the determination of Lead (Pb), Iron (Fe), Zinc (Zn), Cadmium (Cd), Copper (Cu), and Manganese (Mn) so as to predict their source, origin, toxicity and bioavailability tendencies.

## II. MATERIALS AND METHODS

**Study Area**

Ozoro is the administrative headquarters of Isoko North Local Government area of Delta State. Ozoro is located at latitude 5°32'I8''N and longitude 6°12'58"E (www.wikipedia.org). Ozoro is one of the fastest developing communities in Delta State probably because of the presence of the Delta State Polytechnic.

**Sampling and Sample Preparation**

Soil samples were collected from two dumpsites located at Ozoro, January, 2018 with the aid of soil auger at 0 – 15cm depth (Ataikiruet al., 2008).The samples were kept in black polythene bag for onward transportation to the laboratory for heavy metal distribution analysis.The samples were air dried for four days at room temperature and large objects (sticks, stones) were removed accordingly (Asiagwu, et. al., 2007). The dried samples were crushed into fine powder using agate mortar sieved using a 100 mesh screen.

**pH Determination**

Soil pH was determined in water according to the method described by Uwumaronge et al (2008). Ten grams of soil were weighed into a 50ml beaker and 20ml of distilled water was then added. The soil/water mixture (ratio 1:2) was stirred with a glass rod intermittently for 30 minutes. The pH meter was then calibrated using buffer 4 and 7. The pH meter (Cyberson 20) was set with appropriate controls to pH 7.0. The electrodes were rinsed and subsequently immersed into soil/water mixture. The pH was recorded as pH ($H_2O$).

**Determination of Electrical Conductivity**

Electrical conductivity was measured in 1:5 ratio of soil to water with Electrical conductivity meter (Marton 407 S 214) after calibration with 0.01M KCl solution.

**Sequential Extraction**

The method employed for the speciation studies was that introduced by Tessier et al (1979). 1.00g of the sieved samples were used for the sequential extraction processes. A total of two samples representing the top soil of the dumpsites were investigated.

**Exchangeable Fractions**

The soil samples were extracted with 8.0ml of 1M sodium acetate (NaOAc at pH 8.2) in a Teflon beaker for 1 hour with continuous agitation.

**Bound to Carbonates**

The residue from exchangeable fractions was leached at room temperature with 8.0ml of 1M NaOAc solution adjusted to pH 5.0 with acetic acid (HOAc) with continuous agitation for 1 hour.

**Bound to Fe-Mn Oxides (Reducible)**

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The residue from carbonate bound fractions was extracted with 20ml of 0.04M $NH_2OH.HCl$ in 25% (v/v) HOAc at temperature of 96±2˚C with occasional agitation for 5 hours.

**Bound to Organic Matter (Oxidisable)**

3.0ml of 0.02M $HNO_3$ and 5.0ml of 30% $H_2O_2$ adjusted to pH 2 with $HNO_3$ was added to the residues from Reducible fractions and the mixture heated to 85˚C for 2 hours with intermittent agitation. Another 3.0ml aliquot of 30% $H_2O_2$ was then added and the mixture heated to 85˚C for 3 hours with occasional agitation. After cooling, 5.0ml of 3.2M $NH_4OAc$ was added and sample was diluted to 20ml with deionised water and agitated continuously for 30 minutes. The addition of $NH_4OAc$ was designed to prevent the adsorption of extracted metal into the oxidized sediment.

**Residual**

The residues from oxidisable fractions were digested with 5ml HF and 5ml aqua regia and filtered into a 50ml standard flask and made up to mark with deionised water.

Blanks for the successive steps were also prepared. The residues were filtered into a 50ml standard flask and made up to the mark with deionised water after digestion.

The stored supernatant solutions from the various fractions digest sample solutions as well as the blanks were analysed instrumentally for their metal contents using Atomic Absorption Spectrophotometer (Buck 200A model). Total metal concentration was taken as the sum of the metal in the fractions.

## III. RESULTS

The results of heavy metals concentrations and bioavailability are presented in table 1 -.3 as well as the mobility index in table 4.

*Table.1: Physicochemical and Toatal metal concentrations of soil around some waste dumpsites in Ozoro, Delta State (taken as the sum of fractions)*

| Parameter | Kwale Road | Owhelogbo Road |
|---|---|---|
| pH | 81.0 | 81.0 |
| Electrical conductivity (µS/cm) | 9,6 | 10.3 |
| Zn | 0.29±0.025 | 0.28±0.035 |
| Cu | 0.47±0.040 | 0.22±0.040 |
| Cd | 0.21±0.00 | 0.16±0.010 |
| Pb | 0.39±0.040 | 0.33±0.030 |
| Mn | 0.24±0.040 | 0.27±0.035 |
| Fe | 0.53±0.020 | 0.29±0.035 |

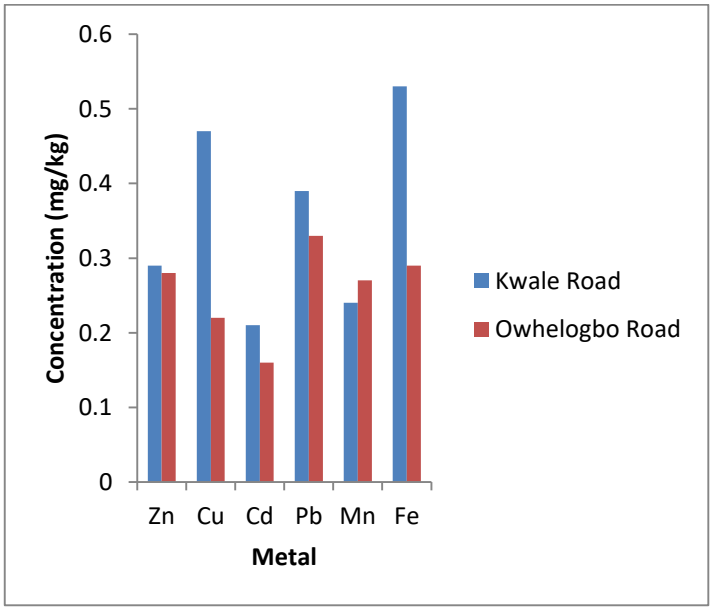


*Fig.4.1: Bar Chart showing the concentration of Zn, Cu, Cd, Pb, Mn and Fe in Soil in two dumpsites in Ozoro, Delta State.*

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*Table.1: Bioavailability of Zn, Cu, Cd, Pb, Mnand Fe in mg/kg in the various geochemical fractions of selected dump sites in Ozoro, Delta State.*

| Metal/Fraction | Kwale Road | Owhelogbo Road |
|---|---|---|
| **Zn** | | |
| Exchangeable | 0.10±0.005 | 0.03±0.010 |
| Carbonate bound | 0.05±0.010 | 0.09±0.010 |
| Fe-Mn Oxide | 0.07±0.000 | 0.07±0.005 |
| Organic matter bound | 0.03±0.005 | 0.05±0.010 |
| Residual | 0.04±0.005 | 0.04±0.000 |
| Sum of extracted metal | 0.29±0.025 | 0.28±0.035 |
| **Cu** | | |
| Exchangeable | 0.13±0.010 | 0.04±0.005 |
| Carbonate bound | 0.12±0.010 | 0.06±0.010 |
| Fe-Mn Oxide | 0.11±0.010 | 0.05±0.010 |
| Organic matter bound | 0.05±0.010 | 0.03±0.010 |
| Residual | 0.06±0.000 | 0.04±0.005 |
| Sum of extracted metal | 0.47±0.040 | 0.22±0.040 |
| **Cd** | | |
| Exchangeable | 0.08±0.010 | 0.02±0.005 |
| Carbonate bound | 0.04±0.005 | 0.06±0.010 |
| Fe-Mn Oxide | 0.06±0.005 | 0.05±0.005 |
| Organic matter bound | 0.01±0.010 | 0.02±0.010 |
| Residual | 0.02±0.005 | 0.01±0.000 |
| Sum of extracted metal | 0.21±0.035 | 0.16±0.030 |
| **Pb** | | |
| Exchangeable | 0.13±0.010 | 0.05±0.000 |
| Carbonate bound | 0.14±0.010 | 0.10±0.005 |
| Fe-Mn Oxide | 0.12±0.005 | 0.08±0.010 |
| Organic matter bound | 0.06±0.010 | 0.06±0.005 |
| Residual | 0.07±0.005 | 0.04±0.010 |
| Sum of extracted metal | 0.39±0.040 | 0.33±0.030 |
| **Mn** | | |
| Exchangeable | 0.09±0.005 | 0.04±0.005 |
| Carbonate bound | 0.05±0.010 | 0.09±0.010 |
| Fe-Mn Oxide | 0.05±0.010 | 0.08±0.005 |
| Organic matter bound | 0.02±0.005 | 0.04±0.010 |
| Residual | 0.03±0.010 | 0.02±0.005 |
| Sum of extracted metal | 0.24±0.040 | 0.27±0.035 |
| **Fe** | | |
| Exchangeable | 0.12±0.000 | 0.03±0.010 |
| Carbonate bound | 0.13±0.005 | 0.09±0.005 |
| Fe-Mn Oxide | 0.11±0.005 | 0.08±0.005 |
| Organic matter bound | 0.08±0.010 | 0.05±0.010 |
| Residual | 0.09±0.000 | 0.04±0.005 |
| Sum of extracted metal | 0.53±0.020 | 0.29±0.035 |

*Table.2: % Bioavailability of Zn, Cu, Cd, Pb, Mg and Fe in the various geochemical fractions of selected dump sites in Ozoro, Delta State.*

| Metal/Fraction | Kwale Road | Owhelogbo Road |
|---|---|---|
| **Zn** | | |
| Exchangeable | 34.48 | 10.71 |
| Carbonate bound | 17.24 | 32.14 |

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| Metal/Fraction | Kwale Road | Owhelogbo Road |
|---|---|---|
| Fe-Mn Oxide | 24.14 | 25.00 |
| Organic matter bound | 10.35 | 17.86 |
| Residual | 13.79 | 14.29 |
| **Cu** | | |
| Exchangeable | 27.66 | 18.18 |
| Carbonate bound | 25.53 | 27.27 |
| Fe-Mn Oxide | 23.40 | 22.73 |
| Organic matter bound | 10.64 | 13.64 |
| Residual | 12.77 | 18.18 |
| **Cd** | | |
| Exchangeable | 38.10 | 12.50 |
| Carbonate bound | 19.05 | 37.50 |
| Fe-Mn Oxide | 28.57 | 31.25 |
| Organic matter bound | 4.76 | 12.50 |
| Residual | 9.52 | 6.25 |
| **Pb** | | |
| Exchangeable | 25.00 | 15.15 |
| Carbonate bound | 26.92 | 30.30 |
| Fe-Mn Oxide | 23.08 | 24.24 |
| Organic matter bound | 11.54 | 18.18 |
| Residual | 13.46 | 12.12 |
| **Mn** | | |
| Exchangeable | 37.50 | 14.81 |
| Carbonate bound | 20.83 | 33.33 |
| Fe-Mn Oxide | 20.83 | 29.63 |
| Organic matter bound | 8.33 | 14.81 |
| Residual | 12.50 | 7.41 |
| **Fe** | | |
| Exchangeable | 22.64 | 10.34 |
| Carbonate bound | 24.53 | 31.03 |
| Fe-Mn Oxide | 20.75 | 27.59 |
| Organic matter bound | 15.09 | 17.24 |
| Residual | 16.98 | 13.79 |

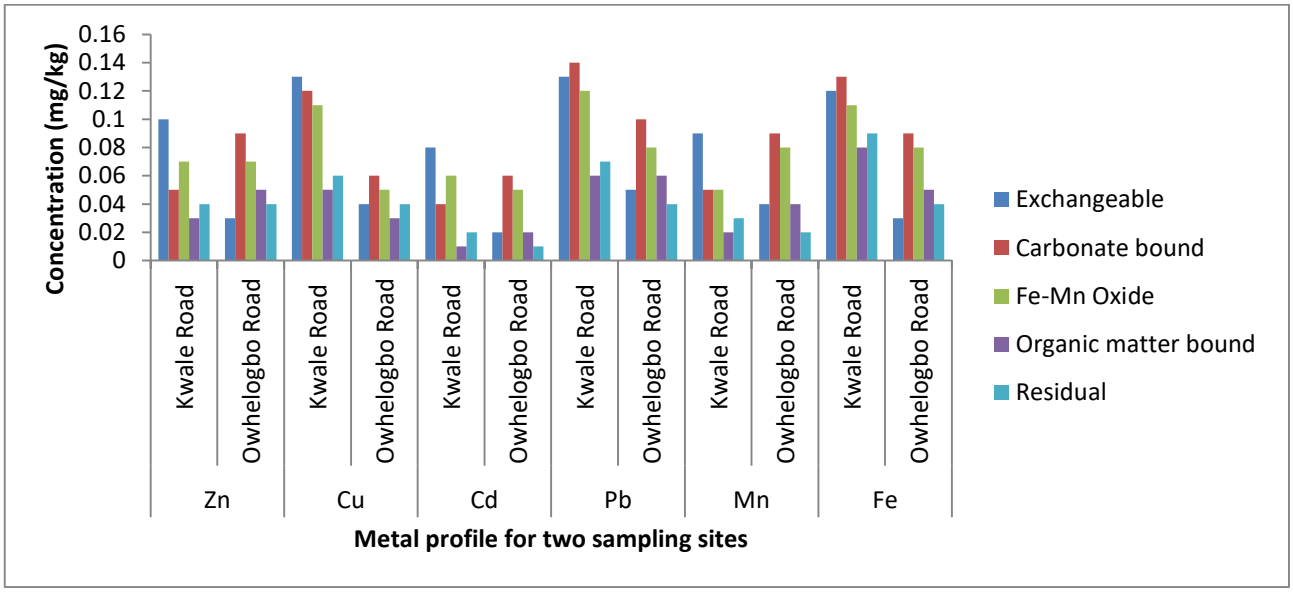


*Fig.2: Bar chart showing the Bioavailability of Zn, Cu, Cd, Pb, Mn and Fe in the various geochemical fractions.*

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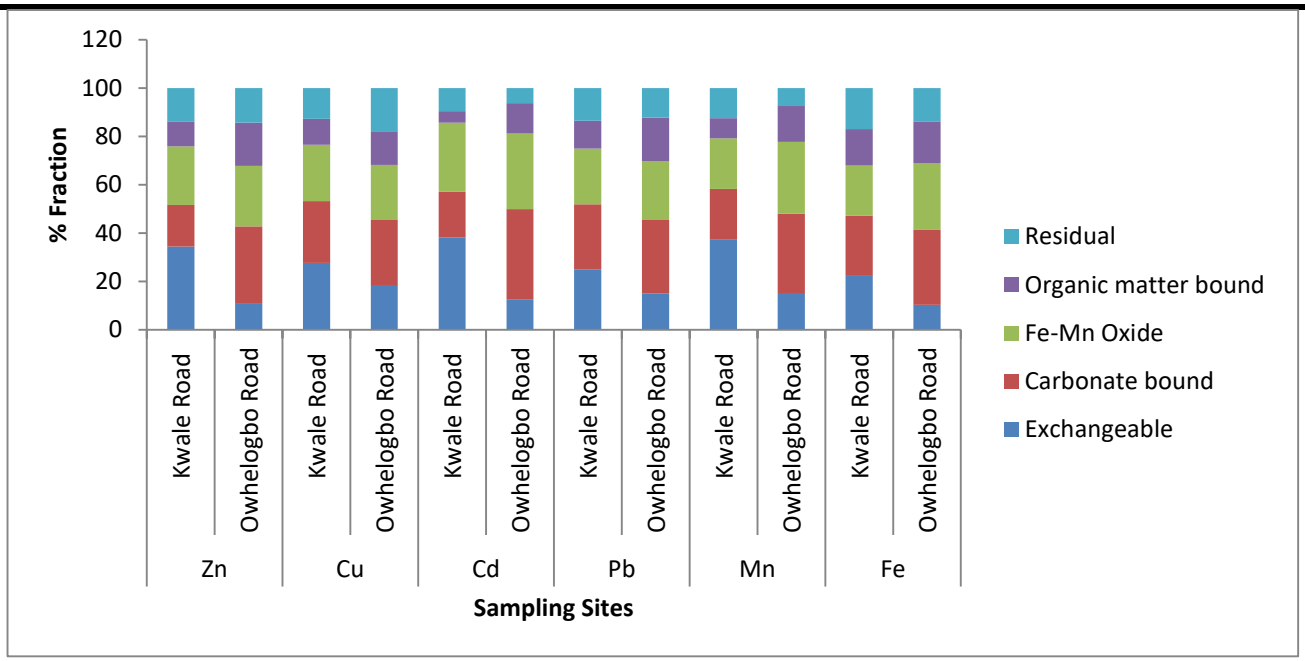


*Fig.2: Bar chart showing the Percentage bioavailability of Zn, Cu, Cd, Pb, Mn and Fe in the various geochemical fractions as a function of total metal concentration in soil dump sites.*

## IV. DISCUSSION

The pH result ranged from 9.6 – 10.3. The pH values were basic in both sampling sites. Hydrolysed metal species increases with pH (Mclean and Bledsoe 1992). The electrical conductivity of the sample station could be attributed to the ionic content of the soil (Table 1). The metal concentration for the two sampling stations is in the order: Fe>Cu>Pb>Zn>Mn>Cd for Kwale road and Pb>Fe>Zn>Mn>Cu>Cd for Owhelogbo road (Table 1 and Figure 1).

The exchangeable is the predominant fraction of zinc (34.48%) for Kwale road whereas carbonate bound is the predominant fraction (32.12%) for Owhelogbo road as shown in Table 2 and 3 and Figure 1 and 2. The results indicate that zinc is readily available. The carbonate associated zinc could be attributed to the relatively high stability constant of $ZnCO_3$ under the pH – (redox) conditions common in inland water, as it characterises co-precipitation with $CaCO_3$ (CSIRO, 1990; Chakarapani and Subramaman, 1993).

The Fe-Mn oxides formed the next predominant fraction of zinc in both sampling sites (24.14%) for Kwale road and (25.00%) for Owhelogbo road as shown in Table 2 and 3 and Figure 2 and 3. The association of zinc with Fe-Mn oxides in soils and sediments has been widely reported (Ma and Rao, 1997; Reddy et al., 2001; Gao et. al., 2008; Ekwumemgbo et al.; 2013). Fe-Mn oxides seems to play an essential role in zinc accumulation in the soil as precipitation or co-precipitation products.

Figure 2 and 3 presents the speciation profiles of copper in both sampling sites. The predominant species of copper is associated with the exchangeable fraction (27.66%) for Kwale road and carbonate bound fraction (27.27%) for Owhelogbo road. The high concentration of copper in the organic matter bound fraction is due to very high values for formation constants of Cu-organic complexes (Stum and Morgan, 1981; Yobout et al., 2010; Obasi et al., 2013). The carbonate bound fraction was the next important fraction of copper (25.53%) for Kwale road and Fe-Mn oxide fraction (22.73%) for Owhelogbo road in this study. Adsorption has been noted as the important control of Copper in soils (Jenne, 1968; Hickey and Kittrick, 1984). The high surface area and adsorbing capacity of Fe-Mn oxide, coupled with the ability of $Cu^{2+}$ to replace $Fe^{2+}$ in some Fe-oxides (Taylor, 1965) may be responsible for such adsorption.

The speciation profile of cadmium is presented in Table 2 – 3 and Figure 2 and 3. The exchangeable fraction forms the predominant form of cadmium (38.10%) and carbonate bound (37.50%) for Owhelogbo road.The next dominant fraction of cadmium is associated with Fe-Mn oxide fraction in both sampling locations. The high level of cadmium in this fraction represents the fact that cadmium could be readily remobilised under reducing conditions.

The predominant species of lead is the organic matter bound (26.92%) for Kwale road and (30.30%) for Owhelogbo road as shown in Table 1 – 2 and Figure 1 and 2. The next predominant fraction of lead is the exchangeable fraction (25.00%) for Kwale road and Fe-Mn oxides fraction (24.24%) for Owhelogbo road. The result agrees with speciation profile reported by several investigators (Salomon and Forstner, 1980; Tessier et al., 1980; Ryan et al., 2002; Abdus-Salam et al., 2011). The

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result indicates that lead may be remobilized under reducing conditions (Zhang et al., 2003).

The predominant species of Manganese is the exchangeable fraction (37.50%) for Kwale road and the carbonate bound (33.33%) for Owhelogbo road as shown in Table 1 and 2 and Figure 1 and 2. The association of Manganese with the carbonate fraction agrees with the findings of Asiagwu et al., (2009). The next predominant fraction of manganese is the carbonate bound fraction (20.83%) and Fe-Mn oxide (20.83%) for Kwale road and Fe-Mn oxides fraction (29.63%) for Owhelogbo road.

The predominant species of Iron is the carbonate bound (24.53%) for Kwale road and (31.03%) for Owhelogbo road as shown in Table 1 and 2 and Figure 1 and 2. The next predominant fraction of Iron is the exchangeable fraction (22.64%) for Kwale road and Fe-Mn oxides fraction (27.59%) for Owhelogbo road as shown in Table 1 and 2 and Figure 1 and 2. T

The mobility index of metals in the soil may be assessed on the basis of absolute and relative contents of fractions weakly bound to the sediment components. Operationally defined extraction sequence fractionates the metals in order of decreasing solubility, as a result early fraction collected ($F_1$and$F_2$) captured the most reactive, and presumably most mobile and bioavailable fraction (Iwegbue, 2007). Fe-Mn oxide bound ($F_3$**)** and organic matter bound ($F_4$**)** may become bioavailable if the redox and pH condition of the soil changes.

The relative index of metal mobility was calculated as a mobility factor (MF) (Salbuet al., 1998; Narwal et al., 1999; Kabala and Singh, 2001; Osakwe, 2010 ) on the basis of the equation given below.

$$MF = \frac{F_1 + F_2}{F_1+F_2+F_3+F_4+F_5} \times 100$$

Where, $F_1$ = Exchangeable; $F_2$ =Carbonate bound; $F_3$ = Fe-Mn Oxide; $F_4$ = Organic matter bound; and $F_5$ = Residual .

A high MF values for heavy metals in the soil or sediment has been interpreted as evidence of relatively high lability and biological availability (Ma and Rao, 1997; Ahumada et al., 1999; Narwal et al, 1999, Kabala and Singh, 2001). The MF for metal in the sampling sites is given in Table 4. Kwale road showed higher mobility factors than Owhelogbo road. The mobility factors of metals in the soil profile follows in the order: Cd>Mn>Cu>Pb>Zn>Fe

*Table.3: Mobility factor of heavy metals in some dump sites in Delta State*

| Metal | Sites | |
|---|---|---|
| | Kwale Road | Owhelogbo Road |
| Zn | 51.72 | 42.86 |
| Cu | 53.19 | 45.45 |
| Cd | 57.14 | 50.00 |
| Pb | 51.92 | 45.45 |
| Mn | 58.33 | 48.15 |
| Fe | 47.17 | 41.38 |

## V. CONCLUSION

This study on the bioavailability of some heavy metals in some waste dumpsites in Ozoro, South-South, Nigeria has revealed the bioavailability of the metals in the various geo-chemical forms. The Exchangeable formed the predominant fraction of zinc, Copper, cadmium, lead and Manganese in Kwale road while carbonate bound fraction was predominant for Zinc, copper, cadmium, lead, manganese and Iron in Owhelogbo road sampling site. Iron and lead were more associated with carbonate bound fraction in both sampling locations. Kwale road showed a higher mobility factors than Owhelogbo road (Table 3).

## VI. RECOMMENDATION

Dumpsites contribute tremendously to the contamination/pollution of soil around the designated sites. This situation demands control because the level of generation of waste is ever on the increase. As a result the following recommendations are made.

I. Dumpsites should be sited far away from residential area
II. Appropriate disposal methods for recycling and reuse should be adopted.
III. The agency on the environment should continue its monitoring activities to ensure safe guideline limit compliance by all and sundry.

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# Efficacy of Biological and Chemical Treatments for the Management of Damping Off (*Pythium Spp.*) of Bitter Gourd in the Nursery

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***Abstract—*** *A field experiment on "Efficacy of Biological and Chemical Treatments for the management of Damping Off (Pythium Spp.) of Bitter Gourd in the nursery" was conducted during March 1st 2017 to April 18th 2017. The experiment was laid out in Randomized Complete Block Design (RCBD) with seven treatments and three replications. The treatments included biocontrol agent as Trichodermaharzianum(1× $10^6$ CFU/ml),Vitavex (0.1%), Bavistin (2 g/kg of seed) and Mancozeb 75 % WP (2 g/kg of seed) as chemical treatments and integration of bio control agent and chemicals were used. The germination percentage, pre and post emergence damping off of bitter gourd seedlings were recorded. The significant; increment in germination percentage (120 %),reduction in pre and post disease incidence (92 % and 89 %) was found in seed treatment of Vitavex(0.1%) + soil treatment with Trichoderma (1× $10^6$ CFU/ml) and it was followed by seed treatment with Bavistin (2 g/kg of seed). It was may be due to the inhibitory effect of fungicide Vitavex on development of seed borne pathogen reducing seedling mortality at an early stage followed by vigorous root and shoot development as affected by the antagonistic and hormonal effect of the Trichodermaharzianum.*

***Keyword—Pythium spp.; damping off; Trichoderma; biocontrol agent.***

## I. INTRODUCTION

Bitter gourd (*Momordicacharantia*) is a tropical and subtropical vine of the family Cucurbitaceaeis known variously as bitter gourd, balsam pear, bitter melon, bitter cucumber and African cucumber (Heiser 1979).It is widely grown in Asia, Africa, and South America (Raj *et. al.*, 1993, Singh 1984). Area cultivated of bitter gourd in Chitwan is 435 ha with the production of 6090 mt and productivity of 14.0 mt/ha. Whereas total area cultivated of bitter gourd in Nepal is 10082.2 ha with the production of 132350.1 mt and productivity of 13.1 mt/ha (MOAD,2016).

Damping off is the most common disease of the seedling vegetables. Buchenauer, 1998; Vogt and Buchenauer, 1997 reported that Damping off is the important soil-borne disease attacking plants. The two fungi that are most often associated with damping-off are *Rhizoctoniasolani* and *Pythium species*. The most aggressive species of *Pythium* that causes important plant diseases is *P.aphanidermatum.* It is a soil as well as seed born pathogen. It is the most important and responsible for pre and post emergence damping off of seedlings. Damping-off disease by *Pythium species* causes more than 60 percent mortality of seedlings both in nursery and main field (Manoranjitham*et. al.*, 2000).

For the management of the damping off disease chemical fungicides is considered to be easiest and the fastest method. Apart from the disease control aspects of pesticide we cannot undermine its detrimental effects in environment. The over application of chemical fertilizer and pesticide reduce farm profits, create a risk of soil degradation and cause environmental pollution (Trisdale, Nelson and Beaton, 1985).Biological control offers environment friendly approach for the management of plant disease and can be incorporated into cultural and physical controls methods and also very limited chemical usage for an effective integrated pest management system (Monte 2010). This research was mainly focused in effective yet environment friendly approach of damping off management to produce healthy seedling of the bitter gourd plant so that farmers can gain more income from the bitter gourd production. The major objective of this study was to find out the efficacy of biological and chemical treatments for the management of damping-off (*Pythium spp.*) of bitter gourd in the nursery.

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## II. MATERIALS AND METHODS

**Selection of treatments**

The commonly grown variety White Long was brought from Anamolbiu Private Limited Company, Bharatpur-12 Chitwan for the experiment. A total of 7 treatments including control were selected for the experiment .The treatments applied in the field were also used in the laboratory for seed treatment. The details of the treatments are as follows:

*Table.1: Details of the treatments used for experiment*

| SN | Treatments | Symbol |
|---|---|---|
| 1 | Seed treatment with 0.1% Vitavex + Soil treatment with *Trichodermaharzianum*@ $10^6$cfu $ml^{-1}$ | T1 |
| 2 | Seed treatment with Vitavex @ 2g $kg^{-1}$ | T2 |
| 3 | Seed treatment with Bavistin (Carbrendazim 50%WP) @ 2g $kg^{-1}$ | T3 |
| 4 | Seed treatment with Mancozeb 75%WP @ 2g $kg^{-1}$ | T4 |
| 5 | Soil treatment with *Trichodermaharzianum*@ $10^6$CFU/ml | T5 |
| 6 | Seed treatment with *Trichodermaharzianum* @ $10^6$cfu/ml | T6 |
| 7 | Control | T7 |

**Collection of fungicide and bio-control agent**

Systemic fungicides like Vitavex, Bavistin and contact fungicide like Mancozeb were collected from the local market of Narayanghat and UnnatBijBriddi Farmers Group Patihani-5 of Chitwan district and the pure culture of bio-control agent (*Trichodermaharzianum*) was acquired from AFU Rampur Chitwan.

**Preparation of Trichoderma solution**

A fully grown Trichoderma PDA plate was scraped with sterilized cotton to collect spores. Those spores were filtered through muslin cloth and then collected on sterilized beaker and diluted using sterilized distilled water. The concentration of the spores was checked using haemocytometer after dilution to obtain the required concentration of $10^6$ conidia $ml^{-1}$. The concentration of $10^6$ conidia $ml^{-1}$ was used for soil and seed treatment.

**Preparation of the chemical fungicides**

Chemical fungicides like Bavistin, Mancozeb and Vitavex at the rate of 2g $kg^{-1}$ were taken for seed treatment and seed were treated accordingly.

**Experimental design**

The experiment was conducted in one factor RCBD with 3 replications. Seven different treatments were used as mentioned on Table no 1 .There were 21 plots with an individual plot size of 50*50 cm. Inter block and inter plot spacing were 50cm and 20cm respectively. Treatments were randomly allocated in experimental units.

**Seed treatment**

Seed treatment was done in the plant pathology lab of NPI. Required amount of *Trichodermaharzianum* spore suspension@$10^6$ CFU/ml and seeds from related treatments were kept in a 250 ml conical flask and were shaken mechanically for 10 minutes for proper coating of bio fungicide. Same procedure was applied for seed treatment with different fungicides.

**Application of treatments in the field**

*Trichodermaharzianum* soil application@$10^6$ CFU/ml was done by soil drenching before the seeds were sown. Spray formulation were sprayed using different hand sprayer for each treatments.

**Observation in field**

Observation was done on regular basis to record the data of germinated seedlings and to record the data of other parameters (seedling height, root weight, shoot weight), ten sample plants were randomly selected and tagged for further observation.

$$Germination\ \% = \frac{No\ of\ seeds\ germinated}{Total\ no\ of\ seeds\ sown} \times 100$$

$$Disease\ incidence\% = \frac{No\ of\ infected\ seedlings}{Total\ no\ of\ seedlings\ in\ a\ plot} \times 100$$

**Data collection and analysis**

The recorded data were tabulated in Excel data sheet and were analyzed by using Gen stat software program. The data entry was done to develop ANOVA table and different treatments were compared through Duncan's multiple range test. All the figures and graphs were prepared by using Microsoft excel 2013.

## III. RESULTS AND DISCUSSION

**Efficacy of chemical fungicide and biological agent seed and soil treatments on germination of bitter gourd seed**

In case of germination percentage of Bitter gourd seedling, analysis of variance (ANOVA) revealed significant difference between the treatments. Mean germination

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percentage of bitter gourd after the application of *Trichodermaharzianum* ($10^6$CFU/ml) in the soil along with vitavex seed treatment was 95%, whereas seed treatment with Bavistin was 84% and control had significantly lowest germination percentage (43%) than other treatments. Combined efficacy of vitavex and Trichoderma resulted in higher germination percentage (Table 1). The *Trichodermaharzianum* was found effective in reducing disease incidence and increasing crop germination (Shanmugam, Varma and Surendran 1999). Also Trichoderma was found most effective in reducing seedling mortality and root infection (increase in plant no.) in cucumber and bottle gourd (Sultan and Ghafar 2013) and this may be due to *Trichoderma species* being capable of producing extracellular lytic enzymes that are responsible for their antagonistic activity (Elad*et. al.*,, 1982). The chemical seed treatment with Carbendazim (Bavistin) also helped for higher germination percentage (85%). Other scientist also reported that Benlate, Carbendazim and Topsin-M completely checked seedling mortality in bottle gourd (Shazad 1994 and Sultan and Ghafar 2013). However, highest increase in germination as compare to control was recorded on Soil treatment with Trichoderma+Vitavex seed treatment followed by Bavistin seed treatment. Present research clearly indicated that combined use of seed treatment with 0.1% Vitavex and soil treatment *with Trichodermaharzianum* is the best option for the management of damping-off disease of bitter gourd seedlings in the nursery among other treatments. It may be due to the inhibitory effect of vitavex and the antagonistic activity of *Trichodermaharzianum*for the reduction of seedling mortality and increment of germination for the production of healthy seedlings in the nursery.

*Table.1: Effect of seed treatment by chemical fungicides and biological agent on germination of bitter gourd seed*

| Treatments | Germination % | Increase in Germination % |
|---|---|---|
| Seed treatment with 0.1% Vitavex +Soil treatment with *Trichodermaharzianum* @ $10^6$CFU/ml | $95.33^{f}$ | 120.01 |
| Seed treatment with Vitavex @ 2g $kg^{-1}$ | $78.00^{d}$ | 80.01 |
| Seed treatment with Bavistin (Carbrendazim 50%WP) @ 2g $kg^{-1}$ | $84.67^{e}$ | 95.41 |
| Seed treatment with Mancozeb 75%WP @ 2g $kg^{-1}$ | $72.00^{dbc}$ | 66.17 |
| Soil treatment with *Trichodermaharzianum* @ $10^6$CFU/ml | $75.00^{cd}$ | 73.09 |
| Seed treatment with *Trichodermaharzianum* @ $10^6$CFU/ml | $69.00^{b}$ | 59.24 |
| Control | $43.33^{a}$ | |
| Grand Mean | 73.9 | |
| SEm(±) | 2.12 | |
| P-value | <.001 | |
| LSD(=0.05) | 3.78 | |
| CV(%) | 2.9 | |

Mean in a column with same letters are not significantly different (p=0.05) according to DMRT, CV= Coefficient of variation, LSD=Least significance Difference,* = significantly different at (P<0.05), ** =highly significantly different at (P<0.01), *** = very highly significantly different at (P<0.001).

**Efficacy of chemical fungicides and biological agent on seed and soil treatments on damping off disease**

In case of pre emergence damping off disease incidence caused by *Pythium spp.*, Analysis of Variance (ANOVA) revealed significant difference between the treatments. The lowest pre emergence damping off disease percentage was recorded in seed treatment with 0.1% Vitavex + soil treatment with $10^6$/ml *Trichodermaharzianum* (3.33%) which was followed by seed treatment with Bavistin (12.67%), seed treatment with Vitavex @ 2g $kg^{-1}$ (18.67%) respectively. Also, in case of post emergence damping off disease incidence caused by *Pythium spp.*, Analysis of Variance (ANOVA) revealed significant difference between the treatments. The lowest post emergence damping off disease percentage was recorded in seed treatment with 0.1% Vitavex + soil treatment with$10^6$/ml *Trichodermaharzianum* (1.33%) which was statistically at par with other treatments viz. Seed treatment with Mancozeb 75%WP @ 2g $kg^{-1}$ (1.33%),Seed treatment with Bavistin (Carbrendazim 50%WP) @ 2g $kg^{-1}$(2.66%), Seed treatment with $10^6$/ml *Trichodermaharzianum* (2.67%), Soil treatment with$10^6$/ml *Trichodermaharzianum* (3.33%), Seed treatment with

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Vitavex @ 2g $kg^{-1}$ (3.33%)except control which had the highest (12.33%) post emergence disease incidence (Table 2).

In the present study, the pre emergence damping off disease incidence reduction was highest in seed treatment with 0.1% Vitavex + Soil treatment with $10^6$/ml *Trichodermaharzianum* (92.49%) followed by seed treatment with Bavistin (Carbrendazim 50%WP) (71.42%). Similarly, in case of post emergence damping off disease incidence, highest reduction in incidence was found in seed treatment with 0.1% Vitavex + Soil treatment with$10^6$/ml Trichoderma (89.21%) followed by Seed treatment with Mancozeb 75%WP (89.21%) (Figure 1) .These results indicated that pre and post emergence damping off of bitter gourd seedling was significantly reduced by using different treatments as compare to control.

The *Trichodermaharzianum* was found effective in reducing disease incidence and increasing crop germination (Shanmugam, Varma and Surendran 1999). Synthetic fungicides bring about the inhibition of pathogens either by destroying their cell membrane or its permeability or by inhibiting metabolic processes of the pathogens and hence are extremely effective (Osman, Alrehiayam, Saudi and Bio 2003). Trichoderma species are capable of producing extracellular lytic enzymes that are responsible for their antagonistic activity (Elad*et. al.*,, 1982). As the findings made by different researchers above in discussion viz. antagonistic behavior of *Trichodermaspp.*due to extracellular lytic enzymes production and inhibitory effect of synthetic fungicide like vitavex by may be reason behind the significant control in pre and post emergence disease incidence with the use of different chemical and biological treatments. Also the combined antagonistic effect of Trichoderma and inhibitory effect of vitavex was found to be best in controlling pre emergence (92%) and post emergence (89%) damping off caused by *Fusarium*and *Pythium spp*.

*Table.2: Effect of seed treatment with chemicalfungicides and biological agent on disease incidence of pre and post damping off disease of bitter gourd in seedling stage*

| **Treatments** | **% Pre-emergence DI** | **% Disease Control** | **% Post emergence DI** | **% Disease control** |
|---|---|---|---|---|
| Seed treatment with 0.1% Vitavex + Soil treatment with *Trichodermaharzianum* @ $10^6$CFU/ml | $3.33^{a}$ | 92.49 | $1.33^{a}$ | 89.21 |
| Seed treatment with Vitavex @ 2g $kg^{-1}$ | $18.67^{c}$ | 57.88 | $3.33^{a}$ | 72.99 |
| Seed treatment with Bavistin (Carbrendazim 50%WP) @ 2g $kg^{-1}$ | $12.67^{b}$ | 71.42 | $2.66^{a}$ | 78.43 |
| Seed treatment with Mancozeb 75%WP @ 2g $kg^{-1}$ | $26.67^{de}$ | 39.84 | $1.33^{a}$ | 89.21 |
| Soil treatment with *Trichodermaharzianum* @ $10^6$CFU/ml | $21.67^{cd}$ | 51.12 | $3.33^{a}$ | 72.99 |
| Seed treatment with *Trichodermaharzianum* @ $10^6$CFU/ml | $28.33^{e}$ | 36.09 | $2.67^{a}$ | 78.35 |
| Control | $44.33^{f}$ | | $12.33^{b}$ | |
| Grand Mean | 22.24 | | 3.86 | |
| SEm(±) | 2.7 | | 1.32 | |
| P-value | <.001 | | <.001 | |
| LSD(=0.05) | 5.89 | | 3.35 | |
| CV(%) | 14.9 | | 42.2 | |

Mean in a column with same letters are not significantly different (p=0.05) according to DMRT, CV= Coefficient of variation, LSD=Least significance Difference,* = significantly different at (P<0.05), ** =highly significantly different at (P<0.01), *** = very highly significantly different at (P<0.001).

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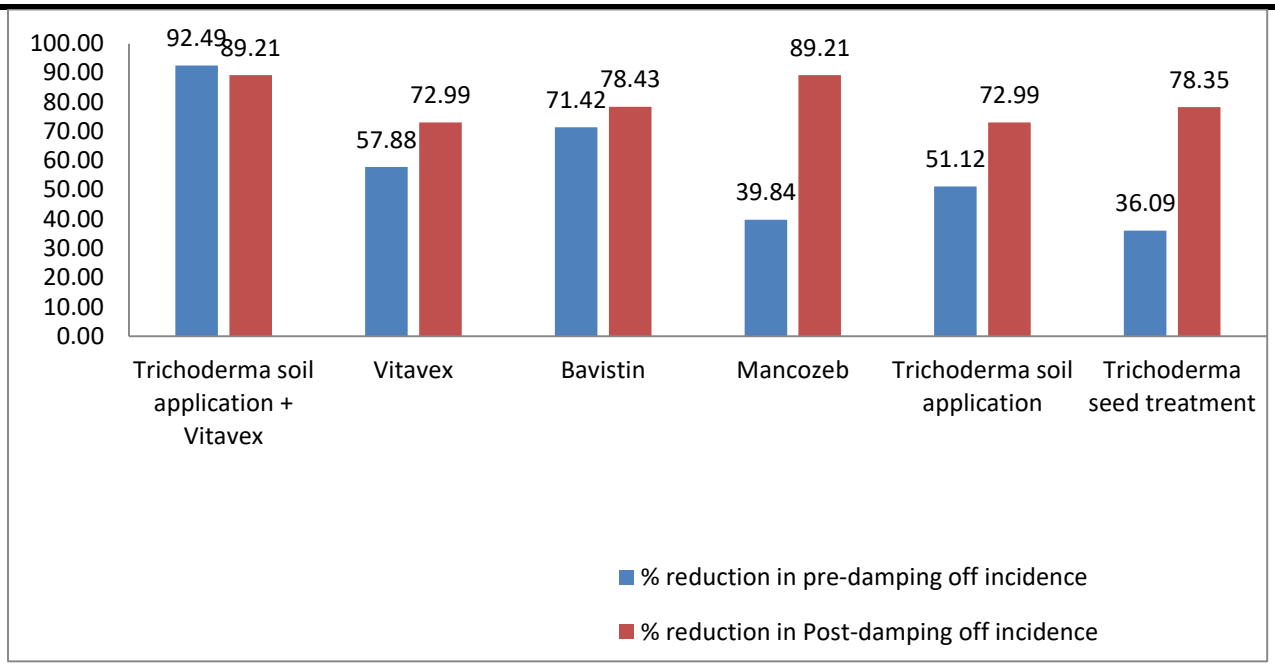


*Fig.1: Percentage reduction in pre and post emergence damping off disease incidence of bitter gourd due to different seed treatments*

## IV. CONCLUSION

The use of *Trichodermaharzianumin* soil along with Vitavex in seed showed the best result among all other treatment. However use of *Trichodermaharzianum* in soil only also showed significant reduction in post and pre emergence disease incidence resulting higher germination percentage, plant height and healthy root system. This may be due to the inhibitory effect of chemical fungicide Vitavex for the growth of pathogen and hormonal effect produce by *Trichodermaharzianum* to stimulate vigorous root and shoot growth of the bitter gourd seedlings. This study was mainly focused on the only one dosage of chemical fungicide and single isolate of Trichoderma on the control of *Pythium spp*. Furthermore, study can be carried out to find out the best dosage of chemical fungicides, best conidial concentration of Trichoderma and best isolate of Trichoderma. Also the best combination and benefit cost ratio could be identified between bio control agent and chemical fungicide.

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# Effect of Biochar and Water Level on Increasing Availability and Water Use Efficiency for Maize in Vertisol from Jeneponto South Sulawesi

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***Abstract*—***This study aims to determine the effect of biochar and water level on improving water retention and water use efficiency of corn crops in vertisol. The soil sample was taken from Jeneponto south Sulawesi. This research used split-plot design. The main plot treatment is a soil amendment consisting of two factors ie without biochar and Biochar,sub plot treatment is a water used level consisting of 4 levels ie 100%, 90%, 80%, and 70% field capacity. Observated parameters include field capacity, permanent wilting point, available water, the crops water consumption, crop matter use efficiency, and water use efficiency. The results showed that biochar was able to increase water retention and water use efficiency at low water used level conditions.*

***Keywords*— Biochar, Water Level, Availability and Water Use, and Vertisol.**

## I. INTRODUCTION

Vertisol is one type of soil that is widely used for agricultural because it has a fairly good fertility rate, characterized by high cation exchange capacity, relatively basic saturation, high water holding capacity , with a neutral to alkaline pH ranging from 6-8.5, but water available low for plants (Deckers*et al.*, 2001; Prasetyo, 2007).

The high water-binding ability of Vertisol is due to the high clay content that may reaches more than 30% in all horizon with montmorillonite as its main mineral (FAO, 1990). May a montmorillonite is a clay mineral that has a very small in size so that the surface area clay becomes high. According to Foth (1998), the fine grain size of the clay affects the pore space and the adsorptive surface area, thereby increasing water storage capacity. The more surface area the more water and ions can be absorbed, 2: 1 clay mineral has surface area of 700-800 m2 $g^{-1}$(smectite) and 57-152 m2$g^{-1}$ (mica-smectiteinterstification), 1: 1 (kaolinite) 7-30 m2 $g^{-1}$, while allophane has surface area of 157 -484 m2 $g^{-1}$ (Tan, 1998).

Hanafiah (2007) reported that groundwater content in the field capacity conditions (1/3 atm) in sand, silt, and clay were 15%, 40%, and 55%, respectively. In Vertisol high water content conditions are also followed by high moisture content at the condition of the permanent wilting point, so the high amount of water available does not guarantee adequate availability for the plant.

Efforts to improve soil properties of vertisol can be done by administering biochar (Gao Lu *et al.*, 2014; Shackley*et al.*, 2012; Atkinson *et al.* 2010; Van Zwieten*et al.*, 2010). Biochar significantly increases the amount of water available in vertisol (Gao Lu *et al.*, 2014; Fangfang and Lu, 2014; Ouyang*et al.*, 2013). One of the ingredients that can be used as a source of biochar is rice husk. Rice husk is an easy agricultural waste in research location and the surrounding area.

Soil improvement in Vertisol is expected to increase water availability for plants and avoid crops from drought. Drought conditions are responsible for 50% crop yield decline in the world (Wood, 2005). Plants with water shortages generally have smaller size compared to normal growing plants (Kurniasari*et al.*, 2010). Lack of water causes a very significant decrease in yield and even the cause of death in plants (Salisbury and Ross, 1992).

Research on the utilization of biochar to improve physical properties and water availability has been widely used (Asai*et al.*, 2009; Atkinson *et al.*, 2010; Chan *et al.*, 2007, 2008; Glaser *et al.*, 2002; Laird *et al.*, 2010; Liu *et al.*, 2012; Major *et al.*, 2010). Based on literature searches, previous studies have studied more appropriate doses of biochar to improve soil properties (Jacek*et al.*, 2017, Pandian*et al.*,2016, Scilowska*et al.*, 2015, Fangfang and Lu, 2014; Gao Lu *et al.*, 2014). So far, further research on how biochar response in improving water availability in various soil moisture conditions has not been done. Based on the above

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description, this study is deemed necessary to determine the effect of soil biochar and moisture level on improving water retention and water used efficiency in vertisol.

## II. MATERIALS AND METHODS

The study was prepared based on a split-plot design with a completely randomized design baseline design. Where the main plot factor is the soil amandement (A) and the plot factor is the water content level (K).The main plot factor consists of A0: no soil enhancer, A1: Biochar. Sub plot factor is K1: 100% field capacity, K2: 90% field capacity, K3: 80% Field Capacity, and K4: 70% Field Capacity. there are 8 treatment combinations and repeated 3 times, so there are 24 units of an experimental block.

Media Planting Preparation

Media planting comes from the Punagaya village Bangkala district Jeneponto. The soil is described as the Vertisol soil developed from the limestone parent material. The soil is taken from a depth of 0-20 cm and then dried, mashed and sieved with a 2 mm diameter strainer. The soil is weighed as much as 12 kg and given the soil enhancer according to the treatment of biochar as much as 6 % of the total weight of the soil (Fangfang and Lu, 2014; Gao Lu *et al.,* 2014). Giving water is done by weighing the pot to know the amount of water that should be given according to the treatment. The initial soil properties can be seen in Table 1.

*Tabel.1: Soil characteristics*

| No | Soil Parameters | |
|---|---|---|
| 1. | pH (H20) | 6,7 |
| 2. | Organic matter | 4,6 % |
| 3. | Organic Carbon | 2,6 % |
| 4. | CEC | 22,5 cmolkg-$^{1}$ |
| 5. | Bulk Density | 1,2 g cm$^{-3}$ |
| 6. | silt | 1,55 % |
| 7. | pasir | 24,9 % |
| 8. | clay | 72,23 % |
| 9. | Porosity | 56.49 % |
| 10. | Field Capacity | 42 % |
| 11. | Permanent Wilting Point | 31 % |

Bulk density analysis was done by gravimetric method. Porosity was determined based on weight value of particle type and weight by using gravimetric method as follows:

Porosity (% volume) = (1- BD (Bulk Density) / PD (Particle Density is 2.65) X 100% (1)

Where BD is bulk density and PD is partikel density use a value of 2.65 for mineral soil. Water retention analysis using Pressure method Plate apparatus at pF 2.54 and pF 4.2 (Capillarity and pF curve equations) (Richards and Fireman, 1943).Water use efficiency in this study used the amount of water given during plant growth (mm) and dry weight of the plant (g) harvested at 60 days old plants, by the formula:

$$WUE = \left(\frac{E_y}{E_t}\right) \times 100 \quad (2)$$

Where WUE is water use efficiency (g.mm-1), EyThe Dry weight of the plant (g), Et is the plant water consumption (mm)

Statistical Analysis

The result were analyzed by using variance analysis and followed by LSD at 5% level using STAR (Statistical Tool for Agricultural Research).

## III. RESULT AND DISCUSSION

**Bulk Density and Soil Porosity**

Statistical analysis showed no interaction between the treatments of soil amendment with the water level. Biochar is able to decrease bulk density and increase soil porosity significantly, whereas biochar treatment decreases bulk density from 0.897 to 0.775 gcm$^{-3}$and increases porosity from 66.13% to 70.72%. Biochar's ability to decrease bulk density and soil porosity was also reported by Jacek*et al.* (2017) in the HaplicPodzol research in which biochar 4,5 and 3 t.ha-1 significantly reduced bulk density after 2 years of application. Biochar 2.5 to 5 t.ha-1 was found to decrease bulk density from 1.41 to 1.3 g.cm$^{-3}$ compared to the application of manure on Alfisol soil (Pandian*et al.,* 2016). Castellini*et al.* (2015) reported that biochar administration significantly balances the amount of liquid phase and gas in the soil and reduces the solid phase in the soil.

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*Table.2: Bulk density and soil porosity*

| Treatments | Bulk Density ($gcm^{-3}$) | Soil Porosity (%) |
|---|---|---|
| | Soil Amendment | |
| A0 (Control) | 0.897 a | 66.13 b |
| A1 (Biochar) | 0.775 b | 70.72 a |
| | Water Level | |
| K1 (100 % FC) | 0.877 a | 67.04 b |
| K2 (90 % FC) | 0.867 a | 67.30 b |
| K3 (80 % FC) | 0.788 b | 70.25 a |
| K4 (70 % FC) | 0.818 ab | 69.12 ab |

Note : numbers followed by different letters are statistically different (P<0.05)

The treatment of moisture level showed that the K3 treatment (80% FC) gave the best result against the decrease of bulk density and porosity increase of 0.788 $g.cm^{-3}$ and 70.25%, which was significantly different with K1 treatment (0.877 g.cm-3 and 67.04 %) and K2 (0.867 $g.cm^{-3}$ and 67.30%) and differed from K4 treatment (0.818 g.cm-3 and 69.12%). The treatment of K1 (100% FC) and K2 (90% FC) caused the soil to become more humid and the air in the soil decreased, whereas on the soil K3 and K4 treatment were drier and the pore of soil was filled with air. In the condition of K3 and K4 is the development of plant roots to be better and affect the decrease of bulk density and increased porosity of the soil.

**Water Retention**

The results of the analysis of variance indicate that there is an interaction between the treatment of soil enhancer and moisture level to field capacity, permanent wilting point, and available water.

**Field Capacity**

Comparison of soil amendment factor (A) at various levels of water content (K) showed that treatment A0 (control) was significantly different from treatment A1 (Biochar) at high water content levels K1 (100%) and K2 (90%), how ever at water content of K3 (80%) and K4 (70%) there is no real difference between A0 (control) and A1 (Biochar).

The comparison of water content (K) factor at soil amendment level (A) shows the field capacity at the highest A0 (control) treatment achieved at K1 treatment (100%) of 0.597% followed by K2 treatment (90%) of 0.56% and significantly different with K3 (80%) and K4 (70%). In Treatment A1 (Biochar) there was no significant difference between the various levels of water content (Table 3).

*Table.3: The effect of soil amendment an water level on field capacity*

| Treatments | Water Level | | | |
|---|---|---|---|---|
| | K1 (100 % FC) | K2 (90 % FC) | K3 (80 % FC) | K4 (70 % FC) |
| Soil Amendment | | | | |
| A0 (Control) | 0.597aA | 0.560aA | 0.433aB | 0.493aB |
| A1 (Biochar) | 0.480bA | 0.430bA | 0.450aA | 0.446aA |

Note : numbers followed by different letters are statistically different (P<0.05). Different small letters indicates the Comparison of A at each level of K. Different capital letters shows Comparison of K at each level of A.

**Permanent Wilting Point**

Comparison of factor A at level K showed that the treatment of A0 (control) and A1 (Biochar) was significantly different at levels of water K1 (100%), K2 (90%), K4 (70%) and not significantly different at K3 level (80 %).

The comparison of factor K at level A shows that there is no real difference of permanent wilting point on treatment A0 (control). At treatment A1 (Biochar), the highest wilting point reached at K1 (100%), but content was not significantly different with K3 treatment (80%) danK4 (70%), but significantly different from K2 treatment (90%)(Table 4).

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*Table.4: The effect of soil amendment an water level on permanent wilting point*

| Treatments | Water Level | | | |
|---|---|---|---|---|
| | K1 (100 % FC) | K2 (90 % FC) | K3 (80 % FC) | K4 (70 % FC) |
| Soil Amendment | | | | |
| A0(Control) | 0.370 aA | 0.356 aB | 0.293 aC | 0.337 aB |
| A1(Biochar) | 0.307 bA | 0.263 bB | 0.283 a AB | 0.283 bAB |

Note : numbers followed by different letters are statistically different (P<0.05). Different small letters indicates the Comparison of A at each level of K. Different capital letters shows Comparison of K at each level of A.

**Water available**

Comparison of A at level K shows a significant difference between A0 (control) and A1 (biochar) occurring at treatment K1 (100%) and K2 (90%) but not significantly different at K3 and K4.

Comparison of K at level A indicated that the highest available water A0 (control) was achieved at the treatment of K1 and K2 and was significantly different from the treatment of K3 and K4. While treatment A1 (Biochar) showed no significant difference in water available at various levels of water content (table 5).

*Table.5: The effect of soil amendment and water level on water available*

| Treatments | Water Level | | | |
|---|---|---|---|---|
| | K1 (100 % FC) | K2 (90 % FC) | K3 (80 % FC) | K4 (70 % FC) |
| Soil Amendment | | | | |
| A0(Control) | 0.267 aA | 0.203 aA | 0.170 aB | 0.157 aB |
| A1(Biochar) | 0.173 bA | 0.170 bA | 0.167 aA | 0.163 aA |

Note : numbers followed by different letters are statistically different (P<0.05). Different small letters indicates the Comparison of A at each level of K. Different capital letters shows Comparison of K at each level of A.

The result of statistic analysis for field capacity, permanent wilting point and water available on the comparison of soil enhancer (A) to water content level (K) showed that there was a significant difference between treatment A0 and A1 at water level K1 and K2, while on treatment K3 and K4 is not significantly different. This shows that at high levels of water content, A0 (control) treatment is able to bind water better than in treatment A1 (Biochar), but in low water content treatments, biochar is able to bind water better than control treatment. This result is in line with the Devereux et al. (2012) study which states that the addition of real biochar increases the water holding capacity when soil conditions dry out.

Comparison of moisture level (K) to the soil enhancer (A), indicating that the field capacity, permanent wilting point, and water available at the A0 treatment (control) decreased as water supply decreased. While in treatment A1 (Biochar) showed no real difference in field capacity, permanent wilting point, and water available at all levels of water content. The results of this study are in line with the results of the Fangfang and Shenggao (2014) study which stated that rice bran biochar on vertisol increases groundwater content in field capacity, permanent wilting point, and water available to plants.

**The Crops Water Consumption**

*Table.6: Effect of Soil Amendment (A) and Water Level (K) on the Plant water Consumption*

| Treatments | Water consumption (mm) |
|---|---|
| Soil Amendment | |
| A0 (Kontrol) | 125.49 b |
| A1 (Biochar) | 163.43 a |
| Water Level | |
| K1 (100 % KL) | 216.72 a |
| K2 (90 % KL) | 146.04 b |
| K3 (80 % KL) | 113.91 c |
| K4 (70 % KL) | 101.18 c |

Note :numbers followed by different letters are statistically different (P<0.05)

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The results of statistical analysis showed no interaction between treatment of soil amendment (A) with water level (K), but there was significant difference between the influence of soil amendment (A) and water content level (K), in which biochar administration increased the amount of water 163.43 mm in maize compared with A0 treatment (125.49 mm).

For the comparison of the treatment of moisture content, the largest amount of water consume by corn crops was achieved at K1 treatment (216.72 mm) followed by K2 (146.04 mm), K3 (113.91 mm), and K4 (101.18 mm) respectively. This is in line with Handayani (2004) study which states that the lower the moisture level of the soil during watering, the less water it will be. The reduced water the treatment responds to the plant by adjusting for water use during its growth phase. The plant responds to drought conditions in two ways by changing the distribution of new assimilates and regulating the level of stomatal opening to reduce the loss of water through transpiration (Mansfield and Atkinson, 1990).

**The Dry weight of the plant**

For the dry weight component of the plant, the statistical analysis shows that there is an interaction between the soil amandment (A) and the water content (K) level (Figure 2). For comparison A at level K, it was seen that treatment A1 (biochar) gave the highest yield and was significantly different from treatment A0 (control).

Comparison of water level (K) at soil amendment level (A) shows that the dry weight of the plant decreases in line with the decreasing amount of water administered both on treatment A0 (control) and A1 (Biochar). Maize is a very sensitive plant with soil moisture, where water is the limiting factor. The Khalili et al. (2014) study showed that the weight of plant biomass treated with drought stress significantly decreased compared to the control treatment. Previous research also proves that the decline in plant biomass is closely related to the decrease in soil moisture (Stone *et al.*, 2001, Osborne *et al.*, 2002).

*Table.6: The effect of soil amendment and water level on dry weight*

| Treatments | Water Level | | | |
|---|---|---|---|---|
| | K1 (100 % FC) | K2 (90 % FC) | K3 (80 % FC) | K4 (70 % FC) |
| Soil Amendment | | | | |
| A0(Control) | 17.346 bA | 15.355 bB | 12.903 bC | 6.489 bD |
| A1(Biochar) | 76.823 aA | 50.795 aB | 46.940 bC | 45.610 aD |

Note : numbers followed by different letters are statistically different (P<0.05). Different small letters indicates the Comparison of A at each level of K. Different capital letters shows Comparison of K at each level of A.

**Water Use Efficiency**

There is an interaction between the treatment of soil enhancer and the level of water content to the efficiency of water use in corn crops. The comparison of the median treatment of factor A at level K showed that treatment A1 (Biochar) gave the highest yield and was significantly different at different levels of water content than the A0 (control) treatment. Biochar's ability to increase the efficiency of plant water use caused biochar to increase the availability of plant nutrients, improve cation exchange capacity so as to improve crop growth and yield (Atkinson *et al.*, 2010; Mukherjee and Lal, 2013). This is in line with Yeboah's (2016) study which stated that 5 ton/ha biochar significantly increased corn yield by 2.5 ton H-1 compared to without biochar. Previous studies also suggest that Biochar can provide nutrients for plants, especially cations such as K, Ca and Mg (Danik***et al.,*** 2011) and ensure nutrient availability for plants (Zhang *et al.*, 2016).

*Table.7: The effect of soil amendment and water level on water use efficiency*

| Treatments | Water Level | | | |
|---|---|---|---|---|
| | K1 (100 % FC) | K2 (90 % FC) | K3 (80 % FC) | K4 (70 % FC) |
| Soil Amendment | | | | |
| A0(Control) | 8.667 bC | 11.420 bB | 14.519 bA | 9.197 bC |
| A1(Biochar) | 33.999 a AB | 32.350 aB | 33.871 a AB | 34.699 aA |

Note : numbers followed by different letters are statistically different (P<0.05). Different small letters indicates the Comparison of A at each level of K. Different capital letters shows Comparison of K at each level of A.

For the treatment of water level (K) ratio at soil enhancer level (A) showed that the efficiency of water use in the K3 treatment gave the best result for corn corp on treatment A0 (control) and K4 level gave the best result of 36.69% for

treatment A1 (Biochar) was significantly different from K2 treatment (32.35%) and was not significantly different with K1 and K3 treatment. The high efficiency of water usage at K3 level for treatment A0 (control) and K4 level on treatment A1 (Biochar) showed that under high humidity conditions (K1 and K2 levels) nutrient absorption did not run optimally, so that water content is appropriate for treatment A0 (Control) is at the level of K2 80% of the field capacity, under the condition of the moisture content the availability of nutrients decreases as the permanent wilting point increases. Provision of biochar is proven to increase the efficiency of water use at low levels of water content.

## IV. CONCLUSION

1. Biochar is able to increase field capacity, reduce permanent wilting points and increase the amount of water available at all levels of water content.
2. Bbiochar feeding increases the amount of water consumption of the plant.
3. There is an interaction between the soil enhancer and the moisture content of the dry weight of the plant. The biochar treatment (A1) gave the best results compared to the treatment without biochar (A0). The dry weight of the plant decreases as the amount of water is decreased.
4. Biochar (A1) was able to increase the efficiency of water use compared to without biochar treatment (A0), and the highest result was obtained in combination of biochar treatment and lowest moisture content (K4).

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# Role of Phytogenic Feed Additives in Swine Production- A Review

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***Abstract*—** *Continuous research is being carried out to attain higher productivity with the available resources since several decades. Feed additives comprising of probiotics, prebiotics, acidifiers, immune modulators, buffering agents, ionophores etc. though are in vogue,in addition to antibiotic growth promoters(AGP), advancement is being aspired through the way of herbs and their products which are called as Phytogenic feed Additives (PFA) or simply Phytobiotics. PFA are said to be having positive effects in improving the performance of poultry and swine. Many reports say that PFA increase the dry matter intake probably due to an increased palatability of the feed.PFA is said to have anti microbial and anti oxidant properties. In addition, PFA have shown to improve the endogenous enzyme secretion, stimulation of appetite, improving the digestibility and absorption of nutrients and also promote the proliferation of beneficial bacteria like Lactobacillus spp. A ban on the use of AGP leads to explore the use of herbs and their products like extracts and residues. Herbal residues are the left over's remained after the active principle is extracted. Reports say that extraction efficiency (%) ranges from 88-97 for different methods. Some of the residues showed considerable anti bacterial property at 2% levels during the Minimum Inhibitory Concentration tests. The use of PFA is restricted to commercial preparations and results are available only for these works, there needs a systemic approach to explain about the function of these PFA in terms of type and dose of each additive. However long term studies will be of added advantage proving the efficacy of these PFA,their safety for animal health and their availability widely in nature. The aim of this review is to explore and explain the multifaceted properties of PFA in terms of elimination of gut pathogens improving the digestibility and palatability and thus enhancing the overall production of the animal.*

***Keywords*— *Feed additives, PFA, performance, pathogen inhibition, nutrient digestibility.***

## I. INTRODUCTION

Any nutrient fed to the livestock is meant for its productivity and the accountability for this nutrient is fulfilled only when it is used to the maximum extent. Proteins and energy, the major nutrients are of critically important and these provide energy to the livestock on metabolism. But there are certain non-nutrient substances used in animal nutrition for getting the better quality of feed, better quality livestock products, for better availability of nutrients in the gut, and also for improving the gut health. Feed additives are the non-nutrient substances which come under this category. Common feed additives used in animal diets include probiotics, prebiotics, immune modulators, antimicrobials, anti oxidants, enzymes, pH control agents, flavonoids in addition to antibiotic feed supplements

There is increasing pressure for livestock producers to minimize the use of antibiotics as growth promoters in food animals. There is a ban on the use of most of the antibiotic feed additives within the European Union in 1999, a complete ban enforced in 2006, due to a speculated risk of generating antibiotic-resistance in pathogenic bacteria.

**Some discussion on other feed additives**

Prebiotics have been described as non-digestible oligosaccharides which selectively stimulate the growth of favourable species of bacteria in the gut, thereby benefitting the host. Because they are not digested and absorbed by the pig, they provide readily available substrates for the normal bacteria to grow. Fructo-oligosaccharides (FOS), Mono-oligosaccharides (MOS) and inulin are the best examples that have been used as prebiotics.

Probiotics are live microbials supplemented in pig diets that can beneficially affect the host animal by improving the microbial balance in the gut.Probiotics commonly used include*Lactobacillus acidophilus*, *Enterococci faecium*, *Bacillus* species, *Bifidobacteriumbifidum*, and the yeast *Saccharomyces cerevisiae*. As feed additives, they are supplemented in diets to improve the balanceof bacteria in the gut.

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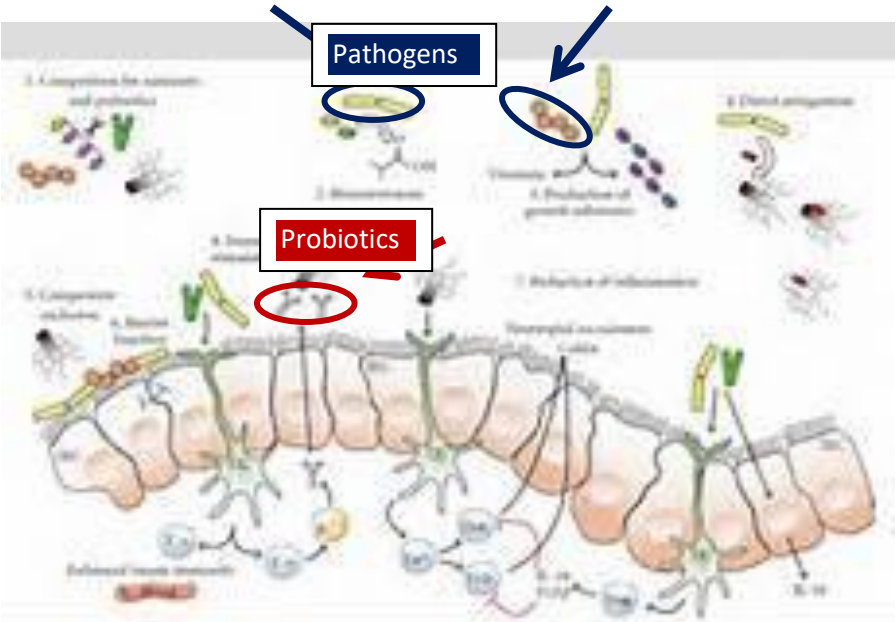
The proposed benefits from probiotics are improved digestion, stimulation of gastrointestinal immunity and increased resistance to infectious diseases of the gut. Probioticsalso changes the permeability of the mucous membrane and increase the nutrient uptake and thus improve the growth performance.




**Binding of probiotic organisms to the intestinal mucosa**

Anotherimportant feed additivesare the antibiotic growth promoters (AGP).These are the substances which are produced by the living organisms (molds, bacteria, fungi or green plants) and which have bacteriostatic /bacteriocidal properties. In addition to their feed addition as growth promoters, antibiotics are used as nutritional stimulants to promote better feed efficiency in ruminants and swine and to increase the egg production, hatchability and shell quality in poultry. They are also added to the feed in substantially higher quantities to remedy pathological conditions. Since there is a ban on the use of AGP in the farm animals to improve the productivity and health status by the European Union and US, the use of other feed additives have come into force.

Other feed additives include those which influence feed stability, those which modify animal growth, which feed efficiency, metabolism and performance and those which modify consumer acceptance. Antifungals, antioxidants, pellet binders, acidifiersfeed flavours, buffers, immune modulators, xanthophylls etc all come under these categories. In spite of very good results obtained using these additives, they are still not comparable to those obtained using antibiotic growth promoters and research is still very actively looking for new alternatives to combat the increased potential for bacterial disease development in growing pigs especially under conditions of average management quality.All these additives either improve the keeping quality of the feed or increase the feed intake and most of them have no role in the nutrient utilization from the feed in the gut. For the nutrients to be utilized to the maximum they have to be attached to the gut mucosa for absorption and utilization.But this sometimes gets minimized due to the presence of pathogenic organisms which compete with the nutrients for absorption cites in the gut mucosa. In this process, some of the nutrients will be eliminated from the gut due to lack of sites. Hence there should be some additive which eliminates the pathogens from the gut and this is to say that the additive should have antibacterial property. Antibiotic feed additives belong to this category. With the introduction of Aureomycin in 1949 as a growth promoter, sub-therapeutic dosage of antibiotics in animal feed has been generalized all over the world and has produced important benefits in productive performance and in the prevention of pathologic processes (Anderson *et al.*, 1999). However after five decades of usage, concerns about bacterial resistance have become an important issue. Since there is a ban on the use of these antibiotics, alternates in the form of Phytogenic feed additives are being explored.

**What are Phytogenic feed additives**

Phytogenic feed additives (often also called ‘phytobiotics’ or ‘botanicals’) are commonly defined as plant-derived compounds incorporated into diets to improve the productivity of livestock through amelioration of feed properties, promotion of the animals’ production performance, as well as improving quality of food derived from those animals.

Herbs, their residues and plant extracts (PE) are one of the oldest additives used by mankind. However during the 20$^{th}$ century, they are left apart because of the production of synthetic drugs. Recently doubts about

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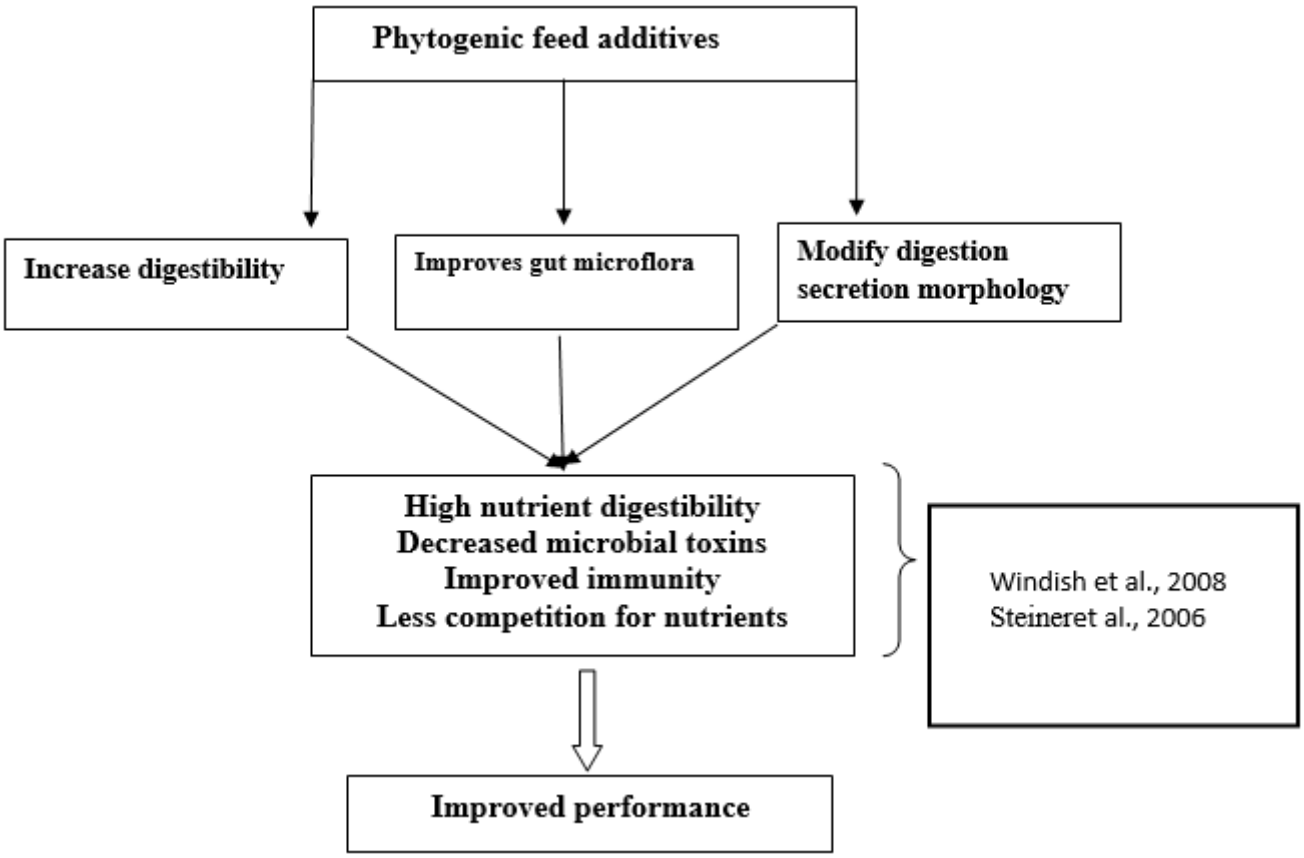
safety of some synthetic drugs, especially antibiotics, have allowed the growth of new interest on the so-called natural products i.e., herbs and plant extracts. These are termed as **Phytogenic feed additives**(often also called 'phytobiotics' or 'botanicals') which are commonly defined as plant-derived compounds incorporated into diets to improve productivity of livestock through amelioration of feed properties, promotion of the animals' production performance, as well as improving quality of food derived from those animals.

Whole herbs contain many active principles used to treat diseases and relieve symptoms. Herbal medicine (botanical medicine), uses the plant's seeds, berries, roots, leaves, bark or flowers for medicinal purposes. Many factors like the type of environment in which the plant grows, the harvesting method of the herb and the way in which the herbal plant is processed influence the efficiency of an active principle. Maceration with solvents like water, alcohol and other solvents will also affect the efficiency of an active principle to work.

**Schematic diagram on the various functions of phytogenic feed additives**




**Probable functions of Phytogenic feed additives**

These feed additives explored after a ban on certain additives is said to have antimicrobial (Guo et al, 2004 a), antioxidant (Hahemi et al, 2009 a), anti-stress (Chattopadhyaya et al,2005), gut flora multiplication (Hahemi et al, 2009 b)and immune enhancement (Guo et al, 2004 b) and over and above feed intake is increased. Photogenic feed additives also comprises of a wide variety of herbal residues, spices and products derived thereof. The mode of action of plant active substances include improvement of the endogenous enzymes secretion, stimulation of appetite, improving the digestibility and absorption of nutrients, promote proliferation of beneficial bacteria like *Lactobacillus* species in the gut.

**Anti microbial property**

Many reports say that these feed additives have antibacterial /anti microbial property which is depicted by the inhibition of many pathogenic bacteria in the gut (Chizzola et al, 2005; Newman et al, 2000; Cowman 1999;Baratta et al, 1999; Namkung et al, 2004). It was also reported that these improve the post weaning performance in pigs (Sulabo et al., 2010).Anti microbial effect is due to the elimination of pathogenic bacteria in the gut and thus making the nutrients more available to the animals and thus improves the performance. This property is mainly attributed to the presence of essential oils in the medicinal plants. Oregano and Thyme are the main essential oils which gained interest in this regard.

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In general, phytogenic feed additives have a strong antibacterial and to some extent antifungal properties. They inhibit the growth of *Escherichia coli*, *Proteus sp*, *Staphylococci*, *Streptococci* and *Salmonella* (Aruoma*et al.,* 1996; Benencia and Courreges, 2000; Garcia *et al.,* 2003) which otherwise compete with the host for nutrients.

The antimicrobial property was attributed to the hydrophobicity (Newbold *et al*., 2004) of plant extracts which facilitates their union to the bacterial surface inducing unstabilization (Tsuchiya *et al.,* 1996; Zhang and Lewis, 1997) or the inactivation of different molecules of the bacteria such as enzymes or receptors through their union to the specific site(Mohammadi et al 2015 a & b).

Residues of Ginger, Emblica and Turmeric were used in the swine rations (Suryanarayana, 2010) and has reported maximum inhibitory effect on pathogenic bacteria in the gut was shown by Ginger followed by Turmeric and Emblica. The antibacterial effect in gut pathogens was in the order of ginger > turmeric >amla.

These products are used in animal production as alternatives to AGP because of their antimicrobial properties. However, many other different effects have been reported such as changes in immune function (Boyaka*et al.,* 2001), enzyme stimulation (Platel and Srivasan, 2000), antiparasitic (Force *et al.,* 2000), antifungal (Mahmoud, 1994), antiviral effects (Aruoma*et al.,* 1996; Benencia and Courreges, 2000; Garcia *et al.,* 2003), anti-toxigenic activity (Sakagami*et al.,* 2001) and antioxidant activity (Dorman *et al.,*2000 ;Teissedre and Waterhouse, 2000). Concerning digestive function, they have important effects upon secretions and motility of the stomach and intestine. Given the enzymatic limitation of the piglet at weaning and also the limited ability of the pigs to digest dietary fibre, these products may be beneficial in improving the digestive capacity of pigs.

*Emblicaofficinalis* (Synonym, Phyllanthusemblica) has been known to have antioxidant, hepatoprotective and immunomodulation effects (Bandyopadhyay*et al.,* 2000; Sai Ram *et al.,* 2002).

Ginger (*Gingeberisofficinale*) has strong antibacterial and to some extent antifungal properties. *In vitro* studies have shown that active constituents of ginger inhibit multiplication of bacteria in colon. These bacteria ferment undigested carbohydrates causing flatulence. It inhibits the growth of *Escherichia coli, Proteus spp*, *Staphylococci*, *Streptococci* and *Salmonella.* The ginger extract has antimicrobial action at levels equivalent to 2000 mg/ ml of the spice. Ginger inhibits aspergillus, a fungus known for production of aflatoxin, a carcinogen. Fresh ginger juice showed inhibitory action against *Asperigillu sniger*, *S.cerevisiae*, *Mycodermaspp.*.and *L. acidophilus* at 4, 10, 12 and 14% respectively, at ambient temperatures, respectively (Windisch*et al*., 2008)

Turmeric (*Curcuma longa*) is a well-known indigenous herbal medicine. It's major constituents, curcumin, various curcuminoids, curcuma oil – particularly dl-ar-turmerone – exhibit a wide range of biological activities like anti-bacterial (Windisch*et al*., 2008), anti-inflammatory, hypolipidemic, hepatoprotective, lipoxygenase, cycloxygenase, protease inhibitory effects, besides being effective active oxygen scavengers and lipid peroxidase inhibitors.

**Antibacterial activity (Inhibition zone, mm) of herbal residues (Suryanarayana, 2010)**

| **Herbal residues** | *Escherichia coli** | *Staphylococcus aureus** | *Salmonella typhimurium** | *Bacillus cereus** | *Campylobacter jejuni* | *Listeria monocytogenes** | *Streptococcus pyogenes** | *Methicillin resistant Staphylococcus aureus*** |
|---|---|---|---|---|---|---|---|---|
| ***Emblica Officinale*** | 18.00 $^{b}$ ± 1.15 | 19.33 $^{b}$ ±0.33 | 13.33 $^{b}$ ±0.33 | 14.00 $^{b}$ ±1.15 | 13.00 ± 0.58 | 16.67 $^{b}$ ±2.33 | 12.00 $^{b}$ ± 0.00 | 13.00$^{c}$ ± 0.57 |
| ***Curcuma longa*** | 21.00 $^{ab}$±2.31 | 25.00 $^{ab}$ ±2.88 | 22.00 $^{ab}$±3.46 | 12.00 $^{b}$ ±1.15 | 13.00 ± 0.58 | 21.00 $^{ab}$ ±1.73 | 18.00 $^{ab}$±2.31 | 18.66 $^{b}$ ±0.68 |
| ***Gingiber Officinale*** | 26.00 $^{a}$ ±1.15 | 30.67 $^{a}$ ±3.48 | 24.33 $^{a}$ ±3.48 | 18.00 $^{a}$ ±1.15 | 13.00 ±1.15 | 25.00 $^{a}$ ± 1.15 | 20.33 $^{a}$ ± 4.09 | 22.67 $^{a}$ ± 1.21 |

$^{abc}$ values in a column not sharing common superscripts differ significantly ** (P<0.01) * (P<0.05)

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| Name of the herb | Properties identified | | |
|---|---|---|---|
| | Antioxidant | Anti viral | Anti bacterial |
| **Black mustard** | 4 | 4 | 5 |
| **Clove** | 3 | -- | -- |
| **Coriander** | 7 | 12 | 20 |
| **Cumin** | 5 | 7 | 11 |
| **Garlic** | 9 | 5 | 13 |
| **Ginger** | 6 | 6 | 17 |
| **Oregeno** | 14 | 11 | 19 |
| **Thyme** | 4 | 3 | 5 |
| **Turmeric** | 3 | 3 | 5 |

**Some of the phytogenic feed additives with number of active principles identified**

**Antioxidant property**

The extracts from the phytogenic plants (herbs & spices) are said to have Anti-oxidative properties (Wei and Shibamoto, 2007). Among a variety of plants the volatile oils from the *Labiatae* family have drawn more interest. These anti oxidant feed additives will prevent the auto oxidation of the cells preventing the cell damage and (Miguel,2010) protects the feed lipids also from cell damage. It was reported that these feed additives protect the cells on par with the feed added antioxidants like tocopheryl acetate or butylated hydroxytoluene (**BHT**).

**Some information on herbal residues**

Herbal residues are the left over remained after the active principle is extracted which is the most common method of getting out the active principle. Generally the organizers follow 2 methods of extraction-(i) until equilibrium exists between drug components and solvent (decoctions, tinctures etc) (ii) extraction of active principle to exhaustion (until all solvent extractables are removed. Extraction efficiency (%) for different methods range from 88-97 and in no case extraction is percent (Chemiloids Pvt Ltd).

Residues o*f Curcuma longa, Emblicaofficinale and Gingeberisofficinale*were able to inhibitthe pathogenic bacteria *Viz- Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, Bacillus cereus, Campylobacter jejuni, Listeria monocytogenes, Streptococcus pyogenes,* Methicillin resistant *Staphylococcus aureu* . These residues were able to inhibit the pathogenic bacteria at 2% level during the studies by Minimum Inhibitory Concentration (MIC) tests (Suryanarayana, 2010). Feeding diets containing herbal residues reduced ($P<0.01$) the Coliform, Staphylococci and Salmonella in the gut of swine (Suryanarayana et al, 2010)

During *in vitro* studies conducted by (Suryanarayana et al, 2010) it was reported that *Zingiberis* residue was effective in inhibiting the growth of pathogens. It was observed that herbal residues are able to check the growth of bacteria during fermentation. Higher Organic matter fermentation, higher acetic acid production, lower pH could be the probable reasons for a lower bacterial count in $T_4$, since these factors can arrest the growth of undesirable bacteria especially *Salmonella*. It is well known that the presence of the SCFA will lead to a drop in pH that can have a negative effect on some potentially pathogenic bacteria (Williams et al., 2005). It has also been shown that SCFA inhibit the growth of *Salmonella* (Van derwiele, 2001). VFA can have an antibacterial effect, thereby preventing the establishment of pathogenic bacteria, such as *Salmonella* spp. (Cummings and Englyst, 1987).

The growth of *Salmonella*, a gram-negative bacterium and more dreadful communicable from humans to animals and vice-versa is checked with certain of the Phytogenic feed additives as mentioned here under…

Extract of *Schezandra efluctus* is effective against 13 strains of *Salmonella* (Zaika, 1988)

Golden seal fights against harmful bacteria especially *Salmonella*

Allian, (from garlic oil) checks *Salmonella* (Johnson and Vanght, 1969)

Turmeric (*Curcuma Longa*) contains curcuma and curcuminoids (phytochemicals) guard the stomach by killing *salmonella* (Vitaminstuff.com)

(Windisch *et al*., 2008) *In vitro* studies have shown that Ginger extract (2000 mg/ml) inhibits *E. coli, Proteus spp. Staphylococcus, Streptococcus* and *Salmonella*.

*Emblica officinalis* (active principle) inhibits pathogenic bacteria Coliforms, Staphylococcus and Salmonella in gut of monogastric animals (Bandyopadhyay *et al*., 2000)

Ginger residue inhibits pathogenic bacteria in the gut followed by turmeric and amla suggesting that ginger has high antibacterial activity (Suryanarayana *et al.,* 2010). These check Coliforms, *Staphylococcus* and *Salmonella*

In general phytogenic feed additives (herbs and their products) have a strong antibacterial and antifungal properties. They inhibit *E. coli. Proteus spp., Staphylococcus, Streptococcus* and *Salmonella.*

## II. CONCLUSION

The primary mode of action of phytogenic feed additives is by beneficially affecting the ecosystem of GI tract through controlling the pathogens. This is benefitted to the animal during stress conditions by not losing the

immunity which otherwise usually occurs. There seems no restriction globally over the use of these phytogenic feed additives with a notion that some resistance will develop for them in the animal body

Even though a product is said to be of natural origin, it is not necessarily better or safer than antibiotics or other synthetic feed additives. It is important to note that various antibiotics also are of natural origin. The fact that some herbs and spices also exhibit antimicrobial properties suggests that phytogenic feed additives may pose similar risks to producers and meat consumers. Similarly, potential overdose that may be harmful to the pig also is possible. All of these considerations warrant further investigation into the safety of phytogenic feed additives both for humans and animals.

PFA should not only look to the profitability and superior quality of livestock products but also should look to food safety and environmental regulation. PFA was said to have reduce the environmental pollution by reducing the release of ammonia, methane and greenhouse gas emission. PFA include essential oils, spices, herbs and then products which improves growth rate, nutrient digestibility and gut health in animals. They can act as an alternate to AGP and the rapid growth of the popularity of organic farming can also considered as the major cause for exploring PFA. In a nut shell, PFA increases feed intake, improves gut function, reduces anti-oxidation of the cell and eliminates pathogens from the gut.

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# Determination of Physiochemical Properties and Lactic Acid Bacteria Presence in Ackee (*Blighia sapida*) Fruit

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***Abstract—*** *This study covers proximate analysis and Antimicrobial activities of cell free extract of lactic acid bacterial from Blighia sapida fruit (Ackee). The proximate analysis of Blighia sapida were determined using standard methods which varied in contents. The proximate analysis of the sample were ash content (6.10±0.03%), moisture content (5.43±0.12%), fat content (14.60±0.02%), crude fibre content (25.12±0.02%), protein content (14.19±0.09%) and carbohydrate content (34.19±0.23%). Probiotic of lactic acid bacteria isolated from Blighia sapida (Ackee) were identified as Lactobacillus leshmanni, and Lactobacillus acidophilus, with Streptomycin sulphate as control. Antimicrobial activities of the lactic acid isolated were determined by the agar well diffusion methods against pathogenic bacteria: Escherichia coli, Staphylococcus aureus, Xanthomonas sp, Pseudomonas aeruginosa, Salmonella typhi and Bacillus subtilis. The zone of inhibition ranged from 3mm to 25mm and control ranged from 23mm to 30mm. Lactobacillus acidophilus displayed high antimicrobial activity compared with Lactobacillus leshmanni showing its probiotic potential of lactic acid bacteria use for food supplement.*

***Keywords— Blighia sapida, probiotic, L. leshmanni, L. acidophilus, proximate analysis.***

## I. INTRODUCTION

Ackee arils have been reported to have comparable proximate composition to many known legumes and oil seeds (Ekue *et al*., 2010; Akintayo *et al*., 2002; Howele *et al*., 2010). However, Ackee arils have little commercial and nutritional significance in the West African sub-region.

The ripe fruit arils are eaten fresh, dried, fried, roasted or made into sauce or soup in some parts of West Africa (Ekue *et al*., 2010).

Various parts of the Ackee tree are employed in traditional medicine for the treatment of fever, malaria, internal hemorrhage, dysentery, yellow fever, diabetes and constipation in West Africa. The roots, bark, leaves, capsules and seeds were identified in the treatment of 22 diseases in Benin (Ekue *et al*., 2010). Consumption of Ackee roots bark extract exerted significant hypoglycemic effect on the normoglycemic albino rats (Saidu *et al*., 2012). However, limited information exists on the health beneficial components of the arils.

The fruit produces lather in water and is therefore used for laundering purpose in some West Africa countries (Saidu *et al.*, 2012). The extract of the flowers is used as cologne while the pulverized bark is mixed with grounded hot peppers and rubbed on the body as stimulant. Probiotic are defined as live microorganism which when consumed in adequate amounts as part of the diet, may play an important role in respiratory, immune and gastrointestinal functions and have a significant effect on the clearance of infectious diseases in children and lactose intolerance (FAO/WHO, 2001). The objectives of this study is to determine the proximate composition and assess antimicrobial activities of lactic acid bacteria against pathogenic microorganisms.

## II. MATERIALS AND METHODS

Fruit used were obtained from Masifa-lle, Ejigbo Local Government, Osun State western part of Nigeria, The fresh sample was sun dried for 3 days and was later oven-dried for another 2 days at 60°C which was immediately blended and was carefully packed in air tight polythene bag and kept in a refrigerator at $4^0$C until further analysis.

### Proximate Analysis of *Blighia sapida*

Moisture content was determined at $105^0$C using air oven, ash content was determined at $550^0$C with muffle furnace while crude protein, fat and crude fibre were determined according to the procedures of AOAC (2000).

**Cultural and Morphological Characteristics of Bacteria from *Blighia sapida***

Lactic acid bacteria were isolated from the fruits using De-Mann Rogosa Sharpe (MRS) agar and incubated for 24 hours. After incubation, the distinct bacterial colonies on the cultured plates were examined for colonial characteristics such as colour, shape, size, elevation, surface and edges. The growth pattern of lactic acid bacteria (LAB) in MRS broth after incubating at $37^0$C and $45^0$C for 24 hours was examined (Dave and Shah, 1996).

**Antimicrobial Activity of the LAB Isolates from *Blighia sapida***

Antimicrobial activities of the sample were determined by the agar well diffusion method. 24 h broth culture of the test isolates such as *Escherichia coli*, *Staphylococcus aureus*, *Xanthomonas* sp *Pseudomonas aeruginosa*, *Salmonella typhi* and *Bacillus subtilis*, were prepared. Two milliliter of the broth was added to molten nutrient agar separately and allowed to solidify under laminar flow. Size 8 mm cork borer was used to carve uniform wells on the surface of the dry seeded plates. 0.5ml of each cell-free extract was introduced into each well separately with the aid of needles and syringes before incubation. The plates were incubated at $37^0$C for 24 h. Zones of inhibition were measured with the aid of digital Vernier caliper and recorded appropriately and control plates were using Streptomycin sulphate.

## III. RESULTS AND DISCUSSION

The moisture content of *Blighia sapida* (5.43±0.12%) was low compared to that of Fallon *et al*., (2014) who reported (8.39±0.25%) for tree-ripened Ackee. It was also a little bit low to the Akintayo *et al*., (2002) that obtained (6.8%) for Ackee pulp and seed flours. Differences in reported moisture content could be due to different methods of ripened. However it was obviously lower than 10% moisture content limit recommended for storage stability of flours (Oladele and Oshodi, 2008). Ash content of (6.10±0.03%) was higher than the (4.9%) reported by Ouattara *et al*., (2010). Fat content of (14.60±0.02%) is considered similar to (14.0±0.60%) reported for tree ripened *Blighia sapida* (Fallon *et al*., 2014). Fat is important in diets because it promotes fat soluble vitamin absorption. Crude fibre of Ackee fruit was very high (25.12±0.02%) compared with (16.14±0.04%) reported by Oyeleke *et al*., (2013) for Ackee pulp and pulp oil. Low crude fibre is undesirable as it could cause constipation and such diets have been associated with diseases. Crude protein content of (14.19±0.09%) was higher compared to (5.89%) obtained by Ureigho and Ekeke (2010) but low in comparison with protein rich foods such as soybeans, cowpea, pigeon peas, melon, pumpkin and gourd seeds ranging between (23.1-33.0%) (Olaofe and Sanni, 1988) and (World Health Organization 2007). The United States recommended daily allowance (RDA) specified that, protein should be consumed at a minimum of 0.45g and maximum of 0.8g per kilogram of an ideal body weight per day and this requirement can be supplemented with the protein content from this sample. Carbohydrate content had higher value (34.19±0.23%) than that of *Chrysophyllum albidum* with (10.38%) reported by Ureigho and Ekeke (2010). Carbohydrates are one of the essential nutrients that provide energy to human body.

*Table.1: Proximate Compositions of Blighia sapida (%)*

| **Parameter** | **Value± S.D** |
|---|---|
| **Ash content** | 6.10±0.03 |
| **Moisture content** | 5.43±0.12 |
| **Fat content** | 14.60±0.02 |
| **Crude fibre content** | 25.12±0.02 |
| **Protein content** | 14.19±0.09 |
| **Carbohydrate content (By difference)** | 34.19±0.23 |
| **N=2** | |

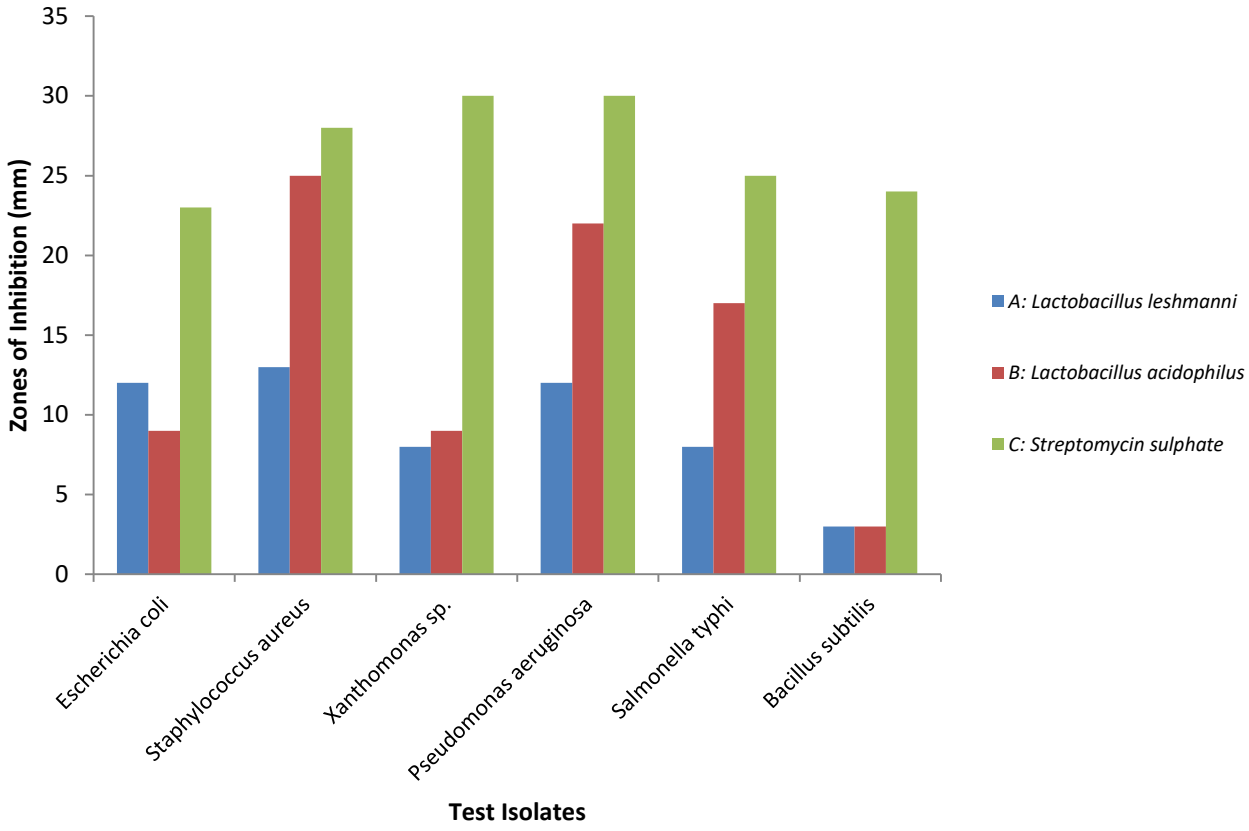


*Fig.1: Antimicrobial Activity of Cell Free Extract of Lactic Acid Bacterial Against Pathogenic Organisms*

The two *Lactobacilli* isolated from *Blighia sapida* were tested for inhibitory potential against *Escherichia coli*, *Staphylococcus aureus*, *Xanthomonas* sp, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Bacillus subtilis* and demonstrated the ability to inhibit all pathogenic bacteria and Streptomycin sulphate used as control for the pathogenic organisms mentioned above. The *Lactobacillus acidophilus* had highest inhibitory value 25 mm in *Staphylococcus aureus* and both *Lactobacillus acidophilus* and *Lactobacillus leschmanni* were lower 3 mm in inhibitory of *Bacillus subtilis* while control Streptomycin sulphate had higher values between 30 mm-23 mm shown in (figure 1). *Staphylococcus aureus* is a Gram positive opportunistic human pathogen it is one of important bacteria isolated from burn patients and is a common cause of commonly and hospital acquired infections involving skin infections and septicemia (Revazishvili *et al*., 2006). The inhibitions of test bacteria by *Lactobacilli* in *Blighia sapida* were between 3 mm-25 mm (figure 1). The *Lactobacillus leschmanni* and *Lactobacillus acidophilus* of lactic acid bacteria inhibited the growth of test organisms and control had higher inhibition between 23 mm - 30 mm. It has higher values 3 mm - 25 mm than the report obtained for the inhibition value of Mango pulp (2.64 mm) (Ravi *et al*., 2011).

## IV. CONCLUSION

The lactic acid bacteria isolated from *Blighia sapida* fruit had high inhibition of pathogenic bacteria.

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# Performance of Medium term Agro-Forest treespecieson hard Laterite Soils

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***Abstract—*** *A long term research was initiated in 1999 using medium term agro-forest trees in a shallow Andigama series soils having a hard laterite gravel layer. The present paper focuses on the growth and survival of the medium term forest tree species planted in 1999 and theirperformance by the year 2016. Acacia species had the fastest (P<0.05) growth in terms of tree diameter at breast height (DBH)during the time period (1999-2016) followed byMacarangapeltata, Gliricidia sepium andTectonagrandis. In contrast,Swieteniamacrophyllahad the lowest (P<0.05) growth during the same period. Further, Brideliamoonii had a lower (P<0.05) growth compared to Acacia species and Macarangapeltatabut not different from other species. Thus Acacia species, Macarangapeltata, Gliricidia sepium andTectonagrandis could be selected as better agroforest tree species for medium term basis to be grown in hard laterite soils in Andigama soil series Shallow Phase.*

***Keywords—agroforest tree species,Andigama soil series,hard laterite soils.***

## I. INTRODUCTION

The soils with laterites, a form of rock found in lowlands, uplands and highlands in Sri Lanka, are rich with ferrous, aluminium and silicon oxides due to the weathering process called laterization*(Dahanayake, 1982)*. Depending on the severity of the weathering process these laterites can be either hard laterites or soft laterites. When it is hard laterites it is difficult to use for agricultural purpose unless the laterites are broken into soft laterites. Likewise,it has been noted that the expected growth and the yield could not be achieved when coconutpalms are established in hard laterite soils*(CRI Advisory Circular No. 1, 2008)*. It is mainly due to the shallow top soil layer followed by a hard lateritic layer obstructing coconut roots to penetrate/break into the deeper soil layers.

The Andigama series soil found in Andigama area is one of soils used for cultivating coconuts in Sri Lanka*(Somasiri et al., 2006)*. This soil series belongs to the Red Yellow PodzolicGreat Soil Groupwitha gravel layer. Andigama soil series is divided into three phases considering the depth; Moderately Deep Phase, Shallow to Moderately Deep Phaseand Shallow Phase. *Parrotta et al.,(1997)* documented thatthere is a possibility of improving soil physical and chemical properties by establishing deep rooted tree plantations.Thus, a research was implemented in 1999 to improve the Andigama soil series Shallow phase using medium term agro forestry tree species.The present paper focuses on the growth and survival of the medium term agro forest tree species planted in 1999.

## II. METHODOLOGY

Experimental site was located at the Rathmalagara Research Centre, Coconut Research Institute (Longitude $7.5^0$ 32' N and Latitude $79^0$ 53' E) in the Puttalam district (North western Province) in agro-ecological zone $IL_{1a}$ in the intermediate low country, Sri Lanka*(Punniyawardena, 2008)*.

Ten medium term agro forest tree species that were commonly found in the area were planted in an area of one hectarein a layout of Randomized Complete Block Design with three replicatesin October 1999 (Table 1).Initially one replicate had 10 plants of the respective species. The soil series at the site was Andigama soil series Shallow Phase with a hard laterite gravellayer at various depths.The initial depth of top soil was 15 cm in average.

*Table.1: The scientific names of 10 forest tree species selected for the study in 1999*

| Species | Family |
|---|---|
| *Acacia auriculiformis* | Fabaceae |
| *Acacia mangium* provenance 1 | Fabaceae |
| *Acacia mangium*provenance 2 | Fabaceae |
| *Calophylluminophyllum* | Clusiaceae |
| *Grewiatiliifolia* | Tiliaceae |
| *Macarangapeltata* | Euphorbiaceae |
| *Gliricidia sepium* | Fabaceae |
| *Tectonagrandis* | Lamiaceae |
| *Swieteniamacrophylla* | Meliaceae |

| | |
|---|---|
| *Brideliaretusa* | Euphorbiaceae |

One year old seedlings of each specieswere planted in a 30x30x30 cm planting hole. Spacingbetween plants varied depending on the plant species i.e.*Gliricidia sepium* 2x1 m,*Acacia* species 2x2m and for other species 2.5x2.5 m between and within row spacing.Plants were monitored closely and irrigated whenever,needed at the seedling stage.

However, by the end of year 2002, only one replicate of *Grewiatiliifolia*and *Calophyllumelatum*species survived at the experimental site. Therefore, neither of those two species considered as a treatment in the present paper. Further,even though all replicates of the three *Acacia* species survived up to the year 2016, all three *Acacia*species were considered as one treatment and randomnly selected any *Acacia* species from any block for data collection.At the beginning of experiment the parameters such as leaf litter content, weed biomass, weed species and soil organic matter contents were measured inconsistently. These data were presented and used in the discussion of this paper for the mere understanding of the growth and survival of these agro forest tree species.Tree girth at breast height (GBH) was measured at two heights (30 cm and 130cm) above ground using a tapeduring the years 2000, 2002, 2003 and 2016. Later, all GBH values were converted to diameter at breast height values (DBH).

Tree diameter measurements were analysed using repeated measure analysis using the procedure for general linear model (proc GLM) in SAS version 9.1 *(SAS, 2002)*. The means were separated using least significant difference (LSD) procedure in proc GLM.

## III. RESULTS AND DISCUSSION

Six medium term forest tree species *Acacia* species, *Macarangapeltata* (Kenda),*Gliricidia sepium, Swieteniamacrophylla* (Mahogany), *Brideliamoonii* (Ketakela) *and Tectonagrandis* (Teak)survived during the period 1999 to 2016 except *Grewiatiliifolia*and *Calophyllumelatum*as mentioned above.

*Calophyllumelatum*(Dombe) *and Grewiatiliifolia* (Damminna)did not survive after the year 2002. The reason could be the hard laterite soils presence at the experimental site was not supportive for their natural growth as *Eldridge et al.,(1994)*observed with*Eucalyptus degluptas*species. The above authors havenoted that *Eucalyptusdegluptawould* not survivein degraded soils as it thrives in well-drained tropical alluvial soils naturally. Similarly, *Calophyllumelatum and Grewiatiliifolia*may not be successful in hard laterite soils.It was documented in*Annual Report(2000), Calophyllumelatum* had the lowest growth rate and was susceptible to drought and pests during the early growth stages. Thus *Calophyllumeletum* being grown in an unfavourable hard laterite soils plus its inability to withstand the drought and pest conditions could be the reasons for not surviving at the experimental site.

*Fig.1: An uprooted Acacia tree at the experimental site in 2016*

By the mid of year 2016, there werenumber of trees of *Acacia* species, *Macarangapeltata (*Kenda), *Gliricidia sepium* (Gliricidia), *Brideliamoonii* (Ketakela) and *Tectonagrandis*(Teak)were fallendue to the effect of a

minor cyclone that swept through the area during May, 2016.Field observations showed that the tap rootwas hard to distinguish in these uprooted trees (Figure 1). It may be due to the hard laterite layer at the experimental site obstructing the downward penetration of the tap root damaging its tip and causing it to branch as it grows as observed by *Dobson(1995).*

Diameter at 30 cm and 130 cm of the agro forest tree species in year 2000 and 2016 are given in Table 2. By the end of year 2000, *Acacia* species and *Macarangapeltata*were having significantly higher diameter at 30 cm and 130 cm heights in comparison with other agro forest tree species.In 2016,*Acacia* species reached the highest (P<0.05) growth rate compared to *Macarangapeltata* which in turn was higher (P<0.05) than that of *Gliricidia sepium, Swieteniamacrophylla, Brideliamooniiand Tectonagrandis. Swieteniamacrophylla*had the lowest (P<0.05) growth rate in 2016 after approximately 17 years of planting. *Gliricidia sepium* and *Tectonagrandis*had similar growth rates in 2016 while, the growth rate of *Brideliamoonii* was different (P<0.05) from *Acacia* species and *Macarangapeltata*but not differ (P>0.05) from other tree species.

During the early stage of the experiment leaf litter content, weed biomass, light availability at ground level and soil organic matter percentage were measured. The data is presented in Table 3. *Annual Report(2001)* showed*that Acacia mangium* provenance 2 had significantly higher (P<0.05) leaf litter content in the year 2001. *Acacia auriculiformis, Swieteniamacrophylla* and *Brideliamooniihad* the lowest (P<0.05) leaf litter content compared to *Acacia mangium* provenance 2 (*Annual Report 2001*). The leaf litter content in *Acacia mangium* provenance 1, *Macarangapeltata, Gliricidia sepium* and *Tectonagrandis* were similar. However, by the year 2016(Table 4) leaf litter content of *Tectonagrandis* was higher (P<0.05) than *Acaia* species, *Swieteniamacrophylla,Brideliamooniiand Macarangapeltata*but not different from *Gliricidia sepium(Bandara et al., 2017). Lugo et al.,(1991)* observed that the floor litter content in a tropical plantation having indigenous tree species ranges from 500 to 2800 g per $m^2$. Further, supporting the above finding,*Stanley and Montagnini,(1999)* stated that leaf litter accumulation in soils varies within a year depending on tree species supporting the findings of the present study.

*Table.2: Tree diameter (cm) measurements of the forest tree species*

| | **Forest tree species** | **Year** | | | |
|---|---|---|---|---|---|
| | | **2000** | | **2016** | |
| | | **Diameter at 30 cm** | **Diameter at 130 cm** | **Diameter at 30 cm** | **Diameter at 130 cm** |
| 1 | *Acacia* species | $13.18^{b} \pm 0.86$ | $11.11^{b} \pm 0.86$ | $40.33^{d} \pm 0.91$ | $35.74^{d} \pm 0.86$ |
| *2* | *Macarangapeltata (Kenda)* | $12.59^{b} \pm 0.86$ | $10.63^{b} \pm 0.86$ | $30.31^{c} \pm 0.86$ | $28.44^{c} \pm 0.86$ |
| *3* | *Gliricidia sepium* | $6.93^{a} \pm 0.86$ | $5.86^{a} \pm 0.86$ | $27.25^{b} \pm 0.86$ | $22.89^{ab} \pm 0.86$ |
| *4* | *Swieteniamacrophylla (Mahogany)* | $4.97^{a} \pm 0.86$ | $3.83^{a} \pm 0.86$ | $24.43^{a} \pm 0.86$ | $21.10^{a} \pm 0.86$ |
| *5* | *Brideliamoonii (Ketakela)* | $5.11^{a} \pm 0.86$ | 4.19a ± 0.86 | $25.40^{ab} \pm 0.89$ | $22.91^{ab} \pm 0.86$ |
| *6* | *Tectonagrandis (Teak)* | $6.97^{a} \pm 0.86$ | $4.08^{a} \pm 0.86$ | $27.32^{b} \pm 0.86$ | $24.28^{b} \pm 0.86$ |

Different superscripts within columns differ significantly (a,b,c,d: P<0.05)

*Table.3: Leaf litter content, weed biomass level and Light availability at ground level and soil organic matter levels in the experimental site during the early stages of growth.*

| | **Forest tree species** | **2001(1)** | **2002(2)** | **2002(2)** | **2005(3)** |
|---|---|---|---|---|---|
| | | **Leaf litter (Dry Weight basis (g/m2)** | **Weed biomass ($g/m^2$)** | **Light availability at ground level ($lumen/m^2$)** | **Soil organic matter (%)** |
| 1 | *Acacia auriculiformis* | $92^{a}$ | 63 | 13 | 2.3 |
| 2 | *Acacia mangium – provenance 1* | $327^{bc}$ | 20 | 16 | 2.9 |
| 3 | *Acacia mangium – provenance 2* | $488^{c}$ | 27 | 18 | 2.4 |
| 4 | *Macarangapeltata* (Kenda) | $188^{ab}$ | 13 | 6 | 2.3 |
| 5 | *Gliricidia sepium* | $156^{ab}$ | 16 | 3 | 3.4 |

| | | | | | |
|---|---|---|---|---|---|
| 6 | *Swieteniamacrophylla* (Mahogany) | 90[a] | 282 | 44 | 2.8 |
| 7 | *Brideliamoonii* (Ketakela) | 120[a] | 197 | 10 | 3.0 |
| 8 | *Tectonagrandis* (Teak) | 175[ab] | 258 | 25 | 3.3 |
| | Level of significance | *** | * | *** | n.s. |
| | LSD (P=0.05) | | 85 | 11 | |

*Note: Data in this table was obtained from the Annual Reports published by the Coconut Research Institute,[1]*and *Annual Report (2001),[2]Annual Report (2002)*and*[3]Annual Report (2005)*
*** P=0.05 *P=0.01

*Table.4: Leaf litter content at different forest tree species at the experimental sitein 2016*

| **Treatment** | **Leaf litter(g/m$^{2)}$** | **Soil Organic Carbon percentage (%)** |
|---|---|---|
| *Acacia spp* | 2085[a] ± 415 | 1.89[c] ± 0.09 |
| *Brideliamoonii* | 2011[a] ± 415 | 1.55[a] ± 0.09 |
| *Swieteniamacrophylla* | 2040[a] ± 415 | 1.78[abc] ± 0.09 |
| *Tectonagrandis,* | 3321[b] ± 415 | 1.61[bc] ± 0.09 |
| *Macarangapeltata* | 2050[a] ± 415 | 1.83 [ab] ± 0.09 |
| *Gliricidia sepium* | 2374 [ab] ± 415 | 1.77[abc] ± 0.09 |

Different superscripts within columns differ significantly (a,b,c: P<0.05)
Source: *Bandara et al.,(2017)*

It had been noted that the weed growth was lower (P<0.05) in the plots with *Acacia* species and *Macarangapeltata*in 2000*(Annual Report, 2000)*. However, by the year 2002, weed biomass per m$^2$(Table 3) were higher (P<0.05) in *Swieteniamacrophylla*, *Tectonagrandis* and *Brideliamoonii* compared to*Macarangapeltata, Gliricidia sepium* and *Acacia* species *(Annual Report, 2002)*.Thus, higher the light availability at ground level, higher the weed biomass allowing favourable conditions for the growth of weeds (Table 3). This is also supported by the slower growth rates of these forest tree species (*Swieteniamacrophylla*and *Brideliamoonii*). Similarly, *Swieteniamacrophylla* has a higher (P<0.05) weed density than *Acacia auriculiformis*which in turnhad a higher (P<0.05) weed density than *Tectonagrandis(Annual Report, 2004)*. Weed density in other forest tree species were significantly lower (P<0.05). Lowest weed density was observed in plots with *Gliricidia sepium(Annual Report, 2004)*. It may be due to the lower light availability at ground level (Table 3) restricting the growth of weeds in the Gliricidia plots.

Soil organic matter content in the year 2005(Table 3) was not significantly different among treatments *(Annual Report, 2005)*. However, by the year 2016(Table 4) *Acacia* species had a higher (P<0.05) soil organic carbon percentage (SOC%) compared to *Brideliamoonii*. It was observed that accumulation of leaf litter has not directly influencedon the SOC%.This may be because depending on the forest tree species the rate of decomposition varies along the year as suggested by *Stanley andMontagnini (1999)*. According to the above Authors even though *Pithecellobiumelegans*and*Vochysiaferrainea*producers a larger amount of floor litter content with higher organic matter content, both species have different decomposition rates.*P. elegans* has a higher decomposition rate whereas, species such as *V.ferrainea* has a slower decomposition rate *(Stanley & Montagnini, 1999)*.

## IV. CONCLUSION

*Calophyllumelatum* and *Grewiatiliifolia* did not survive beyond year 2002 could be due to the hard laterite soils being not their naturally favourable soils, susceptibility for drought and competition from other forest tree and weed species.*Acacia* species had the fastest growth during the time period (1999-2016) followed by*Macarangapeltata, Gliricidia sepium* and*Tectonagrandis.* In contrast, *Swieteniamacrophylla* had the lowest growth during the same period. Further,*Brideliamoonii* had a lower growth compared to *Acacia* species and *Macarangapeltata* but not different from other species.Thus *Acacia* species, *Macarangapeltata, Gliricidia sepium* and*Tectonagrandis* could be selected as better agro-forest tree species for medium term basis to be grown in hard laterite soils in Andigama soil series Shallow Phase.

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# Soil Fertility Characterization in Mvumi and Mbogo - Komtonga Irrigation Schemes in Kilosa and Mvomero Districts, Morogoro Region, Tanzania.

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***Abstract*—** *Soil samples from three (3) mapping units in Mvumi and four (4) mapping units in Mbogo Komtonga representing two irrigation schemes in Kilosa and Mvomero Districts in eastern Tanzania were collected and analyzed for different mineral elements. Using zigzag sampling techniques, 9 composite samples with three replicates were collected at depth 0 – 30 cm from the delineated pedogeomorphic units at a radius of 20 m around the soil pits. Soil samples from each soil type were bulked, thoroughly mixed, sub sampled to obtain a representative composite sample, packed and sent to Mlingano National Soil Service laboratory (NSS), Tanga, Tanzania for the determination of physical chemical fertility indicators. The data showed overall significant ($P \leq 0.05$) difference in fertility status in the selected irrigation schemes. The pH of top soils in Mvumi and Mbogo - Komtonga irrigation schemes ranged from 4.4 to 6.3. These were rated as extremely and/or strongly acid to slightly acid. Of the total area studied in Mvumi and Mbogo Komtonga irrigation schemes, 25.5 % is slightly acid, 40.2 % is medium acid, 31.0 % is extremely acid and 3.3 % extremely acid. Similarly, results of organic carbon (OC) determination from the top soil (0 - 30 cm) samples ranged from 26.6 g $kg^{-1}$ to 51.8 g $kg^{-1}$. This corresponds to 45.7 g $kg^{-1}$ to 89.0 g $kg^{-1}$ SOM in both irrigation schemes. The data showed that % OC in all irrigation schemes was very high in 92.2 % and high in 7.8 % of the surveyed areas. The results show that the top soils of all the surveyed areas in Mvumi and Mbogo - Komtonga irrigation schemes had N in the range of 1.2 to 3.8 mg $kg^{-1}$, 48.7 % had N below the critical limits whereas 51.3 % were above the same. Available P in both schemes range from 0.68 – 6.53 mg $kg^{-1}$. Based on the generally accepted threshold P level, all the observed P values in Mvumi and Mbogo - Komtonga respectively were considered to be below the critical range. Cation exchange capacity values in most topsoil in Mvumi and Mbogo - Komtonga irrigation schemes were rated as medium or high to very high. These values range between 27.0 – 54.6 cmol (+) $kg^{-1}$ and were rated as medium in 25.5 %, high in 35.3 % and very high in 39.2 % of the total surveyed areas. Exchangeable Ca in the topsoil of Mvumi and Mbogo - Komtonga irrigation schemes ranged from 3.99 – 31.3 cmol (+) $kg^{-1}$. These were rated as medium in 0.96 %, high in 34.3 % and very high in 70.2 %. Based on the critical limits, MV – Pa3 in Mvumi is likely to be deficient of $Ca^{2+}$ for most crops as it lies below the proposed critical limits. Exchangeable $Mg^{2+}$ in the irrigation schemes range from 0.28 – 5.07 cmol (+) $kg^{-1}$, rated as high to very high. These data suggests that all the MUs except for MV – Pa3 in Mvumi and Mbogo - Komtonga have sufficient $Mg^{2+}$ supplies for crop growth. Potassium in Mvumi and Mbogo – Komtonga irrigation schemes, range from 0.61 - 2.97 cmol (+) $kg^{-1}$. These were rated as medium in 64.3 % to very high in 35.7 % of the total area. The data shows that in Mvumi K is unlikely to respond similar to Mbogo – Komtonga. The results of $Na_{exch}$ indicates that the levels of $Na^{+}$ in the top soils corresponds to 0.15 – 0.47 cmol (+) $kg^{-1}$ soil in both irrigation schemes. These values were rated as low in 16.4 % and medium in 83.6 % and the corresponding ESP range from 0.5 – 2.2 % in Mvumi considered non-sodic. These results suggest that the surveyed areas have no threat to sodicity problems and the major soil fertility constraints were soil reaction (pH), Nitrogen (N), Phosphorus (P) and poor Soil Organic Matter (SOM).*

***Keywords*— Calcium, cation exchange capacity, paddy production, fertility constraints, management practices, soil organic matter, survey.**

## I. INTRODUCTION

Agriculture is by far the most important sector of the economy for the farmers in Mvomero and Kilosa districts, as

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it provides employment of about 75 % of the population. However, limited irrigation facilities, limited use of modern farming technology, equipment as well as inputs which improves soil fertility, pose the major limitations to increased crop production and productivity. Soil fertility is imperative in enhancing crop productivity including rice production in Mvumi and Mbogo - Komtonga irrigation schemes that were selected for irrigation development. According to FAO [1], rice (*Oryza sativa* L.) is the second most important food crop in Tanzania after maize in terms of area cultivated and production. It is similarly a major source of employment and income for the rural poor farmers [2]. Annual per capita rice consumption increased from 20.5 kg in 2001 to 25.4 kg in 2011 [3]. At a lower scale, rice yields in Kilosa are 44,246 tons $year^{-1}$ [4]. The smallholder farmers in Mvumi and Mbogo – Komtonga villages, depend on pastoralism as well as rice and maize production (as food and cash crop) for their livelihood. It is therefore important to improve rice production. Although rice is grown in almost all regions of the country, the major producers are Coast, Morogoro, Tabora, Mbeya, Mwanza, Shinyanga, Kilimanjaro and Arusha Regions. The national total annual average rice production was reported to be 1.35 million tons [3]. This national average is however very low and cannot meet the demand of the rapid population increase in the country. The low yields of rice have been attributed to low soil fertility and poor water management [2]. Low soil fertility is a function of soil parent materials [5] and poor mineral elements such as macronutrients management practices used by majority of farmers who tend to apply N fertilizers alone and thereby negatively affecting other nutrients through depletion [6]. Macronutrients play significant role during the entire plant life by performing various beneficial activities in plant metabolism as well as protecting plants from various abiotic and biotic stresses including heavy metals, drought, heat, UV radiations, and from diseases and insect pest attacks [7; 8; 9]. These macronutrients also help to increase the yield, growth, and quality of various crops [9]. Nitrogen is required for plants in the greatest amount [10; 11; 12; 13; 14]; most essential component of all existing cells [10; 11; 14] essential component of all proteins and enzymes and various metabolic processes of energy transformation [15]; an essential constituent of chlorophylls, which is closely associated with photosynthetic process [16] and facilitates plant growth and development [17; 18]. Achieving and maintaining appropriate levels of soil fertility, is of paramount importance if agricultural land is to remain capable of sustaining crop production at an acceptable level. This study therefore assesses soil fertility in the selected irrigation schemes which is essential to help identify strategies with less environmental impact in order to achieve more sustainable agricultural systems through irrigation development.

## II. MATERIAL AND METHODS

### 2.1. Description of Study Area

Morogoro Region is one of the high potential agricultural regions in Tanzania Mainland that is located on the eastern side of the country. The Region lies between latitudes 5º58' and 10º00' South of the Equator and between longitudes 35º25' and 38º30' East of Greenwich. It is bordered by seven regions. In the north are Tanga and Manyara while in the eastern side are the Coast and Lindi Regions. On the western side there are Dodoma and Iringa Regions while Ruvuma is located in the southern side of the Region. In Morogoro Region expanded rice production project (ERPP) is implemented in three (3) districts which are Kilombero (Njage and Msolwa Ujamaa), Kilosa (Mvumi) and Mvomero (Mbogo - Komtonga and Kigugu) (Fig.1).with a total of five (5) irrigation schemes. Of the five irrigation schemes, Mvumi and Mbogo – Komtonga were selected for the study since no such studies were conducted before.

The centre of Mvumi Village is located at latitude 06º 35' 48.9" South and longitude 37º 13' 31.5" East at an elevation of 413 m above mean sea level [19]. Mvumi irrigation scheme is also located at a distance of approximately 36 km in the North Eastern direction away from Kilosa town. Administratively, Mvumi irrigation scheme is located in Mvumi village, Mvumi ward, Magole Division, Kilosa District in Morogoro region. The proposed irrigation project is reachable from Morogoro City by all-weather roads, passable throughout the year. However, the road to the scheme was not easily passable particularly during the wet season. On the one hand Mvumi village is bordered by Kisangata River to the North; Wami River to the East; Mvumi prison to the south and private farm known as ''JITU" to the western side. The scheme has the potential area of about 414 ha, of which 200 ha was under development plan through ERPP project. On the other hand, Mbogo – Komtonga irrigation scheme is bordered by Kichangani village to the North, Nguu Mountains in the West, Diwale/Mbulumi River in Kisala village to the East, and Kigugu village to the South. Administratively, the project area is located at Mbogo - Komtonga village, Sungaji ward, Turiani division, Mvomero District, Morogoro Region.

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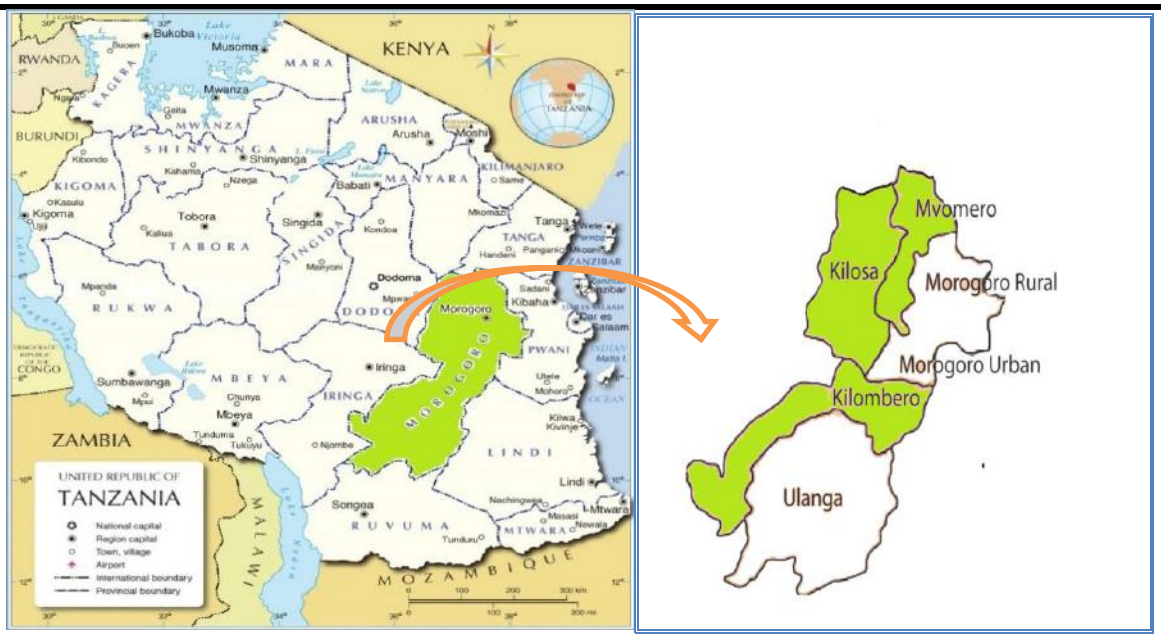


*Fig.1: Tanzania Administrative Map (Left) – Districts under ERPP projects in Morogoro Region (Right)*

### 2.2 Climate characteristics of the area

Rainfall in the study areas is bi - modal with 46.2 % of the total rains falling between March and May and about 44.5 % light rains falling between November and February. The total average annual rainfall is about 970 mm. Temperature, RH (%), potential evaporation ($ET_o$) and other climate variables representative of the study areas are presented in Table 1. The mean temperature varies from 21.8ºC in July to 27.0ºC in February. The monthly average relative humidity (RH) varies from 58.8 (i.e. October) to 77.9 % (i.e. April). The $ET_o$ is about 1,799 mm per annum and varies widely throughout the year from 93.5 to 206.9 mm per month in June and December respectively.

*Table.1: Climatic data representative for Mvumi and Mbogo - Komtonga Irrigation schemes.*

| Description | J | F | M | A | M | J | J | A | S | O | N | D |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rainfall (mm $m^{-1}$) | 119.4 | 107.7 | 163.6 | 204.4 | 80.3 | 16.5 | 9.3 | 14.0 | 11.8 | 38.6 | 81.3 | 132.7 |
| T mean max (ºC) | 32.2 | 32.5 | 31.9 | 29.9 | 28.8 | 28.1 | 27.7 | 28.5 | 30.2 | 31.6 | 32.2 | 32.3 |
| T mean min (ºC) | 21.6 | 21.5 | 21.3 | 20.8 | 19.1 | 16.4 | 15.8 | 16.4 | 17.5 | 18.8 | 20.3 | 21.5 |
| T mean (ºC) | 26.9 | 27.0 | 26.6 | 25.3 | 24.0 | 22.2 | 21.8 | 22.4 | 23.9 | 25.2 | 26.3 | 26.9 |
| Evap. (Pan) mm $m^{-1}$ | 192.0 | 177.1 | 160.5 | 110.3 | 96.3 | 93.5 | 105.4 | 117.5 | 161.2 | 186.1 | 191.7 | 206.9 |
| 0.5ETo (mm $m^{-1}$) | 96.0 | 88.6 | 80.3 | 55.1 | 48.2 | 46.8 | 52.7 | 58.8 | 80.6 | 93.0 | 95.9 | 103.4 |
| RH mean (%) | 65.4 | 65.2 | 69.8 | 77.9 | 75.7 | 70.5 | 68.5 | 65.8 | 60.3 | 58.8 | 60.3 | 63.2 |
| SH (hrs.) | 7.9 | 7.7 | 6.8 | 5.8 | 5.9 | 6.5 | 6.3 | 6.6 | 7.5 | 8.1 | 8.2 | 7.9 |
| WS (km $day^{-1}$) | 252.0 | 232.9 | 172.7 | 89.0 | 85.3 | 99.4 | 120.6 | 150.3 | 185.5 | 187.4 | 238.0 | 261.6 |

Source: Mtibwa, Ilonga, Dakawa, Dakawa Rice farm and Morogoro Meteorological weather stations. Total annual rainfall ≈ 970 mm, Total annual Evaporation (Pan) ≈ 1,799 mm

### 2.3 Soil Sampling

Soil sampling at Mvumi and Mbogo Komtonga irrigation schemes in Kilosa and Mvomero respectively was done after the soils were grouped into similar soil types or pedons following pedogeomorphic approach [20]; [21] whereby a total of seven (7) mapping units were delineated. Of the total (7) mapping units, three (3) were from Mvumi and four (4) from Mbogo - Komtonga irrigation schemes. During the soil survey process, nine (9) disturbed soil samples in four (4) replicates were then collected at a depth of 0 – 30 cm in and around the soil pits representative of the delineated pedogeomorphic units. Soil samples from each soil type were bulked, thoroughly mixed, and sub sampled to obtain a representative composite sample. After the soil samples were

filled in plastic bags and labelled, they were sent for laboratory analysis at the National Soil Service laboratory, Mlingano (NSS), Tanga, Tanzania. In the laboratory, the samples were air dried and then ground to pass through a 2-mm sieve for determination of physical chemical fertility indicators.

### 2.4 Soil Physico - Chemical Analysis

Particle size analysis was determined following the procedure in Day [22] and textural classes were determined using the USDA textural class triangle [23]. Organic carbon (OC) was determined by the Walkley and Black wet oxidation method [24] and was converted to organic matter (OM) by multiplying by a factor of 1.724 [25]. Soil pH was measured potentiometrically in water and 1N KCl at a ratio of 1: 2.5 soils: water and KCl [26]. Total nitrogen (N) was determined using micro - Kjeldahl digestion distillation method [27]. Determination of exchangeable bases (EB) and cation exchange capacity (CEC) depended on soil pH. In soils with pH < 7.5, these parameters were extracted by saturating soils with 1M ammonium acetate ($NH_4OAc$) at pH 7, ethanol and acidified 1MKCl in the first percolate ([28]. The absorbed $NH_4^+$ displaced by $K^+$ using 1M KCl was then determined by Kjeldahl distillation method for the estimation of CEC of the soil. For soils with pH > 7.5 and high carbonates contents, the method recommended by Polemio and Rhoades [29] was followed. Determination of $K^+$ and $Na^+$ was done with flame photometer, $Ca^{2+}$ and $Mg^{2+}$ by Inductively Coupled Plasma Atomic Absorption Emission Spectrophotometer [30]. Cation exchange capacity (CEC) was done following the method by [30]. Potentiometric method was used to determine electrical conductivity (EC) of soil samples following the procedure described in Piper [31]. The total exchangeable bases (TEB) were calculated arithmetically as a sum of the four exchangeable bases ($Ca^{2+}$, $Mg^{2+}$, $Na^+$ and $K^+$) for a given soil sample. Available Phosphorus (Pav) was extracted spectrophotometrically [32] by reacting with ammonium molybdate using ascorbic acid as a reductant in the presence of antimony as in [33]. Per cent base saturation (% BS) was obtained by dividing TEB by CEC and then multiplied by 100 [34]. Likewise, exchangeable sodium percentage (ESP) was obtained by dividing total exchangeable sodium by CEC, and then multiplied by 100. The K: TEB was obtained by dividing K by TEB.

### 2.5 Statistical Analysis

One - Way ANOVA was used to compare soil mineral elements from the different pedogeomorphic units. The analysis was performed using the STATISTICA software of 2016 version (Stat Soft Inc., Tulsa, OK, USA) [35]. The mean values were compared at 5 % level of significance using least significant differences (L.S.D) test.

## III. RESULTS AND DISCUSSION

### 3.1 Soil Reaction

The pH of top soils in Mvumi and Mbogo - Komtonga irrigation schemes ranged from 4.4 to 6.3. These were rated as extremely and/or strongly acid to slightly acid [36]. The chemical properties of these soils are summarized in Tables 2 and 3. The data show that pH varied ($P \leq 0.05$) with mapping units in the studied irrigation schemes. In Mvumi irrigation scheme, pH was ($P \leq 0.05$) highest in MV – Pa1 followed by MV – Pa3 and MV – Pa2 which registered the lowest pH levels. Similarly, in Mbogo – Komtonga irrigation scheme, with the exception of MB – Pa3 mapping unit which showed lowest ($P \leq 0.05$) soil reaction, the other mapping units didn't show any ($P \leq 0.05$) variation. The nature of the observed acidity in these soils threatens the availability of mineral elements such as P which is readily available in soils with pH centred at 6.5. Of the total area studied in Mvumi irrigation scheme, 1.7 % is medium acid (MV – Pa3), 44.4 % is slightly acid (MV – Pa1) and 53.9 % is extremely acid (MV – Pa2). Likewise, in the surveyed area in Mbogo – Komtonga Irrigation scheme, 92.2 % is medium acid and 7.8 % is strongly acid. The extremely/strongly acid to medium acid soils (i.e. pH 4.0 - 5.0) such as those observed in some of the surveyed areas can have high concentrations of soluble Al, Fe and Mn which may be toxic to the growth of some plants owing to the sensitivity of many plant roots to Al toxicity [37; 38; 39]. Additionally, if pH is less than five (pH < 5), the availability of some nutrients such as P, Ca, Mg and Mo is very low, a condition that limits plants mineral elements uptake. For example, when pH was 4.4 as in MV – Pa2, P availability was 0.68 mg $kg^{-1}$ but when pH was 6.3 as in MV - Pa1, P availability was 6.53 mg $kg^{-1}$. However, most plant mineral elements are available in the pH range of approximately 6.5 – 7.0 [40]. Similarly, soil pH can influence plant growth by its effect on the activity of beneficial micro-organisms. For example, bacteria that decompose SOM are hampered in strong acid soils which in turn prevent OM from breaking down [41]. As such, mineral elements required by the plants such as N are tied up and therefore unavailable to plants due to accumulation of un-decomposed or unbroken OM [42; 43]. Soil pH influences the rate of plant nutrient release by weathering, suitability of all materials in the soil, and amount of nutrients ions stored on the cation exchange complex. Before nutrients can be used by plants they must be dissolved in the soil solution. The pH is therefore a good guide for predicting which plant nutrients are deficient. Soils

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tend to become acidic as a result of (1) rainwater leaching away basic ions (Ca, Mg, K and Na); (2) formation of a weak organic acid as a result of $CO_2$ from decomposing OM and root respiration dissolving in soil water; (3) formation of strong organic and inorganic acids, such as nitric ($HNO_3$) and sulphuric acid ($H_2SO_4$), from decaying OM and oxidation of ammonium ($NH_3$) and sulphur (S) fertilizers. Strongly acid soils are usually the result of the action of these strong organic and inorganic acids.

*(See Table 2)*

### 3.2 Total Soil Organic Matter (SOM)

Results of organic carbon (OC) determination from the top soil (0 - 30 cm) samples in Mvumi irrigation scheme ranged from 26.6 g $kg^{-1}$ to 51.8 g $kg^{-1}$ while in Mbogo - Komtonga ranged from 24.7 g $kg^{-1}$ to 49.1 g $kg^{-1}$ (Tables 2 and 3). These tallies with 45.7 g $kg^{-1}$ to 89.0 g $kg^{-1}$ SOM in Mvumi and 42.5 g $kg^{-1}$ to 84.4 g $kg^{-1}$ SOM in Mbogo - Komtonga. The data showed that there was ($P \leq 0.05$) variation between the mapping units in both Mvumi and Mbogo – Komtonga irrigation schemes. For example, % OC and SOM were ($P \leq 0.05$) greatest in MV – Pa2 and MB – Pa4 and ($P \leq 0.05$) lowest in MV – Pa3 and MB – Pa3 respectively. Results also showed that % OC and SOM were very high in 98.3 % and high in 1.7 % of the area surveyed in Mvumi. In Mbogo - Komtonga, % OC and SOM were very high in 84 % and high in 16 % of the studied area. Since SOM content was calculated from SOC [25], these parameters have similar trend and showed systematic trend of decreasing with depth. It is generally accepted that a threshold for SOM in most soils is 34 g $kg^{-1}$ below which decline in soil quality is expected to occur [44]. With the observed data, all values were above the proposed threshold limits, suggesting that no decline in soil quality for both Mvumi and Mbogo - Komtonga irrigation schemes [45]. SOM affects the physico - chemical properties of the soil and its composition and breakdown rate affect: the soil structure and porosity; the water infiltration rate and moisture holding capacity of soils; the diversity and biological activity of soil organisms; and plant nutrient availability. Organic carbon (OC) or SOM in the soil is important because humidified OM molecules may react with mineral colloids and contribute to the stabilization of soil aggregates. Through $Fe^{2+}$ and $Al^{3+}$ oxide, SOM positively affects water retention capacity, adsorption of fulvic and humic compounds and prevents their crystallization, thus, decreasing their fixation power with regards to phosphates at unfavourable pH values. Similarly, SOM provides much of the CEC, and, surface soils contain large quantity of plant nutrients with storehouse considered as slow release of nutrient especially so by N.

### 3.3 Total Nitrogen (TN)

Nitrogen (N) is biologically combined with carbon, hydrogen, oxygen and sulphur to create amino acids, the building blocks of proteins. Amino acids are used in forming protoplasm, the site for cell division and thus for plant growth and development. N is also needed for all of the enzymatic reactions in a plant; a major part of the chlorophyll molecule necessary for photosynthesis and a necessary component of several vitamins. Additionally, N improves the quality and quantity of dry matter in leafy vegetables and protein in grain crops. Results from the study areas showed that N levels were low to medium ranging from 1.2 – 2.5 g $kg^{-1}$ in Mvumi similar to Mbogo – Komtonga irrigation schemes with values ranging from 1.5 – 3.8 g $kg^{-1}$ (Table 2). These levels were rated as low in 46.1 % and medium in 53.9 % of the total area surveyed in Mvumi Irrigation scheme. Similarly, TN was rated as low in 52.4 % and medium in 47.6 % in Mbogo – Komtonga irrigation scheme. It was observed that TN in the study area ($P \leq 0.05$) varied between the pedogeomorphic units. In Mvumi Irrigation scheme for instance, the data showed that TN was greatest ($P \leq 0.05$) in MV – Pa2, that is, 2.5 g $kg^{-1}$ and lowest in MV – Pa1, that is 1.2 g $kg^{-1}$ (Table 2). Results also showed that in Mbogo – Komtonga, TN was ($P \leq 0.05$) highest i.e. 3.8 g $kg^{-1}$ in MB – Pa4 and ($P \leq 0.05$) lowest i.e. 1.5 g $kg^{-1}$ in MB – Pa3 (Table 2). According to NSS [36] guidelines, the proposed threshold value for N in most crops in Tanzania is 2 g $kg^{-1}$ soil. The data indicates that of all the surveyed areas in Mvumi and Mbogo – Komtonga irrigation schemes, 46.1 % and 52.4 % had low TN respectively and were below the threshold value (< 2 g $kg^{-1}$) whereas 53.9 % and 47.6 % in Mvumi and Mbogo – Komtonga respectively had N above the threshold value (Tables 2 and 3). Plants use N primarily for the production and maintenance of leaves in order to maximize carbon fixation. Insufficient N in soils in many parts of the world is the prime factor that limits plant growth and development [46; 47]. The observed low or medium N in the surveyed areas may probably be due to medium or poor quality SOM even though results indicates that SOM values are very high or high. This may greatly be influenced by microbial activity in the soil and the very low or low soil pH [48; 45; Table 3]. Improvement of soil pH, SOM quality as well as microbial activities in the study areas can, subsequently lead to increase in soil N [45]. The low to very low levels of N in the surveyed areas suggests application of N in a form that better resists leaching caused by rainfall or irrigation in the surveyed areas.

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**3.4. C/N Ratio**

There was significant ($P \leq 0.05$) difference between the studied C:N ratio in mapping units of each of the irrigation schemes (Table 2). The data showed that C: N ratio was ($P \leq 0.05$) higher in MV – Pa1 and ($P \leq 0.05$) lowest in MV – MP1. It was likewise observed that the C: N ration in Mbogo – Komtonga was ($P \leq 0.05$) greatest in MB – Pa2 and ($P \leq 0.05$) lowest in MB – Pa3. However, the C:N ratio results of most of the surveyed areas range from 16 – 29 in Mvumi and 13 – 23 in Mbogo - Komtonga irrigation schemes (Table 2) and were rated as good/medium to poor quality SOM (Table 3). Of the surveyed area 98.3 % and 1.7 % is poor and moderate quality SOM [36] respectively in Mvumi. In Mbogo – Komtonga, 44.6 %, 7.8 % and 47.6 % of the surveyed area showed poor, moderate and good quality SOM respectively. Generally, C/N ratios between 8 and 12 are considered to be the most favourable [36], as N from the organic materials is mineralised relatively faster than otherwise. With the exemption of MV – Pa3 in Mvumi which showed a C/N ratio of 16, that is, medium quality SOM, the rest of the mapping units had C/N ratio outside the suggested range and were rated as poor quality SOM. The C/N ratio observed in BM – Pa3 and BM – Pa4 in Mbogo was rated as medium and good quality SOM respectively, and the remaining areas were rated as poor quality SOM. The observed C/N ratio in MB – Pa4 in Mbogo suggests an ideal condition for plant growth, since in this case mineralization is higher than immobilization in the soil. C/N ratios greater than 23 [49], a situation observed in MV – Pa1 in Mvumi and MB – Pa2 in Mbogo - Komtonga, have been shown to favour slow degradation of residues by the associated micro-organisms [50], higher immobilization effects [49] and limited N in the soil that may lead to reduced crop yields [51].

*(See Table 3)*

**3.5 Available Phosphorus (AP)**

Data from the study areas showed that all the top soil samples taken from Mvumi and Mbogo - Komtonga irrigation schemes had ($P \leq 0.05$) low levels of available P (Tables 2 and 3). In Mvumi irrigation scheme, available P was ($P \leq 0.05$) greater in MV – Pa1 and ($P \leq 0.05$) lowest in MV – Pa2. It was similarly revealed that available P in Mbogo – Komtonga was ($P \leq 0.05$) highest in MB – Pa2 and ($P \leq 0.05$) lowest in MB – Pa3. Results indicate that available P in Mvumi range from 0.68 – 6.53 mg $kg^{-1}$ and 0.87 – 5.47 mg $kg^{-1}$ in Mbogo - Komtonga top soils. Phosphorus (P) deficiency is a major abiotic stress that limits crop productivity on 30 – 40 % of the world's arable land [52]. P deficiency symptoms are likely to occur in most crops if an average available P in the soil is below 7 mg $kg^{-1}$ considered as optimal [36]. These results suggests that all the observed P values in Mvumi and Mbogo - Komtonga irrigation schemes respectively are considered to be below the critical range and will definitely need measures to reverse the trend. The generally low P availability revealed in all the mapping units in Mvumi and Mbogo - Komtonga (Tables 2 and 3) also suggests that management of P in these areas is critical for sustainable agricultural development. Phosphorus (P) is an essential macro element for plant growth, hence an important soil fertility indicator. Phosphorus (P) constitutes about 0.2 % of plant's DM and therefore an essential macro element for plant growth [53]. Phosphorus is also required during the process of energy generation and transfer, carbon metabolism, membrane synthesis, enzyme activation, and nitrogen fixation [54] and a constituent of key biomolecules like nucleic acids, phospholipids, and adenosine triphosphate (ATP) [53]. Limited P availability in soils is thus an important nutritional disorder to plant growth and development [55].

**3.6 Exchangeable Bases (K, Mg, Ca)**

Results of the exchangeable bases (Ca, Mg and K) in the top soils of the surveyed areas are presented in Tables 2 and 3. These results indicate that the levels of exchangeable Ca, Mg and K ($P \leq 0.05$) varied between the mapping units and were generally rated as medium or high to very high.

**3.6.1 Exchangeable Ca**

Results of Calcium (Ca) in Mvumi and Mbogo – Komtonga irrigation schemes were ($P \leq 0.05$) different in the studied mapping units. Exchangeable Ca in top soil samples collected from Mvumi irrigation scheme ranged between 3.99 cmol (+) $kg^{-1}$ (MV – MP1) and 13.27 cmol (+) $kg^{-1}$ (MV - Pa2) rated as ($P \leq 0.05$) medium to very high (Tables 2 and 3). In Mbogo - Komtonga irrigation scheme, exchangeable Ca ranged from 12.6 cmol (+) $kg^{-1}$ (MB – Pa3) – 31.3 cmol (+) $kg^{-1}$ (MB – Pa4) rated as ($P \leq 0.05$) high to very high. In Mvumi result show that Ca was very high in 44.4 %, high in 53.9 % and moderate in 1.7 % of the studied areas. Similarly, in Mbogo – Komtonga irrigation scheme, Ca was very high in 92.2 % and medium in 7.8 % of the surveyed areas. Marx et al. [56] proposed that in most of the crops, the recommended threshold level of $Ca^{2+}$ is 5 cmol (+) $kg^{-1}$. It is generally acknowledged that field conditions that limit $Ca^{2+}$ uptake produce lower crop yields than crops grown with adequate $Ca^{2+}$ [57]. These results indicate that mapping unit MV – Pa3 in Mvumi is likely to be deficient of $Ca^{2+}$ for most crops as it lies below the proposed critical limits. Calcium plays an extremely important role in producing plant tissues and enables plants to grow better; increases the plant tissues'

resistance and allows for more erect stems, contributes to normal root system development, increases resistance to outside attack, increases the feed value of forage crops (by enriching the plant in calcium)

### 3.6.2 Exchangeable Mg

Exchangeable $Mg^{2+}$ in Mvumi range from 0.28 cmol (+) $kg^{-1}$ in MV – Pa3 – 4.15 cmol (+) $kg^{-1}$ in MV – Pa2 and was rated as ($P \leq 0.05$) low to high. Mg was high in 98.3 % and low in 1.7 % of the surveyed area in Mvumi irrigation scheme. Exchangeable $Mg^{2+}$ in Mbogo - Komtonga range from 4.25 cmol (+) $kg^{-1}$ in MB – Pa1 to 5.07 cmol (+) $kg^{-1}$ in MB – Pa2, rated as ($P \leq 0.05$) high to very high. The data shows that Mg in 63.6 % of the studied area is high and in 36.4 % of the studied area is very high. Magnesium is a constituent of the chlorophyll molecule, a driving force of photosynthesis; essential for the metabolism and translocation of carbohydrates (sugars); an enzyme activator in the synthesis of nucleic acids (DNA and RNA); regulates uptake of the other essential elements; serves as a carrier of phosphate compounds throughout the plant and enhances the production of oils and fats. The recommended value of $Mg^{2+}$ in most crops is 2 cmol (+) $kg^{-1}$ [58]. These data suggests that all the MUs except for MV – Pa3 in Mvumi and Mbogo - Komtonga have sufficient $Mg^{2+}$ supplies for crop growth.

### 3.6.3 Exchangeable K

Potassium (K) increases crop yield and improves quality. Likewise, it is required for numerous plant growth processes. K increases root growth and improves drought resistance; activates many enzyme systems; maintains turgor; reduces water loss and wilting; aids in photosynthesis and food formation; reduces respiration; preventing energy losses; enhances translocation of sugars and starch; produces grain rich in starch; increases protein content of plants; builds cellulose and reduces lodging; and helps retard crop diseases. Potassium in Mvumi range from 0.61 cmol (+) $kg^{-1}$ in MV – MP1 to 0.83 cmol (+) $kg^{-1}$ in MV – Pa2 and was rated as ($P \leq 0.05$) medium. In Mbogo - Komtonga, K ranged from 0.62 cmol (+) $kg^{-1}$ in MB – Pa3 to 2.97 cmol (+) $kg^{-1}$ in MB – Pa2 rated as ($P \leq 0.05$) medium to very high. Whereas K was medium in all the studied areas in Mvumi irrigation scheme, in Mbogo – Komtonga irrigation scheme, 5.2 % of the surveyed area had medium K and 94.8 % had very high K. In general terms, a response to K fertilizers is likely when a soil has an exchangeable K value of < 0.2 cmol (+) $kg^{-1}$ soil and unlikely when it is above 0.4 cmol (+) $kg^{-1}$ soil [Tables 2 and 4; 59; 36]. The data shows that in Mvumi irrigation scheme, K if applied, is unlikely to respond. Similar trend was observed in Mbogo – Komtonga irrigation scheme.

### 3.7 Cation Exchange Capacity

Cation exchange capacity (CEC) refers to the exchange phenomenon of positively charged ions (cation) at the surface of the negatively charged colloids [60]. It is often used as a characteristic in the determination of the nutrient retention land quality. The higher the CEC, the more capable the soil is to retain nutrients. High CEC means more nutrients are held on the soil, decreasing their mobility and uptake whereas low CEC means that more nutrients are in the soil solution, making them available to plants but also increasing the likelihood of leaching. Studies have shown that soils with CEC values of between 6 - 12 cmol (+) $kg^{-1}$ soil are poor in exchangeable bases [36]. In this study, CEC levels in Mvumi and Mbogo - Komtonga irrigation schemes top soils were rated as ($P \leq 0.05$) medium or high to very high (Tables 2 and 3). In Mvumi, these values range between 17.9 cmol (+) $kg^{-1}$ (MV – Pa1) – 34.64 cmol (+) $kg^{-1}$ (MV – Pa2), and were rated as ($P \leq 0.05$) medium in 44.4 % of the area to ($P \leq 0.05$) high in 55.6 % of the area surveyed. In Mbogo - Komtonga, these values ranged between 27.02 cmol (+) $kg^{-1}$ (MB – Pa3) – 54.64 cmol (+) $kg^{-1}$ (MB – Pa4) and were rated as ($P \leq 0.05$) high in 7.8 % to ($P \leq 0.05$) very high in 92.2 % of the surveyed areas. The high to very high CEC could be related to the clay mineral and soil organic matter (SOM) or organic carbon (OC) present in these soils. However, it is recommended to apply both manure/compost manure and the required amount of fertilizer. When these inputs added to the soil increases the humus content of the soil, consequently resulting into a higher or maintenance of higher CEC hence a better retention of nutrients.

### 3.8 Exchangeable Sodium (Na) or Exchangeable Sodium Percentage (ESP) and Electrical Conductivity (EC)

Results of the levels of exchangeable Na, exchangeable sodium percentage (ESP) and electrical conductivity (EC) in Mvumi and Mbogo - Komtonga surveyed areas are presented in Tables 2 and 3. The data show that the levels of $Na^{+}$ in the top soils corresponds to 0.15 – 0.47 cmol (+) $kg^{-1}$ soil in Mvumi and 0.17 – 0.45 cmol (+) $kg^{-1}$ in Mbogo - Komtonga. These values were rated as ($P \leq 0.05$) low in 1.7 % and medium in 98.3 % of the surveyed area in Mvumi. Similar trend was observed in Mbogo – Komtonga where Na was low in 36.4 % and medium in 63.6 % of the total surveyed area. The corresponding ESP range from 0.5 – 2.2 % in Mvumi and 0.4 – 1.7 % in Mbogo - Komtonga. These were ($P \leq 0.05$) rated as non-sodic. The critical values of ESP above which most crops are affected are established at 15 % [61] suggesting that sodicity in the surveyed areas is not a threat to crop production and productivity.

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### 3.9 Cation Ratios

Mineral elements uptake by plants is dependent on absolute levels and relative amounts of individual elements. Results from this study indicate that Ca/Mg and Mg/K ratios reflect imbalances among the individual mineral elements (Table 4). For example, Ca will reduce plant uptake of Mg even if there is enough Mg in the soil whenever the Ca/Mg ratio is high. However, if Ca/Mg ratio is rated as good, but the total amounts of the individual mineral elements are low, then, such mineral elements should be applied. Previous research works in the tropical areas have suggested that the optimal cation ratio for the optimum growth of most crops is assumed to be equal to 76/18/6 for Ca/Mg/K respectively (i.e. 12.7/3/1). Research has likewise indicated that the Ca/Mg ratios of 3 – 5 in the topsoil are considered optimal for most crops. The top soils in the surveyed areas were found to have Ca/Mg ratios ranging from 2.3 – 14.3 in Mvumi and 2.5 – 6.3 in Mbogo – Komtonga irrigation schemes. As for Mg/K ratio, the values ranged from 0.5 – 5.0 in Mvumi and 1.7 – 8.1 in Mbogo - Komtonga irrigation schemes. The optimal range of Mg/K ratio is between 1 – 4 for most crops. The K/TEB ratio range from 4.4 – 12.2 % in Mvumi and 3.3 – 7.8 % in Mbogo - Komtonga. As the K/TEB ratios are greater than 2 % in all the surveyed areas, problems of K – deficiency in the study areas is unlikely.
*(See Table 4)*

## IV. CONCLUSION

In conclusion, the results of the present study provide soil fertility status in Mvumi and Mbogo – Komtonga irrigation schemes. Data also suggest that soil indicators such as pH, TN, P and poor SOM are the overall major soil fertility constraints to crop production in the areas followed by Ca in some mapping units. This information could be incorporated in the soil fertility management programs in Kilosa and Mvomero Districts, thus, contributing significantly in the efficient utilisation of land resources for maximum production and productivity in the study areas.

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*Table.2: Soil fertility data for Mvumi and Mbogo Komtonga Irrigation schemes in Kilosa and Mvomero Districts (topsoil samples 0 – 30 cm depth).*

| MU | Texture | pH | EC | OC | OM | TN | C/N | Pav | CEC | Ca | Mg | K | Na | BS | ESP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ($H_2O$) | $dS.m^{-1}$ | | $g.kg^{-1}$ | | | $mg.kg^{-1}$ | | | ($cmol (+).kg^{-1}$) | | | (%) | |
| Mvumi Irrigation Scheme | | | | | | | | | | | | | | | |
| MV-Pa1 | SCL | 6.3a | 0.24c | 36.6b | 63.03b | 1.2c | 29a | 6.53a | 17.94c | 8.08b | 3.47b | 0.73b | 0.39b | 74.00a | 2.20a |
| MV-Pa2 | C | 4.4c | 0.29b | 51.8a | 89.02a | 2.5a | 21b | 0.68c | 34.64a | 13.27a | 4.15a | 0.83a | 0.47a | 54.00b | 1.40b |
| MV-Pa3 | CL | 5.9b | 0.32a | 26.6c | 45.71c | 1.7b | 16c | 5.15b | 28.34b | 3.99c | 0.28c | 0.61c | 0.15c | 31.00c | 0.50c |
| One Way ANOVA *(F-Statistics)* | | | | | | | | | | | | | | | |
| F Value | | 240.5*** | 52.1*** | 765.9*** | 764.4*** | 101.6*** | 629.1** | 3021.9** | 688.3*** | 1891.5** | 3273.8*** | 171.28** | 1577.75** | 1113.81** | 2308.51*** |
| CV (%) | | 4.8 | 1.8 | 12.8 | 16.8 | 8.4 | 9.7 | 4.7 | 10.7 | 6.4 | 3.9 | 1.7 | 1.3 | 15.3 | 2.6 |
| Mbogo - Komtonga Irrigation Scheme | | | | | | | | | | | | | | | |
| MB-Pa1 | C | 5.8a | 0.32b | 38.0c | 65.35c | 1.8b | 21.0b | 4.80b | 41.02c | 22.46c | 4.25b | 1.15c | 0.36c | 68.0b | 0.9b |
| MB-Pa2 | CL | 6.0a | 0.24c | 40.3b | 69.33b | 1.8b | 23.0a | 5.47a | 44.84b | 29.64b | 5.07a | 2.97a | 0.17d | 82.0a | 0.4d |
| MB-Pa3 | C | 5.4b | 0.23c | 24.7d | 42.50d | 1.5c | 17.0c | 0.87d | 27.02d | 12.57d | 5.02a | 0.62d | 0.45a | 65.0c | 1.7a |
| MB-Pa4 | C | 5.8a | 0.45a | 49.1a | 84.38a | 3.8a | 13.0d | 4.60c | 54.64a | 31.34a | 4.97a | 2.10b | 0.42b | 69.0b | 0.8c |
| One Way ANOVA *(F-Statistics)* | | | | | | | | | | | | | | | |
| F Value | | 14.4** | 746.3*** | 501.5*** | 499.96*** | 1453.4** | 413.2** | 1728.3** | 530.6*** | 866.8*** | 48.0*** | 2169.9** | 882.0*** | 83.6*** | 1977.8*** |
| CV (%) | | 4.8 | 1.2 | 12.7 | 16.6 | 3.2 | 8.8 | 4.4 | 13.3 | 10.3 | 4.4 | 3.0 | 1.2 | 16.9 | 2.2 |

**: significant at *P*=.01; ***: significant at *P*=.001; ns: not significantly different from each other; CV: Coefficient of variation. Values followed by dissimilar letters in the same column for each parameter are significantly different from each other at *P*=.05 according to Fischer Least significance difference (LSD). EC = Electrical Conductivity, TN = Total Nitrogen; C/N = Carbon/Nitrogen ratio, Pav = Available Phosphorus; K = Potassium; Ca = Calcium; Mg = Magnesium; Na = Sodium, OC = Organic Carbon; CEC = Cation Exchange Capacity; BS = Base Saturation, ESP = Exchangeable Sodium Percentage; SCL = Sand Clay Loam; CL = Clay Loam; C = Clay

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*Table.3: Soil Fertility Data Interpretation for selected Irrigation schemes in Mvumi and Mbogo Komtonga (0 - 30 cm)*

| Soil fertility Unit symbol | Land form characteristics | Area (ha) | S (%) | pH ($H_2O$) | Soil fertility description | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | N | P | K | Ca | Mg | Na | % OC | CEC | ESP | C/N |
| Mvumi Irrigation scheme | | | | | | | | | | | | | | |
| MV-Pa1 | Flat to gently undulating | 183.9 | <2 | SA | L | L | M | VH | H | M | VH | M | NS | PQ |
| MV-Pa2 | Flat to almost flat | 223.5 | <1 | EA | M | L | M | H | H | M | VH | H | NS | PQ |
| MV-Pa3 | Flat | 6.9 | <2 | MA | L | L | M | M | L | L | H | H | NS | MQ |
| | Total | 414.3 | | | | | | | | | | | | |
| Mbogo – Komtonga Irrigation scheme | | | | | | | | | | | | | | |
| MB-Pa1 | Flat to undulating | 25.2 | <2 | MA | L | L | M | VH | H | M | H | VH | NS | PQ |
| MB-Pa2 | Flat | 111.4 | <2 | MA | L | L | VH | VH | VH | L | VH | VH | NS | PQ |
| MB-Pa3 | Flat to undulating | 23.4 | <2 | Str.A | L | L | M | H | H | M | H | H | NS | MQ |
| MB-Pa4 | Flat | 145.8 | <1 | MA | M | L | VH | VH | H | M | VH | VH | NS | GQ |
| | Total | 306.2 | | | | | | | | | | | | |

S = Slope, N = Nitrogen, P = Phosphorus, K = Potassium, Ca = Calcium, Mg = Magnesium, %OC = Per cent organic carbon, CEC = Cation exchange capacity, ESP = Exchangeable sodium percentage, C/N = Carbon to Nitrogen ratio: (Based on Mlingano National Soil Service (NSS) Laboratory guide to general soil fertility evaluation); VL = Very low; L = Low; M = Medium; H = High; VH = Very high; NS = Non Sodic; PQ = Poor quality; MQ = Moderate quality; GQ = Good quality

*Table.4: Summary of results with emphasis on the nutrient balance in Mvumi and Mbogo Komtonga Irrigation schemes in Kilosa and Mvomero Districts (0 – 30 cm)*

| Mapping Unit | Texture | Ca/Mg | Mg/K | K/TEB | TEB |
|---|---|---|---|---|---|
| Mvumi Irrigation Scheme | | | | | |
| MV-Pa1 | SCL | 2.30c | 4.80b | 5.70b | 12.70b |
| MV-Pa2 | C | 3.20b | 5.00a | 4.40c | 18.70a |
| MV-Pa3 | CL | 14.30a | 0.50c | 12.20a | 5.00c |
| One Way ANOVA *(F-Statistics)* | | | | | |
| F Value | | 4568.11*** | 3011.49*** | 1957.87*** | 1979.88*** |
| CV (%) | | 6.7 | 4.3 | 6.0 | 7.7 |
| Mbogo – Komtonga Irrigation scheme | | | | | |
| MB-Pa1 | C | 5.3c | 3.7b | 4.1c | 28.2b |
| MB-Pa2 | CL | 5.8b | 1.7d | 7.8a | 37.8a |
| MB-Pa3 | C | 2.5d | 8.1a | 3.3d | 18.7c |
| MB-Pa4 | C | 6.3a | 2.4c | 5.4b | 38.8a |
| One Way ANOVA *(F-Statistics)* | | | | | |
| F Value | | 805.0*** | 2813.8*** | 986.4*** | 652.5*** |
| CV (%) | | 4.7 | 4.7 | 4.8 | 11.5 |

***: significant at *P* =.001; CV: Coefficient of variation. Values followed by dissimilar letters in the same column for each parameter are significantly different from each other at *P* =.05 according to Fischer Least significance difference (LSD). K = Potassium; Ca = Calcium; Mg = Magnesium; Na = Sodium, TEB = Total Exchangeable Bases, BS = Base Saturation; SCL = Sand Clay Loam; SL = Sandy loam; CL = Clay Loam; C = Clay

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# Insect pests of amaranthus and their management

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***Abstract —*** *Amaranthus (Amaranthus spp.) is one of the most popular leafy vegetables in India. They are grown widely as a source of grain and leafy vegetables. It is rich source of many essential micronutrients like carotene, vitamin A, B, C and D, iron, calcium, amino acid like lysine and minerals especially iron, phosphorous and magnesium. But its production hampers due to the infestation of various insect pests namely Amaranth stem weevils: Hypolixus truncatulus (F.), H. nubilosus (B.), Beetworm Moth: Spoladea recurvalis (F.), Leafminer: Liriomyza huidobrensis (B.), Aphid: Myzus persicae S., Plant Bugs: Cletus sp., which ultimately affect the true potential of the crop. Here, the present article provides recent information regarding different insect pests of amaranthus, their identification, life-history, nature of damage and their management in an eco-friendly way.*

***Keywords— Amaranthus, insect pests, damage, management.***

## I. INTRODUCTION

Amaranthus (*Amaranthus spp.*; Family: Amaranthaceae) is grown mainly as a source of leafy vegetables and as a source of high-protein grain. The leaves are a rich source of calcium, iron and Vitamins A, B and C [4] whereas, grains are rich in dietary fiber, calcium and minerals such as iron, magnesium, phosphorus, copper and manganese and is a good source of essential amino acids especially lysine. It helps in lower blood pressure, cholesterol levels and improves the body's antioxidant status and immunity [6]. However, its production is affected by infestations of various insect pests that feed on various plant parts such as stems, leaves, flowers and seeds. A total of 92 insect pests belonging to 11 orders have been recorded from cultivated amaranthus [10]. Amaranth stem weevils: *Hypolixus truncatulus* (Fabricius), *H. nubilosus* (Boheman), Beet webworm Moth: *Spoladea recurvalis* Fabricius, Leafminer: *Liriomyza huidobrensis* Blanchard, Aphid: *Myzus persicae* Sulzer, Plant Bugs: *Cletus sp.*, are some of important pests which cause damage in amaranthus plant. The infestation and intensity of damage caused by them varies from different crop growth periods, regions and seasons. So, it is very important to know the different insect pests of amaranthus, their identification, life-history, nature of damage and their management in an eco-friendly way for their sustainable productivity.

## II. MAJOR INSECT PESTS ATTACKING AMARANTHUS

### 1. Amaranth stem weevils: *Hypolixus truncatulus*, *H. nubilosus* (Coleoptera: Curculionidae)

It is a major pest of the cultivated amaranthus, larvae tunnel the stems and adult feeds on tender leaves. The maximum number of adults recorded from a single plant (2.6 m height) is eight; however a maximum of 49 specimens at different stages of development recorded from a single plant, in the month of June-July. Percentage infestation varies from 35% to 81% at different regions in India [11].

**Distribution:** It is found in India, Mexico, Nigeria, South Africa and Kenya.

**Host range:** It is a polyphagous pest. Besides various species of Amaranthus it can infests large numbers of other host plants i.e. *Acacia nilotica, Dalbergia sissoo* etc. [11].

**Identification and life cycle:**

*Hypolixus truncatulus* adults are dark brown, variegated with white hairs and several dark patches of dense pubescence [11]. Body medium sized varying from 9.0 - 14.0 mm long and 3.0 -4.0 mm wide. Females are slightly larger than males. Eyes are large, well developed, black, located at the base of rostrum on either side. Antennae are geniculate, fourteen segmented, present on either side of the snout arising from anterior one third of the snout. *H. nubilosus* adults are redish brown, variegated with white patches. Full grown larva are creamish white, apodous, C shaped grub.

Females lay oval, light yellowish eggs singly in excavated holes in stems, branches, petiole or midrib of the leaves. Egg period varies from 3-6 days in June-July. After egg laying holes were covered with the light green colour substances, secreted from the mouth. Hatching period is 3- 4 days and it takes almost 20-45 days to complete its 5 larval instars. After hatching, the larva begins to feed on the internal tissues, making its way into the stem in the form of an irregular zigzag tunnel, filling it with excreta as it bores down. They eaten up the pith region of the plant and go on tunneling downwards until fully fed and gets ready for pupation. Before pupation full grown larva bores its way up to the stem surface, where a small round hole is made leaving

the thin epidermis layer intact. At the same place, subsequently the larva makes elongate oval pupal chamber which afterwards swells up and develops a large gall. Pupae are exarate, creamish white colour in the beginning but gradually turning into pale yellow. Pupal period lasts for 9-24 days and they completed at least three generations from April to November and total life cycle takes almost 58-64 days [11].

**Damage Symptoms:**

- Adults cause damage through feeding on the leaves, making irregular scratches on the tender stem and sometimes eating up all the inner contents of stem leaving behind only the epidermis and hypodermal tissues.
- Larvae cause damage through tunneling within the stems in a zig-zag way which reduces the vitality and vigour of the plants. Many such stems later rupture longitudinally thus exposing to the risk of desiccation; sometimes even 2-3 tunnels may be seen in transverse sections of the stem.
- At the places of pupal chamber, the stem walls become thickened so as to form galls. The adults emerge by biting holes through these galls. As a result, the stem becomes very weak and breaks down at such places during heavy winds; such plants often lie prostrate on the ground and dry up.

**Management of stem weevils:**

- Destroy all wild amaranthus plants in the vicinity.
- As soon as infestation is observed, remove and destroy promptly all the affected plants with grubs inside.
- Spraying with Neem seed extract 5% (w/v) is helpful to reduce the pest infestation [10].

**2. Beet webworm, *Spoladea recurvalis* (Lepidoptera; Pyralidae)**

**Distribution:** It is present in tropical and sub-tropical regions of Asia, Africa and Australia. **Host range:** Other than amaranth, it attacks on beet, beans, spinach and several weed species i.e. *Chenopodium album*, *Portulaca oleracea* and *Trianthema portulacastrum* [7].

**Identification and life cycle:**

Adults are brown in colour with forewing is deep brown with broad white median band and hindwings is deep brown with a broad white median bar. Mature larva is greeninsh with transparent epidermis, head light yellowish-brown with many brown spots.

Female lays almost 200 flattened elliptical eggs singly or in small groups on the lower surface of leaves. It takes 7 days to hatch. The larval stage lasts for 3 to 4 weeks. The most voracious and damaging stage is the third instar larva which prefers tender leaf [1]. Just before pupation, mature larvae turn reddish in colour. The pupa is yellowish brown in colour and pupates inside cocoons just below soil surface. The pupal period lasts for 12 days and it produces many generations in a year.

**Damage:**

- Young caterpillars feed on epidermis and voraciously feed on the green matter.
- Older ones web the leaves together and feed within.
- Severe attack results in complete skeletonisation and drying up of the leaves within a short time.

**Management:**

- Plough around trees to expose and kill pupae
- Collect and destroy the caterpillars
- Conserve the parasitoids such as *Trichogramma*, *Bracon*, *Apanteles*, *campoletis*.
- Spray azadirachtin 0.03% (300 ppm) @ 1000-2000 ml in 200-400 l of water/acre or azadirachtin 5% W/W neem extract concentrate @ 80 ml in 160 l of water/acre.

**3. Leafminer: *Liriomyza huidobrensis* (Diptera; Agromyzidae)**

**Distribution:** The moth is found in America, Asia, Africa and the Oceania.

**Host range:** It is a polyphagous pest and is known to attack host plants from 14 different families, both cultivated and wild including amaranth. Other important hosts are faba beans, onions, melons, garlic and peas [7].

**Identification and life cycle:** Adults are small, greyish-black, compact-bodied, 1.3-2.3 mm in body length, 1.3-2.3 mm in wing length. Females are slightly larger than males. Initially maggots are colourless but later turning pale yellow-orange in colour. It may be distinguished from other *Liriomyza* species by the head and leg yellow parts being a darker orange-yellow, the third antennal segments very dark, sometimes almost black on top, and the mesoplura is largely black.

They inserted almost 117-161 off-white, slightly translucent eggs just below the leaf surface. The number of eggs laid varies according to temperature and host plant. Hatching period is 2-5 days and the duration of larval development is generally 4-7 days. The larval stage consists of three larval instars. The larva leaves the plant to pupate and pupae may be found in crop debris or in the soil [8].

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Females had an average longevity of 3-28 days; male longevity was 2-6 days. It takes almost 17-30 days to complete its life cycle during the summer and in 50-65 days during the winter [5].

**Damage:**

- Maggots produce the serpentine leaf mines which are usually white with dampened black and dried brown areas.
- Several larvae feeding on a single leaf may produce a secondary blotch like mine and leaf wilt may occur.

**Management:**

- Hand picking and destruction of infested leaves with maggots in early stages may be effective in reducing population built-up.
- Spray azadirachtin 0.03% (300 ppm) @ 1000-2000 ml in 200-400 l of water/acre or azadirachtin 5% W/W neem extract concentrate @ 80 ml in 160 l of water/acre.

**4. Aphid,** *Myzus persicae* (**Aphididae: Hemiptera)**

**Distribution:** It is distributed throughout the world except in areas with extreme temperatures or moisture.

**Host range:** It is a serious pest of amaranth, groundnuts, capsicums, carrots, maize, beans, potato, tomato and eggplants. Peach is its primary host for sexual reproduction in colder climates.

**Identification and life cycle:**

Nymphs are about 2 mm long, olive-green, resembling the parthenogenic females, which reach 2.5 mm in length. The apterate viviparous females are yellow-green, with 3 brown longitudinal lines, one at mid-dorsum the others on each side. The siphunculi are pale green, twice as long as the cauda, slightly swollen in their middle. The head, antennae, thorax and cauda of the alate females are dark.

In the cool regions sexual (amphigonic) females appear in the autumn and lay about a dozen fertilized eggs in crevices on their primary hosts, usually peaches. The eggs remain dormant during winter, hatching in the spring and the emerging nymphs feed on opening buds. They then initiate a few parthenogenic cycles of about two weeks each. Later in the season as host quality declines, alate forms appear and migrate to their diverse alternative ("secondary") host-plants, on which they establish colonies of apterate adults. A female may give birth to 75 young during its life. The nymphs moult five times to become adults in 8-12 days. Dry weather conditions are favorable to aphids whereas heavy rainfall decreases their numbers [2].

**Damage:**

- The damage is caused both by the nymphs and adults by sucking plant sap causing yellowing and drying of leaves.
- Severe infestation results in curling of leaves, stunted growth and gradual drying and death of tender region of the plants.
- They produce copious amounts of honeydew, which serves as a medium on which sooty mold grows. Sooty mold blackens the leaf and decreases photosynthetic activity of the plant [3].
- Seed production is hampered by aphid infestation where it may lead to deformed seeds, decreased flower and seed formation or reduced seed viability [9].
- It also transmits many important plant viruses such as such as Potato leaf roll virus (PLRV), Potato virus Y (PVY), Cucumber mosaic virus (CMV), and Pepper veinal mottle virus (PVMV).

**Management:**

- Destroy the infested plant parts.
- Follow clean cultivation.
- Conserve the Parasitoid, i.e. *Aphidius colemani* and Predators i.e. syrphid/hover flies, green lacewings (*Mallada basalis* and *Chrysoperla carnea*), predatory coccinellids (*Stethorus punctillum*) etc.
- Spray azadirachtin 5% W/W neem extract concentrate @ 80 ml in 160 l of water/acre.
- Spraying of *Verticillium lecanii* @ $1\times10^7$ viable spores/ml, reduce the aphid population.

Beside the above mentioned major insect pests there are many minor insect pests causing damage to amaranthus crop at different region and growth stages of the crop.

The incidence of grasshoppers such as *Atractomorpha crenulata* (Pyrgomorphidae: Orthoptera) and *Pyrgomorpha conica* (Pyrgomorphidae: Orthoptera) also found on amaranthus. Both adults and nymphs feed on the leaves and cut the tender shoots.

The plant bug; *Cletus* sp. (Coreidae: Hemiptera) whose population often reaches peak during the critical milky seeds stage. It feeds on the seed causing discolouration, shriveling and premature dying of seeds thereby reducing seed yield and viability.

A B C D

*Fig.1: Insects infesting amaranthus plant (Contai, West Bengal); A: Pyrgomorpha sp., B: Hypolixus nubilosus, C: Cletus sp., D: Webworm infested leaves*

## III. CONCLUSION

Importance should be given to sustainable production of amaranthus by managing the insect pests by integrating different easily available management practices including biological, cultural, mechanical and physical means. Further, care should be given to conserve the indigenous bio control agents.

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# Variation of Drinking Water Quality in Rural Areas of Kurunegala District, Sri Lanka

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***Abstract*—***Due to the human activities, the natural water system gets contaminated with various chemical substances which can be harmful for the human health. In this research, it was attempted to find out the variation of drinkingwater quality in rural areas of Kurunegala district, Sri Lanka. Five GramaNiladari (GN) divisions in Ibbagamuwa Divisional Secretariat (DS) Division, were selected as the study area for this research. Seven water quality parameters were investigated in laboratory scale, which were basically divided in to physical and chemical parameters. Altogether 150 samples were collectedand all the parameters were investigated in the laboratory scaleunder the standard specifications given in SLS 614:2013 part 1.*

*In this research, it was identified that Ibbagamuwa DS Divisionwas badly contaminated with water hardness (exceeded the standard limit of 200 mg/l). Further, several areas were identified as not suitable for drinking purposes. Most of the time, these unsuitable areas were reported around the Kibulwanaoya reservoir, one of the main irrigation reservoirs located inIbbagamuwa DS Division. This area was highly contaminated with chloride and fluoride iron. There were several places that were appropriately identified as safe for drinking purposes because all the water quality parameters in that area were within the standard limit. The areas with suitable water resources have been identified around the rocky areas. It was also identified that the ground water around the highly cultivated areas and irrigation reservoir were not in good condition.*

*The design of separate maps for each water quality parameters were very important for people in this area. By referring these maps, they will be able to identify what are the suitable and unsuitable areas to take water for the purpose of drinking.*

***Keywords*—*Ibbagamuwa Divisional Secretariat Division, North Western Province, Drinking water quality parameters, Kibulwanaoya Irrigation Reservoir.***

## I. INTRODUCTION

Water is the most considerable and valuable wealth for living beings. All the living beings are consuming water for their daily needs. Without having water, there is no existence of lives on planet. In Sri Lanka, most of the time, people use ground water sources as their source of water but in most of the urban areas, people use tap water as their source of water. Tap water is mainly supplied by the Water Board of Sri Lanka and few other relevant organizations.


*Fig. 1: Location of the selected Study Area*

As for rural areas, most of the time people use well water for their daily drinking purposes. In this case, there might be a risk of water borne diseases because in Sri Lanka there is no any regulations or law to determine the quality of these ground water resources. As a result, many rural districts such as Anuradhapura, Polonnaruwa and Kurunagala etc. are highly affected by water borne diseases. Chronic kidney disease, renal disease, Dental fluorosis, Bladder stones are some of the drinking water related long term diseases. These diseases are very dangerous and the ultimateresult can be death. As an outcome of this research, proper solutions can be

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identified in order to safeguard people from these water borne diseases.

The purpose of this research is to identify the variation of drinking water quality in rural areas of Kurunegala District. Fig.1 shows the location of the selected study area. Five GN divisions in Ibbagamuwa DS Division were selected for this investigation of drinking water quality.

The following factors were considered when selecting Ibbagamuwa DS Division as the study area.

- Geographical location
- Population
- Educational standard of the people
- Ground soil condition
- Details of ground water aquifers
- Type of domestic water resources used
- Historical details of water related diseases in this area

The variation of drinking water quality in these five GramaNiladari divisions is discussed in this research. The main objective of this research is to collect information about the drinking water resources of this selected area. In this study area, people are mostly usingnatural water resources or dug wells as theirdrinking water resources as shown in

Table *1*. Therefore the investigation of drinking water in this area was mainly focused on well water.

*Table 1 Main Source of Drinking Water in Rural Area*

| GN Division | Number of Houses | Main Source of Drinking Water | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Well | | Pipe Borne Water | Rural Water | Tube Well | Bottled Water | Other |
| | | Protected well | Unprotected Well | | | | | |
| Neerammulla | 385 | 322 | 19 | 30 | 4 | 2 | - | 8 |
| Leenawa | 224 | 223 | - | 1 | - | - | - | - |
| Bandipola | 248 | 226 | 10 | 1 | - | 6 | - | 5 |
| Pitapahamuna | 250 | 247 | 2 | - | - | 1 | - | - |
| Karadagolla | 431 | 369 | 19 | 7 | 29 | 5 | - | 2 |

### 1.1 Drinking Water Quality and Water quality standards in Sri Lanka

In this research seven water quality parameters were checked in laboratory scale. To determine the selected water quality parameters, the Environmental Laboratory of the General Sir John KotelawalaDefence University was used.These selected parameters were divided in to two different categories, which are physical and chemical parameters. According to the physical characteristics of the drinking water, three main parameters were discussed as follows.

- Colour (Hazen Unit).
- Turbidity (NTU).
- pH at 250 C.

According to the chemical characteristics of drinking water, four main parameters were discussed as follows.

- Chloride (as $Cl^-$).
- Fluoride (as $F^{-}$).
- Total Alkalinity (as $CaCO_3$).
- Total Iron (as Fe).

Table.*2* shows the ranges and the maximum exceeding limit for each and every parameter.

*Table.2: Standard specification requirements*

| PARAMETER | Requirement (SLS 614: 2013 part 1) |
|---|---|
| **A.Physical-Organoleptic Requirements** | |
| Colour, Hazen Units, (max.) | 15 |
| Turbidity, (NTU) (Nephelometric Turbidity Units), (max.) | 2 |
| pH at $25^0C$ +. $2^0C$ | 6.5 to 8.5 |
| **B. Chemical requirements** | |
| Chloride (as $Cl^-$) (mg/l) | 250 |
| Fluoride (as $F^-$) (mg/l) | 1.0 |
| Iron (as Fe) (mg/l) | 0.3 |
| Total alkalinity (as $CaC0_3$) (mg/l) | 200 |

All these parameters were investigated under the standard specifications of SLS 614: 2013 part 1 and the Drinking Water Standards which are followed by the National Water Supply and Drainage Board of Sri Lanka [1].These standards were carried out ensuring conformity to the provisions of the National Environmental Act and its regulations [7].

## II. METHODOLOGY

This research is based on the experimental data analysis method. From the field investigation and the details collected from the statistic division of the Kurunegala district, it was found that most of the rural area people are consuming drinking water from dug wells [4]. Therefore this study was based on these dug wells of this particular area. Several types of dug wells were located in Ibbagamuwa DS division, such as tube wells, agro wells etc.

### 2.1 Sampling

150 Samples were collected from the five GN divisions in this Ibbagamuwa DS division. Nearly 30 samples were collected from each GN division. The sample locations were measured by using a Hand Heal GPS apparatus. A depth between 1m to 12m in shallow wells was selected and 175ml glass bottles were used to collect all the samples. Fig. 2 shows the sampling locations in the study area.

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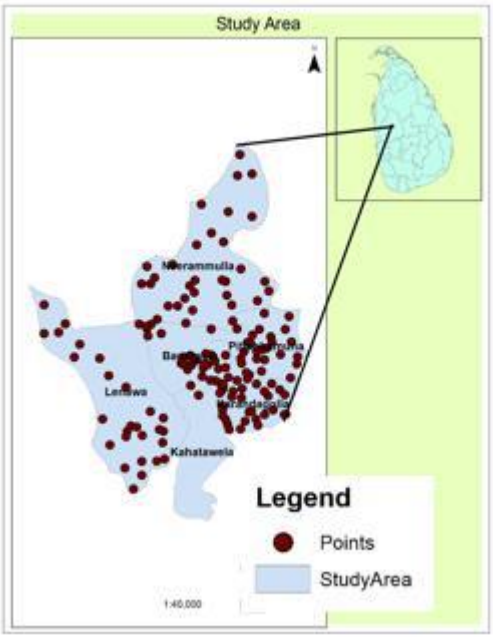


*Fig. 2: Sampling locations in the study area*

**2.2 Laboratory Experiment**

All the collected samples were stored in a thermo freezer under laboratory conditions. To determine the water quality parameters, the following apparatus were used as given in Table 3.

*Table 3 Apparatus and specifications*

| PARAMETER | APPRATUS | SERIAL NO |
|---|---|---|
| Colour, Hazen Units | UV Visible Spectrophotometer (HACH DR6000) | S/N1569293 |
| Turbidity (NTU) | Turbidimeter Model 2100N | S/N14010C031037 |
| pH at $25^0$C +- $2^0$C | HI 2111 pH/ORP Meter | S/N08702309 |
| Chloride (as $Cl^-$) (mg/l) | Digital Titration Apparatus | 16900 |
| Fluoride (as $F^-$) (mg/l) | Digital Titration Apparatus | 16900 |
| Total alkalinity (as $CaCO_3$) (mg/l) | Digital Titration Apparatus | 16900 |
| Iron (as Fe) (mg/l) | Spectrophotometer (HACH DR6000) | S/N1569293 |

All the samples were investigated according to the standards and specifications given by the company. All the experiments were carried out under the room temperature.

After this, tested data were put in to a proper excel worksheet. This worksheet include all the physical and chemical parameters which were tested in the laboratory and the number of samples.

**2.3 Data Analysis Using Arc GIS Model.**

Basically IDW (Inverse Distance Weight) method was used for this analytical part. By using this IDW method, all the samples were reclassified from the exact range. Then the sample points were interpolated to determine all the surface details of the particular data set. All the physical and chemical characteristics were used to design a unique map. The design model is shown inFig. 3.


*Fig. 3: Model of IDW*

The selected study area consists of a main irrigation reservoir and few other small scale streams and lakes. Ground water characteristics of this particular area are not included in this research range. In this Arc GIS map designed for the surface water area,the interpolation values of this surface water resources might not be correct. Therefore it was assumed,that these surface water resources might consist the same ground water table for this IDW interpolation method.

## III. RESULTS AND DISCUSSION

**3.1 Physical characteristics**

To determine the physical characteristic of drinking water there are several types of test procedures to follow.

**3.1.1 Variation of colour (Hazen Unit)**

A simple colorimetric procedure was developed for apparent color determinations using platinum cobalt standards and expressing the results in Hazen units. The method involves measuring the absorbance of a sample with a colorimeter using two broad band fillers. A second absorbance reading is required to obtain a suitable correction for particulates. With this method, the human response factor is minimized, and the accuracy, as estimated by standard deviation, is one Hazen unit [3].

*http://dx.doi.org/10.22161/ijeab/3.3.51* *ISSN: 2456-1878*

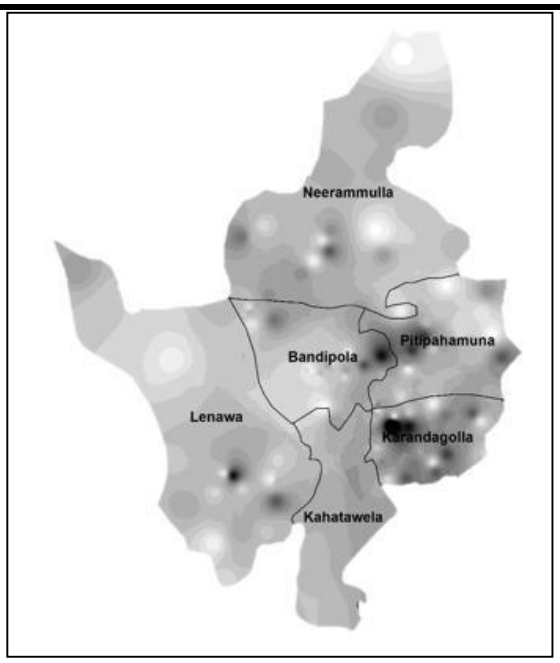


*Fig. 4: Variation of colour in Pitipahamuna, Bandipola, Leenawa, Neerammulla and Karadagolla GN Divisions 2017*

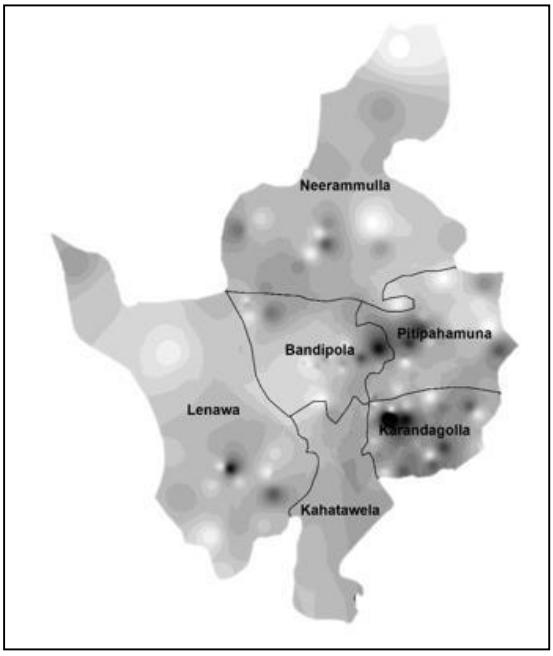


Fig. *4*shows the variation of colour in this five GN divisions by using separate colour variation of White to Black. According to the

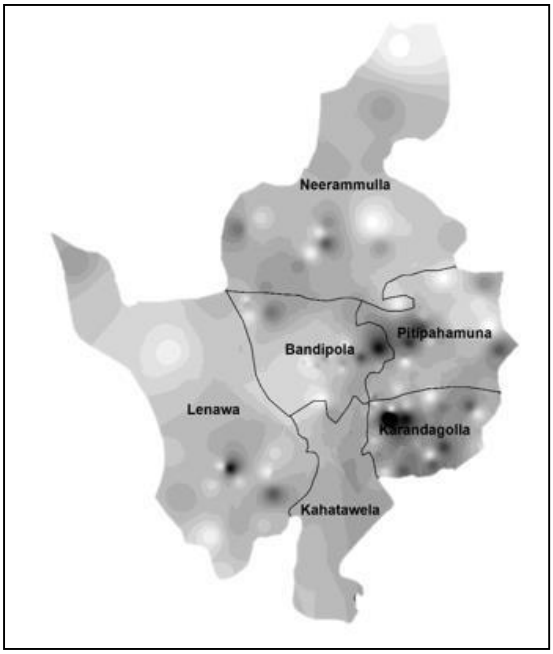


Fig. *4* the black colour represents the area where the colour has exceeded its limit, and grey and white colours represent the safe levels of the drinking water in colour. One Hazen unit represents a specified colour range. 0 to 1 represent white colour, 1 to 2 represent another different colour. According to the results of this study area, no any sample has exceeded the maximum range of colour [3].

**3.1.2 Variation of Turbidity (NTU)**

The definition of Turbidity is the cloudy appearance of a fluid caused by suspended solids that are usually invisible to the normal eye. It is also called as an aggregate optical property of the water and does not identify individual substances. There are several ways to determine turbidity in water. The most commonly used method is the measure of attenuation, or reduction in strength and a light source, as it passes through a water sample. NephelometricTurbidity Units (NTU) are the units of measurement used by a nephelometer meeting EPA design criteria [2].

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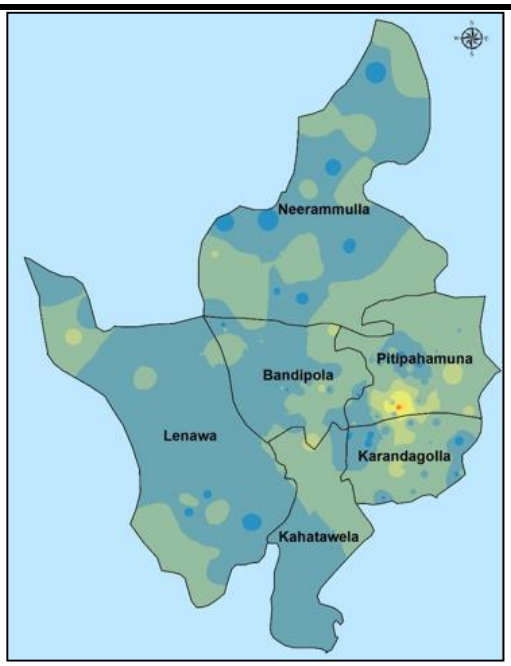


*Fig. 5: Variation of Turbidity in Pitipahamuna, Bandipola, Leenawa, Neerammulla and Karadagolla GN Divisions 2017*

As shown in

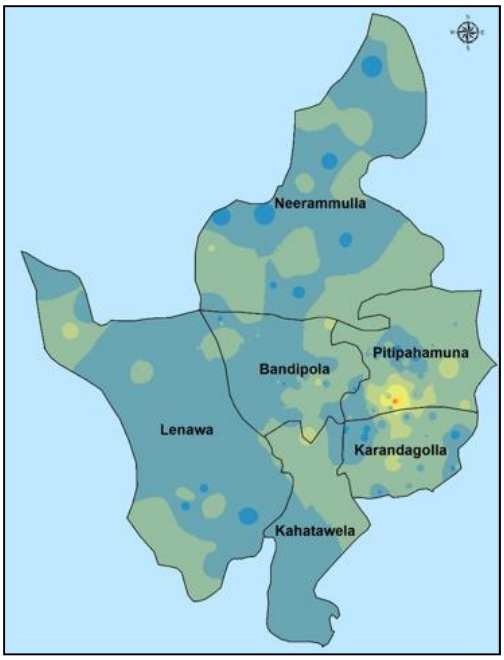


*Fig. 5*, variation of turbidity were represented by using separate colour range. Basically the Turbidity value in drinking water may vary in this selected area in a range of 0 to 2 NTU. The variation of Turbidity was represented in a colour range of Blue Colour to Yellow colour. But according to the test result carried out in KDU laboratory, there were no any sample reported in the exceeded limit of turbidity. Therefore all the values which were included in this map were in safe range for drinking water.

### 3.1.3 Variation of pH at $25^0$C +/_ $2^0$C

Basically the pH value is a good indicator to identify whether water is hard or soft. The pH value of pure water is 7. In general, water with a pH lower than 7 is considered acidic, and with a pH greater than 7 is considered basic. The normal range for pH in surface water systems is 6.5 to 8.5, and the pH range for groundwater systems is in between 6 to 8.5 [5]. The measurement of pH was carried out at the room temperature of $25^0$C +/_ $2^0$C.

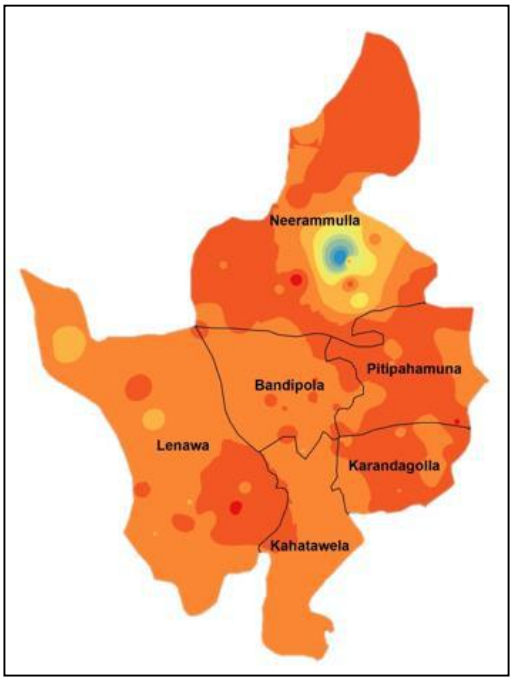


*Fig. 6: Variation of pH at 250C in Pitipahamuna, Bandipola, Leenawa, Neerammulla and Karadagolla GN Divisions 2017*

According to

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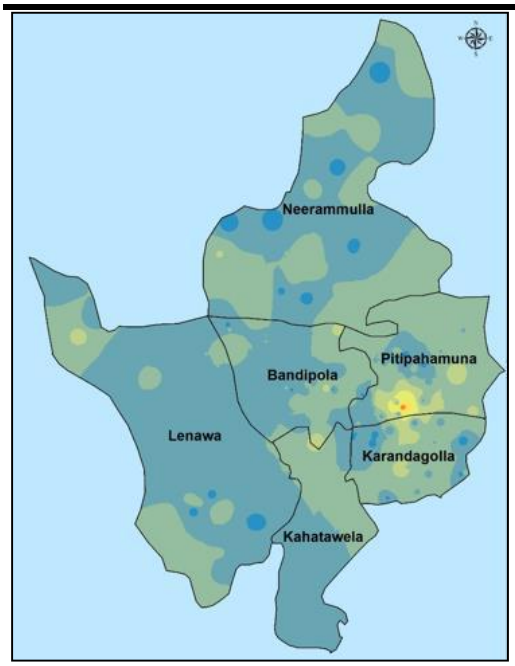


*Fig. 5* there were several cases where the pH value is low. But these values were not below the minimum value of pH according to the standard specifications. By referring this map it can be easily identified what are the vulnerable areas in variation of pH value.

**3.2 Chemical Characteristics**

Distilled water is considered as pure water that is not contaminated with any kind of suspended particles or chemical substances. The chemical substances in the ground surface are diluted in ground water. Rain and surface runoff directly causethe contamination of heavy metals in ground water, as this surface runoff water passes through the cultivated lands in this area. These cultivated lands consists of a high percentage of heavy metals, because the farmers use poison and inorganic fertilizers for their cultivations. Another important parameter to understand the contamination of chemical substances in ground water is,the surface and ground soil condition in this area.

**3.2.1 Variation of Chloride (mg/l)**

Chloride can be found in ground water as diluted salts such as Sodium Chloride (NaCl), Potassium Chloride (KCl), and Calcium Chloride ($CaCl_2$). Chloride can be found in surface and ground water generated from both natural and anthropogenic sources, such as run-off containing road de-icing salts, use of organic fertilizers, landfill leachates, septic tank influents, animal feeds, industrial influent and irrigation drainage[1]. Chloride can also enter a watershed through water softener discharge or sewage contamination. Another important anthropogenic source of chloride in ground water is, fertilizer made with potash, or mined salts. Potassium Chloride (KCl) is the salt most commonly used in potash fertilizer. However, this potash can leach from fertilizer soil to the rivers and streams. Ultimately these chlorides are collected to natural water bodies and diluted ground water sources [6].

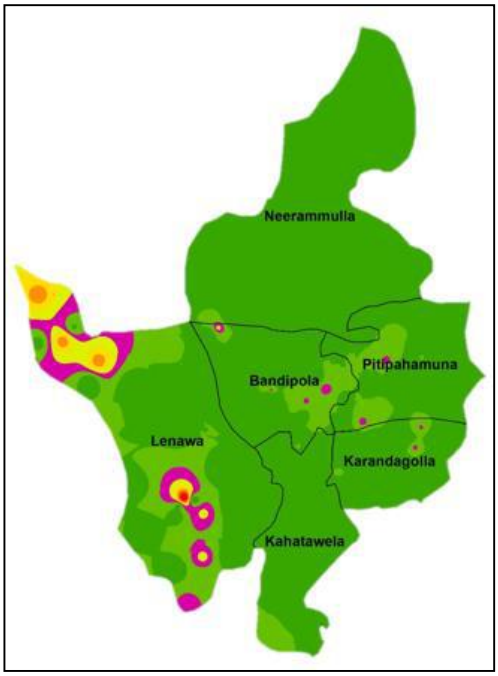


*Fig. 7: Variation of Chloride in Pitipahamuna, Bandipola, Leenawa, Neerammulla and Karadagolla GN Divisions 2017*

As shown in

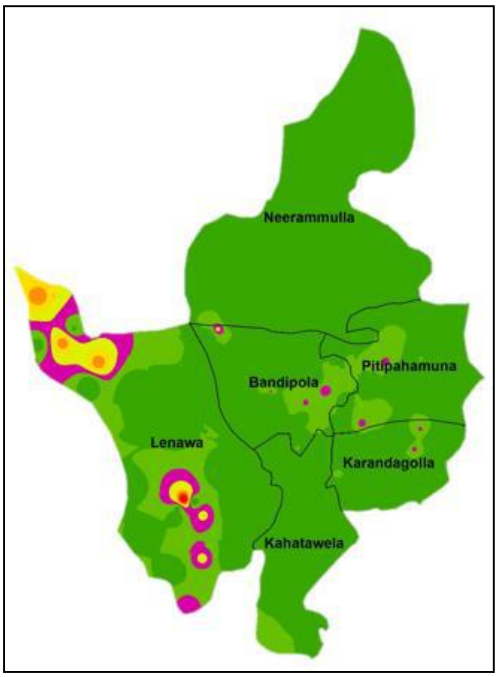


Fig. *7*,the variation of chloride were represented in separate colour ranges. Minimum value of chloride is shown in green colour and maximum chloride ranges were shown in red colour. Purple and yellow colour represent the exceeded values of chloride. Neerammulla GN division was considered as safe for drinking water in

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this variation of chloride. As shown in

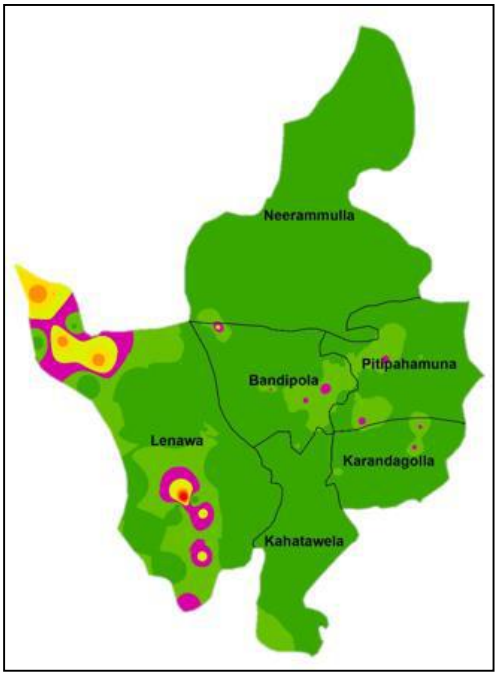


Fig. *7* all other four GN divisions were having some issues in chloride.

**3.2.2 Variation of Total Alkalinity (mg/l)**

Alkalinity of drinking water may be due to the presence of irons. Hydroxides, carbonates and bicarbonates are the iron that case of this alkalinity. Alkalinity of water may be defined as its capacity to neutralize acid. Alkyl substances in water include hydroxide or bases. Most of the times alkalinity of surface and ground water comes from calcium carbonate ($CaCO_3$) generated from rock and soil. This process is enhanced if the rocks and soil have already been broken up before entering the water. When discussing the health effect of alkalinity in drinking water, it causes excessive drying of the skin due to the fact that they tend to remove normal skin oil. Actually this can be happened when high mineralized alkaline is present in water.

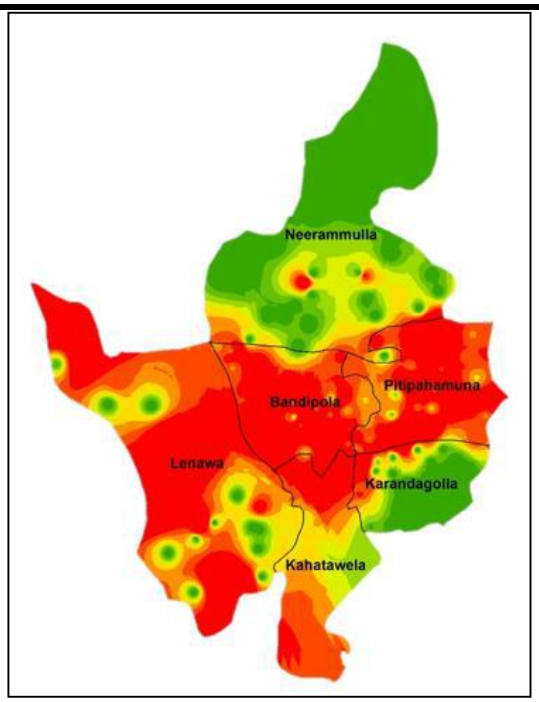


*Fig. 8: Variation of Total Alkalinity in Pitipahamuna, Bandipola, Leenawa, Neerammulla and Karadagolla GN Divisions 2017*

According to the

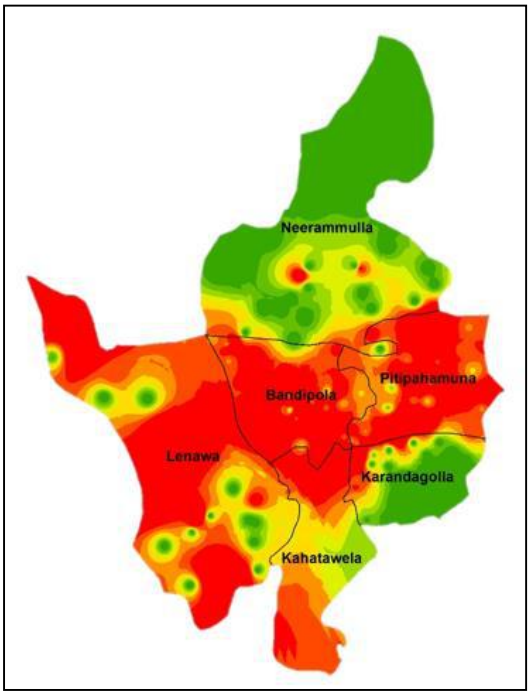


Fig. *8* total alkalinity of this study area was represented in a separatecolour range. Safe drinking water zone was represent in the range of green to yellow and unsafe drinking water zone was represented in dark yellow to red

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colour range. According to the

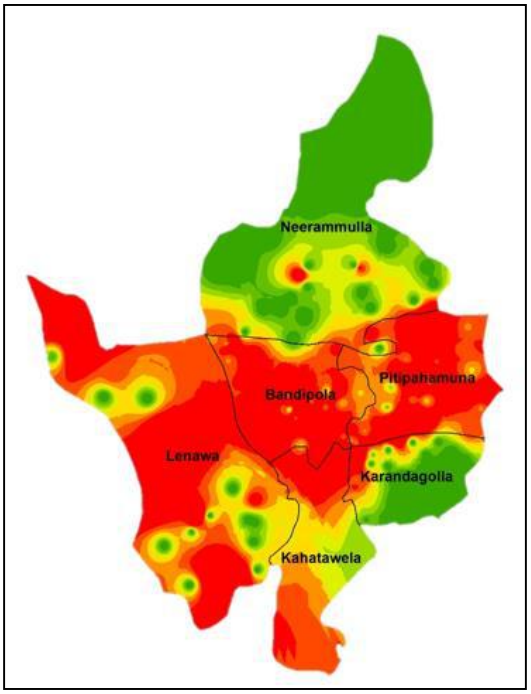


Fig. *8* the variation of total alkalinity in this study area were highly contaminated with $CO^{3-}$and $HCO^{3-}$ions [8]. On the other hand, it was concluded that this area were highly contaminated with $CaCO_3$ or $MgCO_3$ crystalline. By referring this map it was clearly identified that Pitipahamuna, Bandipola, Karadagolla and Leenawa GN divisions were considered as highly contaminatedby hardness (exceeded the maximum standard limit of 200 mg/l). Therefore, people who refer this map would be able to identify the unsafe drinking water resources easily.

### 3.2.3 Variation of Fluoride (mg/l).

Fluoride can be considered as an essential iron for human health. But when fluoride ingested in excessive dose it becomes toxic. Low fluoride in water also causes dental fluorosis which is very common in Central province and North Central province in Sri Lanka. The geochemical pathways of fluoride is strongly influenced by the process involving absorption–desorption and dissolution precipitation reactions [5]. Therefore, fluoride concentration of water mainly depends on the degree of weathering and the amount of leachable fluoride.

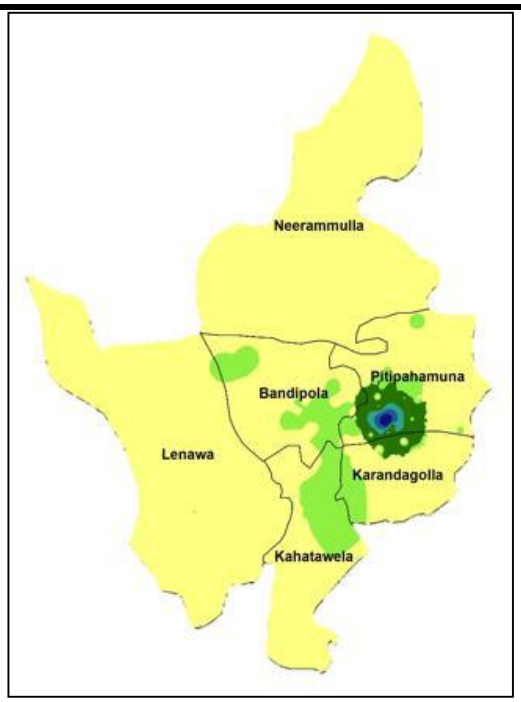


*Fig. 9: Variation of Fluoride in Pitipahamuna, Bandipola, Leenawa, Neerammulla and Karadagolla GN Divisions 2017*

According to the

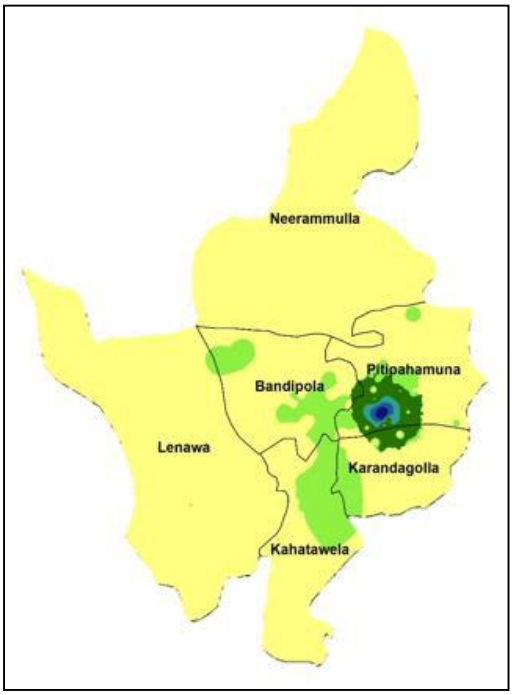


Fig. *9*, the variation of fluoride in this study area was represented in a separate colour range. The accepted fluoride ranges were represented in yellow and light green colour and the exceeded ranges were represented in other colours. Pitapahamua GN division is having an unexpected fluoride variation of 49 mg/l as shown in Fig 9.

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**3.2.4 Variation of Total Iron (mg/l)**

Mainly Iron can control the taste of the drinking water, asIron can produce Humic acids and that exert a positive influence in the presence of Fe Iron in drinking water. The Iron in water is basically in the form of $Fe^{2+}$ which can occur largely under anoxic condition. It has been shown that $Fe^{3+}$ is the only oxidation state which is possible for Iron in oxygen containing water such as domestic water. These forms can be reduced to the soluble $Fe^{2+}$ Ions only under anoxic condition. Further, the oxidation of $Fe^{2+}$ may also be catalyzed by a wide range of micro – organisms [1].

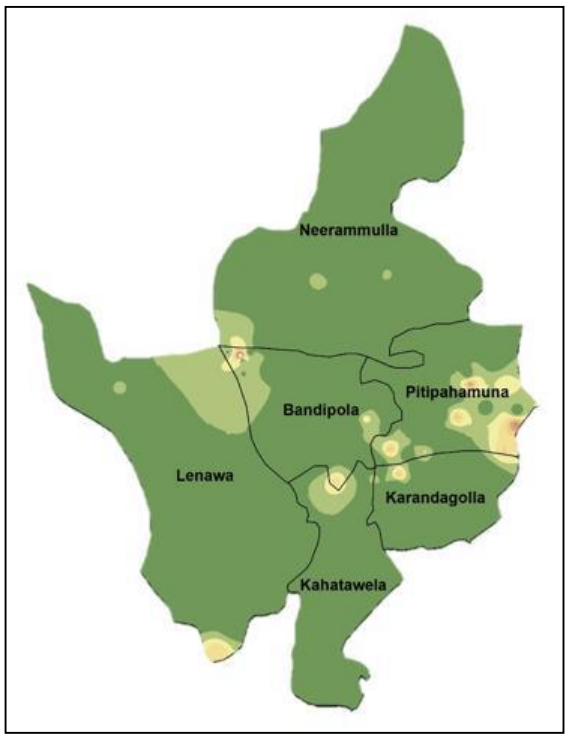


*Fig. 10: Variation of Total Iron in Ptapahamuna, Bandipola, Leenawa, Neerammulla and Karadagolla GN Divisions 2017*

As shown in

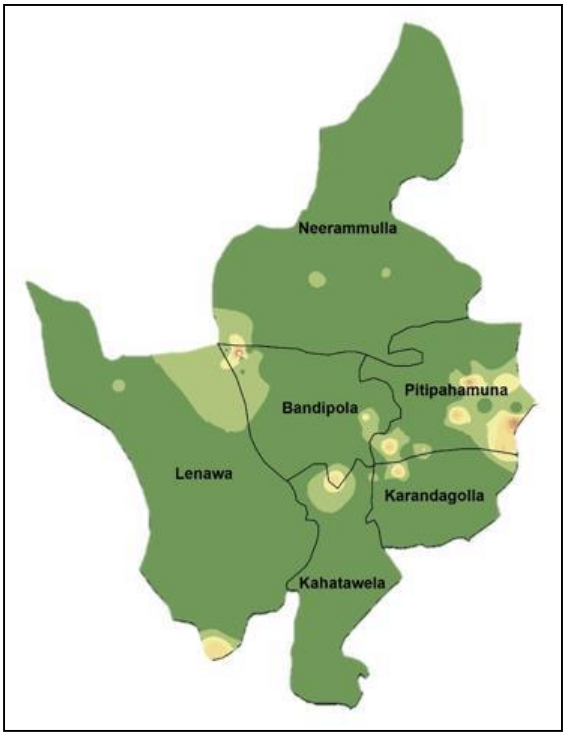


Fig. *10*, the variation of total iron in this study area was represented in a separatecolour range. The acceptable total iron ranges were represented in green and light yellow colours. Except for these two colours, other colours represent the exceeded limit of total iron values. According to the

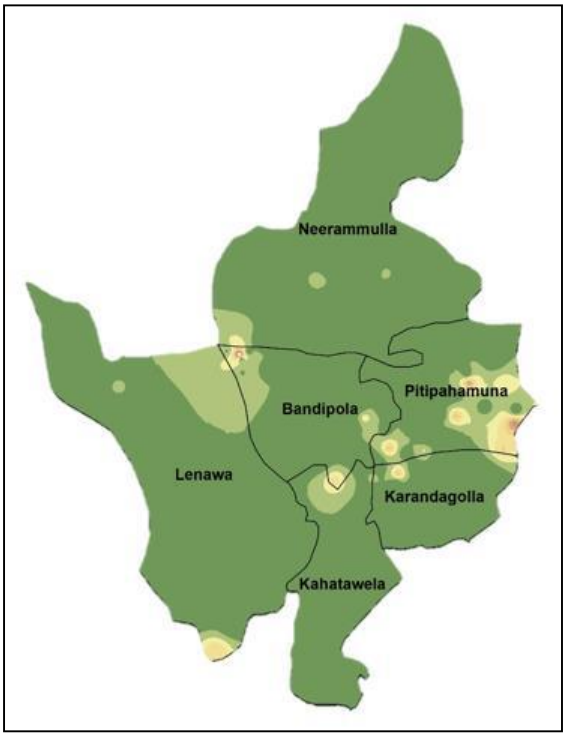


Fig. *10*, the vulnerable and suitable areas for drinking water can be identified by considering the total iron concentration of water.

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## IV. CONCLUSION

According to these different types of maps which were designedto represent the variation of drinking water quality in this study area, the suitable and unsuitable areas for drinking water can be clearly identified. Especially in highly cultivated areas and reservoirs areas, ground water is not suitable for drinking purposes as both chloride and fluoride values in these areas have exceeded the maximum requirement according to the standard specifications. Few samples out of the 150 samples of drinking water was having heavy hardness in this study area. All these values have exceeded the maximum requirement of 200 mg/l according to the standard specifications of SLS: 614-2013 part 1. There were several locations which were identified as safe for drinking water. All the physical and chemical parameters were in between the standard range and below the maximum exceeded limit.

Ultimately it can be concluded that Ibbagamuwa DS division was badly contaminated with the total alkalinity. In the other hand the research area was contaminated with $Ca^{2+}$ or $Mg^{2+}$ irons. But except for the total alkalinity, these areascan be recommended for drinking purposes. To minimize the total alkalinity it was recommended to boil the water before use.

## ACKNOWLEDGEMENTS

We take this opportunity to express our gratitude to the Department of Civil Engineering of General Sir John Kotelawala Defense University and National Water Supply and Drainage Board Sri Lanka for providing all the laboratory facilities and valuable instructions.

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# Oodev Injection Frequency and Time Period in Advancing Gonad Rematuration of Snakehead (*Channa striata* Blkr) in Hapa System

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***Abstract***—*This study provides meaningfully scientific information on the attempt to accelerate the gonad rematuration of snakehead (Channa striata) to support the quality seed production for both commercial and restocking purposes in South Kalimantan, Indonesia. A total of 48 snakehead fish broods (296-312 mm total length and 234-298 g weight) were subjected to different treatment levels using oodev dose of 0.5 ml kg$^{-1}$ fish weight to determine the best frequency and time period for gonad rematuration after spawning. Treatment-A: 3 times/9 days, B: 2 times/6 days, C: 1 time/3 days, and D: no oodev injection (control). The 12 hapas were used comprising 4 individuals per hapa (0.4×0.4×1 m) under the controlled condition. After all, ten snakehead brood samples were dissected to find out the gonad maturity level of fish. The treatment-A showed the best performance in term of mean fecundity (19600±450.00 granules/individuals), egg diameter (1.10±0.15 mm), and gonad somatic index/GSI (3.41±0.90 %) among other treatments. Dealing with hepatosomatic index (HSI), the treatment-C was significantly higher than treatment-A and D, but not differ from treatment-B. The mean HSI was varied from 0.89±0.03 % to 1.53±0.23 %. Temperature, pH and DO during sampling period are considered comfortable for snakehead fish broods.*

***Keywords— Channastriata, gonad rematuration, fecundity, egg diameter, GSI, HSI.***

## I. INTRODUCTION

In the world, the snakeheads comprise two extant genera, namely Channa (36 species native to Asia) and Parachanna (four African species), and most recently, barcoding snakehead is revisited to solve perpertuated taxanomi confiusions (Conte-Grand *et al*., 2017). Snakehead (*Channa striata* Blkr) of family Channidae, is considered as valuable food fish not only in Indonesia (Widodo *et al*., 2013; Irhamsyah *et al*., 2017), but also other countries such as Thailand (Khomsab and Wannasri, 2017), Philippines (Jumawan and Seronay, 2017), Vietnam (Quyen *et al*., 2016), Malaysia (Song *et al*., 2013), Cambodia (Sinh, 2014), Sri Lanka (Wijeyaratne, 1994), Nigeria (Ama-Abasi and Ogar, 2013), Bangladesh (Islam *et al*., 2013), India (Kashyap *et al*., 2014), Pakistan (Najero *et al*., 2015) and China (Gu*et al*., 2015) due to delicious, high-quality meat fish and availability throughout the year. Snakehead can be commercially cultured in fish farming, earthen ponds, or hapa system (Kumar *et al*., 2011; Quyen *et al*., 2016), and culture strategies of them are currently being developed (Xie *et al*., 2002; Xie *et al*., 2017; He *et al*., 2015; Istiyanto and Diana, 2016). They inhabit freshwater such as swamps, rivers, streams, lakes, reservoirs, irrigation canals and paddy fields (Saikia *et al*., 2012; Muthmainnah, 2013; Kashyap *et al*., 2014; Sakhare, 2015; Singh and Serajuddin, 2017). Snakehead locally known as "Haruan", is able to tolerate to adverse environments due to its hardiness and air-breathing capabilities assisted with a suprabranchial chamber, an air-breathing organ (Chandra and Banerjee, 2004). They are carnivorous feeders that consume plankton, aquatic insect, mollusks, fish or frogs. Snakeheads from river or swamps are caught by using hooks, gillnet, castnet or fish pot (Song *et al*., 2013; Irhamsyah *et al*., 2017). Overfishing, pollution, habitat disruption, disease, and growing human intervention on wetlands is very likely threat to this species (Balkhis *et al*., 2011; Uthayakumar *et al*., 2014; Rao *et al*., 2015).

Seasonal reproduction is a strategy adapted by fishes to guarantee better chances of their offspring's survival bytiming reproductive activity with favorable conditions during certain times of the year (Bernal *et al*., 2015). An understanding of the reproductive biology of fishes in relation to external factors (e.g. photoperiod, temperature, rainfall, salinity, and food supply) in their habitat is useful in the propagation of captive fish broodstock (Bromage *et al*., 2001). Snakehead in swamp waters spawning in the early or mid-rainy season. It takes longer time for the gonad growth and reproductive process, meanwhile the culture development is highly depend on the availability of

fish seeds that meet the timeliness, quality and quantity. Seeds can be continuously produced if it is supported by the availability of matured broodstock with good egg quality. One of efforts to meet the need of seeds production is by increasing the spawning frequency of nilem fish broodstock through the acceleration of gonad rematuration period after spawning. The gonad rematuration period usually takes longer time compared to the maturation process. It is clearly found in *Osteochilus hasselti* in which the gonad maturation period takes 10 days (Cholifah, 2016), while its rematuration period takes 17 days (Fitriatin *et al*., 2018) by using oodev hormone 1 ml $kg^{-1}$.

Understanding the propoer function of follicle stimulating hormone (FSH) of oodev content will reduce the artificial spawning failure of the snakehead, because it is direcrtly involved in the acceleration of the vitelogenesis process (Moore and Ward, 1980). The previous studies confirmed that oodev hormone beneficially supports for gonad maturation and spawning in *Pangasius hypophthalamus* (Ernawati, 1999), *Clarias batrachus* (Zairin *et al*., 2000), *Hemibagrus nemurus* (Supriyadi, 2005), and *Osteochilus hasselti* (Cholifah, 2016), as well as for gonal rematuration in *P. hypophthalmus* (Agustinus, 2013), *Anabas testudineus* (Sari, 2015), and *O. hasselti* (Fitriatin *et al*. 2018), but lack of available information on most snakehead species. Therefore, the present study is performed to determine the best frequency and time period (day) for advancing the snakehead gonad maturation through oodev hormone intervention. From all stages of this research, we endeavor to provide a simple technology package of snakehead gonad rematuration to supply the needs of quality seeds for both commercialand restocking purposes.

## II. MATERIALS AND METHODS

### Study site and Experimental condition

The research was carried out in the Wet Laboratory at the Faculty of Marine and Fisheries, University Lambung Mangkurat for three months. A set of tools, equipment and materials arewell-prepared as described in Table 1. About one hundred snakehead broodstocks were collected from local fishers in the monotonous swamp and were kept in the Wet Laboratory. The only fourty-eight fish samples (296-312 mm total length and 234-298 g weight) were used in this study, while the remaining fish was kept in a large hapa (1.5×1.5×1.0 m) as stock in case of the death during the trial periods. The hapas were placed in a concrete tank pond (7×4×1.2 m). Tap water was precipitated one week before treatment. Water lettuce (*Pistia stratiotes* L) was given for shelter and supplemental nutrients in the water. Fish were fed with the frog as 4 % of body weight per day with the frequency of once a day. The twelve hapas were used in this experiment with the density level of 4 individuals per hapa (0.4×0.3×1 m). Water quality parameters recorded during the study were as follows: temperatures from 26.40 to 27.45 ºC, pH from 6.90 to 7.23, DO from 4.59 to 6.10 mg $l^{-1}$ and $NH_3$ from 0.15 to 0.23 mg $l^{-1}$. Temperature, pH and DO were measured using Watercheckker U10 Horiba, while $NH_3$ was determined by Spectofotometer with Spec-Nessler method.

### Experimental Procedures

The experimental design used was Completely Randomized Design with four different treatments (A, B, C, and D) and each treatment was repeated three times. For treatment-A, B and C, the *Oodev*dose was given to the snakehead broodstocks with the same amount of 0.5 ml $kg^{-1}$ fish weight but vary with frequency and time period (day). For instance, the treatment-**A**: 3 times/9 days, **B**: 2 times/6 days, **C**: 1 time/3 days, and **D**: no oodev injection (control) and later to be compared to other treatments. The details of experimental treatment are given in Table 2. At the end of experiments, a total of ten snakehead samples were dissected to find out the gonad maturity level of fish, and reproductive indicators such as egg diameter, fecundity, Somatic Gonad Index (SGI) and Hepato Somatic Index (HSI) were measured. The egg diameter was measured using a micrometer. The fecundity, SGI and HSI were calculated using the following formulas:

$$\mathbf{F = n \times G / g} \qquad (1)$$

F is fecundity; n is the average number of eggs in sub-sample; G is gonad weight (g); and g is the sub-sample weight (g).

$$\mathbf{GSI = Gw / Bw \times 100\%} \qquad (2)$$

GSI is Gonado Somatic Index; Gw is Gonad weight (g); and Bw is Body weight of fish (g)

$$\mathbf{HIS = Lw / Bw \times 100\%} \qquad (3)$$

HSI is Hepato Somatic Index; Lw is Liver weight (g); and Bw is Body weight of fish (g)

### Statistical Analysis

At the beginning, the normality and homogeneity of experimental data obtained were analysed using Lilliefors test and Bartlett test, respectively. Data transformation should be first done if data were found not normal or not homogeneous. If the assumption was fulfilled, then apply for the Analysis of Variants (ANOVA). The Mean differences test was used if there were significantly

differences among the treatments. All tests were analysed at the 0.05 level of significance using SPSS-16 software.

## III. RESULTSAND DISCUSSION

The mean ± standar deviation of fecundity, egg diameter, somatic gonad index (SGI) and hepatosomatic index (HSI) obtained from experiments was presented in Table 3. It is clearly shown that among different injection frequency, the treatment-A was determined as the best performance in term of generating fecundity, egg diameter and SGI rate i.e. 19600 ± 450.00 granules/individuals, 1.10 ± 0.15 mm and 3.41 ± 0.90 % respectively. It was followed by treatment-B with the respective values of 7300 ± 556.77 granules/individuals, 0.90 ± 0.11 mm and 1.39 ± 0.14 %. Meanwhile, the treatment-C showed better performance as compared to treatment-D (the control) in term of fecunditythat is 1367 ± 2367.13 granules/individuals. With regard to the egg production, the mean fecundity obtained ranging from 1033 ± 1789.79 to 19600 ± 450.00 granules/individuals. There were significantly differences in the mean fecundity among the treatments ($p<0.05$). The mean fecundity of treatment-A was considerably higher than other treatments ($p<0.01$). The treatment-B was significantly higher than treatment-C ($p<0.05$); whereas treatment-C and D was not significant different ($p>0.05$).

There were significantly differences in the mean egg diameter among the treatments ($p<0.05$). The mean egg diameter was ranged from 0.20 ± 0.40 mm to 1.10 ± 0.15 mm. The maximum size of egg diameter for injected fish in the treatment-A or treatment-B was found larger than treatment-D without injection in the control group ($p<0.05$), indicating that Oodev had positive effect on the egg maturation of snakehead boodstock. No significant difference was observed in the egg diameter between treatment-C and D ($p>0.05$).

Overall, the result also clearly demonstated that the treatment-A yielded the highest GSI rate among other treatments ($p<0.01$). The treatment-B was considerably higher than treatment-D ($p<0.05$), but not significant different from treatment C ($p>0.05$). There was no significantly difference in the SGI rate between treatment-C and D ($p>0.05$). The mean SGI rate was ranged from 0.40 ± 0.62 % to 3.41 ± 0.90 mm. The GSI rate gradually increases with increased frequency and time given. The other way the HSI rate decreases with increased frequency and time. The treatment-C showed higher HSI rate as compared to treatment-A and D ($p<0.05$), but not differ from treatment-B ($p>0.05$) was observed. No statistical difference in the mean HSI rate was found between treatment-A, B and D ($p>0.05$). The mean HSI rate was varied from 0.89 ± 0.03 % to 1.53 ± 0.23 %.

Table 4 shows the mean ± standar deviation of the three estimated weights i.e. body weight, liver weight and gonad weight, as well as the ratios of these parameters for each treatment. The body weight and the liver weight were ranged from 253.67 ± 21.33 to 266.80 ± 727.78 g and from 2.43 ± 0.61 to 3.68 ± 0.45 g respectively. The ratio of liver weight to body weight obtained from the treatment-Cwas considerably higher than that of treatment-A and D ($p<0.05$), but not differ from treatment-B ($p>0.05$) was detected. There were no statistically significant difference in those ratios between treatment-A, B and D ($p>0.05$). The ratio of liver weight to body weight was varied from 0.0091 to 0.0143. The results also clearly revealed that the treatment-A yielded the highest gonad weight (9.20 ± 2.50 g) among other treatments ($p<0.05$). The ratio of gonad weight to body weight was ranged from 0.0050 to 0.0345. Furthermore, the highest ratio of fecundity to body weight was 73.44 (treatment-A), while the lowest ratio was 3.88 (treatment-D). It was similarly demonstrated, the highest ratio of fecundity to body lenght was 62.88 (treatment-A), while the lowest ratio was 3.32 (treatment-D). Such relationships between fecundity and body weight as well as between fecundity and body length can be seen in Figure 1.

The appropriate hormone preparation should be selected on the basis of the species to be spawned and the availability of the hormones. Many factors which have impact on ability of induced spawning, include: 1) condition of the fish, 2) stage of sexual maturity, 3) size of the fish, 4) previous spawning history, 5) water temperature, 6) season of the year and 7) dosage of hormone use (Rottmann *et al*., 1991). Hormones for induced spawning have been widely used in air-breathing fishes such as human chorionic gonadotropin (HCG) (Mollah and Tan, 1983; Inyang and Hettiarachchi, 1994), luteinizing hormone releasing hormone analogue (LH-RHa) (Fermin, 1992), and ovaprim (Alok *et al*., 1993; Haniffa *et al*., 1996). Oodev is one solution when fish can not perform vitellogenesis and spermatogenesis.

The oodev hormonal treatments have been successfully used for advancing the process of gonad rematuration period in *A. testudineus* for 12-24 days (Sari, 2015). Furthermore, Agustinus (2016) reported that *P. hypophthalmus* being injected with oodev 0.7 ml $kg^{-1}$ fish weight every 7 days for 4 weeks showed the best performance in term of growth rate and egg diameter and also can accelerate gonad rematuration of them for 28 days after spawning. Fitriatin *et al*. (2018) affirmed that oodev hormone dose of 1 ml $kg^{-1}$ fish weight was effective for gonad rematuration of *O. hasselti* broodstock within 17 days with the GSI rate of 14.48 %, average egg diameter of 1.035 mm and body weight of 31.25 mm. For the time being, the effectiveness of oodev works in snakehead is still questionable due to lack of available evidence. Dealing with the presence of oodev itself, it is commonly

known that oodev hormone is a combination of Pregnant Mare's Serum Gonadotropin (PMSG) hormone and Antidopamine (AD) compounds. PMSG hormone contains follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Moore and Ward, 1980). The role of FSH increases the chances of egg cell maturation, while the role of LH stimulates ovulation. PMSG chemical structure is similar to the FSH and LH, whereas FSH structure is greater than LH (Reeves, 1987). The PMSG hormone in oodev is thought to have triggered the gonad maturation of brood fish, where vitelogenesis only takes place when a hormone is present associated with this process. The gonadotropin hormone of PMSG injected from the outside will work on the egg by maturing the existing follicle by stimulating the growth of interstitial cells and the formation of luteal cells. The process of gonadal rematuration starts from the synthesis of vitelogenin which is the precursor of egg yolks (Wiegand, 1982). One of the conditions governing vitelogenesis is the availability of hormones associated with vitelogenesis in the body. It is known that there is a positive correlation between gonadal steroid hormone and vitelogenesis (Mackenzie *et al.* 1998)

It was clear from our findings that snakehead successively induced with 0.5 ml $kg^{-1}$ fish weight for three-frequency and time periods in the treatment-A produced more eggs about 3 times higher than twice, or about 14 times higher than once or about 19 times higher than with no injection. It was significantly different from other treatments ($p<0.05$). The fecundity of fish varied according to the size (length-weight), age, species, food availability and season. Larger fish tends to have greater fecundity than small fish. For a given size, females in better condition exhibit higher fecundity (Kjesbu *et al*., 1991). The relationship between the age of brood female and the size of the egg is the square. The young mother spawning for the first time produces small eggs (about 0.8 mm diameter); the productive females will produce large eggs (about 1 mm) and old females again producing small eggs (about 0.8 mm). A relationship between length, weight, and fecundity for the family Channidae was well-documented in *C. gachua* (Gaikwad *et al*., 2009; Widodo *et al*., 2013), *C. striatus* (Islam *et al*., 2013; Sakhare, 2015), and *C. Limbata* (Khomsab and Wannasri, 2017). Figure 1 clearly shows that the ratio of fecundity to body weight as well as the ratio of fecundity to body length increases proportionally to the time periods of oodev injections given. The longer time period of oodev injections, the higher the ratio of fecundity to body sizes. It also implies that the larger fish the higher ratio of fecundity to body sizes. Such relationships were expressed in the following equations i.e. $y = 3.578\ x^{2.0343}$ and $y = 3.064\ x^{2.0343}$ with the coefisien of determination ($R^2$) was 0.9748, suggesting that approximately 97% of the variation in the fecundity data could be explained by the body weight of the fish. In practical, when researchers endeavor to evaluate the commercial potential of fish stocks, the fecundity estimation must consider a variety of attributes, including size at first sexual maturity, duration of spawning season, daily spawning behavior and spawning fraction (El-Drawany, 2013).

Snakehead fish brood injected with oodev 0.5 ml $kg^{-1}$ fish weight in the treatment-A showing the biggest egg diameter and was significantly different from other treatments ($p<0.05$). However, the average egg diameter of *C. striata* in the present study (1.10 mm) was smaller than *C. striatus* (1.53 mm) in Malaysia and India (Ghaedi *et al*., 2013; Sakhare, 2015.) or *Ophiocephalus striatus* (1.60 mm) in Mekong River, Vietnam (Long *et al*., 2002). The vitelogenesis process can be seen from the value of the enlarged egg diameter. It is the process of vitelogenin synthesis in the liver which is the main precursor of yolk, resulted in the egg diameter increases. The diameter of the eggs increases as the weight of the gonads increases. The sexual maturity in fish is characterized by the development of egg diameter and through the distribution of its egg size (Kuo *et al*., 1974). The egg size can be expressed in many ways. Single diameter is commonly used, but the longest diameter (i.e. egg length and egg width) is also occasionally used. Other egg sizes include the volume of eggs, wet weight and dry weight. From an energetic standpoint, the best term for egg size is the equality of egg calorie (energy content per egg or joule per egg), because it shows the energy available to the developing embryo (Ginzburg, 1972). The large egg size is a guarantee of higher survival (Kamler, 1992), this because the egg contains food reserve to be used by fish larvae for survives. Larvae derived from the large eggwill have more egg yolk reserves as energy source before getting food from outside. The size of the egg diameter can determine the quality of the yolk content. The egg diameter is the accumulation of the vitelogenesis process that is the the absorption of vitelogenin which is going to prospective yolk. Increasing the yolk granules in the number and size resulted in the oocyte volume will begreater until the maximum size then the egg is in "dormant" phase. Egg quality is affected by both internal factors (e.g. brood age, brood size and genetic) and external factors (e.g. feed, temperature, light, density, and population). The first spawning female fish produce small diameter eggs. The egg diameter increases clearly when the second spawning and the rate of increase are slower in subsequent spawning

The results also confirmed that snakehead consecutively injected with oodev 0.5 ml $kg^{-1}$ in the treatment-A increasing the GSI rate about 2.5 times higher than treatment-B, or about 8.5 times higher than treatment-C or

about 7 times higher than with no injection in treatment-D. It is clearly revealed that the GSI increases proportionally towards the time periods of oodev interventions. Also, an increase of GSI value is in conjuction with the increased doses of Oodev hormone given (Fitriatin *et al*., 2018). It is alleged that FSH and LH activity in oodev hormone influences the gonad development as a whole (Moore and Ward, 1980). The GSI of family Channidae varies according the type of species (Gaikwad *et al*., 2009; Kapil *et al*., 2011; Widodo *et al*., 2013; Tiwari *et al*., 2014). For example, the GSI of *C. striata* (3.41 %) in the present study was lower than GSI of *C. gachua* (6.00 %) in Godavari River, India (Gaikwad *et al*., 2009) or *C. marulius* (47.56 %) in Son River Shahdol, India (Tiwari *et al*., 2014), or *C. striatus* (8.00 %) in Badin Sindh District, Pakistan (Narejo *et al*., 2015). The IGS value is used to predict when the fish will be ready for spawning. Increased IGS values can be triggered by oocytes, while vitelogenin is main precursor of yolk which is the main component of the growing oocyte. When the process of vitelogenesis is continuing, the yolk granules increase in number and size, so that the volume of the oocyte enlarges and eventually will lead to increased value of GSI. The process of vitelogenin formation starts from the presence of environmental factor cues such as fotoperiode, temperature, eating activity, and other factors that will all stimulate the hypothalamus to secrete *gonadotropin releasing hormone* (GnRH). GnRH will be secreted into the blood stream will stimulate hypophysis to secrete gonadotrop in hormones (Darwisito *et al*., 2006).

In the reproductive cycle, GSI increases with the maturation process, whereas the HSI in contradicting to GSI (Lodeiros *et al*., 2001). It was clear from our findings that the treatment-A produced the highest GSI among other treatments (3.41 %), contrariwise it provided the lowest HSI among other treatments (0.89 %) in the same amount of oodev content. In other words, the longer time period of oodev injection given, the lower the ratio of liver weight to body weight gained. This is attributable to the use of energy reserves derived from the liver instead of energy sourced from the body. The HIS of *C. striata* (1.53 %) in the present study was lower than HSI of *C. punctatus* (1.64 %) in Syilhet, Bangladesh (Hossain 2013), but slightly higher than *C. striatus* (1.40 %) in Penang, Malaysia (Ghaedi *et al*., 2013). In addition, Bijaksana (2006) argued that most of the snakehead caught during fishing season is allocating their somatic growth for reproductive growth. In line with this; temperature, pH and DO during sampling period are considered to be comfortable for snakehead fish broods.

## IV. CONCLUSION

Oodev hormone had a significant effect in speeding up the process of gonadal rematuration in the snakehead injected. Oodev dose of 0.5 ml $kg^{-1}$ those given in 3 times/9 days (treatment-A) is the most effective treatment to rematurate the gonad of snakehead female broodstock within 30 days, producing fecundity of 19,600 granules/individuals, egg diameter of 1.10 mm, and GSI rate of 3.41 %. The results may applicable when outdoor environmental quality isimproved. Despite these findings, the outdoor environmental quality should be considered into account. Some of the information presented here may be applicable in other geographical areas.

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*Table.1: Tools, intruments and materials used in snakehead gonad rematuration experiment*

| No. | Tools and Materials | Size/Merk | Utility |
|---|---|---|---|
| 1. | Concrete pond | 4×7×1.2 m | Placement of hapa |
| 2. | Small hapa 12 units | 0.4×0.3×1m | Placement of fish brood tested |
| 3. | Large hapa 2 units | 1.5×1.5 m | For brood fish reserves |
| 4. | Watercheckker U10 | Horiba | Measurement of water quality |
| 5. | Scoop net | 30 cm | For collecting the fish samples |
| 6. | Name label | 15×10 cm | Mark offhapa |
| 7. | Digital balance | ACS | Weighing of fish samples |
| 8. | Ruler | 30 cm | Measurement of body length |
| 9. | Washbasin | ∅ 50 cm | Container for brood fish |
| 10. | Knife | 10 cm | Dissection ofthe selected brood fish |
| 11. | Rope | 10 m | Hapa installation |
| 12. | Injection | 1 ml | Oodev injection |
| 13. | Snakehead brood | 48 fish | Experimental fish sample |
| 14. | Frogs | 4% of body weight/day | Feed/bait served for snakehead |
| 15. | Oodev hormone | 0.5 ml $kg^{-1}$ fish weight | Dose of oodevto be injected to snakehead fish brood samples. |

*Table.2: The experimental treatments for investigating the snakehead gonad rematuration*

| Treatment | Oodev dose/fish weight | Frequency | Time period |
|---|---|---|---|
| A | 0.5 ml $kg^{-1}$ | 3 times | Day-3, day-6, day-9 |
| B | 0.5 ml $kg^{-1}$ | 2 times | Day-3, day-6 |
| C | 0.5 ml $kg^{-1}$ | 1 time | Day-3 |
| D | No injection | 0 time | Control |

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*Table.3: The mean ± standar deviation of the parameters observed for each treatment.*

| Treatment | Parameters observed | | | |
|---|---|---|---|---|
| | Fecundity (granule/individual) | Egg diameter (mm) | GSI (%) | HSI (%) |
| A | 19600 ± 450.00 ** | 1.10 ± 0.15 * | 3.41 ± 0.90 ** | 0.89 ± 0.03 |
| B | 7300 ± 556.77 * | 0.90 ± 0.11 * | 1.39 ± 0.14 * | 1.11 ± 0.03 |
| C | 1367 ± 2367.13 | 0.20 ± 0.40 | 0.40 ± 0.62 | 1.53 ± 0.23 * |
| D | 1033 ± 1789,79 | 0.20 ± 0.40 | 0.50 ± 0.54 | 0.93 ± 0.09 |

Significance level: ** ($p<0.01$), and * ($p<0.05$)

*Tabel.4: The mean ± standar deviation of the estimated weights and their ratios for each treatment. A = three time injections, B = two time injections, C = once injection, and D = no injection (control).*

| The estimated weight, and the ratio | Treatment | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Body weight (g) | 266.8 ± 727.78 | 253.67 ± 21.33 | 258.03 ± 10.13 | 266.57 ± 11.60 |
| Liver weight (g) | 2.43 ± 0.61 | 2.84 ± 0.38 | 3.68 ± 0.45 | 2.48 ± 0.16 |
| Gonad weight (g) | 9.20 ± 2.50* | 3.83 ± 0.31 | 2.10 ± 1.49 | 1.33 ± 1.53 |
| Ratio of liver weight to body weight | 0.0091 | 0.0112 | 0.0143* | 0.0093 |
| Ratio of gonad weight to body weight | 0.0345* | 0.0151 | 0.0081 | 0.0050 |

Significance level: ** ($p<0.01$), and * ($p<0.05$)

*Table 5: Comparative fecundity, egg diameter, GSI and HIS of family Channidae from different geographical areas*

| Species | Fecundity (granule/ind) | Egg diameter (mm) | GSI % | HSI % | Locations | Country | References |
|---|---|---|---|---|---|---|---|
| *Channa striata* | 1,033 - 19,600 | 0.20 - 1.10 | 0.40 - 3.41 | 0.89 - 1.53 | Sungai Batang | Indonesia | Present study |
| *C. striata* | - | 1.00 - 1.60 | - | - | Danau Panggang | Indonesia | Fitriliyani, 2005 |
| *C. striatus* | 28,332 - 41,068 | 1.21 - 1.33 | 10.4 - 11.5 | 0.70 - 1.40 | Penang | Malaysia | Ghaedi *et al*., 2013 |
| *C. striatus* | 3,000 - 12,000 | 0.70 - 1.30 | 2.10 - 8.00 | - | Badin Sindh | Pakistan | Narejo *et al*., 2015 |
| *C. striatus* | 4,900 - 14,028 | 1.53 | - | - | Beed District | India | Sakhare, 2015 |
| *C. punctatus* | 2,538 - 32,987 | - | 0.53 - 5.34 | - | Syilhet ditches | Bangladesh | Mian *et al*., 2017 |
| *C. punctatus* | 2,116 - 11,332 | - | - | - | Pond Ilanthakulan | India | Marimuthu and Haniffa, 2006 |
| *C. punctatus* | 3,678 - 27,853 | - | - | - | Varuna River | India | Lalta *et al*. 2011 |
| *C. punctatus* | 2,654 - 26,294 | - | 0.19 - 5.64 | 0.84 - 1.64 | Syilhet | Bangladesh | Hossain, 2013 |
| *C. gachua* | - | - | 5.57 - 6.00 | - | Godavari River | India | Gaikwad *et al*., 2009 |
| *C. gachua* | 2,539 - 7,194 | - | - | - | Bhubaneswar | India | Mishra, 1991 |
| *C. gachua* | - | - | 5.31 - 5.63 | - | Dinoyo District | Indonesia | Widodo *et al*., 2013 |
| *C. limbata* | 956 - 4,652 | - | 1.96 - 3.74 | - | Ta Bo - HuaiYai | Thailand | Khomsab and Wannasri, 2017 |
| *C. marulius* | - | - | 8.21 - 47.56 | - | Son River | India | Tiwari *et al*., 2014. |
| *Ophiocephalus striatus* | - | 0.20 - 1.60 | - | - | Mekong River | Vietnam | Long *et al*., 2002 |

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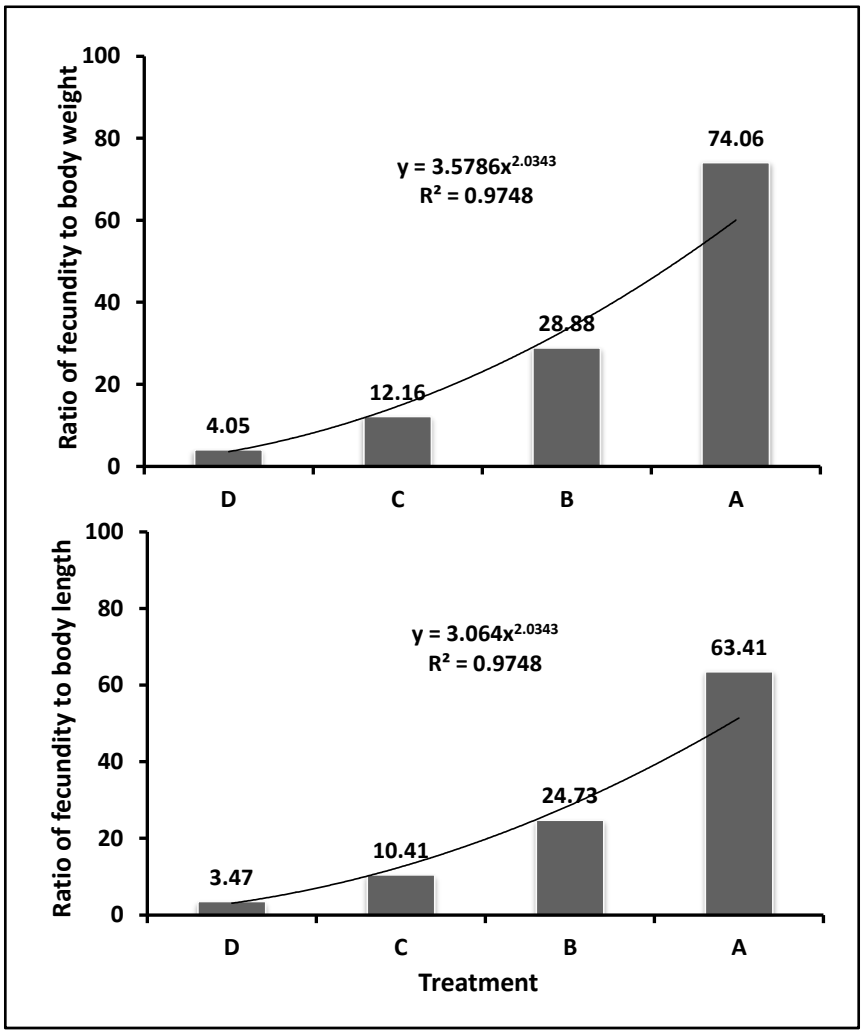


*Fig. 1: The ratio of fecundity to body weight (top) and the ratio of fecundity to body length (bottom) increase proportionally to the time periods of oodev intervention at the same amount of 0.5 ml $kg^{-1}$ fish weight.*
*The treatment-A showed the highest ratios among other treatments ($p<0.05$).*
*A = three time injections, B = two time injections, C = once injection, and D = no injection (control).*

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# The Challenge of Change: A Case of Introduction of Genetically Modified Cotton in the Kingdom of Eswatini

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***Abstract***— *In Eswatini cotton contributes merely 2.1 % of the country's Gross Domestic Product owing to viability challenges.Farmers grow hybrid cottonwhich is now in the decline stage of its life cycle, no longer profitable andcausing persistent challenges to farmers.Other cotton growing countries like United States of America, Canada, Australia and South Africahave replacedhybrid cotton with the more profitable genetically modified cotton. This strategy can be a viable alternative for the Eswatini cotton industry too. The study identified perceptions that Eswatini cotton industry stakeholders have towards genetically modified cotton. In-depth interviews were held with 8 informants selected based on their experience and knowledge about the cotton industry.The study revealed that 2/3 of cotton farmers suspended growing cotton owing to viability problems.Although genetically modified cotton has higher input costs these were easily offset by higher yields and less use of pesticides and labour. The study indicated that farmers required additional capacity to be able to grow genetically modified cotton. The study recommended that industry stakeholders must adapt to change and embrace genetically modified cotton which was successfully implemented in other countries. Liberalisation of the cotton industry was also recommended to pave way for the farmers to try the new product. Training of farmers was recommended as a strategy of capacitating the farmers on how to manage genetically modified cotton and challenges associated with the new technology. Further research is recommended about the modalities of optimising the benefits of genetically modified cotton and how cotton farmers can be supported.*

***Keyword—Adaptable to change; Eswatini cotton industry; Genetically modified cotton; New product development; Product life cycle.***

## I. INTRODUCTION

This paper is about introducing genetically modified cotton in the Kingdom of Eswatini. Discussions in the paper are guided by management processes of introducing a new product or new technology in a market.In the Kingdom of Eswatini, agriculture plays a major role in the economy; it'sa major source of food, and also employs more than 60% of the country's population (Thomson, 2012; ISAAA, 2014). Eswatini's agriculture is mainly dependent on sugar cane, cotton and forestry.Cotton is the second biggest cash crop after sugarcane in Eswatini. It is an important cash crop for most Swazis who live in drought prone areasand smallholder farmers who are reliant on the crop for their livelihood (Central Bank of Swaziland, 2013). Eswatini farmers are still entirely reliant on conventional hybrid cotton seeds. Hybrid cotton seeds have long been used in the industry as the sole means for cotton production. Genetically modified cotton is a variety of cotton that has been modified through a biotechnological process in order to achieve a higher yield. Bollworm resistant, *Bacillus Thuringiensis* (BT) cotton is the most popular genetically modified cotton seed used throughout the world. Genetically modified cotton was first introduced in the early 1990s and has since been adopted by major cotton producing countries such as the USA, India, China and South Africa (James, 2011). Genetically modified cotton seeds are engineered via a biotechnological process to reproduce the soil bacterium *Bacillus Thuringiensis* in a crystal form in order to exterminate certain types of insects and pests which damage the cotton crop and reduce farmer's yields (Craig *et al.*, 2008). The new genetically modified seed has outstripped its traditional hybrid counterparts in terms of yield (Brookes & Barfoot, 2013).

The Eswatini cotton industry is currently facing a decline in production and this has affected the textile industries which relied on Eswatini cotton as their main source of inputs. Most textile industries have closed due to the shortage of cotton. The few textile factories that are operational survive through importing cotton to supplement locally depressed supplies for the daily operations. The government of Eswatini has to revive the cotton industry by introducing a new product in the market. The purpose of this paper is to investigate the

costs and benefits of changing from hybrid cotton to genetically modified cotton in Eswatini. The paper will evaluate business opportunities, capacity requirement, economic benefits and cost associated with adopting genetically modified cotton in the Kingdom of Eswatini.

The cotton industry in Eswatini is currently facing many challenges. The country's largest cotton ginnery which is under the stewardship of the Swaziland Cotton Board (SCB) and located at Big Bend, has a capacity to handle 25 000metric tons of cotton. Currently, a mere 10% of the ginnery's capacity is being utilised owing to unavailability of inputs and decreased cotton production, among other reasons (Mavuso, 2014). The cotton industry is solely dependent on conventional hybrid cotton seeds. All cotton farmers have been using hybrid cotton seed for the past two decades (Cotton Board, 2014). However, the hybrid cotton seed has reached the decline phase of its life cycle which is characterised by a rapid decrease in the yields and it is no longer profitable for farmers to grow cotton. The decrease in cotton production threatens the 90 ginnery employees' jobs at the Big Bend ginnery (Cotton Board, 2014).

Hybrid cotton that is currently grown by Eswatini farmers is no longer producing high yield as it used to do in the past years. The product has reached a decline phase which is characterized by high production cost, low yields, and heavy pesticides application requirements. From a management point of view a product in decline phase needs to be phased out and replaced because it will be fool hardy to rejuvenate the product (Kotler, 2012). Cotton acreage has drastically reduced from 30,000 hectares to merely 3000 hectares (Cotton Board, 2013). Correspondingly, the number of cotton farmers in Eswatini has also decreased from 9000 to 3000 in the past 6 years (Cotton Board, 2013). The sector's capacity to create employment directly, in cotton farms and indirectly,in the textile industry, and ginning, spinning, and weaving of fabric has gone downdrastically. This has been aggravated by labour migration from rural areas to the cities (Thomson, 2012). The country has to find strategies of filling the demand gaps created by dwindling cotton production over the years and cheaper technology to continue producing enough cotton to meet increasing demand. Opportunities that are not utilised when they arise will always be taken up by one's competitors (Bimha & Bimha, 2018). Therefore, it is the researchers' conviction that the introduction of genetically modified cotton seed is one of the viable options to tackle the cotton industry's prevailing challenges. There is an urgent need to ascertain stakeholder willingness, capacity requirements and readiness to adopt genetically modified cotton technologyto replace hybrid cotton seed which has passed the maturity phase and is no longer economically viable. In the public domain, no research has been carried out to ascertain the costs and economic benefits of introducing genetically modified cotton in the Kingdom of Eswatini.

The paper aims to investigate the costs and benefits of phasing out hybrid cotton with introducing genetically modified cotton in the Kingdom of Eswatini. The specific objectives of the study were to:

- Identify stakeholders' perceptions towards growing genetically modified cotton in place of Hybrid cotton in Eswatini.
- Identify challenges associated with the production of genetically modified cotton in Eswatini.
- Provide recommendations on how stakeholders in Eswatini can adopt and implement genetically modified cotton production

Based on the above objectives the research was designed to address the following questions:

- What are the perceptions of cotton industry stakeholders towards adopting genetically modified cotton in the Kingdom of Eswatini?
- What are the benefits of growing genetically modified cotton?
- What are the challenges of growing genetically modified cotton?
- What suggestions could be made to an industry that is considering the adoption of genetically modified cotton technology?

In other developing countries that have already adopted genetically modified seed as alternative technology agriculture contributesup to 11.9 percent to Gross Domestic Product (GDP) of those countries (Central Bank of Swaziland, 2014). Based on the fact that in Eswatini cotton contributes merely 2.1 percent of GDP, any research which seeks to improve cotton production methods may be of value to many stakeholders, including the Swazi farmers, Swazi textile manufacturers, cotton seed crushing companies and the Eswatini economy in general. The findings from this research can assist in generating new information for the farmersto appreciate the potential benefits and probable costs associated with producing genetically modified cotton. The study is also significant since it intends to investigate the capacity requirements and challenges associated with producing genetically modified cotton. In this regard, policy makers can use the research outcomes to plan the adoption of genetically modified cotton production.

In Eswatini, there is no research available in the public domain which discusses genetically modified cotton

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as a concept, cost, benefits, capacity requirements and challenges associated with producing genetically modified cotton. This will be the first of its kind and it is hoped that the study will trigger progressive debate on the growing of genetically modified cotton. It is the researchers' conviction that, with the cotton industry in Eswatini facing a crisis, genetically modified cotton production may be the rational way to resuscitate the ailing industry. Therefore, this study will make an original contribution to the cotton industry, and turning around people's livelihoods.

The following section will critically analyse the literature related to cotton industry stakeholders' perceptions about change and the management processes of introducing a new product in the market. To replace an ailing product it is necessary to evaluate the product's life cycle andto understand the requirements for new product development.

## II. LITERATURE REVIEW

### 2.1 Perceptions towards change and implementing genetically modified technology

Farmers in Eswatini are used to growing hybrid cotton. To them,the introduction of genetically modified cotton constitutes introduction of change or a new product. From a management point of view, people generally resist change (Burnes, 1992). Change management researchers have identified the following factors being quoted as reasons why people resist different forms of change (Burnes,1992; Thuis&Stuive,2012; Brevis&Vrba,2014):

1. Habit-people are not happy to change from something they are used to
2. Security- people feel secure in a situation they know and find moving to a different situation to be threatening
3. Economic-fear of losing income
4. Fear of the unknown-people take freight at any change if they do not know what the change brings about
5. Lack of awareness-people resist things that are introduced without prior notice
6. Social factors- people are afraid of what others will think or say

Because people's perceptions will influence the decisions they will make the introduction of new technology has to be done with caution, taking into account the above issues. Strategies must be in place to deal with any form of resistance to be faced. Generally, this should include effective communication among stakeholders, participation and involvement, facilitation and support, negotiation and consent, and manipulation and cooperation (Brevis&Vrba,2014). Additionally reasons for the change must be explained to the affected. Some of the reasons given for redesigning or changing technology include:

1. Economic-when there is low demand for the old product and it is costly to produce. Brown, Bessant and Lemming (2013) suggest that, if costs are to be driven down then new ways of doing things are required.
2. Social and demographic-there is a lot of migration from rural areas to urban areas. The moves leave aging farmers with less labour hence the need for less labour intensive technology (Mavuso, 2014).
3. Political and legal- The stance that Government takes about the new technology must be understood by those affected so that they strengthen lobbying against policies that are unfavourable. In Eswatini, the Biosafety Act of 2012 is seen as a major hindrance to the procurement and use of genetically modified cotton seeds (Mayet, 2012).
4. Costs or availability of raw materials, components, labourand other inputs. Increase in costs directly or indirectly affects the cost of doing business hence they have an impact of the company's bottom line (Stevenson, 2012; Coyle et al., 2017).

### 2.2 The Concept of Product life cycle

Product life cycle concept describes how a product goes through the four phases of introduction, growth, maturity and decline from the time a product is launched till it is phased out of the market (Palffy, 2015). To evaluate the potential of a new product in the market, organizations must review the sales performance of the product at each stage of the life cycle (Kotler& Keller, 2012; Palffy, 2015). Therefore,the stage where a product is in its life cycle is associated with its performance and profitability.Each life cycle stage requires a different mix of marketing strategies (product strategies, pricing strategies, promotion strategies and distribution strategies) to maximize the lifetime profitability of the product. A product can be phased out when its sales stall and continue to fall. Additionally when old products are no longer grabbing new market share, management should consider launching new product to continue generating revenue for the entrepreneurs (Brown,Bessant& Lemming, 2013). Figure 2.2 below illustrates the product life cycle.

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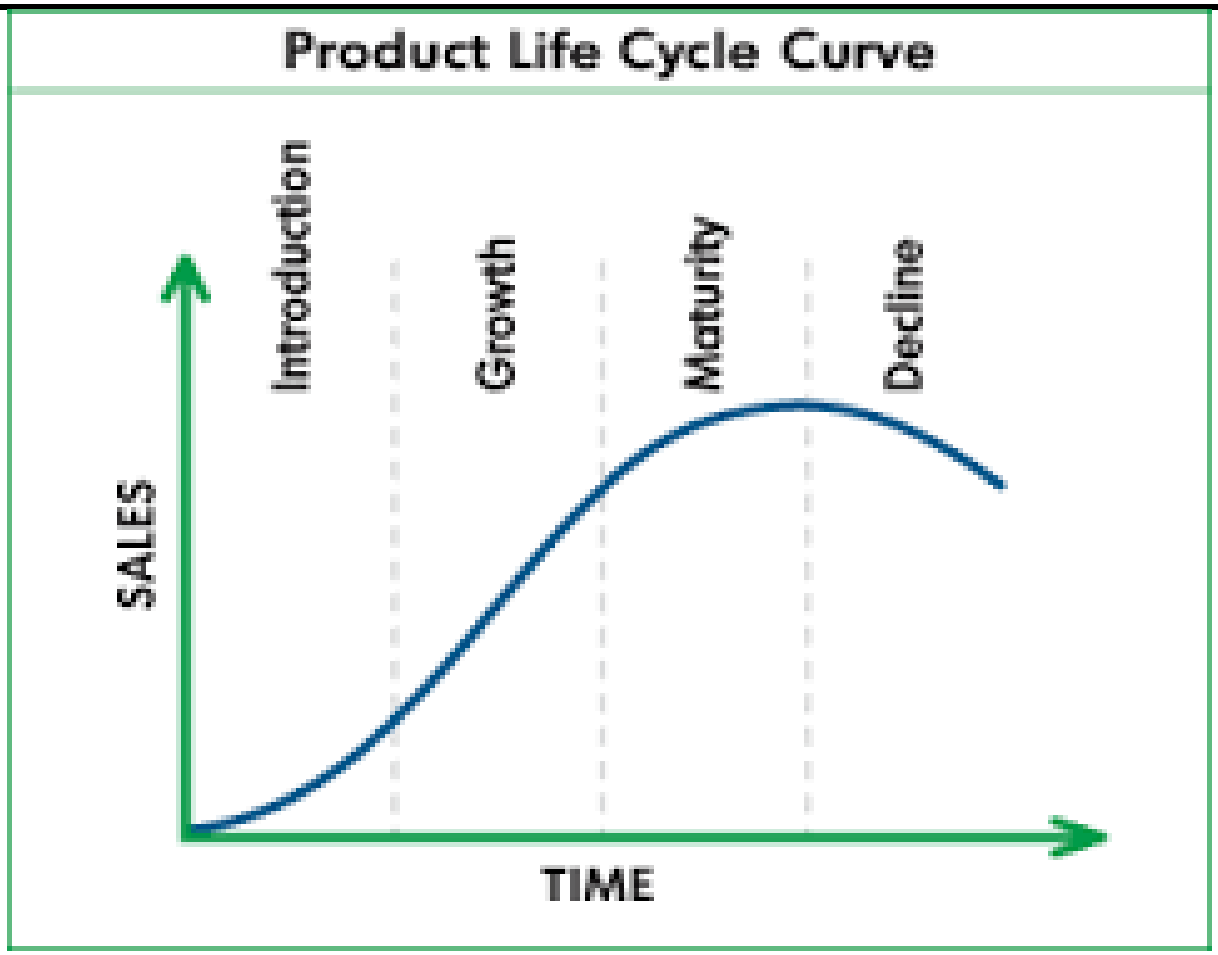


*Fig.2.2: Product Life cycle concept*

**Source: Kotler and Keller (2012)**

While the model does not predict sales, when used alongside carefully analysed sales figures and forecasts, it provides a useful guide to marketing strategies that may be most appropriate at a given time (Kotler& Keller, 2012). Therefore, it is novel and ideal to plan for the exit of a product because no product can survive for ever owing to reasons which includes increasing competition, changing customer tastes and priorities, changing production processes and technology (Brown, Bessant& Lemming,2013).

The cotton industry is currently striving on conventional cotton which is in its decline stage.(Cotton Board, 2014). Farmers are no longer interested in the product due to shortage of labour and high pesticides application requirements. The product has proven to be low yielding and labour intensive. There is a need for a new product in the market. Conventional cotton is at the decline stage of its life cycle, and the product is no longer profitable to the farmers. There is a need for the industry to introduce a new product to the market. This view is supported by Kotler and Keller (2012) who note that a company must have a different set of strategies at each product life cycle phase including phasing out old products which are no longer profitable.

**2.3 New product development concept**

Genetically modified cotton is a new product that will replace conventional cotton; an old product that has reached its decline phase. The product will be successful in the market if it undergoes known stages of product development. Product ideas are consummated and developed into ideas which then go through a screening process. Only those ideas with potential to survive the market go through all development stages like product prototype development,market testing and commercialisation (Kotler, 2012; Stevenson,2012; van Weele, 2018). The genetically modified cotton seed has gone through the rigorous new product development process and Eswatini does not need to repeat these processes again.vanWeele (2018) points out that all these stages should involve strategic planning to provide the necessary infrastructure for future technological collaboration with suppliers and operations management processes that relates to the management of individual developmental projects.

Because genetically modified cotton was consummated in the 1990s in the global market it has already gone through the new product development stages in the above model. Adopting the new technology for Eswatini is a question of introducing the technology already in use in other cotton growing countries such as India,South Africa and Brazil. However, Phipps and Park, (2002) recommended that to create genetically modified cotton seeds, scientists must adhere to 5 stages of genetic engineering:

- Identify the selected gene that requires modification,
- Apply the appropriate gene transfer technology,
- Achieve regeneration ability from tissues (or protoplasts and callus),
- Express the gene of the product at the desired level, and
- Reintegrate the gene in order for it to be carried via reproduction

To date, technology has facilitated the first four stages of genetic modification. These four stages of genetic engineering are critical to the transfer of the foreign gene into the cotton crop. Scientists employ various transfer strategies including, micro-injection, direct DNA absorption, bombardment of particles or the plasmid method (Stone, 2007). Cotton genotypes have proved to be unsusceptible to regeneration and this is considered as a barrier to the reuse of genetically modified seeds. Once sowed and grown and harvested, the cotton bolls produced by genetically modified seeds are indistinguishable from those cotton bolls produced by conventional cotton seeds (Quim, 2009).

**2.4 Benefits that can be derived from the production of genetically modified cotton**

The most important benefit of genetically modified cotton relates to the reduction in pesticide use and weed control using herbicides. Since genetically modified cotton is purposely created to be resistant to many types of worms and insects, cotton farmers find that there is no need to excessively spray different types of pesticides to protect their cotton crops (Morse &Mannion, 2009). The use of fewer pesticides is associated with lower cost of production. This can lead to lower risk exposure for the farmers owing to the handling of fewer harmful chemicals.Organisms in the soil are also preserved due to lower quantities of pesticide use and this ultimately results in better soil quality over time (Anderson, Valenzuela & Jackson, 2008).

In the United States of America (USA), the introduction of genetically modified cotton resulted in a reduction of pesticide consumption of up to 60%, in China, pesticide use decreased by up to 80% after the introduction of genetically modified cotton and in South Africa pesticide use decreased by 66% (Hossain*et al*. 2004). In addition, the reduction in pesticide production, distribution and use reduces environmental impacts of the harmful pesticides throughout the supply chain (Ali &Abdulai, 2010).

Morse and Mannion (2009) note that, cultivating genetically modified cotton instead of traditional cotton leads to increased yields. The increased yields are a result of better soil quality from lower pesticide use as well as the elimination of most crop destroying worms and insects such as Lepidoptera's specie (Ibid). The crop destroying worms are usually responsible for significant yield reduction. Therefore, with the introduction of genetically modified cotton, farmers are able to benefit from increased yields within the same acreage.In India, research determined that, genetically modified cotton produced a yield that was almost double to that of traditional cotton – on the first yield and the study reported increases in earnings of 60% after planting genetically modified cotton. In the same study, the farmers reported increases in earnings by up to 50-60% after planting genetically modified cotton (Bennet et. al., 2005).

Farmers who require fewer pesticides also require less labour since the frequency and application of pesticides decreases. The reduction in pesticide application also implies that farmers are able to lower the costs associated with the maintenance and running of pesticide application equipment. The reduction of pesticide application reduces soil compaction since large equipment is not rolled over the land as many times (Qaim&Zilberman, 2005).

According to Stone (2007), the cultivation of genetically modified cotton is also beneficial from an environmental perspective. Genetically modified cotton is regarded as the eco-friendly alternative to traditional cotton cultivation since it does not have adverse effect on parasites, predators, beneficial insecticides and organisms present in soil. The biodiversity of the cultivated area is preserved for a longer duration, thus reducing the costs associated with rehabilitating soil and the land in general.

Farmers in USA reported a $20 per hectare increase in net income and overall, the growing adoption of genetically modified cotton in USA was estimated to increase cotton income by $103 million in 2010, Chinese farmers reported an increase in income of approximately $350 to $500 per hectare and in South Africa, the return on investment in genetically modified cotton ranged from a $20 to $50 increase in net earnings per hectare (Morse&Mannion, 2009).

It is also important to note that genetically modified cotton cultivation requires less management and involvement. As such, farmers have more time available to

spend with their families as well as engage in other income generating activities (Bennet*et al*., 2005; Qaim&Zilberman, 2006; Morse & Mannion,2009). A similar study conducted by Qaim (2009), found that,cotton farming families were able to plan better financially, based on the dependability and resilience of the crop to yield predictable harvests.

**2.5 Costs associated with Genetically Modified Cotton Production**

Vitale *at al*., (2011) found that the initial costs of producing genetically modified cotton are usually more than the cost of producing traditional cotton. The additional cost is largely attributable to fees related to investment capital. The initial costs, however, are offset over time, due to increased yields and the savings realised through the use of less pesticides. Kambhampati*et al*., (2006) found that typical small to medium scale cotton farmers experience additional costs of modifying the seeds via biotechnology before it is planted. The costs associated with human labour were found to decrease with the use of genetically modified cotton production. This is attributable to the fact that genetically modified cotton requires a lower level of pesticide application, thus reducing the hours of human labour required (Qaim&Zilberman, 2005). Costs associated with the use of tractors increase by almost one third owing to a more technologically inclined approach to growing and harvesting genetically modified cotton, (Kambhampati*et al*., 2006).

Whilst the cost of fertilisers increases significantly (more than 45%), this cost is partially offset by the use of less pesticides (approximately one third less). There is a negligible change in the cost of irrigation, since, in most cases, traditional and genetically modified cotton were found to require similar volumes of irrigation in small to medium operations. This notion, however, is not replicated in larger operations – where it was noted that genetically modified cotton required significantly less volumes of irrigation as compared with traditional cotton irrigation requirements. Other operational costs also increased significantly (more than 250%). However, it is important to interpret this increase in a broader context, where it should be noted that other operational costs only make up a small component of the total costs. The increase in other operating costs could be due to the fact that genetically modified cotton requires more sophisticated equipment, and as a consequence, the associated costs may rise (Kambhampati*et al.*, 2006; Stone,2007).

Anderson, Valenzuela and Jackson, (2008) found that, besides the direct costs associated with the introduction of genetically modified cotton, there are also indirect costs borne by stakeholders along the production value chain. Although it is difficult to actually measure the indirect costs, there is conclusive evidence to support the assertions. Research findings byBennet*et al*., (2006), indicated that the small cotton farmers often stand to lose their holdings when genetically modified cotton is introduced. In the absence of sufficient government support, small scale farmers find it difficult to absorb the high initial costs of adopting the new genetically modified product and the additional capital requirements.

**2.6Capacity requirements of genetically modified cotton production**

Eswatini currently has a ginnery with a capacity of 25 000 metric tons and is currently operating at 10 percent. The introduction of the new product will increase yield and supply of cotton to the ginnery to meet the throughput. Farmers will require training to be able to manage the new product. Genetically modified cotton is different in that it requires planting of a refuge. The refuge harbour susceptible pest for future breeding of the cotton pest.This is required to minimise resistance on the future generation. There will be no additional equipment required for the new product except for training of farmers on management (Hererra-Estrella,2000).

**2.7 Potential challenges associated with the production of genetically modified cotton**

A cursory review of the majority of literature relating to the production of genetically modified cotton indicates that the advantages far outweigh the disadvantages. In fact, very few disadvantages and challenges have been documented with regards to the adoption of genetically modified cotton. In addition, the previous section has alluded to the fact that little to no additions are necessary to the production process once the genetically modified seeds have been procured.The literature relating to genetically modified cotton production indicate that the adoption and change-over process is relatively simple. Furthermore, numerous national research agencies (for example, in the USA, Australia, and China and in India) have concluded that genetically modified cotton should be promoted by governments and that grants and subsidies need to be provided to farmers, given the limited requirements for adoption (Morse &Mannion, 2009).

Despite the minimal challenges identified regarding the adoption of genetically modified cotton, Qaim and Zilberman, (2006) point to a very important consideration and potential challenge to farmers. First, it is important to note that, given the science of genome alteration, scientists have claimed that genetically modified seeds cannot be re-used. This has important implications for farmers, who are now unable to reuse seeds, like they did with the production of traditional cotton crops. Furthermore, with only a few international

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companies specialising in the production and distribution of genetically modified cotton seeds, local farmers are at the mercy of these organisations, should production decrease and prices increase. The other potential challenge is associated with the import of the genetically modified cotton seeds. The global economy is currently extremely volatile and brings with it many potential challenges, especially with regards to exchange rate, which will inevitably influence the costs of seeds and thus the cost of production for cotton farmers (Craig *et al*., 2008).

## III. RESEARCH METHODOLOGY

The phenomenological research philosophy guided the study. Phenomenologyentails the use of qualitative research approaches which endeavour to understand meanings as constructed by participants. It is more reflective of reality for research subjects' opinions and perceptions (Leary, 2011; Creswell, 2014; Smith, 2015; Maree,2016).In the study empirical data was used to understand contemporary phenomena; the introduction of genetically modified cotton) from the perspective of participants (Richey & Klein, 2014). The research therefore, uses the Swaziland cotton industry as a case in order to study the potential costs, benefits, capacity requirements and challenges associated with the introduction of genetically modified cotton.

Selecting the most appropriate research design is important since different designs yield different outcomes (Pickard, 2012). The studywas an exploratory research design. Exploratory research designs promote a broader research scope than other research designs, thus enabling the researcher to explore as many variables as possible (Creswell; 2014;Bryman et al., 2014). The purpose of exploratory research is to gain familiarity with a given phenomenon. More importantly, exploratory research is often conducted in business settings to explore the potential impacts of anticipated phenomena (Pickard, 2012). However,exploratory research findings may not be generalizable to the target population even though it enables the researcher to gain significant insight into the phenomenon being investigated (Leary, 2011).

The target population for this study includes all key informants in Eswatini's cotton value chain who were selected based on their knowledge and understanding of the current dynamics in Eswatini's cotton industry. The study relied of the informants' years of experience and product knowledge. These qualities can enable them to fairly and accurately assessthe potential costs, benefits, capacity requirements and challenges associated with the introduction of genetically modified cotton in the country. There are approximately 18 senior managers employed throughout the cotton value chain in Eswatini. Time and financial limitations prevented the researchers from conducting a census. Thus, only 8 of the 18 managers were purposively sampled and interviewed.

Purposive sampling is entirely guided by the researchers' judgement and ability to select participants whocan contribute to the study in a meaningful way (Maree,2016). Thus, the researcher should be knowledgable about the participants knowledge, capacity and ability to add value before approaching them(Creswell, 2014). The selection process targeted to have at least a representative from each sector in the cotton industry value chain.

Qualitative research employs a variety of research instruments for the collection of raw data. Observations, focus groups and personal interviews are among the more popular qualitative research instruments. Observations would not yield the desired raw data and focus group interviews were irrelevant because the targeted informants were of varied orientations and background. Therefore, the personal, face-to-face interviews with key informants were used as the research instrument (Leary, 2011). A semi-structured, face-to-face interview was used to collect qualitative data for analysis. Semi structured interviews can offer flexibility that allow the researcher to probe and follow-up questions based on the participants' responses.

During the recording key words were marked to be used in word and tree clouds graphical representation.Finally, the transcribed interviews were submitted to informants for verification, to ensure that what they had said during the interview was correctly understood and transcribed by the interviewer (Creswell, 2014).In this studyDedoose-version 6.2.21 Word cloud and Word tree was used to analyse the data. Word and tree clouds are graphical representations of words frequency that give greater prominence to words that appear more frequently in a source text. This allows themes to emerge from the responses of participants that may enable the researcher to answer the research questions. In order to add further value to the analysis identification of sub-themes under each major theme was conducted. Sub-themes assist the researcher in identifying the major variables that influence each major theme (Maree,2016 ).

In qualitative research, it is advisable to sample until saturation; researchers continue to look for information until they are satisfied that all information required has been collected (Creswell, 2014). However, due to time and financial constraints, thesearch for data was limited to 8 key informants. As such, the findings may not be as insightful as a larger sample could have achieved.Additionally, the concept of genetically modified cotton is fairly new, having been discovered in the 1990s. Therefore, there is limited literature about genetically modified cotton's costs, benefits, capacity requirements and challenges associated with introducing genetically

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modified cotton technology. Researchers are therefore forced to be heavily dependent on information and opinions of the sampled informants.

## IV. DISCUSSION OF FINDINGS

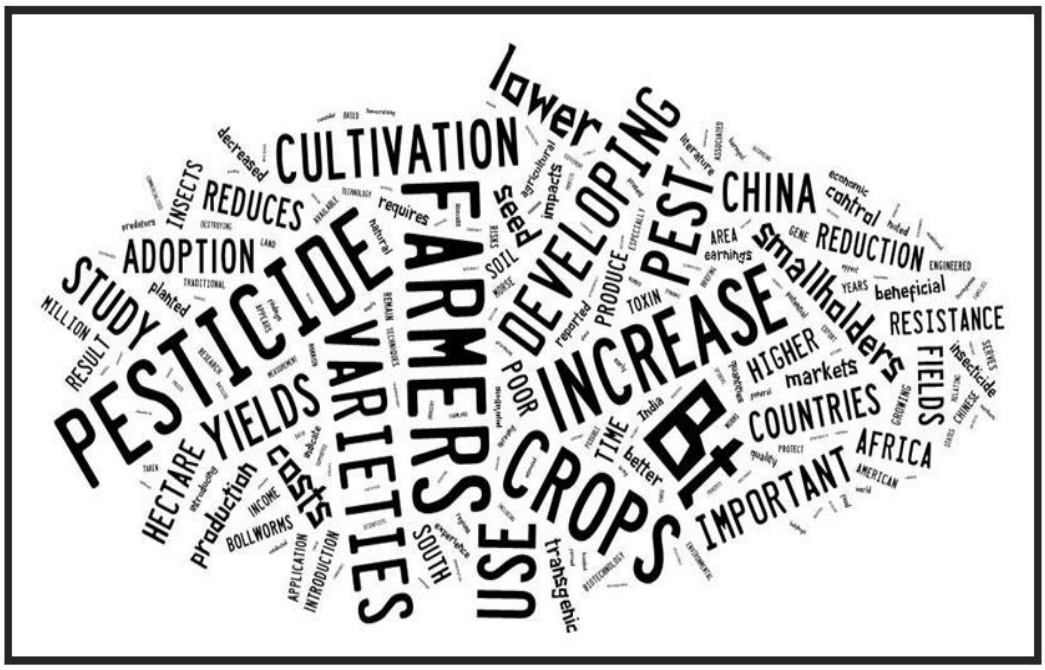
A total of 8 informants representing cotton farmers, seed suppliers, chemical suppliers, ginning sector (primary processors), spinners (secondary processors), the regulator,Government and other stakeholders participated in the study. The informants had cotton industry experience ranging from 5 to 21 years and they hold key positions within the cotton industry. The findings are discussed based on on four themes that emanated from the 8 interviews.The four themes are the benefits of introducing genetically modified cotton, the costs of introducing genetically modified cotton, capacity requirements for introducing genetically modified cotton,and challenges associated with genetically modified cotton. The views given by the informants were personal and did not represent views of the companies they worked.

**Theme 1: The benefits of introducing genetically modified cotton in Eswatini**

Informants were asked to name and explain possible benefits that genetically modified cotton can bring to Eswatini. The key word "pesticide" was used more often by informants.Other common wordswere yield, reduction and lower. Table 4.1 and Figure 4.1 below summarise the analysis of words usedto describe the benefits of introducing genetically modified cotton.

*Table.4.1: Benefits of introducing genetically modified cotton*

| Key Benefits | Frequency |
|---|---|
| Lower use of pesticides | 4 |
| Increased crop yield | 2 |
| Reduction in production cost | 1 |
| Lower labour costs | 1 |
| **TOTAL** | **8** |




*Figu.4.1: Word cloud of potential benefits from introducing genetically modified cotton in Swaziland*

It emerged that informants believed that introducing genetically modified cotton would impact pesticide application and use. This is most probable due to the informants' knowledge of the scientific process behind the genetic modification of cotton seeds as well as their knowledge of other countries' experiences of cultivating genetically modified cotton.The fact that growers of genetically modified cotton use fewer pesticides is the most important benefit cited by the informants.

Informant 1 mentioned that:

*"The introduction of genetically modified cotton will significantly reduce the efforts that farmers invest in pest management and will assist in making their pest management strategies more attainable with fewer resources…"*

Informant 2 explained that:

*"By virtue of their genetic structure, these modified seeds are naturally resistant to certain types of worms which are known to*

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*destroy the crop. Therefore, it is not surprising that many farmers around the world, who plant genetically modified cotton seeds, have noticed that the need for large volumes of pesticides is drastically reduced".*

The above observations are in line with the fact that, since genetically modified cotton is purposely created to be resistant to many types ofworms and insects, cotton farmers find that there is no need to excessively spray different types of pesticides to protect their cotton crops. Thus, the introduction of genetically modified cotton reduces the use of pesticide (Anderson, Valenzuela & Jackson, 2008).

Informants concurred that genetically modified cotton has the potential benefit to increase yield of the cotton crop in a given cultivation area.Informant 8 highlighted that genetically modified cotton tends to produce higher yields because there is less crop damage since the modified crop is resistant to worms.

> *"I will put it to you simply; traditional cotton yields are drastically reduced from damage caused by different pests. In genetically modified cotton plants, there is less damage and therefore more cotton to harvest. "*

Informant 4 added that,

*"Genetically modified cotton seeds are manufactured for high yield. The scientific process involved in the biotechnology is a proven technique worldwide and serves to enhance the capability and potential of each cotton seed. In essence, we are removing, or lowering the chances of crop failure by introducing a seed that is proven. Farmers can expect higher yields per hectare after planting these seeds."*

Perceptions of informants that genetically modified cotton seeds produce higher yields is supported by evidence presented by Arundel and Sawaya (2009) who found that the increased yields are a result of better soil quality from lower pesticide use and the elimination of most crop destroying worms and insects such as Lepidoptera species. These crop destroying worms are usually responsible for significant yield reduction. Therefore, with the introduction of genetically modified cotton, farmers are able to benefit from increased yields within the same acreage. All of the informants agreed that grow traditional cotton faced hardship due to low yields stemming from damaged crops and drought. It also emerged from the interview that as use of pesticides goes down and farmers have better yield there was great potential to boost farmers' earnings and eventually quality of life.

Informants 6 and 2 cited examples of China and India where cotton farmers were able to lower production costs by adopting genetically modified cotton seeds. Informant 6said Chinese farmers managed to reduce their operational costs after adopting genetically modified technology:

> *"China, for example, assisted farmers and encouraged them to adopt genetically modified cotton cultivation techniques. After two years, small farmers reported that their costs had dropped because they used fewer pesticides and didn't need to bear the cost of employing people to apply as much herbicides."*

Informant 2 also explained that:

> *"Indian farmers were extremely successful in the months following the uptake of genetically modified cotton. Farmers fast came to know that the seeds required less water and thus less manpower to grow."*

Informant 8 added that:

> *"…with the introduction of genetically modified cotton, farmers will expect a whole host of benefits. Ultimately, the reduction in stress alone, will improve their quality of life, let alone the improvement in cash stream. "*

The findings of a study conducted by Nita, *et al*., (2013) at the European Commissionconfirm that, the increased yields per hectare translate into more earnings. As a result, farmers enjoy higher profits and can thus experience a better quality of life. It is also important to note that genetically modified cotton cultivation requires less management and involvement. As such, farmers have more time available to spend with family as well as engage in other income generating activities.

**Theme 2:The costs of introducing genetically modified cotton in Eswatini**

Informants identified and explained costs they associated with growing genetically modified cotton. Table 4.2 shows four key elements associated with cost of growing genetically modified cotton and Figure 4.2 below summarises common words and phrases used by participants in describing costs related to growing genetically modified cotton.

*Table.4.2: Costs of introducing genetically modified cotton in Eswatini*

| **Key costs** | **Frequency** |
|---|---|
| Price of inputs | 4 |
| Seed cost | 2 |
| Increasedhectrage | 1 |
| Capacity requirement | 1 |
| **TOTAL** | **8** |

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*Fig.4.2: Word cloud summaryof costs of introducing genetically modified cotton in Swaziland*

The price of genetically modified cotton seeds, will be higher than traditional cotton seeds. This is attributable to the fact that there is a scientific, biotechnological process involved in the genetic modification of cotton seeds. Informant 6 explained thata farmer in KwaZulu Natal, South Africa indicated that while they expected the cost of seed to be higher, there was also an anticipated higher demand for farm land as more people had interest in growing genetically modified cotton.

Interviewee 2 predicted that:

> *"…seed prices are bound to be higher for genetically modified ones…I have witnessed this trend in India, where seed prices increased by almost 110% and I am aware that in Australia, seed prices jumped to almost 260% more than that of normal cotton seeds. I therefore am fairly certain that farmers in Eswatini can expect to pay higher prices for seed if we allow genetically modified seeds…"*

The primary findings relating to the costs associated with introducing genetically modified cotton production techniques in Eswatini are strongly linked with the findings presented by Nita, *et al*., (2013) who found that genetically modified cotton has an impact on all input costs for the typical small to medium scale cotton farmer.

**Theme 3: Capacity requirements of introducing genetically modified cotton in Eswatini**

Table 4.3 and Figure 4.3 below summarise the key words and common words and phrases used by informants in explaining capacity requirements for adopting genetically modified cotton.

*Table.4.3: Capacity requirements of introducing genetically modified cotton in Eswatini*

| **Key word** | **Frequency** |
|---|---|
| Price | 3 |
| Seed | 3 |
| Increased | 1 |
| Requirement | 1 |
| **TOTAL** | **8** |

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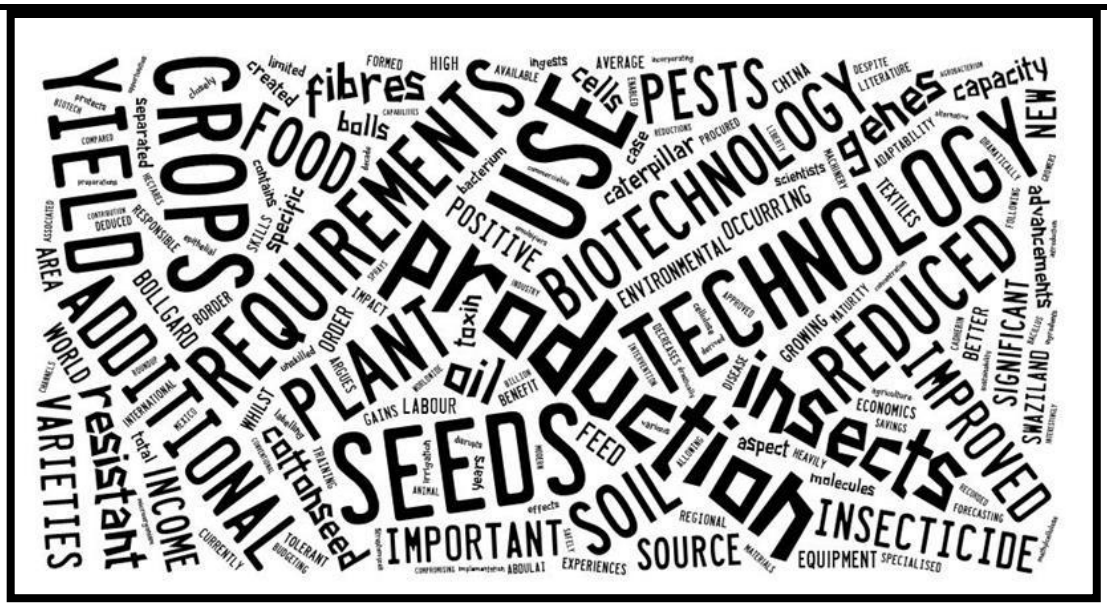


*Fig.4.3: Word cloud relating to capacity requirements of introducing genetically modified cotton in Swaziland.*

Informant 3 hinted that, with the adoption of genetically modified cotton seeds, farmers would be required to modify their production techniques and adopt appropriate technology. Informant 6 also stated that;

> *"Production techniques, including budgeting and forecasting must be improved if we are to be able to afford the cost of new seeds. This is critical because the genetically modified cotton seed cannot be reused. We must therefore consider our capacity to forecast our profits and budget for the cost of new seeds."*

Informant 5 added that;

> *"If we are adopting a new product, we cannot expect to use it or grow it with the old techniques. We must be clearer about how we structure our capacity to accommodate these new seeds. Farmers need to be able to produce cotton using a more advanced approach. They need correct seed to achieve a better yield.*

Informant 8 said ;

"*Although the Government prohibits the use of genetically modified seeds, farmers were no longer interested in traditional cotton. Many farms lie idle because cotton is no longer profitable, people have no money for pesticides and there are no workers to work in the fields. It is not clear how the Government will help farmers on these issues"*

Respondent **7** explained that:

> *"Eswatini's cotton farmers would need to transcend from the traditional methods of cultivation to a newer more sophisticated approach. I am not saying that they need to become scientists to plant genetically modified cotton. But they will need to know how to make the most from the newer and also more expensive seeds if they are to be successful at it. They need to start learning what the requirements are to make the most out of the new seed. For example, they should know that genetically modified seeds require more fertiliser and they must know when to apply this..."*

Informant3 asked some questions about genetically modified cotton

> *"...have we considered what impact higher yield would have on farmer? Would they be able to harvest much more cotton in time with the machinery that they have? Can they get it to the ginnery in time? We know that the ginnery has the capacity, but are the farmers capacitated in this process?...the technology that must be introduced has a critical role to play in ensuring that the smaller guys are efficient in their operations and are not overwhelmed by the higher yields. We don't want a situation where they are incurring high costs to deal with the higher yields. This needs to be considered..."*

Informant 5 who urged for a conscientious approach said,

> *"It may be an exciting opportunity for Eswatini's cotton farmers to improve their quality of life... certainly... but they should also be prepared in terms of the ability to process the higher yields and bigger*

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*volumes of cotton from the harvest...Other countries have been doing this successfully for a long time and we could benchmark the average smallholdings farmer's operations in Eswatini and compare it to those countries...identify the key characteristics ...to be replicated here."*

**Theme 4: Challenges associated with introducing genetically modified cotton in Eswatini.**

Respondents were asked to state the major challenges which they believed would hinder the adoption of genetically modified cotton in Swaziland and how these challenges could be overcome. Table 4.4 summaries key challenges and Figure 4.4 below summarise the key common words and phrases used by participants in responding to questions about key challenges.

*Table.4.4: Challengesof introducing genetically modified cotton in Eswatini*

| Key word | Frequency |
|---|---|
| Price | 3 |
| Seed cost | 2 |
| Increased reliance on suppliers | 2 |
| Capacity requirement | 1 |
| **TOTAL** | **8** |

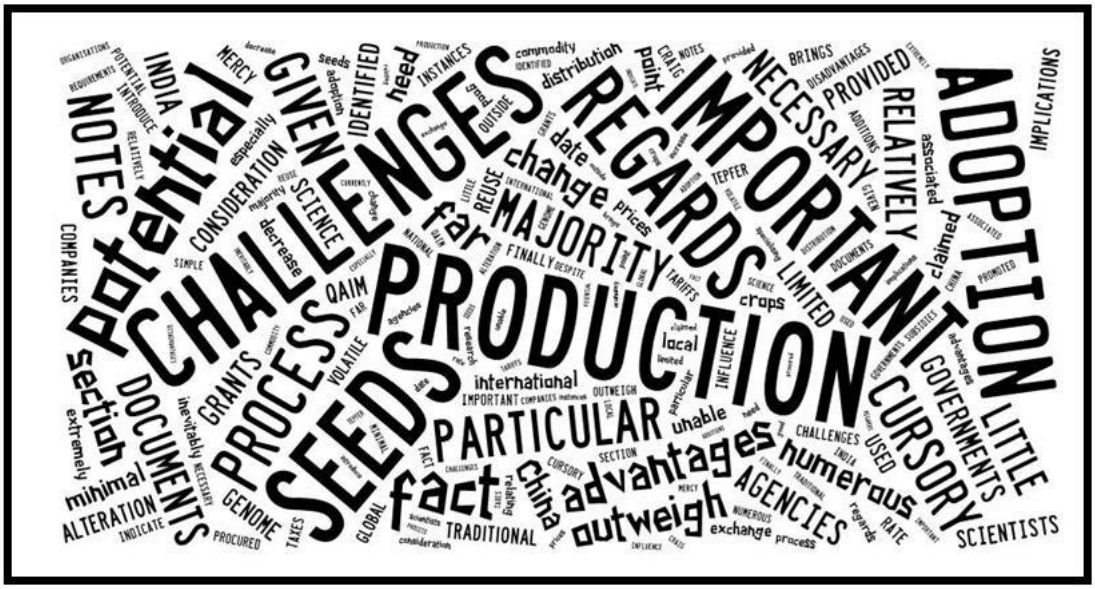


*Fig.4.4: Word cloud of potential challenges associated with introducing genetically modified cotton in Swaziland*

Most important challenges are to do with capacity issues, as per the previous theme.

For example, Informant7 said:

*"I do not think we have enough capacity to handlegenetically modified cotton.…increased costs are the most important challenges and also capacity. These challenges are best managed through a proper strategy which must be created at a national level."*

Informant 8 explained the challenges created by the high cost of the seeds by stating:

*"We all know that cotton farmers are in a bad state at the moment, they are suffering and do not have money to waste. It may be a big challenge for these poor guys to adopt a new product that requires more money to be invested at the beginning, and by that I mean the high cost of the genetically modified seeds. To overcome this potentially disastrous challenge, we must explore ways of getting the new seeds subsidized by the relevant stakeholders, or even explore having small loans being made available to them just to get the running with the new seeds."*

Globally, very few disadvantages and challenges have been documented with regards to the adoption of genetically modified cotton. The literature relating to genetically modified cotton production seems to indicate that the adoption and change-over process is relatively simple. Furthermore, national research agencies in the USA, Australia, China and India have concluded that

genetically modified cotton should be promoted by governments and that grants and subsidies need to be provided to farmers, given the limited requirements for adoption (Arundel &Sawaya, 2009). In line with the current findings,Herrera- Estrella (2002), elaborated the potential challenge created by monopoly of seed producers. This creates a supplier's market where farmers will always be vulnerable to the market conditions created by monopolies.

## V. CONCLUSIONS AND RECOMMEDATIONS

### 5.1 Conclusions

Based on the study's objectives and findings the following conclusions were made regarding the possibility of introducing genetically modified cotton in place of hybrid cotton in the Kingdom of Eswatini:

- Continued farming of hybrid cotton in Eswatini is no longer economically viable owing to high production costs such as prices of cotton seed,cost of pesticidesand rural to urban labour migration.
- Cotton farmers and other cotton supply chain stakeholders in Eswatini are convinced that genetically modified cotton will earn them more revenue than the hybrid crop owing to anticipated higher yields per hectare and reduction in labour and pesticides costs which have been recorded in other countries.
- Farmers get motivation and encouragement fromsuccess stories of cotton farmers from countries such as USA, India, Australia and South Africa who have managed to improve their lives based on growing genetically modified cotton.
- Despite the fact that farmers are willing to adopt a new product in place of hybrid cotton it is clear that the farmers will not be able to engage in serious commercial farming without capital injection from either government or other financiers that need to be organised at national level. The said intervention is necessitated by the fact that genetically modified cotton inputs, especially seed is very expensive because of biotechnological processes involved in preparing the seed.
- Farmers are aware of the surmountable efforts needed to introduce genetically modified cotton for commercial purposes and they are looking forward to get subsidies and other forms of support from Government and other stakeholders.

### 5.2 Recommendations

- In any form of business, survival is not about being strong and / or having unlimited resources; survival is about the business being adaptable to change.It is therefore recommended that, cotton farming strategies must change with time and in light of what is happening in successful cotton farming countries such as USA, India, Australia and South Africa. Farmers in these countries benefitted from genetically modified cotton. Eswatini cotton farmers can adopt the new technology based on careful benchmarking programmes.
- Liberalisation of the cotton industry and related legislative reforms can create opportunities for the cotton farmers because they will be able to try the new technology (genetically modified cotton) and attract investment partners at national, industry and individual levels. Therefore, it is recommended that the adoption of genetically modified cotton be implemented as one of the strategies to fast-track the revival of the moribund cotton industry.
- In light of the several challenges associated with introducing genetically modified cotton, the study recommends a robust training programme for farmers who are interested in genetically modified cotton. The farmers require training on the behaviour of genetically modified cotton and how it must be handled. Short courses on genetically modified seeds can be organised in liaison with colleges that offer agriculture courses, international sponsors and promoters of genetically modified cotton.
- To deal with the restrictive environment, it is recommended that the Government of Eswatini introduces a law that allows farmers to grow genetically modified cotton and provide capital and technical support to the farmers until such a time that the farmers are ready to sustain themselves through cotton farming without there being need for third party support. This recommendation will not require a lot of capital input from the farmers hence it can be implemented as soon as appropriate legislation is put in place.
- Genetically modified crops are resistant to harsh weather patterns and they are capable of flourishing in drier seasons. It is further recommended that the Eswatini government adopts genetically modified crop technology starting in drought prone areas first. The programme can be rolled out to other regions later in a phased approach.
- Finally, but not least, it is recommended that further research be conducted on the different types of support needed in a country that is adopting new product and new technology. Studies on effective strategies to deal with anticipated higher yields from genetically modified cotton and the management of the farmers' sustainability are also recommended.

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# Cattle Farm Development by Forages Cultivation on Coconut Land Based on Carrying Capacity in West Bolangitang, Indonesia

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***Abstract*****—** *West Bolangitang became one of the central areas of livestock development in North Sulawesi, Indonesia. Livestock are usually only kept and grazed under coconut trees, and consumes waste from food crops and even grass that grows wild under coconut trees. The research analyzed the potential of cattle development based on carrying capacity index of forages on coconut land. This research has been conducted using survey method, and data source is primary data. The sample villages were determined by purposive sampling, with 32 respondents. The research material is coconut land and cattle. Analysis of data used is the analysis of the carrying capacity index (IDD). The results showed that the District of West Bolangitang has a coconut land, 3,668 Ha, with a real population of 2,044 AU. PMSL value of 4,744.24, meaning that based on land resources in this district can still accommodate the population of cattle for the value of PMSL. Total feed requirement amounted to 2,330.16 tons with a value of the carrying capacity of 2.04. Based on result of the research, it can be concluded that population of cattle in West Bolangitang District can still be improved by utilizing the land under coconut tree. Suggestion is need introduction of superior forage in supporting the development of cattle farms.*

***Keywords*****— Carrying capacity, cattle, coconut.**

## I. INTRODUCTION

Coconut plants are dominant plants in some areas in Indonesia including North Sulawesi. Coconut plant is one of plantation plants that are able to adapt to the environment, growing in tropics and can be found both in lowlands and highlands. The coconut plant grows and produces well at an altitude of 0-450 meters from sea level, and grow well in coastal areas [1,2].

The coconut plant in this area is a deep coconut that can reach age of 100 years. Coconut starts to produce rather slowly that is 6-8 years after planting[1]. Coconut is known as a plantation commodity, which is export orientation.

Coconut farming is a source of income for some people of West Bolangitang District which are sold in the form of copra. These commodities contribute to farmers' income, as well as the potential employment for agricultural sector growth.

West Bolangitang is one of the central areas of livestock development in North Sulawesi, Indonesia. The cattle developed in this region has potential as a reliable business as a source of income. Cattle can be sold at any time by farmers to meet the needs of family members. In this case cattle farming can provide economic value added for farmers, in rural areas. Cattle in addition to providing a role as a source of income, also plays a role in opening employment opportunities and as a source of animal protein. Cattle product as a reliable commodity in order to meet the demand for beef tends to increase. This can support government programs in meeting the needs of animal protein, whose goal is to improve the nation's intelligence.

The resources of cattle are potential economic commodities to be developed and can be used as a prime commodity because it has the potential to increase economic growth in the research area. Cattle farming, in this case has a strategic role in the life of the economy and the development of Indonesian human resources. The role as described has been seen from the function of cattle products as a provider of animal protein which is essential for the growth and development of the human body. The increasing trend of consumption of food produced by cattle is a challenge as well as opportunities for livestock sub-sector to increase the production of cattle efficiently and competitively.

The priority of cattle development programs in the study areas is still an increase in population. The population increase program is conducted through the introduction of cattle by the government. The fact at the site of the study shows that the source of cattle in this area is still relied on traditional farms, the management of the sideline and simple. In general, the cattle farming is still relies on natural grass that grows freely in the field for agriculture or coconut plantation. The characteristics of traditional livestock farming, among others, the number of cattle

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ownership of 1 to 3 heads per family, limited land area, cattle rearing is done alone and simple relatively and waste management is produced relatively low. The characteristics of traditional livestock farming systems are cattle ownership of 1 to 3 heads per family, limited land area, cow maintenance carried out by farmers and members of their families and relatively simple, and the management of cattle waste produced is relatively low. Development of traditional cattle has been done by utilizing agricultural waste, but still done partially with diversification system [3]. West Bolangitang District is one of the districts in North Bolaang Mongondow Regency that develop cattle. Cattle are grazed in the land under coconut trees. The area under the coconut tree is very potential for development of cattle[4]. Cattle consume food crops and grass that grows wild under coconut trees. Coconut land cultivated many crops, especially maize and used as cattle grazing land, especially cattle [5]. Based on existing conditions, the management of cattle raise as one of the keys to livestock succeed, should no longer be traditional oriented.

The main constraint faced by farmers in the development of cattle is unavailability of adequate and continuous forages, especially in dry season. These constraints that cause some farmers are forced to sell cattle at a relatively cheaper price. Feed is one of the factors that determine the success of the livestock business. Furthermore, related to the increase in population and productivity of cattle, the factors that need attention are feed both of quantity and quality, price and availability.

The increase of cattle population in some areas including in the research area, still oriented to the ability of a region in providing cattle feed (carrying capacity). However, the area of feed source, whether in the form of common pasture from cattle or forage specific fields, shows tend to decrease. The constraints that often found in beef cattle farming is the low productivity of beef cattle because of the quality of feed that does not meet the nutritional needs of livestock[6].

Farmers generally only cultivate forage crops in the lands, fields and on the edge of the irrigation canal. Cultivation of forage grasses of fodder will be more effective with livestock integration systems with [7]. The land under coconut trees can be utilized for quality forage development. This is because forage feed is the main feed ingredient for the life of cattle.

Forage development under coconut trees should be done for the life and growth of cattle. Provision of feed throughout the year is an important factor that must be considered in supporting the population and productivity of cattle. The provision of feed is not only quantity but also must pay attention to the quality of feed. Increasing the quality of the feed is intended so that the needs of cattle food substances can be met and achieved and sustainable. Cattle require food substances with aim that the preservation of life and wholeness of components of the livestock body (basic necessities of life) and the purpose of production (production needs) can be maintained.

Forage feed is basically, as a decisive factor in the development of cattle farms. Forage plays an important role because it contains many nutrients needed for cattle as a source of energy in the activity, growth and benefits for cattle who are breastfeeding. Forage is a source of fiber, even forage in the form of leguminous, into mineral supplementation, and cheap protein for cattle.

The problem is how far the potential development of cattle under coconut tree. The potential of development of cattle is associated with the carrying capacity of feed under coconut trees. This is because feed is one of important factors in increasing the productivity of cattle. The available feed, in addition to quality, should also be available throughout the year, and economically, in order to benefit farmers. Successful development of cattle is determined by the availability of forage in sufficient quantities, and can be sustainable. Based on these problems, we have conducted a study with the aim of analyzing potential of cattle development based on index of carrying capacity of feed in coconut land.

## II. MATERIALS AND METHODS

The subject of this research is cattle farmers in West Bolangitang District of North Bolaang Mongondow Regency. Cattle are farmers' livestock in West Bolangitang District. Coconut land is unused land for forage feed. The research method is survey method with data source is primary data. The sample villages were determined by purposive sampling, with 32 respondents. The data analysis used is ICC analysis[8], such as equation (1).

$$ICC = \frac{PMSL}{TK} \qquad \text{.....} \qquad (1)$$

Description :

ICC = Index of Carrying Capacity

TK = Total feed requirements

TK = k x POPRIL

k = The constant requirement of dried material is digested (DDM) by one animal unit that is: 1.14

## III. RESULTS AND DISCUSSION

Potential of livestock development is effectively analyzed using analysis of the maximum potential of land resources (PMSL), as in Table 1.

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*Table.1: Results from PMSL Analysis*

| No | Coeffisient/Variable | Value of Variable |
|---|---|---|
| 1 | A | 0,80 |
| 2 | LG | 3.668,00 |
| 3 | B | 0,50 |
| 4 | PR | 3.504,48 |
| 5 | C | 1,20 |
| 6 | R | 48,00 |
| | PMSL | 4.744,24 |

Description :

PMSL = Maximum potential in animal unit of cattle (AU based on land resources.

A = The coefficient calculated based on ratio of ruminant livestock population in animal unit (AU) to cultivated land area (Ha), referenced from East Java provincial standard, 1995 was 0.8 AU / Ha.

LG = Area of coconut at research location (Ha)

B = The coefficient is calculated as carrying capacity of natural grasslands (1995 = 0,5 AU/Ha)

PR = Area of grassland (Ha)

C = The coefficient is calculated as carrying capacity to swamp (1,2 AU/Ha)

R = Area of swamp (Ha)

The results of Carrying Capacity Index analysis can be seen in Table 2

*Table.2: Index of Carrying Capacity of Forages*

| No | Coeffisient/Variable | Value of Variable |
|---|---|---|
| 1 | PMSL | 4.744,24 |
| 2 | K | 1,14 |
| 3 | POPRIL | 2.044,00 |
| 4 | TK (kxPOPRIL) | 2.330,16 |
| | ICC | 2,04 |

Description :

POPRIL = The real population of cattle (AU) in the study area

ICC = Index of carrying capacity

TK = Total requirement of feed (TK = k x POPRIL)

K = The constant requirement of dry matter (DM) by one animal unit is: 1.14

The success of cattle farming can not be separated from the role of government, private sector and society in this case cattle farmers. The success also requires the support and utilization of technology so as to ensure the increase in population, productivity and sustainability of cattle farming. Age as characteristics of farmer affect absorption of technology and success of cattle farming in the District of West Bolangitang. Age of respondents is mostly included in the productive age, which ranges from 21 to 60 years (93.75 percent or 30 respondents). The lowest education level of respondents is the elementary level (65.62%), followed by the junior secondary (21.88%), senior high school (6.25%) and the highest level of PT (6.25%). Based on research results, the level of education of farmers is mostly categorized low farmers that have an impact on the success of cattle farming, where education is important for the agricultural sector that is not yet modern and the application of technology is low[9].

Cattle is one component of food fulfillment, plays an important role in relation as a source of animal protein from livestock. The collaboration of various world food institutions to make livestock as an important commodity is seen from the missions launched "livestock to 2020, the next food revolution"[10]. The importance of the development of cattle is in support of food security, so that these animals can be developed with sustainable and environmentally friendly. Cattle development, which is environmentally friendly and based on local resources, is a strategic step in realizing the improvement of the quality and quantity of livestock products [11].

Dry land in the research area has great potential for agricultural development, both food crops and annual crops or plantations (more specifically coconut plants). The development of agricultural commodities in dry land is one of the strategic choices in increasing production and supporting national food security. Type of dry land shows lower productivity, for it requires optimal handling, and sustainable, in the face of dry land issues[8]. The biophysical problems faced by dryland farmers are the destruction of land function as a growing medium, such as soil sensitivity to erosion, minimal nutrients, and limited organic matter content [8]. Land degradation is a problem faced by various countries[12]. Management of agro-ecosystem from coconut and cattle in the research area is done by utilizing the land under coconut for the development of cattle.

The results showed that, the farmers manage coconut plant, on average 1.31 Ha, for 32 respondents as many as 92 heads, the amount of each farmer ownership of about 2-7 cattle. The ownership of cattle according to the results of the study showed that farming cattle managed by farmers, is still a sideline business. In fact, cattle farms in Indonesia, for the most part, are still dominated by small-scale peasant farms, located in rural environments. The technology used is also still simple or traditional. The existing livestock ownership is still as a savings, and as one indicator of the social status of the farmer. The development of cow farming by farmers is now more concerned with livestock productivity, meaning that they

have not considered the environmental aspects or the impact of their livestock activities on the environment.

The cattle belonging to the respondents according to the results of the study were grazed under coconut trees, with food consumed being waste from food crops and wild grasses. This causes productivity of cattle is lower than cattle in other areas. Whereas, is one of factors that determine both the bad growth of cattle[13]. The productivity of cattle is largely determined by quality of feed consumed. The quality of feed includes understanding the content of various nutrients such as energy, protein, minerals, vitamins and the content of anti-nutritional substances such as tannins, lignin, and other secondary compounds. Feed is the main obstacle faced by farmers[8,14]. The forages commonly used as feed for smallholder livestock are field grasses and agricultural by-products as well as some introduced grasses[15]. Low productivity of cattle is due to the feed consumed in low quantity and quality[3]. In addition, about 62 percent of farmers said the provision of forage feed is a limiting factor of cattle farming[16]. Efforts to increase the productivity of cattle to meet the nutritional adequacy standards of community need to be done in several ways, such as by optimizing the utilization of local feed resources under coconut trees, and agroindustry through a system of crop-livestock integration. Another way to do this is to develop a system of cattle farming sustainable, integrated, and environmentally friendly, that can improve farmers' welfare. The carrying capacity of land under the coconut trees can be determined by firstly analyzing the potential for effective livestock development.

The data in Table 1 shows that maximum potential value of land resources, under coconut trees is 4,744.24 AU, meaning that based on the land resources in this area can still accommodate cattle for value of the PMSL. This phenomenon indicates that increase of cattle population in this area needs to be encouraged so that available resources can be utilized optimally. This effort can be done in order to optimize land under coconut, because utilization of land resources in support of agricultural development in future still continues and improved. The goal is to balance the increase in population and food needs. Strategy and effort of land resource utilization can be done by optimizing the utilization of existing land resources to be more productive and sustainable[17]. The results of this study can be used as recommendations for government in an effort to increase growth of cattle. The one of information availability of feed is needed to accelerate the development of cattle farms[18]. Higher cattle population indicates one of potentials and opportunities that can be utilized to provide added value in cattle business. Increasing population of cattle in this case can affect increase in farmers' income. Furthermore, the increase in number of cattle has an impact on improving social status of farmers[19]. Increasing the population of cattle also has an impact on increase of family nutrition consumption in this case the product of cattle is a source of animal protein. The demand for meat shows a significant increase, for last few years, so it is a good market opportunity for cattle.

The value of Carrying Capacity Index of 2.04 (Table 2) indicates carrying capacity of the land is quite high, meaning that maximum potential of land resources is still greater than need for feed. Based on the land potential, the real population can still be increased up to 2.04 times. The results of analysis are based on potential of effective coconut land. The indication of carrying capacity of feed is greater than population of beef cattle in District of West Bolangitang. The capacity of ruminant according to results of his research is greater than livestock population due to rainy season forage production is available in large quantities[20]. Sustainable forage production is an important factor in cattle production systems. The strategy to achieve success of beef cattle farming, one of which is need for technology intake[21]. Forage feed needed by cattle can be given directly in fresh or processed. That is, farmers should be more innovative in feeding forage for cattle, to anticipate if there is a dry season because forage feed more difficult to obtain. Farmers need knowledge of how to store forages in fresh form, until a certain period of time.

Land under coconut trees according to research results, not yet exploited, utilization of plantation land has not been maximized[22]. Land under coconut trees in West Bolangitang District can be used for forage development. Availability of forage land will determine amount of forage feed[23]. Land under coconut trees in the research area, in this case can be utilized for forage development. Utilization of land under coconut trees for forage feed can serve as cover crops[8]. This can be done for the purpose of land closure, so that there is no erosion and can increase soil fertility. Utilization of land under coconut trees in support of development of cattle is an effort in supporting agricultural business in an integrated manner. Integrated farming is better known as integrated farming system as recommended by some researchers. The research related to the integrated farming system whose purpose is to explain the natural resources without any negative impact to environment[24]. The research that integrated farm management is less risky, if managed efficiently can provide benefits and produce environmental health[25]. In relation to the increasingly limited agricultural land, suggested farming system is an integrated farming system. The integrated farming system, is right choice because of the limited ability of agricultural resources[26]. The integrated farming system according is an alternative effort in order to improve the efficiency of cattle business in

farmland[27]. Furthermore, the integrated farming system is an alternative to climate change mitigation[28]. Integration of livestock and crops is often recommended as one of the most promoted solutions related to occurrence of soil fertility decline and a loss in intensification system productivity[29]. Integrated farming system approach as an effort made by pressing production cost, especially on the provision of land for forage, cattle as a source of labor, and can contribute in suppressing the purchase of fertilizer. Optimal benefits in integrated farming system can be achieved if it meets the criteria of agricultural development that can combine economic interests, socio-cultural and environmental sustainability. This system of integration is increasingly important given the availability of dry land for development of livestock and feed source is increasingly limited and expensive.

Introduction of superior forage is needed in supporting development of cattle farm agribusiness. Cattle farming agribusiness is a priority of the government in an effort to increase population and productivity of cattle. The development of cattle farming by rural farmers is expected to be done with agribusiness orientation. Steps that can be pursued among others, by involving aspects of commodities in system of agribusiness and agroindustry[30]. The development can be done through the application of Good Farming Practice with special attention to aspects of cattle breed ownership and strengthening of feed in increasing productivity of beef cattle[31]. Essentially feed is the basis in development of cattle farms.

The development of cattle population in the future will face the challenge of availability of forage feed, where the more cattle population increase, so the demand for feed forage will increase as well. Integrated development of cattle farms with coconut crops, intended in addition to efficient land use, also primarily to implement environmentally sound farming systems.

## IV. CONCLUSION

Based on result of the research, it can be concluded that population of cattle in West Bolangitang District can still be improved by utilizing the land under coconut tree for a program to increase forage feed for cattle production development with integrated coconut plant. Suggestion is need introduction of superior forage in supporting the development of cattle farms

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# The Business Prospect of Climbing Perch Fish Farming with Biofloc Technology at De' Papuyu Farm Banjarbaru

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***Abstract***— *This research aimed at investigating the business prospect of climbing perch (Anabas testudineus) fish farming with biofloc system at De' Papuyu Farm, Banjarbaru of Indonesia, as well as providing the business development strategy by mean of SWOT analysis. Total cost required for this business was IDR 165,218,200 per year and the income gained was IDR 7,880,700 per month with the Payback Period (PBP) value was 2.67. On the basis of 7 % and 9 % of interest rates, the Net Present Value (NPV) value was greater than 0, and the Net Benefit Cost Ratio (Net BCR) was greater than 1. While the Internal Rate of Return (IRR) value obtained was 57.04 %. The business provides the profit more than three times higher than the province minimum wage; thus it is considered feasible and profitable for the future strategic choices. The business has the favorable prospect and the biofloc system for the culture of climbing perch is applicable for other fish farmers.*

***Keywords— Climbing perch, biofloc, business feasibility, NPV, Net BCR, IRR, SWOT.***

## I. INTRODUCTION

The climbing perch (*Anabas testudineus* Bloch, 1792), in South Kalimantan locally called "papuyu" is a very important indigenous fish species not only in Indonesia (Akbar *et al*., 2016) but also in Malaysia (Zalina *et al*., 2012), Vietnam (Van and Hoan, 2009), Lao PDR (Sokheng *et al*., 1999), Cambodia (Sverdrup, 2002), Thailand (Chotipuntu and Avakul, 2010), the Philippines (Bernal *et al*., 2015), India (Kumar *et al*., 2013) and Bangladesh (Hossain *et al*., 2015; Uddin *et al*., 2017). It plays a significant role in fisheries and aquaculture practices due to its high nutrition value as well as for great taste and flavor. It is rich in iron and copper that support hemoglobin synthesis (Sarma *et al*., 2010) and has high quality poly-unsaturated fats and many essential amino acids (Kohinoor *et al*., 1991). It also provides 4.4% of lipid and 19.50% of protein contents (Wimalasena and Jayasuriya, 1996; Ahmed *et al*., 2012). The demand of this species is very high not only for local consumption but also for restaurants and small enterprises of fish processing. It is a native air-breathing fish, typically found in swamps, rivers, streams, lakes, canals, reservoirs, and estuaries (Sarkar *et al*., 2005; Chotipuntu and Avakul, 2010; Rahman and Marimuthu, 2010). It can survive in adverse environmental conditions such as low oxygen due to its air breathing ability, wide range of temperature and other poor water conditions (Rahman and Monir, 2013). However, the presence of them is threatened by the ecological degradation, indiscriminate fishing, use of pesticides and fertilizers, habitat modification, obstruction to breeding migration, and management failure (Misra, 1994, Kalita and Deka, 2013; Hossain *et al*., 2015). Considering the importance of them, the breeding technologies of this species have been developed with varying degrees of success (Kohinoor *et al*., 1991; Sarkar *et al*., 2005; Marimuthu *et al*., 2009; Zalina *et al*., 2011), as well as the attempts to improve the culture management system of ponds or cages with different culture strategies (Mondal *et al*., 2010; Habib *et al*., 2015; Putra *et al*., 2016; Ali *et al*., 2016). All these studies outlined above more or less describing on ecological and biological aspects of this species.

To date, biofloc technology application in aquaculture system is also introduced and had been successfully used for some commercial fish species such as African catfish *Clarias gariepinus* (Ekasari *et al*., 2016), nile tilapia *Oreochromis niloticus* (Nahar *et al*., 2015; Ekasari *et al*., 2015) as well as for shrimp culture such as pink shrimp *Farfantepenaeus duorarum* (Emerenciano *et al*., 2013), the pacific white shrimp *Litopenaeus vannamei* (Da Silva *et al*., 2013), Malaysian prawn *Macrobrachium rosenbergii* (Perez-Fuentes *et al*., 2013). Meanwhile the use of biofloc technology for air-breathing species cultured like climbing perch is still rarely done. Moreover in term of economic importance, very little literature is available describing how the business prospect being executed in these culture systems. The current research will elucidate the secret

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behind successful of climbing perch culture business of De' Papuyu Farm on the basis of biofloc technology application. To get clear picture, we analyzed the total cost, profit, and the feasibility of business, as well as provided the business development strategies for this species.

## II. MATERIALS AND METHODS

### Study site

De' Papuyu Farm Banjarbaru was selected purposely for the study area due to the following reasons i.e. climbing perch cultured with biofloc system was commercially introduced first in this city; secondly, many farmers around interested to learn and have adopted such biofloc technology in their fish farming; and De' Papuyu Farm can be a good model for small-scale culture business development in advance. The total land area for fish farming was about 624 $m^2$ (24×26 m) including for pond area, recycle pond, security house/warehouse, audience hall, park area and space area. The lay-out of De' Papuyu Farm can be seen in Figs. 1, 2. A total of 24 pond units used for the growth-out of climbing perch culture business. The circle-shaped pond made of tarpaulin with the diameter of 3 m, 120 cm height and 90-100 cm depth of water. The fish growth-out period in the ponds appropriated eight months starting from seeding to harvesting. The sorting process was undertaken after rearing for three months, and the only female fish were selected to be grown (50-60 %) due to rapid growth and having the greater size when harvested. The seeds sourced from fish hatchery of Gunung Manau Balangan, about 120 km from Banjarbaru. The research activity was carried out in October 2017 until May 2018.

### Data collection and analysis

The data collected comprising primary and secondary data. Primary data obtained from farm owner by interview and questionnaires as well as direct observation related to the culture system, fish production, operational cost, profit, distribution and marketing. While secondary data in the forms of annual report, literature, and other document related. The followings are formulas used to estimate the total cost, total revenue, and total profit.

$$TC = FC + VC \quad (1)$$

Where TC is total cost, FC is fixed cost, and VC is variable cost

$$TR = Q \times P \quad (2)$$

Where TR is total revenue, Q is quantity (kg), and P is price (IDR)

$$\Pi = TR - TC \quad (3)$$

Where Π is profit, TR is total revenue, and TC is total cost. The profit gained is then compared to the minimum wage of South Kalimantan province, which is equal to IDR 2,455,671 per month in accordance with Governor Decree Number 188.44/0492/KUM/2017 about the Minimum Wage of Regency and City, 2018). The feasibility of business is analyzed using four investment criteria, namely Payback Period (PBP), Net Present Value (NPV), Net Benefit Cost Ratio (Net BCR), and Internal Rate of Return (IRR) with the following formulas:

$$PBP = \frac{InCap}{AnnualCF} \quad (4)$$

Where *PBP* is payback period, *In Cap* is investment value, and *Annual CF* is annual cash flow

$$NPV = \sum_{t=1}^{n} \frac{(B_t - C_t)}{(1+i)^t} \quad (5)$$

Where *NPV* is net present value, $B_t$ is benefit of year-t, $C_t$ is *cost of year-t*, *i* is interest rate, and *t* is investment time.

$$NetBCR = \frac{\sum_{t=1}^{n} NPV^+}{\sum_{t=1}^{n} NPV^-} \quad (6)$$

Where $NPV^+$ is positive net present value, and $NPV^-$ is negative net present value

$$IRR = i_1 + \frac{NPV^+}{NPV^+ - NPV^-}(i_2 - i_1) \quad (7)$$

Where IRR is internal rate of return, $NPV^+$ is positive net present value, $NPV^-$ is negative net present value, $i_1$ is interest rate when NPV positive and $i_2$ is interest rate when NPV negative.

It is assumed that if $NPV \leq 0$; $Net\ BCR \leq 1$; and $IRR \leq 9\%$, indicating the business is unreasonable. Otherwise, if $NPV > 0$; $Net\ BCR > 1$; $IRR > 9\%$, it is therefore the business is reasonable to be developed.

The SWOT analysis is used to formulate the strategy for business development of De' Papuyu Farm starting from the collection of valuable data-information related and internal problems being faced, as well as the external factors that may inhibit the development progress. SWOT Analysis is a tool used effectively to build organizational strategy and competitive strategy. SWOT Analysis has two dimensions: Internal and external. Internal dimension includes organizational factors, also strengths and

weaknesses; external dimension includes environmental factors, also opportunities and threats (Gurel and Tat, 2017). SWOT Analysis is a simple but powerful tool for sizing up an organization's resource capabilities and deficiencies, its market opportunities, and the external threats to its future (Thompson *et al*., 2007).

## III. RESULTS AND DISCUSSION

### Cost Structure and Profit

Investment cost is the initial-capital of De' Papuyu Farm to purchase long-term goods and assets for the climbing perch culture business (more than one year). To run this business, the total investment cost required was IDR 167,634,000 with depreciation cost of IDR 24,337,800 per year. The highest investment cost was for procurement of 24 ponds reached IDR 72,000,000 (Table 1). Total cost for the business was IDR 165,218,200 comprised the IDR 24,337,800 for fixed costs and IDR 140,880,400 for variable costs. Fixed cost is the regular outcome regardless of the production volume (e.g. depreciation cost, salary expense, capital rate). While variable cost is the cost variance depends on the production volume (e.g. wages, seeds, feed, and labor). It was clear from Table 2, the pay for 120,000 fish seeds (50-80 mm total length) was the top rank of variable costs reaching IDR 36,000,000 (46.10 %), followed by the feed expenses of IDR 29,700,000 (40.41 %). About 13.49 % pay for other variable costs regardless employee's wage. The total revenue obtained from the selling of the fish was IDR 228,000,000 per cycle of production (Table 3). There are two types of revenue: (1) the main revenue from the fish being cultured for 8 months and (2) the additional revenue from the selling of male fish after rearing for three months. The main fish were sold to outside of South Kalimantan, especially Central Kalimantan, while the remaining fish were marketed around Banjarbaru City and Banjar Regency. The profit sharing system was 50% for De' Papuyu Farm's owner and 50% for the employees, which is equal to IDR 62,781,800 per cycle of production or IDR 7,880,700 per month. Since total revenue was greater than total cost, it meant that this business was considered effective and profitable. This income is more three times higher than the province minimum wage of IDR 2,454,671 per month.

### Feasibility Analysis

The payback period is a measure of profitability and liquidity (Hajdasinski, 1993). In the present study, the payback period (PBP) value obtained for climbing perch culture business was 2.67, showing that the investment capital can return after having three times of productions within two years. It is generally accepted that investments with shorter payback periods are considered to have lower risk (Lohmann and Baksh, 1993; Lin, 2010). It is also considered reasonable that the shorter the PBP, the more liquid and the more viable the business (Kim *et al*., 2013). The values of NPV investigated at 7 % and 9 % of interest rates were IDR 568,915,905 and IDR 502,791,869 respectively (Table 4), indicating that the business was very feasible. The feasibility of business can also be seen from the Net BCR values of 4.39 at 7 % and 4.00 at 9 % of interest rates. Final evaluation revealed that the IRR value obtained for 10 years of investment period was 57.04 %. It means that this culture business provides financial growth by 57.04 % per year. This IRR value was higher than MARR (minimum attractive rate of return) 9 %. In other word, a business will be acceptable if IRR is greater than opportunity cost of capital. It was clear from our study that the climbing perch culture business with biofloc technology was feasible and profitable.

### Business Development Strategy

The internal factor (strength and weakness) and external factor (opportunity and threats) of De' Papuyu Farm have been identified using a SWOT matrix as presented in Table 5. By Quantified SWOT analysis and revealing of the coordinates (1.15:0.4), we found that the position of De' Papuyu Farm was in the first quadrant (Fig. 3), indicating that the farm has external opportunities for business development (e.g. market expansion, job creation, network building) and internal competing strength (e.g. capacity building, entrepreneur, corporate culture), thus are in the best position for facing future business competition. In line with this, Chang and Huang (2006) suggested that enterprises in the first quadrant can use their strengths to adopt strategies, such as market penetration, market development, and product development to form competitive strength. In this position, enterprise has extra resources, forward, backward and horizontal integration may be efficient strategies. We used the SWOT matrix to systematically prepare for the future strategic choices, such as: (1) the local government should establish the certified community hatchery units to support the high demand of climbing perch fish production, as well as to reduce transportation cost; (2) improvement of business management by training and internship program; (3) network building with other businessmen related to the hatchery, fish processing and marketing business; (4) product advertising of climbing perch culture business with biofloc system through social media should be encouraged.

## IV. CONCLUSION

1. The climbing perch fish culture business with biofloc system provides the profit more than three times higher than the province minimum wage.
2. The business feasibility can be seen from the following criteria: NPV > 0, Net BCR > 1, PBP and IRR value were 2.67 % and 57.04 % respectively.

3. The fish farming with biofloc system has the favorable prospect due to high demand and the biofloc technology package can be adopted by small-medium scale enterprises and other fish farmers.

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*Table.1: Investment cost and fixed cost of climbing perch culture business for eight months (IDR. 000)*

| Cost items | Unit | Unit Price | Total Price | Economic life (year) | Depreciation Cost |
|---|---|---|---|---|---|
| *Investment cost* | | | | | |
| Ponds | 24 | 3,000 | 72,000 | 5 | 14,400 |
| Aerator (100 watt) | 4 | 1,500 | 6,000 | 5 | 1,200 |
| Pipe 1/2 inch (rod) | 28 | 24 | 672 | 3 | 224 |
| Roof frame (wood) | 1 | 4,500 | 4,500 | 10 | 450 |
| Tarpaulin roof (8x10) | 4 | 320 | 1,280 | 1 | 1,280 |
| Hapa (roll) | 1 | 375 | 375 | 1 | 375 |
| Electric cable 2×1.5 mm (roll) | 1 | 195 | 195 | 5 | 39 |
| Security house | 1 | 25,000 | 25,000 | 10 | 2,500 |
| Recycle pond | 1 | 6,000 | 6,000 | 10 | 600 |
| Concrete panel fence | 100 | 400 | 40,000 | 20 | 2,000 |
| Jet pump | 1 | 750 | 750 | 5 | 150 |
| Washbasin | 2 | 55 | 110 | 2 | 55 |
| Plastic bucket-cover 40 l | 2 | 75 | 150 | 2 | 75 |
| Plastic bucket-cover 20 l | 2 | 16 | 32 | 2 | 16 |
| Scoop nets | 2 | 45 | 90 | 2 | 45 |
| Filters | 2 | 13 | 26 | 1 | 26 |
| Fish sorter | 2 | 20 | 40 | 1 | 40 |
| Generator set | 1 | 1,300 | 1,300 | 5 | 260 |
| Aerator tube (50 m roll) | 3 | 50 | 150 | 2 | 75 |
| Reservoir 1500 l | 1 | 1,850 | 1,850 | 10 | 185 |
| Frame of reservoir | 1 | 2,000 | 2,000 | 10 | 200 |
| Water pipe 1 inch | 17 | 42 | 714 | 5 | 143 |
| Training certification | 1 | 400 | 400 | | |
| Land (26×24 $m^2$) | 1 | 4,000 | 4,000 | | |
| Total investment cost | | | 167,634 | | |
| *Fixed cost* | | | | | |
| Total investment depreciation | | | | | 24,337.8 |

*Table 2: Variable cost of climbing perch culture business for eight months (IDR. 000)*

| Cost items | Unit | Unit Price | Total Cost/ Production |
|---|---|---|---|
| Electricity (kwh) | 2,304 | 1.35 | 3,110,4 |
| Fish seeds (50-80 mm) | 120,000 | 0.3 | 36,000 |
| Feed pf 500 (sack) | 6 | 155 | 930 |
| Feed pf 1000 (sack) | 6 | 155 | 930 |
| Feed Cargile 1 (sack) | 100 | 297 | 29,700 |
| Probiotic (l) | 24 | 100 | 2,400 |
| Molasses (l) | 48 | 20 | 960 |
| Lime (kg) | 200 | 5 | 1,000 |
| Salt (kg) | 500 | 3 | 1,500 |
| Water ($m^3$) | 168 | 5.15 | 865,2 |
| Pineapple | 24 | 7 | 168 |
| Litmus paper (pack) | 1 | 35 | 35 |
| Transportation | 1 | 500 | 500 |

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| Cost items | Unit | Unit Price | Total Cost/ Production |
|---|---|---|---|
| Total variable cost without labor wage | | | 78,098.6 |
| Labor wage (share profit system) | | | 62,781.8 |
| Total variable cost with labor wage | | | 140,880.4 |

*Table.3: Total revenue of climbing perch culture business (IDR. 000). The values in the brackets indicate the average fish production and total revenue per each pond achieved.*

| Type of revenues | Production (kg) | Unit Price | Total Revenue |
|---|---|---|---|
| 1. The main revenue gained from fish cultured for eight months. | 2,880 (120) | 75 | 216,000 (9,000) |
| 2. The additional revenue from the selling of male fish that sorted during three months cultivation. | 1,200 (50) | 10 | 12,000 (500) |
| Total | 4,080 (170) | | 228,000 (9,500) |

*Table.4: Comparative NPVs that calculated at 7 % and 9 % of interest rates (IDR)*

| Year | NPV at 7 % | | | NPV at 9 % | | |
|---|---|---|---|---|---|---|
| | Total Cost | Revenues | Profit | Total Cost | Revenues | Profit |
| 0 | 167,634,000 | - | (167,634,000) | 167,634,000 | - | (167,634,000) |
| 1 | 154,409,533 | 213,084,112 | 58,674,579 | 151,576,330 | 209,174,312 | 57,597,982 |
| 2 | 267,358,372 | 398,288,060 | 130,929,688 | 257,637,068 | 383,806,077 | 126,169,009 |
| 3 | 134,867,266 | 186,115,916 | 51,248,650 | 27,578,765 | 176,057,833 | 48,479,069 |
| 4 | 233,521,156 | 347,880,217 | 14,359,060 | 216,847,965 | 323,041,896 | 106,193,931 |
| 5 | 117,798,293 | 2,560,849 | 44,762,556 | 107,380,494 | 148,184,356 | 40,803,862 |
| 6 | 203,966,422 | 303,852,054 | 99,885,632 | 182,516,594 | 271,897,901 | 89,381,307 |
| 7 | 102,889,591 | 141,986,941 | 9,097,350 | 90,380,013 | 124,723,808 | 34,343,795 |
| 8 | 178,152,172 | 265,396,152 | 87,243,980 | 153,620,566 | 228,851,024 | 75,230,458 |
| 9 | 89,867,754 | 124,016,893 | 34,149,139 | 76,071,049 | 104,977,534 | 28,906,485 |
| 10 | 155,605,007 | 231,807,277 | 76,202,271 | 129,299,357 | 192,619,328 | 63,319,971 |
| Total | 1,806,069,566 | 2,374,988,471 | 568,918,905 | 1,660,542,200 | 2,163,334,069 | 502,791,869 |

*Table.5: Identification of internal and external factors that interplay the culture business of De' Papuyu Farm using SWOT analysis.*

| **Internal Factor** | **External Factor** |
|---|---|
| *Strength* | *Opportunity* |
| 1. The only business of climbing perch fish farming with biofloc system in Banjarbaru city.<br>2. Low feeding rate<br>3. Can be used in a limited area.<br>4. Competence of skilled workers who have been trained and have certificate of expertise.<br>5. The probiotics used are resistant to uncertain weather conditions<br>6. Production does not depend on the season. | 1. Public interest towards climbing perch fish farming business.<br>2. High demand for climbing perch fish from both local and regional.<br>3. Fish farming can increase the role of the fishery sector.<br>4. Creating business partnerships such as seeding, processing and marketing business.<br>5. Creating jobs for local people |
| *Weaknesses* | *Threats* |
| 1. Fish farming business with biofloc system is very dependent on the electricity.<br>2. Culture facilities not well maintained.<br>3. The absence of business accounting records.<br>4. Inexperience of workforce.<br>5. Location of fish hatchery is quite far from the fish farming (± 200 km). | 1. The qualified seeds for fish farming activity are still limited in quantity.<br>2. Negative paradigm due to the failure of fish culture business in the community.<br>3. The newly seeds stocked in the pond is threated by wild bird.<br>4. Unstable electricity causes production failure. |

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| Internal Factor | External Factor |
|---|---|
| 6. The price of probiotics making is expensive | 5. Shared concern on fish farming business with biofloc system is still lacking |

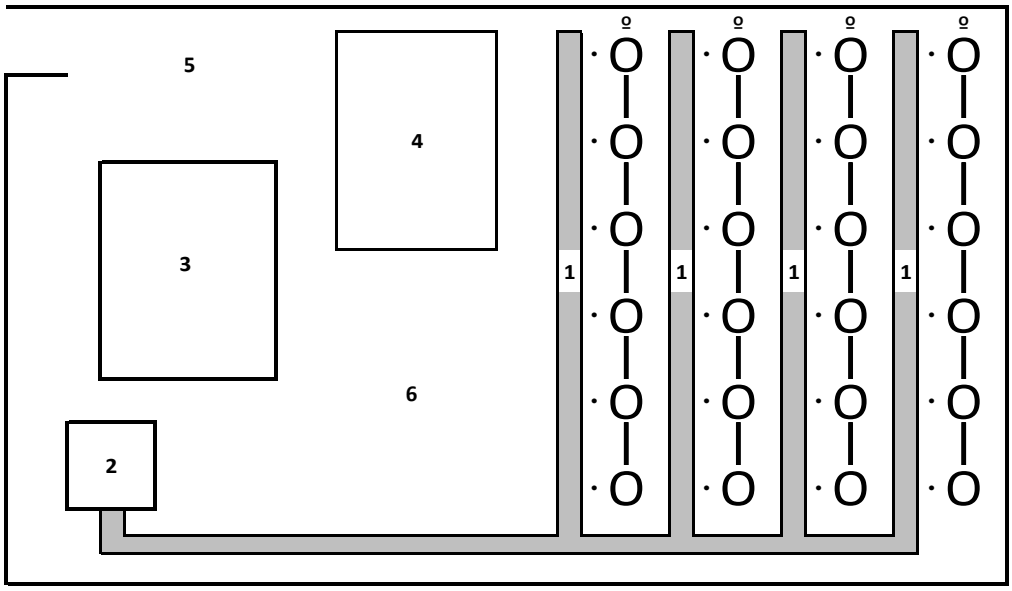


*Fig.1: The lay-out of De' Papuyu Farm Banjarbaru*
*1. Culture pond area, 2. Recycle ponds, 3. Security house/warehouse, 4. Hall, 5. Park area, 6. Space area*


*Fig.2: De' Papuyu Farm's facilities: 1. Typical culture pond, 2. Recycle pond, 3. Climbing perch*

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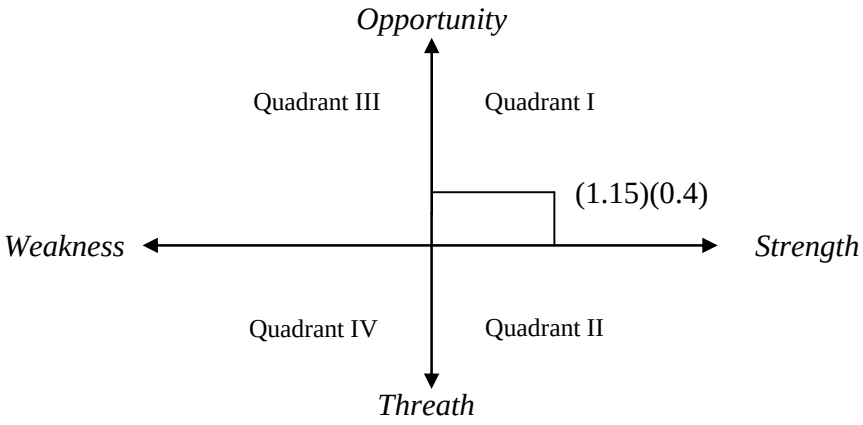


*Fig.3: SWOT analysis presenting the position of De' Papuyu Farm was in the first quadrant*