International Journal of Environment Agriculture and Biotechnology

(IJEAB)
An open access Peer-Reviewed InternationalJournal

International Journal of Environment, Agriculture and Biotechnology

Editor in Chief

Dr. Pietro Paolo Falciglia

Publisher

International Editorial Board/ Reviewer Board

FOREWORD

I am pleased to put into the hands of readers Volume-6; Issue-3: May-June 2021 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: July, 2021

Article   *Peer-Reviewed Journal*

Bioclimate influence on seed germination and seedling morphology parameters in *Pterocarpus erinaceus* Poir., 1804 (Fabaceae)

Beda Innocent Adji[1,2*], Doffou Sélastique Akaffou[1], Kouadio Henri Kouassi[1], Yao Patrice Houphouet[1], Jerôme Duminil[3], Sylvie Annabel Sabatier[2]

[1]Université Jean Lorougnon Guédé, Agroforestry UFR, BP 150, Daloa, Côte d'Ivoire
[2]CIRAD, UMR AMAP, F-34398 Montpellier, France ; AMAP, Université Montpellier, CIRAD, CNRS, INRA, IRD, Montpellier, France
[3]IRD, UMR-DIADE, Montpellier Cedex 5, France ; DIADE, Université Montpellier, CIRAD, IRD, Montpellier, France
*Corresponding Author: adjibedainnocent@gmail.com/beda-innocent.adji@cirad.fr

Abstract— Pterocarpus erinaceus is over-exploited for its multiple uses. Its exploitation and trade are strictly prohibited in many countries, including Côte d'Ivoire, to prevent its extinction. It is nevertheless imperative to restore populations of this species, while accounting for its germinative capacities and its adaptation to changing climates. The aim of this study was to assess the seed germination and seedling development in Pterocarpus erinaceus in three different environments. A total of 2,160 seeds from different seed trees and 540 individuals germinated from seeds were selected and evaluated. The trials were conducted at three sites with distinct microclimates (two nurseries in Côte d'Ivoire and one greenhouse in France). The results showed that the environment had a significant influence on germination parameters (P < 0.05), whereas the seed trees did not (P > 0.05). The environment influenced the height and internodes length of the seedlings (P < 0.05). However, the diameter, number of leaves, and the length and width of the leaves of the seedlings were statistically identical from one site to another (P > 0.05). Seed trees influenced the number and length of seedlings leaves (P<0.05). PCA showed that the seedlings developed better in the Montpellier greenhouse and at the Daloa site than Korhogo site. This information could guide the choice of ideal environments for the implementation of reforestation or agroforestry programs based on Pterocarpus erinaceus in the current context of climate change from a nursery. This study could be extended to other species in order to regenerate important species in disturbed ecosystems.

Keywords— Pterocarpus erinaceus, nurseries, greenhouse, environment, germination and seedling morphology, Côte d'Ivoire.

I. INTRODUCTION

Plant genetic resources, a component of biodiversity, the biological basis of the planet's environmental equilibrium, and a source of economic and ecological security for the future, provide the basis for sustainable development (Houndonougbo et al. 2020; Choat et al. 2012; United Nation 2002). Today, this biodiversity is threatened by overexploitation, yet the survival of all humanity depends on it. In 1992 in Rio de Janeiro, the United Nations adopted the following authoritative but not legally binding

declaration: "Forest resources and woodlands should be managed on a sustainable basis in order to meet the social, economic, ecological, cultural and spiritual needs of present and future generations". These needs relate to the products and services that the forest can provide such as wood and wood-based products, water, food, fodder, medicinal products, fuel, shelter, employment, recreation, wildlife habitat, a source of diversity in the landscape, the role of a carbon sink and reservoir, and many other products from the

forest. Appropriate measures should be taken to protect forests from the damaging effects (Leroy 2015).

In Africa, forest degradation is intensifying because the principles of sustainable forest management are being ignored (Rabiou et al. 2015; Dipelet et al. 2019). Targeted and selective exploitation of certain important forest species severely threatens the forest ecosystems of many West African countries (Segla et al. 2016, 2020; Mbowa et al. 2013). To respond effectively to the current environmental emergency, sustainable forest management must therefore become an integral part of farmers' usual agricultural practices. Regenerating the forest and/or reconciling forest with modern agriculture by associating important forest tree species (leguminous, food, fodder, and medicinal species) with crops is a choice and a major challenge that many African countries including Côte d'Ivoire want to take up (Reed + 2017).

Generally, trees are markers of the identity of the rural populations that use them in West Africa (Mabetty 2018). Among these trees, *Pterocapus erinaceus*., Poir (1804) from Fabaceae family called veneer wood « bois de vène » or Senegal rosewood is a spontaneous, forest fire-resistant, nitrogen-fixing CITES species and is native to fragile semi-arid habitats (CITES 2016; Anonymous 2016; Dumenu 2019). She originates from the Guinean-Sudanian and Sudano-Sahelian zones of West Africa and is highly overexploited for its multiple uses (quality timber, fodder, medicine, dyeing, soil restoration, etc.) (Ouedraogo et al. 2006; Rabiou et al. 2019; Goba et al. 2019; Segla et al. 2020). Indeed, recent years have been characterized by a spectacular increase in the trade of *Pterocarpus erinaceus*. This increase responds on the one hand to the growing demand for rosewood furniture in Asia, and on the other hand to the increasing scarcity of other species officially recognized as "rosewood" (several are listed in the CITES Appendices). It is estimated that exports of *Pterocarpus erinaceus* as logs to China increased by a factor of 2,000 between the third quarter of 2009 and the third quarter of 2015, from 70 m^3 to over 149,000 m^3 (Cop 17 2016). Widespread illegal and unsustainable exploitation of the species within its range has led many States of West Africa (i.e. Burkina Faso, Benin, Togo, Niger, Côte d'Ivoire) to enact total bans on harvesting and trade in the species in recent years, with the aim of preventing its extinction.

In Côte d'Ivoire, the exploitation of *Pterocarpus erinaceus* is prohibited by decree n° 2013-508 of 25 July 2013 (MINEF 2013). Despite these measures, its trade continues to grow steadily so that *Pterocarpus erinaceus* stands are almost non-existent in Côte d'Ivoire today. Given the importance of this species and faced with this problem,

which could lead to a loss of diversity that could lead to its extinction in Côte d'Ivoire, there is an urgent need to develop strategies for the rapid regeneration of this species.

Among several rapid regeneration solutions, domestication through germination tests has proved to be very effective in saving pioneer species that are threatened or overexploited in many countries (Amani et al. 2015; Douma et al. 2019; Adji et al. 2020). However, in the current context of climate change for the domestication of *Pterocarpus erinaceus*, several questions arise: (i) could the ever-changing environment have an effect on seed germination? (ii) are the dendrometric characteristics of the seed tree (ideal choice of seed trees) necessary to obtain a good germination rate? (iii) is the choice of vigorous seedlings resistant to climatic stress necessary? (iv) do seedlings of this species adapt to different types of environment? (v) is seedling development influenced by the type of seed tree? (vi) could the germination or morphology parameters of the seedlings guide the choice of an environment conducive to the implementation of a forest or agroforestry management programme? etc. This study was carried out with the aim of trying to answer all these questions. The objective is to evaluate the germination of seeds and the development of *Pterocarpus erinaceus* seedlings in a changing environment.

II. MATERIAL AND METHODS
2.1. Plant material

The plant material is composed of seeds obtained after shelling of mature fruits, from six seed trees growing more than 300 m apart in the same stand of *Pterocarpus erinaceus* and four-months old seedlings resulting from the germination of seeds collected under the six seed trees. All the seeds were collected from trees in good physiological condition in April 2019 at the experimental station (DeFo) of the CNRA (*Centre National de Recherche Agronomique*) in the Korhogo department of Côte d'Ivoire. The characteristics of the seed trees are listed in Table 1. The plant material used is the property of the CNRA of Côte d'Ivoire and the authorisation to use this plant material was given to us in the framework of this study via a partnership agreement signed and available on request between the said structure and our study project (EFISA). The formal identification of the species was undertaken by Poir in 1804 (Encycl.5 728.1804) (Anonymous 2019). A specimen of this material exists in the public herbarium (N°UCJ010935) of the CSRS (*Centre Suisse de Recherches Scientifiques*) in Côte d'Ivoire (Bakayoko et al. 2020) and the CNF (*Centre National de Floristique*) in Côte d'Ivoire (Koffi et al. 2018).

Table 1 Characteristics of Pterocarpus erinaceus seed trees and seeds used

Seed trees	DBH (cm)	H (m)	Age (year)	GPS coordinates		Number of seeds	Seed mass (g)		
				Longitude	Latitude		Mini	Maxi	Mean
1	24.36	15	23	-5.55112 W	9.5681 N	360	0.02	0.1	0.07 ± 0.01 **a**
2	32.32	18.5	26	-5.55119 W	9.56797 N	360	0.06	0.1	0.08 ± 0.01 **a**
3	31.05	18	26	-5.55134 W	9.56779 N	360	0.04	0.08	0.07 ± 0.01 **a**
4	26.11	15.5	22	-5.55122 W	9.56785 N	360	0.04	0.1	0.07 ± 0.01 **a**
5	29.78	16.5	25	-5.55132 W	9.56781 N	360	0.05	0.1	0.07 ± 0.01 **a**
6	23.88	16.5	25	-5.55131 W	9.56808 N	360	0.02	0.1	0.07 0.02 **a**
Pr > F									0.851

DBH = Diameter at chest height in centimetres; **H** = Height in metres; **W** = West; **N** = North; **Mini** = Minimum in grams; **Maxi** = Maximum in grams

2.2. Methods

2.2.1. Study sites

The trials were implemented April to September 2019 in three sites, two in Côte d'Ivoire and one in France with different microclimates. Of the two sites in Côte d'Ivoire, one was at the CNRA forest experimental station (hereafter DeFo) in Korhogo, and the other at the *Université Jean Lorougnon Guédé* (hereafter UJLoG), in Daloa. The trial in France took place in a controlled environment, in Greenhouse 8 at CIRAD (The French agricultural research and international cooperation organization working for the sustainable development of tropical and Mediterranean regions) in Montpellier. The characteristics of the study sites are listed in Table 2.

Table 2 Geographical location and characteristics of study sites (Guillaumet and Adjanohoun 1971; Perraud 1971; Loupe and Ouattara 1996; Bornand et al. 1999; FAO 2005; Goula et al. 2007; Soro 2011; Djaha et al. 2012; Millan 2016; Barry et al. 2018; AISA 2019; Anonymous 2019; Hérault et al. 2020).

Study sites or Bioclimates	Coordinates	Vegetation	Climate	Temperature	Rainfall (mm/year)	Soil type
Korhogo (DeFo)	9°570'80556''N 5°542'88889''W	Clear forest (wooded and grassy savannah)	Tropical dry	26.6 – 35.7 °C	817 - 1216	Ferruginous (90%) and Ferralitic (10%) superficial gravelly soil, deep gravel with a heavy texture, low in organic matter, highly desaturated.
Daloa (UJLoG)	6° 909'6363''N 6°438'1157''W	Dense rain forest	Wet tropical (sub-equatorial)	21 – 34 °C	1000 - 1900	Ferralitic, deep, acidic and desaturated in exchangeable bases, rich in organic matter
Montpellier (Greenhouse)	43°64981'N 3°86842'W	in the greenhouse	in the greenhouse	24 °C (night) - 32 °C (day)	10 cm³ per week for each pot	Mixture of Substrate Soil 1, Neuhaus N2 Bio, Tref Rice CIRAD 2 and extra-silica sand from bio

W = West; **N** = North; **°C** = degrees Celsius; **mm** = millimetre; **Substrate 1** = Iron, trace elements, perlite and coconut fibre; **Neuhaus N2 Bio** = vegetable co-composting, blond and black peat; **Tref Rice CIRAD 2** = clay, volcanic sand, perlite no. 2, coconut, Irish white peat and fine blond peat

2.2.2. Setting up the tests

Seed harvesting

Mature fruits were harvested in April and May 2019 on the seed trees using long wooden sticks with forks attached or by knocking the top of the tree with stones. The mature fruit collected under each seed tree was husked by hand to remove the thorny shells from the seeds. The seeds were then divided into three batches. Each batch contained seeds from all six seed trees (seed trees), i.e. 120 healthy seeds were selected per seed tree and per study site after sorting all the seeds collected (120 seeds x 6 seed trees x 3 test sites giving a total of 2,160 seeds of *Pterocarpus erinaceus*).

2.2.3. Test preparation and apparatus

Site 1 and 2: Korhogo and Daloa Nursery

Polyethylene black bags with drainage holes measuring 20 x 10 cm were filled with local soil and arranged in one block comprising six sub-blocks. Each sub-block was labelled with the seed tree's serial number and geographic coordinates and contained seeds harvested on and under one seed tree. Each sub-block contained 60 bags of soil prepared to receive two seeds each. The seeds from each seed tree were soaked in water for 12 hours to break seed dormancy and then sowed directly at a depth of approximately 2 cm in the bags at a rate of two seeds per bag. Before planting, the seeds were treated with granulated FURADAN to control rodents and after seedling emergence, the pre-leaves were treated with DECIS to limit insects' attacks. Nursery maintenance consisted of daily watering and manual weeding.

Site 3: Greenhouse at CIRAD, Montpellier

Polyester black pots with drainage holes measuring 30 x 15 cm were filled with a mixture of potting compost as specified above (Table 2). The pots were arranged in labelled blocks and sub-blocks in metal bins arranged in the same way as in Korhogo and Daloa. The seeds were sown in the same way as those from the other two sites in Côte d'Ivoire. Biological protection consisted of treatment with BIOBEST against greenhouse whiteflies. The pots were watered daily (10 cm^3 per week). all the pots occupied an area of about 12 m^2.

2.2.4. Data Collection

Germination parameters

Five parameters were evaluated:

- Latency time, i.e. the time it takes for the first seed to germinate from the sowing of all the seeds (Amani et al. 2015);

- Germination delay refers to the period between the sowing of each seed and the appearance of each seedling (N'golo et al. 2018);

- Germination speed, i.e. the average time after which 50% of the seeds have germinated (Berka and Abdelkader 2001; Diatta et al. 2009; Douma et al. 2019);

- Germination duration or Spread time, i.e. is the period between germination of the first seed and the last seed (Adji et al. 2020);

- Germination rate, i.e. the number of seeds sprouted divided by the number of total seeds sprouted, expressed as a percentage (Zerbo et al. 2010; Gorgon et al. 2015; Akaffou et al. 2019).

Development parameters

Six parameters were evaluated (Adji et al. 2020):

- Seedling height (SH), i.e. the length between the collar and the apex of the seedling;

- Diameter at the collar of seedling (Dcol), i.e. the base thickness of the main stem of the seedling;

- Number of leaves (LN°) corresponds to the number of leaves on the main stem of the seedling;

- Leaf length (LL) corresponds to the length from the beginning of the petiole to the end of the main vein of the leaf blade of each leaf present on the main stem of the seedling;

- Leaf width (LW) corresponds to the width of the leaf blade or to the line perpendicular to the main vein in the centre of the leaf blade;

- Length of the internodes (LIN), i.e. the length connecting two nodes or the length of two points of successive insertions of organs or leaf scars, from the base to the apex of the seedling.

All morphological parameters were measured using a ruler graduated in centimeters and an electronic caliper in millimeters.

2.2.5. Data analysis

Statistical analyses were first performed using one-dimensional descriptive statistics, link analysis (linear regression, correlation and covariance) and multidimensional analysis (principal component analysis, PCA) with XLSTAT 2020 version 7.5. The difference between the germination and development parameters was assessed using a two-factor multivariate analysis (MANOVA) with SAS software version 9.4. The Student-Newman-Keuls test at the 5% threshold was used for post-hoc comparisons.

III. RESULTS

Pterocarpus erinaceus is epigerminated with a hypocotyl remaining in the soil, the epicotyl is on average 8.74 cm long and 1.8 mm in diameter at the base (at an of average 9

days after sowing). The pre-leaves (at an average 10 days after sowing) have a long petiole in the same direction as the stem with two (2) stipules each at the base of the leaf sheath. The cotyledonary leaves remain visible for an average of 58 days. The phyllotaxis is alternate spiral with simple leaves with stipules until an average of 84 days, after which the seedling develops compound- imparipinnate and then compound-paripinnate leaves later (100 days on average). Branching appears on a few seedlings after the appearance of compound leaves (average 118 days).

3.1. Germination and development parameters by each study site

3.1.1. Germination parameters
Site 1: Korhogo

At the Korhogo site, the shortest germination latency was three days and was obtained with the seeds of seed trees 3, 5 and 6 (Fig 1a). The shortest germination delay ranged from four to 13 days to 6.17 ± 2.249 days, they were recorded in the seeds of seed tree 1 (Fig 1a). The seeds of seed trees 1 and 3 showed the fastest speed after nine days (Fig 1a). The shortest germination time was observed in the seeds of seed trees 1 and 2 with a duration of nine days (Fig 1a). The seeds of seed tree 6 had the highest germination rate with a rate of 86.66% (Fig 1b).
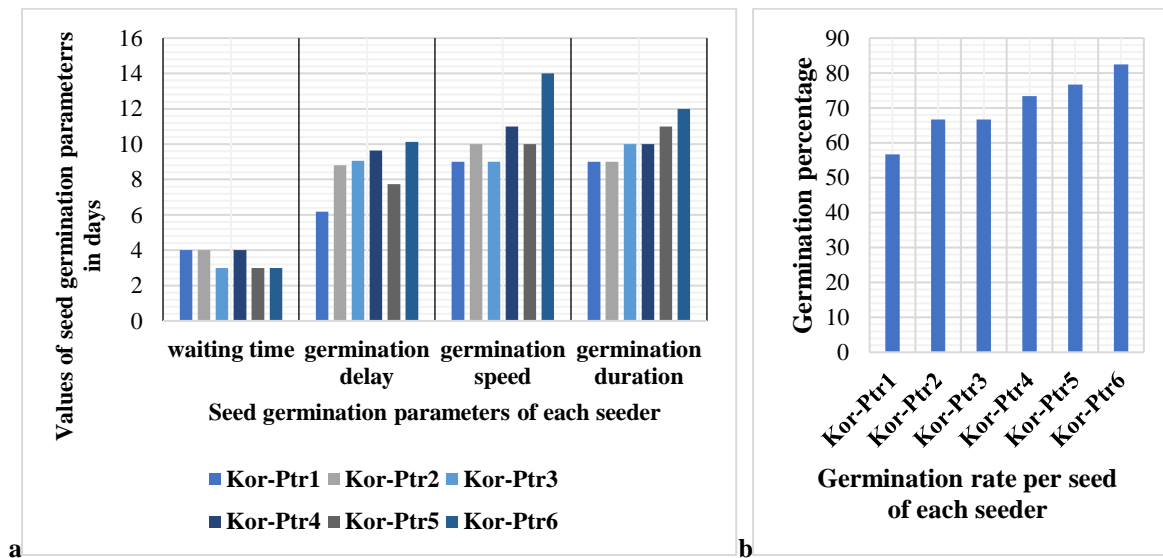
Kor-Ptr1=Seeds from seed tree 1, **Kor-Ptr2**=Seeds from seed tree 2, **Kor-Ptr3**=Seeds from seed tree 3, **Kor-Ptr4**=Seeds from seed tree 4, **Kor-Ptr5**=Seeds from seed tree 5, **Kor-Ptr6**=Seeds from seed tree 6

Fig 1. Distribution of seed germination parameters (a: Waiting time, germination delay, germination speed and germination duration; b: germination rate) of the six seed trees in Korhogo nursery

Site 2: Daloa

At Daloa site, the seeds of seed trees 1, 5 and 6 were characterized by short latencies, i.e., a period of three days (Fig 2a). The germination delays of the seeds of seed tree 1 were minimal ranging, from three to 12 days with an average of 6.357 ± 1.985 days (Fig 2a). The seeds of seed

trees 1 and 2 had the highest germination rate after six days (Fig 2a). The range of germination was smaller in the seeds of seed trees 2, 4 and 5 at 7 days (Fig 2a. Seeds of seed tree 5 had the highest germination rate at 96.66% (Fig 2b).
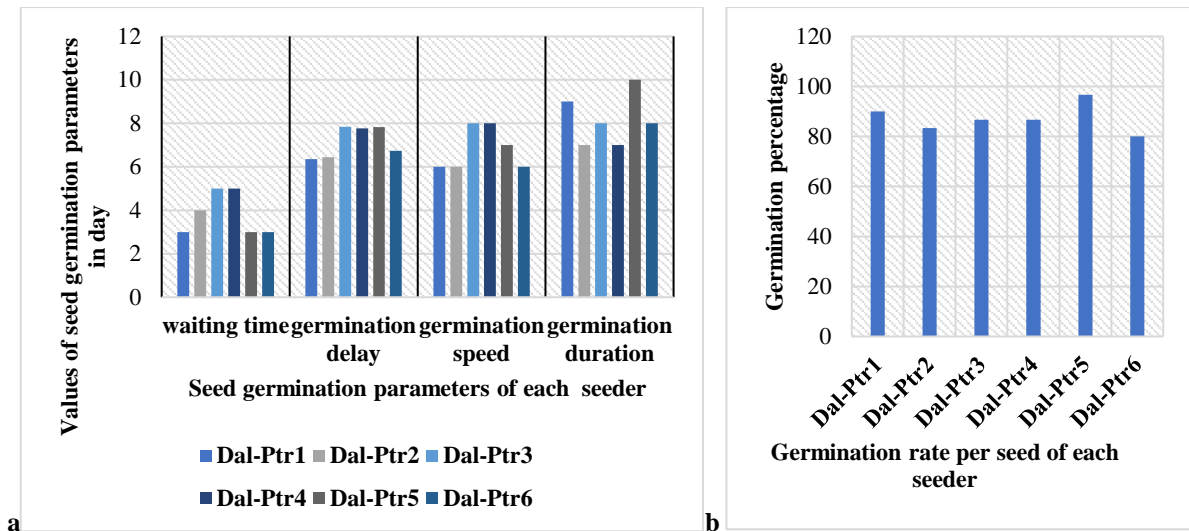
Dal-Ptr1=Seeds from seed tree 1, **Dal-Ptr2**=Seeds from seed tree 2, **Dal-Ptr3**=Seeds from seed tree 3, **Dal-Ptr4**=Seeds from seed tree 4, **Dal-Ptr5**=Seeds from seed tree 5, **Dal-Ptr6**=Seeds from seed tree 6

Fig 2. Distribution of seed germination parameters (a: Waiting time, germination delay, germination speed and germination duration; b: germination rate) of the six seed trees in Daloa nursery

Site 3: Montpellier greenhouse

In the CIRAD greenhouse (controlled environment), the shortest germination latency was seven days and was obtained with the seeds from seed trees 2, 5 and 6 (Fig 3a). The shortest germination delay ranged from seven to 14 days, average 9.33 ± 2.658 days, with the seeds of seed tree 2 (Fig 3a). The seeds of seed tree 2 were also the fastest after 14 days. The shortest germination duration (six days) was observed in the seeds of seed tree 1 with a duration of six days (Fig 3a). The seeds of seed tree 6 had the highest germination rate with a rate of 56.66% (Fig 3b).
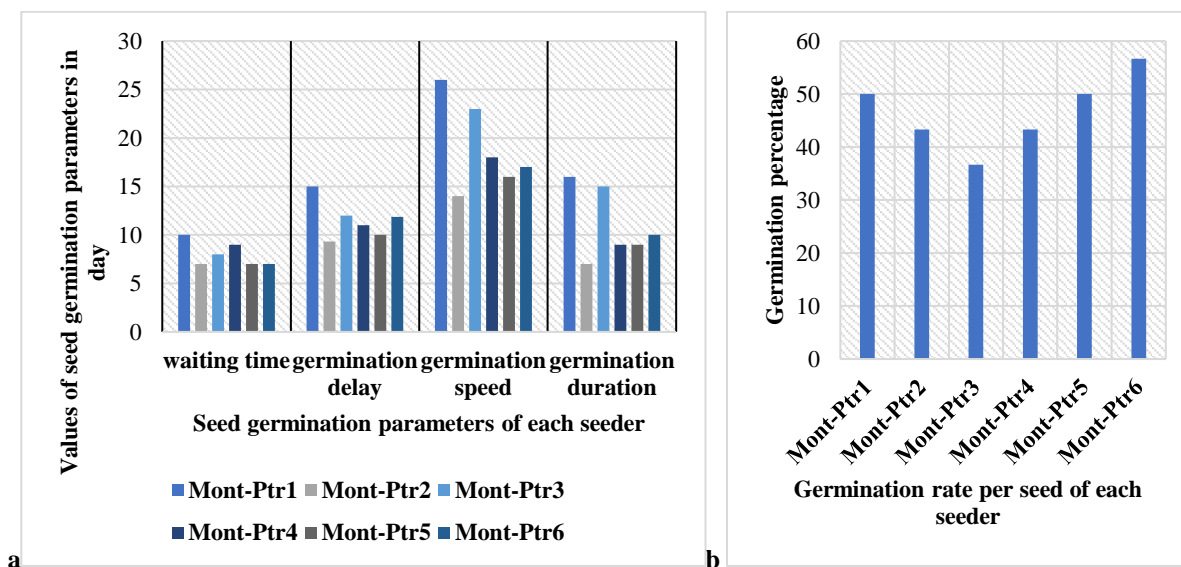
Mont-Ptr1=Seeds from seed tree 1, **Mont-Ptr2**=Seeds from seed tree 2, **Mont-Ptr3**=Seeds from seed tree 3, **Mont-Mont**=Seeds from seed tree 4, **Mont-Ptr5**=Seeds from seed tree 5, **Mont-Ptr6**=Seeds from seed tree 6

Fig 3. Distribution of seed germination parameters (a: Waiting time, germination delay, germination speed and germination duration; b: germination rate) of six seed trees in Montpellier greenhouse

3.1.2.   Development parameters

Comparison of morphological characteristics of the seedlings from each seed tree in Korhogo site (Table 3) showed that all the seedlings from all the seed trees combined had identical collar diameters (P > 0.05). In contrast, the height, number of leaves, length of leaves, width of leaves and length of internodes all differed significantly (P<0.05).

At the Daloa site, comparison of morphological characteristics of seedlings from seed trees (Table 3) showed statistically significant variability of variables among the seed trees' seedlings. Height, number of leaves, the length and width of seedling leaves (P < 0.05). The variables collar diameter and internode length were statistically identical from one seedling to another (P > 0.05).

Comparison of morphological characteristics of the seedlings from each seed tree in Montpellier greenhouse (Table 3) showed that all the morphological characteristics of seedlings grown from each seed tree and observed in the Montpellier site differed from each other (P < 0.05).

Table 3 Comparison of morphological characteristics of seedlings according to the seed trees used per study site

Sites	Seed trees	SH (cm)	Dcol (mm)	N°L	LL (cm)	LW (cm)	LIN (cm)
Korhogo	Seed tree-1	11.34±1.24 ab	1.98±0.21 a	5.6±0.44 b	5.87±0.34 a	5.74±0.61 a	1.61±0.51 b
	Seed tree-2	9.7±1.36 b	2.04±0.51 a	6.31±0.41 ab	6.21±0.56 a	6.02±0.31 a	2.06±0.14 a
	Seed tree-3	12.6±0.74 ab	2.06±0.34 a	7.54±0.53 a	5.37±0.47 ab	5.2±0.22 ab	2.41±0.16 a
	Seed tree-4	15.74±1.51 a	2.62±0.42 a	6.31±0.53 ab	5.64±0.47 ab	5.08±0.34 ab	1.86±0.23 b
	Seed tree-5	12.63±1.47 ab	2.31±0.13 a	7.51±0.62 a	4.62±0.53 b	4.12±0.25 b	2.19±0.24 a
	Seed tree-6	10.63±1.71 b	2.58±0.26 a	5.1±0.33 b	4.23±0.72 b	3.8±0.62 b	1.57±0.06 b
	Pr > F	**0.0001**	**0.652**	**0.001**	**0.0071**	**0.0311**	**0.0114**
Daloa	Seed tree-1	16.38±1.21 ab	2.05±0.25 a	5.4±0.71 b	4.7±0.17 ab	3.98±0.22 b	2.8±0.10 a
	Seed tree-2	14.12±1.11 ab	2.64±0.23 a	7.6±0.62 ab	5.8±0.27 a	5.1±0.41 a	3.8±0.13 a
	Seed tree-3	13.5±1.53 b	2.67±0.32 a	7.58±0.42 ab	5.56±0.31 a	4.54±0.42 ab	3.2±0.21 a
	Seed tree-4	14.57±1.41 ab	2.33±0.22 a	5.65±0.34 b	4.78±0.32 ab	3.68±0.34 b	2.4±0.11 a
	Seed tree-5	18.29±0.84 a	2.71±0.14 a	8.54±0.36 a	5.62±0.12 a	5.55±0.61 a	3.7±0.51 a
	Seed tree-6	16.62±1.35 ab	2.63±0.34 a	7.21±0.44 ab	3.8±0.51 b	3.9±0.31 b	3±0.21 a
	Pr > F	**0.041**	**0.812**	**0.011**	**0.0221**	**0.001**	**0.921**
Montpellier (Greenhouse)	Seed tree-1	14.65±1.22 b	2.95±0.36 a	6.7±0.39 ab	5.24±0.52 a	5.8±0.34 a	3.1±0.41 ab
	Seed tree-2	16.07±1.34 ab	1.55±0.43 b	7.6±0.53 ab	4.79±0.34 ab	4.3±0.41 ab	3.61±0.16 ab
	Seed tree-3	18.7±1.52 a	2.61±0.34 a	7.4±0.47 ab	5.8±0.22 a	5.7±0.16 a	4.31±0.31 ab
	Seed tree-4	16.77±1.37 ab	2.31±0.12 a	5.97±0.63 b	4.31±0.34 ab	3.5±0.24 b	2.34±0.25 b
	Seed tree-5	16.42±1.43 ab	2.92±0.15 a	8.3±0.46 a	4.53±0.51 ab	4.36±0.21 ab	3.42±0.17 ab
	Seed tree-6	15.2±1.65 ab	2.87±0.12 a	6.96±0.26 ab	3.97±0.13 b	3.82±0.34 b	5.08±0.37 a
	Pr > F	**0.001**	**0.04991**	**0.0001**	**0.001**	**0.001**	**0.0001**

For each character, values with the same letters are not statistically different at the 5% threshold.

SH: seedling height; **Dcol**: Diameter at the collar of the seedlings; **N°L**: Number of leaves; **LL**: Leaf length; **LW**: Leaf Width; **LIN**: Length of the internodes; **cm**: centimetres; **mm**: millimetres.

3.2. Global influence of study sites and seed trees on seed germination and seedling development in *Pterocarpus erinaceus*

3.2.1.   Influence on seeds germination

Figure 4 is an overview of all germination parameters observed at the three study sites. She indicates that the highest germination rate was measured at the Daloa site. However, the highest values for the four other parameters

(waiting time, germination delay, germination speed and germination spread time) were recorded in Montpellier greenhouse.
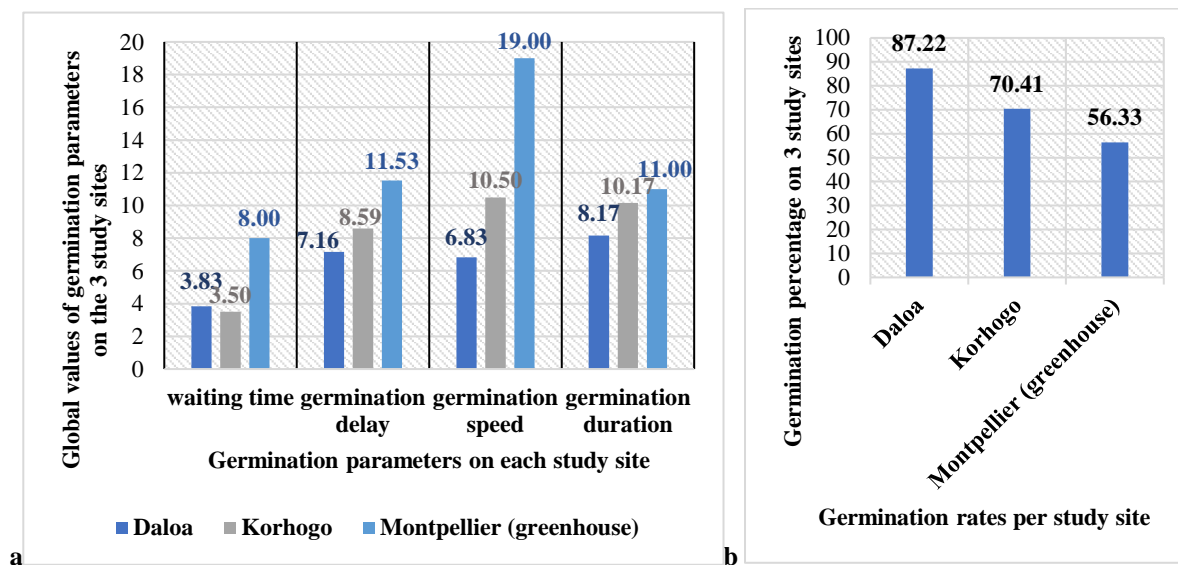
Fig 4. Global distribution of each germination parameter (a: Waiting time, germination delay, germination speed and germination duration; b: germination rate) for the all study sites.

According to Table 4, all germination parameters differed statistically (P < 0.05) from site to site, except for germination spread, which is identical from site to site (P>0.05). The analysis of variance (Table 4) of germination parameters according to the types of seed trees used showed that all observed variables were statistically identical from one seed tree to another (P > 0.05) for all three sites.

Table 4 Comparison of germination parameters according to the bioclimates and seed trees used

Sites or bioclimates/Seed trees	waiting time	germination delay	germination speed	germination duration	germination rate
Korhogo	3.50 ± 0.22 **b**	8.59 ± 0.58 **b**	10.50 ± 0.76 **b**	10.17 ± 0.48 **a**	70.41 ± 3.70 **b**
Daloa	3.83 ± 0.40 **b**	7.16 ± 0.29 **b**	6.83 ± 0.40 **c**	8.17 ± 0.47 **a**	87.22 ± 2.34 **a**
Montpellier (Greenhouse)	8 ± 0.52 **a**	11.53 ± 0.81 **a**	19 ± 1.86 **a**	11 ± 1.48 **a**	56.33 ± 3.36 **c**
Pr > F	**0.0001**	**0.0004**	**0.001**	**0.1251**	**0.0001**
Seed tree-1	5.67 ± 2.18 **a**	9.18 ± 2.91 **a**	13.67 ± 6.23 **a**	11.33 ± 2.33 **a**	65.55 ± 12.37 **a**
Seed tree-2	5 ± 1 **a**	8.19 ± 0.89 **a**	10 ± 2.31 **a**	7.67 ± 0.66 **a**	66.66 ± 9.62 **a**
Seed tree-3	5.33 ± 1.45 **a**	9.63 ± 1.23 **a**	13.33 ± 4.84 **a**	11 ± 2.08 **a**	67.77 ± 10.59 **a**
Seed tree-4	6 ± 1.53 **a**	9.47 ± 0.94 **a**	12.33 ± 2.96 **a**	8.67 ± 0.88 **a**	71.99 ± 8.87 **a**
Seed tree-5	4.33 ± 1.33 **a**	8.52 ± 0.74 **a**	11 ± 2.65 **a**	10 ± 0.58 **a**	78.44 ± 10.05 **a**
Seed tree-6	4.33 ± 1.33 **a**	9.57 ± 1.50 **a**	12.33 ± 3.28 **a**	10 ± 1.15 **a**	77.50 ± 3.82 **a**
Pr > F	**0.9534**	**0.9762**	**0.9843**	**0.5011**	**0.8784**

For each character, values with the same letters are not statistically different at the 5% threshold.

Table 5 shows a strong positive correlation between first germination and germination delay and then germination speed at all three study sites: between germination delay and germination speed and then

germination duration and between germination speed and germination time. However, this matrix indicated a negative correlation between germination rate and waiting time, germination delay and germination speed.

Table 5 Total correlation matrix (Pearson) between germination parameters

Variables	Waiting time	Germination delay	Germination speed	Germination duration	Germination rate
Waiting time	**1**				
Germination delay	**0.796**	**1**			
Germination speed	**0.870**	**0.943**	**1**		
Germination duration	0.396	**0.733**	**0.746**	**1**	
Germination rate	**-0.720**	**-0.609**	**-0.747**	-0.393	**1**

Values in bold are different from 0 at significance level alpha=0.05

3.2.2. Influence on seedling development

The results (Table 6) showed that only the heights and lengths of the internodes differed from one bioclimate to another (P < 0.05). The collar diameter, number of leaves, the length and width of the leaves were the same from one bioclimate to another (P > 0.05).

Analysis of variance of the seedlings from one seed tree to another at all three sites (Table 6) indicated that only the number of seedling leaves and leaf length differed significantly in seedlings from one seed tree to another (P < 0.05). Height, diameter, leaf width and successive internode lengths of seedlings were all statistically identical from one seedling to another (P > 0.05).

Table 6 Comparison of morphological parameters according to the bioclimates and seed trees used

Sites or bioclimates/Seed trees	SH (cm)	Dcol (mm)	N°L	LL (cm)	LW (cm)	LIN (cm)
Daloa	15.58±0.74 **a**	2.50±0.11 a	6.99±0.49 a	5.04±0.31 a	4.46±0.30 a	3.15±0.22 a
Korhogo	12.11±0.86 **b**	2.26±0.12 a	6.39±0.40 a	5.32±0.31 a	4.99±0.36 a	1.95±0.14 **b**
Montpellier (Greenhouse)	16.30±0.58 **a**	2.53±0.22 a	7.15±0.33 a	4.77±0.27 a	4.58±0.39 a	3.64±0.39 a
Pr > F	**0.0024**	**0.4292**	**0.4168**	**0.4455**	**0.5456**	**0.0014**
Seed tree-1	14.12±1.48 a	2.33±0.31 a	5.90±0.40 **b**	5.27±0.34 **ab**	5.17±0.59 a	2.50±0.45 a
Seed tree-2	13.29±1.88 a	2.08±0.31 a	7.17±0.43 **ab**	5.60±0.42 a	5.14±0.49 a	3.16±0.55 a
Seed tree-3	14.93±1.90 a	2.45±0.19 a	7.51±0.05 **ab**	5.58±0.12 a	5.15±0.34 a	3.31±0.55 a
Seed tree-4	15.69±0.64 a	2.42±0.10 a	5.98±0.19 **b**	4.91±0.39 **ab**	4.09±0.49 a	2.20±0.17 a
Seed tree-5	15.78±1.66 a	2.65±0.18 a	8.12±0.31 a	4.92±0.35 **ab**	4.68±0.44 a	3.10±0.46 a
Seed tree-6	14.15±1.81 a	2.69±0.09 a	6.42±0.66 **b**	4±0.13 **b**	3.84±0.03 a	3.22±1.02 a
Pr > F	**0.8629**	**0.4272**	**0.0094**	**0.0335**	**0.1900**	**0.7129**

For each character, values with the same letters are not statistically different at the 5% threshold.

SH: seedling height; **Dcol**: Diameter at the collar of the seedlings; **N°L**: Number of leaves; **LL**: Leaf length; **LW**: Leaf Width; **LIN**: Length of the internodes; **cm**: centimetres; **mm**: millimetres.

Figure 5 shows the projection of bioclimates and morphological parameters for all seedlings on PCA 1-2 (biplot). Analysis of the matrix of factor weights showed that two components explained 100% of the variability and are therefore highly relevant in explaining the total variation between the morphological characteristics of the seedlings and the bioclimates. Plan 1-2 s characterized by eigenvalues of 95.40% for axis F1 and 4.60% for axis F2. The different descriptors contributing to the formation of the first component (F1) and the second component (F2) revealed two groups. The first group consisted of the Montpellier (greenhouse) and the Daloa site characterized by higher heights, collar diameters, numbers of leaves and internode lengths of the seedlings than at the Korhogo site. The second group consisted of the Korhogo site, which was characterized by seedlings with longer and wider leaves than those at the Daloa site and the Montpellier greenhouse.
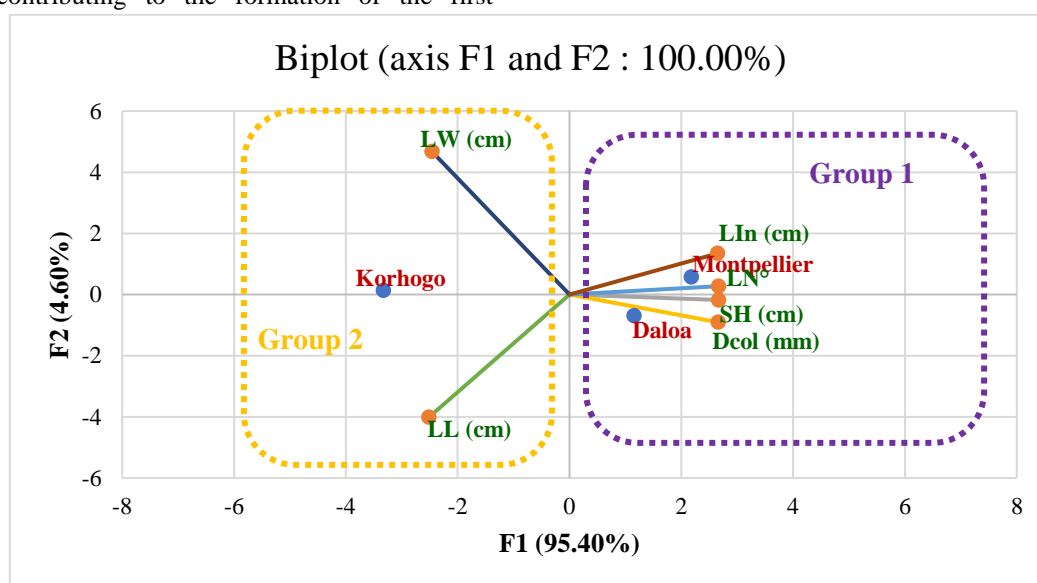
SH: seedling height; **Dcol**: Diameter at the collar of the seedlings; **N°L**: Number of leaves; **LL**: Leaf length; **LW**: Leaf Width; **LIN**: Length of internodes; **cm**: centimetres; **mm**: millimetres.

Fig 5. Projection of bioclimates and morphological parameters observed in PCA Plan 1-2 as a function of axis type

IV.   DISCUSSION

4.1. Germination parameters

The germination of *Pterocarpus erinaceus* is epigeal generally. Our results confirm those of Adou et al., in 2013 and N'golo et al., in 2018, who all found the same type of germination. At germination, the leaves are simple with alternating spiral phyllotaxis and it is over time that compound leaves appear. This is due to the fact that in the very young stage, the seedling depends on the reserve (starch) contained in the embryo (seed) which only serves to emit simple pre-leaves with long petioles. These pre-leaves take over via photosynthetic activity following the disappearance of the embryo and the cotyledonary leaves. The seedling thus becomes independent and expresses its normal organogenesis by producing compound leaves (after 84 days and at 100 days on average). In fact, the cost of building its authentic development is high and therefore requires progressive adaptation. The seeds were shelled in our study because of pericardial dormancy. Indeed, studies by Lauries in 1974, Rajendrudu and Naidu in 2001, Adou et al in 2013, Ameri et al in 2017 and N'golo et al in 2018 respectively on *Pterocarpus angolensis*, *Pterocarpus santalinus*, *Pterocarpus erinaceus*, *Faidherbia albida* and *Pterocarpus erinaceus* showed that the pericarp has a negative effect on germination rate and germination speed.

Our results showed overall that the seeds germination performances in Montpellier were poor; this is due to poor seed storage (long storage at an average temperature of 28 °C). In fact, the seeds were harvested in April, the peeled seeds were stored at room temperature (25 °C night and 32 °C day) for one and a half months until their shipment in Montpellier in mid-June. As the seeds were not stored at a low temperature, we think that the heat had a negative effect on the germination capacity of the seeds like in the study of

Pterocarpus erinaceus seeds by N'golo et al., in 2018 in the Haut Bandama reserve in Côte d'Ivoire and in the study of *Faiherbia albida* seeds by Ameri et al. (2017). However, good performances were recorded in Daloa probably due to the humid tropical climate that favoured the harmonious development of forest species and the relatively rich soil. Indeed, several studies have demonstrated the effect of climate and soil poverty on the germination of certain species (Giordano 1972; Soloviev et al. 2004; Dianda et al. 2009; Sambe et al. 2010). In addition, the seeds were sown immediately after harvesting and shelling at the Korhogo and Daloa sites, which explains the short germination latency times observed at these two sites; moreover, Daloa is a transition zone between forest and savannah, both savannah and forest species are found there, and all crops that depend on the savannah and forest zones develop there without exception. Korhogo follows just after Daloa in terms of recorded performances, indeed Korhogo is part of the distribution area of *Pterocarpus erinaceus* (Adjonou et al. 2010; Ouedraogo et al., 2006; Kossi et al., 2015; Anonymous 2016). In the same distribution area (in Niger), Amani et al. (2015) reported high germination rates ranging from 70 to 100% in four species of Combretaceae with germination duration of 9 to 18 days. In spite of the absence of pre-treatment of the seeds, the results were satisfactory, in contrast to those of Adou et al. (2013), who reported a germination rate of 29%, a latency time of four days and a germination duration of 11 days. In the study by N'golo et al. (2018), the seeds were pre-treated, and the maximum germination rate was 68.5% with a maximum delay of days and a maximum germination speed of 11.42 days. Ameri et al. (2017) obtained a low germination rate (27%) with non-pre-treated seeds of *Faidherbia albida*; whereas Ahoton et al., in 2009 and Amonum et al., in 2016 obtained a high germination rate with treated seeds of *Parkia biglobosa*.

Analysis of variance indicated that there was no significant difference between germination parameters according to the seed trees, however germination was expressed differently from one site to another except for the germination spread parameter which was statistically identical regardless of the study site. This is because although the composition and variability of seeds were practically the same (size and mass) from one seed tree to another, the environmental conditions (climate and soil type) were influenced germination. this is the case for many study (Assogbadjo et al., 2005, 2006; Dianda et al., 2009; Sambe et al., 2010). Indeed, the plant adapts to the conditions imposed by the microclimate (environment of location).

The correlation matrix showed that the germination rate is negatively correlated with all other germination parameters. This means that the higher the germination rate, the lower the germination latency time, the shorter the germination delay, the faster the speed (fewer days) and the shorter the germination spread.

4.2. Development parameters

Generally high values were recorded at the Montpellier greenhouse because of the stable climate (no temperature fluctuations or insect attacks) in the greenhouse and a substrate rich in mineral elements that allowed the seedlings to develop well and flourish. Morphological parameters were moderately poor in Korhogo due to the unstable dry tropical climate and abundant attacks by larvae. Studies by Salazar and Quesada (1987), Dianda and Chalifour (2002), Maranz and Wiesman (2003), Soloviev et al. (2004) have already shown the effect of the original climate or climatic zone, soil, mother trees and soil poverty on the germination, growth and morphological development of several plant species. The results obtained by N'golo et al. (2018) contradict ours, as they indicate that at two weeks, the number of leaves was six, the average height of the seedlings was 7.5 cm and the diameter was 1 mm, and three months later the seedlings reached a diameter of 5 mm with an average height of 40 cm. In the first results of SODEFOR research on *Pterocarpus erinaceus*, Adou et al. (2013) reported average plantlet heights of 4.5 cm and 5.4 cm in, respectively, three weeks and one month. Using five growth accelerators on a legume (*Parkia biglobosa*) of the same family (Fabaceae) as *Pterocapus erinaceus* in Benin at 140 days, Gnanglé et al. (2010) reported an average seedling height of 26.3 cm, an average diameter of 6.6 mm with an average of 8.5 leaves. On the other hand, in Niger, Amani et al. (2015), reported heights ranging from 7.71 cm to 25.8 cm with diameters ranging from 2.68 mm to 3.22 mm in four families of Combretaceae.

Analysis of variance showed that there was no significant difference from one seed tree to another for morphological parameters, and only the number of leaves and leaf length differed from one seed tree to another. However, the site only influenced the height of seedlings and the length of internodes. The climatic conditions of the site affected seedling length. The more favourable the conditions (rich soil and humid tropical climate), the vigor and larger the seedling (Montpellier and Daloa sites). This fact was confirmed by the principal component analysis, which indicated that in Montpellier and Daloa sites, seedling heights, collar diameters, leaf counts and internode lengths were higher than those observed in Korhogo.

V.   CONCLUSION

This study found that a long shelf life of *Pterocarpus erinaceus* seed at room temperature had a negative effect on the performance and germination of dehulled seeds. The study also showed that the germinative expression was

basically the same from one seed tree to another and that only the site significantly influenced the germination expression through the climate and the type of soil. Correlations showed that the higher the germination rate, the shorter the waiting time, the shorter the germination delay, the slower the germination speed and the shorter the germination duration. The study also showed that a stable (greenhouse), healthy climate (with no larval attacks), rich soil and a humid tropical climate are essential for the harmonious development of *Pterocarpus erinaceus* seedlings, as the height, diameter, number of leaves and length of internodes are higher. The seedlings morphological parameters were almost identical from one seed tree to another (p > 0.05), while the site significantly influenced the height and length of the seedling internodes (p < 0.05). Principal component analysis showed that the Montpellier greenhouse and the Daloa site are favorable because they resulted in greater heights, collar diameters, leaf counts and internode lengths than the Korhogo site. these results can be a decision-making guide for the artificial regeneration of *Pterocarpus erinaceus* stands, for the establishment of permanent plots within the framework of reforestation or agroforestry programmes based on this species from a nursery. They are a useful source of information for nurserymen and for the sustainable management of *Pterocarpus erinaceus* forest populations in Côte d'Ivoire and the West African sub-region.

VI. CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Beda I Adji: Conception, methodology, supervision, software, formal analysis, writing the paper, resources, data acquisition and analysis. **Sélastique D Akaffou**: Paper reading, Project administration, methodology, resources, data acquisition, supervision, writing the paper - original project, research and acquisition of funding. **Henri K Kouassi**, **Yao P Houphouet** and **Jerôme Duminil**: writing the paper - original project, research and acquisition of funding. **Sylvie A Sabatier**: Paper reading, Project administration, methodology, resources, data acquisition, supervision, writing the paper - original project, research and acquisition of funding.

VII. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest either between the authors, or between the organisations which financed the work, or on the site used for the experiments. All the authors agree to the publication of the submitted version of the paper.

VIII. DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

REFERENCES

[1] Adji, B.I., Akaffou, S.D., Kouassi, K.H., Houphouet, Y.P., Duminil, J. and Sabatier, S. (2020) Influence of Different Environments on Germination Parameters and Seedling Morphology in Khaya senegalensis (Desr.) A. Juss (Meliaceae). American Journal of Plant Sciences, 11, 1579-1600. https://doi.org/10.4236/ajps.2020.1110114

[2] Adjonou K, Ali N, Kokutse AD, Kokou K (2010) Étude de la dynamique des peuplements naturels de *Pterocarpus erinaceus* Poir. (Fabaceae) surexploités au Togo. Bois et Forêts des Tropiques, N°306 (1), pp 33-43.

[3] Adou k, konan kJc, Diarra F (2013) Note sur le Vène. Premiers résultats de travaux de recherche sur le vène, a la sodefor. 2p. http://ci.chm-cbd.net/biodiversity/fauneflore/flore/flore-terrestre/note-sur-le-vene

[4] Ahoton LE, Adjakpa JB, M'po IM, Akpo EL (2009) Effet des prétraitements des semences sur la germination de *Prosopis africana* (Guill., Perrot. et Rich.) Taub., (Césalpiniacées). Tropicultura 27 (4) : 233-238.

[5] AISA (2019) Association Ivoirienne des Sciences Agronomiques. 10 p. https://pratik.ci/annuaire

[6] Akaffou SD, Kouamé KA, Gore BBN, Abessika YG, Kouassi KH, Hamon P, Sabatier S, Duminil J (2019) Effect of the seeds provenance and treatment on the germination rate and plants growth of four forest trees species of Côte d'Ivoire. J. For. Res. 10 p. DOI : https ://doi.org/10.1007/s11676-019-01064-y

[7] Anonymous (2019) *Pterocarpus erinaceus* Poir. First published in Lamarck's JBAM, Encycl. 5: 728 (1804). In the GBIF Secretariat (2019). Taxonomy of the GBIF Ridge. Checklist dataset https://doi.org/10.15468/39omei accessed via GBIF.org on 2021-03-05.

[8] Anonymous (2019) Https:// www.fr-climatedata.org. Accessed in October 2019.

[9] Anonymous (2016) https://uses.plantnet-project.org/en/*Pterocarpus_erinaceus* (PROTA). https:// www.prota4u.org/database /protav8.asp?fr=1&h=M 4&t=*Pterocarpus,erinaceus*&p =Pterocarpus+erinaceus# Synonyms

[10] Amani MM, Inoussa I, Dan Guimbo A, Mahamane M, Saadou AM, Lykke (2015) Germination et croissance de quatre espèces de Combretaceae en pépinière. Tropicultura 33 (2) : 123-145

[11] Ameri AH, Daldoum AMD (2017) Effect of different pretreatment methods and materials on germination potential of *Faidherbia albida* seeds, Scholars Journal of Agriculture and Veterinary Sciences 4 (3) : 86-90.

[12] Amonum JI, Nyam RT, Gbande S (2016) Effect of pre-treatments on seed germination of *Parkia biglobosa* (Benth). Journal of Research in Forestery, Wildlife and Environment 8 (4) : 364-369.

[13] Assogbadjo AE, Kyndt T, Sinsin B, Gheysen G, Van Damme P (2006) Patterns of genetic and morphometric diversity in baobab (*Adansonia digitata* L.) populations across different climatic zones of Benin (West Africa). Annals of Botany, 97 : 819-830.

[14] Assogbadjo AE, Sinsin B, Codjia JTC, Van Damme P (2005) Ecological diversity and pulp, seed and kernel production of the baobab (*Adansonia digitata*) in Benin. Belgian Journal of Botany. 138(1) : 47 56.

[15] Bakayoko A, Khatelain C, Kone MW, Kone I, Ouattara D, Yao K, Gautier L (2020) Occurrences des échantillons de plantes de l'Herbier du Centre Suisse de Recherches Scientifiques (CSRS) en Côte d'Ivoire. Version 1.2. Centre Suisse de Recherches Scientifiques en Côte d'Ivoire. Occurrence dataset https://doi.org/10.15468/gdbrsj Ajouter au projet Citavi par DOI accessed via GBIF.org on 2021-03-05.

[16] Barry AA, Caesar J, Tank AMGK, Aguilar E, McSweeney C, Cyrille AM, Nikiema MP, Narcisse KB, Sima F, Stafford G, Touray LM, Ayilari-Naa JA, Mendes CL, Tounkara M, Gar-Glahn EVS, Coulibaly MS, Dieh MF, Mouhaimouni M, Oyegade JA, Sambou E, Laogbessi ET (2018). West Africa climate extremes and climate change indices. Int. J. Climatol. 38, e921–e938. https://doi.org/10.1002/joc.5420.

[17] Berka S, Abdelkader H (2001) Effets de quelques traitements physico-chimiques et de la température sur la faculté germinative de la graine d'Arganier. *Revue Forestière Française* 53 (2) :125-130.

[18] Bornand M, Barthès JP, Bonfils P (1999) Carte des pédopaysage de la région languédoc-Roussillon-Département de l'Hérault. INRA Montpellier 2010. 2p

[19] Choat B, Jansen S, Brodribb TJ, Cochard H, Delzon S, Bhaskar R, Bucci SJ, Feild TS, Gleason SM, Hacke UG, Jacobsen AL, Lens F, Maherali H, Martinez-Vilalta J, Mayr S, Mencuccini M, Mitchell PJ, Nardini A, Pittermann J, Pratt RB, Sperry JS, Westoby M, Wright IJ, Zanne AE (2012) Global convergence in the vulnerability of forests to drought. Nature (491) : 752-755. doi:10.1038/nature11688

[20] CITES Secrétariat (2016) Notification of the Parties No. 2016/008. https://cites.org/sites/default/files/notif/E Notif 2016 008.pdf

[21] CoP17 Prop. 57 (2016) Convention sur le commerce international des espèces de faune et de flore sauvages menacées d'extinction. « dix-septième session de la conférence des parties Johannesburg (Afrique du sud) ; 24 septembre 5 octobre 2016 : examen des propositions d'amendement des annexes I et II ». 17 p.

[22] Dianda M, Bayala J, Diop T, Ouédraogo SJ (2009) Improving growth of shea butter tree (*Vitellaria paradoxa* C.F.Gaertn.) seedlings using mineral N, P and arbuscular mycorrhizal (AM) fungi. Biotechnol. Agron. Soc. Environ., 13(1) : 93-102.

[23] Dianda M, Chalifour FP (2002) Effets du N minéral et du génotype de la plante sur la croissance et la nodulation de *Faidherbia albida*. Can. J. Bot., 80(3) : 241-254.

[24] Diatta S, Salifou I, Sy Mo, Kabore-Zoungrana CY, Banoin M, Akpo LE (2009) Évaluation des potentialités germinatives d'un ligneux fourrager sahélien : Maerua crassifolia Forssk., Capparaceae. 1-11p http://www.irrd.org

[25] Dipelet UGB, Doumenge C, Loumeto JL, Jacques F, Gonmadje C, Doyle M (2019) Des confusions entre espèces préjudiciables à la gestion durable des essences forestières : l'exemple des acajous d'Afrique (Khaya, Meliaceae). Bois et forêts des tropiques 339 (1) : p 17-32.

[26] Djaha JA, N'da AAA, Koffi EK, Ballo CK, Coulibaly M (2012) Croissance et aptitude au greffage de deux génotypes d'anacardier (*Anacardium occidentale* L.) élites utilisées comme porte–greffe en Côte d'Ivoire. Int. J. Biol. Chem. Sci. 6(4) : 1453-1466, August 2012 14p. http://ajol.info/index.php/ijbcs. DOI : http://dx.doi.org/10.4314/ijbcs.v6i4.5

[27] Douma S, Adamou MM, Aboubacar K, Alleidi I, Boubacar AN (2019) Effet du régime d'irrigation sur la germination et la croissance en pépinière de *Parkia biglobosa* (Jacq.) G. Don. J.Anim.Plant Sci, 40 (1) : 6573-6583. http://www.m.elewa.org/JAPS ; ISSN 2071-7024

[28] Dumenu WK (2019) Assessing the impact of felling/export ban and CITES designation on exploitation of African rosewood (Pterocarpus erinaceus). Biological Conservation 236 (2019) 124–133. https://doi.org/10.1016/j.biocon.2019.05.044

[29] FAO (2005) State of the World's Forests disponible sur http://www.fao.org/3/a-y5574e.pdf.

[30] Giordano E (1972) Interaction de la sélection et de la culture intensive. Unasylva, 97- 98 : 82-88.

[31] Gnanglè PCR, Glele K, Oumorou M, N'djolosse K, Bonou W, Sokpon N (2010) Tests de croissance de jeunes plants de néré (*Parkia biglobosa*, Jack, R. Br.) en pépinière. *Int. J. Biol. Chem. Sci*. 4(6) : 1939-1952

[32] Goba AE, Koffi G, Raoul SS, Leonie C K, Yeboa AK (2019) Structure démographique et régénération naturelle des peuplements naturelle de *Pterocarpus erinaceus* Poir. (Fabaceae) des savanes de Côte d'Ivoire. Bois et forêt des Tropiques, 341 (3) : 5-14

[33] Gorgon IT, Olga DY, Komla EA, Francois W, Kouami K (2015) Essai de germination et de croissance au stade

juvénile des souches locales de *Jatropha curcas* en république centre africaine. European Scientific Journal ; vol.11, No.15. 260-276.

[34] Goula BT, Brou K, Brou T, Savane I, Vamoryba F, Bernard S (2007) Estimation des pluies exceptionnelles journalières en zone tropicale : cas de la Côte d'Ivoire par comparaison des lois Log normale et de Gumbel. Journal des Sciences Hydrologiques. 52 (1) : 49–67.

[35] Guillaumet JL, Adjanohoun E, Avenard J M, Eldin M, Girard G, Circoulin J, Toucheboeuf P, Perraud A (1971) Le milieu naturel de Côte d'Ivoire. Mém.ORSTOM no50. ORSTOM, Paris. Pp156-264.

[36] Hérault B, N'guessan AK, Ouatara N, Ahoba A, Bénédet F, Coulibaly B, Doua-bi Y, Koffi T, Koffi-Konan JC, Konaté I, Tieoulé F, Wourro F, Zo-Bi IC, Louppe D (2020) The long-term performance of 35 tree species of sudanian West Africa in pure and mixed plantings. Forest Ecology and Management 468 (2020) 118171. https://doi.org/10.1016/j.foreco.2020.118171

[37] Houndonougbo JSH, Kassa B, Mensah S, Salako VK, Glèlè Kakaï R, Assogbadjo AE (2020) A global systematic review on conservation nd domestication of Parkia biglobosa (Jacq.) R. Br. ex G. Don, an indigenous fruit tree species in Sub-Sahara African traditional parklands: current knowledge and future directions. Genet Resour Crop Evol (2020) 67:1051–1066. https://doi.org/10.1007/s10722-020-00892-w

[38] Koffi KJ, Kouassi AF, Ake-assi AE, Yao K, N'goran KSB, N'Goran B (2018) Liste de quelques espèces à statuts particuliers de l'herbier du Centre National de Floristique (CNF) en Côte d'Ivoire. Version 1.2. Centre National de Floristique. Occurrence dataset https://doi.org/10.15468/ckbuue Ajouter au projet Citavi par DOI accessed via GBIF.org on 2021-03-05.

[39] Kossi NS, Kossi A, Abdou RR, Adzo DK, Kouami K, Rabiou H, Pouwisawe K, Babou AB, Ali M (2015) Importance socio-economique de *pterocarpus erinaceus* poir. au Togo. European Scientific Journal, N° 23 (11) : p 135-145

[40] Laurie, M. V., 1974. Tree planting practices in African savannas. Food and Agriculture Organization of the United Nations, Rome. pp. 42-43.

[41] Leroy M, Derroire G, Vendé J, Leménager T (2015) La gestion durable des forêts tropicales. De l'analyse critique du concept à l'évaluation environnementale des dispositifs de gestion. Bois et forêts des tropiques, N° 325 (3) : p 86-87.

[42] Louppe D, Ouattara N (1996) Les arboretums d'espèces locales en Nord Côte d'Ivoire. IDEFOR.

[43] Mabetty T (2018) Le néré, un arbre du patrimoine de la Haute Guinée. Belgeo, 2. 24p, consulté le 3 avril 2020. URL : http://journals.openedition.org/belgeo/21569

[44] Maranz S, Wiesman Z (2003) Evidence for indigenous selection and distribution of the shea tree, Vitellaria paradoxa, and its potential significance to prevailing parkland savanna tree patterns in sub- Saharan Africa north of the equator. J. Biogeogr., 30: 1505-1516.

[45] Mbowa C, Chhinb S, Samboua B, Skolec D (2013) Potential of dendrochronology to assess annual rates of biomass productivity in savanna trees of West Africa. Dendrochronologia 31 : 41– 51. http://dx.doi.org/10.1016/j.dendro.2012.06.001

[46] Millan M (2016). Analyse de la variabilité des traits architecturaux des formes de croissance dans les communautés végétales. Thèse de Botanique. Université de Montpellier. Français. 178 p. https://tel.archives-ouvertes.fr/tel-02489116

[47] MINEF (2013) Décret n° 2013-508 du 25 juillet 2013portant interdiction de l'exploitation, de la coupe, du transport, de la commercialisation et de l'exportation de *Pterocarpus* spp appelé communément « bois de vêne ». Journal Officiel de la République de Côte d'Ivoire, 22 août 2013. pp 532-533.

[48] Nations Unies (2002) Rapport du Sommet mondial pour le développement durable. Johannesburg (Afrique du Sud) 26 aout-4 septembre 2002. 191p.

[49] N'golo B, Noufou DO, Djézou K, Adama B, Fezan H T (2018) Effets de cinq prétraitements sur la germination du vène (*Pterocarpus erinaceus Poir*., Fabaceae) dans la Réserve du Haut Bandama (Côte d'Ivoire). European Scientific Journal, 30 (14) : p 438-453

[50] Ouedraogo A, Adjima T, Hahn-Hadjali K, Guinko S (2006) Diagnostic de l'état de dégradation des peuplements de quatre espèces ligneuses en zone soudanienne du Burkina Faso. Sécheresse, 17 (4) : p 485-491

[51] Perraud A (1971) Les sols. In : Le milieu naturel de la Côte d'Ivoire. Mémoires ORSTOM 50 : 69-390.

[52] Rabiou H, Adjonou K, Issaharou-matchi I, Segla KN, Bationo BA, Kohutse AD, Mahamane A, Kokou K (2019) Influence of anthropogenic and ecological factors on stand structure of *Pterocarpus erinaceus* Poir. in Sudanian and Sahelian zones of Burkina Faso and Niger. J. Ecol. Nat. Environ. Vol. 11(7), pp. 98-107. DOI: 10.5897/JENE2019.0776

[53] Rabiou H, Segla KN, Adjonou K, Radji AR, Moussa MB, Saley K, Kokutse AD, Bationo BA, Mahamane A, Kokou K (2015) Estimate trees potential and wood cubage in natural stands of *Pterocarpus erinaceus* Poir. based on ecological gradient in West Africa. Int.J.Curr.Microbiol.App.Sci (2015) 4(5): 1103-1117

[54] Rabiou H, Diouf A, Bationo AB, Segla NK, Adjonou K, Kokutse DA, Radji R, Kokou K, Mahamane A, Saadou M (2015) Structure des peuplements naturels de *Pterocarpus erinaceus* Poir.dans le domaine soudanien, au Niger et au Burkina Faso. Bois et forêts des tropiques 325 (3) : p 71-125.

[55] Rajendrudu G, Naidu CV (2001) Influence of kinetin and nitrogenous salts on seed germination of red sanders (*Pterocarpus santalinus* Linn. f.). Seed Science and Technology 29 (3) : 669-672.

[56] REDD+ (2017) « Stratégie nationale Redd+, Côte d'Ivoire » 15p. https://chm.cbd.int/api/v2017/documents/

[57] Salazar R, Quesada M (1987) Provenance variation in Guazuma ulmifolia L. in Costa Rica. Commonwealth Forestry Review, 66: 317-324.

[58] Sambe M, Sagna M, Sy MO (2010) Seed germination and in vitro plant regeneration of *Parkia biglobosa* (Jacq.) Benth. African Journal of Biotechnology, 9(21): 3099-3108.

[59] Segla KN, Adjonou K, Rabiou H, Bationo BA, Mahamane A, Guibal D, Kokou K, Chaix G, Kokutse AD, Langbour P

(2020) Relations between the ecological conditions and the properties of *Pterocarpus erinaceus* Poir. wood from the Guinean-Sudanian and Sahelian zones of West Africa. Holzforschung aop. 11 p. https://doi.org/10.1515/hf-2019-0250

[60] Segla NK, Rabiou H, Adjonou K, Moussa BM, Saley K, Radji RA, Kokutse AD, Bationo AB, Mahamane A, Kokou K (2016) Population structure and minimum felling diameter of Pterocarpus erinaceus Poir in arid and semi-arid climate zones of West Africa. South African Journal of Botany 103 (2016) 17–24. http://dx.doi.org/10.1016/j.sajb.2015.09.005

[61] Soloviev P, Niang TD, Gaye A, Totte A (2004) Variabilité des caractères physicochimiques des fruits de trois espèces ligneuses de cueillette, récoltés au Sénégal : *Adansonia digitata*, *Balanites aegyptiaca* et *Tamarindus indica*. Fruits, 59 : 109-119.

[62] Soro GE (2011) Modélisation statistique des pluies extrêmes en Côte d'Ivoire. Thèse unique. Abidjan (Côte d'Ivoire) : Université Nangui Abrogoua. 173 p

[63] Zerbo P, Belem B, Mllogo-Rasolodimby J, Van Damme P (2010) Germination sexuée et croissance précoce d'Ozoroainsignis Del., une espèce médicinale du Burkina Faso. Cameroon Journal of Experimental Biology 2010 Vol. 06 N°02, 74-80.

Article   *Peer-Reviewed Journal*

Effects of Season on Disease Frequency and Mortality of Poultry in Owerri Urban South-Eastern Nigeria

Akagha, N. U[1]*, Nwagbara, M. O[2]

[1]Department of Agricultural Technology, Imo State Polytechnic, Umuagwo-Ohaji, Nigeria.
[2]Department of Soil Science and Meteorology, Michael Okpara University of Agriculture Umudike, Abia State Nigeria.

Author's contributions

This work was carried out in collaboration among all authors. Author ANU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author NMO managed the analyses of the study, literature searches and helped with the discussions. Authors read and approved the final manuscript.

Abstract— The study examined effects seasons have on diseases frequency and mortality of poultry birds in Owerri urban south-eastern Nigeria. The study therefore employed the use of secondary and primary data. Climatological data on rainfall, temperature, relative humidity and mortality of birds was collected from Nimet, Sam Mbakwe International cargo Airport, Imo State and ministry of livestock development Owerri respectively for a period of 16 years (2004-2019). Also 83 copies of questionnaire were used in the analysis. Results showed positive correlation between rainfall and relative humidity (0.517 and 0.747) and an R² of 39.98% with implies that, the higher the rainfall the higher the relative humidity, and by extension the higher the frequency of deadly poultry diseases (Coccidiosis, Gumboro, Fowl cholera, Fowl typhoid) and mortality especially for broilers as the cell immune response of layer birds in defending itself against diseases and viruses are greater than that of broilers. While Newcastle diseases, Fowl pox, Fowl typhoid, chronic respiratory diseases mostly affect birds in the dry season (November- March). While Newcastle diseases, Fowl pox, Fowl typhoid, chronic respiratory diseases mostly affect birds in the dry season (November- March).In conclusion, seasons of the year affect poultry and its production in particular in Owerri urban. It is recommended that farmers should make sure they adopt proper roofing methods, as well as maintain good sanitation/hygiene practices to minimize heat and the occurrence and spread of diseases which may adversely affect birds across seasons.

Keywords— Season, poultry birds, diseases, mortality.

I. INTRODUCTION

Poultry are birds such as the domestic fowl, Turkey, Duck, Gesse, Ostrich etc which render not only economic services but contributes significantly to human food as a primary supplier of meat, egg and raw materials to industries (feathers, waste production). It also serves as source of income and employment to people compared to other domestic animals, their production is one that plays significant contribution to food for human consumption (Demeke, 2004). Poultry flocks are particularly vulnerable to climatic variations because there is a range of thermal conditions within which animals are able to maintain a relatively stable temperature (Adesiji, Baba and Tyabo, 2013).

In many African countries, poultry generally and its production in particular make substantial contribution to relive the protein insufficiency, about 80% of poultry production in Africa is found in rural and peril-urban areas where birds are raised in small numbers by the traditional extensive or semi – intensive, low input, low output systems (Faostat, 2012). Poultry production in Nigeria is an important component of the livestock sub-sector

and has developed to the level of enterprise. Involving thousands of birds that provide employment, income, animal protein for rural and urban dwellers as well as manure for production of crop (Arhyel,*et al.,* 2014). Poultry plays an important role for alleviating challenges associated with poverty in Nigeria (food security and malnutrition) and significantly contributes to women's income and help meet some level of household protein needs (Arhyel *et al.,* 2014). It plays an important role in the diet and economy of Nigeria. Poultry birds are efficient converters of feed to egg and meat within a short period of time and in terms of nutritive value, poultry egg ranks second to cow milk (Adesiji *et al.,* 2013). Agriculturists and nutritionists have generally agreed that developing the poultry industry of Nigeria is the fastest means of bridging the protein deficiency gap presently prevailing (Amos, 2006).

In Nigeria, there are two seasons, dry and rainy seasons. The rainy season begins by April and lasts till October whereas the dry season begins November and lasts till March (Odekunle, 2004). These seasons have their various effects not only on living things, poultry inclusive but also on non living things (Osaguona , *et al.,* 2007). Season comes with various pathogenic disease occurrence , morbidity and mortality rate is majorly a factor of the type of management practices and vaccination of flocks (Kelly-Hope and Thomson , 2008).). Poultry flocks are particularly vulnerable to seasonal changes because there is a range of thermal conditions within which animals are able to maintain a relatively stable body temperature in their behavioural and physiological activities (Ayo-Enwerem , Ahaotu, Nwogu and Opara, 2017).

Seasonal variation is one of the major threat to poultry production and also an important factor associated with incidences of diseases and mortality (Singh, *et al.,* 2005; Nayak, *et al.,* 2015). Disease and climate problems are seen as major limitations to modern animal husbandry in the tropical areas, coupled with high relative humidity in mostly chicken's pen, leads to mortality of young poultry birds (Smith, 2001). Variation due to season affects the severity and distribution of livestock diseases and parasites which adversely influence poultry production (Thornton, 2010). Diseases remains a major threat affecting the boost of the industry in Nigeria (Adewole, 2012). Major diseases are Newcastle diseases, Avian influenza, Avian pox, infectious burals diseases, coccidiosis, colisepticemia and worm infestation (Usman and Diarra, 2008). Due to diseases, productivity is reduced resulting to less meat, fewer eggs and high mortality. Therefore this study was carried out to investigate if seasons of the year have influence on disease frequency and mortality of chickens in the study area.

II.   MATERIALS AND METHODS

Study location

Owerri, the Imo State capital lies at the intersection of six major roads of regional importance namely: Aba road, Okigwe road, Mbaise/Umuahia road and Orlu road. It is located within latitude $5^{\circ}25^{'10}N$ and $5^{\circ}30'15N$ and longitude $7^{\circ}10E$ and $7^{\circ}40'E$ and occupies a land area of about 104 square kilometers. It lies within the humid tropical climate with annual rainfall and temperature of over 2000 mm and 20ºC respectively. And occupies a land area of about 104 square kilometers.
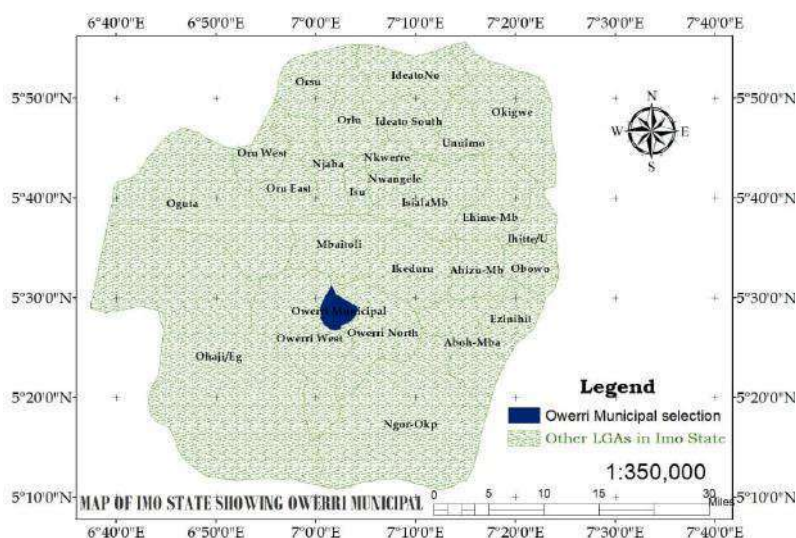
Fig.1: Map of Imo State showing Owerri Municipal
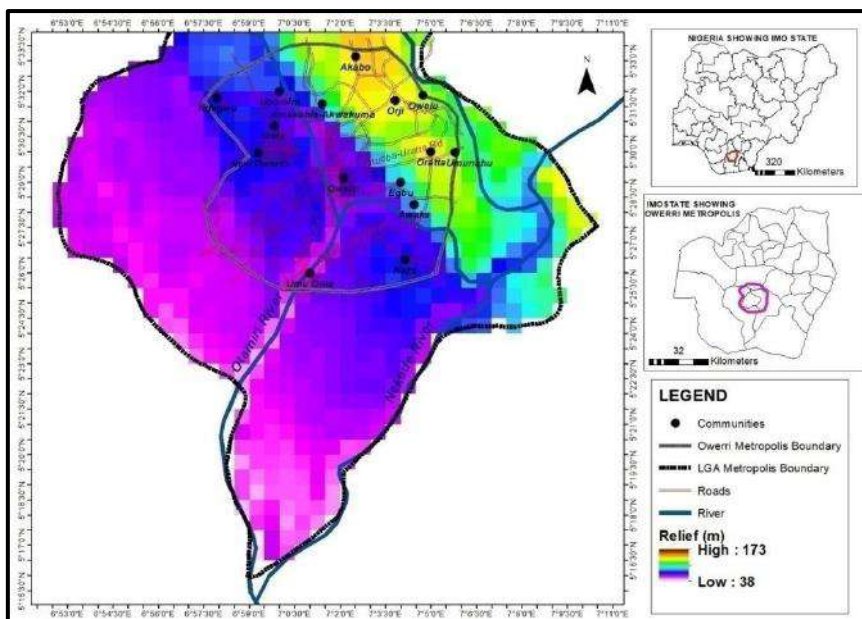
Fig.2: Relief and Drainage Map of Owerri Urban.

Method of data collection

The study deployed the quasi experimental research design. Data for rainfall, temperature, relative humidity and mortality was collected from Nigerian Meteorological Agency (NIMET) Sam Mbakwe International Cargo Airport Imo State and Ministry of Livestock Development Owerri for a period of 16 years respectively. Also 83 copies of questionnaire retrieved from farmers was used. Data was analyzed using Pearson's moment correlation, stepwise regression and linear regression to ascertain variations in disease/mortality across seasons from(2004-2019). The analysis was done using statistical package for social sciences (Spss version 21).

III.   RESULTS AND DISCUSION

The table 1 shows monthly climatic data of maximum temperature, minimum temperature, mean temperature, relative humidity, total rainfall and number of birds mortality.

Table 1: Monthly climatic data and poultry mortality between 2004-2019

Months	Max Temp (^{0}C)	Mini Temp (^{0}C)	Mean Temp (^{0}C)	Relative humidity (%)	Total rainfall (mm)	Number of birds mortality
JAN.	31.7	18.1	24.9	78.6	22.1	75.1
FEB.	34.4	22.9	28.7	71.8	40.2	66.0
MARCH	34.7	21.9	28.3	76.3	85.6	67.9
APR.	33.8	23.3	28.6	68.9	168.4	65.5
MAY	32.4	23.1	27.7	80.6	272.5	71.6
JUN	32.6	22.7	27.6	85.6	296.5	75.5
JUL	30.1	22.6	26.4	87.3	342.7	77.6
AUG	30.0	22.4	26.5	85.4	359.7	96.6
Sept	31.5	22.6	26.8	85.2	339.1	69.8
OCT.	31.7	22.6	27.2	79.1	219.6	72.0
NOV.	32.9	21.3	27.1	69.9	55.6	55.9
DEC.	33.5	21.0	27.2	69.4	42.6	64.3

Source: Author's computation 2019

The mean temperature, relative humidity and total rainfall shows significant variation in the seasons of the year, which has effect on diseases and mortality of poultry birds in Owerri Imo state, Nigeria. The months of rainy season (April-October), shows higher rainfall amount as follows 168.4mm, 272.5mm, 296.5mm, 342.7mm, 359.7mm, 339.1mm, and 219.6mm and corresponds to increase in the mortality of poultry birds in the months of rainy season (April-October) at 65.5, 71.6, 75.5, 77.6, 96.6, 69.8 and 72.0. The month of August recorded the highest rainfall amount of 359.7mm which corresponds with month of highest mortality rate of bird's at 96.6. Relative humidity revealed higher percentage in the months of rainy season (April-October), at 71.4%, 80.6%, 85.6%, 87.3%, 85.4%, 85.2% and 79.1%, while the months of the dry season (November-March) shows 69.9%, 69.4%, 56.6%, 68.9% and 76.3%. Thus increase in relative humidity percentage concise with the months of rainy season (April-October), which shows that bird's mortality is higher in the rainy season.

3.1: Effects of season on disease frequency and mortality of chickens

The positive correlation value of (0.517 and 0.747), shows that the higher the relative humidity and rainfall for the years under investigation, the higher the probability of frequency of diseases and mortality of poultry birds. Relative humidity and rainfall which are indicators of rainy season are less than the alpha level of 0.05 and indicate that the two variables are statistically significant with disease frequency and mortality of poultry birds. Stepwise regression model was carried out to ascertain which predictor variables has more effect on diseases frequency and mortality of poultry birds. The regression model obtained has an R square value of 0.3998 which means about 39.98% variations in disease frequency and mortality of bird's is determined by relative humidity and rainfall.

Table 2: Regression model summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.302[a]	0.3998	.175	577.37060	.973

Table 3: Anova for regression model

Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	1776987.162	2	592329.054	1.7768620	.0043[b]
	Residual	2666854.505	9	333356.8131		
	Total	4443841.667	11			

The regression model obtained in table 2 is statistically significant since the Sig. value of 0.0043 in table 3 is not greater than alpha level of 0.05. We therefore conclude that relative humidity and rainfall amount have effect on disease frequency and mortality of poultry birds in the study area within the years under investigations.

The stepwise regression model obtained from the statistically significant regression coefficient is as follows:

$$\text{diseases frequency and birds mortality} = 10361.83 + 0.171 * \text{relative humidity} + 22.79 * \text{rainfall} + error$$

3.2: Trends of seasonal variations in disease frequency and mortality

The trend analysis shows seasonal variation in disease frequency and mortality of birds, for the months of January and July for the span under investigation. The months of January represents the dry season while the months of July represents the rainy season for a period of sixteen years (2004 – 2019). The mortality plot also was used to access outbreak of poultry disease.

Figure.3 and figure.4 indicates trend analysis for diseases and mortality of birds in January and July for all the years under investigation.
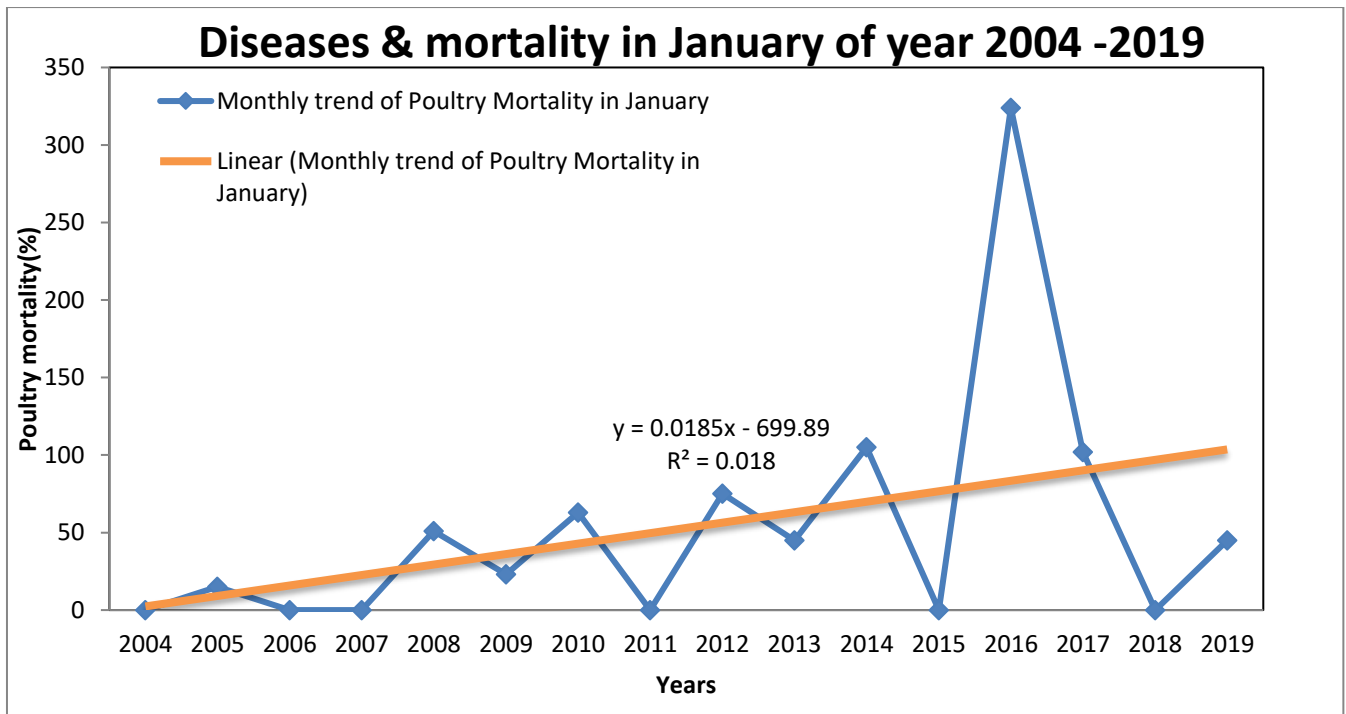
Fig.3: Trend analysis of diseases and mortality in the month of January for all the years
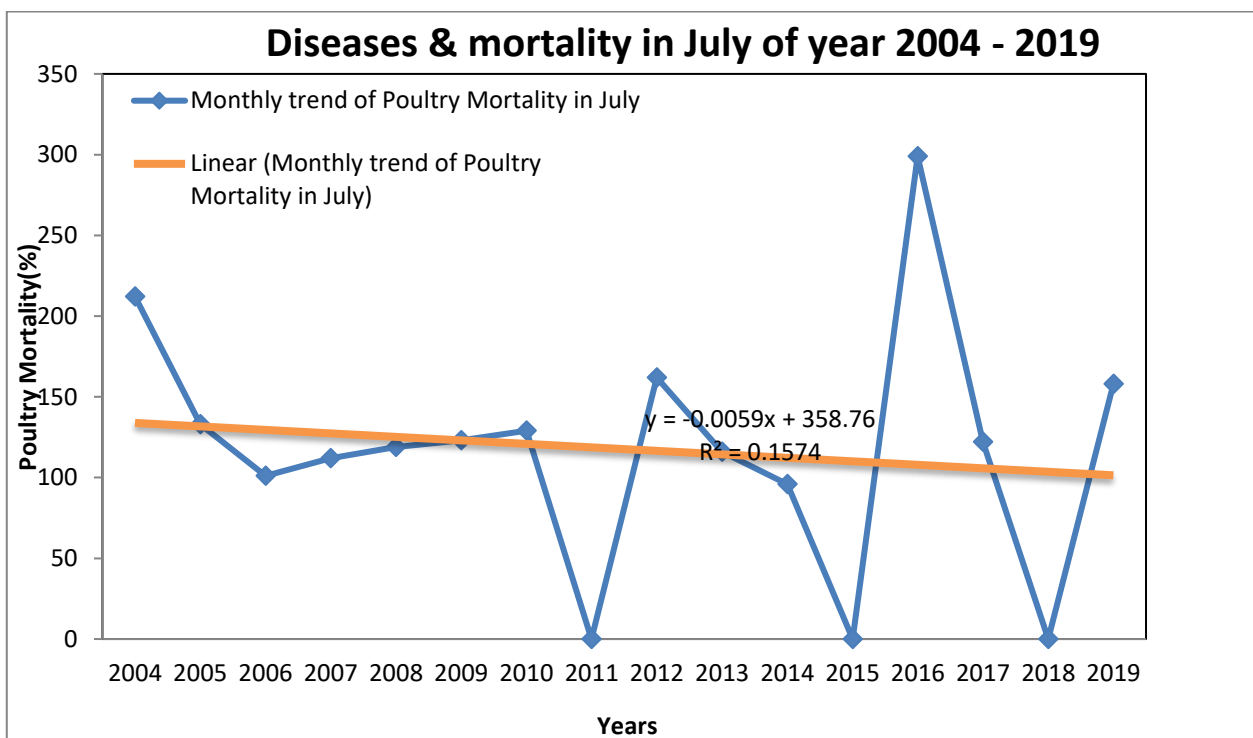
Fig.4: Trend analysis of diseases and mortality in the month of July for all the years

The trend analysis in figure 3 (January) and figure 4 (July), shows fig 3 possess a downward trend which is a decrease in disease frequency and mortality of birds in January. While fig 4 shows an upwards trend which is an increasing trend in disease frequency and mortality of birds in July.

The months of January revealed a trend equation of y=0.0185x – 699.89 with an R^2 of 0.018. While the months of July revealed a trend equation of y=0.0059x+358.76 with an R^2 of 0.1574. The trends shows seasonal variation

in disease frequency and mortality rate affecting poultry birds across the seasons.

Table 4 shows statistics report of the answers provided by the respondents who attended to the structured questionnaire for this research purpose.

Table 4: Percentage effects of seasons on frequency of diseases and mortality

Question Code	Effects of seasons on frequency of poultry diseases and mortality	(UD)% UNDECIDED	(SD)% STRONGLY DISAGREED	(D)% DISAGREED	(A)% AGREED	(SA)% STRONGLY AGREED
D1	In dry season (Nov-March)birds are mostly affected by Newcastle diseases , fowl pox, fowl typhoid, chronic respiratory diseases.	0.069	0.000	0.000	1.000	1.793
D2	In rainy season (April-Oct) birds are mostly affected by coccidiosis, Gumboro, fowl cholera, fowl typhoid.	0.310	0.000	0.000	0.690	1.862
D3	During the harmattan (Dec-Feb) mortality rate is high.	0.138	0.000	0.310	1.034	1.379
D4	Increase in mortality rate after harmattan (Feb – March) due to high environmental temperature.	0.276	0.000	0.517	0.552	1.517
D5	Newcastel disease, fowl pox, fowl typhoid, fowl cholera is frequent in rainy season.	0.345	0.793	1.724	0.000	0.000
D6	Coccidiosis, Gumboro, fowl cholera, fowl typhoid is frequent in dry season.	0.414	1.552	0.897	0.000	0.000
D7	Mortality of poultry birds is higher in dry season (Nov – Oct).	0.310	2.000	0.552	0.000	0.000
D8	Mortality of poultry birds is higher in rainy season (April – Oct).	0.172	0.000	0.000	0.483	2.207
D9	Fowl pox, fowl typhoid, fowl cholera, chronic respiratory diseases, can occur in any season, but frequent in the dry season (Nov – March)	0.448	0.862	1.552	0.000	0.000
D10	Fowl pox, fowl typhoid, fowl cholera, chronic respiratory disease can occur in any season but frequent in the rainy season (April – Oct.)	0.379	0.000	0.000	0.966	1.517

The table 4 shows the percentage of different ways the farmers in the study area responded on the effects of seasons on frequency of poultry diseases and mortality. Out of 83 respondents, 2.207 % which is the highest strongly agreed that mortality of poultry birds is higher in the rainy season (April-Oct), while 0.483% agreed and 0.172% are undecided on the effect. 1.862% of respondents strongly agreed that Coccidiosis, Gumboro, Fowl cholera, Fowl typhoid are mostly frequent in the rainy season (April-Oct), 0.690% agreed and 0.310% are undecided of any season. 1.793% strongly agreed that

Newcastle diseases, Fowl pox, Fowl typhoid, chronic respiratory diseases mostly affect birds in the dry season (Nov- March), 1.000% of respondents agreed and 0.069% are undecided of any season. 1.517% of respondents agreed that Fowl pox, Fowl typhoid, Fowl cholera, chronic respiratory disease can occur in any season but frequent in the rainy season (April – Oct.),0.966% agreed, while 0.379% are undecided of any season. The block diagram shows responses on effects of seasons on the frequency of disease and mortality.
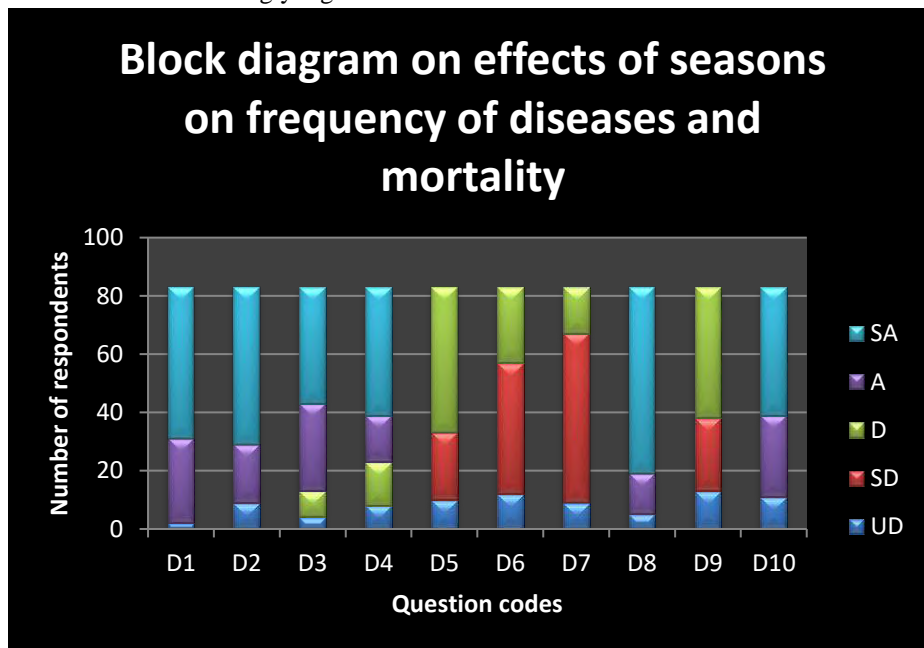
Fig.5: Responses on effects of seasons on frequency of disease and mortality.

IV. CONCLUSION

The correlation analysis on effects of seasons of the year on disease frequency and mortality of chickens was carried out and it revealed a positive (r=0.517 and r= 0.747) with relative humidity and rainfall which are indicators of season. It implies that the higher the relative humidity and rainfall for the years under consideration, the higher the frequency of diseases and mortality of poultry birds, therefore seasons of the year have influence on disease frequency and mortality of chickens in the study area. The co-efficient of determination (CD) obtained, which is in percentage implies that 39.98% of disease frequency and mortality is explainable by relative humidity and rainfall which are indicators of the rainy season. This report is in agreement with (Uddia *et al.*, 2010) who noted that rainy season are most vulnerable to various poultry diseases.(Sato *et al.,* 2002), also noted that, due to cold stress birds increase their susceptibility to a number of infectious and non –infectious agents. Also (Nayak *et al.,*

2015), noted that higher rainfall and relative humidity increases diseases incidence on poultry birds.

V. RECOMMENDATION

It is recommended that farmers should make sure they select species that are suitable and can resist the prevailing conditions due to variability in season in the study area as well as to the prevailing diseases. There is also the need for appropriate stocking of birds for free movement and space, adoption of proper roofing methods, vaccinating the birds when due, maintaining good sanitation/hygiene practices to minimize heat and the occurrence and spread of diseases which may adversely affect birds across seasons.

VI. COMPETING INTERESTS

Authors have declared that no competing interest exits.

REFERENCES

[1] Adesiji, G.B., Tyabo, I.S., Bolarin, O., Ibrahim, M. and Baba, S.T. (2013) Effects of climate change on poultry production in Ondo State, Nigeria. *Ethiop. J. Environ. Stud. Manage.*, 6(3): 242-248.

[2] Adewole, O. (2012). The efficacy of drugs in the treatment of coccidiosis in chicken in selected poultries. Acad. Res. Int., 2: 210-220.

[3] Amos, T. T. (2006). Analysis of Backyard Poultry Production in Ondo State Nigeria. *International Journal of Poultry Science* volume 5(5): 247- 250

[4] Arhyel, G. B., Ndahi, J. I. Zaifada, A. U., Mustapha, M. Jarafu, D. J., Asogwa, N. T. and Hajara, S. (2014). A retrospective study of poultry diseases diagnosed in Maiduguri, North-East, Nigeria. POUHFISH wildiSci ISSN: 2375-46.

[5] Ayo-Enwerem, M.C; Ahaotu, E.O; Nwogu, C. M. and Opara, J. (2017a). Growth performance of starter broilers fed diets containing red sandalwood (Pterocarpussantolinoides) leaf meal. Direct Research Journal of Veterinary Medicine and Animal Science. 2 (4): 106-109.

[6] Demeke, Solomon, (2004). Egg production and performance of local and white Leghon hens under intensive and rural household conditions in Ethopia. LRD 16:2.

[7] Faostat(2012). http://faostat.fao.org/site/340/default/as_px, viewed on 19- 22/03/2007.

[8] Kelly-Hope and Thomson (2008). Climate and Infectious Diseases.springer Nertherlands 31-71.

[9] Nayak, G. D. Behura, N. C., Sardar, K. K. and Mishra, P.K (2015) Effect of climatic variables on production and reproduction traits of colored broiler breeder poultry. Veterinary world, ESSN: 2231 – 0416.

[10] Odekunle, T.O.(2004). Rainfall and the length of growing season in Nigeria. International journal of Climatology 24(4), 467-479.

[11] Osaguona, P.O, Fajobi, E. A, Meduna, A.J ,Irokanulo,U.O, Ayeni, S.M and Ogunjobi, J.A.(2007). Effect of climate variation on the environment. Proceedings of the Association of women in Forestry and Environment 167-170.

[12] Sato, T, Tezuka Shibuya, H., Watanabe, T. Kamata, H. and Shirai, W. (2002). Cold induced ascities in broiler chickens and its improvement by temperature controlled rearing. Avian Diseases, 46, 989-996.

[13] Smith, T. (2001). Caring for plants in the Retail Setting. University Massachusetts extension service Leaflet (accessed November, 2014).

[14] Singh , R, Shanker, H, Arora, B.M ,Singh, V.P.(2005). Studies on morbidty and mortality pattern in cattle of the organized farms of different agro- climatic conditions in ultra Pradesh. Indian Journal of Animal Health . 44: 47-53.

[15] Thornton , P.K(2010). Livestock production : recent trend ,future prospects. PhilosTrans .R. SocBiolosci 365: 2853-2867.

[16] Uddia, M. B. Ahemd, S. S. U., Hassan, M. M., Khan, S. A. and Mamum, M. A. (2010) Prevalence of poultry diseases at Narsingdi Bangladesh. *Int. J. Bio. Res*. 1(6): 09-13.

[17] Usman, B. A. and Diarra, S. S. (2008). Prevalent diseases and mortality in egg type layers. *An overview. int. j. poulty. sci.* 7:304-310.

Article   *Peer-Reviewed Journal*

Study of genetic variability and character association for yield and yield related traits in f₃ generation of blackgram (*Vignamungo* (L.) Hepper)

Mr. K. Vinay Teja[1]*, Dr. G.M. Lal[2]

[1]Department of Genetics and Plant Breeding, Naini Agriculture Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, U.P, INDIA.
[2]Professor, Department of Genetics and Plant Breeding, Naini Agriculture Institute, SHUATS, Prayagraj, U.P, INDIA.
*Corresponding author E-mail: kvinayteja@gmail.com

Abstract— *The present investigation was carried out with a view to study the magnitude of variability, correlation and path analysis excluding reciprocals involving 27 genotypes in blackgram during Kharif - 2018. The experimental material was planted in randomized block design with three replications at the Field Experimentation Centre, Department of Genetics and Plant Breeding, SHUATS, Prayagraj, U.P. The analysis of variance for all the characters revealed that parents were significant for all the characters, indicating presence of considerable amount of genetic variability in the germplasm tested. Harvest index, Number of primary branches, Number of pods per plant, Number of seeds per pod exhibited high GCV, PCV and genetic parameters revealed that heritability (broad sense) and genetic advance as % of mean values were high for Number of primary branches, Number of seeds per pod, biological yield, harvest index, seed yield per plant indicating that selection would be fruitful for improvement of these traitsAmong 27 genotypes studied MU-06 X KPU-13-192 showed high mean performance for seed yield per plant followed by MU-06 X NDUK-13-6, PU-31 and NDUK-13-6 Correlation coefficient analysis revealed that seed yield per plant exhibited positive and significant correlation associated with Plant height, Number of pods per plant, Biological yield and harvest index at both genotypic and phenotypic levels. Path coefficient analysis revealed that characters plant height, number of primary branches, days to maturity, biological yield, and harvest index have positive direct effect on seed yield per plant at genotypic and phenotypic levels.*

Keywords— *Blackgram, GCV, PCV, Variability, Heritability, Correlation and Path analysis.*

I.   INTRODUCTION

Blackgram (*Vigna mungo* [L]. Hepper) is commonly known as urad, mesh or kalai. India is primary center of origin of Blackgram and Central Asia is a secondary center of origin. It is one of the most important legumes of India which belongs to family leguminosae, sub order Papilionaceae and the tribe phaseoleae with chromosome number (2n=22). It is India is the world's largest producer as well as consumer of Blackgram. It produces about 1.5 to

1.9 million tonnes of Blackgram annually from about 3.5 million hectares of area, with an average productivity of 500 kg per hectare. Black gram output accounts for about 10% of India's total pulse production. Urdbean crop is also gaining momentum since 2015-16 and there has been phenomenal increase in its coverage. During 2017-18 the crop was cultivated in an area of > 54 Lha. The success of this crop was released with a harvest of about 36 Lt at an ever-highest yield levels of 655 kg/ha. About 95 per cent of Urdbean production comes from 10 states of Madhya

Pradesh, Rajasthan, Andhra Pradesh, Uttar Pradesh, Tamil Nadu, Maharashtra, Jharkhand, Gujarat, Karnataka and West Bengal. In U.P. Blackgram is grown in about 6.14 lakh hectares with a total production of 3.15 lakh tones. Blackgram has a wide range of economic value. It is well known that 50 g pulses/person/day should be consumed in addition to other sources of protein such as cereals, milk, meat and egg which is very difficult task to achieve as the production and productivity of pulse crops including the

blackgram is very low. Hence, there is a strong need to improve the productivity of crop. This could be achieved by studying the genetic architecture of this crop for yield improvement. Therefore, the aim of present study is to identify suitable. Heritability is an important parameter which determines the extent of expressivity of a trait in a setup of environment or agro-climatic conditions. Therefore, heritability estimates are useful in predicting genetic advance under different intensities of selection. High heritable estimates together with high genetic advance are more valid for selection than heritability estimates alone (**Johnson *et al.,* 1955**). Estimation of genetic variability in conjunction with heritability and genetic advance gives an idea of the possible improvement of the character through selection.

II. MATERIALS AND METHODS

The present investigation was carried out at the field experimentation center, Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, U.P. during *kharif-*2018. All types of facilities necessary for cultivation of successful crop including field preparation, inputs and irrigation facilities were provided from the Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, U.P. The recommended dose of fertilizer N:P:K @ 20:40:40 kg/ha was applied in the form of Urea and Di-Ammonium Phosphate as basal dose at the time of sowing

Five competitive plants from each genotype were randomly selected for recording observations on fifteen characters, viz. Days to 50 per cent flowering, Days to 50 per cent pod setting, Plant height (cm), Number of primary branches per plant,Number of days to maturity, Number of pods per plant, Number of seeds per pod, Number of seeds per plant, Biological yield per plant (g), Harvest index (%), 100 Seed weight (g), and Seed yield per plant (g).Correlation coefficient is the mutual association between variables without implying any cause and effect relationship. Single correlation coefficient were computed at genetopic and phenotypic levels between pair of characters adopting following formula given but (**Al-**

Jibouri *et al*., 1958). Biometrical methods were followed to estimate genotypic and phenotypic coefficient of variation Heritability Broad Sense (**Burton and De Vane, 1953**)Genetic Advance (**Johnson *et al*. 1955**)Correlation coefficient analysis (**Al- Jibouri *et al*. 1958**) The test of significance for association between characters was done by comparing table 'r' values at (n-2) error degrees of freedom for phenotypic and genotypic correlations with estimated values, respectively Path coefficient analysis (**Dewey and Lu, 1959).**

III. RESULTS AND DISCUSSION

Analysis of variance showed highly significant differences for all the 13 characters used under study at 1% level of significance. This indicates that there is ample scope for selection of genotypes for yield and its components and also indicating the presence of considerable variability among the all traits (Table 1).

Estimation of genetic parameters: A wide range of Genotypic Coefficient of Variation (GCV) was observed for all the traits ranged from 3.71 (days to 50% pod setting) to 24.56 (harvest index). The magnitude of Genotypic Coefficient of Variation (GCV) were recorded high for 24.56 (harvest index) followed by number of primary branches per plant (23.68), number of seeds per pod (20.69) and number of pods per plant (20.68).Medium magnitude of Genotypic Coefficient of Variation (GCV) were recorded for number of clusters per plant (18.02), biological yield per plant (17.57), plant height (14.33), seed yield per plant (12.14) and pod length (10.35), whereas the magnitude of Genotypic Coefficient of Variation (GCV) were recorded low for days to maturity (9.95), seed index (7.29), days to 50% flowering (4.40) and days to 50% pod setting (3.71).

Heritability: The heritability estimates varied from 79.14 (number of pods per plant) to

95.11 (harvest index). All the characters *viz,*days to 50% flowering (91.27), days to 50% pod setting (88.94), plant height (81.57), number of primary branches per plant (92.35), number of clusters per plant (89.39), number of pods per plant (79.14), number of seeds per pod (84.58), pod length (83.38), seed index (81.89), biological yield (89.90), harvest index (95.11), seed yield per plant (84.75) and days to maturity (92.38) were identified with high heritability. High heritability of these traits indicates that there is a close correspondence between the genotype and phenotype and selection can be done for these characters

Genetic advance: Improvement in the mean genotypic value of selected plants over the parental population is known as genetic advance. It is the measure of gain under

selection. Heritability estimates along with genetic advance are normally more helpful in predicting the gain under selection (**Johnson *et al.,* 1955**). The estimate of genetic advance ranged from

18.65 (harvest index) to seed index (0.42), whereas it is high for plant height (17.77). Moderate genetic advance was recorded number of pods per plant (5.99), biological yield per plant (5.62), number of seeds per pod (4.82), days to 50% pod setting (4.79), days to maturity (3.99), days to 50 % flowering (3.73), number of clusters per plant (1.92), number of primary branches per plant (1.63), pod length (1.42) and seed yield per plant (1.38) and low genetic advance was recorded for seed index (0.42).

IV.   CONCLUSION

From the present investigation it is concluded that among 27 genotypes of Blackgram on the basis of mean performance MU-06 X KPU-13-192 showed high mean performance for seed yield per plant followed by MU-06 X NDUK-13-6, PU-31 and NDUK-13-6 were found to be superior and showed possessed maximum seed yield. Harvest index, Number of primary branches, Number of pods per plant, Number of seeds per pod exhibited high GCV, PCV and genetic parameters revealed heritability (broad sense) and genetic advance as % of mean values were high for Number of primary branches, Number of seeds per pod, biological yield, harvest index, seed yield per plant indicating that selection would be fruitful for improvement of these traits. Correlation coefficient analysis revealed that seed yield per plant exhibited positive and significant correlation associated with Plant height, Number of pods per plant, Biological yield and harvest index at both genotypic and phenotypic levels. Path coefficient analysis revealed that characters plant height, number of primary branches, days to maturity, biological yield, and harvest index have positive direct effect on seed yield per plant at genotypic and phenotypic levels.

ACKNOWLEDGEMENT

REFERENCES

[1] Ali, M.N., Gupta Suparna, Bhattacharyya Somnath and Sarkar, H.K. (2008). Evaluation of blackgram (*Vigna mungo* (L.) Hepper) germplasm using multi-varietal analysis *Environment and Ecology,* 26(2): 943-945.

[2] Bakshi, A., Bala, S. and Dostisder, K.G. (2006). Character association for seed yield components in blackgram (*Vigna mungo* (L). Hepper). *Environment and Ecology,*24(3): 943-945.

[3] Chauhan, M.P., Mishra A.C. and Singh A.K. (2007). Correlation and path analysis in urd bean. *Legume Research* 30(3): 205-208

[4] Dadepeer, P., Ravikumar, R.L. and Salimath, P.M. (2009). Genetic variability and character association in local greengram (*Vigna radiata (*L.)Wilczek.) genotypes of Karnataka. *Environment and Ecology*, 27(1): 165-169.

[5] Deepshikha, Lavanya, G.R. and Kumar, S. (2014). Assessment of GeneticVariability for Yield and Its Contributing Traits in Blackgram (*Vigna mungo* (L.) Hepper). *Trends in Biosciences*, 7(18): 2835-2838

[6] Deepalakshmi, A.J. and Anandakumar, C.K.(2004). Creation of genetic variability for different polygenic traits in black gram (Vignamungo (L.) Hepper) through induced mutagenesis. *Legume Research.* 3:188-192.

[7] Fisher, R.A. (1918). The correlation between relatives of the supposition of mendelian inheritance. *France Royal Society of Edinburgh,* 52: 399-433.

[8] Gupta, S., Gupta, D. S., Anjum, K., Pratap, A and Kumar, J. (2013). Transferability of simple sequence repeat markers in black-gram (Vigna mungo (L.) Hepper). *Australian Journal of Crop Science,* 7(3): 345.

[9] Gowsalya, P., Kumaresan, D. Packiaraj, D. and Bapu, K.J.R. (2016). Genetic variability and character association for biometrical traits in blackgram (*Vigna mungo* L. Hepper). *Electronic Journal of PlantBreeding.* 7(2): 317-324.

[10] Hemalatha, K., Sapna, S.L. and Gabrial, M.L. (2017). Study on Genetic Variability and Correlation in Blackgram (*Vigna mungo* L. Hepper). *Journal of Pharmacognosy and Phytochemistry,*6(4): 674-676

[11] Isha Parveen, S., Reddi Sekhar, M., Mohan Reddy, D. and Sudhakar, P. (2011).Correlation and path coefficient analysis for yield and yield components in blackgram (*Vigna mungo* (L.) Hepper). *International Journal of Applied Biology and Pharmaceutical Technology,*2(3): 619-625.

[12] Jayamani, P. and Sathya.(2013). Genetic diversity in pod characters in black-gram. *Legume Research,* 36(3): 220-223.

Table 1: Analysis of variance for 13 characters in 27 genotypes of blackgram

Characters	Mean Sum of Squares		
	Replications	Treatments	Error
	(df= 2)	(df= 26)	(df=52)
Days of 50 % flowering	8.346	4.642**	2.641
Days of 50% pod setting	12.605	3.354**	1.810
Days of maturity	6.90	6.69**	2.30
Plant height	9.072	151.955**	3.772
Number of clusters per plant	0.396	1.784**	0.170
Number of pods per plant	2.734	27.511**	1.306
Pod length	0.008	0.051**	0.016
Number of seeds per pod	0.413	0.350**	0.135
Number of primary branches plant	0.135	0.169**	0.077
Seed index	0.117	0.128**	0.066
Biological yield	1.305	17.553**	2.536
Harvest index	24.167	151.143**	16.230
Seed yield per plant	0.130	2.061**	0.152

Table 2: Estimation of components of variance and genetic parameters for 13 characters in black gram genotypes

S. No	Character	Genotypic Coefficient of variation (%)	Phenotypic Coefficient of variation (%)	Heritability of (h2)(%) (broad sense)	Genetic advance	Genetic advance as percent of mean (5%)
1	Days to 50% flowering	4.40	4.61	91.27	3.73	8.66
2	Days to 50% Pod setting	3.71	3.93	88.94	4.79	7.21
3	Days to Maturity	14.33	15.87	81.57	17.77	26.66
4	Plant height	23.68	24.64	92.35	1.63	46.87
5	No.of primary branches	20.68	23.24	79.14	5.99	37.89
6	No.of clusters per plant	18.02	19.06	89.39	1.92	35.09
7	No.of pods per plant	10.35	11.34	83.38	1.42	19.48
8	No.of seeds per pod	20.69	22.49	84.58	4.82	39.19

9	Pod length	17.57	18.53	89.90	5.62	34.31
10	seed index	7.29	8.05	81.89	0.42	13.58
11	Biological yield	24.56	25.19	95.11	18.65	49.35
12	Harvest Index	9.95	10.36	92.38	3.99	19.71
13	Seed yield	12.14	13.18	84.75	1.38	23.02

Vg = Genotypic variance; Vp = Phenotypic variance; GCV = Genotypic coefficient of variation; PCV = Phenotypic coefficient of variation; h^2(bs) = Heritability at broad sense;

GA = Genetic advance; GAM = Genetic advance as percent mean.

Table 3: List of genotypes used in present investigation

S. No	Genotypes	S. No	Genotypes
1.	KPU-13-189 X IU-02-1-3	16.	KPU-13-189
2.	KPU-63-189 X NDUK-13-4	17.	IU-02-1-3
3.	IU-02-1-3 X PU-31	18.	NDUK-13-4
4.	IU-02-1-3 X PU - 09-37	19.	PU-31
5.	IU-02-1-3 x PU-38	20.	PU-09-37
6.	IU-02-1-3 X AZAD-1	21.	PU-38
7.	IU-02-1-3 X MU-06	22.	MU-06
8.	MU-06 X PU-09-37	23.	KPU-13-192
9.	MU-06 X KPU-13-192	24.	KU-96-7
10.	MU-06 X PU-31	25.	NDUK-13-6
11.	MU-06 X KU-96-7	26.	KPU-63-189
12.	MU-06 x PU-28	27.	T9(CHECK)
13.	MU-06 X IU-02-1-3		
14.	PU-09-37 X MU-06		
15.	AZAD-1		

Table 4. Analysis of variance for 13 characters in Blackgram

| S. No | Character | Mean sum of squares | | |
		Replication(d.f=2)	Treatment(d.f=26)	Error (d.f=52)
1	Days of 50 % flowering	8.346	4.642**	2.641
2	Days of 50% pod setting	12.605	3.354**	1.810
3	Days of maturity	6.90	6.69**	2.30
4	Plant height	9.072	151.955**	3.772
5	Number of clusters per plant	0.396	1.784**	0.170
6	Number of pods per plant	2.734	27.511**	1.306
7	Pod length	0.008	0.051**	0.016
8	Number of seeds per pod	0.413	0.350**	0.135

9	Number of primary branches plant	0.135	0.169**	0.077
10	Seed index	0.117	0.128**	0.066
11	Biological yield	1.305	17.553**	2.536
12	Harvest index	24.167	151.143**	16.230
13	Seed yield per plant	0.130	2.061**	0.152

Table 5 Mean performance of 27 genotypes of Blackgram for yield and component characters

S. No.	Genotypes	Days to 50% Flowering	Days to 50% Pod setting	Days to Maturity	Plant Height (cm)	No. of Primary Branches	No. of Clusters per Plant	No. of Pods per Plant	No. of Seeds per Pod	Pod Length (cm)	Seed index (g)	Biological Yield (g)	Harvest Index (%)	Seed yield per Plant (g)
1	KPU-13-189 X IU-02-1-3	44.33	54.67	67	63.4	6.33	18.8	4.39	6.58	3.4	3.96	14.42	30.36	5.23
2	KPU-63-189 X NDUK-13-4	43.67	52	70	58.1	6.17	24.33	4.51	7	3.13	4.21	16.4	36.58	4.9
3	IU-02-1-3 X PU-31	45.33	52	69.33	50.07	6.27	17.33	4.86	7.4	3.6	3.97	15.57	37.23	5.85
4	IU-02-1-3 X PU -09-37	43	52.67	70.33	65.6	6.07	25.65	4.54	6.74	3.2	3.83	17.87	25.43	5.41
5	IU-02-1-3 x PU-38	46	53	69	57.47	6.87	23.33	4.48	6.71	3.4	3.85	16.6	35.25	6
6	IU-02-1-3 X AZAD-1	44	52.33	69	53.53	6.96	17.53	4.51	7.33	3.28	4.08	15.67	47.44	5.47
7	IU-02-1-3 X MU-06	44.33	52.33	68	45.17	7.6	16.73	4.48	6.44	3.47	3.78	20.33	31.58	5.15
8	MU-06 X PU-09-37	44	51.33	73	46.53	5.13	24.73	4.68	6.92	3.6	4.02	15.07	22.1	5.31
9	MU-06 X KPU-13-192	43	53.33	68	52.2	5.53	24.13	4.48	6.78	3.2	3.79	16.13	30.33	7.66
10	MU-06 X PU-31	45.33	56	67	45.4	5.53	18.93	4.29	6.41	3.6	3.78	17.27	47.33	5.44
11	MU-06 X KU-96-7	44.67	53	70.33	62.57	6.6	19.47	4.3	6.56	2.8	3.85	25.4	39.89	5.61
12	MU-06 x PU-28	46	54.33	69	51.9	7.27	20.6	4.54	7.04	3.4	3.73	18.4	24.56	5.56
13	MU-06 X NDUK-13-6	43	53.33	69	51.4	6.13	18.73	4.34	6.96	3.27	3.68	15.8	33.54	7.21
14	MU-06 X IU-02-1-3	43.67	51.67	69.67	67.6	6.2	20.07	4.53	7.11	3.33	4.02	13.67	32.54	5.44
15	PU-09-37 X	46	51.67	68.67	60.63	6	23.47	4.34	6.63	3	3.56	16.13	44.87	4.27

	MU-06													
16	**NDUK-13-6**	41.67	54	67.67	56.47	7	25.2	4.46	6.67	3.67	3.73	18.8	36.89	6.84
17	**AZAD-1**	45	52.67	69	46.17	5.93	20.47	4.55	6.89	3.67	4.27	16.87	28.84	5.48
18	**KPU-63-189**	45.33	53	69.33	50.53	5.8	21.07	4.39	5.93	3.6	4.05	14.53	49.72	6.51
19	**IU-02-1-3**	41.67	53.33	67.67	42.37	6.6	19.8	4.49	6.74	3.33	3.76	16.67	33.97	4.87
20	**NDUK-13-4**	45.33	52.67	68.33	51.83	4.47	18.1	4.57	6.96	3.47	3.46	16.07	35.27	4.61
21	**PU-31**	42.67	51.67	65	45.7	5.27	20.6	4.34	6.74	3.4	3.62	13.93	25.43	6.93
22	**PU-09-37**	43.67	52	66.33	42.6	6.2	18.13	4.4	6.48	3.07	3.58	16.67	36.79	5.65
23	**PU-38**	43.67	53	68.33	62.53	5.2	18.53	4.35	6.45	3.07	3.55	14.53	38.72	5.12
24	**MU-06**	43.33	52	69.67	53	6.2	21.07	4.31	6.15	3.27	3.88	19.67	34.36	4.74
25	**KPU-13-192**	42.33	52.33	68.67	54.17	6.27	16.4	4.28	6.33	3.07	3.69	15.67	41.41	5.7
26	**KU-96-7**	44.33	52.67	68	55.11	7.53	27.4	4.4	6.26	3.8	3.66	18.07	36.92	6.62
27	**T9(CHECK)**	44.33	51.67	69.33	47.33	7.39	23.27	4.5	6.7	3.33	3.59	18.87	29.44	6.41
	Mean	44.41	52.66	68.42	53.31	6.24	20.88	4.46	6.7	3.35	3.81	16.85	35.07	5.7
	SE	1.33	1.1	1.24	1.59	0.34	0.93	0.1	0.3	0.23	0.21	1.3	3.29	0.32
	CD5%	**2.66**	**2.2**	**2.48**	**3.18**	**0.68**	**1.87**	**0.21**	**0.6**	**0.45**	**0.42**	**2.61**	**6.6**	**0.64**
	CV	3.69	2.55	2.21	3.64	6.61	5.47	2.86	5.47	8.28	6.75	9.45	11.49	6.84
	Max	46	55	72.33	67.6	7.6	27.4	4.86	7.41	3.8	4.27	25.4	49.72	7.66
	Min	42	51.33	64	42.37	4.47	16.4	4.29	5.93	2.8	3.46	13.67	22.11	4.27

Table 6 Genetic parameters for 13 seed yield characters in Blackgram

Characters	**Genotypic Coefficient Variation**	**Phenotypic Coefficient Variation**	**Heritability (%)**	**Genetic Advance**	**GA as % Mean**
Days to 50% flowering	4.40	4.61	91.27	3.73	8.66
Days to 50% pod setting	3.71	3.93	88.94	4.79	7.21
Plant height (cm)	14.33	15.87	81.57	17.77	26.66
No. of primary branches / plant	23.68	24.64	92.35	1.63	46.87
No. of pods per plant	20.68	23.24	79.14	5.99	37.89
No. of clusters per Plant	18.02	19.06	89.39	1.92	35.09
Pod length (cm)	10.35	11.34	83.38	1.42	19.48
No. of seeds per pod	20.69	22.49	84.58	4.82	39.19
Biological yield per plant (g)	17.57	18.53	89.90	5.62	34.31
Seed index(g)	7.29	8.05	81.89	0.42	13.58
Harvest Index	24.56	25.19	95.11	18.65	49.35
Days to maturity	9.95	10.36	92.38	3.99	19.71
Seed yield per plant	12.14	13.18	84.75	1.38	23.02
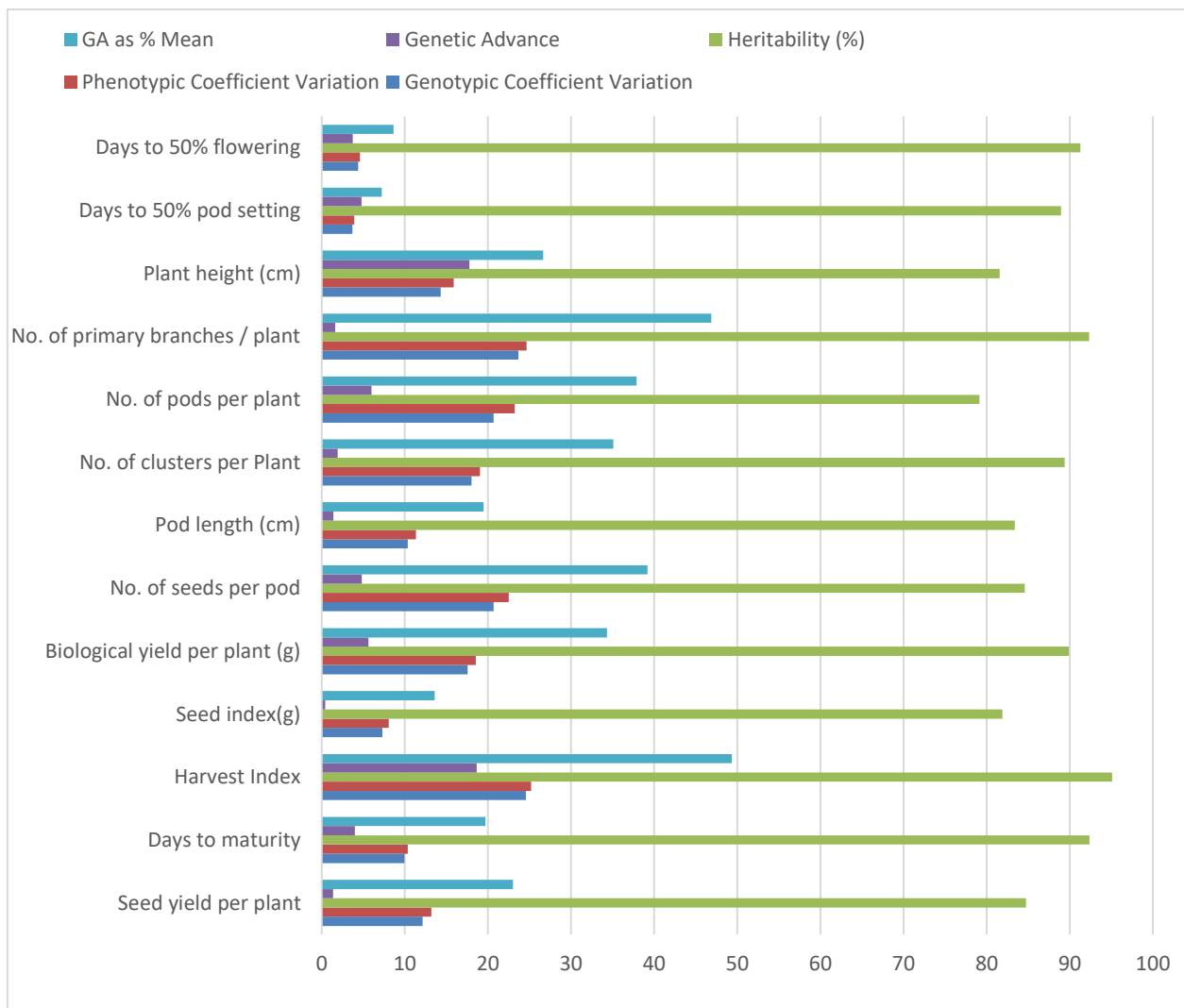
Fig. 1.Bar chart representing the relationship among the GCV, PCV, heritability and genetic advance as percent of mean in blackgram

Table 7 Genotypic correlation coefficient of seed yield and its component traits in Blackgram

Characters	Days to 50% flowering	Days to 50% pod setting	Plant height	No. of primary branches	No. of pods per plant	No. of clusters per Plant	Pod length	No. of seeds per pod	Biological yield	Seed index	Harvest Index	Days to maturity	Seed yield per plant
Days to 50 % flowering	1.000	0.242*	0.297**	0.126	-0.434**	-0.113	-0.035	-0.029	-0.047	-0.055	-0.023	-0.027	-0.04
Days to 50% pod setting		1.000	0.025	0.305**	-0.541**	0.037	-0.086	0.217	-0.085	0.008	0.02	0.202	-0.142
Plant height			1.000	-0.031	-0.015	-0.275*	0.091	0.18	-0.223	-0.243*	0.391**	0.027	0.471**
No. of primary branches				1.000	0.135	0.118	0.17	0.146	0.308**	0.318**	-0.246*	-0.011	0.027
No. of pods per plant					1.000	0.355**	0.381**	-0.046	0.128	-0.106	0.035	-0.053	0.291*
No. of clusters						1.000	-0.284*	0.14	0.054	0.272*	0.059	0.243*	0.151

Characters								1.000	-0.032	0.251*	-0.616**	-0.320**	-0.072	-0.264*
per Plant														
Pod length								1.000	-0.032	0.251*	-0.616**	-0.320**	-0.072	-0.264*
No. of seeds per pod									1.000	0.500**	0.16	-0.337**	0.435**	-0.007
Biological yield										1.000.00	0.166	-0.878**	0.308**	0.177**
Seed index											1.000	0.017	-0.212	0.189
Harvest Index												1.000	-0.391**	0.702**
Days to maturity													1.000	-0.22

*significant at 5% level of significance   ** significant at 1% level of significance

Table 8. Phenotypic correlation coefficient of seed yield and its component traits in Blackgram

Characters	Days to 50% flowering	Days to 50% pod setting	Plant height	No. of primary branches	No. of pods per plant	No. of clusters per Plant	Pod length	No. of seeds per pod	Biological yield	Seed index	Harvest Index	Days to maturity	Seed yield per plant
Days to 50% flowering	1.000	0.12	0.235*	0.113	-0.276*	-0.168	-0.004	-0.037	-0.009	-0.002	-0.005	0.039	-0.02
Days to 50% pod setting		1.000	0.062	0.155	-0.428**	0.015	-0.102	0.156	-0.001	-0.032	-0.037	0.11	-0.133
Plant height			1.000	0.087	-0.012	-0.171	0.086	0.166	-0.181	-0.12	0.341**	0.007	0.411**
No. of primary branches				1.000	0.16	0.091	0.102	0.163	0.205	0.189	-0.141	-0.029	0.06
No. of pods per plant					1.000	0.182	0.287*	-0.075	0.118	-0.149	0.032	-0.045	0.245*
No. of clusters per Plant						1.000	-0.253*	0.105	0.058	0.098	0.056	0.168	0.162
Pod length							1.000	0.001	0.222	-0.328**	-0.271*	-0.029	-0.187
No. of seeds per pod								1.000	0.441**	0.09	-0.309**	0.369**	0.012
Biological yield									1.000	0.053	0.853**	0.260*	0.194*
Seed index										1.000	0.026	-0.139	0.055
Harvest Index											1.000	-0.345**	0.670**
Days to maturity												1.000	-0.177

*significant at 5% level of significance   ** significant at 1% level of significance

Table 9. Direct and indirect effects of yield and its component traits in Blackgram at genotypic level

Characters	Days to 50% flowering	Days to 50% pod setting	Plant height	No. of primary branches	No. of pods per plant	No. of clusters per Plant	Pod length	No. of seeds per pod	Biological yield	Seed index	Harvest Index	Days to maturity	Seed yield per plant
Days to 50% flowering	**0.141**	-0.041	-0.051	0.029	-0.024	0.020	0.005	0.001	-0.074	0.010	-0.051	-0.006	-0.04
Days to 50% pod setting	0.034	**-0.169**	-0.004	0.071	-0.030	-0.007	0.012	-0.009	-0.133	-0.001	0.045	0.049	-0.142
Plant height	0.042	-0.004	**-0.171**	-0.007	-0.001	0.050	-0.013	-0.008	-0.349	0.045	0.881	0.007	0.471
No. of primary branches	0.018	-0.051	0.005	**0.233**	0.007	-0.021	-0.024	-0.006	0.482	-0.060	-0.554	-0.003	0.027
No. of pods per plant	-0.061	0.091	0.003	0.031	**0.055**	-0.064	-0.053	0.002	0.200	0.020	0.080	-0.013	0.291
No. of clusters per Plant	-0.016	-0.006	0.047	0.028	0.019	**-0.180**	0.039	-0.006	0.085	-0.051	0.134	0.059	0.151
Pod length	-0.005	0.015	-0.016	0.040	0.021	0.051	**-0.139**	0.001	0.392	0.115	-0.722	-0.017	-0.264
No. of seeds per pod	-0.004	-0.037	-0.031	0.034	-0.003	-0.025	0.004	**-0.044**	0.782	-0.030	-0.760	0.105	-0.007
Biological yield	-0.007	0.014	0.038	0.072	0.007	-0.010	-0.035	-0.022	**1.564**	-0.031	-1.977	0.074	0.177
Seed index	-0.008	-0.001	0.042	0.074	-0.006	-0.049	0.085	-0.007	0.259	**-0.187**	0.038	-0.051	0.189
Harvest Index	-0.0032	-0.0033	-0.0670	-0.0574	0.0019	-0.0107	0.0444	0.0147	-1.3723	-0.0032	**2.2525**	-0.0944	0.702
Days to maturity	-0.0037	-0.0340	-0.0046	-0.0025	-0.0029	-0.0438	0.0103	-0.019	0.4808	0.0397	-0.881	**0.2414**	-0.22
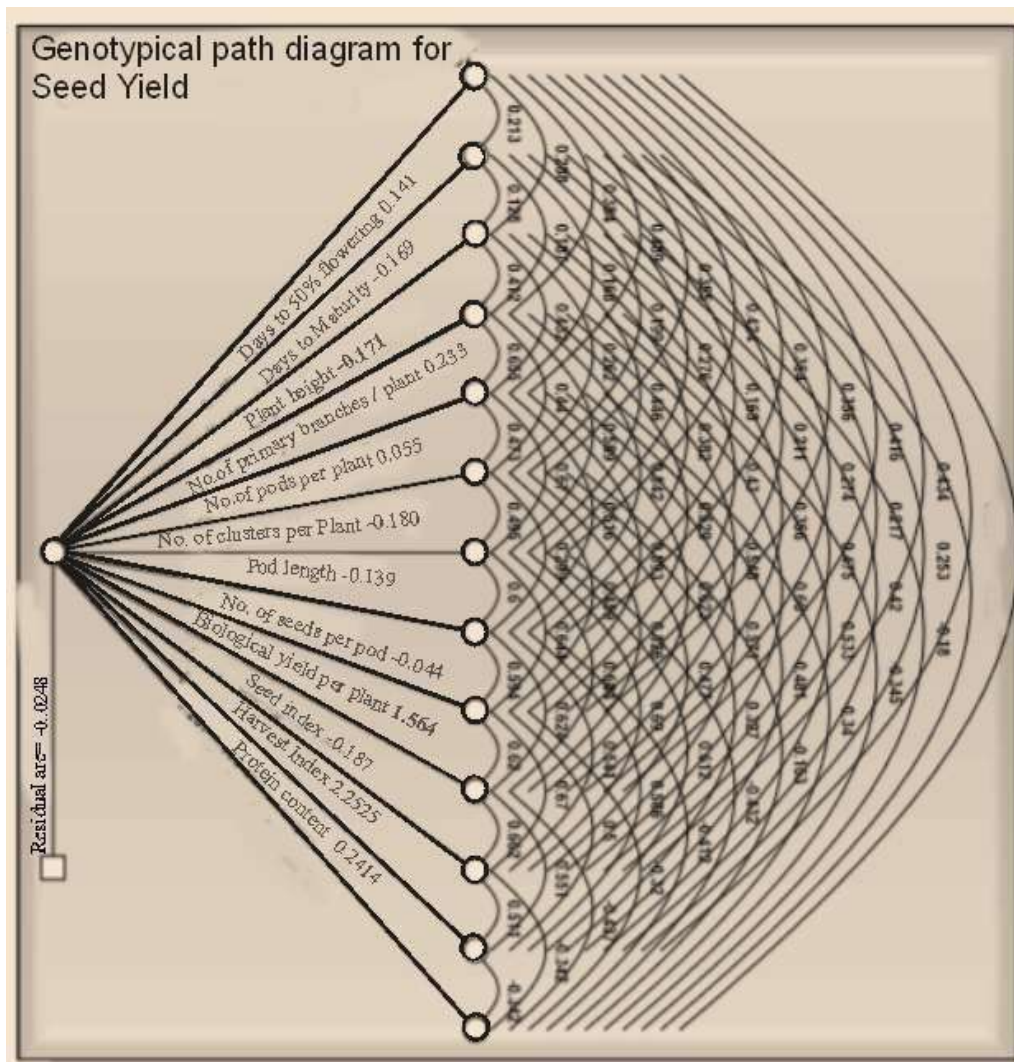
Fig 2: Genotypical path diagram for seed yield.

Table 10 Direct and indirect effects of yield and its component traits in Blackgram at Phenotypic level

Characters	Days to 50% flowering	Days to 50% pod setting	Plant height	No. of primary branches	No. of pods per plant	No. of clusters per Plant	Pod length	No. of seeds per pod	Biological yield	Seed index	Harvest Index	Days to maturity	Seed yield per plant
Days to 50% flowering	**-0.025**	-0.012	0.009	0.011	0.004	0.005	0.000	0.001	-0.011	0.000	-0.008	0.005	-0.02
Days to 50% pod setting	-0.003	**-0.100**	0.002	0.015	0.006	0.000	0.003	-0.006	-0.001	0.002	-0.064	0.014	-0.133
Plant height	-0.006	-0.006	**0.040**	0.008	0.000	0.005	-0.003	-0.007	-0.224	0.007	0.595	0.001	0.411
No. of primary branches	-0.003	-0.016	0.003	**0.097**	-0.002	-0.003	-0.003	-0.007	0.253	-0.011	-0.246	-0.004	0.06
No. of pods per plant	0.007	0.043	-0.001	0.015	**-0.014**	-0.005	-0.009	0.003	0.146	0.009	0.056	-0.006	0.245
No. of clusters per Plant	0.004	-0.001	-0.007	0.009	-0.002	**-0.030**	0.008	-0.004	0.072	-0.006	0.099	0.022	0.162

Pod length	0.000	0.010	0.003	0.010	-0.004	0.007	**-0.030**	0.000	0.273	0.020	-0.474	-0.004	-0.187
No. of seeds per pod	0.001	-0.016	0.007	0.016	0.001	-0.003	0.000	**-0.041**	0.544	-0.005	-0.539	0.048	0.012
Biological yield	0.000	0.000	-0.007	0.020	-0.002	-0.002	-0.007	0.018	**1.233**	-0.003	-1.090	0.034	0.194
Seed index	0.000	0.003	-0.005	0.018	0.002	-0.003	0.010	-0.004	0.065	**-0.061**	0.046	-0.018	0.055
Harvest Index	0.00012	0.0037	0.01351	-0.01364	-0.00044	-0.00167	0.00816	0.01251	-1.05246	-0.00161	**1.74624**	-0.04457	0.670
Days to maturity	-0.00097	-0.01107	0.00028	-0.00278	0.00062	-0.00496	0.00087	-0.01495	0.32048	0.00845	-0.60259	**0.12917**	-0.177

Fig 3: Phenotypical path diagram for seed yield

Article   Peer-Reviewed Journal
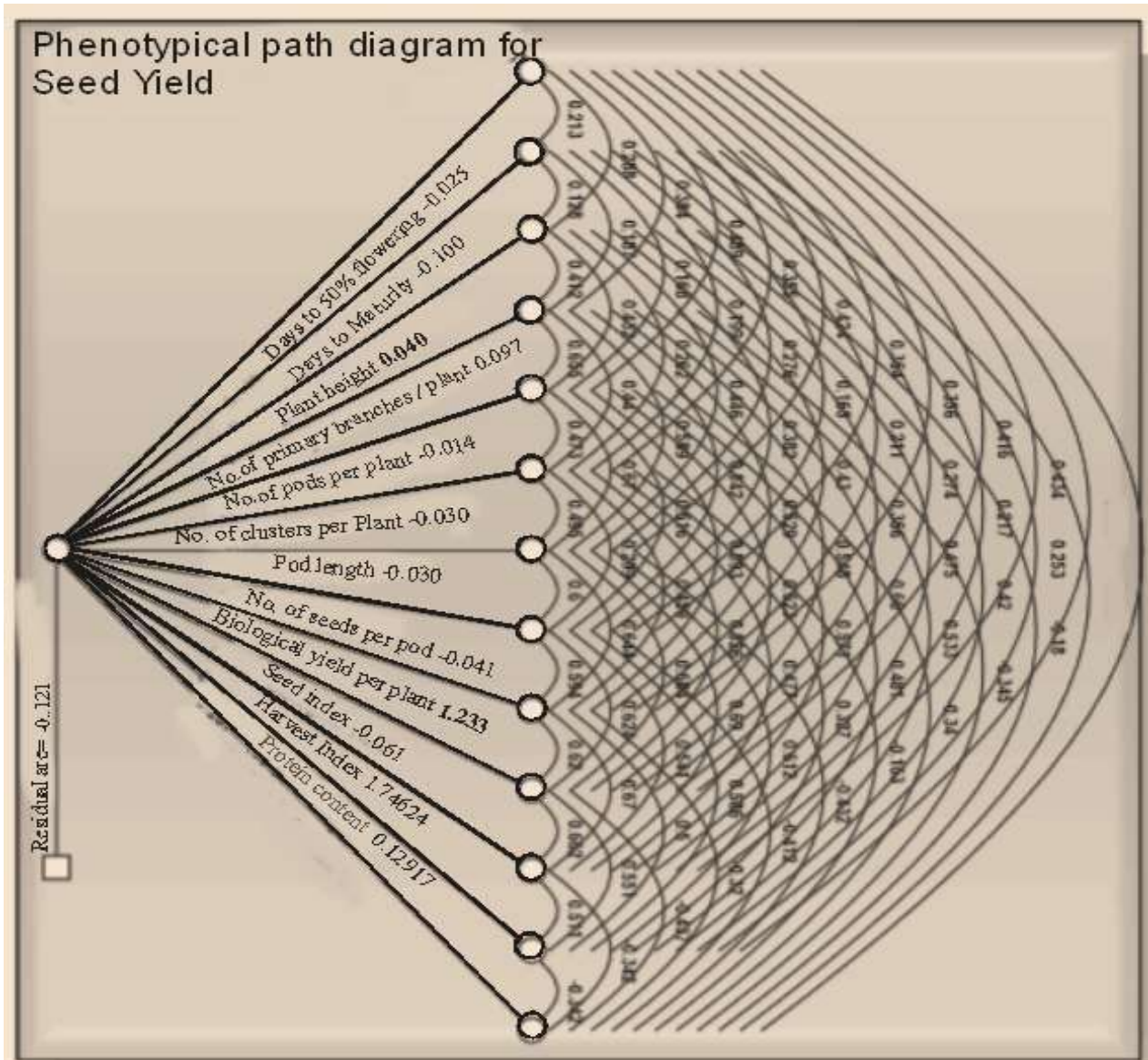
The effect of adding *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* on the acidity and overall consumer acceptability of Lebanese Kishk

Soumaya Obeid[1], Ali Alkhatib[1], Sami Tlais[2], Hassan Hajj Hussein[2*]

[1]Department of Nutrition and Food Science, Lebanese International University, Lebanon
[2]Department of Biological and Chemical Sciences, Lebanese International University, Lebanon
*Corresponding author

Abstract— Kishk is a fermented cereal product made traditionally from bourghul (cracked wheat) and yogurt. Being a fermented product, which gets stored and used over a period of a year, moisture and acidity are two critical components of the preparation method and storage/spoilage. We investigated the effect of a pure Lactic acid bacteria (LAB) starter culture (Streptococcus thermophilus and Lactobacillus delbrueckii subsp. Bulgaricus) on the acidity and consumer acceptability of Lebanese Kishk. Traditional Lebanese Kishk was prepared and used as a control in this study. Three additional test samples were prepared by adding the following to bourghul; fresh cow milk "Milk", milk and LAB "Milk+LAB", and yogurt prepared by inoculating fresh cow milk with LAB. Samples containing yogurt exhibited higher acidity and lower moisture content during the preparation and fermentation phase, both of which are required parameters for safety. Even though the final product of all samples were comparable to the control in terms of moisture, the "Milk+LAB" sample was more acidic than the control. None of the tested samples had a significant impact on consumer overall acceptability of the final product; however, the "Yogurt+LAB" sample was significantly preferred over the control for its odor.

*Keywords— **Kishk, cereal, LAB, fermentation.***

I. INTRODUCTION

Modern consumers are becoming increasingly aware of the benefits of fermented foods in a healthy diet and their overall impact on the quality of life. Fermentation is a metabolic process in which beneficial microorganisms create desirable changes in food. During this process, beneficial microbes break down sugars and starches into alcohol and acid [1,2]. These metabolites might cause various changes in the food product including:

1. Changes in the pH and texture of fermented dairy products due to the production of lactic acid and other organic compounds [3,4].

2. Changes that provide a pleasant taste and flavor in food. The proteolytic enzymes from the starter cultures may produce flavor compounds such as acetaldehyde, acetone, acetoin and/or diacetyl, which are important in some dairy products. In addition, some degree of fat degradation may take place, which significantly contributes towards the flavor of the product [5].

3. Changes that make food more nutritious [6,7].

4. Changes that extend the shelf life of the product, especially highly perishable foods [8,9], by producing antimicrobial compounds such as organic acids and bacteriocins [10].

Foods are fermented through two main methods. First, natural/spontaneous fermentation, which is carried out by microorganisms naturally present in raw food. Secondly, foods can be fermented via the addition of starter cultures, known as "culture-dependent ferments" [11]. One method

of performing a culture-dependent ferment is "back-slopping", in which a small amount of a previously fermented batch is added to the raw food (e.g., adding a small amount of previously fermented yogurt to a fresh batch of milk in order to make fresh yogurt) [12]. Starters used to initiate fermentation can either be natural starters (e.g., back-slopping) or selected starters to standardize the organoleptic characteristics of the final product [13].

Fermented food products such as fermented milk (e.g., yogurt and yogurt-related products) are becoming more and more appealing to a wider base of consumers [14]. Among the many products made with yogurt, special attention should be given to Kishk. While methods for preparing this product may differ from country to another, as well as within the same country, cereal and fermented milk are systematically the two main ingredients [15,16]. It is widely consumed by people indigenous to the eastern Mediterranean region and the Indian subcontinent. It is known as Kishk in Lebanon, Syria, Palestine, Jordon, and Egypt [17], as Tarhana in Turkey, Kushuk in Iraq [16], Kashk in Iran, Trahanas in Greece, and Tahonya/Talkuna in Hungary and Finland [18]. Kishk has a considerable dietary potential as a good source of minerals, vitamins, and amino acids [19]. It contains nutrients from both milk and cereal, a combination of these two balanced and highly complementary food products, where minor constituents that are deficient in cereal such as amino acids, are supplemented by milk, and vice versa [17]. In fact, due to the high nutritive value and the interesting organoleptic characteristics of Kishk, it became one of the most famous traditional Asian fermented foods regularly implemented in the diets of children and the elderly [16].

Lactic acid bacteria (LAB) starters are the most commonly used microorganisms for the fermentation and preservation of foods. They have a long history of safe use in food and are considered as GRAS (Generally Recognized as Safe) for human consumption [20]. In industrial production, selected yogurt starters, namely *Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus*, are often used to ferment pasteurized milk [21]. The use of these thermophilic starter cultures during the manufacture of yogurt leads to a more rapid acid production which reduces spoilage, suppresses the growth of potentially pathogenic bacteria, and is essential for the proper development of the product's flavor and aroma [22,23]. The traditional method for manufacturing fermented food products was the "inoculation" of the food, via back-slopping. This method has certain drawbacks, mainly a great fluctuation in the quality of the product, but it is still used for homemade products [24], especially in the production of traditional Lebanese Kishk.

The traditional method for manufacturing Kishk in Lebanon starts with a mixture of these two main components: yogurt and cracked wheat (bourghul). This method involves daily addition of plain yogurt to bourghul for a final ratio of 4:1. The amount of plain yogurt added each time depends upon the pre-mix absorption rate, which is governed by the fermentation pace as affected by ambient temperature, relative humidity, bacterial culture, and moisture content. This is followed by heavy handling for five days of uncontrolled fermentation. During the first four days of soaking with yogurt, the mix is kneaded by hand on a daily basis to make sure that the yogurt is fully absorbed by the coarse bourghul grains and left to ferment at room temperature (23-25°C). On day 6, the resulting dough is formed into small nuggets to expedite the drying process and is sun-dried until the moisture content becomes 6 to 12 %. The nuggets are then milled into powder that is ready to be used and that may be stored over a year in dry containers at room temperature [17]. The necessary frequent and heavy handling of Kishk during processing increases the risk of contamination with undesirable microorganisms. However, the final product characteristics, such as low moisture content (< 12%), salinity, and acidity (pH ~3.8) may help ensure the relative safety of the final product [25].

Therefore, this traditional process runs the risk of abnormal or secondary fermentation due to the presence of undesirable microorganisms. In addition, such an uncontrolled fermentation process is not suitable for the industrial production of Kishk [26]. To overcome these disadvantages and to improve the quality and safety of traditional Lebanese Kishk, we investigated several alternative methods to determine if it were possible to expedite the fermentation process without altering the final products organoleptic qualities.

II. METHODS AND MATERIALS

2.1 Materials

All raw materials used for Kishk preparation were obtained from local markets located in Bekaa, Lebanon. Fresh Cow milk was obtained from a local farm, Lactic acid bacteria (LAB) starter culture (Proquiga), which is a mixed culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* was purchased from a local supplier. Previous day's yogurt, white coarse bourghul, and salt were purchased from the local market.

2.2 Production of Kishk

To make the yogurt that was used in the experiment, fresh cow milk was heated at 85°C and stirred vigorously for 15 min (to destroy microorganisms that might compete with the starter culture during fermentation) and allowed to cool

down to 45°C in preparation for inoculation. Once the temperature of the milk reached 45°C, it was immediately inoculated with either a sample of homemade yogurt (CTR) or LAB (Yogurt+LAB). After thorough mixing, the milk was incubated at 40°C for 8 hours then kept refrigerated at 4°C until used**.**

For the "Milk" experiment, the pasteurized milk was left undisturbed for 24 hours in a large stainless-steel container covered with a tray and wrapped up in a blanket until the cream rose to the top and the milk coagulated.

To produce the dough for the three samples, yogurt, milk, or yogurt+LAB was mixed with pre-washed and dried coarse bourghul (Table 1). For the "Milk+LAB" experiment, pasteurized milk was mixed with 1kg of washed and dried bourghul and heated to 95°C for 10 seconds, then rapidly cooled to 45°C before adding the LAB culture. The ingredients (Milk + bourghul+ starter culture) were mixed thoroughly to produce a dough while gradually adding salt. All mixtures were kneaded twice daily, maintained at room temperature, and allowed to ferment for 5 days (Fig. 1). On day 6, the dough was formed into small nuggets 3-5cm in size and spread on clean cloth sheets on the rooftop to dry under the sun while rubbing it every morning to break it into smaller pieces and make sure it dries well. On day 8, the dried pieces were milled into a fine powder and packed into glass jars (Fig. 2) [27].

Table 1. Different samples of Kishk and their ingredients

	Yogurt (CTR)	*Milk*	*Yogurt +LAB*	*Milk +LAB*
White coarse bourghul (1000g)	✓	✓	✓	✓
Fresh cow milk (4L)	✓	✓	✓	✓
Previous day's yogurt (1 cup)	✓	✗	✗	✗
LAB (0.104g)	✗	✗	✓	✓
Salt (60g)	✓	✓	✓	✓
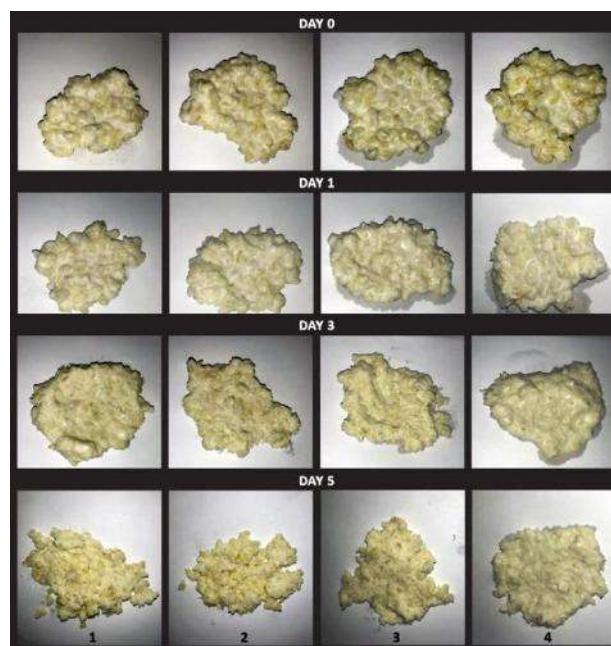
Fig.1: Pictures of the four Kishk dough samples during the fermentation period extending between days 0 and 5.

Column 1 represents "Yogurt+LAB" sample, column 2 represent the control "Yogurt" sample, column 3 represents the "Milk+LAB" sample, and column 4 represents the "Milk" sample.

Fig.2: Pictures of the final products of all four sample.

Sample 1 represents "Yogurt+LAB", sample 2 represents the control "Yogurt", sample 3 represents "Milk+LAB" and sample 4 represent "Milk".

2.3   Chemical analysis

The moisture content of Kishk samples was determined by drying the samples to constant weight at 105°C for 3 hours using the oven method according to standard methods of the Association of Official Analytical Chemists (AOAC 2000) [28]. The titratable acidity (TTA) was calculated as percentage of lactic acid according to the method of AOAC (1996) [29]. The pH values were measured after mixing a 5g of the sample with 100 ml distilled water using a pH meter (AOAC, 1995) [30].

2.4   Sensory analysis

Sensory analysis consisted of describing the organoleptic properties of the Kishk. Twenty panelists aged 20 to 45, regular consumers of Kishk soup, were advised to rate the soups in terms of odor, texture in the mouth, sour taste, saltiness, color, and overall acceptability using an interval Likert scale ranging from 1 to 5 (where "1" was "least liked" and "5" was "most liked"). Kishk soups were prepared by mixing 20 g of Kishk powder with 80 ml of drinking water with continuous stirring until dissolved.

The sample preparation, presentation, temperature, and serving container shape were the same for all tested samples. Kishk soup was served in plastic cups identified by a three-digit code number. Mineral water was used as palate cleanser (offered to the panelists between samples). Each sample was blindly analyzed by the sample panelist for a total of three replications.

2.5   Statistical analysis

Data were expressed as the mean ± SEM values from three independent replicates. Two-way analysis of variance was conducted using Graphpad Prism 8.3.0 and the differences were analyzed by Dunnett's Multiple comparisons test.

III.   RESULTS AND DISCUSSION

3.1   Chemical analysis

Considering that Kishk is a fermented product that is stored for periods that extend beyond a full year, we calculated the moisture content (Table 2), TTA (Table 3), and pH values (Table 4) of each of the samples included in the experiment and compared the progress of each sample at different days to itself at Day 0 (Fig. 3; *p<0.05; **p<0.01; and ***p<0.001) and to the control sample (Yogurt) at all 8 days (Fig. 4; *p<0.05; **p<0.01; and ***p<0.001 vs. "Yogurt" sample on the same day).

When inspecting the moisture content of all samples throughout the experiment, we noticed a significant drop in moisture content at day 1 in the "Milk" and "Yogurt+LAB" samples, only; however, by day 3 and until day 8, all samples exhibited a significant drop in moisture content

(Fig. 3A). Only the "Milk" sample started with a significantly higher moisture content from Day 0 compared to the control and continued as such all throughout the fermentation period that extended up to day 5 (Fig. 4A). On days 5 and 7, the moisture content of the "Yogurt+LAB" sample was significantly below that of the control; however, the moisture content of the final product was the same for all 4 samples (Fig. 4A). Since milk has a consistency that is more water than solid compared to yogurt, it is possible that milk did not mix very well with bourghul during the fermentation period (days 1 through 5) and drained down to the bottom of the container; while the thicker yogurt could very easily stick to bourghul grains and get absorbed by the grains in a more consistent manner.

Table 2. Moisture content ± SEM as a percentage for all 4 samples from day 0 to day 8.

	Yogurt (CTR)	Milk	Yogurt +LAB	Milk +LAB
DAY 0	69.39±0.53	74.14±2.04	69.26±1.47	69.42±0.59
DAY 1	67.71±0.31	71.24±0.35	65.60±0.24	68.71±0.36
DAY 3	62.81±0.18	68.47±0.45	61.84±0.08	64.34±0.24
DAY 5	57.91±0.29	67.61±0.23	54.92±0.14	62.15±0.07
DAY 7	21.77±0.53	22.42±0.56	19.13±1.00	22.44±0.16
DAY 8	6.38±0.16	4.59±0.02	4.936±0.61	4.86±0.74

Table 3. TTA values ± SEM for all 4 samples from day 0 to day 8.

	Yogurt (CTR)	Milk	Yogurt +LAB	Milk +LAB
Day 0	0.80±0.01	0.52±0.02	0.69±0.01	0.51±0.02
Day 1	0.89±0.01	0.58±0.01	0.75±0.01	0.58±0.01
Day 3	1.00±0.03	0.66±0.01	0.90±0.03	0.68±0.02
Day 5	0.94±0.03	0.68±0.01	0.89±0.05	0.68±0.01
Day 7	0.84±0.05	0.44±0.03	1.00±0.03	0.30±0.01

| Day 8 | 1.78±0.07 | 1.11±0.02 | 2.08±0.14 | 0.96±0.05 |

Table 4. pH values ± SEM for all 4 samples from day 0 to day 8.

	Yogurt (CTR)	*Milk*	*Yogurt +LAB*	*Milk +LAB*
Day 0	3.74±0.04	4.53±0.05	3.80±0.05	4.37±0.06
Day 1	3.83±0.04	4.45±0.01	3.97±0.04	4.34±0.01
Day 3	3.65±0.03	4.31±0.02	3.82±0.02	4.21±0.03
Day 5	3.64±0.01	4.13±0.01	3.74±0.01	4.03±0.01
Day 7	3.79±0.02	4.18±0.01	3.73±0.01	4.04±0.03
Day 8	3.81±0.02	4.18±0.02	3.65±0.03	3.96±0.03
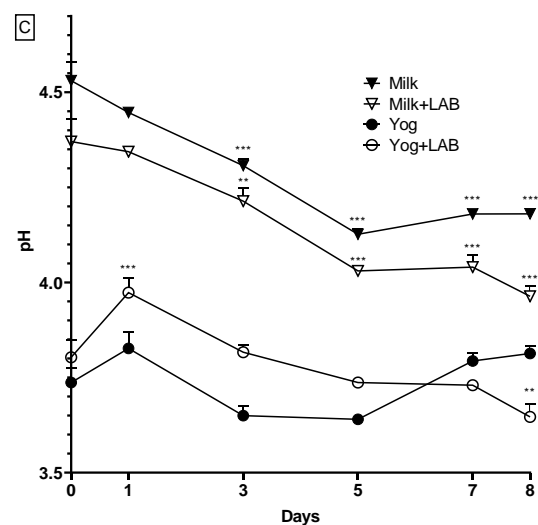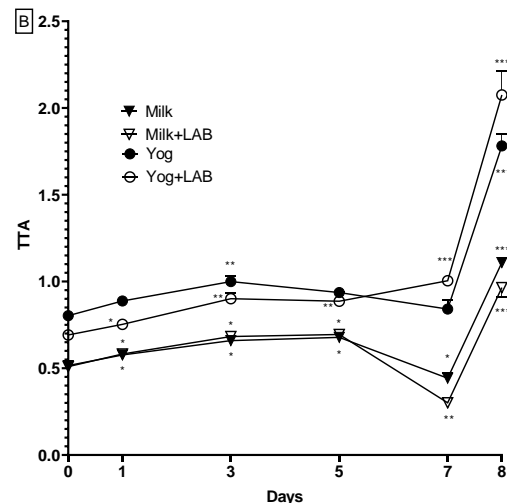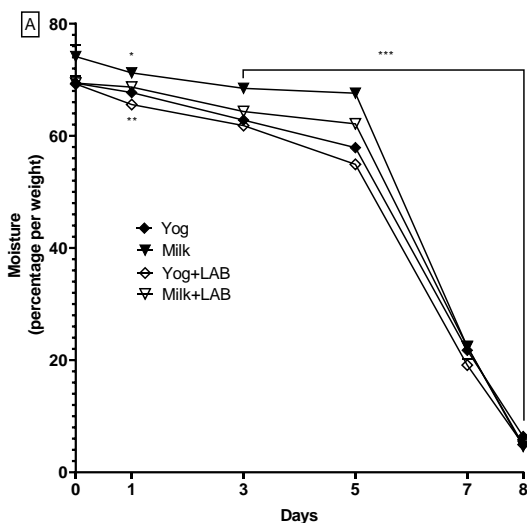
Fig.3: Comparing moisture content, TTA, and pH of each sample to itself on day 0.

Figure 3A represents the moisture content of each sample reported as a percentage of weight. The values for days 1 through 8 are compared to day 0, per sample. Numerical values are shown in Table 2. Figure 3B represents the TTA values of each sample. The values for days 1 through 8 are compared to day 0, per sample. Numerical values are shown in Table 3. Figure 3C represents the pH values of each sample. The values for days 1 through 8 are compared to day 0, per sample. Numerical values are shown in Table 4.

An increase in TTA was detectable starting at day 1 and continuing to day 5, for all 3 samples. For the control sample "Yogurt", an increase in TTA was detected at day 3 but then it dropped back to day 0 levels by days 5 and 7 (Fig. 3B). Interestingly, both milk samples dropped to below day 0 levels on day 7, while the "Yogurt+LAB" sample continued

to increase until day 7. However, the final product (day 8) of all 4 samples exhibited a very highly significant increase in TTA compared to day 0 (Fig. 3B). This is most likely because these powders contained between 4 and 6% water at this level, which made them very highly concentrated. When comparing the TTA values of all 3 samples to the control, the TTA values for the "Milk" and "Milk+LAB" samples were significantly lower than the control throughout the entire experiment and even in the final product (Fig. 4B). However, the TTA value of "Yogurt+LAB" sample increased above the control on day 7 and was even more significantly higher in the final product (Fig. 4B).

Looking at the pH values of the samples, no changes were detected in the pH level of the control sample throughout the experiment (Fig. 3C). Indicating no further fermentation took place. For the "Yogurt+LAB" sample, the pH values increased significantly at day 1, then dropped back to day 0 levels between days 3 and 7, and then dropped significantly in the final product at day 8. This was not consistent with our TTA data; however, we consider TTA to be a more accurate representation of the acidity. Both milk samples exhibited a significant drop in pH values starting at day 3 and extending to day 8 (Fig. 3C). When comparing the pH values of all 3 samples to the control, both "Milk" and "Milk+LAB" samples had pH values significantly higher than the control throughout the entire experiment and even in the final product (Fig. 4C).

This is to be expected for the "Milk" sample; however, the results of the "Milk+LAB" sample could be interpreted that the culture was somewhat diluted when it was mixed with bourghul instead of being allowed to ferment the milk alone before being added to bourghul. The pH value of the "Yogurt+LAB" sample was the same as the control sample throughout the entire experiment; however, the pH of the final product was significantly lower than that of the control (Fig. 4C).
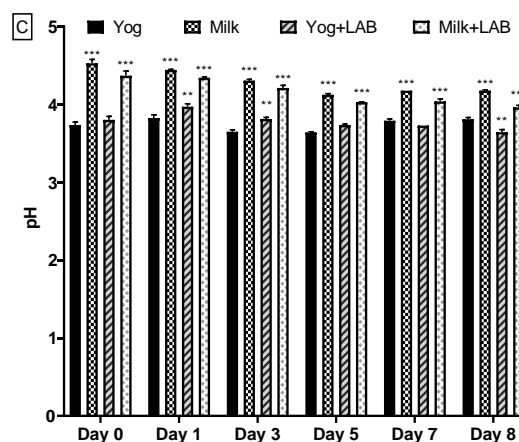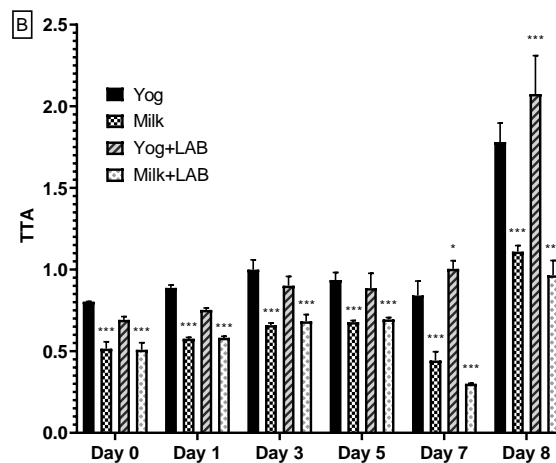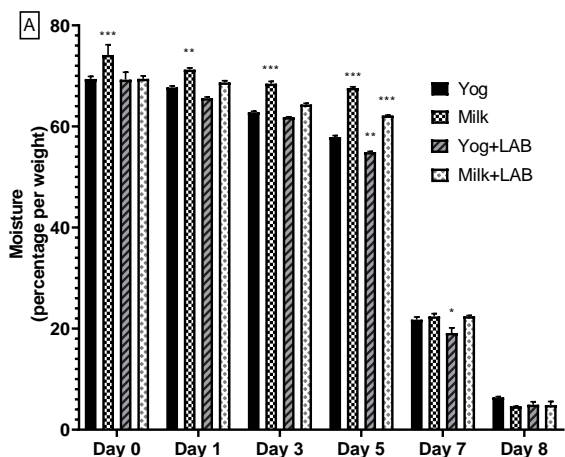
Fig.4: Comparing moisture content, TTA, and pH of each sample to the control on the same day of the experiment.

Figure A represents the moisture content of each sample reported as a percentage of weight. The values for days 0 through 8 for each sample is compared to the control on the same day. Numerical values are shown in Table 2. Figure 3B represents the TTA values of each sample. The values for days 0 through 8 for each sample is compared to the control on the same day. Numerical values are shown in Table 3. Figure 3C represents the pH values of each sample. The values for days 0 through 8 for each sample is compared to the control on the same day. Numerical values are shown in Table 4.

3.2   Sensory evaluation of prepared Kishk samples

Sensory evaluation of all 4 samples of Kishk was carried out and the results were reported as mean ± SEM of an interval Likert scale from 1 to 5 where "1" is least liked and "5" is most liked for each of the reported characteristics (Table 5 and Fig. 5; *p<0.05' **p<0.01 compared to the control). In terms of odor, only the "Yogurt+LAB" sample was significantly more appealing compared to the control. The panelists reported no difference between the control and "Milk" or "Milk+LAB" samples. In terms of saltiness, only

the "Milk" sample was reported to taste significantly less salty. In terms of sourness, only the "Milk" sample was reported as less sour than the control. In terms of texture, color, and overall acceptability, the panelists did not report any differences for any of the "Milk", "Yogurt+LAB", and "Milk+LAB" samples in comparison to the control.

Table 5. Sensory evaluation data reported as mean ± SEM for all 4 samples based on an interval Likert scale from 1 to 5 where 1 is "least liked" and 5 is "most liked".

	Yogurt (CTR)	Milk	Yogurt +LAB	Milk +LAB
Odor	2.85±0.30	2.95±0.22	3.35±0.30	3.95±0.18
Texture	3.20±0.25	2.65±0.26	3.35±0.25	3.50±0.17
Sourness	3.65±0.30	3.15±0.24	4.10±0.18	3.95±0.21
Saltiness	3.75±0.23	2.80±0.20	3.30±0.19	3.60±0.17
Color	3.95±0.18	3.35±0.21	3.85±0.20	3.65±0.20
Overall Acceptability	3.45±0.26	3.20±0.26	3.55±0.29	4.05±0.21
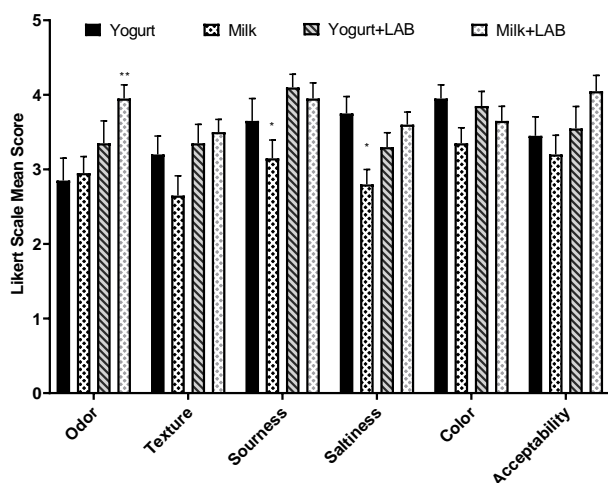
Fig.5: Comparing organoleptic properties of each sample to the control.

Values are mean ± SEM of the scores given by the 20 panelists for each of the listed characteristics.

IV. CONCLUSION

Our results showed that the milk containing samples exhibited higher moisture content and lower acidity

compared to the yogurt containing samples (during the fermentation phase) from day 0 to day 5. This is not ideal for a fermented product as it runs the risk of allowing the growth of undesirable microorganisms. The acidity of the "Yogurt+LAB" sample was higher than the control during day 7 and in the final product. It is possible that the purity of the LAB culture in comparison to the natural culture of the control sample allowed the fermentation process to be extended longer. Overall, using a pure LAB culture in the preparation of Kishk has the potential to provide a more controlled and safer fermentation process without reducing final product acceptability.

REFERENCES

[1] Ansorena, D., & Astiasar, I. (2016). *Fermented foods: Composition and health effects encyclopedia of food and health* (pp. 649–55). Oxford: Academic Press.

[2] Sharma, R., Garg, P., Kumar, P., Bhatia, S. K., & Kulshrestha, S. (2020). Microbial Fermentation and Its Role in Quality Improvement of Fermented Foods. *Fermentation*, 6(4), 106. https://doi.org/10.3390/fermentation6040106.

[3] London, L. E., Chaurin, V., Auty, M. A., Fenelon, M. A., Fitzgerald, G. F., Ross, R., & Stanton, C. (2015). Use of Lactobacillus mucosae DPC 6426, an exopolysaccharide-producing strain, positively influences the techno-functional properties of yoghurt. *International Dairy Journal*, *40*, 33–38. https://doi.org/10.1016/j.idairyj.2014.08.011.

[4] World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO) (2011). Fermented milks (CODEX STAN 243-2003), in: Milk and Milk Products -Codex Alimentarius. Rome.

[5] Ngassam Tchamba, C. (2007). Caracterisation de la flore lactique des laits fermentes artisanaux au Senegal: Cas de la cone des Niayes. Universite Cheikh Anta Diop de Dakar, Dakar.

[6] Altay, F., Karbancıoglu-Güler, F., Daskaya-Dikmen, C., & Heperkan, D. (2013). A review on traditional Turkish fermented non-alcoholic beverages: Microbiota, fermentation process and quality characteristics. *International Journal of Food Microbiology*, *167*(1), 44–56. https://doi.org/10.1016/j.ijfoodmicro.2013.06.016.

[7] Hwang, J., Kim, J. C., Moon, H., Yang, J. Y., & Kim, M. (2017). Determination of sodium contents in traditional fermented foods in Korea. *Journal of Food Composition and Analysis*, *56*, 110–114. https://doi.org/10.1016/j.jfca.2016.11.013.

[8] Nuraida, L. (2015). A review: Health promoting lactic acid bacteria in traditional Indonesian fermented foods. *Food Science and Human Wellness*, *4*(2), 47–55. https://doi.org/10.1016/j.fshw.2015.06.001.

[9] Terefe, N. S. (2016). *Food fermentation reference module in food science*. Elsevier.

[10] Nout, M. J. R. (2014). *Food technologies: Fermentation A2 – Motarjemi, Yasmine Encyclopedia of food safety* (pp. 168–177). Waltham: Academic Press.

[11] Rezac, S., Kok, C. R., Heermann, M., & Hutkins, R. (2018). Fermented Foods as a Dietary Source of Live Organisms. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.01785.

[12] Marco, M. L., Heeney, D., Binda, S., Cifelli, C. J., Cotter, P. D., Foligné, B., Gänzle, M., Kort, R., Pasin, G., Pihlanto, A., Smid, E. J., & Hutkins, R. (2017). Health benefits of fermented foods: microbiota and beyond. *Current Opinion in Biotechnology*, *44*, 94–102. https://doi.org/10.1016/j.copbio.2016.11.010

[13] Yann, D., & Pauline, G. (2014). Usefulness of Natural Starters in Food Industry: The Example of Cheeses and Bread. *Food and Nutrition Sciences*, *05*(17), 1679–1691. https://doi.org/10.4236/fns.2014.517181

[14] Gupta, S., & Abu-Ghannam, N. (2012). Probiotic Fermentation of Plant Based Products: Possibilities and Opportunities. *Critical Reviews in Food Science and Nutrition*, *52*(2), 183–199. https://doi.org/10.1080/10408398.2010.499779

[15] Platt, B.S. (1964). Biological ennoblement: Improvement of the nutritive value of foods and dietary regimes by biological agencies. *J. Food Technol.*, 18: 662-670.

[16] Van Veen. A. G., Graham, D. C.W. & Steinkraus, K. H. (1969). Fermented milk-wheat combination. *Trap. Geogr. Med., 21* (3)47-52.

[17] Tamime, A., & O'Connor, T. (1995). Kishk—A dried fermented milk/cereal mixture. *International Dairy Journal*, *5*(2), 109–128. https://doi.org/10.1016/0958-6946 (95)92205-i.

[18] Campbell-Platt, G. (1987). *Fermented Foods of the World*. Butterworths, London, UK, p. 290.

[19] Tamime, A. Y., Barclay, M. N., Amarowicz, R., & McNulty, D. (1999). Kishk - a dried fermented milk / cereal mixture. 1 Composition of gross components, carbohydrates, organic acids and fatty acids. *Le Lait*, *79*(3), 317–330. https://doi.org/10.1051/lait:1999327

[20] The maintenance of the list of QPS microorganisms intentionally added to food or feed - Scientific Opinion of the Panel on Biological Hazards. (2008). *EFSA Journal*, *6*(12), 928. https://doi.org/10.2903/j.efsa.2008.923

[21] Fisberg, M., & Machado, R. (2015). History of yogurt and current patterns of consumption. *Nutrition Reviews*, *73*(suppl 1), 4–7. https://doi.org/10.1093/nutrit/nuv020

[22] Beukes, K.M. (1999). Lactic acid bacteria in South African indigenous fermented milks and the evaluation of selected strains for application in the manufacturing of cultured milk. MSc (Agric). Department of Food Science. University of Pretoria, Pretoria. pp. 3-38.

[23] Akpemado, K. M., & Bracquart, P. A. (1983). Uptake of Branched-Chain Amino Acids by Streptococcus thermophilus. *Applied and Environmental Microbiology*, *45*(1), 136–140. https://doi.org/10.1128/aem.45.1.136-140.1983.

[24] Bintsis, T., & Athanasoulas, A. (2015). Dairy starter cultures. *Dairy Microbiology* (pp. 114–154). CRC Press.

[25] Tamime, A., Muir, D., Khaskheli, M., & Barclay, M. (2000). Effect of Processing Conditions and Raw Materials on the Properties of Kishk 1. Compositional and Microbiological Qualities. *LWT - Food Science and Technology*, *33*(6), 444–451. https://doi.org/10.1006/fstl.2000.0686.

[26] Hajj, E., Dib, H., Yaacoub, R., Al-Amin, M., & Mcheik, Z. (2019). Effect of modified manufacturing procedure on the overall quality attributes and safety of Kishk. *Lebanese Science Journal*, *20*(2), 215–229. https://doi.org/10.22453/lsj-020.2.215-229.

[27] FAQ (1982). Food Composition Tables for the Near East. *Food and Nutrition paper No. 26,* Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 100-3, 226, 231.

[28] Association of Official Analytical Chemists. (2000). Official Methods of Analysis of AOAC international. 17th ed, Vol. II, Gaithersburg, MY, USA.

[29] Association of Official Analytical Chemists. (1996). Official Methods of Analysis of AOAC international. 16th edition. Washington, DC.

[30] Association of Official Analytical Chemists. (1995). Official Methods of Analysis of AOAC international, 16th edition. ed. Cunniff, P., Washington, DC.

Article   *Peer-Reviewed Journal*

Fungal Laccase as a Green Catalyst

Ram Sahay

Department of Chemistry, Maharana Pratap P. G. College, Jungle Dhusan, Gorakhpur, India

***Abstract**— Laccases belongs to multinuclear copper containing oxidase and can act as a variety of aromatic and non-aromatic compounds. Due to their broad substrate specificity, they are considered as a promising candidate in various industrial and biotechnological sectors. They are regarded as a green catalyst in biotechnology. The present review focuses on structure, reaction mechanism, categories, applications, economic feasibility, limitations, and future prospects of fungal laccases. Thus, this review would help in understanding laccases along with the areas, whichhas not been focused and requires attention. Since past, immense work has been carried out on laccases: yet, new discoveries and application are ever increasing which includes biofuel, biosensor, fiber board synthesis, bioremediation, clinical, textile industry, food, cosmetics, and many more.*

Key words**—**Bioremediation, Catalyst, Mediator, Oxidoreductase, Multicopper, Antibiotics.

I. INTRODUCTION

Laccases are p-diphenol:dioxygen oxidoreductase [EC 1.10.3.2] belonging to the family of multicopper proteins. It was first isolated from the sap of Japanese lacquer tree *Rhus vernicifera* [1] and are widely distributed in nature being found in plants,

Fungi [2-4] insects and bacteria first bacterial laccase was discovered in *Azospirillum lipoferum*. Since bacterial laccases have low redox potential [5] fungal laccases [6] are more preferred owing to their high redox potential [7-9]. Laccases have the ability to oxidize a wide range of aromatic and non-aromatic compounds which includes substituted phenols, some inorganic ions, and variety of non phenolic compounds [10] Due to its low substrate specificity it can act on a broad range of substrates and has attracted considerable attention in different environmental, industrial and biotechnological sectors [11-12]. Laccases have been regarded as a Green Tool, because they require molecular oxygen (O_2) as the only co-substrate for bio-catalysis and not hydrogen peroxide H_2O_2. Laccases have the capability to reduce dioxygen to water by oneelectron oxidation of substrate [13] which is mainly substituted phenolic compounds.

Laccases have high catalytic efficiency and are used for technical applications in various industrial and biotechnological domains [14-15] which includes improving properties of fibers, biosynthesis, energy exploitation, environmental protection, biodetection, degradation of synthetic dyes, printing and dyeing industry, biopulping in paper industry, conversion of aromatic compounds [16] and removal of phenols which causes cancer and teratogenicity when present in waste water [17]. In addition, it is also used in fast moving consumer goods as toothpaste, mouthwash, detergent, soap, and diapers in cosmetics as deodorants; in beverage and food industry for wine and juice stabilization [18-19] in dough or baked products to increase strength of gluten structures in pharmaceutical industries as anesthetics, anti inflammatory drugs, antibiotics, and sedatives and in nanobiotechnology as nanoparticle based biosensors. However, laccases are not able to exhibit full efficiency under harsh conditions. Therefore, novel strains which can tolerate harsh conditions and give maximum enzyme production with minimum energy consumption are in huge demand.

The present review is an attempt to provide cumulative information on various aspects of fungal laccases, which includes information pertaining to the structure, reaction mechanism,categories, and industrial and biotechnological

application of laccase.

II.   SRUCTURE AND REACTION MECHANISM OF LACCASE

Laccases are known to exist in a variety of forms; they can be monomeric, homotetrameric, heterodimeric, and multimeric. Their molecular weight ranges from 50 to 130 kDa depending upon the organism [20]. The plant laccases approximately contains 45% carbohydrate content, whereas for fungal laccases, it is 10-30% of molecular weight [21]. It is assumed that the carbohydrate portion of laccase ensures the conformational stability of the protein part and protects the enzyme from proteolysis and inactivation by radicals. The primary structure of laccases consists of Greek key β barrel topology which constitutes of approximately 500 amino acid residues organized in three consecutive domains. These amino acids are distributed in three domains: first domain with 150 initial amino acids, second domain with 150 and 300 amino acid, and third domain with 300-500 amino acids. The stabilization of laccase structure is due to the presence of disulphide bonds between domains I and II and between domains I and III [22]. However, in *Melanocarpus albomyces,* three disulfide bridges are present of which one is inside domain I, another between domain I and domain III and the last one between domain II and III [23]. Laccases are known to exist in four different Cu catalytic forms per protein unit. These four Cu ions are divided into three types of structures:

TYPE-1: PARAMAGNETIC BLUE COPPER

Copper type-1 confers blue color to multicopper proteins, which is due to the intense electronic absorption caused by the covalent copper cysteine bond. Due to its high redox potential of +790 mV substrate oxidation takes place at the type-1 copper site and has an absorbance at 610 nm type-1 copper has a trigonal coordination, with two histine and a cysteine as conserved equatorial ligands and one position usually variable, and in case of fungal laccases, the axial ligand is leucine or phenylalanine. It has even been argued that the axial position ligand influences the oxidation potential of the enzyme, which possibly provides the mechanism for regulating its activity [24].

TYPE-2: PARAMAGNETIC NORMAL/NON-BLUE COPPER

Copper tpe-2 or normal Cu site is characterized by the lack of strong absorption features in the visible region and reveals usual electron paramagnetic resonance (EPR) spectra. Type-2 copper is coordinated by two histidines residues and is strategically positioned close to type-3 copper.

TYPE-3: DIAMAGNETIC SPIN COUPLED COPPER-COPPER PAIR

It is a binuclear center regulated by six histidines and spectroscopically characterized by an electron adsorption at 330 nm oxidized form and absence of an EPR signal due to the strong antiferromagnetical coupling between the two type-3 copper atoms which is related to the presence of a hydroxyl bridge. The type-2 copper and type-3 copper form a trinuclear cluster where molecular oxygen is reduced and release of water takes place. An example of one electron oxidation of phenolic hydroxyl groups, while reducing oxygen and forming phenoxy radicals along with water are represented below [25]:

$$4\text{Phe}{-}\text{OH} \; + \; O_2 \quad \xrightarrow{\text{Laccas}} \quad 4\text{Phe}{-}\text{O}^\circ + 2H_2O$$
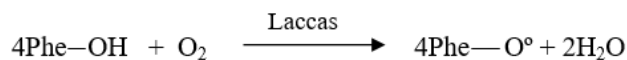
The Type-3 copper centers also have common feature of another protein superfamily which includes the tyrosinases and haemocyanins [26] figure-1.

REACTION MECHANISM OF LACCASE: DIRECT AND INDIRECT OXIDATION

The basic reactions catalyzed by laccase can be of two types: direct oxidation and in direct oxidation. The direct oxidation involves the oxidation of substrate to the corresponding radical as a result of direct interaction that occurs with copper cluster. However, in certain reactions, direct oxidation is not feasible as laccase can only oxidize those compounds whose ionization potential does not exceed redox potential of T1 copper ion. Nevertheless, the limitation can be overcome by the use of mediator which is a two step process: first enzyme catalyzes the oxidation of the mediator and then the oxidized mediator oxidizes the substrate. However, for the reaction to occur without any obstruction, certain features should be exhibited by the mediator: (a) the reaction should occur without any hindrance it must be good substrate for laccase both in its oxidized and reduced forms, should be stable; it must not inhibit enzymatic reaction, and conversion must be cyclic in nature [27] shown in figure.2
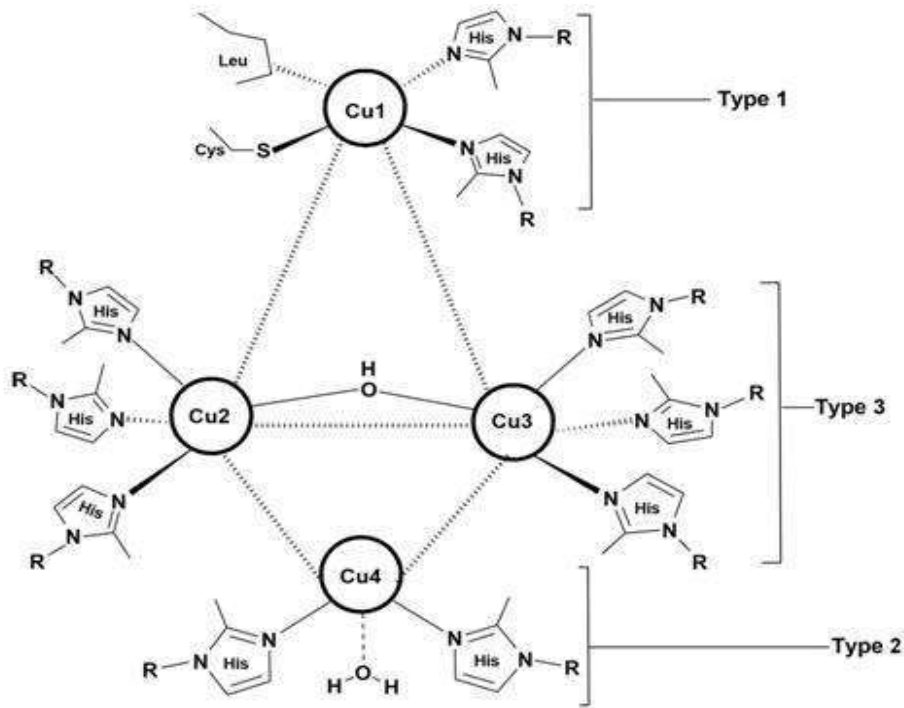
Fig: 1 Copper centers of laccase: Type-1, Type-2, and Type-3 (Adapted and modified axial ligand as leucine as per fungal laccases)
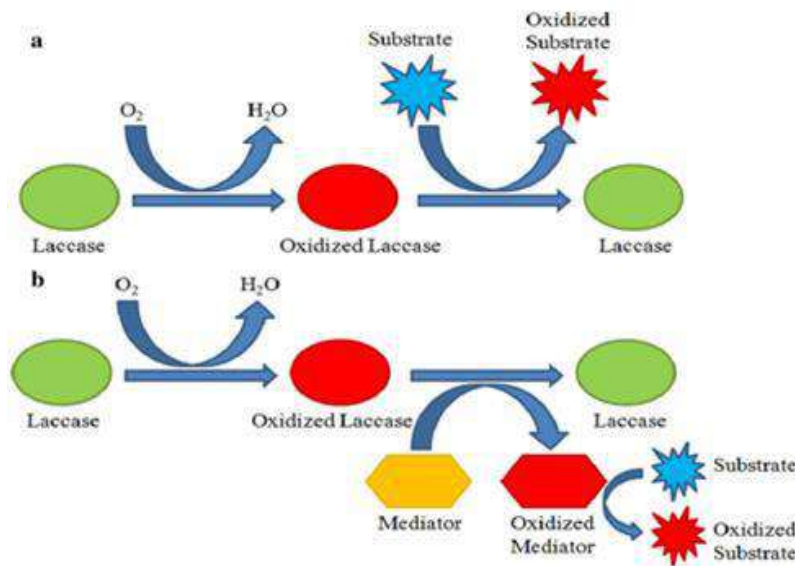
Fig: 2 Schematic representation of reaction catalyzed by laccase; a direct oxidation: the substrate is oxidized to the corresponding radical as a result of direct interaction and b indirect oxidation: the substrate is oxidized in the presence of a mediator

Besides mediators, the use of inducer to enhance laccase production has been widely practiced in fungi especially in the white rots where metals, aromatic compounds, and phenolic compounds [28] have been used as inducers. Conversely, there are certain substances which can inhibit the production of laccase and are known as the inhibitors such as sodium azide and dithiothreitol DTT.

YELLOW LACCASE

Yellow laccase is artificially reduced blue laccase as it does not have absorption at 600 nm and EPR spectrum [29]. Alteration of yellow to blue laccase can occur by the reduction of type-I copper site by aromatic product of lignin degradation or binding of specific amino acid of enzyme polypeptide to a molecule of modified product

produced by lignin degradation; it can also be due to heterogeneity induced by glycosylation. The modified molecule bound to the apoenzyme performs the function of electron transfer mediator analogous to the role of 2,2-azinobis (3-ethylbenzthiazoline -6-sulfonic acid) diammonium salt (ABTS) or other compounds in the reaction of blue laccase, hence having high redox potential which allows them to oxidize non phenolic compounds without any mediatorsand having greater industrial potential [30]. The change in protein conformation may explain the sensitivity of yellow laccase to CO and other inhibitors, e.g., *P. tigrinus*. At present, information about the purification and characterization of yellow laccase is extremely limited and their catalytic properties are still seldom investigated. Much work has not been done on the yellow laccase, but a few strains which are reported for the production of yellow laccase are as follows *P. tigrinus* [29], *S. aeruginosa, G. fornicatum* [30], *P. ostreatus* [31], *Panustigrinus, Phlebia radiate, Phlebia tremellosa, Pleurotus ostreatus* D1 (YLPO), *Sclerotinia sclerotiorum, and Panustigrinus* [30].

WHITE LACCASE

The white laccase exhibits neutral pH and has anomalous metal content which is responsible for its unique characteristic [32]. It exhibits absorption peak at 400 nm but absence of peak at 605 nm T1 copper site and 330 nm (T3 binuclear copper). White laccase does not exhibit EPR spectra as well as T1 and T2 signals [33]. The reason for the colorlessness iron, but it was conferred that the lack of absorbance at 605 nm can be due to the incomplete oxidation state of copper which has fully occupied electron configuration of d10 and no d-d transition [33] and may be responsible for extra high activity of protein. White laccase has been considered under laccase family, because the primary structure of the white laccase is identical to those of known laccase and it uses oxygen (O_2) as an oxidative substrate. There is the absence of the formation of hydrogen peroxide (H_2O_2) as the product of catalyzed reaction and substrate specificity exhibited is also the same as that of known laccase [32], e.g., *Lepista nuda* molecular mass of 56 kDa [34].

Few strains reported for white laccase production include *Pleurotus ostreatus* [32] and *Myrothecium verrucaria* NF-05 [33]. These strains besides single copper atom consist of various.

LACCASE MEDIATOR

The efficiency of substrate oxidation by a laccase depends on the difference between the redox potentials [35] of the substrate and the T1 Cu. Due to the lower redox potentials of laccases ($\leq 0.8V$) compared to ligninolytic peroxidases ($>1V$) [36] laccases are originally thought to

be able to oxidize only the phenolic ligninmoiety, with the majority of lignin being nonphenolic and with higher redox potentials. Low molecular- weight redox mediators are used to expand the laccase substrate range or increase the reaction rate, especially for substrates with higher redox potentials or too large to fit in the enzyme's active site. Commonly used laccase mediators include synthetic mediators such as 2,2$'$-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) and 1-hydroxybenzotriazole (HBT) as well as natural phenolic mediators such as syringaldehyde and acetosyringone. Despite the proven efficiency of artificial mediators, natural mediators (believed to be true mediators of fungal laccases in nature) are considered to be alternatives to the artificial ones because they are more economically feasible and environmentally friendly. Laccase oxidation of the substrate may proceed differently with a mediator. However, it is not always the case. Malachite green degradation products in the presence and absence of ABTS have been shown to be identical or different, depending on the enzyme.

Different types of mediators have different catalytic mechanisms ABTS mediated substrate oxidation proceeds via an electron transfer route. ABTS is first oxidized to its radical cation ($ABTS^{.+}$) and then to the dication ($ABTS^{2+}$) with redox potentials of 472 and 885 mV, respectively. Unlike ABTS, an N-OH type mediator (such as HBT and violuric acid) forms the N-oxy radical upon laccase oxidation and subsequent deprotonation the radical in turn abstracts the benzylic hydrogen atom from the substrate. Similarly, phenolic mediators also follow a radical hydrogen abstraction mechanism, but with the intermediate being a phenoxy radical. The effect of a mediator on laccase oxidation varies with the laccase and substrate and depends on the radicals formed, recyclability of the mediator and stability of the laccase in the presence of the mediator [37]. Regardless of the reaction mechanism, mediators incur additional costs, and can cause toxicity [38] and laccase inactivation [39]. Although, laccases without the requirement for facilitating mediators, the laccase/mediator system is regarded as a feasible industrial solution, ideal mediators that are cheap, green, effective, stable, recyclable, not toxic, or enzyme-inactivating should be ascertained [40].

LACCASE IMMOBILIZATION

Laccases are immobilized for recycling, operational stability, and resistance to application conditions. Immobilization techniques include entrapment, adsorption, covalent binding, self immobilization as well as combinations of the aforementioned techniques. Activity recovery varies based on the enzyme, the

immobilization method of choice, and preparation parameters. Compared with their free counterparts, immobilized laccases are more tolerant to high temperatures and storage and can be reused multiple times [41-42] they are also more resistant to inhibitors such as NaCl. Immobilization sometimes improves the catalytic activity of laccases [43-45] despite the common concern of reduced enzyme flexibility, steric hindrance and diffusion limitations [46]. Readers can refer to reviews on preparation and applications of immobilized laccases [41-42].

III. LACCASE APPLICATIONS IN BIODEGRADATION OF PPCPs

The value of fungi as well as fungal enzymes in pollution control and environment management has been recognized. Examples of environmentally important enzymes comprise hydrolases, laccases, lyases, peroxidases, tyrosinases, and P450 cytochrome mono oxidases [47-50]. The ability of laccases to effectively degrade and detoxify a variety of persistent organic pollutants (POPs) has received considerable attention in the field of bioremediation [51-53] and laccases can also be used in enzymatic biosensors for environmental pollution monitoring [48]. A summary of environmental contaminants as laccase substrates is provided in (Table-1). The contaminants investigated include dye stuffs [54-55], polycyclic aromatic hydrocarbons (PAHs) [56], endocrine disrupters [51, 57] and pesticides [58] shown in figure-3.
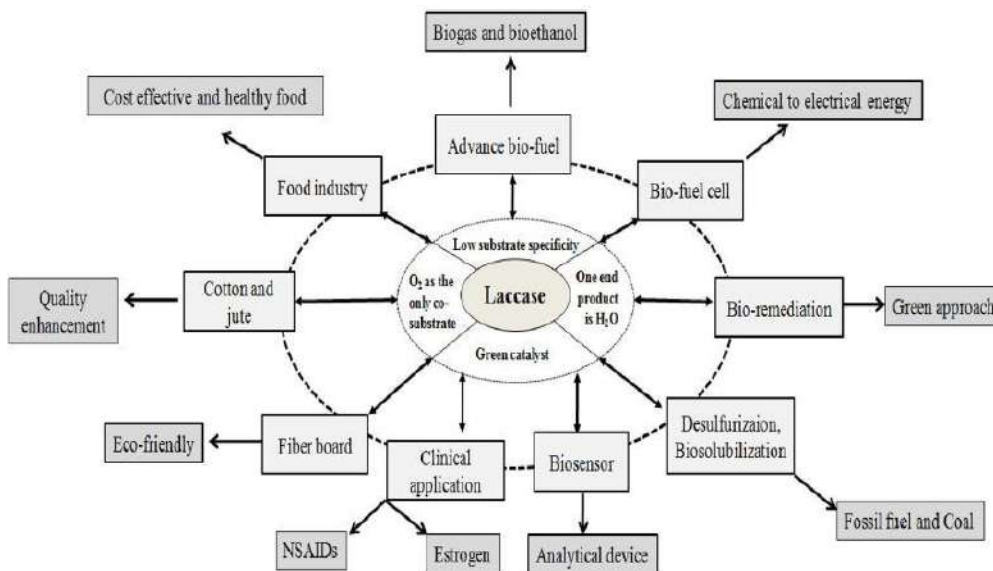
Fig: 3 Applications of laccase in various industries and biotechnological sectors

Pharmaceuticals and personal care products (PPCPs) are becoming ubiquitous in the environment and are recognized as emerging trace organic contaminants [59-61]. Laccases can be employed for their removal [51]. Laccases have been used in PPCPs as an ingredient; many products generated by laccases have antimicrobial, anticancer, antioxidant, detoxifying, or other activities [62]. Specifically, laccases can be used to synthesize novel antibiotics and laccase based antimicrobial formulations are considered as a green alternative to chemical decontamination. Nonetheless, the focus of this review lies in the degradation and detoxification of PPCPs contaminants with laccases.

DEGRADATION OF ANTIBIOTICS

Antibiotics constitute one of the most used classes of drugs in the world; they are used in human and veterinary medicine as well as livestock farming. Antibiotics that are not metabolized enter the environment. Conventional water treatment processes cannot effectively remove antibiotics [61], while more efficient advanced treatment methods have disadvantages such as high costs and secondary pollution. Antibiotics pose health risks by selecting for antibiotic resistance bacteria (ARB). Antibiotics, ARB, and antibiotic resistant genes have been detected in soil, sediments, and water bodies including waste water drinking water and marine water. There has been a fast growth in the literature describing laccase

utilization in antibiotic removal, especially within the past 2 years, but this topic has not been properly reviewed.

Target antibiotics under investigation include penicillins, tetracyclines, sulfonamides, uinolones, trimethoprim, sulfamethoxazole and tetracycline are two most studied (Table-2). The removal time ranges from minutes to hours, depending on the laccase, antibiotic and treatment parameters. Mediators such as HBT, ABTS, and SA are often used to enable or accelerate antibiotic conversion by laccases. Infact, significant antibiotic removal within 1 hrs usually requires involvement of an appropriate mediator [63]. Manganese peroxidase was more efficient in tetracycline conversion than laccase, but the addition of HBT can promote laccase catalysis to a rate higher than that of manganese peroxidase although still slower than that of lignin peroxidase (95%) degradation efficiency in 5 min. Interestingly, mediators, i.e., ABTS, SA, and AS, are consumed without observed catalytic activity during degradation of sulfamethoxazole [64]. Sulfonamides and tetracyclines are more easily attacked by laccase compared with quinolones [65].

Table1. Laccase Applications in Biodegradation and Bioremediation

Compound	Laccase	Enzyme From	Mediator	Reference
Phenol				
Chlorophenols, cresols, nitrophenols	*Trametessanguineu slaccase expressed in Trichoderma atroviride*	In culture	-	[54]
Technicalnonylphenol	*Phoma*sp. UHH5-1-03	Free	SA	[76]
Oxybenzone, pentachlorophenol	*P.ostreatus*	Free	ABTS, HBT, HPI, TEMPO, SA, VA, VAN	[30]
4-tert-butylphenol,4-tert-octylphenol	*Myceliophthorather mophila* laccase expressed in *Aspergillus oryzae* (Novozyme)	Enzymatic membranereactor	SA	[72]
2,4-dichlorophenol	*Pycnoporuss anguineus* CS43	Free	-	[37]
DYESTUFFS				
Bromo phenol Blue, Congo Red, Coomassie Blue, Tripan Blue	*T. sanguineus* laccase expressed in *T. atroviride*	Free	-	[54]
Acid Black 172, Congo Red, Crystal Violet, Direct Fast Blue FBL, Indigo Blue, Naphthol Green B, Methylene Blue, Neutral Red, Reactive Brilliant BlueX-BR, Remazol Brilliant Blue Reactive (RBBR)	*T. pubescens*	Chitosanbeads	-	[10]
Acid Orange 67, Basic Red18, Basic Yellow 28, Direct Black166, Direct Yellow107, Disperse Yellow 79	*Paraconiothyrium variabile*	Free	HBT	[64]

Brilliant Blue-G, Brilliant Blue-R, Bromo phenol Blue, Coomassie Blue R250, Crystal Violet, Malachite Green, Methylene Blue, Xylene Cyanol, RBBR	*P.sanguineus*	Free	VA	[30]
RBBR	*Cerrena*sp.HYB07	Cross-linked enzyme aggregates	-	[43]
Coomassie Blue-R 250	*Cerrena*sp. HYB07	Cross-linked enzyme aggregates	ABTS, AS, HBT, SA, SYA	[73]
ENDOCRINE DISRUPTERS				
Bisphenol-A (BPA)	*Coriolopsis gallica, Bjerkandera adusta, T. versicolor*	Free	HBT	[38]
BPA	*T.sanguineus* laccase expressed in *T. atroviride*	In culture	-	[54]
BPA	*T.versicolor* laccase expressed in *S.Cerevisiae*	Surface display	ABTS	[61]
BPA	*M.thermophila* laccase expressed in *A. oryzae* (Novozyme)	Ongranular activated carbon, continuous flow packed-bed reactor	-	[72]
BPA, 17α-ethinyl estradiol	*T.versicolor*	Polyamide- 6 /chitosannao fibers	-	[72]
BPA, 17α-ethinyl estradiol	*Phoma*sp. UHH5-1-03	Free	SA	[77]
BPA, 17α-ethinyl estradiol, 17α-estradiol, 17α-estradiol 17–acetate, estriol, estrone	*M.thermophila* laccase expressed in *A. oryzae* (Novozyme)	Enzymatic membrane reactor	SA	[72]
POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)				
All-15 USEP AprriorityPAHs	*B.subtilis* Cot Aexpressedin *E.coli*	Free	ABTS	[56]
Naphthalene, phenanthrene	*T.versicolor* (Sigma-Aldrich)	Non ionic surfactant-modified clay	-	[56]
Benzo [a] pyrene, phenanthrene	*T.sanguineus* laccase expressed in *T. atroviride*	Free	-	[54]
PESTICIDES				
Atrazine	*P.ostreatus*	Free	ABTS, HBT, HPI, TEMPO, SA, VA,VAN	[30]
Atrazine, isoproturon	*O.sativa* laccases expressed in *P. pastoris*	Inculture	-	[58]

Ametryn, atrazine, clofibric acid, fenoprop, pentachloro phenol, propoxur	*M.thermophila*l accase expressed in *A. oryzae* (Novozyme)	Enzymatic membrane reactor	SA	[72]

Table:2 Laccase Treatments of Antibiotics

Compound	Laccase	Enzyme from	Reaction parameters	Efficiency	Toxicity after treatment	References
PENICILLINS						
Amoxicillin , ampicillin, cloxacillin, penicillin-G, penicillinV, oxacillin	*T. versicolor* (Sigma-Aldrich)	Enzymatic membrane reactor	10µg/L antibiotics, 1m MSA, starting pH 6, 25°C, 0.07m/s flow, tangential configuration	54–100% after 24h	Increased (*B.subtilis*and*V. fischeri*)	[39]
SULFONAMIDES						
Sulfapyridine, sulfathiazole	*T.versicolor* (Sigma-Aldrich)	Free	16-20 mg/L antibiotic, 50-55 U/L laccase, 0.8 mM VA, pH 4.5, 25°C, 135 rpm	100% after 8h	NR	[74]
Sulfadimet hoxine, sulfamonometh oxine	*Perenni poria*strain TFRI 707	Free	50 mg/L antibiotic, 6 U/mL, 1 mM ABTS or VA, pH4.1,30°C, 8% glycerol	T (1/2) (min):1.8–4.1	NR	[79]
Sulfadimet hoxine, sulfamonometh oxine	*Perenni poria*strain TFRI 707	Free	50 mg/L antibiotic, 6U/mL laccase, 8% glycerol; 1 mM ABTS, pH 4, 50-60°C; 1 mM VA,pH4,40-60°C; 2m MSA, pH 6, 50°C	100% after 30 min with ABTS; 100% after 15 min withVA; >95% after 60 min with SA	Reduced (*V.fischeri*) withVA and HBA; increased with ABTS andSA	[40]
Sulfametho xazole	*T.versicolor*	Free	1,100 µg/L antibiotic, 1mM HBT, 25°C, 70 rpm	41% after 22 h	NR	[48]
Sulfametho xazole	*M.thermop hila* laccase expressed in *A.oryzae* (Novozyme)	Enzymati c membrane reactor	830 µg/L d antibiotic, 70-100 µM/ min laccase, 5µ MSA, 3g/L granular activated carbon	65%	Increased (Tox Screen3 assay with *Photobacterium leiognathi*), which can be reduced by granular activated carbon addition	[69]
Sulfadiazine ,sulfamethazin, sulfamethoxaz	*Echinod ontiumtaxo dii*	Oriented immobilizati onon	50 mg/L antibiotic, 0.2 U/mL laccase, 1m MAS, SA or SYA, pH5	>95% after 30 min	Reduced (*E.coli*and*S. aureus*)	[67]

ole		Fe3O4 nanoparticles				
Sulfamethoxazole	*T. versicolor* (Sigma-Aldrich)	Free	73-93 µ Mantibiotic, mediator/laccase ratio: 1.1 (ABTS), 1.7 (SA) or 2.4 (AS), pH 6, 25°C, static	100%	Reduced (algae *Pseudokirchneri ellasubcapitata*)	[68]
Sulfamethoxazole, sulfathiazole	*T.versicolor*	On poroussilica beads	50 mg/L antibiotic, 1U/mL laccase,1mMHBT,pH 5,40°C, 50rpm	76-85% after1h	Reduced (*E. coli, P. aeruginosa, H. influenza,S. enterica, S. aureus, S. pneumoniae*)	[75]
Sulfadimethoxine	*T.versicolor*	Free	Pergram soil: 2µ g antibiotic,10 U laccase, 8µmol ABTS or HBT, 1mg peat; room temperature	>90% after 72 h	NR	[78]
Sulfamethoxazole	*T. versicolor* Lac3 expressed in *S. cerevisiae*	Surfaced isplay	30 µM antibiotic, 0.25 U/mL laccase, pH5, 37°C, 250 rpm	44% after 30h	NR	[61]
TETRACYCLINES						
Tetracycline	*T. versicolor* (Sigma-Aldrich)	Enzymatic membrane reactor	20 mg/L antibiotic, 0.002g/L laccase, pH 6, 25°C,batch	0.34 mg/h for10d	NR	[71]
Tetracycline	*T. versicolor* (Sigma-Aldrich)	Enzymatic membrane reactor	20 mg/L antibiotic, 10 g/L laccase, 1.4µm poresize, 25 cm length, tangential (10L/h), 25°C, 8 $L/h/m^2$permeation	>200 $mg/h/m^2$ for 24h	NR	[71]
Tetracycline	*T. versicolor* (Sigma-Aldrich)	Free	100 µg/mL antibiotic,17.5 µg/mLlaccase,pH7 ,20°C	78% after18h	Reduced(*B.subtilis*)	[66]
Chlortetracycline, doxycycline, oxytetracycline	*T. versicolor* (Sigma-Aldrich)	Enzymatic membrane reactor	10µg/L antibiotics, 10µ MSA, starting pH 6, 25°C, 0.07 m/s flow, tangential	85–98% after 24h	Increased (*B.subtilis*and*V. fischeri*)	[39]

, tetracycline			configuration			
Oxytetracycline, tetracycline	*Cerrena* sp.HYB07	Magnetic cross-linked enzyme aggregates	100 µg/mL antibiotic, 40U/mL laccase, pH 6, 25°C	80% after12h	Reduced (*E.coli* and *B. licheniformis*)	[66]
QUINOLONES						
Flumequine	*T. versicolor* (Sigma-Aldrich)	Free	90 mg/L antibiotic, 6U/mL laccase, 1.35mM ABTS, pH4, 39°C, 150 rpm	98% after 2h	NR	[65]
Ciprofloxacin	*A. oryzae*	Free	10 mg/L antibiotic, 0.02% (w/v) laccase, pH 6, 60°C, 200 rpm ultrasound (75 W, 22 kHz, 50% duty cycle)	51% after 5 h	NR	[76]
Cinoxacin, ciprofloxacin, danofloxacin, difloxacin, enoxacin, enrofloxacin, flumequine, marbofloxacin, nalidixic acid, norfloxacin, ofloxacin, orbifloxacin, oxolinic acid, pipemidic acid	*T. versicolor* (Sigma-Aldrich)	Enzymatic membrane reactor	10 µg/L antibiotics, 10 or 1,000 µM SA, starting pH 6, 25°C, 0.07 m/s flow, tangential configuration	0-84% after 24 h with 10 µM SA; 15-93% after 24 h with 1,000 µM SA	Increased (*B. subtilis* and *V. ischeri*)	[39]
DIHYDROFOLATE REDUCTASE INHIBITOR						
Trimethoprim	*T. versicolor* (Sigma-Aldrich)	Enzymatic membrane Reactor	10 µg/L antibiotic, 1 mM SA, starting pH 6, 25°C, 0.07 m/s flow, tangential configuration	66.8% after 24 h	Increased (*B. subtilis* and *V. fischeri*)	[39]
Trimethoprim	*T. versicolor* (Fluka)	Magnetic cross-linked enzyme aggregates	100 µg/L antibiotic, 1 U/mL laccase, 0.1 mM ABTS, pH 7, 20°C, 125 rpm	47% after 6 h; 60% after 12 h	NR	[69]
NITROIMIDAZOLE						
Metronidazole	*T. versicolor* (Sigma-Aldrich)	Enzymatic membrane reactor	10 µg/L antibiotic, 10 µM SA, starting pH 6, 25°C, 0.07 m/s flow, tangential configuration	25.9% after 24 h	Increased (*B. subtilis* and *V. fischeri*)	[39]

This is presumably due to the strong electron donating aromatic amine group in sulfonamides and the phenol group in tetracyclines, which are not found in quinolones.

However, identified tetracycline transformation intermediates suggest that the phenol group is not the primary target for laccase oxidation, and that oxygen

addition, demethylation, water elimination reactions occur during laccase treatment [66]. For sulfonamides, increasing electro negativity of the substituents is accompanied by decreased degradation. Two sulfonamides, namely sulfapyridine and sulfathiazole, are desulfonated by laccase. Covalent cross coupling of sulfonamides is observed with laccase and mediator SA or AS [67], but not ABTS [68]. Trimethoprim has 2 amine groups and 3 methoxy groups and is usually administered in combination with sulfamethoxazole. Little [69] to over 60% [70] degradation of this antibiotic without a mediator have been reported. Furthermore, SA at 1,000 µM, but not 10 µM, increases trimethoprim removal from 27 to 67%; nearly complete elimination of sulfamethoxazole is achieved under the same conditions [71]. Some antibiotics (e.g., penicillins) are unstable in aqueous solutions, and attention should be paid to sample preservation and quantification [71]. Laccase from *T.versicolor*, especially the product sold by Sigma-Aldrich, is most frequently used in biodegradation studies of antibiotics as well as other trace organic contaminants. Other laccases include laccases from basidiomycetes *Cerrena*sp. HYB07, *Echinodon tiumtaxodii, Perenniporia* strain TFRI 707 and *P.sanguineus*, from ascomycetes *Phoma*sp. And *Myceliophthora thermophila* (recombinantly expressed in *Aspergillus oryzae*) and from action bacteria *Streptomyces ipomoeae* (expressed in *E. coli*). Laccases immobilized by different methods have been used for antibiotic degradation; including enzymatic membrane reactors [72-74] granular activated carbon [75] silica beads oriented immobilization [67] magnetic cross linked enzyme aggregates and cell surface display [65]. In particular, enzymatic membrane reactors (gelatin ceramic membranes grafted with commercial *T. versicolor* laccase) in tetracycline degradation have been evaluated in depth with respect to membrane preparation, efficiency, kinetics, and economics [71]. Mathematical cost estimation indicates that the enzymatic process is still economically uncompetitive. Improvements should be made in terms of enzyme kinetics, reactor effective lifetime and regeneration costs. For example, a pore diameter of 1.4 µm, in contrast to 0.2 µm, increases enzyme loading of the membrane reactor, avoids extensive membrane area, and facilitates tetracycline degradation.

Occasionally, laccases do not participate in antibiotic removal by white rot fungi; for instance, laccase was not responsible for oxy tetracycline degradation by *P. ostreatus* or *T. versicolor* or sulfamethoxazole degradation by aquatic ascomycete *Phoma*sp. UHH 5-1-03. In these cases, other enzymes, such as cytochrome P450, may be resorted to for biodegradation. It should still be pointed out that even when extracellular laccase is not able to

directly oxidize sulfamethoxazole, when a mediator is added significant removal is achieved. Laccases are also applied in combination with other processes in antibiotic treatment, such as ultrasound [76] and soil adsorption. The involvement of other processes facilitates degradation of antibiotics, e.g. quinolone antibiotics, which are recalcitrant to laccase oxidation. Laccase can also improve efficiency and stability of antibiotic removal by other organisms. When sulfamethoxazole is the transformed by non laccase producing bacteria *Alcaligenes faecalis*, the efficiency drops when some metabolites such as N4 acetyl sulfamethoxazole are transformed back to the parent compound. The removal efficiency does not decrease when the coculture of *A.faecalis* with laccase producing *P. sanguineus*is used or when cell free laccase was added to *A. faecalis* culture [77]. Toxicity of antibiotics after laccase treatment is commonly accessed via growth inhibition assay or bioluminescence inhibition test. Antibiotic degradation by laccase mostly leads to reduced toxicity. A good example comes from the comparison of the sulfamethoxazole transformation products and their toxicity by *A. faecalis* with or without exogenous laccase. N-hydroxy sulfamethoxazole (HO-SMX), a toxic and recalcitrant intermediate of sulfamethoxazole, is formed upon *A. faecalis* treatment. Additional laccase, on the other hand, eliminates HO-SMX along with the toxicity. However, sometimes laccase/mediator catalyzed antibiotic transformation results in even higher toxicity and this seems to frequently associate with the mediator SA [77]. It is postulated that the enhanced toxicity can be derived from oxidation of aromatic structures, especially phenols, to quinonoids.

The majority of studies on antibiotic degradation were carried out in aqueous environments, but there have been a few studies on remediation of soil, river sediment and sludge [78]. Laccase containing extract from spent mushroom compost of *Pleurotus eryngii* and extract containing micro capsules enhanced degradation of three tetracyclines in river sediment as well as degradation of three sulfonamides in sewage sludge. Sulfonamide antibiotics can form stable covalent bonds with humic constituents, and laccase can catalyze unreactive hydroquinone moieties in humic acid to reactive, electrophilic quionone moieties which in turn react with the antibiotic. This will affect the fate, bioactivity, and extractability of sulfonamides in soils.

IV. CONCLUSION

This review clearly professed that laccase is one of the standout enzymes being used in peculiar areas of biotechnology such as medicine, bioremediation,

mediator which includes improving properties of fibers, biosynthesis, energy exploitation, environmental protection, biodetection, degradation of synthetic dyes, printing and dyeing industry, biopulping in paper industry, conversion of aromatic compounds and removal of phenols which causes cancer and teratogenicity when present in waste water. In addition, it is also used in fast moving consumer goods (FMCG) as tooth-paste, mouthwash, detergent, soap, and diapers in cosmetics as deodorants; in beverage and food industry for wine and juice stabilization.

REFERENCES

[1]. Yoshida H. Chemistry of lacquer (*Urishi*) part-1. J. Chem. Soc. (Tokyo)1883; 43,472-486.

[2]. Sahay R, Yadav RSS, and Yadav KDS. Purification and Characterization of extracellular laccase secreted by *Pleurotussajor-caju* MTCC-141.C. J. of Biotechnology. 2008; 25: 24 (12), 2068-2073.

[3]. Dwivedi UN, Singh P, Pandey VP, Kumar A. Structure function relationship among bacterial, fungal and plant laccases, J. Mol. Cat B: Enzyme 2011; 68, 117-28.

[4]. Buddolla V, Bandi R, Avilala J, Arthala PK, and Golla N. Fungal lccase and their applications in Bioremediation, Hindawi Publishing corporation: Enzyme Research. 2014; doi:10.1155/2014/163242.

[5]. Surwase SV, Patil   SA, Srinivas S, Jadhav JP. Interaction of small molecules with fungal laccase: a surface plasmon resonance based study. Enzyme.Microb. Technol.2016; 82, 110-114.

[6]. Akpinar M, Ozturk UR. Induction of fungal laccase production under solid state bio processing of new agro industrial waste and its application on dye decolorization, Biotech. 2017;7, 98.

[7]. Sahay R, Yadav RSS, and Yadav, KDS. Purification and Characterization of laccase secreted by *L. lividus*. Applied Biochemistry and Biotechnology. 2009; 157, 311-320.

[8]. Songulashvili G, Flahaut S, Demarez M, Trico TC, Bauvois C, Debaste F, Pen, NMJ. (2016) High yield production in seven days of Coriolopsis gallica-1184 laccase at 50 L scale; enzyme purification and molecular characterization. Fungal Biol. 120 (4), 481-488.

[9]. Feng, Wang, Ling Xu, Liting Zhao, Zhongyang, Ding, Haile Ma, and Norman T. Fungal Laccase Production from Lignocellulosic Agricultural Wastes by Solid-State Fermentation, Microorganisms. 2019; 7, 665.

[10]. Zheng F, Cui BK, Wu XJ, Meng G, Liu HX, Si J. Immobilization of laccase on to chitosan beads to enhance its capability to degrade synthetic dyes. Int.

Biodeterior. Biodegrad.2016; 110:69-78.

[11]. Afreen S, Anwer R, Singh RK, Fatma T. Extracellular laccase production and its optimization from Arthrospira maxima catalyzed decolorization of synthetic dyes, J. Biol. Sci. 2016; https:// doi.org /10.1016/j. sjbs. 2016.01.015.

[12]. Chenthamarakshan A, Parambayil N, Miziriya N, Soumya PS, Lakshmi MSK, Ramgopal A, Dileep A, Nambisan P. Optimization of laccase production from Maras miellus palmivorus LA1 by Taguchi method of Design of experiments, BMC Biotechnol. 2017; 17: 12.

[13]. Sahay R, Yadav RSS, Yadava Sudha, and Yadav, KDS. A Laccase of *Fomes durissimus* MTCC-1173 and its role in the conversion of methylbenzene to benzaldehyde. Applied Biochemistry and Biotechnology. 2012;166:563-575.

[14]. Xenakis A, Zoumpanioti M, Stamatis H. Enzymatic reactions in structured surfactant free micro emulsions. Curr. Opin. Colloid. Interface. Sci. 2016;22:41-45.

[15]. Nguyen KA, Wikee S, Lumyong S. Brief review: Ligno cellulolytic enzymes from polypores for efficient utilization of biomass. Mycosphere, 2018; 9: 1073-1088.

[16]. Zheng F, Cui BK, Wu XJ, Meng G, Liu HX, Si J. Immobilization of laccase on to chitosan beads to enhance its capability to degrade synthetic dyes.Int. Biodeterior. Biodegrad.2016; 110:69-78.

[17]. Pang S, Wu Y, Zhang X, Li B, Ouyang, DingM. Immobilization of laccase via adsorption onto bimodal mesoporous Zr-MOF. Process Biochem. 2016; 51: (2):229-239.

[18]. Piacquadio P, DeStefano G, Sammartino M, Sciancalepore V. Applejuice stabilization by laccase immobilized on metal chelate regenerable carriers. Industrie. Delle. Bevande. 1998; 27:378-383.

[19]. Alper N, Acar J. Removal of phenolic compounds in pomegranate juices using ultrafiltration and laccase ultrafiltration combinations, Food/Nahrung.2004; 48:(3), 184-187.

[20]. Jaiswal N, Pandey VP, Dwivedi UN. Purification of a thermostable alkaline laccase from papaya (Carica papaya) using affinity chromatography, Int. J. Biol. Macromol. 2015; 72:326-332.

[21]. Baldrian P. Fungal laccase occurrence and properties. FEMS Microbiol. Rev. 2006; 30:(2), 215-242.

[22]. Plácido J, Capareda S. Ligninolytic enzymes: a biotechnological alternative for bioethanol production. Bioresour. Bioprocess. 2015; 2:(1), 23.

[23]. Hakulinen N, Kiiskinen L, Kruus K, Saloheimo M, Paananen A, Koivula A, Rouvinen J. Crystal structure of a laccase from Melanocarpus albomyces with an intact trinuclear copper site, Nat. Struct. Mol. Biol. 2002; 9: 601-605.

[24]. Enguita FJ. Structural biology of fungal multicopper oxidases. In: Leitao AL (ed) Mycofactories. Bentham Science Publishers, Emirate of Sharjah, United Arab Emirates, pp. 2011; 57-72.

[25]. Felby C, Thygesen LG, Sanadi A, Barsberg S. Native lignin for bonding of fiber board evaluation of

bonding mechanisms in boards made from laccase-treated fibers of beech (Fagussylvatica), Ind.Crops. Prod. 2004; 20:(2):181-189.

[26]. Decker H, Terwilliger N. Cops and robbers: putative evolution of copper oxygen binding proteins, J. Exp. Biol. 2000; 203:1777-1782.

[27]. Johannes C, Majcherczyk A. Natural mediators in the oxidation of polycyclic aromatic hydrocarbons by laccase mediators ystems, Appl.Environ. Microbiol. 2000; 66(2):524-528.

[28]. Terrón MC, González T, Carbajo JM, Yagüe S, Arana-Cuenca A,Téllez A, Dobson AD, González AE. Structural close-related aromatic compounds have different effects on laccase activity and on lcc gene expression in the ligninolytic fungus Trametes sp. I-62. Fungal Genet. Biol. 2004;41:(10), 954-962.

[29]. Leontievsky A, Myasoedova N, Pozdnyakova N, Golovleva L. Yellow laccase of Panus tigrinus oxidizes non-phenolic substrates without electron transfer mediators, FEBS. Lett. 1997; 413:(3), 446-448.

[30]. Iracheta-Cárdenas MM, Rocha-Peña MA, Galán-Wong LJ, Arévalo-Niño K, and Tovar-Herrera OE. A Pycnoporus sanguineus laccase for denim bleaching and its comparison with an enzymatic commercial formulation. J. Environ. Manage. 2016; 177:93-100.

[31]. Pozdnyakova NN, Rodakiewicz-Nowak J, Turkovskaya OV. Catalytic properties of yellow laccase from Pleurotus ostreatus D1. J. Mol. Catal. B: Enzym. 2004: 30:(1), 19-24.

[32]. Palmieri G. GiardinaP, Bianco, C Scaloni A, Capasso A, Sannia G. A novel white laccase from Pleurotus ostreatus. J. Biol. Chem. 1997; 272: (50), 31301-31307.

[33]. Zhao D, Zhang X, Cui D, Zhao M. Characterisation of a novel white laccase from the deuteromycete fungus Myrothecium verrucaria NF-05 and its decolourisation of dyes. PLoS.ONE. 2012; 7:(6), 38817.

[34]. Zhu M, Zhang G, Meng L, Wang H, Gao K, Ng T. Purification and characterization of a white laccase with pronounced dye decolorizing   ability and HIV-1 reverse transcriptase inhibitory activity from Lepistanuda. Molecules. 2016; 21:(4), 415.

[35]. Ashe B, Nguyen L N, Hai FI, Lee DJ, Vande Merwe JP, Leusch FDL. et al. Impacts of redox mediator type on trace organic contaminants degradation by laccase: degradation efficiency, laccase stability and effluent toxicity, Int. Biodeterior. Biodegradation.2016; 113:169-176.

[36]. Sitarz AK, Mikkelsen JD, and Meyer AS. Structure, functionality and tuning up of laccases for lignocellulose and other industrial applications. Crit. Rev.Biotechnol. 201636,70-86.

[37]. Rodríguez-Delgado M, Orona-Navar C, García-Morales R, Hernandez-Luna C, Parra R, Mahlknecht J, Ornelas-Soto N. Biotransformation kinetics of pharmaceutical and industrial micropollutants in groundwaters by a laccase cocktail from Pycnoporus sanguineus CS43 fungi. Int. Biodeter.Biodegr.2016; 108:34-41.

[38]. Daassi D, Prieto A, Zouari-Mechichi H, Martínez MJ, Nasri M, and MechichiT.. Degradation of bisphenol A by different fungal laccases and identification of its degradation products. Int. Biodeterior. Biodegradation. 2016; 110: 181-188.

[39]. Becker D, Varela Della, Giustina S, Rodriguez-Mozas S, Schoevaart R, BarceloD, de Cazes M.Removal of antibiotics in wastewater by enzymatic treatment with fungal laccase Degradation of compounds does not always eliminate toxicity. Bioresour. Technol. 2016; 219:500-509.

[40]. Weng SS, Liu SM, and Lai HT. Application parameters of laccase-mediators ystems for treatment of sulfonamide antibiotics. Bioresour.Technol. 2013; 141: 152-159.

[41]. Fernández-Fernández M, Sanromán MÁ, and Moldes D. Recent developments and applications of immobilized laccase, Biotechnol. Adv. 2013; 31: 1808-1825.

[42]. Asgher M, Shahid M, Kamal S, and Iqbal HMN. Recent trends and valorization of immobilization strategies and ligninolytic enzymes by industrial biotechnology, J. Mol. Catal. B Enzym. 2014; 101: 56-66.

[43]. YangJ, Xu X, YangX, Ye X, and Lin J. Cross linked enzyme aggregates of Cerrena laccase: preparation, enhanced NaCl tolerance and decolorization of Remazol Brilliant Blue Reactive. J. Taiwan.Inst. Chem. Eng. 2016c; 65: 1-7.

[44]. Sinirlioglu ZA, Sinirlioglu D, and Akbas F. Preparation and characterization of stable cross-linked enzyme aggregates of novel laccase enzyme from Shewanell aputrefaciens and using malachite green decolorization.Bioresour.Technol.2013; 146: 807-811.

[45]. Kumar VV, Sivanesan S, and Cabana H. Magnetic cross-linked laccase aggregates bioremediation tool for decolorization of distinct classes of recalcitrant dyes, Sci. Total. Environ. 2014; 487: 830-839.

[46]. Talekar S, Joshi A, Joshi G, Kamat P, Haripurkar R, and Kambale S. Parameters in preparation and characterization of cross linked enzyme aggregates (CLEAs). RSC Adv. 2013; 3: 12485-12511.

[47]. Demarche P, Junghanns C, Nair RR, and Agathos SN. Harnessing the power of enzymes for environmental stewardship, Biotechnol. Adv. 2012; 30: 933-953.

[48]. Yang S, Hai FI, Nghiem LD, Roddick F, and Price WE. Removal of trace organic contaminants by nitrifying activated sludge and whole cell and crude enzyme extract of Trametes versicolor. Water Sci.Technol. 2013b; 67,:1216-1223.

[49]. Yadav S, and Yadav JS. Applications of ligninolytic enzymes to pollutants, water, dyes, soil, coal, paper and polymers. Environ. Tech. 2015; 13:309-318.

[50]. Martinkova L, Kotik M, Markova E, and Homolka L. Biodegradation of phenolic compounds by Basidiomycota and its phenol oxidases: a review, Chemosphere. 2016; 149:373-382.

[51]. Gasser CA, Ammann EM, Shahgaldian P, Corvini PFX. Laccases to take on the challenge of emerging organic contaminants in waste water. Appl Microbiol. Biotechnol. 2014; 98:9931-52.

[52]. Viswanath B, Rajesh B, Janardhan A, Kumar AP, and

Narasimha G. Fungal laccases and their applications in bioremediation. Enzyme Res. 2014; 1-21.

[53]. Catherine H, Penninckx M, and Frederic D. Product formation from phenolic compounds removal by laccases: a review, Environ. Technol. Innov. 2016;5: 250-266.

[54]. Balcazar-Lopez E, Mendez-Lorenzo LH, Batista-Garcia RA, Esquivel-Naranjo U, Ayala M, Kumar VV, et al. Xenobiotic compounds degradation by heterologous expression of a Trametes sanguineus laccase in Trichoderma atroviride. PLoS ONE. 2016;11: 14-29.

[55]. Senthivelan T, Kanagara J, and Panda RC. Recent trends in fungal laccase for various industrial applications: an ecofriendly approach a review. Biotechnol. Bioprocess. Eng.2016; 21:19-38.

[56]. Zeng J, Zhu Q, Wu Y, Lin X. Oxidation of polycyclic aromatic hydrocarbons using Bacillus subtilis CotA with high laccase activity and copper independence. Chemosphere. 2016; 148:1-7.

[57]. Husain Q, and Qayyum S. Biological and enzymatic treatment of bisphenol A and other endocrine disrupting compounds: a review, Crit. Rev. Biotechnol. 2012; 33: 260-292.

[58]. Huang MT, Lu YC, Zhang S, Luo F, and Yang H. Rice (Oryzasativa) laccases involved in modification and detoxification of herbicides atrazine and isoprotur on residues in plants. J. Agric. Food. 2016; 64: 6397-6406.

[59]. Jie Yang, Wenjuan Li, Tzi BNG, Xiangzhe Deng, Juan L, and Xiuyu Ye. Laccases Production Expression Regulation and Applications in Pharmaceutical, Biodegradation. Frontiersin Microbiology. 2017; doi:10.3389/fmicb.2017.00832.

[60]. Onesios KM, Yu JT, and Bouwer EJ. Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: a review. Biodegradation.2009; 20: 441-466.

[61]. Chen Y, Stemple B, Kumar M, and Wei N. Cell surface displayfungal laccase as are new able biocatalyst for degradation of persistent micro pollutants bisphenol A and sulfamethoxazole. Environ. Sci. Technol. 2016; 50: 8799-8808.

[62]. Upadhyay P, Shrivastava R, and Agrawal PK. Bioprospecting and biotechnological applications of fungal laccase. Biotech. 2016;6: 15.

[63]. Mikolasch A, Manda K, Schluter R, Lalk M, Witt S, Sefeldt S. Comparative analyses of laccase catalyzed amination reactions for production of novel α-lactam antibiotics. Biotechnol. Appl. Biochem. 2012; 59: 295-306.

[64]. Forootanfar H, and Faramarzi MA. Insights into laccase producing organisms, fermentation states, purification strategies, and biotechnological applications. Biotechnol. Prog. 2015; 31: 1443-1463.

[65]. Becker D, Rodriguez MS, Insa S, Schoevaart R, Barcelo D, Cazes M, Belleville MP, Sanchez-Marcano J, Misovic A, Oehlmann J, Wagner M. Removal of endocrine disrupting chemicals in wastewater by enzymatic treatment with fungal laccases. Org. Process. Res. Dev. 2017; 21:(4), 480-91.

[66]. Llorca M, Rodríguez-Mozas S, Couillerot O, Panigoni K, de Gunzburg J, Bayer S, et al. Identification of new transformation products during enzymatic treatment of tetracycline and erythromycin antibiotics at laboratory scale by anon-lineturbulent flow liquid-chromatography coupled to a high resolution mass spectrometer LTQ-Orbitrap. Chemosphere. 2015; 119: 90-98.

[67]. Shi L, Ma F, Han Y, Zhang X, Yu H. Removal of sulfonamide antibiotics by oriented immobilized laccase on Fe$_3$O$_4$ nanoparticles with natural mediators. J. Hazard. Mater. 2014; 279: 203-211.

[68]. Margot J, Copin PJ, Von-Gunten U, Barry DA, Holliger C. Sulfamethoxazole and isoproturon degradation and detoxification by a laccase-mediator system: influence of treatment conditions and mechanistic aspects. Biochem. Eng. J. 2015; 103: 47-59.

[69]. NguyenL N, Hai FI, Price WE, Leusch FD, Roddick F, Ngo HH, et al. The effects of mediator and granular activated carbon addition on degradation of trace organic contaminants by an enzymatic membrane reactor. Bioresour. Technol. 2014b;167:169–177.

[70]. De Cazes M, Belleville MP, Mougel M, Kellner H, and Sanchez-Marcano J. Characterization of laccase-grafted ceramic membranes for pharmaceuticals degradation. J. Membr. Sci. 2015; 476: 384-393.

[71]. Nguyen LN, Vande Merwe JP, Hai FI, Leusch FD, KangJ, Price WE. Laccase syringaldehyde mediated degradation of trace organic contaminants in an enzymatic membrane reactor: removal efficiency and effluent toxicity. Bioresour. Technol. 2016b; 200: 477-484.

[72]. YangJ, YangX, Ye X, and Lin J. Destaining of Coomassie Brilliant BlueR-250-stained polyacrylamide gels with fungal laccase.Anal.Biochem.2016d ;493: 27-29.

[73]. Rodriguez-Rodriguez CE, Garcia-Galan MA, Blanquez P, Diaz-Cruz MS, Barcelo D, Caminal G. Continuous degradation of a mixture of sulfonamides by *Trametes versicolor* and identification of metabolites from sulfapyridine and sulfathiazole. J. Hazard. Mater. : 2012; 213 347-354.

[74]. Rahmani K, Faramarzi MA, Mahvi AH, Gholami M, Esrafili A, Forootanfar H. Elimination and detoxification of sulfathiazole and sulfamethoxazole assisted by laccase immobilized on porous silica beads. Int. Biodeterior. Biodegradation. 2015; 97:107-114

[75]. Sutar RS, and Rathod VK. Ultrasound assisted Laccase catalyzed degradation of Ciprofloxacin hydrochloride. J. Ind. Eng. Chem. 2015;31: 276-282.

[76]. Hofmann U, and Schlosser D. Biochemical and physicochemical processes contributing to the removal of endocrine disrupting chemicals and pharmaceuticals by the aquatic *ascomycete Phoma sp.* UHH 5-1-03. Appl. Microbiol. Biotechnol. 2016; 100: 2381-2399.

[77]. Weng SS, Ku KL, and Lai HT. The implication of mediators for enhancement of laccase oxidation of sulfonamide antibiotics. Bioresour. Technol. 2012; 113: 259-264.

[78]. Singh G, Kaur K, Puri S, and Sharma P. Critical factors affecting laccase mediated biobleaching of pulp in paper industry. Appl. Microbiol. Biotechnol. 2015; 99: 155-164

Assessing Awareness about Volta Basin Authority, Code of Conduct and Water Governance Challenges in the Volta Basin

Abdul-Razak Zakaria[1], Kenichi Matsui [2]

[1]Graduate School of Life and Environmental Sciences, the University of Tsukuba, Japan.
[2]Faculty of Life and Environmental Sciences, the University of Tsukuba, Japan.

Abstract— River basin organizations have taken shape in different parts of Africa partly under the influence of international aid institutions that aimed to help prevent water conflicts among basin countries. The code of conduct and the Volta Basin Authority (VBA) exemplify one of most recent efforts to collaboratively govern a major transboundary basin of western Africa. The question, however, remains as to the extent to which culturally diverse local communities can be mobilized and integrated in water governance. No study has clarified this point. This paper, therefore, attempts to better understand local stakeholders' perceptions and needs to collaboratively govern transboundary water between Ghana and Burkina Faso. For this research, we conducted preliminary field visits and questionnaire surveys in both countries. We found that 84% of the respondents in Burkina Faso and 91% in Ghana did not know about the VBA. Some respondents, especially educated ones in both countries, did hear about the code of conduct. Regarding local needs for water governance, most respondents in Ghana expressed concerns about frequent flooding due to Bagre Dam spillage in upstream Burkina Faso that devastated their farms. This research then discusses how transboundary water governance in the Volta River Basin can be improved in the future.

Keywords— Volta River Basin, riparian awareness, water governance challenges, Burkina Faso, Ghana.

I.   INTRODUCTION

Proponents of transboundary water management have long sought for an effective cooperation mechanism among basin countries partly to prevent or mitigate water conflicts (Islam and Susskind, 2013). As early as 1908, the United States and Canada established the International Joint Commission (IJC) by signing a treaty to collaboratively govern shared water resources (Chacko, 1932). Since then, many transboundary water agreements were signed partly to establish river basin organizations (RBOs), which, to some extent, affected how nations and jurisdictions may share water sources (Priscoli and Wolf, 2009). These organizations also have institutionalized water conflict resolution mechanisms (Schmeier, 2013).

Wolf et al. (2003) identified 17 water conflict prone river basins in the world, of which eight basins belonged to the African continent. Somewhat following American and European examples, African nations have formulated several transboundary water organizations, such as the Lake Chad Basin Commission (1964), Niger Basin Authority (1980), Permanent Okavango River Basin Water Commission (1994), and Zambezi Watercourse Commission (2004), among others. UN organizations were often involved in the formulation of these organizations. For example, in 1997 the UN Watercourses Convention emphasized international cooperation in managing transboundary watercourses. In western Africa, the World Bank, the Economic Community of West African States, and the European Union Water Initiative have supported the establishment of a cooperation mechanism among six

riparian countries of the Volta River Basin since the 1990s (Opoku-Ankomah et al., 2006; World Bank, 2015; Yankey, 2019).

Partly in response to this transboundary collaboration movement, Burkina Faso and Ghana established the code of conduct for the sustainable and equitable management of the Volta Basin. This was facilitated under the Project for Improvement of Water Governance in the Volta Basin (PAGEV). To faithfully follow this code, the two countries established the Joint Technical Committee on Integrated Water Resource Management (JTC-IWRM) in 2005. At a follow-up validation meeting in 2006, the JTC-IWRM finalized the language in the code (Welling et al., 2014). The directorate of water resources in both countries jointly spearheaded the development of the code of conduct.

This code of conduct defined tasks and obligations of riparian states. It contains eight sections and 59 articles. Some articles provide guidelines for decision making and the implementation of water governance interventions. It laid out nine principles for the rational and sustainable use of basin resources which are somewhat similar to international water law principles on shared water resources (Ampomah et al., 2008). These principles are: (1) equitable resource use and participation, (2) cooperation, (3) regular data and information exchange, (4) notification of planned activity, (5) ecosystem protection and preservation, (6) non-maleficence, (7) emergency notification, (8) freedom for navigation, and (9) precaution and prevention (Yankey, 2019). The code of conduct, however, is not published for the readership of the public.

Despite this agreement, water resource use in both countries remains virtually unchanged partly due to lack of institutional frameworks to ensure the implementation of the code. The Volta Basin Authority (VBA) was expected to fill this gap. In 2004 a joint declaration agreement was signed by Burkina Faso and Ghana. A few months after the enforcement, these nations invited all the other riparian countries of the Volta Basin to join and formulate a similar transboundary water governance framework (Mali, Togo, Côte d'Ivoire, and Benin). In November 2004, all responsible ministers of the six basin countries came together and signed an agreement that established the Volta Basin Technical Committee (VBTC). On December 6, 2005, these countries also signed a memorandum of understanding (MOU) to establish the Volta Basin Authority (VBA) (Opoku-Ankomah et al., 2006; World Bank, 2015; Yankey, 2019). These efforts resulted into the establishment of the Volta Basin Authority (VBA) in 2007 (which came into effect in 2009) (Global Water Partnership, 2014; Amuquandoh, 2016).

The establishment of this Authority was a major step toward a collaborative governance. The Volta River Basin affects water supplies for about 24 million people (as of 2010) and this number is expected to reach 33.9 million in 2025. Riparian communities in this Basin are largely rural and poor, engaging in small-scale agriculture (UNEP/GEF-Volta Project, 2013; Global Water Partnership, 2014). Considering this soci-economic situation along with cultural diversity, the fundamental question is how the VBA can implement its policies effectively among its diverse members, and to what extent local participation can be achieved. Article 6 of the Volta Convention mandates it to promote consultation and partnership among these people. It also encourages the implementation of integrated water resources management (IWRM) and the equitable distribution of benefits. As water conflicts tended to emerge due to dam construction and its release of reservoir water, which can flood lower basin communities, the VBA further instructed member nations to obtain its assessment and approval in order to execute infrastructure developments that have the potential to inhibit water flow (Yankey, 2019; IEADB, 2020). The 2015-2019 Strategic Action Plan of the VBA further aimed at enhancing stakeholder participation through enhanced communication (World Bank, 2015).

Gerlak and Schmeier (2018) noted that RBOs are effective when they contribute to behavioral change of riparian communities towards sustainable transboundary water resource governance. The effectiveness of the VBA, however, has been questioned by scholars. Gao and Margolies (2009) found that the Basin had suffered increasingly from deteriorating water quality in the last ten years. Several other scholars stressed poor coordination among the six countries for flood risk assessment and planning (Obrecht and Mead, 2014; World Bank, 2015; Yankey, 2019). The World Bank (2015) also observed increasing extreme climate events, continuing deforestation, and soil degradation. Yankey (2019) further noted that the VBA was not respected by stakeholders. IUCN (2012) encouraged the VBA to show tangible results and improvements to water users in order to have good community participation at transboundary levels.

One of the most contentious issues that gained attention from scholars was the controversy over Bagre Dam spillage near the Ghana-Burkina Faso border (Amuquandoh, 2016; Yankey, 2019). Some studied the effect of Bagre Dam spillage on downstream watershed areas in Ghana (Matthews, 2012; Mul et al., 2015; Ampomah, 2017). Another group of researchers focused on water allocation between the two countries (Andreini, 2002; Leemhuis et al., 2009; Baah-kumi and Ward, 2020). Some studies looked at institutional arrangement for

undertaking integrated water resource management in this border area (Opoku-Ankomah et al., 2006; Agyenim, 2011).

However, for the VBA to engage in meaningful consultation and induce active participation among smallholders, it is imperative to better understand the level of awareness and needs among riparian communities (Koop et al., 2017). Awareness means a good comprehension of causative factors as well as effects and dangers associated with governance challenges. A lack of awareness of government policies on flood management by local communities in Ghana was identified as a major barrier to stakeholder participation in water resource protection (Gyireh and Nunbogu, 2015). They recommended the adherence of the principle of free, prior, and informed consent (FPIC) in engaging with the public regarding water governance. The international water law principles on shared water resources also emphasized the need for prior consultations with all stakeholders and the duty to cooperate and to negotiate, among others (Ampomah et al., 2008).

Heeding to these suggestions and partly attempting to fill out research gaps we identified above, this paper seeks to find factors that have prevented the effective implementation of transboundary governance in the Ghana-Burkina Faso border area. As the eight principles of the code of conduct generally emphasize the importance of meaningful participation and consultation among stakeholders, this paper tries to better understand riparian communities' awareness of critical water governance issues in the study areas. This examination can help identify factors that have prevented policymakers from effectively coordinating water policies in these two countries.

II.   METHODS

2.1 Study area

As one of the most critical issues of the Volta River water use has been related to Bagre Dam spillage, we chose to focus on two main areas that have been reported to be most affected by the spillage, including Bagre District of the Eastern Central Region of Burkina Faso and the so-called Bawku zone of the Upper East Region in Ghana (Figure 1).

In 2019, we conducted a preliminary field visit in this area and interacted with some community members in order to collect information about dam spillage and flood impact issues. We found that downstream communities below Bagre Dam both in Burkina Faso and Ghana had

frequently suffered from flooding due to its regular spillage (Gao and Margolies, 2009; IIED, 2020).

The town of Bagre is known for its agribusiness projects in connection to the dam. It is home to 19,164 people from various ethnic groups (City Population, 2020). The climate in this area is characterized by dry (harmattan) and wet (rainy) seasons with an average annual rainfall of 1,000 mm (Coche, 1998). Its vegetation is predominantly covered with scattered shrubs, short grasses, and acacia trees. Crop farming, livestock breeding, and fishing constitute main livelihood activities. Rice, maize, vegetable, and fruit plantations are main farming activities (City Population, 2020).

The economy of the Bawku zone largely depends on agriculture, which employs about 80% of the population (Ghana Statistical Service-Bawku Municipality, 2014). The climate here is characterized by the wet season from May to October and the dry season from November to April. An average annual rainfall ranges from 950 mm to 1,100 mm. The vegetation here is like the study area in Burkina Faso. Riparian communities conduct limited irrigation by drawing water from the Volta River. Major food crops here include maize, rice, sorghum, pepper, watermelon, and onion (Ghana Statistical Service-Garu, 2014; MOFA, 2015). The construction of Kpalugu multi-purpose dam in downstream Ghana commenced in April 2020. Once completed, it will be the first storage dam in this part of the Basin to provide irrigation water to farmers (Construction Review Online, 2020).
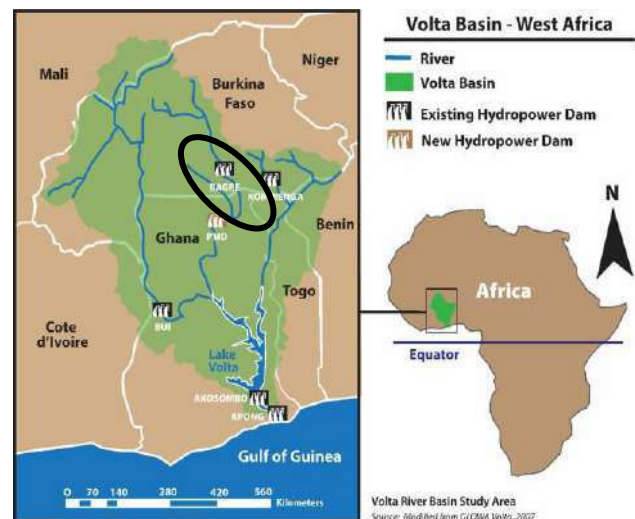
Fig. 1. Map of the Volta River Basin showing the study area in circle

(Source: Baah-kumi and Ward, 2020).

2.2 Data collection and analysis

In December 2019 and January 2020, we conducted questionnaire surveys in the two study areas along the Volta River. In Burkina Faso's Bagre District, we randomly sampled 30 residents each at Poanga, Benkaku and Dirlakou communities. In the Bawku zone of Ghana, we randomly distributed the questionnaire to 50 residents each at Azum-Sapeliga, Gentiiga and Songo communities. Our selection of sampling sizes was based on the population differences between these two areas. A population of Bagre District was 19,164 (CityPopulation, 2020) and that of Bawku zone was 290,117 (Ghana Statistical Service, 2014). Due to their limited reading and writing skills, we obtained support from local enumerators and administered the questionnaire by translating English into local languages called *Mossi* in Burkina Faso and *Kusaal* in Ghana. The response rate was 100% in Burkina Faso and 99% in Ghana. Altogether we collected 238 valid responses.

The questionnaire had three parts. The first part attempted to identify the socio-demographic characteristics of our respondents. The second part tried to understand community members' awareness about water governance in the Volta River Basin. The third part focused on water governance challenges. The questionnaire responses were coded and entered in the Statistical Package for Social Sciences (SPSS version 23) worksheet for analysis. Descriptive statistics in the form of frequencies and percentages were largely used to discuss the results. We also used the Pearson Chi-Squared to understand correlations between respondents' socio-demographic characteristics and their awareness (Kent State University Libraries, 2020).

The null hypothesis (Ho) of the Pearson Chi-Squared (χ2) analysis was that there is no significant difference between respondents' socio-demographic characteristics and their awareness about the code of conduct, VBA, and being key stakeholders of VBA. The alternate hypothesis (Ha) was that there is significant difference between respondents' socio-demographic characteristics and their awareness about the code of conduct, VBA, and being key stakeholders of VBA. The null hypothesis was tested at 0.05 level of significance. Ho was rejected if the p-value is lower than the significance level. However, when the p-value is higher than the significance level, then we accept Ho.

III.   RESULTS AND DISCUSSION

3.1 Socio-demographic characteristics of the respondents

Among the 238 valid responses, 148 were from Ghana and 90 were from Burkina Faso (Table 1). In terms of gender 66% in Ghana and 50% in Burkina Faso were males. The mean age among the Ghanaian respondents was 39.7 years old whereas that in Burkina Faso was 40.5 years old. About 93% of the Ghanaian respondents belonged to either the 40-59 age group (57%) or the 18-39 age group (36%). In Burkina Faso, the 40-59 age group consisted of 37% whereas the 18-39 age group had 53%. To place these age differences in a context, according to the World Bank (2020), average life expectancy in Burkina Faso is 61 years old, and that of Ghana is 64 years old (World Bank Group, 2020). Among them, 62% in Ghana and 30% in Burkina Faso were household heads.

The results on economic aspects show that the Bawku respondents (Ghana) were largely farmers (98%). In Burkina Faso, 70% was farmers. The rest was mainly engaged in trading (18%) and teaching (7%). Only 1% of the Ghanaian respondents were engaged in trading on the contrary. These differences mean that, in Bagre District, the Bagre Dam irrigation project had induced occupation diversity. The residents here typically engage in fishing, rice farming and vegetable cultivation throughout the year. This town has attracted a small number of traders and artisans.

Regarding the duration of their residency in the study areas, we found that about 75% of the Ghanaian respondents lived for 5-20 years in the same community, whereas 65% of the respondents in Burkina Faso did so. About 5% of the Ghanaian respondents lived in the same community for more than 40 years whereas none did so in Burkina Faso.

The education level of the respondents was low in both countries as 57% in Ghana and 60% in Burkina Faso had no formal education. In Burkina Faso, a small portion of the respondents had completed primary education (20%) and junior high school education (10%). In Ghana, the percentages of primary education (16%) and junior high school one (12%) did not show much difference from Burkina Faso counterparts. Also, 5% of the respondents in Burkina Faso had tertiary education whereas 4% in Ghana did.

Table 1. Socio-demographic characteristics of the respondents

Socio-demographics	Response category	Burkina Faso (%)	Ghana (%)
Age	18-29	18 (20%)	9 (6%)
	30-39	30	44 (30%)

		(33%)	
	40-49	19 (21%)	53 (36%)
	50-59	14 (16%)	32 (21%)
	60+	9 (10%)	10 (7%)
Gender	Male	45 (50%)	97 (66%)
	Female	45 (50%)	51 (34%)
Education	No formal education	55 (60%)	84 (57%)
	Primary	18 (20%)	24 (16%)
	Junior high school	9 (10%)	18 (12%)
	Senior high school	4 (5%)	17 (11%)
	Tertiary education	4 (5%)	5 (4%)
Occupation	Artisan	2 (2%)	0 (0%)
	Farmer	63 (70%)	146 (98%)
	Student	3 (3%)	1 (1%)
	Teacher	6 (7%)	0 (0%)
	Trader	16 (18%)	1 (1%)
How long (years) have you lived in this area?	5-10	9 (10%)	55 (37%)
	11-20	50 (55%)	55 (37%)
	21-30	24 (27%)	17 (11%)
	31-40	7 (8%)	14 (10%)
	41-50	0 (0%)	3 (2%)
	51-60+	0 (0%)	4 (3%)
Total		90 (100%)	148 (100%)

3.2 Awareness about the Volta Basin Authority and the code of conduct

In the second part of the survey, we attempted to understand respondents' awareness of transboundary water governance in the Volta Basin. First, we asked the respondents whether they knew of the Volta Basin Authority (VBA). We also asked them whether they were aware of the code of conduct between Ghana and Burkina Faso. Finally, we asked them whether they knew of their status as key stakeholders of the VBA.

In response to the first question about awareness of the VBA, which began its operation three years after the formulation of the code of conduct, only 16% of the respondents in Burkina Faso and 9% in Ghana answered positively. With regards to the awareness of the code, 69% of the respondents in Burkina Faso knew of it. In Ghana, only 11% answered positively. Among those respondents who were aware of the VBA, only 9% knew of their status as key stakeholders in both countries, indicating the low awareness level of the respondents (Figure 2). That only 9% in both countries recognized themselves as stakeholders means that the VBA had largely operated as a top-down institution without much public engagement or participation. Regarding the higher level of awareness about the code of conduct among the respondents in Burkina Faso than those in Ghana, the reason can be at least partially attributable to the fact that the study area in Burkina Faso houses the headquarters of the Project for Improvement of Water Governance (PAGEV) that facilitated the establishment of the code (IUCN, 2009). This agreement preceded the establishment of the VBA in 2009.
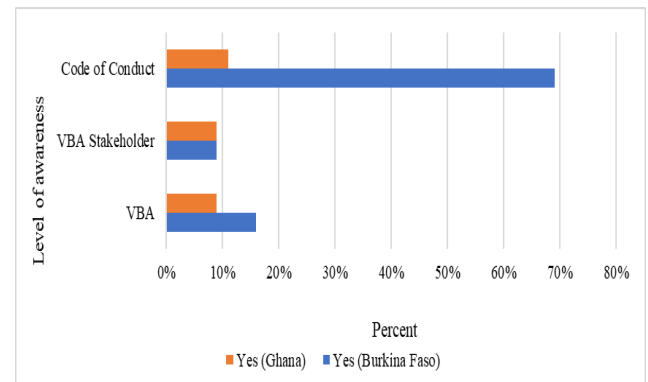
Fig. 2. Riparian communities' knowledge about the VBA and the code of conduct

Considering these results on awareness, we conducted a Chi-squared analysis to gain further insights on correlations with socio-demographic characteristics. A crosstabulation revealed a p-value less than 0.05 ($\chi^2=4.939$, df=5 and p<0.05) for gender (Table 2; Appendix 1). This means that gender had a significant correlation with respondents' awareness. More males (87.5%) than females were aware of their status as key VBA stakeholders in Burkina Faso. Similar gender differences exist in Ghana, but it was not statistically significant. This regional deviation could partly be

explained by the fact that men generally dominate in community meetings and external workshops that discuss issues about the Volta River Basin. Also, the presence of teachers and traders in Burkina Faso might have affected this result to some extent.

Table 2. Correlations between gender and respondents' knowledge about the VBA

Cross-tabulation	Gender (Ghana)	Gender (Burkina Faso)
Are you aware of the VBA?	0.903 (0.342)	3.045 (0.081)
Are you a VBA stakeholder?	0.903 (0.342)	4.939 (0.026*)
Have you heard about code of conduct?	2.014 (0.156)	0.210 (0.647)

About correlations between education and awareness, our analysis found a p-value of less than 0.05 in Ghana (χ^2=36.181, df=5 and p<0.05). This means that education had a significant correlation with respondents' awareness. Among those Ghanaian respondents without formal education, 99% was not aware of the VBA. Those with senior high school and tertiary education, 35% and 75% knew of the VBA, respectively. Similarly, regarding the awareness of the code among the Ghanaian respondents, 95% of those without formal education did not know about it while 30% and 75% of those with senior high school and tertiary education did. Regarding their awareness of being key VBA stakeholders, 99% of those without formal education answered negative whereas 35% and 75% of those with senior high school and tertiary education gave positive answers.

In Burkina Faso, however, we found that education had a significant correlation only with respondents' awareness of being key stakeholders. Here, 98% of those without formal education did not know of their status of being key stakeholders, compared with 33% of those with junior high school education. Among those with tertiary education, 50% did not know of being stakeholder (Table 3; Appendix 2).

Table 3. Correlations between education and respondents' knowledge about the VBA

Cross-tabulation	Education (Ghana)	Education (Burkina Faso)
Are you aware of the VBA?	36.181 (0.000*)	7.406 (0.116)
Are you a VBA stakeholder?	36.181 (0.000*)	14.771 (0.005*)
Have you heard about code of conduct?	22.024 (0.000*)	2.421 (0.659)

Years of residency also significantly influenced respondents' knowledge as being key stakeholders. Among those who had lived in the study area for 11-20 and 21-30 years, 6% and 8% knew of their status as stakeholders, respectively. Also, 33% of those who had lived for 5-10 years knew about their status. Regarding the knowledge about the code of conduct, 92% of the respondents who had lived in the Basin for 21-30 years knew about it whereas 62% of those who had lived for 5-10 years were also aware (χ^2=8.443, df=5 and p<0.05) (Table 4; Appendix 3). In Ghana however, no significant correlation was found with their years of residency regarding all three questions. This result suggests that, overall, all residents were not well-informed about the VBA.

Table 4. Correlations between years of residency and respondents' knowledge about the VBA

Cross-tabulation	How long have you lived along the Volta river? (Burkina Faso)
Are you a VBA stakeholder?	0.342 (0.049*)
Have you heard about code of conduct?	8.443 (0.038*)

Since stakeholder's awareness is essential for effective behavioral change (Koop et al., 2017) and appropriate and timely information dissemination enhances public awareness (Mayunga, 2007), these results may help water managers of the Volta Basin identify social aspects of water governance challenges to forestall possible conflict situations in the future. In particular, at least junior high school education is essential for all residents. For adult and elderly populations, community durbars, mosques, churches, and local chiefs (town crier) may play important roles in better informing riparian communities.

3.3 Riparian community's challenges in water governance

In addition to awareness, we tried to identify challenges the respondents face when they participate in water governance. Based on past studies and our field observation, we formulated a list of possible water governance challenges and presented these to them. The

respondents were asked to rank the challenges in order of importance (important, not important, not sure). The challenges presented to the respondents are: (1) inadequate enforcement of environmental regulations, (2) poor community participation in governance, (3) insufficient flood prevention, and (4) untimely information about spillage from Bagre Dam.

The result shows that the respondents in Ghana (96%) and Burkina Faso (97%) ranked the timeliness of spillage information as the most important. Our in-person interviews also found that farmers located below the Bagre Dam were left without sufficient information about the spillage. As a result, their farms were overly flooded, and their livelihoods were significantly affected. The second most important challenge was flood prevention with 92% in Ghana and 95% in Burkina Faso. The challenge of inadequate legal enforcement was also found high in Ghana (90%) and Burkina Faso (92%). Poor community participation was identified by 88% of the Ghanaian and 91% of the Burkinabe respondents (Table 5). Overall, these answers suggest serious governance failure in the two study areas. These findings positively correspond with past studies that found governance problems under the Volta Basin Authority (Opoku-Ankomah et al., 2006; Gao and Margolis, 2009; Obrecht and Mead, 2014; World Bank 2015; Yankey, 2019).

Table 5. Ranking of water governance challenges by the respondents

Challenges	Burkina Faso Ghana		
	Not important	Important	I don't know
Enforcing regulation	5 (6%)	83 (92%)	2 (2%)
	10 (7%)	134 (90%)	4 (3%)
Public participation	6 (7%)	82 (91%)	2 (2%)
	12 (8%)	131 (88%)	5 (4%)
Flood prevention	4 (4%)	85 (95%)	1 (1%)
	6 (4%)	136 (92%)	6 (4%)
Untimely information of spillage	2 (2%)	87 (97%)	1 (1%)
	1 (1%)	143 (96%)	4 (3%)

IV. CONCLUSIONS

This research assessed the level of awareness and perceptions among Volta Basin Authority (VBA) stakeholders in Ghana and Burkina Faso. Our findings revealed that only a small portion of the respondents had a knowledge of the Volta Basin Authority and the existence

of the code of conduct. Concerning the awareness, most of the respondents in Burkina Faso (84%) and Ghana (91%) did not know about the VBA. Also, 89% of them in Ghana did not know about the code of conduct. Furthermore, 91% in both countries did not know that they were key stakeholders of the VBA.

Through statistical analyses, we found correlations between awareness and gender, education, and years of residency with some regional variations. More males in Burkina Faso were aware of being key VBA stakeholders. In Ghana, education appeared to have affected respondents' awareness. The respondents with senior high and tertiary education in Ghana tend to be aware. Years of residency in Bagre District of Burkina Faso showed a significant correlation to their awareness. Among those who had lived for 21-30 years in the study area, 92% knew of the code of conduct.

Regarding the four pre-identified challenges of water governance, more than 90% of the respondents in both countries similarly found seriousness of all these challenges. In particular, water spillage from Bagre Dam was the most pressing concerns. The other challenges are related to inadequate flood prevention structures and the inadequate enforcement of environmental regulations. All these suggest that the residents knew well about what challenges needed to be addressed to secure their livelihood, but they did not know how their voice can be represented on transboundary water governance matters.

If properly executed, the VBA can provide a powerful avenue for residents to express their needs and monitor progress. We recommend that the VBA regularly disseminate sufficient information about its potential roles to its stakeholders. Local transboundary committees already exist in these two study areas for different purposes. Therefore, a similar committee for water governance, especially on flood protection policies, should be formed by inviting representation from local communities. More women should be invited in local transboundary water governance committees and other water governance programs to improve their participation.

CONFLICT OF INTEREST

We declare no conflict of interest.

DISCLOSURE STATEMENT

REFERENCES

[1] Agyenim, J. B. (2011). Investigating institutional arrangements for integrated water resource management in developing countries: The case of white Volta Basin, Ghana. PhD thesis, Vrije Universiteit.

[2] Ampomah, B. Y., Adjei, B. A., and Youkhana, E. (2008). The transboundary water resources management regime of the Volta Basin (No. 28). ZEF Working Paper Series.

[3] Ampomah, O. (2017). Modeling the Effect of the Bagre Dam on the Water Levels of the Akosombo Dam: Time Series Intervention Analysis. PhD thesis, University of Ghana.

[4] Amuquandoh, M. K. (2016). An Assessment of the Effects of the Bagre Hydro Dam Spillage on Ghana-Burkina Faso Relations. Master's thesis. University of Ghana. https://pdfs.semanticscholar.org/3ad4/2e5ebdd15055b4f2bc9cf52847ba73373be6.pdf (accessed 2 May 2020).

[5] Andreini, M. (2002). Water sharing in the Volta basin: Bridging the Gap between Research and Practice. *Regional Hydrology*. IAI IS publ. No. 274.

[6] Baah-kumi, B., and Ward, F. A. (2020). Poverty mitigation through optimized water development and use: Insights from the Volta Basin. *Journal of Hydrology*, *582*, 124548. https://doi.org/10.1016/j.jhydrol.2020.124548.

[7] Chacko, C. J. (1932). *The International Joint Commission between the United States and the Dominion of Canada*. New York: Columbia University Press.

[8] CityPopulation. (2020). https://citypopulation.de/php/burkinafaso-communes-admin.php?adm2id=BF480101 (accessed 15 April 2020).

[9] Coche, A. G. (1998). Supporting Aquaculture Development in Africa: Research Network on Integration of Aquaculture and Irrigation. CIFA Occasional Paper. No. 23. Accra. http://www.fao.org/3/X5598E/X5598E00.htm (accessed 8 July 2020).

[10] Construction Review Online. (2020). Construction of Pwalugu multipurpose dam in Ghana to start in April 2020. https://constructionreviewonline.com/2020/03/construction-of-pwalugu-multipurpose-dam-in-ghana-to-start-in-april-2020/ (accessed 13 July 2020).

[11] Gao, Y., and Margolies, A. (2009). Transboundary water governance in the Volta River Basin. https://wikis.uit.tufts.edu/confluence/display/aquapedia/Transboundary+Water+Governance+in+the+Volta+River+Basin (accessed 2 May 2020).

[12] Gerlak, A.K., and Schmeier, S., (2018). River Basin Organizations and the Governance of Transboundary Watercourses. In the Oxford Handbook of Water Politics and Policy, edited by K. Conca and E. Weinthal, pp. 532-548. Oxford University Press.

[13] Ghana Statistical Service-Bawku Municipality. (2014). District Analytical Report-Bawku Municipality. Population and Housing Census. 1-71. Retrieved from http://www.statsghana.gov.gh/docfiles/2010_District_Report/Upper East/BawkuMunicipality.pdf.

[14] Ghana Statistical Service-Garu. (2014). District Analytical Report-Garu Tempane District. Population and Housing Census. 1-79. Retrieved from http://www2.statsghana.gov.gh/docfiles/2010_District_Report/Upper%20East/GARU%20TEMPANE.pdf.

[15] Global Water Partnership. (2014). Outlines and Principles for Sustainable Development of the Volta Basin. https://www.gwp.org/globalassets/global/gwp-waf_files/wacdep/brochure_outlines_principles_wacdep_abv_en.pdf (accessed 17 December 2019).

[16] Gyireh, P. F., and Nunbogu, A. M. (2015). Sustainable management of flood disasters in the Upper East Region, Ghana. *International Journal of Development and Sustainability. 4(5)*, 549-562.

[17] IEADB (International Environmental Agreements Database Project). (2020). Convention on the status of the Volta River and the Establishment of Volta Basin Authority. https://iea.uoregon.edu/treaty-text/2007-voltabasinauthorityentxt (accessed 5 August 2020).

[18] International Institute for Environment and Development (IIED). (2020). Global Water Initiative: Burkina Faso Project 2008-2017. https://www.iied.org/global-water-initiative-burkina-faso (accessed 9 July 2020).

[19] Islam, S., and Susskind, L. (2013). *Water Diplomacy: A Negotiated Approach to Managing Complex Water Networks*. Routledge.

[20] IUCN. (2009). Improving Water Governance in the Volta Basin-Phase 2, PAGEV (2). https://www.iucn.org/sites/dev/files/import/downloads/pagev_ii.pdf (accessed 9 August 2020).

[21] IUCN. (2012). Volta River Basin: Ghana and Burkina Faso. Transboundary water management through multi-level participatory governance and community projects. IUCN Water Programme, Demonstration Case study No.4. IUCN, Water and Nature Initiative (WANI). https://portals.iucn.org/library/node/10108 (accessed 22 April 2020).

[22] Kent State University Libraries. (2020). SPSS Tutorials: Chi-square Test of Independence. https://libguides.library.kent.edu/SPSS/ChiSquare (accessed 26 April 2020).

[23] Koop, S. H. A., Koetsier, L., Doornhof, A., Reinstra, O., Van Leeuwen, C. J., Brouwer, S., … and Driessen, P. P. J. (2017). Assessing the Governance Capacity of Cities to Address Challenges of Water, Waste, and Climate Change. *Water Resources Management*, *31(11)*, 3427-3443. https://doi.org/10.1007/s11269-017-1677-7.

[24] Leemhuis, C., Jung, G., Kasei, R., and Liebe, J. (2009). The Volta Basin Water Allocation System: Assessing the impact of small-scale reservoir development on the water resources of the Volta Basin, West Africa. *Advances in Geosciences*, *21*, 57-62.

[25] Mathews, M. (2012). The Volta Convention: An effective tool for transboundary water resource management in an era of impending climate change and devastating natural

disasters. *Denver Journal of International Law & Policy*, *41(2)*, 273-308.

[26] Mayunga, J. S. (2007). Understanding and applying the concept of community disaster resilience: a capital-based approach. *Summer Academy for Social Vulnerability and Resilience Building*, *1(1)*, 1-16.

[27] MOFA (Ministry of Food and Agriculture). (2015). Facts and Figures, 2015. http://agricinghana.com/wp-content/uploads/2017/07/AGRICULTURE-IN-GHANA-Facts-and-Figures-2015.pdf (accessed 11 December 2019).

[28] Mul, M., Obuobie, E., Appoh, R., Kankam-Yeboah, K., Bekoe-Obeng, E., Amisigo, B., and McCartney, M. (2015). Water resources assessment of the Volta River Basin (Vol. 166). International Water Management Institute (IWMI).

[29] Obrecht, A., and Mead, N., (2014). *Cross border risks and transboundary risk governance in West Africa: Case study of the Volta River Basin*.

[30] Opoku-Ankomah, Y., Dembélé, Y., Ampomah, B. Y., and Somé, L. (2006). Hydro-political assessment of water governance from the top-down and review of literature on local level institutions and practices in the Volta Basin (Vol. 111). International Water Management Institute (IWMI).

[31] Priscoli, J. D., and Wolf, A. T. (2009). *Managing and Transforming Water Conflicts*. Cambridge University Press.

[32] Schmeier, S. (2013). *Governing International Watercourses: River Basin Organizations and the Sustainable Governance of Internationally Shared Rivers and Lakes*. Routledge.

[33] UNEP-GEF Volta Project. (2013). Volta Basin Transboundary Diagnostic Analysis. UNEP/GEF/Volta/RR 4/2013.

[34] World Bank Group. (2020). Life Expectancy at Birth, total years. https://data.worldbank.org/indicator/SP.DYN.LE00.IN (accessed 8 July 2020).

[35] Welling, R., Cartin, M., Baykono, D., and Diallo, O. Transboundary Water Management Through Multi-Level Participatory Governance and Community Projects. Ghana and Burkina Faso. Water and Nature Initiative (WANI) Case Study.

[36] Wolf, A. T., Yoffe, S. B., and Giordano, M. (2003). International Waters: Identifying Basins at Risk. *Water Policy*, *5(1),* 29-60.

[37] World Bank. (2015). Project appraisal document on proposed grants to the Volta basin authority from the cooperation in international waters in Africa trust fund and the global environment facility for a Volta river basin strategic action programme implementation project. Global Water Practice (GWADR), Africa Region.

[38] Yankey, I. K. (2019). Ghana's Water Relations with Burkina Faso: Hydropolitical Standpoint (Master's thesis, Sosyal Bilimler Enstitüsü). http://www.openaccess.hacettepe.edu.tr:8080/xmlui/handle/11655/6050 (accessed 2 May 2020).

Appendix 1. Cross-tabulation on gender, riparian awareness of VBA, being key a stakeholder?

Cross-tabulation	Response	Ghana		Burkina Faso	
		male	female	male	female
Are you aware of VBA?	no	86	48	35	41
	yes	10	3	10	4
Sub-Total		96	51	45	45
Are you aware you are stakeholder of the VBA?	no	86	48	38	44
	yes	10	3	7	1
Sub-Total		96	51	45	45
Have you heard about Code of conduct?	no	83	48	15	13
	yes	13	3	29	31
Sub-Total		96	51	44	44

Appendix 2. Cross-tabulation on level of education, riparian awareness about VBA, code of conduct and status as key stakeholder.

Cross-tabulation	Response	Burkina Faso (Ghana)				
		no formal education	JHS	SHS	Tertiary	Primary
Are you aware of the VBA?	no	51 (83)	6 (17)	3 (11)	3 (1)	13 (22)
	yes	4 (1)	3 (0)	1 (6)	1 (4)	5 (2)
Sub-total		55 (84)	9 (17)	4 (17)	4 (5)	18 (24)
Are you aware you are a VBA stakeholder?	no	54 (83)	6 (17)	3 (11)	2 (1)	17 (22)
	yes	1 (1)	3 (0)	1 (6)	2 (4)	1 (2)
Sub-total		55 (84)	9 (17)	4 (17)	4 (5)	18 (24)
Have you heard about Code of conduct?	no	14 (80)	4 (16)	2 (12)	1 (1)	7 (22)
	yes	39 (4)	5 (1)	2 (5)	3 (4)	11 (2)
Sub-total		53 (84)	9 (17)	4 (17)	4 (5)	18 (24)

Appendix 3. Cross-tabulation on years of residency, riparian awareness as key stakeholders and the code of conduct.

Cross-tabulation	Response	How long have you lived along the Volta River?				Total
		5-10	11-20	21-30	31-40	
Are you aware you are a stakeholder of the VBA?	no	6	47	22	7	82
	yes	3	3	2	0	8
Sub-total		9	50	24	7	90
Have you heard about Code of conduct?	no	3	20	2	3	28
	yes	5	29	22	4	60
Sub-total		8	49	24	7	88

Article   *Peer-Reviewed Journal*

Insect Defense System and Immunosuppression Strategies of Entomopathogenic Nematodes - An Overview

Gitanjali Devi

Department of Nematology, Assam Agricultural University, Jorhat-13, Assam, India

Abstract— Studies on host-parasite interaction and immune responses in insects will greatly benefit human health from biocontrol point of view. Role and relationships between insect hosts and entomopathogenic nematodes are elaborated where the efficacy of the entomopathogenic nematodes depends on the stability between the parasitic strategies and the immune response of the host. Entomopathogenic nematodes are potential biocontrol agent. The cellular and humoral responses are avoided by the nematode-bacterium complexes by producing immunodeficiency in insects. The review outlines the mechanisms of immune recognition and defense of insects as well as immune evasion strategies of Entomopathogenic nematodes (EPNs).

Keywords— Insect immune response, Entomopathogenic nematodes, Cellular and humoral immune response, Immunosuppression, evasion.

INTRODUCTION

Innate immunity is common to all metazoans and serves as first-line defense against foreign antigens. Insect possess a potent innate immune system by which they attempt to resist microbial infections and parasitic invasions. Host innate immunity plays a central role in detecting and eliminating microbial pathogenic infections in both vertebrate and invertebrate animals. Entomopathogenic nematodes (EPNs) are used as biological control agents against wide range of insect pests and vectors of pathogen. EPNs are classified into two genera: *Steinernema* and *Heterorhabditis*. The EPNs *Steinernema* spp. and *Heterorhabditis* spp. infective juvenile stage (IJ) harbors the symbiotic bacteria *Xenorhabdus* spp. and *Photorhabdus* spp., respectively in their intestine. Once IJs infect a host through natural openings such as the mouth, anus, and spiracles, they can release symbiotic bacteria into the haemocoel of the host, causing insect death within 24-48 h post infection. To survive within the insect and complete their life-cycle, EPNs use some tactics to suppress the host immune responses.

The suppression of the host immune system is essential for successful infection and the death of the host. Biological control agents may affect ecological fitness of the insects due to behavioral, morphological, and physiological changes (Girling *et al.*,2010; Kunc *et al.*,2017).

1.1. Behavioral resistance: Behavioral resistance occurs when the insect actively avoids or repels the nematode.

- Extremely active mosquito species had a lower prevalence of infection by the mermithid *Romanomermis culicivorax* than less active ones. Petersen (1975).
- A high defecation rate that reduces infection via the anus (scarab grub). Low CO_2 output or CO_2 released in bursts that minimize chemical cues (lepidopterous pupae and scarab grubs (Potter and Held , 2002).
- Walling-off nematode killed individuals that avoid or reduce contamination toother insects in a termites mound ; When nematodes are applied to termite colonies, the workers are able to recognize infected individuals and isolate them behind earthen barriers (Baimey *et al.*,2017).

- Fire ants (*Solenopis invicta*) display avoidance behavior and move their colonies elsewhere as a result of nematode treatment .In field trials in which mounds were drenched with nematode suspensions, the entire colony vacated the treated mound within 48 hrs and created satellite mounds (Drees *et al*., 1992).
- Grooming behaviours including rubbing and using the mandibles to scrape the cuticle can remove nematodes attached to the surface of the insect (scarabaeid white grubs) (Gaugler *et al*.,1994; Koppenhofer *et al*.,2000).

1.2. Physical resistance: Physical barriers such as cuticle,the intestinal wall including the peritrophic membrane , and the tracheas which restrict the entry of nematodes into some insects (Ishibashi and Kondo, 1990).

- Mouth may be obstructed by oral filters (wireworms) or be too narrow (insects with sucking/piercing mouthparts or small insects with chewing mouthparts).
- Having forward projecting hairs in the preoral cavity (elaterid wireworms) or a thick peritrophic membrane protecting the midgut epithelium (white grubs).
- Well developed proventriculus inhibits penetration of infective juveniles.
- The anus may be constricted by muscles or other structures (wireworms).
- Heavily scleritized spiracles, narrow, slit-like openings of the spiracles (wireworms) or fine sieve-like plates covering the spiracles (white grubs) or simply be too narrow (some dipterans and lepidopterans) may limit access to the hemocoel via the tracheal system (Triggiani and Poinar,1976; Eidt and Thurston ,1995).
- The formation of impenetrable cocoons before pupation (lepidopterans and scarabs). Dauer juveniles of *Steinernema carpocapsae* cannot penetrate the silken cocoons of hymenopteran parasitoids (Kaya and Hotchkin, 1981), but if a hole is made in the cocoon, infection occurs .
- *Romanomermis culicivorax* has difficulty in penetrating the integument of older mosquito larvae (Peterson and Willis, 1970). Younger instars of black fly larvae are resistant to infection by *S.carpocapsae* because the comparatively large nematode is excluded from the insect's mouth (Gaugler and Molloy, 1981).

1.3. Physiological resistance: Hemolymph of insects is a medium for several physiological processes like immune responses and intermediary metabolism. Enzymatic

changes in infected larvae would envisage the metabolic stress of the insect experience during the development of pathogen. Insects exhibit cellular and humoral immune responses against various pathogens including microorganisms and multicellular parasites. Hillyer (2016) indicated that the insects have developed sensitive mechanisms for detecting the presence of microbial infections and activating signalling pathways that control the production of molecules with antimicrobial activity. Innate immune response of insects is traditionally divided into two main group factors including the following (i) humoral factors i.e., melanization , synthesis of antimicrobial peptides(AMPs) and (ii) cellular defense reactions (i.e., nodule formation, phagocytosis, or encapsulation by hemocytes) (Vilmos and Kurucz ,1998).

By recognition of non self (microorganisms or metazoans) and rapid effector mechanisms that involve several cell mediated and humoral processes. All the processes are triggered by free and membrane-bound Pattern Recognition Receptors (PRRs) capable of specifically binding to Pathogen Associated Molecular Patterns (PAMPs). PAMPs are molecules that are common to groups of pathogens and are recognized by free or cell associated receptors (PRRs) in all animal species. The prototypical PAMPs are the molecules secreted or derived from the surface of bacteria or fungi.

Host defenses and immune reactions in response to EPN infection have been studied only in a few EPN species-insect species combinations (Lewis and Clarke, 2012; Shapiro-Ilan *et al*., 2018).

The innate immune system in insects comprises two central and several peripheral tissues

A. The central tissues are:

1. The circulating fluid is called hemolymph which is freely distributed in an open circulatory system.The insect immune system consists of the fat body, which secretes effector molecules into the hemolymph and several classes of hemocytes, which reside in the hemolymph and of protective border epithelia. The main function of the fat body within the immune system is to release soluble factors into the hemolymph. Some of the factors are produced constitutively others only after immune stimulation. Humoral defences were also reported which includes the production of antimicrobial peptides (e.g., cecropins, attacins) (Lowenberger 2001; Manniello *et al*., 2021); the pattern recognition protein lysozyme , reactive intermediates of oxygen and nitrogen (Bogdan *et al*.,2000; Shreehan *et al*.,2020); activation of the prophenoloxidase cascade and phospholipase A$_2$ (PLA$_2$) (Hoffmann *et al*., 1996; Gillespie *et al*., 1997; Söderhall and Cerenius 1998; Kanost *et al*., 2004; Müller *et al*., 2007). Induction of their

transcription is achieved via the Toll and imd pathways which are located downstream of recognition molecules that bind microbial elicitors such as peptidoglycan and beta 1,3 glucan (Davis and Engstrom, 2012). Peptidoglycan recognition protein binds to its respective elicitor and results in the production of inactive prophenoloxidase (proPO). Phenoloxidase (PO) is one of the key enzymes activated via prophenoloxidase (PPO) cascade in the cuticle or the hemolymph of many insects in response to the immune challenge (Marmaras *et al.*, 1996; Gillespie *et al.*, 1997; Gillespie *et al.*, 2000; Castillo *et al.*,2011). PO catalyzes the melanin coat around encapsulated pathogens and produces chemically reactive quinones that are toxic to microbial pathogens (González-Santoyo and Córdoba-Aguilar, 2012). Glutathione S-transferase (GST) and esterase (EST) are the major enzymes involved in detoxifying penetrating xenobiotics in insects (Fan *et al.*, 2013). Dunphy and Halwani(1997) isolated two LPS-binding proteins (LBP-1 and LBP-2) in the hemolymph, that are specific for the bacterial surface and acts as endotoxin detoxifier, thus protecting hemocytes from damage in *Galleria mellonella*. Increased detoxifying enzyme activities against mycoses and other infections represent the insect's response to bodily intoxication by metabolites or the host-tissue-degrading products of pathogens (Serebrov *et al.,* 2001).

2. In insects, hemocytes freely circulate in the hemolymph, or are localized in specific regions of the body. The highly variable composition of hemocyte types amongst insect species reflects an adaption to their respective environment and its specific pathogens. Thus the prevalence of a particular set of immune cell types appears as an ecological trade-off indicating the necessity to allocate resources to the dominant immune challenges. Pro-hemocytes, granulocytes, plasmatocytes, spherulocytes and oenocytoids are common type of hemocytes in Lepidoptera.In Dipteran insect lamellocytes, cells with crystalline inclusions and plasmatocytes are present. In *Drosophila*, two prophenoloxidase (PPO1 and PPO2) are harbored by a specialized class of hemocytes (crystal cells) while a third one (PPO3) is produced by lamellocytes. Certain TEPs in *D. melanogaster* were shown to play a regulatory role of modulating phenoloxidase and melanization reactions responses by inducing humoral and cellular immune activities against *Photorhabdus* pathogens, these molecules also form a reliable indicator for their potential multipurpose involvement in linking host immunity and metabolism in the presence of pathogenic bacteria. Cellular immunity in *D. melanogaster* larvae and adult flies is controlled by the different types of hemocytes, which specialize in various

immune activities that mainly include the detection, phagocytosis, and encapsulation of pathogens.

In *S. exigua*, the major haemocyte types reacting against bacteria include the granulocytes and plasmatocytes which respond to particulate antigens by phagocytosis and nodulation. Lavine and Strand (2002) reported plasmatocytes and granulocytes are known to be capable of recognize, adhere to and spread on foreign surface that are phagocytic in Lepidoptera. Six types of haemocytes were identified in *G. mellonella* by Boman and Hultmark (1987). Physiological defenses in chrysomelid beetles and mosquitoes frequently result in encapsulation and melanization of infective juveniles after penetrating the hemocoel . Haemocytes in presence of foreign targets are activated by the presence of PAMPs and /or endogenous soluble factors and initiate complex mechanisms such as intracellular signal transduction which activate the specific immune genes and initiates defense mechanisms such as phagocytosis, nodulation ,encapsulation , synthesis of antimicrobial peptides and cell-mediated melanization (Chapman,1998; Schmidt et al., 2001; Williams ,2007; Strand 2008; Krzemien *et al.*,2011;Li *et al.*,2021).

Melanization also termed as humoral encapsulation is an efficacious defense mechanism in insect. Melanization is due to the activity of an oxidoreductase called phenoloxidase (Kanost and Gorman 2008). This molecule is the terminal enzyme of a complex system of proteases (protease cascade), proteases inhibitors (serpins) and PRRs, constituting proPO-AS (Freitak *et al.*,2007;Castillo *et al.*,2011) . ProPO-AS is the key element in the recognition of foreign bodies, an integral component of the insect immune system. Prophenoloxidase is converted into its active form by a limited proteolysis, and when activated phenoloxidase can oxidize phenols into quinines that in turn autocatalyze into melanin. *S.feltiae* infection in *G.mellonella* suppresses PO activity by interfering with LPS-mediated ProPO activation pathway in *G.mellonella* larvae (Brivio *et al.*, 2002).

Phagocytosis is a process that can be envisioned as a specialized form of receptor-mediated endocytosis resulting in the internalization of foreign body. Apolipophorin III (apoLp-III) , and Arylphorin, heat stable protein, isolated from the haemolymph of *G.mellonella* larvae enhances the phagocytic activity of isolated haemocytes(Gotz *et al.*,1997).

Nodulation: In the presence of many bacterial cells or fungi, hemocytes degranulate releasing humoral factors that form aggregates, called nodules, this process lead to the entrapping of foreign cells. Such nodular aggregates may adhere to host tissues and larger nodules may be encapsulated by the hemocytes.

Encapsulation: When the foreign invaders are too large to be phagocytized , they can be encapsulated by multiple layers of hemocytes.These hemocyte can produce a coat of melanin .The humoral PRRs are needed to stimulate the aggregation of plasmatocytes on the surface of the target by formation of multicellular layered thick capsule that segregates the foreign organisms.The toxic effects of melanin,which is present inside the inner layers of the capsule,may contribute to kill the entrapped organism.

Cellular encapsulation and capsule melanization of EPNs in CPB is documented (Ebrahimi *et al*.,2011). Hemocytes from the Japanese beetle strongly encapsulated and melanized the *H.bacteriophora* HP88 strain, *S.glaseri* FL strain, *S.scarabaei* and *S.feltiae*. *H.bacteriophora* was intensively melanized in *E.orientalis*, *P.japonica* and *C.borealis*. *S.glaseri* NC strain suppressed the immune responses in *M.sexta*, *E.orientalis* and *P.japonica* , where as *S.glaseri* FLstrain was less successful(Li *et al*.,2007). A *Heterorhabditis* species avoids encapsulation in tipulid larvae by exsheathing from the second-stage cuticle during host penetration (Peters *et al*.,1997). Peters and Ehlers (1997) examined the pathogenicity of *S.feltiae* and its symbiont *Xenorhabdus bovienii* to the crane fly (*Tipula oleracea*). *X.bovienii* is triggering the encapsulation response.

B. Peripheral tissues comprising the tracheae, the epidermis, the gonads, and the gut epithelium rely on the more locally restricted release of effectors such as prophenoloxidase and antimicrobial peptides and on the production of reactive oxygen species to varying extent.

Immunosuppression strategies of Entomopathogenic nematodes:

Entomopathogenic nematodes have developed strategies to avoid or suppress the insect immune system by preventing or disrupting the activation of immune responses to promote their survival in the host (Cooper and Eleftherianos ,2016). EPNs species shared immunosuppresion strategies, mainly mediated by their symbiotic bacteria, but there are differences in mechanism of evasion and interference of the nematode with the insect host immune pathways. Once a host has been located, recognized, and penetrated, the nematode's attack still may not succeed if the insect is able to respond with an effective immune response.

Penetration into the insect host is the first step of the EPN infection process. The infective juveniles have to penetrate through the cuticle (including the trachea) or gut to enter the hemocoel. To enter through the cuticle, the nematodes employ physical force such as body thrusting to rupture through the thin trachea or, as with *Heterorhabditis*, use an anterior tooth to penetrate directly. To enter through the

gut, they use physical force and/or proteolytic secretions to digest the midgut tissues to gain access into the hemocoel (AbuHatab *et al*., 1993) EPNs produce bioactive molecules referred to as excreted/secreted products (ESPs). ESPs contain various products that have functions related to other biological processes, e.g., nematode development, social behavior and nematode communication. Some of the molecules described in *S. carpocapsae* play a role in the penetration of a host (e.g., aspartic protease Sc-asp113 and Sc-asp155). It has been reported that *S. carpocapsae* was able to suppress the immune response by secreting proteins, which may facilitate the release of their symbionts (Bowen *et al*., 1998 ;Elias *et al*.,2020). However, it was unknown whether similar proteins were produced by *Heterorhabditis* (Forst and Clarke, 2002). Different species of nematodes induce various immune responses in different insect hosts, which probably are correlated with the differences in surface coat proteins of the nematodes. *S. glaseri* is initially encapsulated by larvae of the Japanese beetle, *Popillia japonica*, but it escapes from the capsule and successfully infects its host (Wang *et al*., 1995) because the nematode has surface coat proteins (SCP) that suppress the host's immune response and lyse the hemocytes (Wang and Gaugler ,1998). Once inside the host, IJs may overcome the host's immune response by shedding of the second-stage-juvenile cuticle (sheath). Within the insect's hemocoel, the nematodes and bacteria overcome the host's immune response (Dunphy and Thurston,1990; Kaya and Gaugler ,1993) that involves interacting humoral and cellular factors. Infective juveniles of *S. carpocapsae* and *H. bacteriophora* release protease secretions which destroy the antibacterial factors of vaccinated *G. mellonella* larvae (Gotz *et al.*, 1980). Balasubramanian *et al*., (2010) purified a trypsin-like secreted protease from *S. carpocapsae* that suppresses the prophenoloxidase (pro-PO) in *G. mellonella*. ESPs produced by *H. bacteriophora* have the ability to inhibit the melanization of *G. mellonella*. The enzymatic activity of ESPs remained the same regardless of nematode age. In *S. carpocapsae*, inhibitors of both humoral and cellular immune responses have been described. SCP protect *H.bacterophora* from immune response in *Popillia japonica* and *Exomala orientalis* (Li *et al*., 2007) and some act as immune modulators (e.g., metalloprotease Sc-AST, chymotrypsin serine protease, BPTI-Kunitz family inhibitor and Sc-SP-3. Genes sc-asp113 and sc-asp155, encoding aspartic proteases, are up regulated at the beginning of the parasitic phase, and are probably involved in the disruption of the host tissue. Additionally, the astacin metalloprotease Sc-AST, could participate in the parasitic process of *S. carpocapsae*, .Chymotrypsin serine protease, identified in the ESPs of *S.*

carpocapsae, can inhibit prophenoloxidase and the subsequent encapsulation and activation of melanization of *Galleria mellonella* (Gulley *et al*., 2013; Veillard *et al*., 2016). Haemolysin activity was shown by both genera (Brillard *et al*., 2002). Similarly, the BPTI-Kunitz family of inhibitors (Sc-KU-4), not only causes inhibition of encapsulation, but also impairs the aggregation of hemocytes. Furthermore, some molecules produced by nematodes can contribute to virulence through their role in the regulation of development, e.g., lamanin, structurally diverse derivates of the 3,6-dideoxysugar ascarylose , acyl-CoA oxidases   and the small pheromone molecule ascaroside C11 ethanolamide . The Mexican strain of *Neoaplectana carpocapsae* help the bacteria *X.nematophilus* by excreting an immune inhibitor that selectively destroys both forms of P9 and P5 immune protein of diapausing pupae of *Hyalophora cecropia* .ESPs produced by *H. bacteriophora* can inhibit PO-catalyzed melanization in *G. mellonella* larvae. *H. bacteriophora* produces a spectrum of ESPs with different functions, and some play a role in virulence.

Following host penetration, the release of bacteria by nematodes is usually delayed in the host by 30 min for *Heterorhabditis* species and several hours for *Steinernema* nematodes .There is thus a possibility for the insect to neutralize its parasite before the bacterial challenge. Many immune factors have been shown to vary in the hemolymph of the host following the entry of nematodes, including both humoral and cellular responses. Bacteria can then suppress immune attacks of insect hosts to protect themselves and their symbiotic nematodes. Under immunosuppressive conditions, these bacteria can multiply in the hemocoel and kill insects by septicemia or toxemia. Secretion of insect toxins, outer membrane proteins, other extracellular products, and the release of lipopolysaccharide (LPS) molecules from the bacterial envelope lead to the death of the host (Owuama,2001). Symbiotic bacterial toxins have been shown to cause actin polymerization, destabilizing the cytoskeleton architecture of haemocytes (Li *et al*., 2009). The decline in the density of all haemocyte types in *Galleria mellonella* Linneaus larvae resulted from the lipid A moiety of *X. nematophila* and *P. luminescence* LPS action triggering haemocytes lysis and inhibiting PO activation but not activity. Brillard *et al*., (2001) reported that haemocyte monolayer from *S. littoralis* has shown two distinct haemolytic activities in supernatants from cultures of *X. nematophila*. Au *et al*., (2004) reported that *Photorhabdus* supernatants reduced haemocyte viability. Production of LPS was shown by both the genera i.e., *P. luminescens* and *X. nematophila* ,where LPS of *X. nematophila* inhibits PO activity and in both systems the

lipid A moiety of LPS was thought to be cytotoxic to haemocytes (Dunphy and Webster 1991). *Photorhabdus* used LPS modification to resist the action of the host-derived AMPs (Eleftherianos *et al*.,2006), but *X. nematophila* prevents induction of insect AMP expression altogether .

Subsequently, nematodes can develop and reproduce in the insect cadaver. To induce immunosuppression, symbiotic bacteria of EPNs can inhibit phospholipase A2 (PLA2) to shutdown eicosanoid biosynthesis of target insects (Stanley and Kim, 2018). Eicosanoids affecting aggregation of haemocytes, haemocyte migration, and release of prophenoloxidase from oenocytoids. The OMPs of *X. nematophila* and *P. luminescens* decreased PLA_2 activity and probably prevented eicosanoid biosynthesis, since Anti microbial peptide (AMP) expression in *S. exigua* by eicosanoid pathway is inhibited by intact *X. nematophila* . Brivio *et al*., (2004) suggested that *S.feltiae* body surface plays an important role in the early parasition phase. *S.feltiae* alone activated the enzyme, a GroEL-like toxin from *Xenorhabdus budapestensis* which activates PO in *G. mellonella* larvae. Yang *et al*., (2012) implies in *H. armigera*, *X. nematophilus* complex to activate the enzyme. Yamanaka (1995) examined pathogenicity of several species and strains of *Xenorhabdus* spp. against *Spodoptera litura*. Pathogenicity varied depending on phase of the bacteria as well as production of biochemical exudates. Previous immunological studies of the *X.nematophila-S.carpocapsae* interaction have focused on their ability to jointly kill an insect (Goodrich-Blair and Clarke, 2007). Specifically, *X.nematophila* produces compound, rhadbduscin which inhibits phenoloxidase and benzylidene acetone, which suppresses antimicrobial peptide production in insects (Hwang *et al*., 2013). Reproduction of entomopathogenic nematodes requires that they escape recognition by a host's immune system or that they have mechanisms to escape encapsulation and melanization. In pathogenic bacteria, some OMPs have been identified as virulence factors overcoming host immune activities (Darsouei *et al*., 2019). Inducible OMPs in *Xenorhabdus* and *Photorhabdus* were identified, including the stress response proteins *skp* in *P. temperata* . *X. nematophila* produces Opns, an inducible protein of provide growth advantage in insect hemolymph. Several bacterial insecticidal factors characterized in *X. nematophila* and *P. luminescens* (Txp40 toxin, Tc toxin, 17-kDa pilin protein) have important roles bacterial virulence and hence EPNs efficacy(Bowen *et al*., 1998). The toxin complex a (Tca) purified by Blackburn *et al*., (1998) from *P. luminescens* has specific effect on the midgut epithelium of the insect *Manduca sexta*. Barbieri *et*

al., (2002) have showed that bacteria have evolved numerous toxins and delivered type III effector molecules which can interfere with the actin cytoskeleton and inhibit phagocytosis.

The insect cadaver becomes deep red but does not putrefy, apparently because of an antibiotic(s) produced by the bacteria (Webster *et al*., 2002) viz., stilbene antibiotic, 3,5-dihydroxy-4-isopropylstilbene.Anthraquinones are metabolites of bacteria and only 1,3,8-trihydroxy-9,10-anthraquinone and two of its monomethyl ether derivatives, 1,8-dihydroxy-3-methoxy-9,10-anthraquinone and 3,8-dihydroxy-1-methoxy-9,10-anthraquinone, have been recorded from *P. luminescens* . These pigments have antimicrobial activities; function as antagonistic agents against colonization from other microorganisms in the insect cadaver.

Dowds and Peters (2002) reported that the bacteria and nematodes cooperate with each other to overwhelm the host's immune response, permitting the bacteria to multiply vegetatively. Binda-Rossetti *et al*., (2016) demonstrated in their experiments with *S. carpocapsae* and *X. nematophila* that infection with live nematodes and bacteria can suppress the antibacterial peptide immune response of red palm weevil *Rhynchophorus ferrugineus*, but the inhibitory effect was not present when insects were injected with dead microorganisms. ESPs of *H. bacteriophora* suppress the expression of the Diptericin gene in *D. melanogaster*. This suppression could help the symbiotic bacteria *P. luminescence* to survive and overcome the insect immune defenses.

Secondary metabolites produced from symbiotic bacteria result in the activity of insect PO and generation of reactive oxygen species (ROS).These free radicals are highly reactive and result in harmful effects on cells and tissues in organisms. For example, in *Manduca sexta*, *P. luminescens* cells secreted an antiphagocytic factor that permitted the bacterial cells to obstruct their own phagocytosis (Silva *et al*., 2002), whereas in *S. exigua*, *X. nematophila* cells were able to hamper nodule formation (Park and Kim 2000; Park *et al*., 2003). Additionally in *S. exigua* and *M. sexta*, *X. nematophila* inhibits transcription of insect genes encoding antimicrobial peptides (Ji and Kim 2004; Park *et al*., 2007). The transcripome resource of insect exposure to nematode challenge will help to support studies on host –parasite interactions.

CONCLUSION

The characterization of specific molecules produced by nematodes could over new possibilities for EPNs in field applications, as well as in improved efficacy of the previously used nematode-based pesticides. Accumulating knowledge on host-parasite relationships will lead to the discovery of novel nematode-bacterial strategies for targeting specific host immune-related components as well as host defense systems (Akhurst and Dunphy, 1993 ;Brivio and Mastore, 2018) designed to oppose deadly attacks by entomopathogens.

REFERENCES

[1] AbuHatab MA, Selvan S and Gaugler R. 1993.Role of proteases in penetration of insect gut by the entomopathogenic nematode *Steinernema glaseri* (Nematoda: Steinernematidae). J. Invertebr.Pathol. 66: 125-130.

[2] Akhurst, R.J.and Dunphy, G.B. 1993. Tripartite Interactions between symbiotically associated entomopathogenic bacteria, nematodes, and their Insect Hosts, In Parasites and Pathogens of Insects; Beckage, N.E., Thompson, S.N., Federich, B.A., (Eds.) Academie Press: San Diego, CA, USA, pp. 1-23.

[3] Au C, Dean P., Reynolds SE.and Ffrench-Constant RH(2004).Effect of insect pathogenic bacterium *Photorhabdus* on insect phagocytes. Cell Microbiol. 6(1):89-95.

[4] Baimey H.,Zadji,L.,Afouda,L.,Fanou A.,Kotchofa R.(2017).Searching for better methodologies for successful control of termites using entomopathogenic nematodes.In: Shah MM and Mahamood M(eds.). Nematology-Concepts, Diagnosis and Control. IntechOpen. doi:10.5772/ intechopen. 69861.

[5] Balasubramanian N,Toubarro D,Simoes N(2010).Biochemical study and *in vitro* immune suppression by a trypsin like secreted protease from the nematode *Steinernema carpocapsae*.Parasite Immunol.32:165-175.

[6] Barbieri JT, Riese MJ, Aktories K (2002) Bacterial toxins that modify the actin cytoskeleton. Annu Rev Cell Dev Biol 18:315–344

[7] Binda-Rossetti S.,Mastore M.,Protasoni M.,Brivio MF(2016).Effects of an entomopathogen nematode on the immune response of the insect pest red palm weevil:focus on the host antimicrobial response. J.Invertebr Pathol.133:110-119.

[8] Blackburn M,Golubeva E,Bowen D.,Ffrench-Constant RH(1998).A novel insecticidal toxin from *Photorhabdus luminescens*,toxin complex a(Tca),and its histopathological effects on the midgut of *Manduca sexta*.Appl.Environ Microbiol.64(8):3036-3041.

[9] Bogdan C.,Rollinghoff M,Diefenbach A.(2000).Reactive oxygen and reactive nitrogen intermediates in innate and specific immunity. Curr.Opin Immunology 12:64-76.

[10] Boman HG, Hultmark D. 1987. Cell-free immunity in insects. *Annu Rev Microbiol.* 41:103-126.

[11] Bowen D, Rocheleau TA, Blackburn M, Andreev O, Golubeva E, Bhartia R and ffrench-Constant RH, (1998). Insecticidal toxins from the bacterium Photorhabdus luminescens. Science (Washington) 280:2129-2132.

[12] Brillard J,Duchaud E., Boemare N,Kunst F., Givaudan A(2002).The Phl a hemolysin from the entomopathogenic bacterium Photorhabdus luminescens belongs to the two partner secretion family of hemolysins.J Bacteriol.184:3871-3878.

[13] Brillard J.,Ribeiro C.,Boemare N,Brehelin M,Givaudan A(2001).Two distinct hemolytic activities in *Xenorhabdus nematophila* are active against immunocompetent insect cells.Appl.Environ Microbiol.67:2515-2525.

[14] Brivio MF,Mastore M,Moro M(2004).The role of *Steinernema feltiae* body surface lipids in host-parasite immunological interactions. Mol Biochem Parasitol.135:111-121.

[15] Brivio MF,Pagani,M.,Restelli,S.(2002).Immune suppression of *Galleria mellonella* (Insecta,Lepidoptera) humoral defenses induced by *Steinernema feltiae* (Nematoda,Rhabditida): involvement of the parasite cuticle. Exp Parasitol.101:149-156.

[16] Brivio, M.F.; Mastore, M. 2018.Nematobacterial complexes and insect hosts: Different weapons for the same war. Insects, 9:117

[17] Castillo JC.,Renolds SE,Eleftherianos I.2011.Insect immune responses to nematode parasites.Trends Parasitol.27:537-547.

[18] Chapman RF(1998).Insects structure and function.Cambridge University Press,Cambridge .pp.94-127.

[19] Cooper D, El eftherianos I (2016). Parasitic nematode immuno modulatory strategies: recent advances and perspectives. Pathogens 5:58.

[20] Darsouei, R.; Karimi, J.; Dunphy, G.B. 2019. Functional Characterization of Outer Membrane Proteins (OMPs) in *Xenorhabdus nematophila* and *Photorhabdus luminescens* through Insect Immune Defense Reactions. Insects, 10, 352.

[21] Davis M. M., Engstrom Y. (2012). Immune response in the barrier epithelia: lessons from the fruit fly *Drosophila melanogaster*. *J. Innate Immun.* 4 273–283 10.1159/000332947

[22] Dowds B.C.A., Peters A. Virulence mechanisms. In: Gaugler R., editor. Entomopathogenic Nematology. CABI; New York, NY, USA: 2002. pp. 79–98.

[23] Drees BM, Miller RW, Vinson SB, Georgis R. 1992 Susceptibility and behavioral response of red imported fire ant (Hymenoptera: Formicidae) to selected entomogenous nematodes (Rhabditida: Steinernematidae & Heterorhabditidae) . *Journal of Economic Entomology.* 85:265–370

[24] Dunphy GB and Halwani A.1997.Haemolymph proteins of the larvae of *Galleria mellonella* detoxify endotoxins of the insect pathogenic bacteria *Xenorhabdus nematophilus* (Enterobacteriaceae).Insect Physiology 43:1023-1029.

[25] Dunphy GB and Thurston GS.1990.Insect immunity. In: Entomopathogenic nematodes in Biological control. R. Gaugler and HK Kaya (Eds.), pp.301-323.CRC Press, Boca Raton,FI.

[26] Dunphy GB. and Webster JM.(1991).Antihemolytic surface components of *Xenorhabdus nematophilus* var.*dutki* and

[27] their modification by serum of non-immune larvae of *Galleria mellonella*. Journal of Invertebrate Pathology 58:40-51.

[27] Ebrahimi L., Niknam G., Dunphy G.B. 2011.Hemocyte responses of the Colorado potato beetle, *Leptinotarsa decemlineata*, and the greater wax moth, *Galleria mellonella*, to the entomopathogenic nematodes, *Steinernema feltiae* and Heterorhabditis bacteriophora. J. Insect. Sci.;11:75. doi: 10.1673/031.011.7501.

[28] Eidt DC, Thurston GS. 1995.Physical deterrents to infection by entomopathogenic nematodes in wireworms (Coleoptera, Elateridae) and other soil insects. *Canadian Entomologist.* 127:423–429.

[29] Eleftherianos ,I.,Millichap,P.,Constant R.F.,Reynold SE.(2006).RNAi suppression of recognition protein mediated immune responses in the tobacco hornworm Manduca sexta causes increased susceptibility to the insect pathogen Photorhabdus.Developmental and Comparative Immunology,30(12):1099-107.
DOI: 10.1016/j.dci.2006.02.008.

[30] Elias, S.; Hurychová, J.; Toubarro, D.; Frias, J.; Kunc, M.; Dobeš, P.; Simões, N.; Hyršl, P. 2020. Bioactive excreted/secreted products of Entomopathogenic nematode *Heterorhabditis bacteriophora* inhibit the phenoloxidase activity during the Infection. Insects, 11:353.

[31] Fan J, Xie Y, Xue J, Liu R (2013). The effect of *Beauveria brongniartii* and its secondary metabolites on the detoxification enzymes of the pine caterpillar, *Dendrolimus tabulaeformis*. J Insect Sci 13:1:13

[32] Forst, S., and D. J. Clarke. 2002. Nematode-bacterium symbiosis, p. 57–77. In R. Gaugler (ed.), Entomopathogenic nematology. CABI Publishing, Wallingford, United Kingdom.

[33] Freitak, D. ,Wheat CW.,Heckel DG,Vogel H.(2007). Immune system responses and fitness costs associated with consumption of bacteria in larvae of Trichoplusia ni. BMC Biol. 5: 56

[34] Gaugler R.,Lewis E.,Stuart,R.J.1994.Ecology in the service of biological control: the case of entomopathogenic nematodes.Oecologia,109:483-489.

[35] Gaugler, R., Molloy, D., 1981. Instar susceptibility of *Simulium vittatum* (Diptera: Simuliidae) to the entomogenous nematode *Neoaplectana carpocapsae*. J. Nematol. 13: 1-5

[36] Gillespie JP, Burnett C, Charnley AK (2000). The immune response of the desert locust *Schistoceria gregaria* during mycosis of the entomopathogenic fungus, *Metarhizium anisopliae* var acridum. *J Insect Physiol* 46:429–437

[37] Gillespie JP, Kanost MR, Trenczek T (1997). Biological mediators of insect immunity. Annu Rev Entomol 42:611-43 11

[38] Girling R.D.,Ennis,D.,Dillon,A.B.,Griffin,C.T.(2010). The lethal and sub-lethal consequences of entomopathogenic nematode infestation and exposure for adult pine weevils, *Hylobius abietis* (Coleoptera: Curculionidae). Journal of Invertebrate Pathology.104:195-202.

[39] González-Santoyo I, Córdoba-Aguilar A (2012) Phenoloxidase: a key component of the insect immune system. Entomol Exp Appl 142(1):1–16

[40] Goodrich-Blair H, Clarke DJ (2007) Mutualism and pathogenesis in *Xenorhabdus* and *Photorhabdus*: two roads to the same destination. Mol Microbiol 64: 260–268.

[41] Gotz P.,Weise C, Kopacek,P.,Losen S,Wiesner A.1997. Isolated Apolipophorin III from Galleria mellonella stimulates the immune reactions of this insect..J.Insect Physiol. 43(4): 383-391.

[42] Götz, F., Elstner, E. F., Sedewitz, B., and Lengfelder, E. (1980). Oxygen utilization by *Lactobacillus plantarum*. II. Superoxide and superoxide dismutation. *Arch. Microbiol.* 125, 215–220. doi: 10.1007/bf00446879;

[43] Gulley, M. M., Zhang, X., and Michel, K. (2013). The roles of serpins in mosquito immunology and physiology. *J. Insect Physiol.* 59, 138–147. doi: 10.1016/j.jinsphys.2012.08.015;

[44] Hillyer J.F.(2016).Insect immunology and hematopoiesis. Developmental and Comparative Immunology.58:102-118.

[45] Hoffmann JA.,Reichhart JM.,Hetru C.(1996).Innate immunity in higher insects.Current Opinion in Immunology 8(1):8-13.

[46] Hwang J.,Park Y.,Kim Y.,Hwang J.,Lee D.2013.An entomopathogenic bacterium,Xenorhabdus nematophila,suppresses expression of antimicrobial peptides controlled by Toll and Imd pathways by blocking eicosanoid biosynthesis. Arch.Insect Biochem.Physiol.83:151-169.

[47] Ishibashi, N and Kondo, E. 1990. Behavior of infective nematodes. In *Entomopathogenic nematodes in biological control*, Gaugler, R and Kaya, H K.(Eds.) 139-150. Boca Raton, , Fl, USA: CRC Press.

[48] Ji, D.; Kim, Y. 2004.An entomopathogenic bacterium, *Xenorhabdus nematophila*, inhibits the expression of an antibacterial peptide, cecropin, of the beet armyworm, *Spodoptera exigua*. *J. Insect. Physiol.* , 50, 489–496.

[49] Kanost M.and Gorman MJ.(2008). Phenoloxidases in insect immunity. Insect Immunology (ed. by N Beckage), pp. 69-96. Academic Press, San Diego, CA, USA. DOI:10.1016/B978-012373976-6.50006-9.

[50] Kanost MR, Jiang H and Yu XQ (2004) Innate immune responses of a lepidopteran insect, *Manduca sexta*. Immunological Reviews 198: 97–105

[51] Kaya HK and Gaugler R. 1993. Entomopathogenic nematodes. Ann. Rev.Entomol. 38: 181-206.

[52] Kaya HK.and Hotchkin PG(1981).The nematode Neoaplectana carpocapsae(Weiser) and its effect on selected ichneumonid and braconid parasites.Environ Entomolgy 10:474-478.

[53] Koppenhofer,AM.,Ganguly,S.,Kaya,HK.(2000).Ecological characterization of Steinernema monticolum,a cold–adapted entomopathogenic nematodes from Korea.Nematology 2(4):407-416.

[54] Krzemien, J., Crozatier, M., and Vincent, A. (2011). Ontogeny of the Drosophila larval hematopoietic organ, hemocyte homeostasis and the dedicated cellular immune response to parasitism. Int. J. Dev. Biol. 54, 1117–1125. doi: 10.1387/ijdb.093053jk

[55] Kunc,M.,Badrul,A.,Pavel,H.,Ulrich,T.(2017). Monitoring the effect of pathogenic nematodes on locomotion of *Drosophila* larva.Fly:3:1-10

[56] Lavine ,MD.and Strand ,M.(2002). Lavine MD, Strand MR. Insect hemocytes and their role in immunity. Insect Biochem Mol Biol 32: 1295-1309

[57] Lewis EE, Clarke DJ. 2012. Nematode parasites and entomopathogens. Pp. 395–424 *In:* F. Vega and H. K. Kaya, eds. Insect Pathology, 2nd ed. Amsterdam, The Netherlands: Elsevier.

[58] Li E.,Qin J.,Feng H.,Li J.,Li X.,Nyamwasa,I.,Cao Y.,Ruan W.,Li k. and Yin J.(2021).Immune related genes of the larval *Holotrichia parallela* in response to entomopathogenic nematodes *Heterorhabditis beicherriana* LF.BMC Genomics 22:192.doi:org/10.1186/s12864-021-07506-4.

[59] Li XY,Cowles RS, Cowles EA,Gaugler R, Cox-Foster DL. 2007. Relationship between the successful infection by entomopathogenic nematodes and the host immune response. Int. J.Parasitol.37: 365-374.

[60] Li XY., Cowles EA., Cowles RS., Gaugler R., Cox-Foster, Dl.(2009). Characterization of immunosuppressive surface coat proteins from *Steinernema glaseri* that selectively kill blood cells in susceptible hosts. Mol Biochem Parasitol 165:162-169.

[61] Lowenberger C.(2001).Innate immune response of Aedes aegypti.Insect Biochemistry and Molecular Biology.31(3):219-229.

[62] Manniello MD.,Moretta A.,Salvia R.,Scieuzo C.,Lucchetti D.,Vogel H.,Sgambato A. and Falabella P.(2021).Insect antimicrobial peptides:potential weapons to counteract the antibiotic resistance.Cellular and Molecular Life Sciences.doi.org/10.1007/s00018-021-03784-z.

[63] Marmaras VJ.,Charalambidis ND.,Zervas CG.(1996).Immune response in insects: the role of phenoloxidase in defense reactions in relation to melanization and sclerotization.Arch Insect Biochem Physiol.31(2):119-133.

[64] Müller P, Donnelly MJ, Ranson H (2007). Transcription profiling of a recently colonised pyrethroid resistant *Anopheles gambiae* strain from Ghana. BMC Genomics 8:36

[65] ni. – BMC Biol. 5: 56

[66] Owuama,CI.(2001).Entomopathogenic symbiotic bacteria,Xenorhabdus and Photorhabdus 17:505-515.

[67] Park, Y., Herbert, E. E., Cowles, C. E., Cowles, K. N., Menard, M. L., Orchard, S. S., and H. Goodrich-Blair. 2007. Clonal variation in Xenorhabdus nematophila virulence and suppression of Manduca sexta immunity. Cellular Microbiology 9:645-656.

[68] Park, Y., Kim, Y., Putnam, S. M., and D. W. Stanley. 2003. The bacterium Xenorhabdus nematophilus depresses nodulation reactions to infection by inhibiting eicosanoid biosynthesis in tobacco hornworms, Manduca sexta. Archives of Insect Biochemistry and Physiology 52:71-80.

[69] Peters A, Gouge DH, Ehlers RU.and Hague NGM. (1997). Avoidance of encapsulation by *Heterorhabditis* spp. infecting larvae of *Tipula oleracea*. J.Invertebr. Pathol. 70: 161-164.

[70] Peters A., Ehlers R.U. 1997. Encapsulation of the entomopathogenic nematode Steinernema feltiae in Tipula oleracea. J. Invertebr. Pathol.69:218-222. doi: 10.1006/jipa.1996.4648.

[71] Petersen, J. J. (1975). Penetration and development of the mermithid nematode Reesimermis nielseni in eighteen species of mosquitoes, J. Nematol., 7: 211.

[72] Petersen, J. J. and Willis, O. R. 1970. Some factors affecting parasitism by mermithid nematodes in southern house mosquito larvae, J. Econ. Entomol., 63: 175

[73] Potter DA.,and Held D.W.(2002).Biology and management of the Japanese beetle.Annual.Rev.Entomology.47:175-205.

[74] Schmidt O.,Theopold U.,Strand M.(2001).Innate immunity and its evasion and suppression by hymenopteran endoparasitoids.Bioessays 23:344-351.

[75] Shapiro-Ilan, D. I., Hiltpold, I., and Lewis, E. E. (2018). Ecology of invertebrate pathogens: nematodes, In *Ecology of Invertebrate Diseases*, ed A. E. Hajek and D. I. Shapiro-Ilan (Hoboken, NJ: John Wiley & Sons, Ltd.), 415–440. doi: 10.1002/9781119256106.ch11

[76] Shreehan G.,Farrell G. and Kavanagh K.(2020).Immune priming: the secret weapon of the insect world.Virulence 11:238-246.

[77] Silva CP.,Waterfield NR.,Daborn PJ.(2002).Bacterial infection of a model insect: *Photorhabdus luminescens* and *Manduca sexta*. Cellular Microbiology 4(6):329-339.

[78] Soderhall, K. and Cerenius, L. (1998). Role of the prophenoloxidase-activating system in invertebrate immunity. Curr. Opin. Immunol. 10: 23-28.

[79] Stanley, D.; Kim, Y. 2018. Prostaglandins and other Eicosanoids in Insects: Biosynthesis and Biological Actions.Front. Physiol., 9, 1927.

[80] Strand MR (2008) .The insect cellular immune response. Insect Sci. 15(1):1-14.

[81] Triggiani, O. and Jr. G.O. Poinar, 1976. Infection of adult lepiodptera by *Neoaplectana carpocapsa* (Nematoda). J. Inv. Pathol., 27: 413-414

[82] Veillard F.,Troxler L.,Reichhart JM.(2016).*Drosophila melanogaster* clip-domain serine protease: structure,function and regulation.Biochimie 122:255-269.

[83] Vilmos P.and Kurucz E.(1998).Insect immunity:evolutionary roots of the mammalian innate immune system.Immunol Lett 62(2):59-66.

[84] Wang Y and Gaugler R. 1998. *Steinernema glaseri* surface coat protein suppresses the immune response of *Popillia japonica* (Coleoptera:Scarabaeidae) larvae. Biol. Control 14:45-50,

[85] Wang Y, Campbell JF and Gaugler R. 1995.Infection of entomopathogenic nematodes *Steinernema glaseri* and *Heterorhabditis bacteriophora* against *Popillia japonica* (Coleoptera: Scarabaeidae) larvae. J. Invertebr. Pathol. 66:178-184,

[86] Webster JM, Chen G, Hu K, Li J. 2002. Bacterial metabolites. Pp. 99-114 *in* R. Gaugler, ed. Entomopathogenic Nematology. Oxon, UK: CAB International.

[87] Williams MJ. (2007). *Drosophila* hemopoiesis and cellular immunityJ Immunol.178(8):4711-4716.

[88] Yamanaka, S., Takeuchi, K., Tanabe, H., 1995. Host wounding ability, vertical migration and infectivity of *Steinernema glaseri*, *S. anomali* and *S. kushidai* (Nematoda: Steinernematidae). Jpn. J. Nematol. 25, 24-32

[89] Yang J,Zeng HM,Lin HF.,Yang XF.,Liu Z.,Guo LH.,Yuan JJ.,Qiu DW.(2012).An insecticidal protein from *Xenorhabdus budapestensis* that results in prophenoloxidase activation in the wax moth, *Galleria mellonella.* J.Invertebr Pathol 110(1):60-67.

Article

Analysis of Sustainable Potential of Kite Fish (Decapterus Spp) in the Waters of bone bay of South Sulawesi province

Muh. Arfah Mustari, Aris Baso, Hamzah Tahang

Master of Fisheries Sciences Faculty of Fisheries and Marine Hasanuddin University, Perintis Kemerdakaan Km 10 Makassar 90245 Indonesia

Abstract— *The purpose of this study is to analyze the level of exploitation of kite fish in the Waters of Bone Bay, South Sulawesi Province. This study was conducted in July to September 2021. The method used is Descriptive Quantitative by using questionnaire tools. The sampling method used is a method with a total of 10 units of Kite Fishing business each in the Bone Bay Waters area. The data analysis used is an estimate of biological parameters and Maximum Sustainable Yield. The results showed that business conditions Condition of maximum sustainable yield potential in the waters of bone bay has not been indicated to experience over fishing with the value of effort can be 64,315.46 units / year, yield of 70,161.10 tons / year and Biomass of 180,332 tons/year.*

Keywords— *Maximum Sustainable Yield, Kite Fish, Bone Bay Waters area.*

I. INTRODUCTION

Kite Fish (Decapterussp) is one of the pelagic fish caught in these waters that contributes the most. Kite fish are caught using fishing gear such as charts, gillnets, payang, and ring trawls (purse seine). This kite fish has an important role and has economic value in the fishery purse seine so much sought after and captured by the fleet of seine purse as the main target of catches (Prihartini, 2004). The approach of the biological aspect is generally used to evaluate fish resources but without neglecting the economic aspects. While fishermen as businesses and resource users are oriented to economic principles by prioritizing the greatest profit without regard to sustainability. Therefore, it is necessary to analyze the utilization rate of Catch Per unit Effort (CPUE), determination of Sustainable Potential Level, Optimum Effort, and Utilization Rate of kite fish resources in the Gulf of Bone Sea of South Sulawesi, where the information is needed in sustainable fishery management (Sangaji, et al. 2017).

In general, fishermen of the Fishing Unit operating in the Waters of Bone Bay are fishing businesses that still rely on their habits such as fishing methods, fishing areas, and fishing seasons. This resulted in the value of the production of volatile catch fisheries. Business feasibility analysis is a criterion for investment investment for a certain production period. The analysis is necessary to determine the future development of the Arrest Unit business. To support the analysis, it is necessary to calculate economic aspects such as capital, financing, revenue, and sustainability in a certain production period (Karningsih, 2014).

II. RESEARCH METHODOLOGY

A. Location and Time of Research

This research was conducted during 3 July – September 2020. The location of this study is in Palopo City, Bone Regency and Sinjai Regency. The samples in this study were kite fish. This research was conducted in Bone Bay by taking place in 3 (three) districts that serve as fishing bases, namely Palopo City, Bone Regency and Sinjai Regency, South Sulawesi Province. The three locations are location points that represent the waters of Bone Bay, While Paying Attention to the Covid-19 Health Protocol.

B. Types of Research

This research uses quantitative methods. Quantitative is the process of finding knowledge that uses data in the form of numbers as a tool to find information about what is the problem of research (Sugiyono, 2013). This research was conducted by conducting survey methods by conducting observations in the field and interviewing directly with respondents, as well as using questionnaires as a data collection tool. Then the data that has been obtained is analyzed quantitatively.

C. Sampling Method

Research methods are basically a scientific way to obtain data with specific purposes and uses. To achieve these goals, a method is required that is relevant to the goal to be achieved. The method used in this study is the survey method. Survey method is research conducted using questionnaires as a means of research conducted in large and small populations, but the data studied is data from samples taken from the population, so that relative events, distribution, and relationships between variables are found. The purpose of the survey research is to provide a detailed picture of the background, traits, and characteristics typical of the case or occurrence of a common thing (Sugiyono, 2013).

Sampling techniques that will be used in this study are determined by consideration of the terms of representation of aspects of the study and analysis needs, Sampling methods used are non probability sampling that is by purposive sampling where, data collection techniques from interview results and direct observations purposively to several stakeholders related to kite fishing activities respondents are considered to have the ability to answer questions asked well and can understand the problems that exist.

Samples are part of the number and characteristics owned by the population (Sugiyono, 2008) Purposive sampling is a technique of determining samples with certain considerations that is based on the purpose of research. This is done to obtain samples that fit the criteria or research objectives to obtain and obtain accurate data. The criteria in this study focused on Fishermen, Collectors, and Retailers.

The respondents selected are those who know internally and externally the condition and are able to provide answers and confirmations about the questions asked by way of interview (depth interview)

.D. Population and Research Samples

The data required in this study are information related to kite production data, number of kite fishing fleet, operational costs and others. In order to obtain these data, the first thing to do is to determine the research population so that this research gets clarity on the subject of the research. Population is a group consisting of objects or subjects that have certain qualities and characteristics set by researchers to be studied and then drawn conclusions (Sugiyono, 2013).The population of this study is fishermen who have a kite fishing business using seine purse fishing equipment. Based on data from the Department of Marine Affairs and Fisheries, the number of kite fishing efforts with seine purse fishing equipment in Bone 9 units, Palopo City 12 units and Sinjai Regency 120 units.

The sample is a portion of the population that has the same characteristics as the population. Sampling techniques in this study using purposive sampling technique (Sugiyono, 2013). The selection of this technique is based on a specific research study, namely the bioeconomic assessment of kite fish in the waters of Bone Bay. The sample is part or representative of the population to be studied (Suharsimi, 2014). Sampling for research according to Suharsimi (2014), if the subject is less than 100 people should be taken all of them, if the subject is large or more than 100 people should be taken 10-15 % or 20-25% or more. So for Sinjai district exceeds 100 units, researchers take 10% of the population so that the sample used as many as 10 units.

E. Data Analysis

Data Analysis used in this study as follows:

To answer the formulation of the first problem about the exploitation of fish Data used in the method of Surplus Production in the form of catches and efforts, then conducted data processing through the Dynamic Model approach which is a model of regression analysis of CPUE to the amount of effort.

1. Catch per Unit Effort (CPUE)

After the production and effort data (input or effort) is arranged in the form of time sequence according to the type of capture tool, the next step is to look for the catch per unit of effort (CPUE).. CPUE values can be notified as follows:

$$CPUE_t = \frac{Catch_1}{Effort_1}$$

$$t = 1,2,...n$$

Where:
CPUEt = catches per capture attempt in the t-year
Catch1 = catch in the t-year

2. Standardization of fishing gear

Standard fishing equipment is based on the amount of catch obtained as well as the

amount of fishing power index value (FPI) with input (effort/ effort) of standardized tools.

$$E_{std} = Y_{tot}/ CPUE_{std}$$

Where:

Estd : Efforts /efforts fishing standards

CPUEstd : CPUE Standard Capture Tool

Ytot : CPUE that is used as standard

3. Estimation of Biological parameters

Biological parameters include water carrying capacity constant (K), natural growth constant (r), technology parameter (q). While the economic parameters include the cost per catching effort (c/p), the price of IkanLayang per unit, the catch (p), and the rate of deduction / discoun rate. There are several approaches in estimating biological parameters, but in this study used is the model of estimation of CYP (Clark, Yoshimoto and Pooley) with the approach and development of the formula model Fox (1970) and Schunate (1977), systematically the equation is written as follows: Clark et al., (1992) with model 4:

$$Ln (CPUE_{t+1}/CPUE_t) = \beta_0 + \beta_1 CPUE + \beta_2 E_t + e$$

Where:

CPUEt+1 = CPUE at t+1 time

CPUEt = CPUE at time t

Et = Effort at time t

Et+1 = Effort at time t+1

β0 = coefficient of intercept rgresi result

β1 = coefficient X variable 1 regression result

β2 = coefficient X variable 2 regression result

Estimation of Biological parameters

Biological parameters include water carrying capacity constant (K), natural growth constant (r), technology parameter (q). While the economic parameters include the cost per catching effort (c/p), the price of kite fish per unit, catches (p), and the rate of deduction / discoun rate. There are several approaches in estimating biological parameters, but in this study used is the model of estimation of CYP (Clark, Yoshimoto and Pooley) with the approach and development of the formula model Fox (1970) and Schunate (1977), systematically the equation is written as follows: Clark et al., (1992)

4. Maximum Sustainable Yield (MSY) Analysis

Calculation of the Maximum Sustainable Yield (MSY) model using the equation:

$$EMSY = r/2q$$
$$YMSY = Kr/4$$

$$XMSY = K/2$$

where:

EMSY : Attempts to capture MSY's condition

YMSY : MSY condition capture

XMSY : Estimating optimal stock of MSY conditions

III.   RESULTS AND DISCUSSION

A. Kite Fishing Equipment

Purse seine is often referred to as a bag net because the shape of the net when operated is shaped like a pouch Where in determining the design is based on the fish that is the purpose of catching, the catching area, as well as the method of catching that will later be applied and the seine purse ship to be used. Purse seine itself is the most effective fishing tool to catch hordes of fish that are on the surface (pelagic fish) which in operation can be done by chasing hordes of fish, or by using fish collecting aids in the form of lamps or rumpons.

Purse seine itself has a rectangular shape with pockets on the edges, this shape is called a type of shawl. Most of the net material is made of polyamide measuring 400 meters with a depth of 100 meters and a mesh size of 25 mm (1 inch). Has a weight of 180 pieces made of lead with a weight of 2 kg / piece each. In addition, it is equipped with a buoy sign and 2300 net buoys made of plastic with a ball-like shape that has a diameter of 10 cm (4 inches).

In the operation of purse seine departure to the location of arrest is carried out in the morning (09.00 Wita) and there is also during the day (13.00 – 16.00 WITA) depending on the distance or nearby fishing ground. If the location of the fishing ground is classified as far away then the departure is done in the morning and if the fishing ground is relatively close then the departure is done during the day. After arriving at the fishing ground, the light boat was removed from the ship and the ABK tethered the rope to the rumpon, and in the afternoon at 18.00 Wita the main light was turned on to attract hordes of fish.

B. Catch Per Unit Effort

Can be seen from 2010-2019 CPUE some fishing tools tend to be volatile. CPUE some of the highest fishing gear in 2017 was 0.4779 tons per unit and the lowest occurred in 2010 at 0.0408 tons per unit.

The possibility of decreased CPUE is also due to the increasing distance of the catchment area and due to the influence of changes in natural/environmental conditions (weather, wind, salinity, seasons) on population and resource communities.

Table 1. The amount or value of the catch per unit effort (CPUE) describes or reflects the productivity level of the effort.

Tahun	Payang	PukatCincin	JaringInsangHanyut	JaringInsangTetap	Bagan Perahu	Bagan Tancap	Pancingulur
2010	0.0363	0.0408	0.0209	0.0001	0.0049	0.0003	0.0079
2011	0.1554	0.0715	0.0086	0.0014	0.0281	0.0016	0.0024
2012	0.1143	0.0836	0.0028	0.0011	0.0251	0.0019	0.0009
2013	0.0278	0.0888	0.0026	0.0013	0.0190	0.0038	0.0000
2014	0.0965	0.4094	0.0083	0.0062	0.0958	0.0243	0.0002
2015	0.0828	0.3477	0.0071	0.0052	0.0813	0.0207	0.0002
2016	0.0947	0.3936	0.0080	0.0059	0.0921	0.0234	0.0002
2017	0.1024	0.4779	0.0086	0.0064	0.0986	0.0251	0.0002
2018	0.1045	0.4600	0.0087	0.0065	0.1006	0.0256	0.0002
2019	0.1086	0.4391	0.0091	0.0067	0.1046	0.0266	0.0002

Source : Secondary Data after processing, 2021

C. Standardization of Fishing Gear

Standardization of fishing equipment is required to homogenize kite fishing steam consisting of various types of fishing equipment. Determination of standardization of effort units in this study is the total effort per year of several fishing equipment with time series data in 2010- 2019 obtained from the Fisheries Service palopo city, Bone regency and Sinjai regency of South Sulawesi Province. The standard fishing gear used in this study is Purse Seine on the grounds that it has a greater catch perunit effort value compared to other fishing gear.

Standardization of Kite Fishing Equipment in the Waters of Bone Bay, South Sulawesi Province in 2010-2019

Tahun	Payang	PukatCincin	JaringInsangHanyut	JaringInsangTetap	Bagan Perahu	Bagan Tancap	Pancingulur
2010	0.8889	1.0000	0.5127	0.0025	0.1203	0.0082	0.1943
2011	2.1733	1.0000	0.1200	0.0195	0.3935	0.0227	0.0341
2012	1.3664	1.0000	0.0340	0.0132	0.2999	0.0227	0.0113
2013	0.3135	1.0000	0.0291	0.0141	0.2139	0.0426	0.0005
2014	0.2358	1.0000	0.0203	0.0151	0.2339	0.0594	0.0005
2015	0.2382	1.0000	0.0203	0.0151	0.2339	0.0594	0.0004
2016	0.2406	1.0000	0.0203	0.0151	0.2339	0.0594	0.0004
2017	0.2144	1.0000	0.0179	0.0133	0.2064	0.0525	0.0004
2018	0.2271	1.0000	0.0190	0.0141	0.2187	0.0556	0.0004
2019	0.2473	1.0000	0.0206	0.0153	0.2382	0.0605	0.0005

Source : Secondary Data after processing, 2021

Table 4. Productivity level of (effort) after strandarization of kite fishing equipment in the Waters of Bone Bay, South Sulawesi Province in 2010-2019

Total	Produksi (Ton)	Total Effort Standard	CPUE (Total/Unit)
2010	2,918.60	71,456	0.0408
2011	5,670.10	79,305	0.0715
2012	6,132.00	73,321	0.0836
2013	5,859.30	65,990	0.0888
2014	22,815.30	55,732	0.4094
2015	19,571.70	56,285	0.3477
2016	22,377.80	56,848	0.3936
2017	24,449.80	51,163	0.4779
2018	25,183.29	54,745	0.4600
2019	26,442.46	60,219	0.4391

Source : Secondary Data after processing, 2021

3. Estimation Parameter Biology

From the value obtained is then incorporated into the equation of biological parameters so that it can be suspected constants of fish growth rate, coefficient of fishing and constants of water carrying capacity.

The estimated result of the three parameters presented in table 1, The value can be seen in table 5

Table.5: estimated result Value

No	Estimasi	Parameter RegresiIkan Layang		
		β_0	β_1	β_2
1	Coefficients	1.97509529	4.1489335	1.1713E-05
2	Standard Error	0.67503034	0.4744975	8.7529E-06
3	t Stat	2.9259356	8.743847	1.33812742
4	F	293.705984		
5	R Square	0.98988898		

Source : Secondary Data after processing, 2021

Ordinary Least Squares (OLS) models from Table 5 for Kite Fish are as follows

Y = y = -2E-05x + 1.9750 R2 0.99 From the data contained in table 5 it appears that the R2 value of IkanLayang is 0.99, this indicates that independent variables in the equation have a strong influence and association with dependent variables. From the value obtained is then incorporated into the equation of biological parameters so that it can be suspected constants of fish growth rate, coefficient of fishing and constants of carrying capacity of fish waters itself. The estimated results of the three parameters presented in table 5, useful for determining the level of sustainable production such as maximum sustainable yield (MSY)

4. Maximum Sustainable Yield

The key assumption of the sustainable potential model or sustainable harvest in maximum sustainable yield (MSY) is that the population of IkanLayang grows and replaces itself, in the sense that the population of IkanLayang is a renewable resource. The concept of sustainable catchment or Maximum Sustainability Yield (MSY), aims to maintain the size of the population at the maximum point where the growth rate with harvesting is will usually be added to the population and allow the population to be productive forever (Hertiniet al., 2013). To determine sustainable catches or sustainable potentials, a maximum sustainable yield management regime is used.

Table 6. Maximum Sustainable Yield of Kite Fish.

Model	Effort (Unit)	Yield (Y) (Ton)	Biomass (X) (Ton)
MSY	64,315.46	70,161.10	180,332.20
Aktual	62,506.41	16,142.04	

Source : Secondary Data after processing, 2021

Based on Table 6 that has been analyzed using the maximum sustainable yield management regime model, the effort value can be 64,315.46 units / year, yield of 70,161.10 tons / year and Biomass of 180,332.20 tons / year, while in actual production conditions of 62,506.41 tons

Table 2. Standardization of Kite Fishing Equipment in the Waters of Bone Bay, South Sulawesi Province in 2010-2019

5. Level of Kite Fish Exploitation in Bone Bay Waters
The availability of fish is also influenced by environmental factors (oceanographic condtions) so that changes in oceanographic conducives affect the produtivtas of the waters which then impact the distribution of fish. Fish distribution will affect fishermen's actions in the allocation of fishing (Nelwan, 2016).

To prevent excessive fishing, a fishery management effort is needed to keep fish resources sustainable. In fishery management it is not easy to regulate and change the existing circumstances so that the way that is possible is to conduct a surveillance system on ships entering the waters, especially for catchers who enter the waters that are not the catchment area and conduct a data collection system on fish production as a whole whether it is against fish that are worth selling, consumption, or wasted. From the results found in the field, there are still many fishermen's catches that have not been recorded thoroughly.

Fishing efforts can be known from the data of kite production although has been described data recorded in various fisheries services three regions such as Bone, Sinjai and Palopo have not been thoroughly or not fully recorded. Fluctuations in the utilization rate of kite fish (Decapterussp) seen from the data of kite fish production can be caused by various factors, the decrease in catch may be due to the decrease in population size due to high fishing efforts in previous years. On the contrary, increased catches can be due to increased population size due to low arrest attempts in the previous year, or increased efforts themselves due to price pushes.

To determine the level of exploitation of kite fish, where the level of exploitation of kite fish is directly related to the sustainable potential (MSY) of fishery resources and kite fishing efforts. By knowing and analyzing the level of exploitation, it is expected that a balance between the sustainable potential (MSY) of fishery resources and their utilization so that the sustainability of fishery resources can be guaranteed.

IV.   CONCLUSION
The condition of maximum sustainable yield potential in bone bay waters has not been indicated to be over fishing with the value of effort can be 64,315.46 units / year, yield of 70,161.10 tons / year and Biomass of 180,332.20 tons / year

REFERENCES
[1] Fujii, Y., Iriana, W., Okumura, M., Lestari, P., Tohno, S., Akira, M., & Okuda, T. (2012, December). Characterization of biomass burning aerosols from forest fire in Indonesia. In *AGU Fall Meeting Abstracts* (Vol. 2012, pp. A21A-0030).
[2] Gemaputri, A. A. 2013. Tingkat Pemanfaatan Sumber DayaIkan Hasil Tangkapan Di PerairanJember Utilization Rate Of Fish Resources In Jember Water. JurnalPerikanan (J. Fish. Sci.) Xv (1): 35-41 ISSN: 0853-6384

[3] Hamka, E dan M. Rais. 2016. Penentuan Musim PenangkapanIkan Layang (Decapterus Sp.) Di Perairan Timur Sulawesi Tenggara Determination Of

[4] Hertini, E dan N. Gusriani. 2013. Maximum Sustainable Yield (MSY) pada PerikanandenganStruktur Prey-Predator. Prosiding Seminar Nasional Sains dan Teknologi Nuklir. PTNBR – BATAN Bandung.

[5] Kawarnidi, F., Labaro, I. L., &Silloy, F. (2018). Komposisi Hasil Tangkapan Jaring Insang Dasar di perairan Desa Talise Tambun, Kecamatan Likupang Barat (Composition catches of bottom Gill Net in Talise Tambun Waters of Likupang Barat District). JurnalIlmu dan Teknologi Perikanan Tangkap. https://doi.org/10.35800/jitpt.3.1.2018.18906

[6] King M. 1995. Fishery Biology, Assessment, and Management. Fishing News Books. London.

[7] Latuconsina, Husain.2010. Eksistensi Sasi Lautdalam Pengelolaan Perikanan Berkelanjutan Berbasis Komunitas Lokal Di Maluku.Jurnal Triton Volume 5, Nomor 1, April 2010, Hal. 63 – 71.

Article   *Peer-Reviewed Journal*

Profitability Assessment with the application of BRASS model of Small-scale Rubber Plantation at Chittagong Hill Tract, Bangladesh

A.K.M. Abul Kalam Azad[1][3], Md. Najmus Sayadat Pitol[2]* and Yonosuke Hara[3]

[1]Bangladesh Planning Commission, Ministry of Planning, Agargoan, Dhaka
[2]Bangladesh Forest Research Institute, Ministry of Environment, Forest and climate change, Bangladesh.
[3]National Graduate Institute for Policy Studies (GRIPS), Tokyo, Japan.
*Corresponding author(s) e-mail: najmus.sayadat@gmail.com

Abstract— *This study concentrated on the profitability assessment of the small-scale rubber production by applying the Bioeconomic Rubber Agroforestry Support System (BRASS) model of Small-scale Rubber Production at Chittagong Hill Tract, Bangladesh. Considering the present market conditions of the cost of materials, land costs, labor costs, rubber prices and discount rate, the results of the Discounted Cash-flow (DCF) analysis indicate that the investment on small-scale rubber plantations in the Chittagong Hill Tracts is not profitable in the short run but in the long run, it is profitable. Rubber production is highly profitable if the government provides an interest-free loan to the poor farmers, and the farmers can reduce their investment costs. The farmers can control the investment costs by co-management. Rubber cultivation can be made more profitable if the farmers cultivate intercropping in their garden for the first few years. Considering the present market situation, the DCF analysis for the study shows that 28 years is the optimum rotation in small-scale rubber plantations with intercropping. With this optimum rotation, the CHT area can be financially most profitable for small-scale farmers. Investment in rubber plantations gives decent economic returns to the small-scale farmers. With the high discount rate, the investment in small-scale rubber plantations is no more profitable. Therefore, the government should allow interest-free credit or very low-interest rate credit support to small-scale rubber cultivators to make the maximum investment profit. With no intercropping, small-scale rubber cultivation is no more profitable. So, in line with the government policy of restricting shifting cultivations, the government should allow small-scale farmers to cultivate intercrops along with rubber cultivations.*

Keywords— *Discounted cash-flow (DCF), labor cost, net present value (NPV), rotation, value added tax (VAT).*

I. INTRODUCTION

In Bangladesh, the increasing demand for rubber and rubber products insisted on the new moneymaking plantation of rubber. Other hands**,** create any plantation near and within a natural forest or hill decreases the diversity of the forest (Rahaman et al. 2020). But still, rubber plantation proved as an excellent tool to cope with the food, wood, energy, ecology, poverty crises and lessen the deforestation rate of forest lands in Bangladesh. At present, both the

governmental and private sectors 18,954 ha plantation of *H. brasiliensis* has been developed and planned to expand its further 1214 ha during 2020 (BFIDC, 2015; Hossain, 2016). The artificial plantations are storing a huge amount of carbon from the environment (Pitol et al. 2019) where rubber trees also acting a carbon soak with latex production (A.K.M Azad et al. 2021). Rubber cultivation is environmentally suitable compared to shifting cultivation. Some people also argue that rubber cultivation can help to

eradicate poverty in the Chittagong Hill Tracts (CHT) in Bangladesh (Nath et al., 2013). For this reason, the government (then Pakistan) first introduced commercial rubber cultivation in 1961 engaging the forest department (BFIDC, 2013). 20 years later since the 1980s, the Bangladesh government took various programs and projects to promote rubber cultivation involving the local people. In 1985, with the help, ADB's financial support government took another project to enhance the involvement of local farmers in rubber cultivation (Nath et al., 2005). However, all these initiatives fail to achieve their expected goals (Rasul, 2005). Nonetheless, later the government took another policy decision to lease out fallow land to the farmers to cultivate rubber (BFIDC, 2012). To find out the reasons for previous government policy failure, this study concentrates on the profitability assessment of the small-scale rubber production by applying the Bioeconomic Rubber Agroforestry Support System (BRASS) model. This model is now widely used in other Asian countries like Indonesia and Lao PDR.

II. MATERIALS AND METHODS

2.1 Study area

Much more time and labors are required to carry out the detailed study of above issues because many small-scale rubber gardens were located in different regions that was impossible to complete the study. In this study, only one village named Rajbila was studied which is located at Bandarban upazila in Chittagong Hill Tracts (CHT) area. Rajbila village, is located in the Central Union (lower-level local government unit) at Bandarban Central (Sadar) Upazila in Bandarban District of Bangladesh. The total area of the village is 4.6 square kilometer where the small-scale rubber cultivation activities are going on. The total

population of the Rajbila village is about 2570 with 492 households (BBS 2011).

2.2 Data Collection and Analysis

To find out the general overview of the small-scale rubber cultivators and shifting cultivators, both qualitative and quantitative data on rubber production, their income, resources, and other status were used. Data was collected by group discussion, direct observation, and a questionnaire survey of the households. There were 48 registered small-scale rubber cultivators and 38 shifting cultivators were interviewed. The focus group consultations were held with the village authorities to understand the general circumstances of the study area by information gathering on rubber and shifting cultivation. Lastly information regarding the quantity and cost of the labor force used for the rubber and shifting cultivation was collected. During the focus group discussion, several field observations were conducted with their cordial help.

III. RESULT AND DISCUSSION

3.1 Undiscounted Cash Flow (UDCF)

The estimated intercrop and latex production undiscounted cash flow of one-hectare rubber plantations from the year 1-31 years (Fig-1). Between 2-4 years, it was found that only intercrop revenue could be generated and hence, it showed positive. It was further found that the undiscounted net return of latex production was negative in the first year and 5-9 years. In the 10th year, it was started to positive flow and continue rising and become highest at the 20th year and again become declining slowly. At the age 16, it becomes less because some more extra cost was done due to fencing purposes. At the final 32nd-year timber harvesting cash flow is shown in Figure-4.
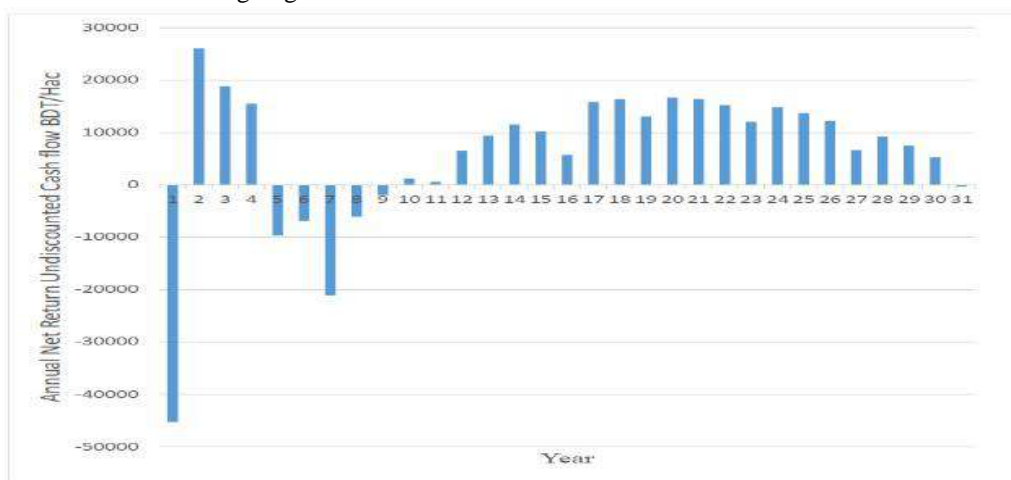
Fig.1: Undiscounted annual cash flow over 31 years lifetime

3.2 Discounted Cash Flow (DCF)

All the costs and revenues are discounted at 8% and the estimated discounted net return of latex production during the year 7-31 was presented separately (fig-2). First two year after starting latex collection, discounted cash flow was negative. At the 10th year starting positive cash flow that was highest at the year 19th and later on again decreasing the

undiscounted cash flow because of decreasing the latex production. In the 17th year, it shows less cash flow because some more management expenses like fencing were done in this year. From the 1st to 6th year, we have some discounted cash flow because 1-4 years we have some revenue from intercrop in the rubber plantations. There was also timber production in the final year of the production cycle that was presented in the next figure of cumulative NPV.
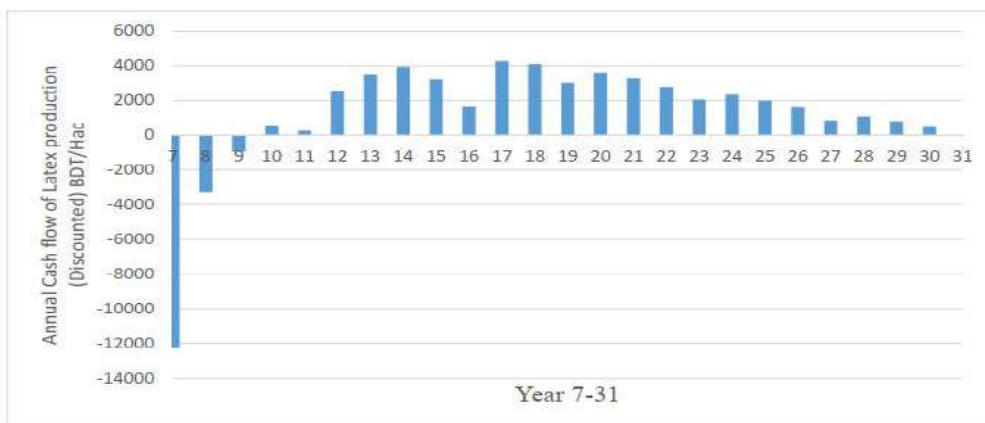
Fig.2: Discounted annual net cash flow of rubber (latex) production (7-31 years)

3.3 Cumulative Net Present Value (NPV)

The cumulative NPV becomes positive from the year 19th that reflects that it requires at least 19 years of planning horizon investment in rubber production to be profitable (Fig 3). In terms of applied three investments criteria, the

NPV of rubber production was 147735 BDT, the IRR was 15.67% that was above the discount rate at 8%, and the BCR was 1.30. Therefore, the DCF analysis of small-scale rubber production shows that the cumulative NPV was negative up to the 18th year and then it became positive and at the final year, it becomes highest because of timber harvesting.
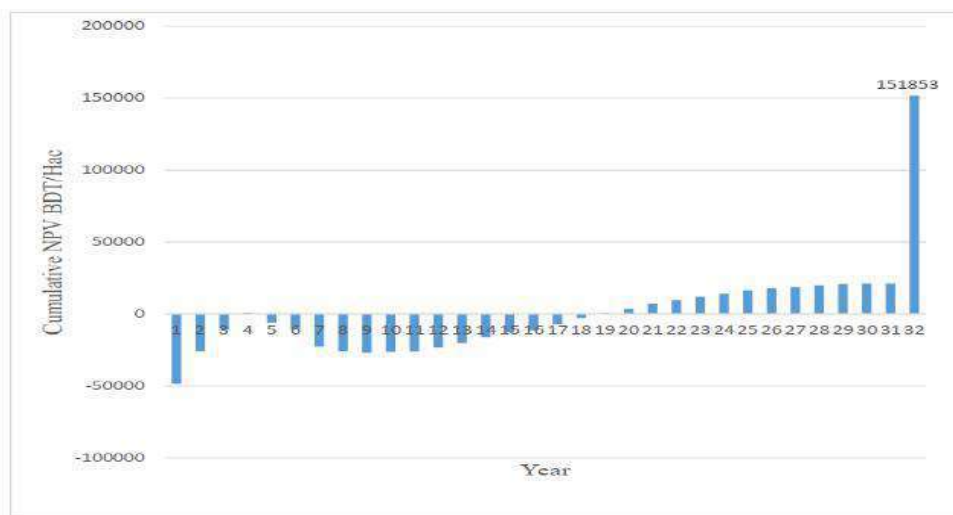
Fig.3: Cumulative NPV over 32 year's lifetime of rubber production

3.4 Cumulative NPV Without Considering Unpaid Family Labor Costs

The cumulative NPV becomes positive from the year 3rd and never goes beyond negative later on (Fig-4). The labor

cost of unpaid family labor is estimated to be at the opportunity cost of family member because in CHT there exist the job opportunities for them. However, there are many areas of small-scale rubber production in Asia, where such job opportunities are not available (Manivong and

Cramb, 2008 a b). Of course, in such cases, the small farmers can get revenue from selling latex. By taking into account this, cumulative NPV without considering unpaid family labor costs is estimated here. The total labor requirement for 32 years of rubber production is 4200 man-days (MDs) of which 2311 MDs (55%) were from unpaid family labors, and 1889 MDs (45%) were from hired labors. The wage rate of unpaid family labor cost was evaluated as zero and for the hired labor was calculated as the local wage rate 340 BDT/man-day. The discount rate (8%) and the rubber price (175 BDT/KG) was evaluated same as the base calculation. In this criterion the NPV of small-scale rubber production was 365756 BDT, the IRR was 36% that was much above the discount rate at 8%, and the BCR was 2.33. From the above DCF analysis of small-scale rubber production, it can be said that how to evaluate the opportunity cost and unpaid family labor cost is a very crucial issue in DCF analysis.
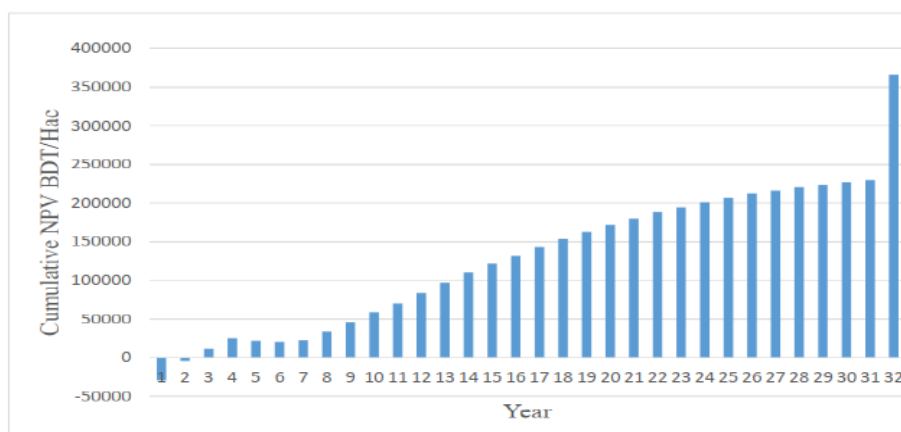
Fig.4: Cumulative NPV over 32 year's lifetime of rubber production without family labor cost

3.5 Sensitivity Analysis

The small-scale rubber cultivation is a long-term investment and may not assure the exact estimated NPV, IRR, and BCR. As the management strategies, different costs, prices, wages and rate of interests are subject to change over time, the probable result of these changes should be estimated (Gregory, 1987). There is no guarantee that the exact estimated NPV, BCR, and IRR will be obtained for the long-time investment. In prediction, uncertainty may be involved in future, and it should be considered in the DCF analysis. There are various methods to incorporate uncertainty into the DCF analysis. The most common method is the sensitivity analysis. The main factors that may greatly affect the overall outcomes of small-scale rubber plantations in the Chittagong Hill Tracts are the management, material cost, labor cost, value added tax, wage rate of labor, the price of latex, and the discount rate by changing different controllable variables.

3.5.1 Sensitivity Analysis of Different Controllable and Uncontrollable Variables

All of the variables of the model associated with the small-scale rubber production can be divided into two groups such as controllable variables and uncontrollable variables. The variables on which there is no control by the government or the farmers is termed as uncontrollable variables. Examples of such uncontrollable variables are climate variables (rainfall, temperature, solar radiation, and potential evapotranspiration), and soil and topographic variables (slope of the land, soil texture, soil nutrients, soil moisture content, soil pH). The variables on which the farmers or the government has the control to change are controllable variables, such as value added tax (VAT), rate of interest, rubber management (number of plants per unit area, types of species, co-management, fencing, fertilizing, tapping, harvesting), labor costs and material costs (reducing cost by co- management, preparation of land, quantity of materials used, reducing labor costs, family labor etc). The controllable variables are again divided into two groups such as government controllable variables and farmer's controllable variables. The variables that control depends on the decision of the government is government controllable variables such as VAT and interest rate. On the other hand, the variables that control depends on the farmers are called farmers controllable variables such as some variables of rubber. management, intercrop management, labor cost and material cost. The controllable variables play a significant role in profitability assessment and policy decision.

3.5.2 Results of Changing Controllable Variables

The analysis was done with DCF model application, changing some government controllable variables and farmers controllable variables and found relations between them. The differences of baseline calculation and changing government and farmers' controllable variables are as follows: The government controllable variables are value

added tax (VAT) and an interest rate of the government bank. The farmers have to pay 15% VAT on the revenue of rubber products. If the government does not impose any VAT (0%) on rubber products, then the NPV result may change. The baseline calculation, the discount rate was 8%. For the government controllable variable analysis, it may be considered as 5%. So, the main changes are VAT (0% instead of 15%) and discount rate (5% instead of 8%) separately in case of government controllable variables.

In the case of farmers controllable variable some labor costs and material costs are separately controlled and analyzed. In the baseline calculation, the rubber cultivation was evaluated as an individual. The major differences in this analysis were changing of some material costs and labor cost by applying co-management. In this case, several farmers (in this case 4 farmers) cultivate rubber plantations together and sharing some materials for raising their gardens. For the baseline analysis the total material cost for whole life cycle was 149560 BDT, but in this calculation, the total material cost was 132015 BDT that could save 17545 BDT for the whole life cycle of rubber plantations. The major portion of material costs reduces in the initial year of rubber plantation that is 3015 BDT. This cost reduces for sharing materials that are axe, knife, wire, posts, sharpening stone, handy saw, etc. Similarly, when the farmers cultivate the rubber plantations together, then they can also reduce some labor costs as well. The baseline total labor requirement for whole life cycle was 4200 man-days but, in this case, the total labor requirement for one unit of rubber garden is 3902 man-days that can save 298 man-days labor cost. The main activities which reduce the labor costs are land slashing, burning, cleaning, and fencing tapping and final tree harvesting. It shows that if the government does not impose value added tax on rubber products or reduce the rate of interest and the farmers reduce some labor costs or reduce some material cost that is under their control. Then the result shows that the small-scale farmers achieve highest NPV (4,26,667 BDT) and BCR (1.65) among the four variables when the government reduces the rate of interest on the loan and farmers reduce some labor costs (Table-1). The second highest NPV (3,83,882 BDT) and BCR (1.55) receive when the government reduces the VAT, and the farmers reduce some labor costs. The lowest NPV (2,48,828 BDT) and BCR (1.52) come when the government does not impose VAT and farmers reduce some Table material costs (Table-1). The analysis also shows the result of combined control of farmer's and government controllable variables. In this case, if the farmers control the labor and material cost together then the highest NPV (4,32,402 BDT) and BCR (1.67) achieved when the government reduces the interest rate. On the other hand, if the government do not impose any VAT and reduces interest rate then the highest NPV (5,94,873 BDT) and BCR (1.91) receives when the farmers can reduce some labor costs. The best NPV (6,00,609 BDT) and BCR (1.93) can be achieved when farmers can control both labor costs and materials costs, and government reduces the interest rate and VAT on products (Table-1). If the farmers do not pay VAT, then it will also be profitable on base discount rate. The farmers can reduce the labor costs and material costs by doing co-management for small-scale rubber farming. So, from the result, it can be summarized that interest rate is the most crucial variable for the small-scale rubber cultivators to obtain maximum benefits from rubber cultivation.

Table-1: NPV and BCR relation between government and farmers-controlled variables

Farmer's controllable variables

		Labor	Material	Labor and material
Government controllable variables	VAT	279515	248828	284272
		(1.62)	(1.52)	(1.64)
	Interest rate	426667	383882	432402
		(1.65)	(1.55)	(1.67)
	VAT and interest rate	594873	552089	600609
		(1.91)	(1.79)	(1.93)

3.5.3 Sensitivity Analysis of Changing of Price, Wage Rate and Discount Rate

As prices, wages and rate of interests are subject to change over time, the probable result of these changes should be estimated. The baseline calculation presented the DCF analysis of a typical one-hectare small-scale rubber production at 8% discount rate, 334 BDT per man-day of wage rate and the current local market price of rubber 175 BDT/KG. The main factors that may greatly affect the overall outcomes of small-scale rubber plantations in the

Chittagong Hill Tracts are the wage rate of labor, the price of latex, and the discount rate. The highest wage rate was 500 BDT per man-day, and the present discount rate was 8%. The present market price of latex was 175 BDT/Kg. Last five years lowest price of the latex was 150 BDT/KG and the highest price was 200 BDT/KG. So, the lowest and the highest price of latex was assumed as 150 BDT/KG and 200 BDT/KG for the sensitivity analysis. At the same time highest discount rate was assumed as 17% because some private bank allocate loan at higher interest rate. The lower discount rate was assumed as around 35% lower than the present discount rate. The sensitivity analysis was conducted in two different wage rates. The basic wage rate was 334 BDT per man-day, and the highest wage rate was 500 BDT per man-day. The results are presented separately in two different tables. Table-2, shows the sensitivity analysis at three different market prices of latex and three different discount rates with present wage rate. The three different

prices are 150 BDT/Kg, 175 BDT/Kg and 200 BDT/Kg. The three different discount rates are 5%, 8% and 17% with the base wage rate 334 BDT/day (Table-2). The result shows that rubber production investments are profitable on 5% and 8% discount rates. The result also demonstrates that the investment is profitable (NPV=10075, BCR=1.04, and IRR=18.78) at the high-level of the discount rate (17%) if the rubber price is higher (200 BDT/Kg) (Table-2). Conversely, if there is no change of rubber price (baseline rubber price at 175 BDT/Kg) but the discount rate is increased up to 17%, the investment on small-scale rubber production will be at a loss. The investment on small-scale rubber plantations cann ot be profitable in this case because, the BCR is less than 1 (0.97), and IRR is 15.74% that is less than 17%. Perhaps this situation could be managed by increasing the latex yields (by using fertilizer) and by reducing the labor costs and material costs as far as possible.

Table 2: Sensitivity (DCF) analysis on the basis of wage rate 334 BDT/MD

Price of Rubber BDT/KG	NPV (BDT/Ha) and B/C at different discount rates			IRR %
	5%	8%	17%	
150	289473	97231	(-23868)	12.67
	1.41	1.20	0.90	
175	377126	151853	(-6897)	15.74
	1.54	1.31	0.97	
200	464780	206475	10075	18.78
	1.66	1.42	1.04	

It also found that, the result of sensitivity analysis for highest wage rates (500 BDT/man-day) using three discount rates (5%, 8% and 17%) and three latex prices (150 BDT/KG, 175 BDT/KG and 200 BDT/KG) for a typical one-hectare rubber production (Table-3). This analysis revealed that the small-scale rubber production is not profitable at all three kinds of latex prices at the baseline discount rate (8%) and higher discount rate (17%). The small-scale rubber production is only profitable at the lower discount rate (5%) at baseline prices of latex (175 BDT/KG) and increased price of latex (200 BDT/KG) (Table-3). So, small-scale rubber production is not profitable at all kinds

of discount rates when the price of latex is low. It is also unprofitable at baseline and increased discount rates at all prices of latex. In this situation, the farmers should stop the tapping activities temporary or trying to reduce management costs and labor costs by using family labors. Co-management may be very helpful to reduce the labor costs and management costs. So, when wage rate and interest rate increase together, then the investment on small-scale rubber plantation is not viable. In this case, the government should offer subsidies to the farmers and take action to reduce the interest rate.

Table-3: Sensitivity (DCF) analysis on the basis of higher wage rate 500 BDT/Man-day

Price of Rubber BDT/KG	NPV (BDT/Ha) and B/C at different discount rates			IRR %
	5%	8%	17%	
150	(-15896)	(-110970)	(-118593)	4.73
	0.98	0.84	0.64	
175	72023	(-56240)	(-101613)	6.28

		1.07	0.91	0.69	
200	159942	(-1510)	(-84634)	7.95
	1.16	0.998	0.74	

3.6 Optimum Rotation Calculation

The government of Bangladesh is allocating fallow land in hill districts for rubber cultivation to reduce shifting cultivation due to soil erosion. The government has already established a rubber standing committee to excel the program of establishing private rubber cultivations. Under this scheme, a total 3915.40-hectare khash land (fallow land) were distributed in Bandarban Hill District for 40-50 years lease (Khisa, 1991). The government allowed 5 years to start rubber plantation after handover the lease of land to the farmers. Though 32 years are considered to be normally for the rubber cultivation, there is no study to show that the optimum rotation of rubber production in Bangladesh. Some cases it was found that the farmers do not start plantation in time just after getting the lease of land. Some farmers use the land for other purposes instead of rubber cultivation such as ginger cultivation, garlic cultivation as they got few years facility to start rubber cultivation which do not fulfill the main objective of government policy to reduce shifting cultivation and soil erosion (Rasul, 2005). Sometimes, farmers require more than 32 years because they cannot start plantation just after allocation of land. In this situation, it is very necessary to find a solution how can solve this misuse of leased land. Hence, tried to find out the optimum rotation of rubber plantation to minimize the land lease period and maximize the land use. Some cases farmer's rubber plantations are uneven, and they cannot harvest the timber at the same times. Here in this study, considered that all of the rubber seedlings plant in the same year of a particular garden, and it became all the trees are the same age, so the farmer can harvest all the timber at the same time. Different farmers have a different year of plantation.

The land allocation and rubber plantations may not be the same time for all farmers. In this section, an optimum rotation for small-scale rubber plantations is estimated based on the revenue that is generated from the latex and wood price. From the BRASS model, total life cycle latex and wood production were calculated to find the net present value (NPV) which was combined to estimate the optimum rotation for rubber plantations in the study area. Wood prices are combined with annual cash flow to calculate the optimum rotation that maximizes the net present value for the rubber farmers. The NPV of rubber production with intercropping and without intercropping are calculated at different possible rotation cycle in the yield table to find out the optimum rotation at which the NPV is maximized.

3.6.1 NPV of Small-scale Rubber Cultivation with Intercrop Production

The small-scale rubber plantations with intercrop become positive NPV at the plantation age of 13 years (Fig-5). The NPV becomes positives over the 13 years due to wood products that become mature to harvest at this age. It is clearly found that at 33 years of the production period, the NPV increases from the 13 years highest (178193 BDT) at the 28th year (Fig-5). After that, its NPV value decreases. Therefore, 28 is the optimum rotation of rubber cultivation of small-scale rubber cultivation with intercropping to derive optimum benefit from the study area under the present conditions. Presently, Bangladesh is following 32 years of rotation of rubber plantations. According to this result, the rotation can be reduced by four more years to yield the optimum profit from the rubber cultivations if intercrops are cultivated along with rubber plantations for early three or four years.
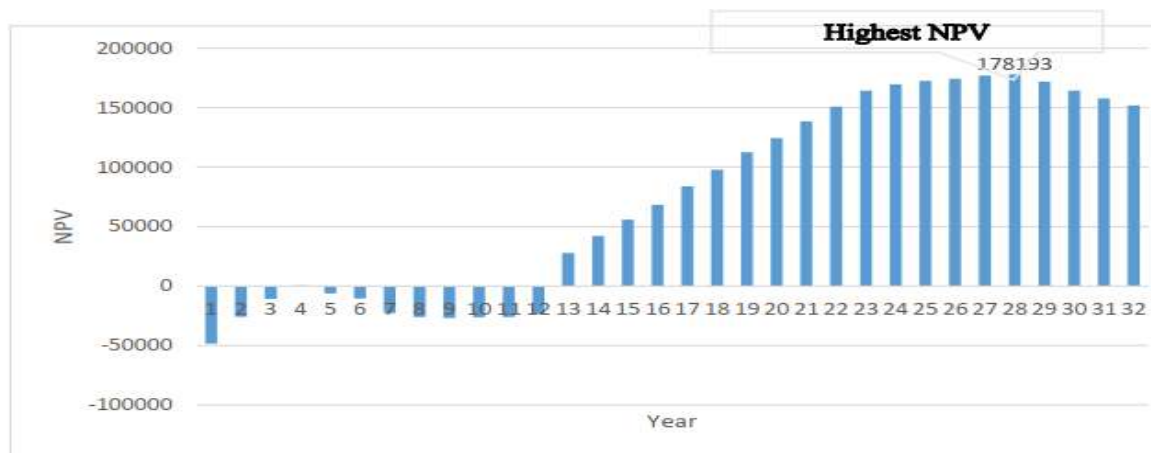
Fig.5: Cumulative NPV up to 32 years rubber production with intercrop production

3.6.2 NPV of Small-scale Rubber Cultivation Without Intercrop Production

The result shows that without intercrops in rubber farm, the NPV of small-scale rubber production is negative up to 17th years (Fig-6). At the 18th year, the NPV (6790 BDT) become positive and increase up to 28th year (87197 BDT).

This result indicates that there should be a minimum of 28 years to increase the NPV value (Fig-6). It is clear that without intercrop production in the rubber garden in the initial 17 years, there is no profitability. It also shows that the optimum rotation is 28 years with highest NPV for rubber cultivation without intercropping. Thus, the rotation should be 28 years to get maximum profit.
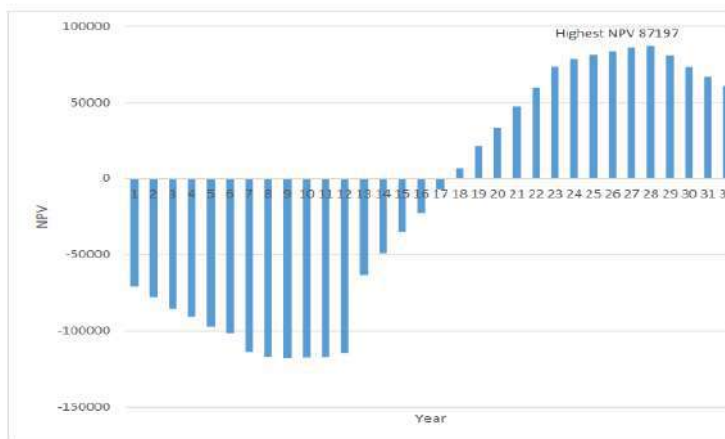
Fig.6: 32 years Cumulative NPV of rubber production without intercrop

3.6.3 Comparison of NPV with and without intercropping when there is no VAT

The small-scale rubber cultivation with intercropping is more profitable system than rubber monoculture or small-scale rubber cultivation without intercropping under the current market conditions (Fig-7). The order does not depend on age since there is no cross point of the NPVs of the two small-scale rubber production system.
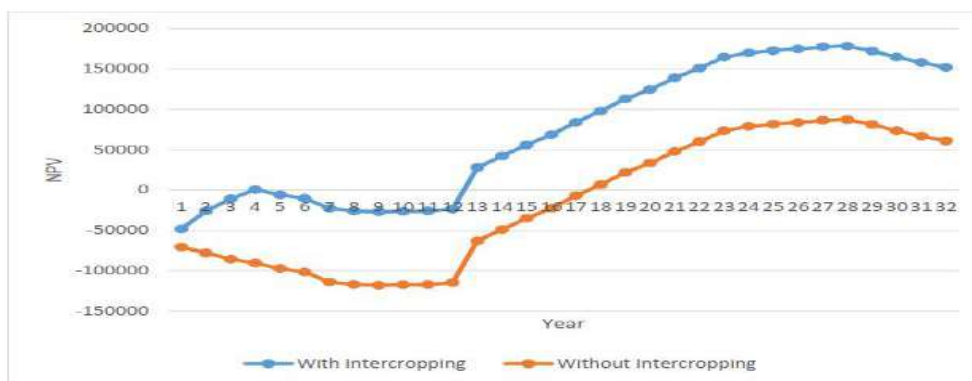
Fig.7: Comparison of NPV with intercropping and without the intercropping

3.6.4 Comparison of NPV with and without Intercropping in the case of VAT and no VAT

The optimum rotation with intercrop is 28 years if there is VAT or no VAT. Similarly, optimum rotation without intercrop is 28 years if the farmers pay VAT or no VAT (Fig-8). The result reflects that VAT has no effect on optimum rotation, but it has a significant impact on profitability or NPV. If the farmers do not pay VAT, they achieve maximum output, but they achieve minimum output in case of paying VAT. The difference of NPV varies significantly between intercropping and no- intercropping because intercrop productions do not need to pay any VAT for the farmers.
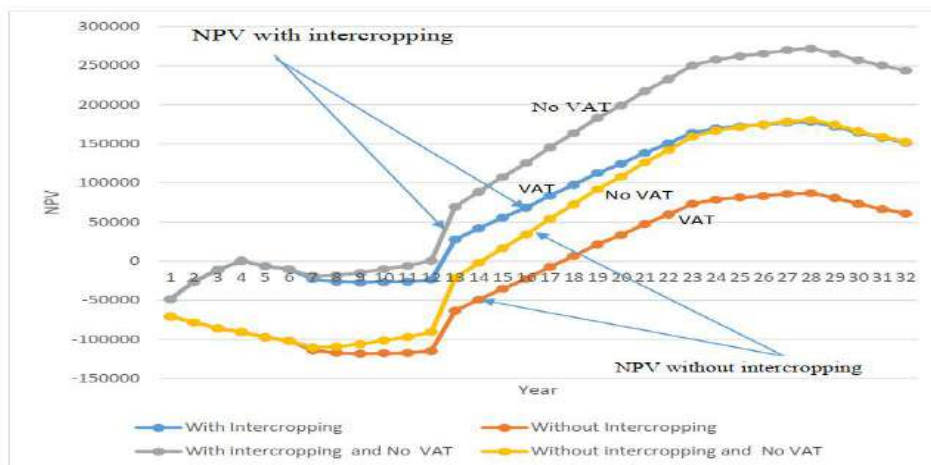
Fig.8: Comparison of NPV with and without intercropping considering VAT and no VAT on latex

3.7 Sensitivity Analysis of Optimum Rotation

Since rubber cultivation is a long-time investment, hence there is no assurance on the exact estimated NPV and optimal rotation will be obtained. Since intercrop cultivation, rubber prices and rate of interests are subject to change over time, the probable result of these changes should be estimated (Gregory, 1987). Therefore, sensitivity analysis plays a significant role in systematically testing how the NPV and rotation or life cycle would change when actual events in the future should differ from the present. In the study area, got the result using a base discount rate 8%, wage rate 334 BDT and latex price 175 BDT/Kg. The main factors affecting the outcome of the production of the small-scale farmers are the price of latex and discount rate. In the study area, the lowest and highest price of 150 BDT/Kg and 200 BDT/Kg, which is around 20% below and above the present market price (Table 4). The lowest discount rate of

5% and highest discount rate of 17% are considered for the sensitivity analysis. The result of sensitivity analysis on small-scale rubber cultivation without intercropping as shown in Table-4, that with all base, lowest, and highest price of latex, the optimum rotation age is 28 years on both 5% and 8% of discount rate and any rubber price. From the both sensitivity analysis of small-scale rubber cultivation with and without intercropping, it was found that discount rate and rubber price are crucial to increasing the NPV and get more profit. Intercropping practice does not have any effect on optimum rotation. The optimum rotation can be 28 years in all cases of rubber prices with the base (present) and lower discount rate. On the contrary, if the discount rate increased then the farmer should stop to invest in rubber cultivation. Thus, if the government plans to produce rubber and to eradicate shifting cultivation, the small-scale farmers should be given low-interest rate loan and subsidies to invest in this sector.

Table 4: Optimum rotation and NPV of rubber production without intercropping

Price of Rubber BDT/KG	Optimum Rotation and NPV at different discount rates		
	5%	8%	17%
150	28 Yr 222225	28 Yr 35228	∞ (-84306)
175	28Yr 302930	28 Yr 87197	∞ (-68359)
200	28 Yr 383636	28 Yr 138866	∞ (-52413)

IV.   LIMITATION AND FUTURE STUDY

Some limitations must be pointed out. The data was

collected from one village. Further study needs to be done not only to include other villages and locations but also with

different types of intercropping cultivation. Another important fact that needs to be considered but not addressed is the environmental benefit. The environmental benefit is much broader than the soil property, how to combine other environmental services to create an integrated ranking is another technical challenge. Many other methodologies could be applied to assess the ecological and economic benefits of small-scale rubber cultivation.

V.   CONCLUSION

The modification or extension of the BRASS model can be more applicable for formulating the rubber policy, not only in CHT but also in other areas of Bangladesh and other Asian countries. More importantly, the proper land use with rubber cultivation will change the livelihood of the tribal people. To allocate fallow land to the small farmers, the land lease period should be considered which is also crucial for the farmers as well as the government. Intercropping is another important element for the small-scale farmers to get some initial benefits when there is no return from latex. The small-scale rubber cultivation could be a profitable investment in the long term and the optimum rotation is 28 years which generate an alternative livelihood for the shifting cultivators. The government controllable rate of interest on a bank loan and value added tax (VAT) and farmer's controllable labor costs and material costs are the most crucial variables that make the rubber cultivation more profitable. The government can assist the farmer by providing low-interest rate credit support or in some cases interest- free financial credit. So, if the government wants to reduce shifting cultivation, must come forward with a low-interest funding for the rubber cultivators.

REFERENCES

[1] A.K.M. Abul Kalam Azad, Md. Najmus Sayadat Pitol and Yonosuke Hara. *"The role of Rubber (Hevea brasiliensis) plantation in carbon storage at Bandarbans Hill Tract, Bangladesh", 2021. International Journal of Current Research, 13, (05), 17373-17377. DOI: https://doi.org/10.24941/ijcr.41365.05.2021*

[2] Bangladesh Forest Industries Development Corporation (BFIDC). (2012). *Ministry of Environment and Forest.* Dhaka: Government of the People's Republic of Bangladesh.

[3] BBS. (2011). *Bangladesh Census.* Dhaka: Bangladesh Bareu of Statistics, Government of the Peoples   Republic of Bangladesh.

[4] BFIDC. (2013). Establishment of rubberwood treatment plant in project. Dhaka, Bangladesh: Ministry   of Environment and Forest, Government of the Peoples Republic of Bangladesh.

[5] BFIDC. (2013). *Rubber Statistics.* Dhaka: Bangladesh Forest Industries Development Corporation.

[6] BFIDC. 2015. Garden and Industries, Bangladesh Forest Industries Development Corporation. (BFIDC) Government of the People's Republic of Bangladesh. http://www.bfidc.gov.bd/site/page/fa44e577-8362-4cbd-ac31-b0fd58445537/-Accessed   on 26 December 2019.

[7] Gregory, G. R. (1987). *Resource economics for foresters.* New Jersey, USA: John Wiley and Sons Inc.

[8] Hossain MK. 2016. Plantation forestry- paradigm to meet the demand of the forestry resources in Bangladesh. In: Monoculture Farming- Global Perspectives, Ecological Impact and Benefits/Drawbacks (T.K. Nath and Patrick O'Reily, Eds.), New York, Nova Publishers. Edition: 1st, Chapter: 3.

[9] Khisa, S. K. (1991). *Parbatto Chattogram Hevea rubber chash abong prokriyajatkoron (in   Bengali) (Chittagong Hill Tracts Hevea rubber cultivation and processing)* (Vol. 336). Chittagong: Simanta Press.

[10] Manivong, V., & Cramb, R. (2008b). The adoption of smallholder rubber production by shifting cultivators in Northern Laos: A village case study. *Advances in Agroforestry, 5,* 117-137.

[11] Manivong, V., & Cramb, R. A. (2008a). Economics of smallholder rubber production in Northern Laos. *Agroforestry System, 74*(2), 113–125.

[12] Nath, T. K., Inou, M. & Mangala, D. Z. (2013). Small-scale rubber planting for enhancement of people's livelihoods: A comparative study in three South Asian countries, society & natural resources. *An International Journal, 26*(9), 1066-1081.

[13] Nath, T. K., Inoue, M., & Myant, H. (2005). Small-scale agroforestry for upland community   development: a case study from Chittagong Hill Tracts, Bangladesh. *J For Res, 10*, 443-452.

[14] Pitol MN, Khan MZ, Khatun R. 2019. Assessment of Total Carbon Stock in Swietenia macrophylla Woodlot at Jhenaidah District in Bangladesh. Asian Journal of Research in   Agriculture   and   Forestry, 2(3),   1-10. https://doi.org/10.9734/AJRAF/2018/46922

[15] Rahaman, M. T., Gurung, D. B., & Pitol, M. N. S. (2020). Comparative Study of Understory Between Exotic Monoculture Plantation (Acacia Sp.) and Adjacent Natural Sal (Shorea Robusta) Forest. *European Journal of Agriculture   and   Food   Sciences*, 2(6).   DOI: https://doi.org/10.24018/ejfood.2020.2.6.204

[16] Rasul, G. (2005). State policies and land use in the Chittagong Hill Tracts of Bangladesh.   International Institute for Environment and Development. *Gatekeeper series* (119), 112-120.

The Impact of COVID-19 on the Atmosphere, Hydrosphere and Biosphere: A global scenario

Atreyi Ghosh[*], Arghya Nath, Parna Dey Banik, Ratnadeep Chowdhury, Debanita Mandal

Department of Microbiology and Biotechnology, Sister Nivedita University, India
*Corresponding Author

Abstract— *The human life had come to a stagnant condition as many countries had to shut down all sorts of internal and external communication from due to the spread of extremely contagious disease of COVID-19. Social distancing of different levels has been a common scenario in all the countries which had made the whole world to a standstill. The industries, transportation, and other activities suddenly came to a pause for the developed and developing countries all across the globe. The atmosphere, hydrosphere and the biosphere have been polluted for last few decades due to various anthropogenic activities all around the world. The air and water pollution got reduced due to less carbon emission and the reduced decarbonization rate first time observed in the modern Anthropocene. The water quality of rivers, lakes, fresh water and sea water has been reported to be improved during this pandemic situation. The drastic reduction in human activity resulted the reduction of atmospheric NO_2, CO_2 and particulate matters in air which led to clean air and pollution free water in many countries and also the wildlife took the opportunity to regain their natural space. Although the situation was in favor of environment, the water in-fluent and the waste water has been reported to have SARS-CoV-2 RNA and also,it's been a great deal of concern for the increased use of medical and plastic waste and their inappropriate disposal which is followed by the health hazards. This review article provides evidence-based analysis of good and bad effect on the global environment during the worldwide lockdown situation. A summary of the published articles of the environmental effects of COVID-19 pandemic have been discussed and the significant facts have been discussed focusing on several relevant aspects like, air quality, water quality, waste management, wildlife and conservation. An attempt has been made to visualize the world scenario.*

Keywords— *COVID-19, Atmosphere, Hydrosphere, Biosphere, Anthropocene.*

I. INTRODUCTION

COVID-19 is the novel coronavirus which causes unusual pneumonia had first occurred in Wuhan, China on December, 2019. The highly contagious nature of the novel coronavirus had been the threat worldwide since then. The disease crossed Chinese borders and spread almost in all countries across the globe [1][2][3].

The spread of the virus is still continuing in all the affected countries. Till 28th of August 2020, COVID19 positive cases was more than 24,373,497 and worldwide death toll had crossed more than 830, 518. Transmission of the novel corona virus had spread to remote countries like Italy, Iran,

South Korea, and many other countries. The speedy spread of the disease had made the epidemiologists sure that he virus could not be confined to the borders of China. [4][5][6].

The symptoms of the novel corona virus include fever, dry cough, fatigue and respiratory choke and also loss of smell and nasal congestion. Severity of the symptoms have been reported to be dependent on the patient's innate immunity. Also, it has been found that the elderly people and patients who are already suffering from other diseases or disorders like cancer, diabetes, hypertension, asthma, cardiac

diseases because their innate immunity is weaker than a normal person [7][8][9][10].

COVID-19 pandemic has made a strong footprint on almost each and every aspect of Anthropocene already. To restrict the spread of the infection, every country had to impose many quarantine rules and had to shut down local transports, small and large business, offices, educational institutions etc. above all the national and international aviation had come to complete halt during the worldwide lockdown period in early 2020.

Although the COVID-19 pandemic had caused a disaster worldwide in terms of death toll, economic loss and spike in deuteriation of mental health, the global environment has been observed to have improved significantly. The complete shutdown of the transportation had led a drastic increase in decarbonization which in turn have been the reason of the significant improvement in Air and water quality in many countries. A significant reduction in air and water pollution has been reported by many researchers. The concentration of NO_2, CO and particulate matterhas been reported to be reduced in many countries around the world. The impact of COVID-19 on the betterment of overall quality of water bodies have also been reported. Also, the lockdown condition has restricted the human invasion in the natural habitats of wild animals and as a result the wildlife got more space to be inhabited and breed freely without the fear of human interference.

In this current review, the direct and indirect impact of COVID-19 in the global aspect has been discussed in a detailed manner. The effect on atmosphere, water, waste-water and wildlife has been analyzed including the current challenge of medical and other solid waste disposal.

COVID-19 and Air pollution:

The lockdown situation had a positive impact on air quality specially in metro cities all around the globe. COVID-19 lockdown has led to an overall drop in carbon emissions in several cities.

Because of the lockdown situation, $NO_2$was found to be reduced by 22.8 µg/m^3 and 12.9 µg/min Wuhan and China, respectively. Particulate matter (PM 2.5) was found to be reduced by 1.4 µg/m^3 in Wuhan and also reduced by 18.9 µg/m^3 in 367 cities in China. Data collected from the Copernicus Sentinel-5P satellite has revealed a decrease in NO_2 concentrations in Rome, Madrid, and Paris which are the first European cities where strict quarantine measures have been implemented [11].

In a recent study carried out by [12] the environmental impact of COVID-19 outbreak has been studied using the spatio-temporal satellite-based products to measure the amount of NO_2, CO, and Aerosol optical density (AOD). In that study it has been mentioned that there has been a significant decrease in all these three parameters which are responsible for air pollution. In another study it has been stated that there has been a significant reduction in particulate matter pollution among European cities like Greece, Portugal, Finland, Norway, Poland, Sweden, and Spain during the lockdown period. The decrease in the amount of NO2 and AOD were attributed to the reduction of fossil fuel usage in those areas during that period.

In a study done by some scientists [13] in the changes in the air quality parameters have been studied extensively in the city of Ghaziabad. The city of Ghaziabad is situated in Western Uttar Pradesh, India. The environmental condition of the city is almost similar to that of Delhi. Fig. 1 shows the amount of AOD in the atmosphere of India during the period of March 31st and April 5th for six subsequent years (2016–2020) which has been mentioned in the study. In the figures the yellow pixel and bluish pixels indicates a little or very low concentration of aerosol respectively and the dark brown pixels indicates very high concentration. From the figure it is very obvious that the AOD concentration has decreased drastically in 2020 which is a clear indication the positive impact of COVID 19 situation on air quality in that particular region. Similar findings have also been reported by European Space agency where the atmospheric NO_2 had reached to a very low level of around 40–50% across major cities in India. The satellite images also revealed the drastic reduction in the particulate matter (PM). The views taken by satellite clearly shows the reduction of concentration of PM2.5 and PM10 in the span of one month in the Northern and eastern states of India including the capital city of New Delhi. Air Quality Index is an overall scheme proposed by Central Pollution Control board (CPCB) [14] which includes the weighted values of seven air pollutants (PM2.5, PM10, CO, NH_3, NO_2, SO_2 and Ozone) in to a single number or set of numbers. The study shows that the air quality index has been improved significantly in India after the COVID 19 outbreak.

According to the study by [12] countries which has average temperatures between 4 °C ± 2 °C to ~19 °C ± 2 °C and atmospheric humidity (AH): 4 to 9 g m^{-3} are at a greater risk of the spread of novel corona virus.
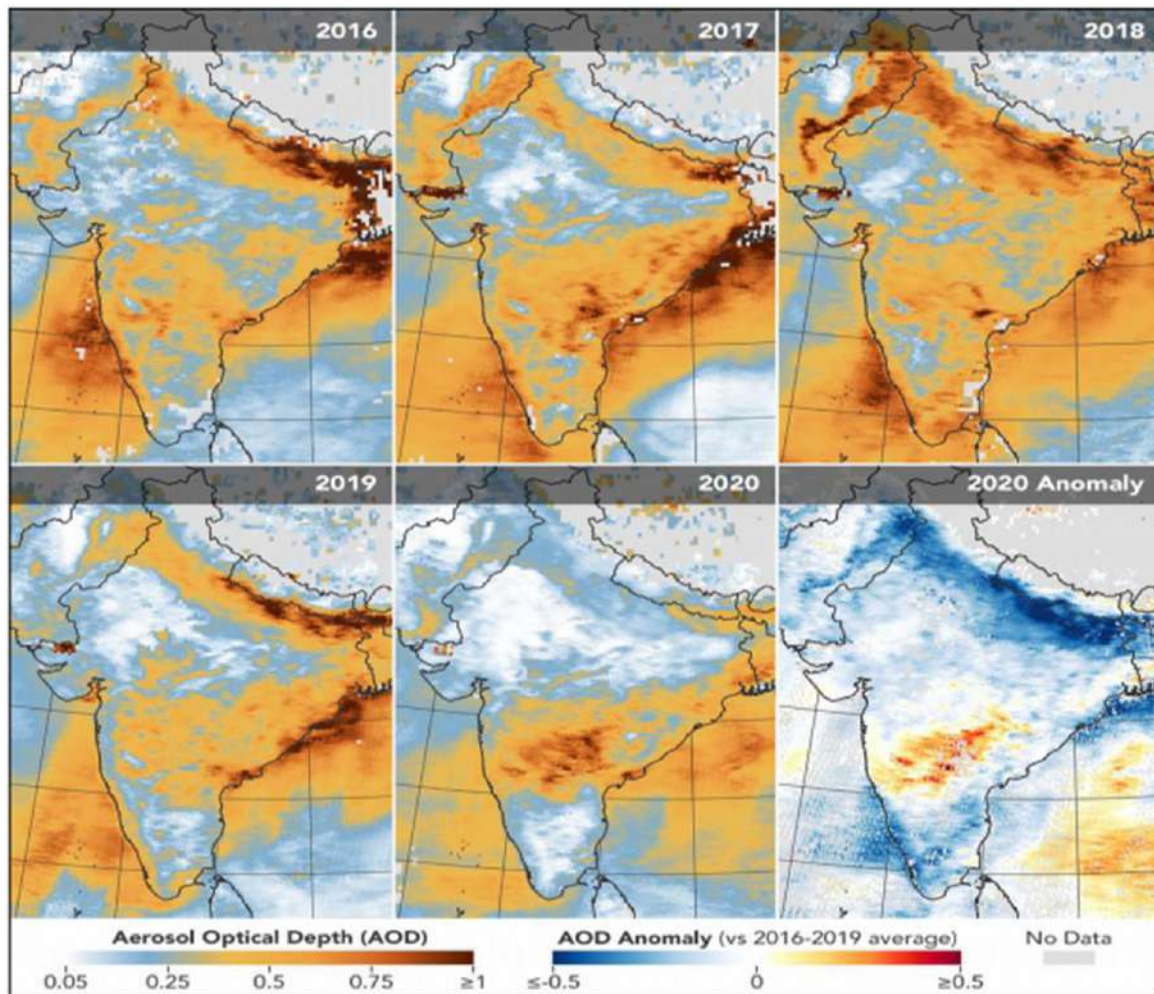
Fig 1: AOD concentration in India from the period March 31 to April 5 in 2016–2020

II.   COVID-19 AND WILDLIFE

COVID-19 crisis has stopped people's activities in a great extent. Restrictions on business, traveling, public transport etc. have created a direct impact on the activities of foraging wildlife, illegal poaching, conversion of habitat for agriculture, and other activities of resource extraction to support livelihoods have been the reason for creation of constant threat towards biodiversity [15][16][17][18][19]. A data published by Wildlife Institute of India entitled as "Lockdown Wildlife Tracker" shows uninterrupted movement of wildlife in people reigned areas. This data has encouraged the volunteers for documentation of wildlife actions in those areas and the collected data can be available to the research community for investigation [20]. There have been many reports of movements of wild animals in the human-dominated space. Wild boars were observed in Italy, wild coyotes and dears have been spotted in the USA, peacocks in Bangor, goats in Wales and rare insects in the UK. wild boar in Barcelona and Bergamo, Italy. Also, it has been observed that the Moles have been crawling the footpaths. Locals of UK have

noticed the highest blooming of wild flowers [21][22]. Also, the wildlife killing through road-accidents have been reported to be reduced drastically. Number of trucks and cars have been very less in number during the lockdown situation which has caused the lower toll for road kills [20]. Those instances of expanding natural habitats of wildlife have proved how the environment can rejuvenate itself within a short period of time and it also the extent of distortion of environment has occurred due to human interference. The observation indicates the necessity of strict administrative actions to be adapted in post-COVID-19 period to implement prohibition in human interference to nurture the natural environment [20].

During the lockdown period, increase in the activity of alien species has been reported by a study by [6]. Eastern cottontail (*Sylvilagus floridanus*) has been funding to intrude in the protected areas. In the same study, there has been reports of probable invasion by alien species from surrounding sites because of the absence of barrier of roads and presence of humans there. Also, they have reported that the lack of invasion of problem-causing

mammals for example, wild boars Sus scrofa increasing the threat of endangered animals and plants in those areas. The reduction of conservation of native species have been accompanied with the reduction of alien species eradication activities. In a nutshell, the ongoing conservation actions have been facing a high risk of failure because of the lockdown [6].

During the pandemic situation, many researchers have encouraged governments to prohibit the wildlife trade to eradicate the chance of spreading infectious diseases like COVID-19. Governments of many countries are implementing rigid efforts to forbid wildlife trade for example, China has forced restrictions on wildlife trade specifically wolfs, civets, pangolin and bats [23].

Although in the present scenario there is a probability of the poorer countries of the southern hemisphere to be in fear because of an increased threat to wildlife due to the decreased economic capacity in pandemic situation. Those countries are likely to have less money and officials to provide necessary conservation of the endangered and vulnerable species and their habitats [21].

III.   COVID-19 ON WATER

The betterment of the environmental quality of atmosphere and hydrosphere have been observed during the worldwide lockdown situation. There has been reports of cleaning of surface water like, lakes, rivers etc. from many countries around the world. Apart from the decrease in the level of greenhouse gases in the atmosphere, the solid particulate matter (SPM) of surface water like, lakes and rivers has been reported to be decreased in a significant level in India, Italy and UK [23][24]. The drastic decrease in the water way travelling through the water bodies has caused the water to settle and the SPM to decrease in a significant level. This resulted the return of the aquatic organisms to return to previously traveled waters at the coastal areas and more birds in the

surrounding area with fewer human interventions. At the end of March 2020, the boats and ships were restricted in the sea coast in Britain which transformed the dark waters of the coastline into much clearer blue hue [14]. Pictures of a cleaner River Ganga could be taken from both the regions of Kanpur and Varanasi at the state of Uttar

Pradesh, India. The clean water. Also, there has been reports of the visibility of fishes at the banks of the river at Varanasi, which could be attributed to the closure of industries releasing toxic effluents into the water and also overpopulated ghats at the river banks [24].

In a very recent article [13] they have reported that the overall water quality of main rivers of India namely Ganga, Cauvery, Sutlej and Yamuna have been improved during the nationwide lockdown period. The causes of the improvement are lack of industrial effluents entering the rivers, reduction of demand of irrigation water, increased level of rainfall and also reduction of religious and cultural activities on the banks of the rivers during the pandemic situation. The dissolved oxygen (DO) levels of river Ganga has become above 8 ppm and biological oxygen demand (BOD) levels down below 3 ppm at some parts [26].

Scientists have claimed that water quality has remarkably improved up to drinking standards at some region of the banks of river Ganga. River Yamuna also in most parts of Delhi has been observed to be clearer and blue after decades. The toxic foam carrying detergents and chemicals effluents of industries and sewage have disappeared [13]. Also, in the southern part of India, the water quality of Cauvery and tributaries like, Hemavati, Kabini, Shimsha and Lakshamanathirtha was also improved.

A group of scientists [25] studied the effect of lockdown situation on the lake of Vembanad, the longest freshwater lake in India situated in southern India. They have suggested the lake Vembanad which is surrounded by densely populated areas (810 inhabitants/km^2) have shown to reduce the SPM concentration by 15.9% compared to the pre lockdown period. Fig.2 shows the results of the mentioned study through remote sensing images of the SPM. The data of SPM analyzed on April 2020 was the lowest for 11 out of 20 zones of the Vembanad lake. It was concluded from the study that the sustainability of the improvement depends on the consideration of the reduction of human intervention such as tourism, socioeconomic and religious activities [25].
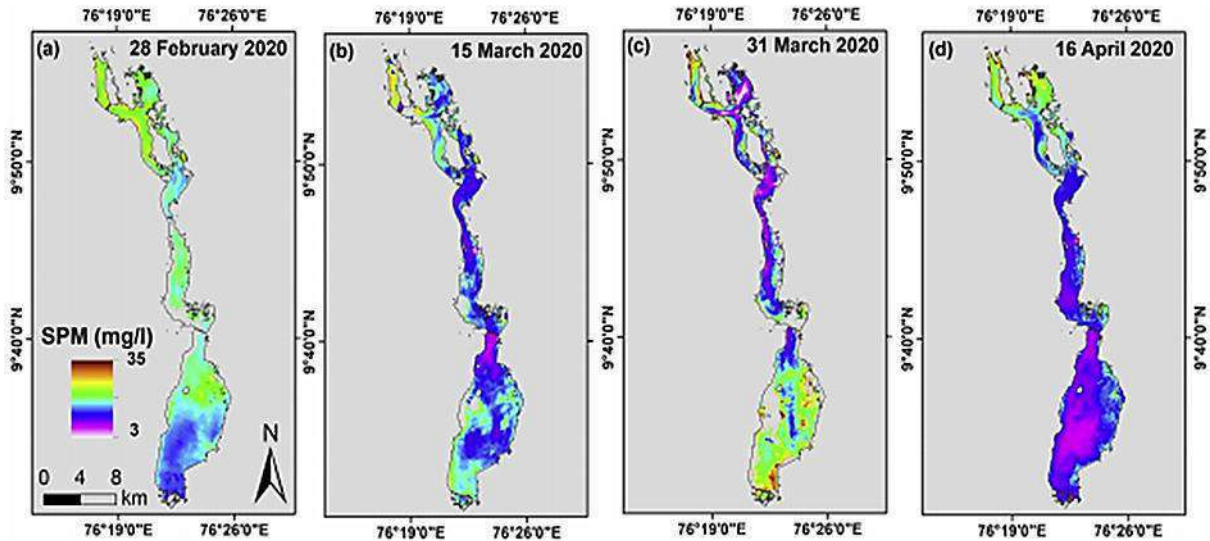
Fig. 2. Suspended particulate matter (SPM) concentrations estimated for the Vembanad lake on 28th February, 15th and 31st March, and 16th April 2020 (values in mg/l; violet is low and dark red is the highest concentration).

COVID-19 on waste-water

There have been worldwide reports of presence of novel corona virus in the waste-water systems [27][28][29]. The occurrence of the virus has been reported in stool samples collected from symptomatic and asymptomatic patients as well as in municipal waste-waters across the globe including western and oriental countries [30][31][32][33]. RNA fragments of SARS-CoV-2 have been reported to be present in the feces of infected individuals. The findings indicates that the virions are getting liberated from the gastrointestinal tracts of the infected patient. Also, it has been reported that fecal samples remain positive for the novel corona virus for a significantly longer period of time in comparison with the samples collected from upper respiratory tract [34]. The fate of SARS-CoV-2 in the water supply and areas of potential human exposure has been depicted in figure 3 which has been adopted from the study carried out by [34][35].
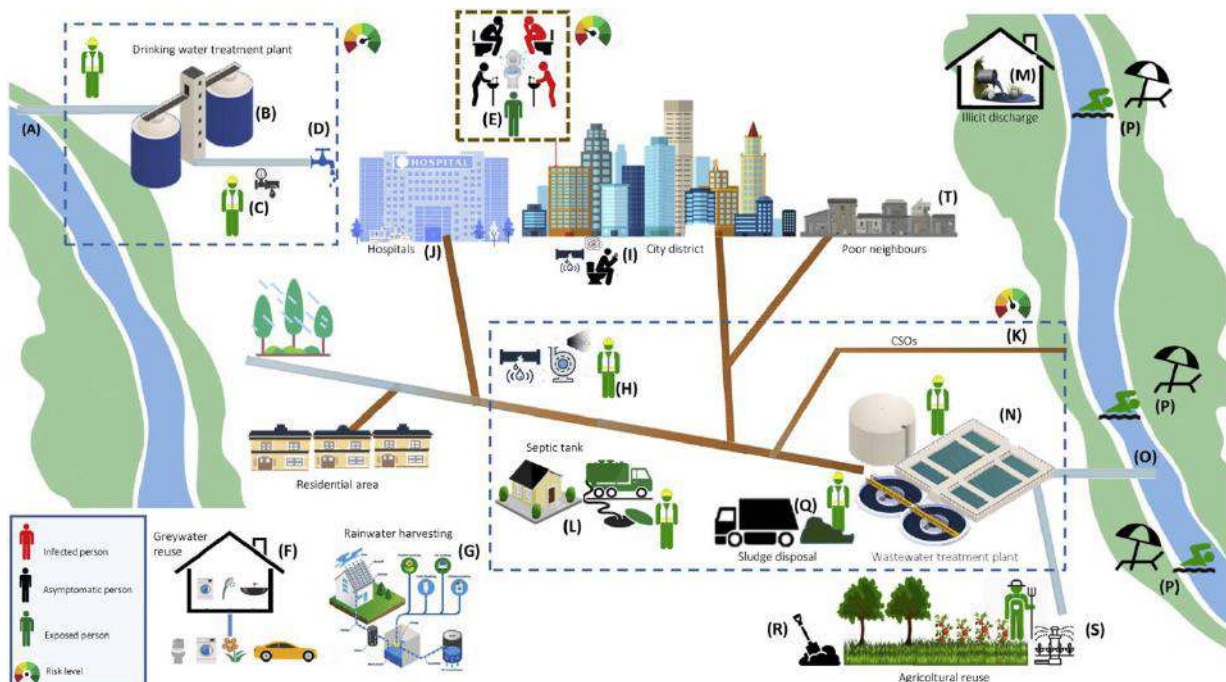
Fig.3: Potential fate of SARS-CoV-2 in the water service and locations of potential human exposure (Wigginton et al. 2015 and Langone 2020)

In a recent study, it has been found that SARS-CoV-2 RNA have been found to be present in the urine sample of an infected individual. Also, two adult urine samples have been detected to be positive for 3 days and up to 4 days for one child urine sample [36]. These results indicates that the waste-waters may contain both RNA fragments and viable virus.

SARS-CoV-2 have been found in waste-water samples in several cities worldwide including Milan, Rome, Massachusetts, Paris, Brisbane and Murcia. The detection of the virus has been done using different methods. Although the presence of high number of copies of RNA, the longevity of SARS-CoV-2 in raw waste-water below significant level [37]. Effluent coming out of the plants treating waste-water by peracetic acid or high intensity UV lamps, were mostly found to be negative for SARS-CoV-2. This indicates the effectiveness of waste-water treatments [37].

There are some reports of the inactivation of SARS-CoV-2 in ethanol (70%) and chlorine-based disinfectants with active chlorine of 0.1% for a contact time of 5 min in room temperature. Ultraviolet light has also been reported to be effective against Coronaviruses. Antagonist microorganisms present in water can also reported to increase the extent of thevirus's inactivation [34].

The most commonly used methods for the detecting SARS-CoV-2 is the reverse transcription-polymerase chain reaction (RT-PCR) and the reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) in water and waste-water [38][39][40] have reported the effectiveness of RT-PCR to detect SARS-CoV-2 in the different specimens like feces, urine, sputum, blood, and nasal discharge of infected patients. Also, viral RNA detection using RT-PCR has been used commonly as the standard method for detecting RNA of SARS-CoV-2 from clinical specimens.

Solid waste and COVID-19:

During the pandemic situation, the sudden increment of the use of single use plastics and the medical wastes has been a serious concern of the cities and urban areas all across the globe. Cities which are facing high COVID-19 infection rates are struggling hard to manage the sudden increase in medical waste production [41][42][10]. The King Abdullah University Hospital in Jordan produced tenfold higher medical waste (~650 kg per day, when considering an occupation of 95 COVID-19 patients) than the average generation rate during the regular operational day of the hospital [10]. Different types of medical and hazardous substances have been generated during the COVID 19 pandemic, including infected masks, gloves,

used bottles of hand and surface sanitizers along with the existing wastes of the same nature. Therefore, it has become a prime necessity to manage the biomedical and healthcare waste and also the appropriate identification, collection, separation, storage, transportation, treatment and disposal of those waste and also other significant aspects like training related to personal protection [42]. A drastic increase in medical waste was also reported in Catalonia, Spain, and in China, with an increment of 350% and 370%, respectively. The sudden increase in medical waste is far beyond the capacity of any country or municipality, to manage or treat it properly. Many countries are classifying all hospital waste as infectious Keeping in mind the high contagious nature of SARS-CoV-2. The medical wastes are subjected to be incinerated under high temperature and after that the residual ash is used as land-filling [10].

Incineration and steam sterilization in 120ºC for 90 mins are the common methods for thermal disposal of hazardous medical waste. Recommendation of the incineration by the WHO for health-care waste is between 900 and 1200ºC [43]. The countries like Germany, Spain, U.S.A have set their own priorities and guidelines for disposals of the solid wastes which includes single used plastics, health-care and hospital waste etc. keeping in mind the extensively increased production of those during the pandemic situation. For example, several states in the U.S. have also stopped recycling programs. The authorities have been concerned about the risk of spreading of COVID-19 in recycling centers and both incineration and land-filling have been given priority [42].

IV. CONCLUSION

Based on the present study it it very clear that the COVID 19 pandemic situation has caused a positive impact on the environment. The atmosphere, hydrosphere and the biosphere have received a cleansing treatment which has been observed throughout the globe. The individual parameters of air and water quality has been improved during the lockdown period in all the big cities all over the world mainly due to drastic decrease of decarbonization and release of industrial and agricultural effluents. Also, the wildlife had been benefited by the expanded ecological niche due to restrictions of human interventions during the worldwide lockdown. On the other hand, the pandemic situation has given a huge challenge of waste disposal especially medical and single use plastic wastes in front of every cities and urban areas across the globe. And also, there is an unavoidable challenge of eradicating SARS-CoV-2 from the water and

waste-water systems. In a nut shell the COVID-19 situation has been a boon and at the same time a bane keeping in mind all the environmental aspects across the planet earth. The sustainability of the improvement of air and water quality depends on the mass awareness about the rejuvenation achieved during the COVID-19 situation.

REFERENCES

[1] Guerrero-Latorre, L., Ballesteros, I., Villacrés-Granda, I., Granda, M. G., Freire-Paspuel, B., & Ríos-Touma, B. (2020). SARS-CoV-2 in river water: Implications in low sanitation countries. The Science of the total environment, 743, 140832. https://doi.org/10.1016/j.scitotenv.2020.140832

[2] Collivignarelli, M. C., Collivignarelli, C., Carnevale Miino, M., Abbà, A., Pedrazzani, R., & Bertanza, G. (2020). SARS-CoV-2 in sewer systems and connected facilities. Process safety and environmental protection : transactions of the Institution of Chemical Engineers, Part B, 143, 196–203. https://doi.org/10.1016/j.psep.2020.06.049.

[3] Muhammad Farhan Bashir, Benjiang Ma, Bilal, Bushra Komal, Muhammad Adnan Bashir, Duojiao Tan, Madiha Bashir (2020). Correlation between climate indicators and COVID-19 pandemic in New York, USA. The Science of the total environment, 728, 138835.

[4] Shefali Arora, Kanchan Deoli Bhaukhandi, Pankaj Kumar Mishra (2020). Coronavirus lockdown helped the environment to bounce back. The Science of the total environment, 742, 140573.

[5] Saeida Saadat, Deepak Rawtani, Chaudhery Mustansar Hussain (2020). Environmental perspective of COVID-19. The Science of the Total Environment, 728 ,138870.

[6] Saoul Manentia , Emiliano Morib , Viola Di Canioa , Silvia Mercurioa , Marco Piconec , Mario Caffid , Mattia Brambillae , Gentile Francesco Ficetolaa , Diego Rubolini, The good, the bad and the ugly of COVID-19 lockdown effects on wildlife conservation: Insights from the first European locked down country, Biological Conservation 249 (2020) 108728

[7] Mohammad Arif, Rajesh Kumar, Shagufta Parveen (2020). Reduction in Water Pollution in Yamuna River due to lockdown under COVID-19 Pandemic. ChemRxiv. Preprint. https://doi.org/10.26434/chemrxiv.12440525.v1

[8] Biswaranjan Paital, Kabita Das, Sarat Kumar Parida (2020). Inter nation social lockdown versus medical care against COVID-19, a mild environmental insight with special reference to India. Science of the Total Environment. 728. 138914. 10.1016/j.scitotenv.2020.138914

[9] Sulaman Muhammad, Xingle Long, Muhammad Salman (2020). COVID-19 pandemic and environmental pollution: A blessing in disguise?. The Science of the total environment, 728, 138820.

[10] Ana L. Patrício Silva, Joana C. Prata, Tony R. Walker, Armando C. Duarte, Wei Ouyang, Damià Barcelò, Teresa Rocha-Santos. Increased plastic pollution due to COVID-19 pandemic: Challenges and recommendations. Chemical engineering journal (Lausanne, Switzerland: 1996) vol. 405 (2021): 126683.

[11] Manuel A. Zambrano-Monserrate, María Alejandra Ruano, Luis Sanchez-Alcalde (2020). Indirect effects of COVID-19 on the environment. The Science of the Total Environment 728, 138813.

[12] Preet Lal, Amit Kumar, Shubham Kumar, Sheetal Kumari, Purabi Saikia, Arun Dayanandan, Dibyendu Adhikari, M.L. Khan (2020). The dark cloud with a silver lining: Assessing the impact of the SARS COVID-19 pandemic on the global environment. The Science of the total environment, 732, 139297.

[13] Snehal Lokhandwala, Pratibha Gautam (2020). Indirect impact of COVID-19 on environment: A brief study in Indian context. Environmental Research, 188, 109807.

[14] CPCB, 2020. Report on Impact of lockdown on River Yamuna Water Quality. https://cpcb.nic.in/index.php.

[15] Amanda E. Bates, Richard B. Primack, Paula Moraga and Carlos M. Duarte (2020). COVID-19 Pandemic and Associated Lockdown as a "Global Human Confinement Experiment" to Investigate Biodiversity Conservation. Biological conservation, 248, 108665.

[16] Bakar, N.A., Rosbi. S., 2020. Effect of Coronavirus disease (COVID -19) to tourism industry. Int. J. Adv. Eng. Res. Sci. 7, doi:10.22161/ijaers.74.23

[17] Gray, R.S., 2020. Agriculture, transportation, and the COVID - 19 crisis. Can. J. Agri. Econ. 2020; 1 -5. doi:10.1111/cjag.12235

[18] Pepe, E., Bajardi, P., Gauvin, L., Privitera, F., Lake, B., Cattuto, C., Tizzoni. M., 2020. COVID -19 outbreak response: a first assessment of mobility changes in Italy following national lockdown. medRxiv. 2020.03.22.20039933. doi:10.1101/2020.03.22.20039933

[19] Rutz, C., Loretto, M -C., Bates, A.E., Davidson, S.C., Duarte, C.M., Jetz, W., Johnson, M., Kato, A., Kays, R., Mueller, T., Primack, R.B., Ropert -Coudert, Y., Tucker, M., Wikelski, M., Cagnacci, F., 2020. COVID -19 lockdown allows researchers to quantify the effects of human activity on wildlife. Nat. Ecol. Evo. (accepted)

[20] Paital B. (2020). Nurture to nature via COVID-19, a self-regenerating environmental strategy of environment in global context. The Science of the total environment, 729, 139088. https://doi.org/10.1016/j.scitotenv.2020.139088.

[21] Loring, K., 2020. In San Francisco, coyotes are your wildest neighbors. https://www.kalw.org/ post/san-francisco-coyotes-are-your-wildest-neighbors (retrieved on 23.042020).

[22] Child, D., 2020. The positive impacts on the environment since the coronavirus lockdown began. https://www.standard.co.uk/news/world/positive-impact-environment-coronavirus-lockdown-a4404751.html (retrieved on 23.042020).

[23] Rupani P. F., Nilashi M, Abumalloh R. A., Asadi S., Samad S., Wang S. Coronavirus pandemic (COVID-19) and its natural environmental impacts. International

Journal of Environmental Science and Technology (2020) 17:4655–4666.

[24] Pratibha Sharma, Dr. Manpreet Kaur and Gaurav Narwal. Other side of the COVID-19 Pandemic: A Review. The Pharma Innovation Journal 2020; 9(5): 366-369.

[25] Yunus, A. P., Masago, Y., & Hijioka, Y. (2020). COVID-19 and surface water quality: Improved lake water quality during the lockdown. The Science of the total environment, 731, 139012. https://doi.org/10.1016/j.scitotenv.2020.139012

[26] Pathak, S.S., Mishra, P., 2020. A review of the Ganga river water pollution along major urban centres in the state of Uttar Pradesh, India. Int. Res. J. Eng. Technol. 7 (3), 1202–1210.

[27] Bhowmick, G.D., Dhar, D., Nath, D. et al. Coronavirus disease 2019 (COVID-19) outbreak: some serious consequences with urban and rural water cycle. npj Clean Water 3, 32 (2020). https://doi.org/10.1038/s41545-020-0079-1

[28] Corburn, J., Vlahov, D., Mberu, B., Riley, L., Caiaffa, W. T., Rashid, S. F., Ko, A., Patel, S., Jukur, S., Martínez-Herrera, E., Jayasinghe, S., Agarwal, S., Nguendo-Yongsi, B., Weru, J., Ouma, S., Edmundo, K., Oni, T., & Ayad, H. (2020). Slum Health: Arresting COVID-19 and Improving Well-Being in Urban Informal Settlements. Journal of urban health : bulletin of the New York Academy of Medicine, 97(3), 348–357. https://doi.org/10.1007/s11524-020-00438-6

[29] Daughton C. G. (2020). Wastewater surveillance for population-wide Covid-19: The present and future. The Science of the total environment, 736, 139631. https://doi.org/10.1016/j.scitotenv.2020.139631

[30] Hart, O. E., & Halden, R. U. (2020). Computational analysis of SARS-CoV-2/COVID-19 surveillance by wastewater-based epidemiology locally and globally: Feasibility, economy, opportunities and challenges. The Science of the total environment, 730, 138875. https://doi.org/10.1016/j.scitotenv.2020.138875

[31] Kumar, M., Patel, A. K., Shah, A. V., Raval, J., Rajpara, N., Joshi, M., & Joshi, C. G. (2020). First proof of the capability of wastewater surveillance for COVID-19 in India through detection of genetic material of SARS-CoV-2. The Science of the total environment, 746, 141326. https://doi.org/10.1016/j.scitotenv.2020.141326

[32] La Rosa, G., Iaconelli, M., Mancini, P., Bonanno Ferraro, G., Veneri, C., Bonadonna, L., Lucentini, L., & Suffredini, E. (2020). First detection of SARS-CoV-2 in untreated wastewaters in Italy. The Science of the total environment, 736, 139652. https://doi.org/10.1016/j.scitotenv.2020.139652

[33] Randazzo, W., Truchado, P., Cuevas-Ferrando, E., Simón, P., Allende, A., & Sánchez, G. (2020). SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. Water research, 181, 115942. https://doi.org/10.1016/j.watres.2020.115942

[34] Langone M, Petta L, Cellamare CM, et al. SARS-CoV-2 in water services: Presence and impacts. Environmental Pollution (Barking, Essex: 1987). 2020 Oct;268(Pt A):115806. DOI: 10.1016/j.envpol.2020.115806. 268 (2021)115806.

[35] Wigginton, K.R., Ye, Y., Ellenberg, R.M., 2015. Emerging Investigators Series: the source and fate of pandemic viruses in the urban water cycle. Environ. Sci. Water Res. Technol. 1, 725e924. https://doi.org/10.1039/C5EW00125K.

[36] Liu, Y., Li, T., Deng, Y., Liu, S., Zhang, D., Li, H., Wang, X., Ji, L., Han, J., Bei, Z., Zhou, Y., Li, L., Li, J., 2020b. Stability of SARS-CoV-2 on environmental surfaces and in human excreta. Prepr, 20094805

[37] Rimoldi, S.G., Stefani, F., Gigantiello, A., Polesello, S., Comandatore, F., Mileto, D., Maresca, M., Longobardi, C., Mancon, A., Romeri, F., Pagani, C., Moja, L., Gismondo, M.R., Salerno, F., 2020. Presence and vitality of SARS-CoV-2 virus in wastewaters and rivers. Prepr, 20086009.

[38] Hai Nguyen Tran, Giang Truong Le, Dong Thanh Nguyen, Ruey-Shin Juang, Jörg Rinklebe, Amit Bhatnagar, Eder C. Lima, Hafiz M.N. Iqbal, Ajit K. Sarmah, Huan-Ping Chao (2020). SARS-CoV-2 coronavirus in water and wastewater: A critical review about presence and concern. Environmental Research, 110265.

[39] Wang, W., Xu, Y., Gao, R., Lu, R., Han, K., Wu, G. and Tan, W. 2020. Detection of SARS-CoV-2 in 799 different types of clinical specimens. JAMA 323(18), 1843–1844.

[40] Wang, P., Kaiyu, C., Zhu, S., Wang, P., Zhang, H., 2020. Severe air pollution events not avoided by reduced anthropogenic activities during COVID -19 outbreak. Resour. Conserv. Recy.158, 104814.

[41] Hale R. C. and Song B. (2020). Single-Use Plastics and COVID-19: Scientific Evidence and Environmental Regulations. Environmental science and technology, 54(12), 7034−7036.

[42] Jiří Jaromír Klemeŝ, Yee Van Fan, Raymond R. Tan, Peng Jiang (2020). Minimising the present and future plastic waste, energy and environmental footprints related to COVID-19. Renewable and Sustainable Energy Reviews, 127, 109883

[43] WHO – World Health Organisation, Treatment and disposal technologies for healthcare waste accessed 07.04.2020.

Article

Analysis of Quality of Landrace Rice: AG4 variety in An Giang, Vietnam

Nguyen thi Lang, Le Hoang Phuong, Bui Chi Hieu, Bui Chi Buu

High Agricultural Technology Research Institute for Mekong delta, VietNam

Abstract— Landrace rice genotypes AG4 were evaluated inTri Ton, Tinh Bien, AnGiang Province with three replications in a field experiment during 2019 to 2020. The analysis revealed significant differences among the genotypes against all the characters studied. In general, phenotypic variance is higher than the corresponding genotypic variance for all the characters studied. AG4 rice is considered as the unique landrace varietal group because of its aroma and superior grain quality.To confirm the presence or absence of fragrance in AG 4,a set of 11 lines was phenotypedby using gas chromatographic separation to quantify 2AP content in milled rice samples. KOH tested and PCR method with two directives RM223 and FMU1-2 are recorded to select the lines with the best fragrance followed by line7, 10 ,14 and 20.The shape is determined by the length and width ratio.From shape evaluation, length and width ratio of AG4 are high. Level of chalkiness is. There are mostly low contents on AG4, when doing rice quality analysing. It is the evidence for the deliciousness on rice. Milling quality determines the final yield and fracture rate of milled rice. Recorded line 7 for high milling rate is over 50%. Protein content of rice varieties ranges from 6.9 to 8.6%. Lines 7 and 56 have the highest protein content (8.5-8.6%). Characters like number of panicles per plant, panicle weightg, number of grains per panicle, and grain yield recorded are high. Grain yield analysis revealed significant differences among lines. Selected AG4, lines number 7 can be used in breed selection program in the nearest future to provide the local need.

Keywords— aroma, amylose, chalkiness, genotypic, phenotypic, milling quality.

I. INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food of more than three billion people, over half the world population. It provides 27% of dietary energy and 20% of dietary protein in the developing world. Rice is cultivated in at least 114 countries, mostly developing, and is the primary source of income and employment of more than 100 million households in Asia and Africa (FAO, 2004). Landraces, traditional varieties or local varieties, form the foundation on which to build better rice plants.Landraces are generally considered to be a rich source of genetic variation. Furthermore, local varieties provide farmers with alternatives, where modern rice varieties are not well adapted in order to contribute to diversity at the field level. However, the number of traditional varieties being planted has reduced, with a few productive and relatively uniform high-yielding varieties dominating the rice landscape. Traditional rice varieties though had been documented to have contributed to the origin of 1,709 modern varieties in Asia which can be traced to 11,592 traditional varieties. The pedigrees of IRRI breeding lines and varieties until 1994 can be traced to hundreds of traditional varieties most from Asian countries (Tran, 2000). Genetic diversity is the basic foundation forspecies survival. The processes of recombination and gene mutation guarantee to the continuous inputs for new variants, as well asthe processes of environmental adaptation and random drift shaping the distribution of genetic diversity in time and space (Brown et al., 1989).

Rice grain quality is the total number of features and characteristics of rice or rice products, which meet the needs of final use. The concept of particle quality includes

many characteristics from physical properties to biomedification, and milling efficiency, grain shape, ease of cooking, appetite and nutritional properties. In general, it consists of four types, i.e. milling quality, appearance quality, cooking and eating quality, and nutritional quality. Rice quality is evaluated based on various characteristics, which can be classified in several ways. Product characteristics can be tasted,grainy texture or color; or outside the product, such as packaging, branding, or labels. Different market segments can be distinguished between continents, regions, countries and even between socio-economic groups [Rutsaertet al 2013]. Rice quality experts in 23 countries have identified the top three popular rice varieties in their countries, in some countries, at different sub-national levels; the most commonly rated cooking and eating properties of these varieties have been reported [Calingacionet al 2014]. Consumers may not be able to state clearly the reasons behind their preferences or describe what they like or dislike in food items, but they show the appreciate or value they attach on food in other ways [Spiller et al 2012] such as willingness to pay higher prices for rice with certain quality attributes. The difference in rice price of rice samples of different types of quality shows that grain quality attributes contribute to the price. Product production, processing, and content are good examples of the credit type attributes of that rice variety [Rutsaertet al .2013]. Characterization and evaluation of diversity among traditional varieties will provide plant breeders information that necessary in the identification of initial materials for hybridization to produce varieties with improved productivity and quality.The objective of the study is to evaluate genetic diversity of the traditional rice varieties in HATRI'sgenebank(Vietnamusing morphological characters and microsatellite markers for aroma and yield components AG 4 lines from An Giang province).

II. MATERIALS AND METHODS

Plant Materials

A total of 12 accessions of traditional varieties collected from An Giang province, Vietnam, and conserved in genebank of *High Agricultural Technology Research Institute for Mekong delta (HATRI)*in Vietnam. Theoriginal information of these accessions is presented in Table 1.

Agro-morphology Analysis

11 lines from AG4 traditional varieties were planted in the field at *High Agricultural Technology Research Institute for Mekong delta (HATRI)*, during the wet season from 2019 to 2020. Seeds were sown in the raised seedbeds, and 30-day old seedlings were transplanted at one seedling per

hill. Hills were established at distances of 20 x 20cm. The standard cultural management practices for rice were followed (Bui, 1986).

Quality traits

A total of 12 lines varieties were evaluated (Table 1) and the following quantitative traits were considered: Panicle length (cm), length of panicle at maturity measured from the base of the plant to the tip of the panicle (taken from 10 random selected primary panicles per accession per replication). Panicles per plant (number), the total number of panicles per plant (from 10 random selected primary panicles per accession per replication). 1000-grain weight (g), weight of 1000 welldeveloped grains at 14% moisture content (from 5 random selected primary panicles per accession per replication). Days to maturity, days from seeding when 80% of the grains are fully ripened per replication basis (from 5 random selected primary panicles per accession per replication). Filled grains (number), obtained from counts of total number of filled grains per panicle (from 5 random selected primary panicles per accession per replication). Unfilled grains (number), obtained from counts of total number of unfilled grains per panicle (from 5 random selected primary panicles per accession per replication). Yield obtained from the harvested plants in each replication. Harvested grains were threshed, cleaned, dried, and weighed for each accession per replication. Moisture content per plot was determined immediately after weighing using a moisture meter. Yield = weight of harvested grain(g)/number of hills harvested x number of possible hills x MF (of the harvested grain).

Grain quality analyses

Milled grains underwent assessment of physical traits (grain dimensions, proportion of head rice in milled rice, and chalkiness) and then a test portion of each sample was ground into fine flour(100-mesh) using a Udy Cyclone Sample Mill (model 3010–30, Fort Collins, CO). Reverse osmosis (RO) water and reagent-grade chemicals were used for the chemical analyses.

Physical traits (length, width, and degree of chalkiness) of the milled rice grains were determined using the Cervitec™ 1625 Grain Inspector (FOSS, Denmark). Grain shape was calculated based on the length-to-width ratio of the grains. The proportion of head rice (%) in the milled rice was determined by measuring the number of grains that are 75% intact after a test portion (100g) of milled rice was sorted using a shaking sieve; the rest are broken grains (%).

The AAC of isolated rice starch was analysed by using the iodine reagent method [AACC International.1999]. Briefly, exactly 25mg rice flour was gelatinized overnight in 2ml of 1.0N NaOH in a water bath set at 50°C. The

solution was boiled in the water bath for 10 min and then cooled to room temperature. The cooled solution was extracted three times with 5ml of butanl:petroleum ether (1:3) to remove the lipid, after which 1.5ml of 0.4N KI was added to the solution and mixed. The ACwas determined in duplicating with an ART-3 Automatic Titrator, according to the manufacturer's instruction (Hirama Laboratories, Japan) in which 1.57mM KIO$_3$ was titrated at a speed of 2.5μl per s to the starch solution. The titration terminal was automatically detected with a sensitivity setting of 3, and the used volume of KIO$_3$ was transformed into amylose content. Standard amylose solutions were prepared as checks by dissolving pure amylose and amylopectin in distilled water [Tan YF et al 1999].

Gelatinisation temperature

GT was determined using the alkali digestion test [Little RR et al 1958]. A duplicate set of six whole-milled kernels without cracks was selected and placed in a plastic box (5×5×2.5cm). 10mL of 1.7% (0.3035M) KOH solution was added. The samples were arranged to provide enough space between kernels to allow for spreading. The boxes were covered and incubated for 23h in a 30°C oven. The starchy endosperm was rated visually based on a seven-point numerical spreading scale as a standard evaluation system for rice [IRRI .2013]. According to the ASV score, GT of rice grains can be classified into four groups: high (1–2), high-intermediate (3), intermediate (4–5), and low (6–7) [Juliano B et al .1985].

Gel consistency

Gel consistency was determined as previously described [34].Rice flour (100mg) was mixed with ethyl alcohol (0.2mL)containing 0.025% thymol blue and 0.2M potassium hydroxide (2mL) and heated in a boiling water bath for 8minutes. After heating,the sample tubes were allowed to cool in an ice-water bath and immediately laid horizontally on the table. Gel consistency was measured by the length of the cold rice paste in the culture tube held horizontally for one hour. Hard, medium, and soft gel standards such Nang Nhen, Khoadawmali105, are respectively included in every set.

Aroma

The current definition of aromatic rice is the presence of the volatile compound 2-acetyl-1-pyrroline (2AP). This was quantified at HATRI using gas equipped with a flame ionisation detector [35]. For those rice samples not measured at IRRI, aroma was determinedby smelling and tasting cooked grains.Volatile analysis of aromatic rice by gas chromatographymassspectrometry (GCMS).Volatile compounds in the aromatic rice from Iran, Pakistan,India, and the Greater Mekong Sub-region (GMS) were analysed.Headspace volatile compounds of selected aromatic rice werecollected by solid phase microextraction using a 65mm polydimethylsiloxane-divinylbenzenefibre (Supelco, Bellefonte, USA)and analysed using GC-MS (GC 8000, Fisons Instruments,Cheshire, UK)[36]. GCMS raw data were processed usingMetAlign [37] to extract and align the mass signals, and MSClust[38] to remove signal redundancy per metabolite and reconstructmass spectra. The PCA plot was constructed using SIMCA-P 12.0(Umetrics AB, Umeau, Sweden).The seeds from AG4 plants were manually dehulled. The seedsfrom each line were treated by Satakedehuller. They were milled by testmiller for one hour. Ten seeds from each line plant were individually ground for 10 seconds witha medium speed by Wil grinder. Rice powder of each grain was placed in an individual 5x5cmplastic box. To each box, 500ul of diluted alkali (1.7%) was added and covered immediately. Thetreated samples were placed at room temperature for 30 minutes. The boxes opened one by one, andaroma was scored by smelling. The heterozygotes were recognized based on the presence of aromaticand non-aromatic grains in lines progeny test. When all ten seeds of individual plant werearomatic, the individual was considered as homozygous for aroma. If the ten seeds of individual plant were non-aromatic otherwise, the individual would be considered as homozygous for non-aroma. Presence of aromatic and non-aromatic seeds inlines indicated heterozygous nature of plant. Due toimportance and accuracy of the phenotyping in mapping process, particularly in bulk-segregantanalysis, additional 30 seeds from each homozygous aromatic and homozygous nonaromaticplants were analyzed. It was done to assure the accuracy of phenotyping. Due to the importance and accuracy of phenotying in mapping process, rice leaves were also evaluatedat tillering stage. Ten leaves were sampled from individual plants at tillering and cut into 5mm longpieces. They were put into a capped glassware and stored at -20ºC before aroma evaluation. Onehour was measured from each frozen leaf sample, by putting into a capped test tube, and mixed with5ml of 1.7% KOH solution for 10minutes at 50ºC. Four to five panelists were asked to classify thesamples as either aromatic or non-aromatic by their own smell.

III.   DATA ANALYSIS

Analysis of variance.

The agro-morphological data collected were initially analyzed through analysis of variance to verify genetic variation in the traits measured. The few traits with insignificant genetic variation, based on the F-test, were not considered for further analyses.

IV.   RESULTS AND DISCUSSION

Without understanding of consumer preference for rice grainquality, wide adoption of any newly developed rice variety is notguaranteed. Hence, identifying the grain traits that governacceptance is important to guide a successful breeding program.Quality attributes of the most popular rice varieties consumed inthe countries and provinces in Asia, as well as for some of the ricegrowing countries in other continents have been collected.Currently, rice grain quality is classified in terms of its physical, taste, and visual characteristics. The physical appearance ofthe grain defines its price in the market.

A gene aroma located on chromosome 8 has been identified as a fragrance gene (Bradbury et al 2005; Shu et al 2008), Lang and ctv. in 2008, confirmed the close link between the RG28 directive and fgr (5.8 cM) on chromosome 8 and identified two locus for the RM223 and RG28 fragrances. The target gene chosen to perform this experiment was the aroma gene on chromosome 8. Gene that is tightly linked on chromosome 8 marked by the molecular marker RM 223. This marker is 200-210bp in size and is used as a DNA mold to establish specific primer pairs. These pairs will amplify smaller pieces of DNA,crediting for PCR method. These small pieces of DNA are called SSR. Then conduct an amplification test on the 3% agarose gel in a TBE 1X solution. The result is shown in Figure 1a. On Figure 1A there is only 1 line of 100 for fragrance along with the molecular size of KDM 105.

A   B
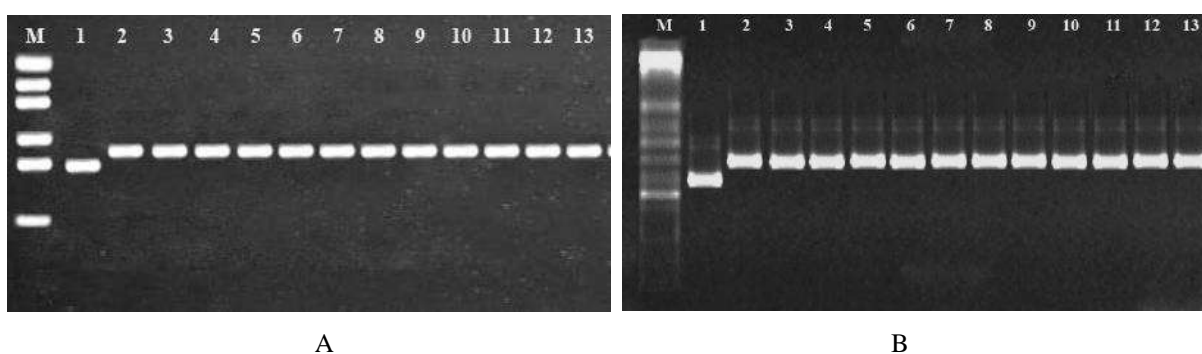
Fig.1: (A) PCR product of the RM223 detected 13 AG lines4(3-13) linked to the aroma gene on chromome 8, positioning two size 200bp (1: Nang Nhen); and 210bp(2: KDM 105), on the agarose gel 3 %. (B) PCR product of the RM223 molecular detected on 13 AG lines 4(3-13) linked to the aroma gene on chromome 8, positioning two size 190bp (1: Nang nhen); and 210bp(2: KDM 105), on the agarose gel 3 %.

Similarly, [Bradburyet al., 2005] identifying the aroma gene between the Badh2 bymolecular markers. Further studies show that the difference between fragrant rice and non-aromatic rice is due to two molecular markers on the gene that encodes betaine dehydrogenase (BADH2). Indeed, after copying based on the map and sequence of the fgr region, it was found that there is a significant difference in the BADH2 gene sequence between fragrant rice and non-fragrant rice, also there is a mutation in fragrant rice in the 7th exon region of the BADH2 gene, which leads to the function of losing BADH2 protein. Therefore, BADH2 is likely related to the fgr gene, which controls rice fragrance [Bradburyandet al., 2005]. To verify the function of the BADH2 gene, 3 candidate genes

in the fgr region were applied to 8 ag3 seasonal rice selective lines recorded (figure 1B). FMU1-2 Allele Analysis [He and ctv 2015]used as a marker, this marker is sized (190-210bp) and is used as a DNA mold to establish specific primer pairs. On figure 1B recorded the breed of non-fragrant, molecularly sized (190bp) and fragrant KDM 105, bearing molecular size (210bp).   All lines give a molecular size (210bp) of the same molecular size as the fragrant KDM 105.   Fragrance is an important grain quality feature in rice, controlled by mutations in the BADH2 gene. Recorded of lines presence or absence of fragrance. A form set using gas chroma separation to quantrate 2AP content in milled rice samples (Table 1). 2AP content ranges from 0.323 to 3.325 ppm.

Table 1. Evaluation of fragrance on AG 4 landrace rice varieties by rice reaction with KOH, 2AP and PCR method.

	Lines ofAG4	2AP Concentration Test (ppm)b	aroma	RM223(bp)	FMU1-2 (bp)
1	7	3,325	aroma	210	210
2	9	1,256	aroma	210	210

3	10	2,912	aroma	210	210
4	14	0,345	no	210	210
5	18	0,323	no	210	210
6	20	0,855	no	210	210
7	27	1,569	aroma	210	210
8	31	1,324	Aroma	210	210
9	48	2,195	Aroma	210	210
10	56	0,768	No	210	210
11	92	1,234	Aroma	210	210
12	KDM(Đ/C)	3,229	Aroma	210	210
13	NNĐ/c	0,000	No	200	190

Evaluation of yield and components yield AG4 in field

The rice landraces revealed a wide range of phenotypic variation in 8 agronomic traits (Table 1). 1000-grain weight, showed similar at 11 lines .Assessing the *yield and components yield*of the AG4 variety in the same field, through a model assessment of 13 elite lines of styling. The AG4 rice variety was also analyzed for aeration properties, the results also showed that the population gave a high genetic purity value on the breed. Particularly, the yieldand panicle numbers per plant , **spikelet** / hill . are statistically significant, so the conditions of cultivation and conditions of care and fertilizer for the full development of the variety are very important.

Table 2: Yield and components yield   of 11 lines from AG4 with two chected

Lines	Length panicle (cm)	germination(%)	No. panicle/ hill	spikelet/hill	unfulling/ hill (%)	1000-grain weight (gram)-	Grain yield/ hill(g)	yield (ton/ha)
7	27,70ab	98,33 a	9,76ab	1104,33a	21,62d-e	25,49a	19,32a	4,62a
9	26,05b	97,33 a	9,50ab	974,33c	23,25a-c	25,04 a	19,33a	4,47 a
10	29,16a	98,00 a	10,00ab	991,00b	24,86a	25,51 a	18,37a-d	4,41 a
14	26,55ab	96,66 a	9,00ab	977,00c	20,16de	25,94 a	18,28a-d	3,39 b
18	29,05a	96,00 a	9,66ab	952,33de	24,47ab	25,18 a	19,10ab	3,47 b
20	29,61a	97,33 a	10,00ab	913,53h	22,46a-d	25,68 a	18,15a-d	4,51 a
27	26,16ab	96,33 a	9,00ab	913,33h	20,24de	25,56 a	18,04a-d	4,24 a
31	26,33ab	96,33 a	10,00ab	960,00d	21,50d-e	25,10 a	18,03a-d	4,19 a
48	29,00a	98,33 a	10,33ab	933,33f	23,84a-c	25,26 a	17,83b-d	4,21 a
56	26,94ab	98,00 a	9,53ab	956,00de	23,20a-c	25,39 a	18,73a-d	4,25 a
92	26,55ab	97,33 a	8,67b	887,33i	19,10e	25,34 a	18,08a-d	4,20 a
KDM(cheked)	28,99a	95,3b	12,2a	1074,5a	29,8b	27,4a	17,70ab	4,10a
NN(Checked)	26,40ab	96,53a	9,00ab	879,40i	22,00b-d	25,00a	17,35d	3,37 b
CV (%)	**3,95**	**2,36**	**8,48**	**0,57**	**6,90**	**3,16**	**5,14**	**13,17**

The promising lines of the AG 4 variety are planted and assess the yield and yield components. The results showed that these lines had more equal and evener length panicle than the opposition, the lines were quite good dust blooms, the number of particles / dusts was quite good, and the ratio of panicle was average. In terms of recorded

productivity: AG 4 lines have quite high productivity, higher than NangNhen (3.37 tons/ha) and KDaw Mali 105 is (4.1 tons/ ha), of which line 7 gives the highest yield (4.62 tons / ha).

Evaluation of rice quality appearance Homity in physical characteristics, such as the length and width of the rice sample can play an important role in the willingness of consumers to pay for rice. When analyzing the size of rice grains is evaluated according to the IRRI standard scale.

AG4 seed record size has a long rice grain size fluctuation of 11.22-11.48mm rice grains fluctuation from 7.28-8.67mm. This is a very long group of rice grains. Analysis of the chalkiness ratio of AG 4 lines noted: most lines do not have chalkiness except lines 9 and 10 for a 0-word (99%) chalkiness ratio in order(Table2). Evaluation of rice and rice grain sizes of 12 samples of AG 4 varieties served and 2 types of confrontation.

Table 2. Evaluation rice grain sizes of 12 lines from AG 4 and 2 varieties checked

lines	Hull length(mm)	Hull wide(mm)	Kernel length(mm)	Kernel wide(mm)	chalkiness			
					Score0	Score1	Score5	Score9
7	*11.36*	*2.62*	*7.49*	*2.18*	100	0	0	0
9	*11.18*	*2.57*	*7.82*	*2.09*	99	1	0	0
10	*11.48*	*2.74*	*8.05*	*2.17*	100	0	0	0
14	*11.29*	*2.68*	*7.28*	*2.1*	99	1	0	0
18	*11.42*	*2.61*	*8.05*	*2.1*	100	0	0	0
20	*11.36*	*2.62*	*7.99*	*2.08*	100	0	0	0
27	*11.31*	*2.78*	*7.67*	*2.21*	100	0	0	0
31	*11.4*	*2.5*	*7.99*	*2.16*	100	0	0	0
48	*11.29*	*2.56*	*7.93*	*2.11*	100	0	0	0
56	11.48	2.36	8.67	2.16	100	0	0	0
92	11.42	2.5	7.99	2.22	100	0	0	0
KhaoDawMali 105(Checked)	11,48	2,65	8,98	2,22	100	0	0	0
NàngNhen(Checked)	7,15	2,56	6,10	2,11	20	15	34	31

Cooking characteristics

Besides the quality of appearance, AG4 lines are also rice qualities. In 12 selective line samples, through amylose content analysis and noted: amylose content most lines give low levels. The lowest recorded amylose content was line 10 (17.3%). Gel strength (AC) is a good measure of

ground rice plasticity and determines softness after cooking. It is a simple and sensitive quick inspection to determine the quality of rice when ingested supplements for AC. AC rice can be distinguished by softness measured by gel barrier (associated with amylose content). Gel strength barrier directly affects the texture of rice, so

cooked rice, gel durability hardens faster than soft gel properties. The line noted good gel durability soft rice. Lake temperature (GT) determines the absorption of water and time for cooking. GT is the temperature at which starch particles suck up water and begin to bulge . Most lines have special characteristics that were preferred in the polished grain of AG 4 rice: (1) a 'greasy' look without any abdominal white, (2) an entire rice grain, (3) fully developed and uniform kernel, and (4) neither too soft nor too hard when crushed under the teeth. The characeristics of the cooked rice for which it was valuable: (1)individuality of the cooked grain without bursting, and (2) sweetness and a special fragrance of the cooked product.

Grinding Quality Assessment - Analysis of Milling Rate: A review of the percentage of raw rice shows that line 7 has a fairly high percentage of raw rice 52.1%, quite good compared to the control variety of 45.7%. The percentage of raw rice is also affected by post-harvest treatment, storage time and conditions, and milling.

Nutritional Qualities Assessment - Protein Analysis: The quantity and type of protein are important factors in rice nutrition. Various factors affect the content of rice proteins: climate and environment, and the number of fertilizers applied, the duration of maturity, the degree of milling, and the characteristics of the breed. The protein content of rice varieties ranges from 6.7 to 8.6%. Lines 7 and 56 have the highest protein content (8.5-8.6%) in order.

Table3: Quality analysis of 8 lines of recovered AG 3 and 2 control lines

lines	amylose (%)	Gelatinisation temperature (cấp)	Gel consistency (mm)	% brown rice	% white rice	% head rice	protein (%)	chackiness (cấp)
7	18.6bc	5	79.1a	85.3a	77.6a	52.1a	8.5a	0
9	18.5bc	5	76.5cd	84.1ab	74.8a-c	45.6e	6.7b	0
10	17.3b	5	74.7ef	82.1ab	76.5ab	51.2ab	8.0a	0
14	18.5bc	5	75.2de	80.1b	74.2a-c	50.3bc	8.2a	0
18	18.6ab	5	74.5ef	84.3ab	76.5ab	42.3f	7.5ab	0
20	19.4c	5	78.5ab	80.2ab	74.2a-c	50.6bc	7.6ab	0
27	18.3ab	5	77.2bc	80.3ab	73.6a-c	49.5cd	7.9a	1
31	18.6ab	5	79.5g	83.4ab	74.5a-c	48.7d	7.8ab	0
48	18.5ab	5	75.4h	84.5ab	75.6a-c	44.6e	7.4ab	0
56	18.8bc	5	79.8a	81.6ab	70.3c	45.6e	8.6a	0
92	18.8bc	5	75.7h	82.5ab	71.2bc	40.5g	7.5ab	0
KDM105(Đ/C)	18.7bc	5	79.2a	82.7a	72.4a	45.5bc	8.1bc	0
Nang nhen (Đ/C)	24.9a	3	48.6c	82.6a	74.2a	45.7bc	8.2bc	0

V. DISCUSSION

Preference for grain size and shape vary from one group of consumers to the other. Some ethnic groups prefer short bold grains, some prefer medium long grains, and long slender grains are highly priced by others. In general, long grains are preferred in Vietnam subcontinent,but in Southeast Asia, the demand is for medium-to-medium long rice. In temperate areas, short grain varieties are prevalent. There is a strong demand for long grain rice for the international market (Singh et al 2000). Usually, the quality of rice grains is evaluated through consumers and is therefore used as some first choice criteria in

improvement programs [Grahamandctv 2002; Tomlins and et al 2007]. Fragrant rice varieties (aromatic rice) account for a significant proportion of the exported rice market with many different levels, including Jasmine rice and Basmati rice. These two types of rice play a key role in the production of world fragrant rice (Mahajan, and ctv. 2018) on AG4 lines analysis recorded all three analyses identifying line 7 for the best aroma. On the other hand, the particle shape is based on the ratio of length to width [Grahamandet al 2002]. The classification of rice samples based on size and shape is not standardized across different countries and different markets [Council of the

European Eunion .2003; Dela Cruz and et al 2000]. The classification system is regularly used by breeding programs.Thus, according to the breed standard AG 4 is enormously long breed (the longest is line 10 and line 56). Rice grain shapes, likewise, can be described based on the usual range of values used in IRRI: tapering ($\leq$ 2.0), average (2.1-3.0) and slender (> 3.0) [Dela Cruz and ctv 2000]. For most lines belong to the average group in terms of particle width. The chalkinessrepresents poor quality in many segments of the rice market; therefore, these types of rice take the lower market price [Fitzgerald and ctv 2009]. Rice grains are classified based on the ratio ofchakiness: none (0%), small (< 10%), average (10-20%), and large (> 20%) [Fitzgerald and ctv 2009; Lang et al 2015]. Rice grain size is measured using photo enlargers and transparent rulers [Lang et al 2015]. AG4 rice-like samples are considered vastly lowchakinessratio.

The three parameters considered the most important in evaluatiing the cooking quality of many types of rice are: amylose content (AAC), gel durability (GC) and lake temperature (GT). As AAC increases, cooked rice grains tend to get harder and harder [Lang .2015]. Subgroup AAC, rice can be grouped into five layers: wax (0-2%), very low (3-9%), low (10-19%), medium grade (20-25%), and high (> 25%) [26].For seasonal rice line 100 was rated to have a lower amylose content than the other lines in table 3. Although a recent study suggests that these AAC classes can be broken down [Calingacionet al 2014]. There are cases where rice materials of the same type of AAC are very different in hardness. In these cases, GC is used as an additional test for the level of rice hardness when cooked. Rice can be classified into three groups based on GC: hard rice ($\leq$ 40mm), medium (41-60mm), and soft (> 61mm) [Graham, and ctv 2002]. Analysis of 8 AG 4 lines recorded shows that most belong to the rice soft group.

On the other hand, milled rice/brown rice kernel appearance and dimensions determine the price in the market. Milling quality determines the final yield and fracture rate of milled rice, which is a concern of consumers and farmers. The three main parameters, the recovery of brown rice (the ratio of brown rice to raw rice), the recovery of ground rice (the ratio of ground rice to raw rice) and the recovery of raw rice (the ratio of raw rice to raw rice) are used to assess the quality and effectiveness of the milling process. Line 7 gives a high percentage of rice over 50%. Pure lines of selection from AG 4 submited at NCBI with sequences were determined directly using thedideoxynucleotide chain-termination method with a DNA Sequencer (ABI PRISM 3130xl; Applied Biosystems/) and BigDye Terminator (version 3.1) cycle sequencing kit (RR-100, Applied Biosystems), according to the manufacturer's instructions. Obtained

rbcL gene sequence was submitted to NCBI /GenBank database(Accession no. MT177967:AG4).

VI.   CONCLUSION

Through aroma analysis on 11 lines from AG 4 series recorded all three evaluation methods for aroma: rice reaction test with KOH, calculated 2-acetyl1-pyrroline (2AP) and PCR method with two markers RM223 and FMU1-2 recorded to select the best fragrant line, which is line 7 followed by line 10 and line 48. When calculating qualities through shape evaluation, the length and width of AG 4 are high. As well as a good level chakiness. Rice quality analysis recording amylose content is recorded that most of the low content on AG4 lines. This proves the delicious lines of rice. Milling quality determines the final yield and fracture rate of milled rice. Recorded lines of 7,10,14, and 20 for high milling rates above 50%. Protein content of rice varieties ranges from 6.7 to 8.6%. Lines 7 and 56 have the highest protein content (8.5-8.6%) inrespectively. The protein content of rice varieties ranges from 6.7 to 8.6%. Lines 7 and 56 have the highest protein content (8.5-8.6%) in respectively. –Analyzing yield and yield component, line 9 gives the highest yield (4.47 tons / ha, next is line 7 (4.62t / ha).

VII.   ACKNOWLEDGEMENTS

REFERENCES

[1] AACC International.1999. Approved Methods of Analysis, 11th Ed. Method 61–03.01. *Amylose content of milled rice*. Approved October 15, 1997; reapproved November 3, 1999. AACC International, St. Paul, MN, U.S.A.

[2] Bradbury MT, Fitzgerald TL, Henry RJ, Jin QS and Waters LE .2005. The gene for fragrance in rice. Plant Biotechnol J 3:363-370.

[3] Buttery RG, Ling LC, Juliano BO. 1982. 2-acetyl-1-pyrroline: an important aroma component of cooked rice. Chem Ind 23:958–959.

[4] Council of the European Eunion. Council Regulation (EC) 1785/2003 on the common organisation of the market in rice. Official Journal of the European Union. 2003;270: 96–113.

[5] Cuevas RP, Fitzgerald MA. Genetic Diversity of Rice Grain Quality In: Caliskan M, editor. Genetic Diversity in Plants. InTech; 2012. pp. 285–310. Available: http://www.intechopen.com/books/genetic-diversity-in-plants

[6] Cuevas RP, Daygon VD, Corpuz HM, Nora L, Reinke RF, Waters DLE, et al. Melting the secrets of gelatinisation temperature in rice. Functional Plant Biology. 2010;37: 439 doi: 10.1071/FP09258

[7] Calingacion M, Laborte A, Nelson A, Resurreccion A, Concepcion JC, Daygon VD, et al. Diversity of global rice markets and the science required for consumer-targeted rice breeding. PloS one. 2014;9: e85106 doi: 10.1371/journal.pone.0085106 [PMC free article] [PubMed]

[8] Dela Cruz NM, Khush GS. Rice Grain Quality Evaluation Procedures In: Singh RK, Singh US, Khush GS, editors. Aromatic Rices. New Delhi, India: Mohan Primlani; 2000. pp. 15–28.

[9] Fitzgerald MA, Resurreccion AP. Maintaining the yield of edible rice in a warming world. Functional Plant Biology. 2009;36: 1037 doi: 10.1071/FP09055

[10] Graham R. A Proposal for IRRI to Establish a Grain Quality and Nutrition Research Center. Los Baños, Philippines; 2002. Report No.: 44.

[11] He, Q., & Park, Y. J. (2015). Discovery of a novel fragrant allele and development of functional markers for fragrance in rice. Mol Breed, 35, 217. https://doi.org/ 10.1007/s11032-015-0412-4.

[12] Mahajan G, Matloob A, Singh R. 2018. Basmati Rice in the Indian Subcontinent: Strategies to Boost Production and Quality Traits. Advances in Agronomy. Elsevier Ltd, pp 159–213.

[13] Little RR, Hilder GB, Dawson EH. Differential effect of dilute alkali on 25 varieties of milled white rice 1958. *Cereal Chemistry*. 1958;35:111–26. [Google Scholar]

[14] IRRI. Alkali digestion In: IRRI, editor. *Standard Evaluation System (SES) for rice*, 5th Ed. Los Banos, Manila, Philippines: International Rice Research Institute (IRRI); 2013. p. 46. [Google Scholar]

[15] Nguyen Thi Lang and Bui Chi Buu.2008.DEVELOPMENT OF PCR-BASED MARKERS FOR AROMA (fgr) GENE IN RICE (*Oryza sativa. L.)*.Omonrice 16: 16-23 (2008).

[16] Nguyen thiLang .2002. Basic methods in biotechnology research, Agriculture publish , Ho Chi Minh City HCM.

[17] Nguyen thi Lang.2015. The report: Research on selecting rice varieties for export for mekong delta 2011-2015. Ministry-level summary report

[18] Petrov M, Danzart M, Giampaoli P, Faure J, Richard H. 1996. Rice aroma analysis: discrimination between a scented and a non-scented rice. Sci Aliments (16):347–360

[19] Rutsaert P, Demont M, Verbeke W. Consumer Preferences for Rice in Africa In: Wopereis MCS, Johnson D, Ahmad N, Tollens E, Jalloh A, editors. Realizing Africa's Rice Promise. Boston: CABI Publishing; 2013. pp. 293–301.

[20] Singh N, Sodhi NS, Kaur M, Saxena SK. Physico-chemical, morphological, thermal, cooking and textural properties of chalky and translucent rice kernels. Food Chemistry. 2003;82: 433–439. doi: 10.1016/S0308-8146(03)00007-4.

[21] -Sing .K.R , US Singh, G.S.Khush .2000. Aromatic rices .Published by Mohan Primlani for Oxford & IBH Publishing Co. Pvt. Ltd., 66 Janpath, New Delhi 110 001.

[22] -Spiller K. It tastes better because … consumer understandings of UK farmers' market food. Appetite. 2012;59: 100–7. doi: 10.1016/j.appet.2012.04.007 [PubMed]

[23] Shu Xia Sun; Fang Yuan Gao; Xian Jun Lu; Xian Jun Wu ,Xu Dong Wang; Guang Jun Ren; Hong Luo.2008.Genetic analysis and good gene mapping of fragrance in rice (Oryza sativa L. Cyperales, Poaceae). Genet. Mol. Biol. vol.31 no.2 2008. *https://doi.org/10.1590/S1415-48572008000300021.*

[24] Tan YF, Li JX, Yu SB, Xing YZ, Xu CG, Zhang Q. The three important traits for cooking and eating quality of rice grains are controlled by a single locus in an elite rice hybrid, Shanyou 63. TAG Theoretical and applied genetics Theoretische und angewandte *Genetik*. 1999;99:642–8. [PubMed] [Google Scholar]

[25] -Tomlins K, Manful J, Gayin J, Kudjawu B, Tamakloe I.2007.. Study of sensory evaluation, consumer acceptability, affordability and market price of rice. Journal of the Science of Food and Agriculture. 2007;87: 1564–1575. doi: 10.1002/jsfa.2889.

[26] Juliano B. Criteria 1985.Test for rice grain quality *Rice chemistry and technology*. Saint Paul: American Association of Cereal Chemists (AACC) 1985. p. 443–513. [Google Scholar]

Article   *Peer-Reviewed Journal*

Analysis of the functioning of the Local Development Fund (FDL) of the Pokola Community Development Series (Congo)

Analyse du fonctionnement du Fond de Développement Local (FDL) de la Série de Développement Communautaire de Pokola (Congo)

Pierre Mbete[1], Gilles Freddy Mialoundama Bakouetila[2], Ayessa Leckoundzou[1,4], Gildas Ricklin Chablys Obimbola[1], Rodolphe Aristide Goma[3], Henri Boukoulou[2]

[1]Laboratoire d'Ecologie Appliquée et de l'Environnement, Ecole Nationale Supérieure d'Agronomie et de de Foresterie, Université Marien NGOUABI, Congo. Tél : (+242) 06 661 18 98, BP : 69, Brazzaville, Congo. Email : mbetedavid@gmail.com
[2]Laboratoire d'Economie et Sociologie Rurales, Ecole Nationale Supérieure d'Agronomie et de de Foresterie, Université Marien NGOUABI, Congo. Tél : (+242) 06 603 67 67, BP : 419, Brazzaville, Congo. Email : gmialoundama2016@gmail.com
[3]Centre National d'Inventaire et d'Aménagement des Ressources Forestières et Fauniques, Ministère de l'Economie Forestière, Congo. Tél : (+242) 06 950 13 71. Email : rodolphegoma@yahoo.fr
[4] Institut national de Recherche Forestière (IRF), Ministère de la Recherche Scientifique et de l'Innovation Technologique Tél : (+242) 055648805/ 066389495, BP : 419, Brazzaville, Congo. Email : ayessa03@gmail.com
Auteur correspondant :ayessa03@gmail.com&mbetedavid@gmail.com / (+242) 06 661 18 98

Abstract— This study aims to assess the functioning of the Local Development Fund (FDL) of the UFA Pokola Community Development Series in Congo. It is based on the documentary review, semi-structured interviews with resource persons, household managers as well as the holding of focus groups. The results indicate that the local communities and indigenous populations (CLPA) are involved in the management bodies of the Community Development Series, bodies responsible for the management of the FDL. This fund is mainly supplied by royalties from the exploitation of forest species, it is intended to finance the micro-projects of the CLPAs. As a result, he is well known to the CLPAs of UFA Pokola (81%). Between 2007 and 2016, the FDL mobilized 182,214,333 FCFA, including 145,274,472 FCFA, made it possible to finance nearly 69 micro-projects for the benefit of the CLPAs. Plant production remains the main sector where investments have been directed (64%). There is also a spatial disparity in the investments of the FDL, the commune of Pokola benefited from 54% of the funds invested. The natives have benefited little from the spin-offs of this mechanism for sharing the benefits of logging.

Keywords— Local development fund, local communities and indigenous populations, royalty, exploitation, Congo.

Résumé— La présente étude vise à évaluer le fonctionnement du Fonds de Développement Local (FDL) de la Série de Développement Communautaire de l'UFA Pokola au Congo. Elle se base sur la revue documentaire, des entretiens semi-structurés avec les personnes ressources, les responsables de ménages

ainsi que la tenue des focus groups. Les résultats indiquent que les communautés locales et populations autochtones (CLPA) sont impliquées dans les organes de gestion de la Série de Développement Communautaire, organes responsables de la gestion du FDL. Ce fonds est essentiellement alimenté par les redevances de l'exploitation des essences forestières, il est destiné à financer les microprojets des CLPA. De ce fait, il est bien connu des CLPA de l'UFA Pokola (81 %). Entre 2007 et 2016, le FDL a mobilisé 182 214 333 FCFA dont 145 274 472 FCFA a permis de financer près de 69 microprojets aux profits des CLPA. La production végétale reste le principal secteur où les investissements ont été orientés (64 %). On note également une disparité spatiale des investissements du FDL, la commune de Pokola a bénéficié de 54 % des fonds investis. Les autochtones ont peu bénéficié des retombées de ce mécanisme de partage de bénéfice de l'exploitation forestière.

Mots clés— Fonds de développement local, communautés locales et populations autochtones, redevance, exploitation, Congo.

I. INTRODUCTION

Depuis la Conférence des Nations Unies sur l'Environnement et le Développement ayant abouti à un programme d'action pour le XXIème siècle (Ansallem, 2015) et le sommet de la terre tenu à Rio de Janeiro, en 1992, des nouveaux concepts de gestion durable et d'aménagement forestier durable sont apparus (FAO, 1996). Des politiques forestières sont formulées au niveau de chaque nation pour pérenniser et développer les fonctions économiques, écologiques et sociales des forêts ceci dans le cadre d'une gestion dite intégrée et participative qui assure de façon soutenue et durable la conservation et l'utilisation des ressources forestières et fauniques (Anonyme, 1999).

Dans le contexte de changement de paradigme dans l'aménagement forestier, la forêt se trouve au cœur des préoccupations environnementales et est aussi à la charnière de nouvelles attentes sociales (Boutefeu, 2005). Les modes de gestion appropriés seraient par conséquent ceux qui obéissent aux notions de démocratisation à la base, de libéralisation politique, de responsabilité collective (Nguinguiri, 1999) ; la gestion participative devient une nécessité.

La politique nationale en matière de gestion des ressources naturelles de nombreux pays est fortement influencée par les conventions internationales relatives à l'environnement et au développement durable qui sont ratifiées (Séne, 2014). Les projets d'aménagement forestier et de biodiversité comportent donc aujourd'hui une dimension sociale, question d'impliquer les populations dans la gestion durable des ressources naturelles (Robbin et *al.*, 2006).

La forêt congolaise est productrice de valeur écologique, économique, sociale et joue un rôle essentiel dans la vie de l'économie nationale. Les activités de gestion forestière relèvent à la fois de l'administration publique (Etat, propriétaires des terres), du secteur privé ou parapublic (propriétaire des outils de production) et des communautés rurales qui possèdent des droits d'usages (N'zala, 2002). Le processus d'aménagement durable des forêts a démarré dans les années 2000 plus précisément dans le Secteur Forestier Nord (Nkeoua et *al*, 2005). Dans les concessions aménagées, on note un effort d'implication des communautés locales et populations autochtones, à travers des mécanismes appropriés, notamment la mise en place des conseils de concertation des Séries de Développement Communautaire (SDC), la création des Fonds de Développement Local, etc.

L'Unité Forestière d'Aménagement de Pokola est une concession forestière concédée à la société forestière Congolaise Industrielle des Bois en sigle CIB (Anonyme, 2007), du groupe international Olam. Le modèle de gestion de la société CIB-OLAM, entreprise pionnière en matière d'aménagement forestier au Congo, et de certification de gestion durable peut être pilote pour comprendre la problématique du Fond de Développement Local (FDL). Il avait été institué en 2007 un fonds de développement local d'une valeur 200F CFA / m^3 indexée à la production de bois commercialisable réalisée par l'exploitant forestier au cours d'une année civile. Ce fonds est géré par un conseil de concertation de la SDC, comprenant différentes parties prenantes, et date depuis plus de dix ans. Une décennie après la mise en œuvre du FDL de la Série de Développement Communautaire de l'Unité Forestière d'Aménagement (UFA) Pokola, son fonctionnement et son bilan restent encore mal connu et peu d'informations scientifiques sont disponibles. C'est pourquoi, la présente étude vise à évaluer le fonctionnement de ce FDL. De manière spécifique, il s'agit : de décrire l'organisation et le fonctionnement du FDL, analyser les ressources financières mobilisé et investit par ce fonds au profit des CLPA.

II.   METHODOLOGIE

Localisation de la zone d'étude

L'étude a été réalisée dans la Série de Développement Communautaire de l'Unité Forestière d'Aménagement (UFA) de Pokola, une entité située dans le département administratif de la Sangha au nord de la République du Congo(Figure 1). La concession forestière attribuée à la société CIB-Olamen 2002 possède une superficie de 452 200 ha.L'UFA Pokolaest repartie en cinq (5) séries d'aménagement : production, conservation, protection, développement communautaire, recherche. Les Communautés Locales et Populations Autochtones vivent et réalisent les activités socioéconomiques dans la Série de Développement Communautaire. La SDC est un espace affecté aux activités villageoises et au développement local, il est composé d'un ensemble de terroirs et finages villageois comprenant les forêts naturelles et artificielles, des terres agricoles, les jachères, les zones de pêche et de chasse. L'objectif de la SDC est de satisfaire les besoins des populations forestières en produits forestiers et d'améliorer leur revenu.

La SDC de l'UFA Pokola comprend une surface de 26 490 hectares pour une population estimée à 15 582 habitants. Elle compte 12 villages et une commune de plein exercice au sein de laquelle on trouve la scierie et les bâtiments administratifs de la société forestière CIB-Olam.
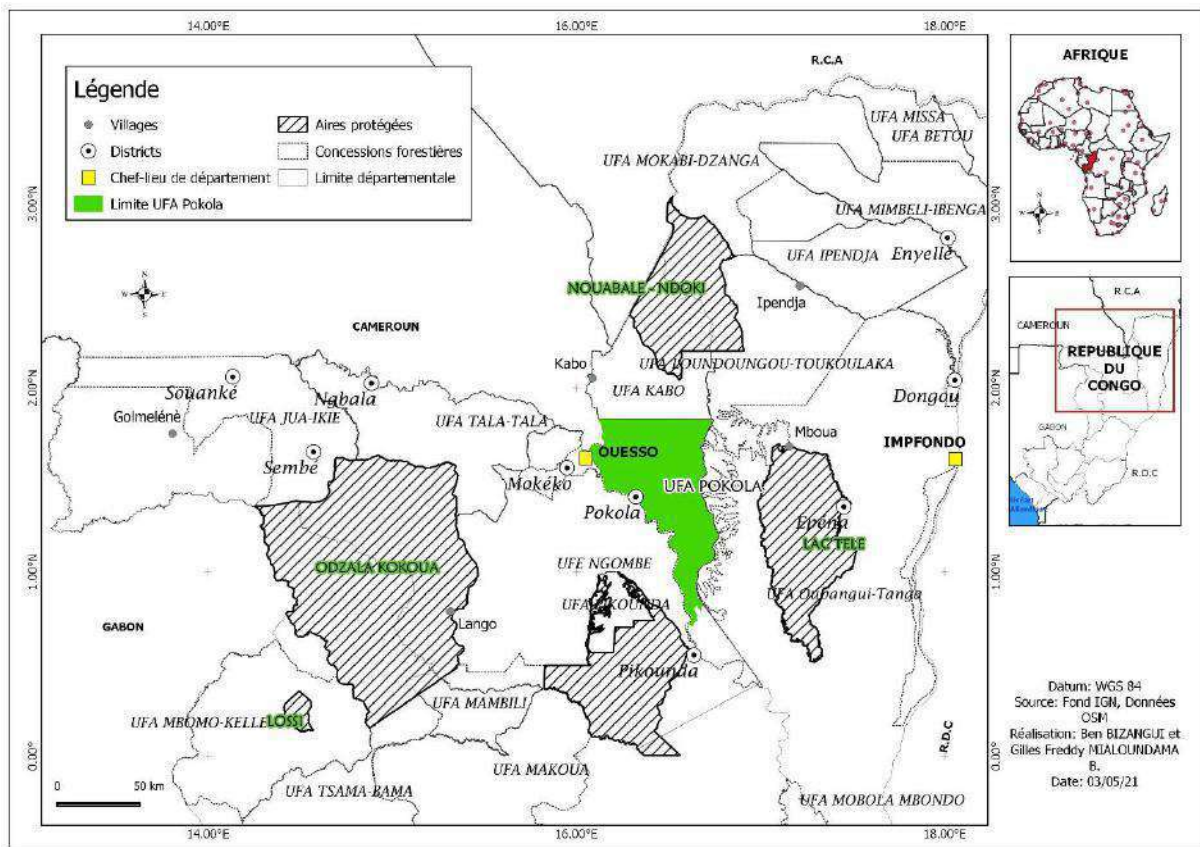
Fig.1 : Localisation de l'UFA Pokoladans le secteur forestier nord Congo

Collecte des données

La revue de la littérature a porté sur la documentation en lien avec l'objet de l'étude, notamment :

-   les données juridiques et officielles sur l'exploitation forestière et la décentralisation au Congo (lois, décrets et arrêtés, économie forestière, etc.) ;
-   les documents de la CIB (plan d'aménagement, rapport d'études et de missions, etc.

-   les documents de gestion de la Série de Développement Communautaire et le conseil de concertation (plan de gestion de la SDC, procédures de gestion des fonds, règlement intérieur, compte rendu des sessions, preuves de calcul de la redevance et alimentation du compte du FDL, etc.) ;
-   les autres études portant sur la gestion participative et l'implication des communautés locales et populations autochtones (CLPA) dans la gestion des ressources naturelles.

L'exploitation de ces documents a permis une meilleure connaissance de la zone d'étude, une compréhension du cadre d'intervention du FDL dans la SDC ainsi que son bilan.

La revue de la littérature a été complétée par des entretiens individuels avec les personnes ressources ayant une connaissance du FDL et/ou ayant été impliquées dans le dispositif de sa mise en œuvre (agents de la cellule d'aménagement de la CIB-Olam, Organisation de la Société Civile, membres du conseil de concertation, responsables de la préfecture de la Sangha, maire de la commune de Pokola, chef de brigade de l'économie forestière ainsi que son Directeur départemental, chef de secteur agricole ainsi que son Directeur départemental, etc.). Ces entretiens semi-structurés ont été menés à l'aide des guides d'entretiens. Les données portaient sur le fonctionnement du FDL, son bilan et les contraintes de développement. La période concernée par la présente étude est celle allant de 2007 à 2016.

Des focus group ont été également tenus dans 8 villages sur les 12 que compte l'UFA Pokola. Ils se tenaient en présence du représentant du programme social de la CIB. Ils ont permis de recueillir des opinions relatives au fonctionnement du FDL, le financement des microprojets, les forces et les faiblesses de l'intervention du FDL. Des observations directes de quelques réalisations du FDL ont été également effectuées. Pour évaluer le niveau de connaissance des CLPA sur le FDL de Pokola, 331 personnes ont été interrogées dont 169 communautés locales (bantous) et 162 autochtones.

Analyse des données

La saisie et l'analyse des données quantitatives ont été réalisées au moyen du logiciel Excel 2013. Cependant, les données qualitatives issues des entretiens et de la revue documentaire ont fait l'objet d'une analyse decontenu. Cette analyse a consisté en un examen systématique et méthodique des documents textuels afin de décortiquer le texte en énoncé plus court et significatif pour en faire la synthèse des idées principales et secondaires.

III.   RESULTATS

3.1 Les parties prenantes de la SDC de l'UFA Pokola

D'après les dispositions de l'arrêté n°2668/ MDDEFE/CAB du 15 avril 2010, portant institution, organisation et fonctionnement du conseil de concertation de la SDC de l'UFA Pokola, les différents organes de gestion de la SDC sont : (i) le conseil de concertation, (ii) la coordination technique, (iii) le comité de suivi et d'évaluation, (iv) le bureau exécutif, (v) le comité d'achat. Le tableau 1 présente les différentes parties prenantes des trois premiers organes de gestion

Tableau 1 : Organisation du conseil de concertation, la coordination technique, le comité d'évaluation de la SDC de l'UFA Pokola

	Conseil de concertation	Coordination technique	Comité de suivi et d'évaluation
Présidence/ coordination	Président : représentant du conseil départemental	Chef de brigade de l'économie forestière	Président : représentant de la préfecture
	Premier vice-président : représentant des communautés villageoises		Vice-président : représentant DDEF
	Deuxième vice-président : représentant de la société forestière		
	Rapporteur : chef de brigade de l'économie forestière, coordonnateur technique de la SDC		
Membres	Représentant de la préfecture	Chef de secteur agricole	Représentant de la CIB
	Sous-préfet du district de Pokola	Chef de poste agricole	Représentant des ONG locales -
	Directeur départemental de l'économie forestière	Représentant des ONG œuvrant dans l'UFA	
	Directeur départemental de l'aménagement du territoire	Représentants des communautés de village	
	Directeur départemental de l'environnement	Représentant de la société forestière	

	Directeur départemental de l'agriculture	Personne ressource	
	Directeur départemental de l'élevage		
	Directeur départemental de la pêche		
	Deux représentants de la société forestière CIB		
	Des représentants des communautés des villages, dont certains semi-nomades et femmes		
	Des représentants d'ONG en activité dans l'unité forestière		
	Toute personne appelée pour sa compétence		

Le bureau exécutif est composé d'un président (Représentant du Conseil départemental de la Sangha) et de deux membres, le chef de brigade de la Direction Départemental de l'Economie Forestière ainsi qu'un représentant de la société forestière. Cependant le comité d'achat est présidé par un représentant des ONG, assisté de 4 membres (un représentant de la société forestière, 3 personnes des villages bénéficiaires).

La gestion de la série de développement communautaire incombe au *conseil de concertation* impliquant tous les acteurs communautaires et présidé par un représentant du conseil départemental. Ce conseil de concertation a pour objectifs :(i) adopter le plan de gestion de la SDC de l'UFA, (ii) examiner et approuver les microprojets et activités prévues dans la SDC, (iii) examiner et approuver le budget du fonds de développement local, (iv) approuver les critères d'éligibilité des microprojets, (v) adopter le plan de gestion de la SDC et les critères d'éligibilité des microprojets, (vi) examiner et faciliter le règlement des différends entre les parties prenantes impliquées dans la gestion des ressources naturelles et le développement socio-économique de la SDC de l'UFA, (vii) examiner et adopter le programme et les rapports d'activités de la coordination technique du Conseil de concertation.

La participation des CLPA aux décisions de la gestion forestière est plus effective avec le fonctionnement du conseil de concertation et ses organes. Le conseil de concertation est une forme de démocratie participative au niveau locale, c'est une plateforme de concertation multi-acteurs.

La coordination technique est le second organe de gestion de la SDC, elle est chargée de : (i) suivre la réalisation des microprojets, (ii) assister les populations dans la réalisation de leurs activités, (iii) préparer les documents à soumettre au conseil de concertation, (iv) suivre la mise en œuvre du plan de gestion de la SDC de l'UFA Pokola, (v) informer et sensibiliser les populations sur la mise en œuvre du plan d'aménagement et du plan de gestion de la SDC de l'UFA Pokola, (vi) mettre en place et gérer la base de données.

Le comité de suivi et évaluation est l'organe de gestion de la SDC de l'UFA Pokola, chargé d'assurer le suivi des activités menées dans la SDC et de procéder à une évaluation technique et financière des activités menées.

Dans le but de lutter contre la pauvreté et de contribuer à l'amélioration des conditions de vies des populations, un Fonds de Développement Local (FDL) est créé. Il est alimenté par une redevance de 200 FCFA par m^3 indexée sur le volume de bois commercialisable exploité dans l'UFA, la subvention du Conseil Départementale de la Sangha, les dons et legs. Le FDL est destiné à :

- financer les microprojets d'intérêt communautaire dans les limites de la SDC à hauteur de 85 % des recettes annuelles ;

- assurer le fonctionnement du Conseil de Concertation et des organes techniques qui composent à hauteur de 15 % des recettes annuelles.

3.2 Niveau de connaissance du FDL par les Communautés Locales et Populations Autochtones

Les Communautés Locales et Populations Autochtones (CLPA) de la Série de Développement Communautaire de l'UFA Pokola ont une connaissance du Fonds de Développement Local (FDL). La proportion de 91 % des populations locales bantous connait le FDL, contre 9 % qui affirment ne pas connaitre ce mécanisme de partage de bénéfice de l'exploitation forestière. Cela témoigne d'une bonne sensibilisation faite pour vulgariser cet outil de financement des microprojets des CLPA.

Le FDL est globalement bien connu des populations autochtones (70 %). Toutefois 30 % des autochtones interrogés ont aucune connaissance de ce mécanisme de financement des microprojets. Le faible niveau de connaissance du FDL (30%)estmarqué chez les autochtones, soit 36 femmes parmi les 48 autochtones n'ayant pas connaissance de ce fonds (tableau 2).

Tableau 2 : Evaluation du niveau de connaissance des CLPA sur le FDL

Genre	Connaissance du FDL par la population locale (Bantou)		Connaissance du FDL par les autochtones	
	Oui	**Non**	**Oui**	**Non**
Homme	101	9	67	12
Femme	53	6	47	36
Total	**154**	**15**	**114**	**48**

3.3 Evaluation des ressources financières mobilisées par le FDL

Pendant la période décennale (2007-2016), le FDL a été alimenté par les redevances payées par la société forestière CIB-Olam, la redevance de l'exploitation du bois de valeur marchande dans la zone de développement communautaire de Pokola de la SDC, les contributions du conseil départemental de la Sangha et de l'Organisation Internationale des Bois Tropicaux (OIBT) à travers le Projet de Gestion des Ecosystèmes Périphériques au Parc (PROGEPP). Au cours de cette période un montant de 182.214.333 FCFA a été mobilisé. La proportion de 85 % de ce montant était orientée vers l'investissement à travers le financement des microprojets, soit 154.882.183 FCFA. Le reste (15 %) était consacré au fonctionnement des organes de gestion de la Série de Développement Communautaire, soit 27.332.150 FCFA.

L'exploitation du bois dans les Unités Forestières de Production (UFP) 1 et 2 (618.632.724 m^3 de volume de bois roulés) a généré au FDL un montant de 123.726.543 FCFA.L'exploitation du bois de valeur marchande dans la SDC a été formalisée en 2013 à travers un protocole d'accord signé entre la société forestière CIB-Olam et le conseil départemental de la Sangha. La récupération de bois de valeur économique par la CIB-Olam dans la zone de développement communautaire de Pokola a eu lieu en 2014 et a généré une somme de 53.256.625 FCFA. La redevance forestière payée varie entre 1000 FCFA et 2000 FCFA par essence forestière exploitée. La base de taxation des essences forestières de haute valeur varie entre 1500 et 2000 FCFA/m^3, c'est le cas du Doussié (*Afzeliabipindensis*) taxé à 2000 FCFA/m^3, du Sapelli (*Entandrophragmacylindricum*) et du Sipo (*Entandrophragma utile*) taxés à 1500 FCFA/m^3. Les autres essences forestières sont taxées à 1000FCFA/m^3, c'est le cas du Wengué (*Milletialaurentii*), du Tali (*Erythrophleumivorense*), du Padouk (*Pterocarpussoyauxii*), etc.

La redevance issue de l'exploitation du bois dans l'UFP 1 et 2 ainsi que dans la SDC par la CIB-Olam ont contribué à près de 97 % des fonds mobilisés par le FDL au cours de la période 2007-2016. La CIB-Olam est donc le principal pourvoyeur des ressources financières du FDL. Cependant les apports de l'OIBT à travers le PROGEPP et du conseil départemental de la Sangha restent très marginal, soit respectivement 3.231.165 FCFA et 2.000.000 FCFA (figure 2).
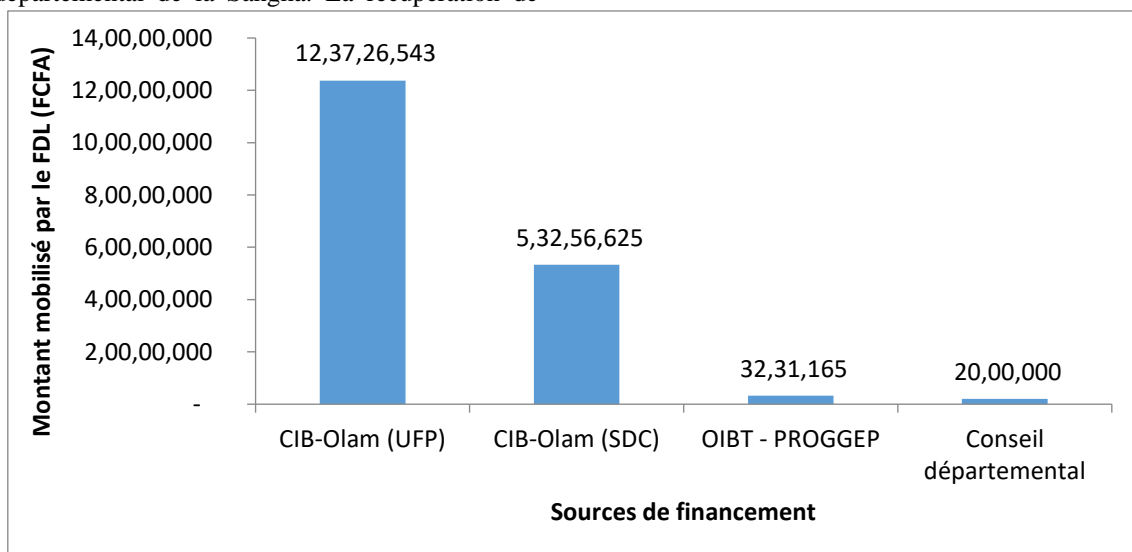

Fig.2: Répartition des sources de financement mobilisées par le FDL de l'UFA Pokola

3.4 Analyse des investissements du FDL de l'UFA Pokola

3.4.1 Répartition des investissements du FDL par secteur d'activité

Dans la période de 2008 à 2016, le FDL a financé 69 microprojets aux bénéfices des communautés locales et populations autochtones, soit un montant investi de 145.274.472 FCFA. Les investissements dans les microprojets de production végétale (45 projets) représentent 93.577.872 FCFA, soit 64 % de l'ensemble des investissements. Dans ce secteur, les microprojets de la culture de cacaoyer (*Theobroma cacao*) et du manioc (*Manihotesculenta*) sont les plus financés, soit

respectivement 55.709.350 FCFA et 33.868.522 FCFA. Le secteur de transport (transport par pirogue motorisée) représente le second secteur d'investissement de ces fonds, mobilisant 20 % des investissements (28.580.000 FCFA). Près de 9 microprojets ont été financés dans ce secteur.Les investissements dans le secteur de l'élevage (aviculture, production ovine et caprine, production porcine) représentent 10 % de l'ensemble des investissements, soit 14.436.600 FCFA pour 8 microprojets (tableau 3). Les investissements dans les autres secteurs d'activité sont faibles.

Tableau 3 : Secteur d'investissements du FDL et coût de réalisation des microprojets

Secteur d'investissement	Montant en FCFA	Nombre de microprojet	Pourcentage des investissements (%)
Production végétale	93 577 872	45	64
Elevage	14 436 600	8	10
Pêche	6 180 000	5	4
Transport	28 580 000	9	20
Habitat	1 500 000	1	1
Pisciculture	1 000 000	1	1
Total	**145 274 472**	**69**	**100**

3.4.2 Répartition inégale des investissements du FDL par localité de la SDC de Pokola

Le FDLa réalisé des investissements dans toutes les localités de la Série de Développement Communautaire (SDC) de l'Unité Forestière d'Aménagement de Pokola (UFA). La commune de Pokola a bénéficié pratiquement de la moitié des fonds investit à l'échelle de l'UFA Pokola, soit 54 % (tableau 4). On note une forte disparité entre les financements accordés par le FDL à la commune de Pokola et les villages de la SDC de l'UFA Pokola. Cette pratique prouve le dynamisme économique de la commune de Pokola, où le FDL est influencé par les acteurs clefs des organes de gestion de la SDC. Ces inégalités spatiales dans les investissements du FDL en faveur de la commune de Pokola semblent être en déphasage avec les recommandations des études socioéconomiques de bases ayant servi à l'élaboration du plan d'aménagement de l'UFA. Ces études ont fortement recommandé

d'encourager les activités socioéconomiques dans les localités qui manquent cruellement d'infrastructures de base (école, centre de santé, etc.).

Les communautés rurales qui ont bénéficié des investissements les plus importants (6 % chacune) sont Ikélemba, Ngatongo, Ngangassa et Ngandzikolo. L'investissement du FDL pour les autres communautés rurales reste faible, soit 4 à 3 %. Les investissements du FDL dans l'ensemble des localités ontcontribué à atténuer les revendications vis-à-vis de la société forestière CIB-Olam pour leurs droits sociaux (emploi, appui aux activités alternatives, etc.) mais aussi à faire asseoir la paix sociale dans l'UFA. En effet, 3 ans après la mise en œuvre du FDL, aucun conflit social ouvert n'a été enregistré dans l'UFA comparativement aux années précédentes (2006 à 2009) où l'on avait enregistré 4 conflits ayant entrainé l'interruption des activités de la CIB en forêt.

Tableau 4 : Répartition des investissements du FDL par localité de l'UFA Pokola

Localité	Statut	Superficie (ha)	Investissement du FDL (FCFA)	Part des investissements (%)
Pokola	Commune	15 810	78448215	54
Ngangassa	Village	365	8716468	6

Ikélemba	Village	1 640	8716468	6
Ngatongo	Village	1 410	8716468	6
Ngandzikolo	Village	895	8716468	6
Mbirou	Village	1 705	7263724	5
Djaka	Village	1 085	5810979	4
Konda	Village	385	5810979	4
Matali	Village	1 705	4358234	3
Bossendé	Campement	-	-	3
Matoto	Village	490	4358234	3

3.4.3 Répartition des investissements du FDL entre les communautés locales Vs populations autochtones

L'approche de financement des microprojets FDL dans les villages ne permet pas de faire une comparaison aisée entre les communautés Bantou et populations autochtones. Le village étant mixte, généralement un seul projet est financé pour ces deux communautés. La mise en œuvre semble être difficile et la répartition des bénéfices pas assez aisée du fait des relations de domination des bantous sur les populations autochtones. Les autochtones affirment n'être pas bénéficiaires des retombées des financements.

Dans les villages de Molembé, Ngangassa, Ngatongo, Djaka et Ikélemba par exemple, les autochtones sont parfois assistés en cas de maladies et lors des funérailles par les bénéfices issus de l'exploitation des pirogues motorisées. Si l'analyse comparative des investissements entre bantou et autochtones est difficile à établir dans les villages, cela est plus aisé à réaliser à Pokola, car l'approche de financement a été séparée. Le FDL a privilégié les individualités, les groupements et associations. L'analyse de la figure 3 montre une faible prise en compte des préoccupations des groupes sociaux défavorisés (autochtones) par le dispositif FDL (figure 3). L'argumentaire avancé par certains gestionnaires n'est pas soutenable, semble-t-il que les populations autochtones n'adressent pas leur demande au FDL. Cette analyse devrait interpeller les décideurs du FDL, en vue de se conformer aux engagements de la CIB prévus dans les documents d'orientations (plan d'aménagement, mesures sociales et de gestion).
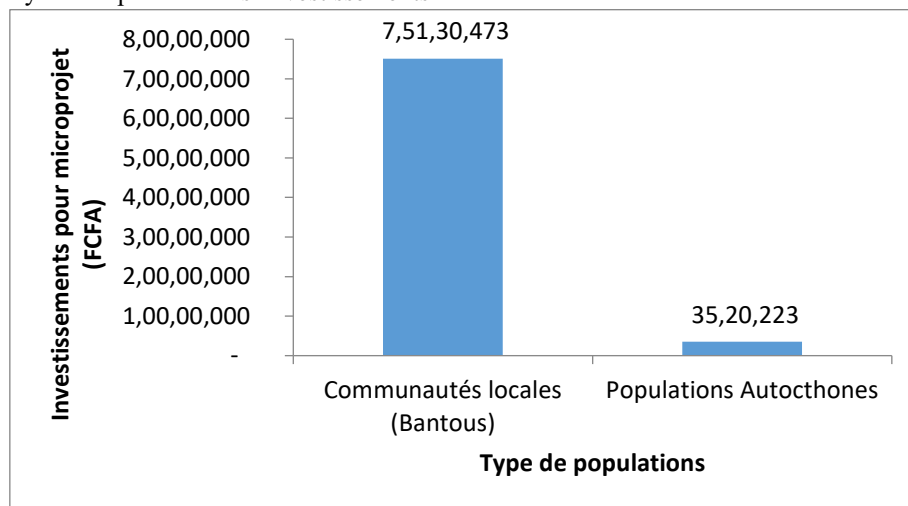
Fig.3: Répartition des investissements du FDL entre organisations bantous et autochtones

3.4.4 Analyse des impacts du FDL

Les investissements réalisés par le FDL visent l'autonomisation des communautés et ainsi contribuer à l'amélioration des conditions de vie des populations. Les microprojets dans le secteur du transport (pirogue motorisé) présentent un impact important. En effet les CLPA ne peuvent pas se procurer d'une pirogue motorisée à cause du faible pouvoir d'achat. L'acquisition des pirogues motorisées a permis d'évacuer des malades des villages vers les centres de santé mieux équipés, d'assurer

la circulation des personnes et des biens le long de la rivière Sangha. Les bénéfices générés par la gestion des pirogues ont contribué à la construction d'une école, la prise en charges des frais scolaires et autres produits de premières importances, etc.

L'agriculture et la pêche sont les secteurs d'activités qui ont un impact significatif pour des raisons suivantes : contribuent à la sécurité alimentaire des villages, possibilité d'acquisition des intrants et de production. Cependant les microprojets dans les secteurs de l''élevage, l'habitat, et la pisciculture sont considérés comme des investissements à impacts limités du fait de leurs faibles apports dans les ménages.

L'initiative du FDL reste une bonne approche pour l'implication et la responsabilisation des CLPA, dans la gestion forestière. Il constitue une opportunité pour encourager la diversification de l'économie locale en s'appuyant sur des projets communautaires, gage du développement socioéconomique de la SDC. Toutefois les effets du financement de ces microprojets restent parfois mitigés et quasiment nul pour les populations autochtones. Au stade actuel, aucun microprojet n'a la capacité de s'autofinancer et se pérenniser à la fin de la subvention accordée. Après 10 ans de mise en œuvre, les microprojets FDL ont de la peine à assurer leurs visibilités.

Tableau 5:Impact des types d'investissementdu FDL sur les conditions de vie des CLPA

Secteur d'activité	Appréciations des impacts sur l'amélioration des vies des CLPA			
	* impact limité	*** impact significatif*	**** impact important*	***** impact très important*
Agricole		**		
Elevage	*			
Pêche		**		
Transport			***	
Habitat	*			
Pisciculture	*			

Le FDL représente un modèle innovant de mécanisme de développement local, intégrant les différents niveaux de planification et d'exécution, de la communauté locale villageoise aux autorités départementales. C'est un outil financier pour le partage de bénéfices de la rente forestière. A travers le financement des microprojets, le FDL a contribué efficacement à faire asseoir la paix sociale entre la société forestière CIB-Olam et les CLPA de l'UFA Pokola. On assiste également à une nette prise de conscience, par les populations à la base, de leur nouveau rôle dans la gestion forestière et cela est vécu comme un impact positif à travers le dialogue permanent institué entre les acteurs parties prenantes à la gestion forestière. Le conseil de concertation apparait aujourd'hui comme un organe régulateur permettant de concilier les logiques des différents acteurs (Etat, exploitant forestier, ONG et CLPA) dans la gestion des ressources forestières dans l'UFA Pokola.

Les microprojets financés par le FDL ont également entrainé des faibles impacts négatifs. Ils ont été à l'origine de l'émergence de petits conflits sociaux entre les bénéficiaires et non bénéficiaires du financement des microprojets ou du partage des avantages

socioéconomiques obtenus. Ces conflits peuvent être atténués par les actions d'accompagnement des acteurs dans la gestion des microprojets.

La réalisation des microprojets dans le secteur de la production végétale (cacaoyer, manioc, etc.) a entrainé la perte de la couverture végétale, de la biodiversité et l'émission des gaz à effet de serre. La pratique d'agriculture itinérante sur brûlis a contribué à la déforestation, le taux de déforestation sur la période de 2007 à 2016 a été évalué à 0,96 % de la superficie de la SDC. Une superficie de 106 ha a été défrichée dont 56 ha pour les cultures vivrières et 50 ha pour la culture du cacaoyer.

IV.   DISCUSSION

La fiscalité forestière décentralisée contribue à améliorer la participation et l'implication des populations locales (Fomété, 2001). Le Fonds de Développement Local (FDL) est un instrument de gestion participative des ressources naturelles, qui permet d'impliquer les Communautés Locales et Populations Autochtones (CLPA) dans la gestion durable d'une concession forestière aménagée. La

présente étude a montré que le FDL contribue au développement socioéconomique local en finançant les microprojets des CLPA vivant dans la Série de Développement Communautaire (SDC) de l'Unité Forestière d'Aménagement (UFA) Pokola. Ce fonds est donc un modèle innovant de mécanisme de développement local, intégrant dans les organes de gestion du FDL (conseil de concertation et la coordination technique)les CLPA ainsi que les autorités du conseil départemental de la Sangha.

Le FDL de la SDC de l'UFA Pokola est alimenté principalement (97 %) par les redevances payéespar la société forestière CIB-Olam à la suite de l'exploitation des unités forestières de production (UFP) et des essences forestières dans la SDC de l'UFA Pokola. Ce fonds permet aux CLPA de bénéficier tant soit peu des retombées de l'aménagement forestier. Dans le secteur forestier Camerounais, deux types de taxes décentralisées contribuent au développement local, notamment la redevance forestière annuelle (RFA) et la taxe dite de 1000 FCFA (Fometé, 2001). Ce même auteur, affirme que la redevance forestière annuelle est un outil de fiscalité décentralisé où 50 % du montant est destiné aux communes dont 10 % versés aux communautés riveraines. Cependant, la taxe dite des 1000 FCFA (1000 FCFA/m^{3}de bois exploités) ne s'applique qu'aux titres d'exploitation de vente et de coupe de bois, elle est destinée à des projets locaux d'œuvres sociales (écoles, routes, etc.).

Les résultats de l'étude montrent que le financement des microprojets par le FDL dans l'ensemble des localités de la SDC de Pokola a permis d'atténuer les revendications vis-à-vis de la société forestière et instaurer la paix sociale. L'implication des CLPA dans les organes de gestion du FDL permet d'augmenter leur participation dans la gestion de l'UFA Pokola. La participation ou l'implication des communautés dans la prise de décision de manière formelle et informelle est l'un des principes de bonne gouvernance des forêts (FapaNanfack et *al.*, 2020). L'approche participative est un instrument innovant de gouvernance forestière qui prend en compte toutes les parties prenantes, notamment les populations locales (Koffi Kouamekan, 2013). Elle permet aux populations concernées par l'exploitation du domaine forestier d'en être les acteurs, au même titre que l'Etat et les exploitants forestiers (Collas de Chatelperron, 2005). Dans ce contexte, le FDL alimenté principalement par les redevances de l'exploitation des essences forestières dans les Unités forestière de Production (200 FCFA/m^3) et dans la SDC (1000 à 2000 FCFA/m^3), est donc une véritable opportunité pour les CLPA de bénéficier des retombées de l'exploitation forestière et de susciter leur participation

dans les organes de gestion de la SDC ; organes comprenant différentes parties prenantes.

En République du Congo, l'effort d'implication des CLPA dans la gouvernance forestière est surtout constaté dans les concessions forestières aménagées. C'est le cas de l'UFA Ngombé où les CLPA sont impliqués dans la gestion du FDL à travers le conseil de concertation (MankeriNdobolo, 2020). Cependant dans les concessions forestières non aménagées dont la plupart se situe dans les secteurs forestiers centre et sud, la gestion participative est loin d'être une réalité. Par conséquent, les CLPA ne sont pas impliquées dans la gestion des ressources naturelles et bénéficient très peu des retombées de l'exploitation forestière, elles ne possèdent pas de FDL (Goma, 2020).

Malgré la présence d'un FDL doté d'une expérience d'une décennie, sa contribution réelle au développement local reste mitigée. La lenteur administrative dans la gestion opérationnelle de la SDC, le retard dans la livraison des matériels et intrants par le comité d'achat (décalage avec le calendrier agricole), la faible compétence des animateurs sur les outils de conception et de pilotage des projets, la faible prise en compte des préoccupations des groupes défavorisés (autochtones, femmes, etc.), la faible adhésion des CLPA aux microprojets communautaires sont parmi les problèmes organisationnels des investissements du FDL dans la mise en œuvre des microprojets. De plus, le protocole d'accord relatif à l'exploitation de bois de valeur commerciale dans la SDC de l'UFA Pokola entre la CIB-Olam et le Conseil de concertation n'a pas été respecté totalement. Le protocole d'accord conclu exige le paiement de 2000 FCFA/m^3 de bois exploité par la CIB pour les essences de catégorie 1 (Doussié et Wengué). Cependant lors du paiement de la taxe, le Wengué a été évalué et payé à 1000 FCFA/m^3 au lieu de 2000 FCFA/m^3, ce qui suppose un manque à gagner pour le FDL.

V.   CONCLUSION

Le but de l'étude était d'évaluer le fonctionnement du FDL de la Série de Développement Communautaire (SDC) de l'UFA Pokola. Les deux objectifs spécifiques poursuivis étaient de décrire l'organisation et le fonctionnement du FDL, et analyser les ressources financières mobilisées et investit par ce fonds au profit des communautés locales et populations autochtones. Il ressort de l'étude que les trois principaux organes de gestion de la SDC sont le conseil de concertation, la coordination technique et le comité d'évaluation. Dans ces organes de gestion, les CLPA sont des parties prenantes au même titre que les représentants des acteurs institutionnels (conseil départemental de la Sangha, service déconcentré de l'état, la société forestière CIB-Olam, etc.). Le financement de la SDC est assuré le

FDL, géré directement par le conseil de concertation. Ce fonds est créé dans le but de lutter contre la pauvreté et de contribuer à l'amélioration des conditions de vie des populations vivant dans la SDC de l'UFA Pokola. Il est alimenté principalement par les redevances issues de l'exploitation des essences forestières dans les unités forestières de production (200 FCFA/m^3 de bois commercialisable exploité dans l'UFA) et dans la SDC (1000 à 2000 FCFA/m^3 de bois exploité). Le FDL est bien connu des communautés locales et populations autochtones de la SDC de l'UFA Pokola (81%). Pendant une décennie de fonctionnement, un montant de 182 214 333 FCFA a été mobilisé dont 145 274 472 FCFA a permis de financer près de 69 microprojets aux profits des CLPA de l'ensemble des localités de la SDC de l'UFA Pokola. L'investissement dans les microprojets de production végétale représente 64 % de l'ensemble des investissements du FDL, avec une prédominance pour les cultures de cacaoyer et du manioc.Des insuffisances d'ordre institutionnel, organisationnel et les capacités techniques des acteurs sont à corriger afin d'optimiser l'utilisation des fonds disponibles et augmenter l'impact socioéconomiques de ces investissements.

VI.   REMERCIEMENTS

REFERENCES BIBLIOGRAPHIQUES

[1] **Anonyme, 1999.** Principes et pratique de cogestion forestière : témoignages d'Afrique de l'ouest. Document n°2 de l'Union Européenne sur la foresterie tropicale. OverseasDevelopment Institut Londres, Commission européenne, Bruxelles, 37 p.

[2] **Anonyme, 2007.** Plan d'aménagement de l'Unité Forestière d'Aménagement (UFA) de Pokola (2007-2035). Congolaise Industrielle des Bois (CIB), Terea, GTZ, FFEM, Groupe Agence Française de Développement, OIBT, 382 p.

[3] **Ansallem I.**, 2015. Conventions internationales formelles. In : Mémento du Forestier Tropical. Editions Quae, pp. 110-130.

[4] Arrêté n°2668 du 15 avril 2010 portant institution, organisation et fonctionnement du Conseil de Concertation de la Série de Développement Communautaire de Pokola. *Journal Officiel de la République du Congo*, 16 : 343-345.

[5] **Boutefeu B. 2005.** La demande sociale de nature en ville : enquête auprès des habitants de l'agglomération lyonnaise. Lyon, Edition Puca-Certu, collection Recherches n°154, 81 p.

[6] **Collas de Chatelperron P., 2000.** Fondements de la démarche du projet en matière de classement des forêts. In gestion durable des forêts au Cameroun : vers une foresterie responsable, contributions du projet forêts et terroirs. Actes de l'atelier d'échanges 4-6 juillet 2000, Yaoundé, CIRAD, 78 p.

[7] **FAO, 1996.** Critères et indicateurs pour l'aménagement des forêts de l'Afrique tropicale sèche. FAO, Rome, Italie, 35 p.

[8] **FapaNanfack R., Gélinas N., Bobo Kandiri S., Asselin H., HiolHiol F., Tatsoula A., 2020.** Détermination de la gouvernance forestière dans les forêts communautaires de l'Est Cameroun. *Bois et forêts des Tropiques*, 343, pp. 53-66.

[9] **Fomété T., 2001.** La fiscalité forestière et l'implication des communautés locales à la gestion forestière au Cameroun. Document du Réseau de foresterie pour le développement rural, pp : 18-29.

[10] **Goma A.R., 2020.** Analyse de l'implication des communautés locales et populations autochtones dans la gestion de l'Unité Forestière d'Exploitation Louvakou. Mémoire de Master en Sciences et Techniques Forestières, Ecole Nationale Supérieure d'Agronomie et de Foresterie, Université Marien Ngouabi, 67 p.

[11] **Koffi Kouamékan J. M., 2013.** Gestion participative, capabilités et résilience dans les forêts classées de Côte d'Ivoire.*Ethics and Economics*, 10(1): 119-152.

[12] **MankeriNdobolo L.N., 2020.** Etat de mise en œuvre du plan de gestion de la série de développement communautaire de l'UFA Kabo. Mémoire de Master en Sciences et Techniques Forestières, Ecole Nationale Supérieure d'Agronomie et de Foresterie, Université Marien Ngouabi, 43 p.

[13] **N'zala D., 2002**. Conservation et gestion durable des écosystèmes des forêts tropicales humides de l'Afrique Centrale. Exemple des concessions de Pokola-Kabo-Loundoungou, Congo. Etude FAO, 21 p.

[14] **Nguinguiri J. C., 1999.** Les approches participatives dans la gestion des écosystèmes forestiers d'Afrique centrale. Revue des initiatives existantes. Occasionalpaper n°23, CIFOR (Center For International ForestryResearch), 24 p.

[15] **Nkéoua G. et NgoyaKessi A. M., 2005.** L'aménagement des forêts au Congo.URL : http://www.c3ed.uvsq.fr/cdg.ecorev/fr/pdf/t4/Nkéoua-Ngoya.pdf.Consulté le 10 avril 2021.

[16] **Robbin, Mcsweeney, Waite, Rice, 2006.** Even Conservation Rules Are Made to Be Broken: Implications for Biodiversity. *Environmental Management*, 37 (2): 162-169.

[17] **Séne A., 2014.** Implication des acteurs non étatiques dans la gouvernance des ressources naturelles au Sénégal : cas des ressources forestières à Zinguinchor et halieutiques à Mbour. Dans Kobor, D. (Ed.) Energie renouvelables et développement durable. *Revue science Lib*, Toulouse, Editions Mersenne, pp : 109-130.

Article   *Peer-Reviewed Journal*

Investigating the capacity of hydroponic system using lettuce (*Lactuca sativa* L.) in the removal of pollutants from greywater while ensuring food security

Drissa Sangare[1*], Lassina Sandotin Coulibaly[1], Harinaivo Anderson Andrianisa[2], Joel Zie Coulibaly[3], Lacina Coulibaly[1,3]

[1]University of Man, Côte d'Ivoire, Agronomic, Forestry and Environmental Engineering Department, P.O. Box 20 Man, Côte d'Ivoire
Email: dsangare2012@gmail.com
[2]International Institute for Water and Environmental Engineering, Water and Sanitation Department, 2iE, 01 Rue de la Science, 01 P.O. Box 594, 01 Ouagadougou, Burkina Faso
[3]University of Nangui Abrogoua, Environmental Science and Management Department, Abidjan, 02 P.O. Box 801 Abidjan 02, Côte d'Ivoire
*Corresponding Author

Abstract—The study aims to investigate the removal of nutrients, organic pollutants and fecal bacteria from greywater while evaluating the growth and yields of lettuce using hydroponic system. Lettuces grow in hydroponic systems irrigated with untreated greywater, and fresh well water, separately. Nutrients, organic pollutants, and fecal bacteria of dishwashing greywater were monitored. Agronomical and microbiological parameters of lettuce were investigated. The results showed that removal efficiencies for ammonia (NH_4^+), nitrate (NO_3^-), and total phosphorus (TP) were 78 %, 87 % and 56 %, respectively. The organic pollutants removal efficiencies were higher than 50% for Total Suspended Solids (TSS), 5-days Biochemical Oxygen Demand (BOD_5) and Chemical Oxygen Demand (COD). The removal efficiencies are 88.68 % and 81.94 % for E. coli, and fecal coliforms, respectively. The fecal bacteria were not observed in the lettuce leaves. Besides, the lettuces irrigated with greywater produced higher biomass than the ones irrigated with fresh well water. The hydroponic system could be an alternative for greywater treatment and ensuring food security.

Keywords—Dishwashing greywater, Fecal bacteria, Hydroponic systems, Lettuce, Removal efficiencies.

I. INTRODUCTION

Wastewater reuse for irrigation is a long-standing practice and has many advantages especially in those areas where water resources are limited (Jaramillo and Restrepo, 2017). Treated wastewater contains nutrients that are useful for plant growth and help to reduce fertilizers needs (Sangare et al., 2017; Marzougui et al., 2018), thus ensuring a closed and environmentally favourable nutrient cycle that avoids the indirect return of nutrients, such are nitrogen and phosphorus to water bodies. Therefore, eutrophication conditions in water bodies would be reduced, as would the

expenses for agrochemicals used by farmers (Candela et al., 2007). Greywater, which represents more than 75% of total global wastewater, and include wastewater from baths, showers, kitchen, hand wash basins and laundry, is the most popular fraction used for agriculture, as it contains low levels of pathogens (Morel and Diener, 2006; Hernandez et al., 2011). Based on such properties, greywater reuse for agricultural irrigation is receiving more and more attention (Li et al., 2009; Rodda et al., 2001). However, direct recycling of greywater is not recommended. Greywater may contain various microbial

pathogens and hazardous chemicals depending on the nature of the raw greywater and the treatment efficiency (Maiga et al., 2018). There are several methods for the treatment of these generated domestic wastewater like Aerated Lagoons (Grady et al., 2011), High Rate Algal Ponds process (Sangare et al., 2017), Electrocoagulation Process (Moosavirad, 2017). However, these methods are very expensive, complex and energy intensive for use semi-urban, and rural areas of developing countries. To address these gaps, it is imperative to design green alternative domestic wastewater treatment technologies linked to crop production to control the long-term effects on water resources, and to minimize the pressures of population growth on food stock. Indeed, wastewater reuse in agriculture through hydroponic systems can be a viable technology to avoid environmental, public health impacts, and food security (Magwaza et al., 2020). The hydroponic systems are agricultural systems in which the development and growth of plants occur without the use of soil, and their roots are immersed directly in the nutrient solution (Martinez-Mate et al., 2018). A literature review conducted by the FAO (2010) showed that the application of the system has improved city environment, food security, and cities economy by providing wide variety of fresh fruits and vegetables through the year. In fact, the systems are widely used for the treatment of nutrient rich wastewaters (Prazeres*et al.,* 2017; Bawiec, 2019). For instance, removal efficiencies ranging from 47 to 91% for both nitrogen and phosphorus was reported when using hydroponic system with different plant species (López-Chuken, 2012; Haddad and Mizyed, 2011). The nutrient removal efficiency was found to be dependent on the life interactions of various species of bacteria, plant roots, gravel, water and sunlight. Indeed, the plant root system modifies the living conditions for the microorganisms present in municipal wastewater by releasing various metabolites, and thus enhancing the biological treatment of wastewater through nitrification and denitrification processes (Tsao, 2003). Moreover, by using hydroponic system with Romaine Lettuce, the removal efficiency of COD, BOD and TSS were 37.36%, 82.31 % and 89.42 %, respectively according to study conducted by Cui et al. (2003). Besides, hydroponic systems have been reported to enhance the removal efficiency of pathogens, in wastewater treatment system (Ndulini et al., 2018). These researchers showed a removal efficiency of 92.77% for faecal coliforms removal, using of *Bidenspilosa* L. and *Amaranthushybridus* L. In their study, Ottoson et al. (2005) have also been shown that hydroponics can be an alternative to conventional wastewater treatment through the virus and (oo)cyst removal. On the other hand, hydroponic systems can help in biomass production for

value-added crops thereby improving food security and income generation among poor communities (Eregno et al., 2017; Jesse et al., 2019). Further, this system is particularly suitable for agricultural reuse of domestic wastewater because it minimizes the health risks to workers, harvested crop and consumer that occur through contact with wastewater (Qadiret al., 2010). In addition, hydroponic greenhouses use 13 fold less water to produce comparable lettuce yields compared to field operations, according to Barbosa et al. (2015). Thus, wastewater reuse in hydroponic system represents a choice of production that can be adapted to the demand of high quality, high production and higher recovery of water and nutrients. However, limited studies have been conducted on potential cultivation of lettuce (*Lactuca sativa* L.) with greywater using hydroponic system, still need some investigations. Lettuce is among vegetables commonly used for hydroponic production due to their short growth cycle allowing better control and standardization of the cultivation process (Magwaza et al., 2020). Moreover, lettuce is popular edible leaf vegetable in tropical countries with a relatively high market value (Palada et al., 1999). In view of all the above, the aim of this study was to explore the treatment of dishwashing raw greywater by hydroponic system cultivated with lettuce (*Lactuca sativa* L.). It will focus on (i) the characteristics of raw and treated dishwashing greywater, (ii) the removal efficiency of nutrients, pollutant loads, and fecal bacteria, and (iii) the effect on the yield and microbiological quality of lettuce in hydroponic systems.

II. MATERIAL AND METHODS

2.1 Experiment design and plant material

Experiments were conducted at the University of Nangui Abrogoua (5.38 °N, 4.01°W) in Abidjan, Côte d'Ivoire. A schematic view of the experimental setup is presented in Fig. 1. It consists of four vertical 25 liters tanks and two horizontal bins as hydroponic reactors where the lettuce were grown. Two of the vertical tanks were used to receive raw dishwashing greywater (RGW), whereas the other two tanks received fresh well water (FWW). RGW was collected from the University of Nangui Abrogoua restaurant and FWW from a well nearby the university which is used by local population for gardening. These water sources were used as an irrigation/fertigation source for the production of lettuce. Each hydroponic reactor was made up of six (6) racks. The hydroponic reactors were fed in batch every two days. 50 liters of RGW and FWW were first poured into the receiving tanks, and then transferred into the hydroponic reactors at a controlled flowrate of 50 mL/min. The theoretical hydraulic residence time in the

hydroponic reactors was estimated at 225 min. To determine the effectiveness of the treatment, greywater samples were collected from the entry and exit of each reactor, equipped with an outlet valve.

After 14 days of seeding, lettuces were transferred to the hydroponic reactors. Each reactor accommodated 6 lettuce plants. The lettuce plants measurements were taken at 49 days after planting (DAP). Plant height was measured as the vertical distance between the top of the reactor and the highest living part of the plant. The number of leaves was measured by counting all the leaves on each plant and the mean of the six plants assumed as the number of leaves. Thereafter, the dry weights were obtained by drying the shoot and root for 48 h at 65°C.
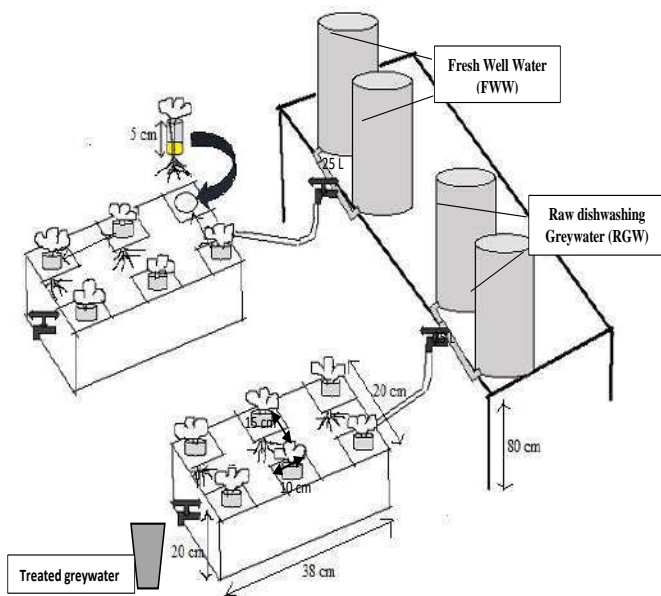

Fig 1. Greywater treatment and lettuce production using hydroponic system

2.2 Characteristics of greywaters

During one month of the experiment, raw greywater (RGW) and treated greywater (TGW) samples were collected every two days, to give a total of 15 samples. The samples were collected in 2.5 L bottles and stored at 4 °C in a thermal box before being transported to the laboratory for analysis. The analyses of pH and Electrical Conductivity (EC) were carried out in situ using a portable device (WTW Multi 350i). Physico-chemical characteristics were evaluated through assessment of ammonia (NH_4^+), nitrate (NO_3^-), and total phosphorus (TP) in raw and treated greywater using spectrophotometer UV visible (DR 6000). Total Suspended Solids (TSS), Chemical Oxygen Demand (COD) and 5-days Biochemical Oxygen Demand (BOD_5) were determined from homogenized samples to assess the removal

efficiency of organic parameters. TSS were measured by a gravimetric method using glass microfiber filters Whatman (porosity 0.45 μm). All analyses were performed according to the Standard APHA methods (APHA, 2012).

Escherichia coli (*E. coli*) and fecal coliforms were monitored as indicator bacteria for microbiological pollution assessment. The spread plate method was used after an appropriate dilution of the samples in accordance with the procedure in Standard Methods (APHA, 2012). Chromocult Coliform Agar (Merck KGaA 64271, Darm-stadt, Germany) was used as the culture medium for both *E. coli* and fecal coliforms.

2.3 Assessment of fecal bacteria on lettuce leaves

For fecal bacteria assay, 10 g of lettuce leaves sample was weighed into separate, sterile bottles (6). 1 N NaCl solution was added to each 10 g sample in each bottle and homogenized for 15 min. The homogenised supernatant of 10-1, and appropriate dilutions were made. A suspension of 0.1 mL of the stock solution and of each of the dilutions were inoculated. The Chromocult coliform agar plates were incubated at 37°C to 24 h, and E. coli and fecal coliforms colonies were counted(APHA, 2012).

2.4 Treatment Efficiency

The removal efficiencies of nutrient (NO_3^-, NH_4^+, TP) and organic pollutants (TSS, BOD_5, COD) were determined. The removal rate of each parameters was calculated according to equation (1):

$$P\ (\%) = \frac{(C_0 - C_i)}{C_0} \times 100 \qquad (1)$$

Where C_0 is the initial parameter concentration and C_i is the parameter concentration at the time i. All parameters will be measured using this method for all the tests.

2.5 Data Analysis

The data were processed using Excel and R software (3.0.1 version). Student's t-test was used to compare data sets. Lettuce growth and yield determined at each treatment were compared using one-way ANOVA followed by a post hoc comparison of means using Tukey's post hoc test ($p < 0.05$).

III. RESULTS AND DISCUSSION

3.1 Raw greywater and treated greywater characteristics

The characteristics of RGW and treated greywater (TGW), and fresh well water (FWW) are presented in Table 1. The mean pH was decreased to values from alkaline to neutral from raw greywater (8.14) to treated greywater (7.37). In their study, Maiga et al. (2014) showed that the

dishwashing raw greywater exhibited the mean pH of 9. This finding is explained by the use of potassium hydroxide for cooking in study area. Furthermore, dishwashing activity are carried out using soaps and detergents such as OMO which show an alkaline pH when diluted in water (Ojo and Oso, 2008). Electrical conductivity (EC) decreased from an average of 2028.25 µS/cm in the raw greywater to 1154.67 µS/cm in the treated greywater. The nutrient analysis in raw dishwashing greywater showed that the mean concentration of ammonia (NH_4^+), nitrate (NO_3^-), and total phosphorus (TP) was 8.55, 14.90 and 8.33 mg/L, respectively, and was largely higher as compared to those of treated dishwashing greywater and fresh well water. Del Porto and Steinfeld(2000) have reported nitrogen in greywater originates from ammonia and ammonia-containing cleansing products as well as from proteins in meats, vegetables, protein-containing. Further, dishwashing is the main source of nitrogen in domestic greywater. And, the primary source of phosphorus found in greywater comes from washing detergents, according to Eriksson et al. (2002). On the other hand, the results of pollutant load analysis in the raw greywater showed that the mean values of TSS, COD and BOD_5 was 416.67 mg/L, 346.33 mg/L and 216.47 mg/L, respectively, and was relatively higher as compared those of treated greywater. However, these pollutant loads were less polluted in terms of organic matter pollution than mixed laundry-dishwashing greywater (SS = 1230 mg/L, COD = 3916 mg/L and BOD5 = 1375 mg/L) reported by Maiga et al. (2014). BOD and COD concentrations in greywater strongly depend on the amount of water and products (especially detergents, soaps, oils and fats) used in the household (Morel and Diener, 2006).

As possible indicators for fecal contamination, *E. coli* and fecal coliforms were enumerated. The mean values in the raw dishwashing greywater were from 2.78×10^5 CFU/100 mL, 1.15×10^5 CFU/100 mL for *E. coli* and fecal coliforms, respectively (Table 1). The highest values of up to 2.3×10^7 CFU/100 mL for *E. coli* and up to 3×10^7 CFU/100 mL for fecal coliforms have been reported from dishwashing of household in rural area (Maiga et al., 2014). Fecal contamination in dishwashing greywater is due to contaminated vegetables and raw meat used during the cooking process (Ottosson, 2003). Based on WHO (2006) guideline for greywater reuse for restricted (*E. coli* < 10^5 CFU/100 mL) agricultural irrigation, the results from the present study demonstrated that dishwashing raw greywater and after hydroponics treatment should be microbiologically acceptable. However, for non-restricted (*E. coli* < 10^3 CFU/100 mL) agricultural irrigation, our greywaters are not safe using for irrigation (WHO (2006).

Table 1. Characteristics of Raw greywater, Treated greywater and Fresh well water

Parameters	Raw Greywater n=15	Treated Greywater n=15	Fresh Well Water n = 15
pH	8.14±0.44	7.37±0.34	6.89±0.78
Temperature	25.6±0.65	26.48±1.25	27.18±1.38
EC (µS/cm)	2028.27±926.43	1154.67±435.18	2974±586.79
NH_4^+ (mg/L)	8.55±2.68	1.88±0.47	3.16±1.98
NO_3^- (mg/L)	14.90 3.60	1.79±1.03	8.05±2.13
TP (mg/L)	8.33±2.04	3.63±1.59	5.98±3.48
TSS (mg/L)	416.67±112.58	115.25±50.26	878.15±128.64
COD (mg/L)	346.33±178.16	164.33±88.28	428.12±125.43
BOD_5 (mg/L)	216.47±124.44	77.76±22.35	398.27±107.85
E. coli (CFU/100mL)	$(2.78±2.1)×10^5$	$(2.84±1.02)×10^4$	$(3.46±0.45)×10^5$
Fecal coliforms (CFU/100mL)	$(1.15±1.01×10^5$	$(1.05±0.88)×10^4$	$(2.68± .58)×10^4$

Values are means ± standard deviations, n: number of sample

3.2 Nutrients removal efficiencies of hydroponic system

The average removal efficiencies of nutrients of hydroponic systems are shown in Fig. 2. They are 78 % for ammonia (NH_4^+), 87 % for nitrate (NO_3^-) and 56 % for total phosphorus (TP) during 48 hours. According to the study conducted by Cui et al. (2003), a hydroponic system to treat wastewater with Romaine lettuce removed 66.76% of N and 47.62 % of P. Further, Haddad and Mizyed, 2011) have reported that total nitrogen removal was 34% for cut flowers and vegetables planted in hydroponic channels and 21% for trees planted in hydroponic barrels, and also the average removal efficiencies of total phosphorous was 37%. The different nutrient removal efficiencies could be explained by the combination of physical, chemical and biological processes may take place synchronously in hydroponic wastewater treatment systems (Magwaza et al., 2020). These processes include sedimentation, microbial biodegradation, adsorption, nitrification as well as plant uptake (Haddad and Mizyed, 2011). Regarding the phosphorus, their removal have greatly improved by the presence of bacterial in raw greywater. Indeed, the organic phosphorus is converted by the bacterial activity into mineral phosphorus that can be assimilated by the plants. It was also reported by Shelef et al. (2013), that biological removal of phosphorus was achieved by the combination of cell uptake for growth, macrophytes uptake as well as biofilm growth. On the other hand, the removal efficiency of NO_3^- was higher than those NH_4^+ and TP in this present study. High removal rate of NO_3^- could be attributed to microorganisms break down inorganic nitrogen mostly through the process of denitrification which converts nitrate to nitrogen gas, resulting so in the removal of nitrate (Gebeyehu et al., 2018). Furthermore, the average pH of raw greywater was 8, which is the optimum pH level, to contribute the nutrient removal through ammonification, nitrification and

denitrification in our treatment system. Moreover, the biological nitrogen removal is most efficient at temperatures between 20-25 °C (Lee et al., 2009). These ranges were similar to those found in our study, which support our findings that temperature to favour nutrients removal. However, the contradicts studies have shown that temperature and pH did not have much effect in the reduction of contaminants (nutrients and faecal coliforms) for treatment of wastewater using hydroponic system (Ndulini et al., 2018). According to these authors, the high removal efficiencies of nutrient were probably due to the increase in hydraulic retention time (Ndulini et al., 2018). Indeed, they have reported that the high removal of nutrient, such are ammonia, nitrate, and total phosphorus reached 87%, 99%, and 87% respectively, with raw wastewater using beggar's tick and green amaranth between 24-240 hours.
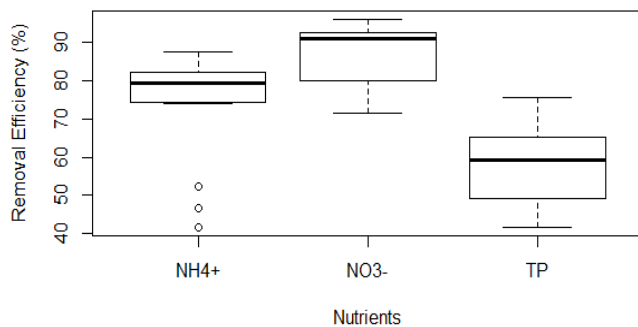

Fig 2. Average removal efficiency of nutrient from raw greywater in a hydroponic system

3.3 Organic pollutants removal efficiencies of hydroponic system

In the present study, the removal efficiencies of the organic pollutants are presented in Fig. 3. Globally, the average removal efficiency of the pollutant organics was higher than 50% during 48 hours (2 days). The removal efficiency were of 67, 53 and 72% for biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), and total suspended solids (TSS), respectively. The results of this study corroborates with the results of Cui et al. (2003), in which a hydroponic system using Romaine lettuce for treating domestic wastewater, and the resulting removal efficiencies were 82.31 % for BOD_5 and 89.42 % for TSS. COD was also reduced, nevertheless, this organic pollutant only presented a percentage of removal around 37.36%. In addition, these authors were also used the water spinach for treating wastewater, and they concluded that hydroponic systems with romaine lettuce showed a good performance in removing organic pollutant compared to the water spinach. Vaillant et al. (2003) achieved depletions of organic matter (BOD_5 = 91%%; COD=82%

and TSS =98%), when treating municipal wastewater using hydroponic system for the growth of *Daturainnoxia* plants. The organic pollutants removal could be attributed by the filtration and adsorption, thus the solids trapped in the root systems are then decomposed and mineralized by bacteria. On the other hand, COD removal were low compared to that of the BOD_5 and TSS, which exhibited very high removal efficiency, likely due to the ability of microorganisms to decompose BOD_5 and TSS. The low COD removal efficiency compared those COD and BOD_5, could be explained by short hydraulic retention time. In that way, the study of Keeratiurai(2013) have shown that the COD removal efficiency was correlated by the hydraulics retention times. The authors reported that the long hydraulics retention times could be increased COD removal efficiency.
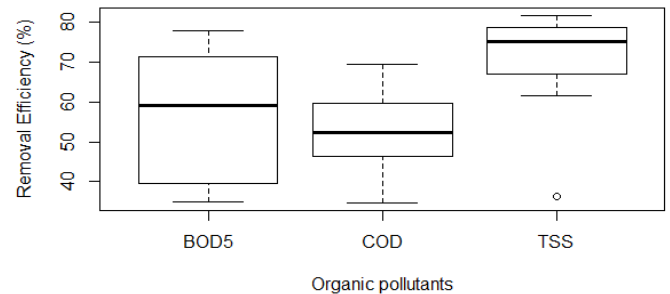
Fig 3. Average removal efficiency of organic pollutants from raw greywater in a hydroponic system.

3.4 Fecal bacteria removal efficiencies of hydroponic system

The average removal efficiencies for the hydroponic system are 81.94 % and 88.68 %, showing 1.00 log units and 1.06 log units for fecal coliforms and *E. coli*, respectively (Fig. 4). The results obtained by Eregno et al. (2017), showed that the lettuce can successfully be produced with treated greywater without posing a substantial health risk for pathogens, while also providing a 5.1 log units. reduction of E. coli in the final effluent. The Hydroponic *Bidenspilosa* L and *Amaranthushybridus* L. production using domestic wastewater has been investigated and found a removal efficiency of 92.77% for faecal coliforms (Ndulini et al., 2018). As constructed wetlands, hydroponic systems are generally considered to feature a combination of biological and physical factors, including mechanical filtration and sedimentation in terms of bacterial removal (Morató et al., 2014). Indeed, plant uptake and absorption to the root could be contributed microbial removal hydroponic cultivation (Ottoson et al, 2005). This could be due the exudation of oxygen through roots increases the concentration of dissolved oxygen in the system, and the elevated level of dissolved oxygen is

reported to have a direct correlation with pathogen reductions (Wu et al., 2016). On the other hand, Moriarty et al. (2019) demonstrated that UV light successfully reduced natural levels of total coliforms by 3 log CFU/mL in aquaponics system. In this way, there is a direct relationship present between the intensity of sunlight that reaches a pathogen and its inactivation rate (Nguyen et al., 2015).
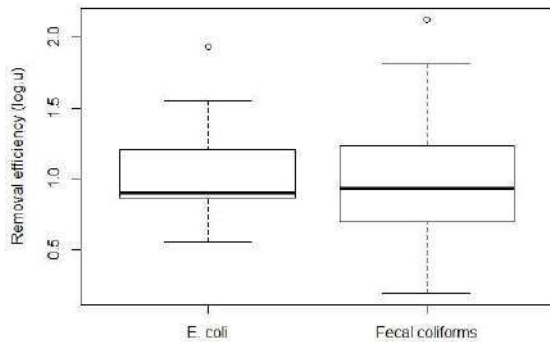

Fig 4. Average removal efficiency of Fecal bacteria from raw greywater in a hydroponic system

3.5 Indicators of fecal bacteria on lettuce leaves

The result shows that no *E. coli* and fecal coliforms were observed in the lettuce leaves samples collected. This indicates that the contamination of the lettuce leaves from the dishwashing greywater is very limited due to the hydroponic irrigation system. This is in accordance with previous reports that there was no detectable E. coli and fecal coliforms in any of the hydroponic lettuce production using treated effluents from a compact WWTP (Keller et al., 2005), and treated post-hydrothermal liquefaction wastewater (Jesse et al., 2019). Same results were obtained by Eregno et al. (2017), who are no *E. coli* found in the lettuce biomass with treated greywater using hydroponic system. This is probable due for the fact that hydroponic cultivation allows different forms of technique such as water culture, drip irrigation technique, which are capable at reducing risks compared to field applications (Oyama et al., 2008). Contrary to leaves, the plant roots with the organic matter provide a large surface for these bacteria (Keller et al., 2005). This indicate that great care must be taken at the moment of harvesting to avoid contamination of leaves with the effluent.Globally, the hydroponic system is particularly suitable for agricultural reuse of wastewater because it minimizes the health risks to workers, harvested crop and consumer that occur through contact with wastewater (Qadir et al., 2010).

3.6 Growth and yield of lettuce from hydroponic systems

The growth (number of leaves and height) and yield (dry matter) of lettuce plant are shown in Table 2. The lettuce irrigated with raw greywater (RGW) possessed higher number of leaves (22), dry matter (97.58 g) and taller (15.50 cm) than the ones irrigated with the fresh well water (FWW). Indeed, the number of leaves and dry yield from RGW were significantly higher than those FWW. There was not a significant difference in lettuce plant height between RGW (15.50 cm) and FWW (12.32 cm). The highest lettuce plant yield with raw greywater using hydroponic system could be attributed to the nutrients solution sourced from greywater/wastewater are considered quick release fertilizers because the nutrients are present as dissolved ionic form and thus directly available for plant uptake (Magwaza et al., 2020). Nevertheless, Tomasi et al. (2015) showed that several important factors (i.e. pH, nitrogen forms) are considered when using treated wastewater as nutrient solutions in wastewater hydroponic system. Contrary to our study, wastewater using hydroponic system showed a significant difference reduction in fresh and dry weight weights compared to those treated with water supply and mineral fertilizers, and effluent treated + mineral fertilizers or /and Hoagland nutrient solution (Carvalho et al., 2018). Globally, several studies reported that wastewaters are used in hydroponic systems as nutrient solutions, provides nutrients needed for the development and growth of fruit-producing plant and vegetables that have commercial value in the market (Jin et al., 2020).

Table 2: Effects of RGW and FWW irrigations on the growth and yield of lettuce

Water irrigation	Number of leaves	Plant height (cm)	Dry matter (g)
RGW	22a	15.50a	97.58a
FWW	13b	12.32a	63.17b

IV.   CONCLUSION

The results obtained in this research has shown suitability of lettuce plant (*Lactuca sativa* L.) for removal of nutrients, pollutant organics, and fecal bacteria in the untreated dishwashing greywater hydroponic system, in addition to production of valuable biomass. Indeed, the average removal efficiencies for ammonia (NH_4^+), nitrate (NO_3^-), and total phosphorus (TP) were 78 %, 87 % and 56 %, respectively. The removal efficiency of the pollutant organics was higher than 50% for TSS, COD and BOD_5. Further, the average removal efficiencies for the hydroponic system are 88.68 % and 81.94 % for *E. coli*, and fecal coliforms, respectively. The results also

indicated that no E. coli and fecal coliforms were observed in the lettuce leaves samples collected. On the other hand, the lettuce irrigated with dishwashing raw greywater possessed higher number of leaves (22), shoot dry matter (97.58 g) and taller (15.50 cm) than the ones irrigated at the fresh well water. The effluent after passing through this hydroponic system met the guidelines for open irrigation/ disposal or further use. The hydroponic system proved to be a method of greywater treatment and offer an useful for plants growth and help to reduce fertilizer needs. The process requires little technical knowledge to operate and can be utilized easily, and it is also very cost-effective for use semi-urban, and rural areas of developing countries.

REFERENCES

[1] American Public Health Association (APHA) (2012). *Standard methods for the examination of water and wastewater.* 22th ed. Washington (DC):American Public Health Association/ American WaterWorks Association/Water Environment Federation.

[2] Barbosa, G.L., Gadelha, F.D.A., Kublik, N., Proctor, A., Reichelm, L., Weissinger, E., Wohlleb, G.M., and Halden, R.U. (2015). Comparison of land, water, and energy requirements of lettuce grown using hydroponic vs. conventional agricultural methods. *International Journal of Environmental Research and Public Health,* 12, 6879–6891.

[3] Bawiec, A. (2019). Efficiency of Nitrogen and Phosphorus Compounds Removal in Hydroponic Wastewater Treatment Plant. *Environmental Technology,* 40 (16), 2062-2072.

[4] Candela, L., Fabregat, S., Josa, A., Suriol, J., Vigués, N. and Mas, J. (2007). Assessment of soil and groundwater impacts by treated urban wastewater reuse. A case study: Application in a golf course (Girona, Spain). *Science of the Total Environment,* 374, 26–35. DOI: 10.1016/j.scitotenv.2006.12.028

[5] Carvalho, R.S.C., Bastos, R.G., and Souza, C.F. (2018). Influence of the use of wastewater on nutrient absorption and production of lettuce grown in a hydroponic system. *Agricultural Water Management*, 203 (30), 311–321.

[6] Cui, L.H., Luo, S.M., Zhu, X.Z., and Liu, Y.H. (2003). Treatment and utilization of septic tank effluent using vertical flow constructed wetlands and vegetable hydroponics. *Journal of Environmental Sciences,* 15 (1), 75–82. PMID: 12602607.

[7] Del Porto, D. and Steinfeld, C. (2000). *What about Graywater, The composting toilet system book Massachusetts* The Center for Ecological Pollution Prevention (CEPP), Concord, Massachusetts. pp. 167–193.

[8] Eregno, F.E., Moges, M.E., and Heistad, A. (2017). Treated greywater reuse for hydroponic lettuce production in a green wall system: Quantitative health risk assessment. *Water,* 9, 454. DOI: 10.3390/w9070454.

[9] Eriksson, E., Auffarth, K., Henze, M., and Ledin, A. (2002). Characteristics of grey wastewater. *Urban Water,* 4, 85–104. DOI :10.1016/S1462-0758(01)00064-4.

[10] Food and Agricultural Organization FAO. 2010. Growing Greener Cities. http://www.fao.org/ag/agp/greenercities/

[11] Gebeyehu, A., Shebeshe, N., Kloos, H., and Belay, S. (2018). Suitability of nutrient removal from brewery wastewater using a hydroponiv technology with Typhalatifolia. *BMC Biotechnology*, 18, 174. DOI: 10.1186/s12896-018-0484-4.

[12] Grady C. P. L., Jr., Daigger G. T., Love N. G., Filipe C. D. M. (2011). *Biological Wastewater Treatment:* Third Edition, London: IWA Publishing, CRC Press, 1022 p. https://doi.org/10.1201/b13775

[13] Haddad, M. and Mizyed, N. (2011). Evaluation of various hydroponic techniques as decentralized wastewater treatment and reuse systems. *International Journal of Environmental Studies,* 68 (4), 461-476.

[14] Hernandez Leal, L., Temmink, H., Zeeman, G., and Buisman, C.J.N. (2011). Characterization and anaerobic biodegradability of grey water. *Desalination,* 270, 111–115. DOI: 10.1016/j.desal.2010.11.029

[15] Jaramillo, M.F. and Restrepo, I. (2017). Wastewater Reuse in Agriculture: A Review about Its Limitations and Benefits. *Sustainability,* 9, 1734. DOI :10.3390/su9101734.

[16] Jesse, S.D., Zhang, Y., Margenot, J.A., and Davidson C. P. (2019). Hydroponic Lettuce Production Using Treated Post-Hydrothermal Liquefaction Wastewater (PHW). Sustainability 11, 3605. DOI: 10.3390/su11133605.

[17] Jin, E., Cao, L., Xiang, S., Zhou, W., Ruan, R., and Liu, Y., (2020). Feasibility of using pretreated swine wastewater for production of water spinach (Ipomoea aquatic Forsk.) in a hydroponic system. Agricultural Water Management 228, 105856. DOI: 10.1016/j.agwat.2019.105856.

[18] Keeratiurai, P. (2013). Efficiency of wastewater treatment with hydroponics. *RPN Journal of Agricultural and Biological Science,* 8 (12), 801– 805.

[19] Keller, R., Perim, K., Semionato, S., Zandonade, E., Cassini, S., and Gonçalves, R.F. (2005). Hydroponic cultivation of lettuce (*Lactuca sativa*) using effluents from primary, secondary and tertiary + UV treatments. *Water Science & Technology Water Supply*, 5 (1), 95–100.

[20] Lee, C., Fletcher, T.D., and Sun, G. (2009). Nitrogen removal in constructed wetlands systems. *Engineering in Life Sciences,* 9 (1), 11–22. DOI: 10.1002/elsc.200800049.

[21] Li, F., Wichmann, K., and Otterpohl, R. (2009). Review of the technological approaches for grey water treatment and reuses. *Science of the Total Environment*, 407(11), 3439–3449.

[22] López-Chuken, U.J. (2012). *Hydroponics and environmental clean-up. In: Asaso, T. (Ed.), Hydroponics – A Standard Methodology for Plant Biological Researches.* In Tech press, Croatia. 181-198. ISBN: 978-953-51-0386-8.

[23] Magwaza, T.S., Magwaza, S.L., Odindo, O.A., and Mditshwa, A. (2020). Hydroponic technology as decentralized systemfor domestic wastewater treatment and vegetable production in urban agriculture: A review.

Science of the Total Environment, 698, 134–154. DOI: 10.1016/j.scitotenv.2019.134154.

[24] Maiga, Y., Moyenga, D., Ushijima, K., Sou, M., and Maiga, A.H. (2014). Greywater Characteristics in Rural Areas of Sahelian Region for Reuse Purposes: The Case of Burkina Faso. *Revue des Sciences de l'Eau*, 27, 39–54. DOI :10.7202/1021981ar.

[25] Maiga, Y., Ndiaye A., Sangare, D., Bitié, E., and Ushijima, K. (2018). Effect of Slanted Soil Design and Filter Media Distribution on the Removal of Fecal Bacteria and Organic Matter from Greywater. *International Journal of Current Microbiology and Applied*, 7 (7), 2317–2329.

[26] Martinez-Mate, M.A., Martin-Gorriz, B., Martínez-Alvarez, V., Soto-García, M., and Maestre-Valeroa, J.F. (2018). Hydroponic system and desalinated seawater as an alternative farm-productive proposal in water scarcity areas: Energy and greenhouse gas emissions analysis of lettuce production in south east Spain. *Journal of Cleaner Production*, 172, 1298–1310. DOI: 10.1016/j.jclepro.2017.10.275.

[27] Marzougui N., Sabbahi S., Guasmi F., Hammami A., Haddad M. and Rejeb S., P. (2018). Effects of Wastewater quality on Henna (Lawsoniainermis L.) germination and plant growth: a case study, Tunisia. *International Journal of Environment, Agriculture and Biotechnology*, 3 (1), 147-157.

[28] Moosavirad, S. M. (2017). Treatment and operation cost analysis of greywater by electrocoagulation and comparison with coagulation process in mining areas. *Separation Science and Technology*, 52 (10), 1742-1750. DOI: 10.1080/01496395.2016.1274761.

[29] Morató, J., Codony, F., Sánchez, O., Pérez, L. M., García, J., and Mas, J. (2014). Key design factors affecting microbial community composition and pathogenic organism removal in horizontal subsurface flow constructed wetlands. *Science of The Total Environment,* 481, 81–89. doi: 10.1016/j.scitotenv.2014.01.068.

[30] Morel, A. and Diener, S. (2006). *Greywater management in low and middle-income countries, review of different treatment systems for households or neighbourhoods.* Dübendorf: Swiss Federal Institute of Aquatic Science and Technology (Eawag), 96p.

[31] Moriarty, M.J., Semmens, K., Bissonnette, G.K., and Jaczynski, J. (2019). Internalization assessment of E. coli O157:H7 in hydroponically grown lettuce. *LWT - Food Science and Technology*, 100, 183–188. DOI: 10.1016 / j.lwt.2018.10.060.

[32] Ndulini, S.F., Sithole, G.M., Mthembu, M.S. (2018). Investigation of nutrients and faecal coliforms removal in wastewater using a hydroponic system. *Physics and Chemistry of the Earth,* 106, 68–72. DOI: 10.1016/j.pce.2018.05.004.

[33] Nguyen, M.T., Jasper, J.T., Boehm, A.B., and Nelson, K.L. (2015). Sunlight inactivation of fecal indicator bacteria in open-water unit process treatment wetlands: modeling endogenous and exogenous inactivation rates. *Water Research*, 83, 282–292.

[34] Ojo, O.A. and Oso, B.A. (2008). Isolation and characterization of synthetic detergent degraders from wastewater from wastewater. *African Journal of Biotechnology*, 7, 3756–3763.

[35] Ottoson, J., Norstrom, A., and Dalhammar, G. (2005). Removal of micro-organisms in small-scale hydroponics wastewater treatment system. *Letters in Applied Microbiology,* 40, 443–447.

[36] Ottosson, J. (2003). *Hygiene aspects of greywater and greywater reuse*. Stockholm, Royal Institute of Technology, Department of Land and Water Resources Engineering. Stockholm, Sweden, 50 p.

[37] Oyama, N., Nair, J., Ho, and G.E. (2008). *Comparison of pathogen die- off patterns of tomatoes grown in two hydroponic systems.* 1st International Conference on Technologies & Strategic Management of Sustainable Biosystems. Fremantle, pp. 6–9.

[38] Palada, M.C. (1999). *Crossman, S.M.A. Evaluation of tropical leaf vegetables in the Virgin Islands.* In: Janick J, ed. Perspectives on new crops and new uses. ASHS Press, Alexandria, VA. pp.388–393.

[39] Prazeres, A. R., Albuquerque, A., Luz, S., Jerónimo, E. and Carvalho, F. (2017). Hydroponic System: A Promising Biotechnology for Food Production and Wastewater Treatment. *Food Biosynthesis*, 317–350. DOI :10.1016/B978-0-12-811372-1.00011-7.

[40] Qadir, M., Wichelns, D., Raschid-Sally, L., McCornick,P.G., Dreschel, P., Bahri, A., and Minhas, P.S. (2010). The challenges of wastewater irrigation in developing countries. *Agricultural Water Management*, 97, 561–568. DOI: 10.1016/j.agwat.2008.11.004.

[41] Rodda, N., Salukazana, L., Jackson, S.A.F., and Smith, M.T. (2011). Use of domestic greywater for small-scale irrigation of food crops: Effects on plants and soil. *Physics and Chemistry of the Earth,* 36, 1051–1062.

[42] Sangare, D., Sawadogo, B., Sou/Dakoure, M., Ouedraogo, M. D., Hijikata, N., Yacouba, H., Coulibaly, L. and Funamizu, N. (2018). Short Term Effects of Treated Greywater by High Rate Algal Ponds Process on Vegetable Yield and SoilProperties under Sudano-Sahelian Climate. *Environmental Progress & Sustainable Energy,* 37 (1), 465–470. DOI: 10.1002/ep.12658.

[43] Shelef, O., Gross, A., and Rachmilevitch, S. (2013). Role of Plants in a Constructed Wetland. Current and New Perspectives. *Water*, 5 (2), 405–419. DOI: 10.3390/w5020405.

[44] Tomasi, N., Pinton, R., Costa, L.D., Cortella, G., Terzano, R., Mimmo, T., Scampicchio, M., and Cesco, S. (2015). New 'solutions' for floating cultivation systems of ready to eat salad: A review. *Trends in Food Science & Technology,* 46, 267–276. DOI: 10.1016/j.tifs.2015.08.004.

[45] Tsao, D. (2003). Overview of phytotechnologies. Advances in Biochemical Engineering/Biotechnology. 78, 1–50.

[46] Vaillant, N., Monnet, F., Sallanon, H., Coudret, A., and Hitmi, A. (2003). Treatment of domestic wastewater by a hydroponic NFT system. *Chemosphere,* 50, 121–129.

[47] WHO (2006). *Guidelines for the safe use of wastewater, excreta and greywater, Vol. 4, Excreta and Greywater Use in Agriculture.* WHO Press, Geneva, Switzerland, 204 p.

[48] Wu, S., Carvalho, P.N., Müller, J.A., Manoj, V.R., and Dong, R. (2016). Sanitation in constructed wetlands: a review on the removal of human pathogens and fecal indicators. *Science of the Total Environment,* 541, 8–22.

Article   *Peer-Reviewed Journal*

Adding Functional Foods to Fermented Snake Melon (*Cucumis melo* var. *flexuosus*) to Increase Consumption and Reduce Crop Loss

Lynn Sleiman[1], Ali Alkhatib[1], Sami Tlais[2], Hassan Hajj Hussein[2*]

[1]Department of Nutrition and Food Science, Lebanese International University, Lebanon
[2]Department of Biological and Chemical Sciences, Lebanese International University, Lebanon
*Corresponding author. Email address: hassan.hajjhussein@liu.edu.lb

Abstract—Snake melon (Cucumis melo var. flexuosus) is a member of the melon family. It is a seasonal fruit that is high in water and low in calories, fat, and cholesterol. It is a high-yielding crop that has a short shelf-life, which results in the loss of a high percentage of the annual yield. In order to reduce the percentage of lost crop, we proposed several new recipes for making pickled snake melon by adding functional foods to enhance the flavor and nutritional values of the pickled snake melon product while evaluating consumer acceptance using sensory evaluation. The different samples of snake melon pickles were analyzed for their sensory attributes (color, odor, texture, taste, saltiness, sourness, and overall acceptability) using a Likert scale ranging from 1 "least liked" to 5 "most liked". While none of the tested recipes were preferred over the control, four of the new recipes were similar to the control in overall acceptability indicating a clear potential for incorporating functional foods to increase diversification of snake melon pickles and in essence increase the potential for snake melon consumption while reducing crop loss.

Keywords—Snake melon, Armenian cucumber, Mekte, Cucumis melo, functional foods.

I. INTRODUCTION

The snake or Armenian cucumber (*Cucumis melo* var. *flexuosus*), also known as snake melon, is a member of the melon family [1]. It is a type of long, curved, slender fruit which resembles and tastes like cucumber. It is known as Fakous in North Africa and some Arabic countries, Alficoz in Spain, Tortarello, Citrangolo, or Cucummaru in Italy, Hiti in Turkey, Tirozi in Kurdistan, Uri in Japan, and Mekte in Lebanon and Syria [2]. It originated in the Mediterranean basin, North Africa, Middle East, and Anatolia [3]. It is grown in house gardens as well as large scale farms [4].

Snake melon is an ancient crop that flourishes in sunny and warm climates [4]. It is characterized by a variety of external colors, which can be light green, dark green, or a mixture of both [2]. Other differences can be detected by the presence or absence of pubescence on the skin and by

the longitudinal ribbing on the fruits, which ranges from very shallow to very deep [2]. It is used in the same applications as cucumber and therefore is considered an alternative to cucumber in many parts of the world [3]. Snake melon seeds contain 15.57% protein, 16.19% carbohydrate, and 5.69% ash. It is a good source of oil since the seed is composed of 22.33% oil. Snake melon seed oil is characterized by its odor, good color, good appearance, and good physicochemical properties [5].

Snake melon is characterized by a high yield and a short shelf-life [3]. According to the Biotechnology Research Institute of the Iranian Ministry of Agriculture, more than 17 % of the 106 million tons of snake melon produced in Iran became unusable in different stages from production to supply, and 7% perished in stores due to inappropriate packaging and unsuitable storage methods [1].

There is an increase in public interest in functional foods due to their positive impact on health [6]. Functional foods are foods or food ingredients that benefit the health in more than the nutrients it contains [6]. Garlic is characterized by the highest fructan fractions compared to many fruits and vegetables and could be considered as a potential source of prebiotics to be used to functional food products [7]. Garlic contains high amounts of sulfur-containing compounds compared to onions, broccoli, cauliflower and apricots [8]. It was suggested that incorporating garlic into food products such as bakery and meat products rendering them the characteristics of functional foods [9]. Beetroot (Beta vulgaris rubra) could be considered as functional food because of its content of betalains and phytochemicals [10].Jalapeno pepper (Capsicum annuum L) is one of the key foods in the Mexican Diet. Its high concentrations of antioxidants and functional compounds gives it a significant role in human health [11]. Black pepper (Piper nigrum) contains piperine that inhibits lipid peroxidation and it improved the memory impairment and neurodegeneration in hippocampus [12]. Also, its rich phytochemistry includes volatile oil, oleoresins, and alkaloids. Its antioxidant, antimicrobial potential, and gastro-protective modules make it a significant healthy food [13]. Ginger (Zingiber oficinale) is a potential functional food and its addition to food may reduce chronic diseases and mineral deficiency [14]. It is considered as a rich source of raw fibers, phenolic compounds, organic acids, terpenes, lipids, and polysaccharides. Its content of phenolic compounds such as shogaols and gingerols contributes to its health benefits [15].

The objective of this study is to develop new recipes of snake melon pickles in an effort to decrease food wasting by adding functional foods to enhance the flavor and nutritional values of the pickled snake melon product while evaluating consumer acceptance using sensory evaluation with emphasis on odor, color, mouth texture, taste, saltiness, sourness, and overall acceptability. This will help in creating additional ways of consuming an otherwise perishable product while introducing new products on supermarket shelves to accommodate the ever-growing complexities of consumer palates.

II. METHODS AND MATERIALS

2.1 Materials

In September, fresh snake melon fruits of equal maturity were collected from the fields of Bednayel, Lebanon. Any damaged, bruised, or infected fruits were discarded. Pickling salt, white crystalized sugar, vinegar (5% acetic acid), packaged and sealed spices, and garlic were purchased from the local market.

2.2 Sample preparation

Nine recipes for snake melon pickle were standardized (Table 1). Fruits were washed and cut into equally sized pieces to better fit into the pickling jars. Snake melon was added first. Salt and vinegar were mixed with water in a large bowl and used to fill up the glass jars until the fruits were completely covered. Additional flavoring ingredients were added to the jars before they were capped tightly and stored at room temperature.

Table 1:Recipes for snake melon pickle including the name of the flavor and amount added

	Snake melon	Salt	Vinegar	Water	Amount of flavoring
CTR	200g	25g	33ml	350ml	
Black Pepper	200g	25g	33ml	350ml	10g
Ginger	200g	25g	33ml	350ml	10g
Garlic	200g	25g	33ml	350ml	10g
Jalapeno Pepper	200g	25g	33ml	350ml	2.5g
Sugar	200g	25g	33ml	350ml	10g
Citric Acid salt	200g	25g	33ml	350ml	2.5g
Chickpeas	200g	25g	33ml	350ml	10g
Red Beets	200g	25g	33ml	350ml	40g

2.3 Sensory analysis

After 20 days of fermentation, the samples were subjected to sensory evaluation. Sensory analysis consisted of describing the organoleptic properties of the samples. Twenty panelists aged 20 to 45 years, who are regular consumers of pickled snake melon, were advised to rate the samples in terms of odor, color, texture in the mouth, taste, saltiness, sourness, and overall acceptability using an interval Likert scale ranging from 1 to 5 (where "1" was "least liked" and "5" was "most liked").

Sample preparation, presentation, temperature, and serving container shape were the same for all tested samples. Samples were served in plastic cups identified by a three-digit code. Mineral water was used as palate cleanser (offered to the panelists between samples). Each sample was blindly analyzed by the sample panelist for a total of three replications.

2.4 Statistical analysis

Data were expressed as mean $\pm$ SEM values from three independent replicates. One way analysis of variance was conducted using GraphPad Prism 8.3.0 and the differences were analyzed by Dunnett's Multiple comparisons test.

III. RESULTS AND DISCUSSION

3.1 Odor

For the test of odor, the panelists evaluated all samples and reported their findings on a Likert scale ranging from 1 to 5 (where "1" was "least liked" and "5" was "most liked").

The panelists did not favor any of the samples above the control in terms of the odor; however, they expressed a significant decrease in the liking of the odor of "Ginger" (3.20±0.17; p<0.01) and "Citric Acid" (3.10±0.15; p<0.01) vs. control (4.00±0.15; Fig. 1). Considering that the panelists were all Lebanese, it is possible that the panelists did not appreciate the taste of ginger since ginger is not a product that is usually used in Lebanese pickled products. However, for some unknown reason, the panelists seemed to find the smell of citric acid salt somewhat off-putting.
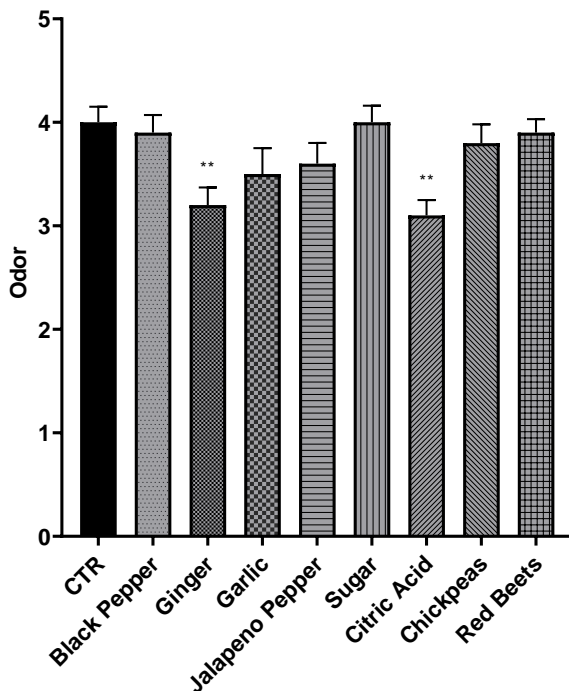
Fig.1: Comparing odor evaluation of all 8 flavors to the control.

3.2 Color

For the test of color, the panelists evaluated all samples and reported their findings on a Likert scale ranging from 1 to 5 (where "1" was "least liked" and "5" was "most liked"). The panelists did not favor any of the samples above the control in terms of the color; however, they expressed a significant decrease in the liking of the color of "Citric Acid" (3.30±0.18; p<0.001) and "Red Beets" (3.60±0.32; p<0.01) vs. control (4.40±0.11; Fig. 2). We tried to mimic the pinkish color of pickled ginger and pickled turnips. While Lebanese are used to pickled turnips being colored pinkish-red, they are not used to seeing pickled snake melon in that color, which probably explains why the panelists reported a decrease in the liking of the pinkish-red colored snake melon pickles compared to the control. In addition, the samples that contained citric acid looked a little more pale and yellow compared to the control.

3.3 Texture

For the test of texture or mouthfeel, the panelists evaluated all samples and reported their findings on a Likert scale ranging from 1 to 5 (where "1" was "least liked" and "5" was "most liked"). The panelists did not report any difference in the texture of any of the eight samples compared to the control (4.30±0.16; Fig. 3).

Fig.2: Comparing color evaluation of all 8 flavors to the control.

3.4 Taste

For the test of taste, the panelists evaluated all samples and reported their findings on a Likert scale ranging from 1 to 5 (where "1" was "least liked" and "5" was "most liked"). The panelists did not favor any of the samples above the control in terms of the taste; however, they expressed a significant decrease in the liking of the taste of "Ginger" (2.90±0.18; p<0.001) and "Citric Acid" (3.30±0.18; p<0.001) vs. control (4.30±0.16; Fig. 4). This is probably due to the same reasons explained in the section about the "Color".

3.5 Saltiness

For the test of saltiness, the panelists evaluated all samples and reported their findings on a Likert scale ranging from 1 to 5 (where "1" was "least liked" and "5" was "most liked"). The panelists did not favor any of the samples above the control in terms of the saltiness; however, they expressed a significant decrease in the liking of the saltiness of "Citric Acid" (3.60±0.15; p<0.05) vs. control (4.30±0.16; Fig. 5). Sourness and saltiness are commonly

called the electrolytic tastes where in both instances small soluble inorganic cations are the stimulants [6]. In our experiment we used sodium citrate as citric acid salt. Since it contains sodium, this would explain why the panelists perceived the sample to be salty and therefore was less liked.
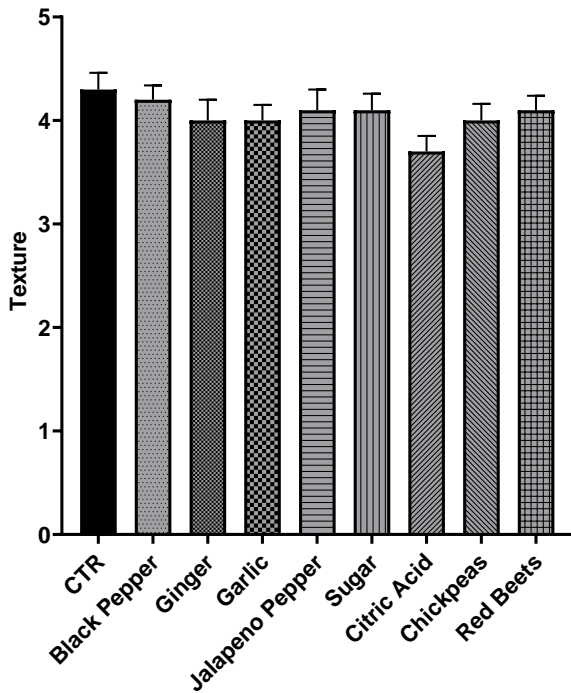
Fig.3: Comparing texture evaluation of all 8 flavors to the control.
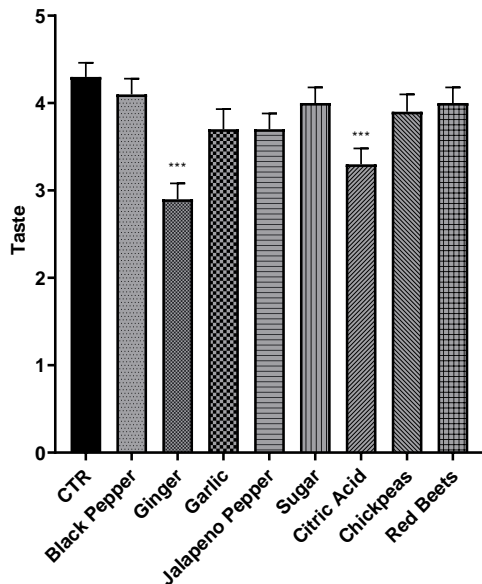
Fig.4: Comparing taste evaluation of all 8 flavors to the control.
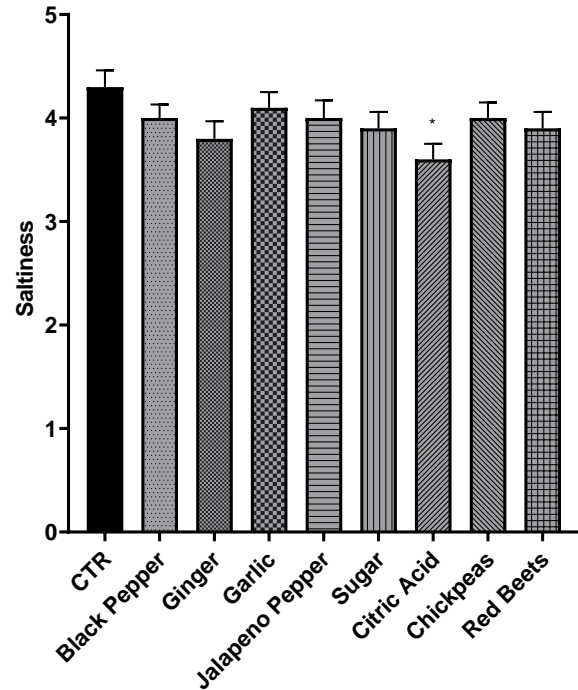
Fig.5: Comparing saltiness evaluation of all 8 flavors to the control.

3.6 Sourness

For the test of sourness, the panelists evaluated all samples and reported their findings on a Likert scale ranging from 1 to 5 (where "1" was "least liked" and "5" was "most liked"). The panelists did not report any difference in the sourness of any of the eight samples compared to the control (4.20 ± 0.16; Fig. 6).
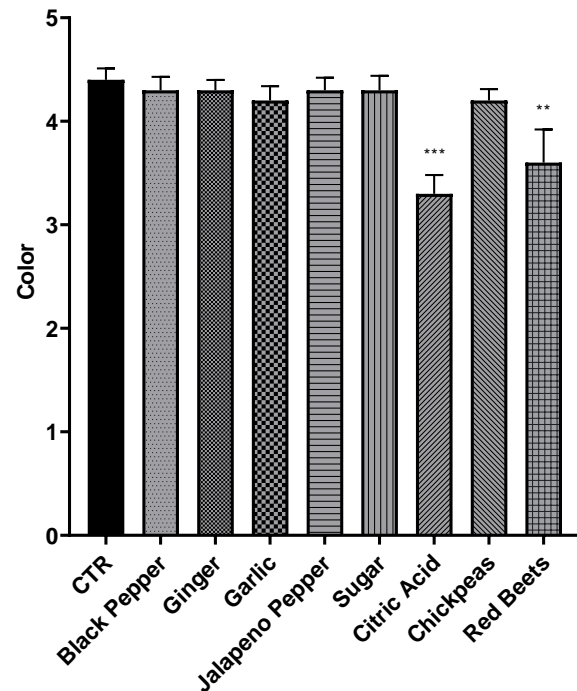
3.7 Overall acceptability

For the test of overall acceptability, the panelists evaluated all samples and reported their findings on a Likert scale ranging from 1 to 5 (where "1" was "least liked" and "5" was "most liked"). The panelists did not favor any of the samples above the control in terms of overall acceptability; however, they expressed a significant decrease in overall acceptability of the "Ginger" (3.30 ± 0.16; $p < 0.001$), "Garlic" (3.60 ± 0.23; $p < 0.01$), "Jalapeno Pepper" (3.70 ± 0.19; $p < 0.01$), and "Citric Acid" (3.40 ± 0.21; $p < 0.001$) vs. control (4.50 ± 0.14; Fig. 7).

3.8 Correlation test

In a Pearson's Correlation test between the different sensory characteristics and overall acceptability, we determined that taste, odor, and texture had the highest correlation with the overall acceptability of the product (94%, 91.5%, and 82.3%, respectively; Table 2). Saltiness had a slightly lower correlation with the overall acceptability of the product (70.9%); however, color and

sourness did not exhibit a significant correlation with the product's overall acceptability.
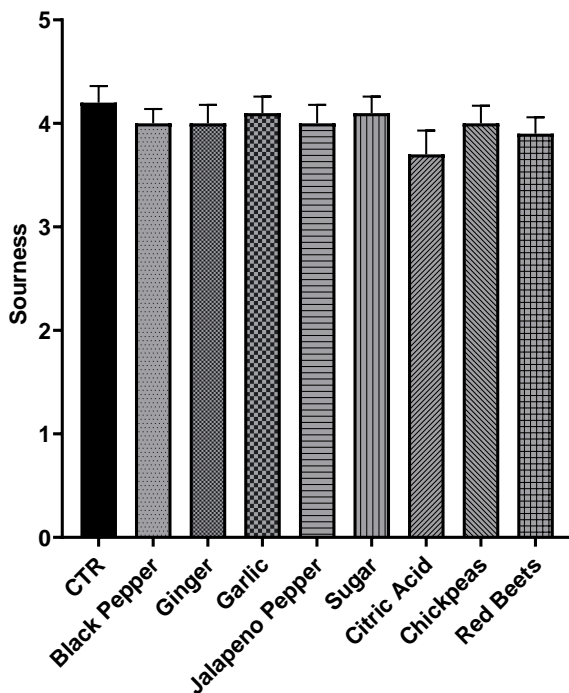
Fig.6: Comparing sourness evaluation of all 8 flavors to the control.
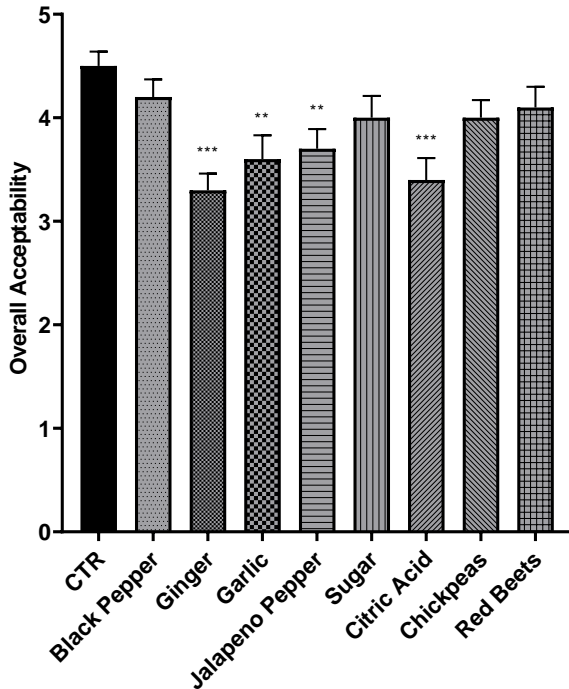
Fig.7: Comparing overall acceptability of all 8 flavors to the control.

Table 2: Pearson's Correlation test between the different sensory characteristics of snake melon pickles

	Color	Texture	Taste	Saltiness	Sourness	Overall Acceptability
Odor	0.478	.876**	.900**	.743*	.674*	.915**
Color		.697*	0.259	.735*	.831**	0.317
Texture			.729*	.880**	.805**	.823**
Taste				.706*	0.508	.940**
Saltiness					.878**	.709*
Sourness						0.505

**: correlation is highly significant (at 0.01 level)
*: correlation is significant (at 0.05 level)

IV. CONCLUSION

Our results indicate a clear and strong positive correlation between taste, odor, and texture with the overall acceptability of snake melon pickle products. The more noticeable off-putting flavors that were used where ginger and citric acid salt, it seemed that both additives did not agree well with the Lebanese palate. Even though it is a common practice to use garlic and green peppers as additives with pickling products, and although the panelists did not report any impact of both additives on the odor, taste, and texture; it was surprising to detect a reduction in the overall acceptability of both garlic and Jalapeno pepper containing samples. It was clear that new snake melon pickle recipes can be made and introduced to the public in an effort to increase consumption of a functional food fermented product and reduce the loss of fresh crops by means of creating new product that has an extended shelf-life.

REFERENCES

[1] Jahanbakhshi, A. (2018). Determination of some engineering properties of snake melon (Cucumis melo var. flexuosus) fruit. *Agricultural Engineering International: CIGR Journal*, 20(1), 171-176

[2] Merheb, J., Pawełkowicz, M., Branca, F., Bolibok-Brągoszewska, H., Skarzyńska, A., Pląder, W., & Chalak, L. (2020). Characterization of Lebanese Germoplasm of Snake Melon (Cucumis melo subsp. melo var. flexuosus) Using Morphological Traits and SSR Markers. *Agronomy*, *10*(9), 1293. https://doi.org/10.3390/agronomy10091293

[3] Abed, M. Y. (2018). Genetic Improvement of Yield and Fruit Traits in Snake Cucumber (Cucumis melo var. flexuosus L.) by Individual Selection. *Asian Journal of Biotechnology and Genetic Engineering*, *1*(2), 1-10. Retrieved from https://www.journalajbge.com/index.php/AJBGE/article/view/426

[4] Paris, H. (2011). Semitic-language records of snake melons (Cucumis melo, Cucurbitaceae) in the medieval period and the "piqqus" of the "faqqous". *Genetic Resources and Crop Evolution*, *59*(1), 31-38. https://doi.org/10.1007/s10722-011-9664-y

[5] Mariod, A., Mirghani, M., & Hussein, I. (2017).Unconventional Oilseeds and Oil Sources: *Cucumis melo var. flexuosus The Armenian Cucumber or Snake Melon, (*pp.85-88), 10.1016/B978-0-12-809435-8.00015-9

[6] Milner, J. A. (2000). Functional foods: the US perspective. *The American Journal of Clinical Nutrition*, 71(6), 1654S-1659S. https://doi.org/10.1093/ajcn/71.6.1654s

[7] Jovanovic-Malinovska, R., Kuzmanova, S., & Winkelhausen, E. (2014). Oligosaccharide Profile in Fruits and Vegetables as Sources of Prebiotics and Functional Foods. *International Journal of Food Properties*, 17(5), 949–965. https://doi.org/10.1080/10942912.2012.680221

[8] Shi, J., Mazza, G., & Maguer, L. M. (2002). Functional Foods: Biochemical and Processing Aspects, Volume 2 (*Functional Foods and Nutraceuticals*) (1st ed.). CRC Press.

[9] Mazza, G. (1998). Functional Foods: Biochemical and Processing Aspects, Volume 1 (*Functional Foods and Nutraceuticals*) (1st ed.). CRC Press.

[10] Ninfali, P., & Angelino, D. (2013). Nutritional and functional potential of Beta vulgaris cicla and rubra. *Fitoterapia*, 89, 188–199. https://doi.org/10.1016/j.fitote.2013.06.004

[11] Moreno-Escamilla, J. O., de la Rosa, L. A., López-Díaz, J. A., Rodrigo-García, J., Núñez-Gastélum, J. A., & Alvarez-Parrilla, E. (2015). Effect of the smoking process and firewood type in the phytochemical content and antioxidant capacity of red Jalapeño pepper during its transformation to chipotle pepper. *Food Research International*, 76, 654–660. https://doi.org/10.1016/j.foodres.2015.07.031

[12] Chonpathompikunlert, P., Wattanathorn, J., & Muchimapura, S. (2010). Piperine, the main alkaloid of Thai black pepper, protects against neurodegeneration and cognitive impairment in animal model of cognitive deficit like condition of Alzheimer's disease. *Food and Chemical Toxicology*, 48(3), 798–802. doi:10.1016/j.fct.2009.12.009

[13] Butt, M. S., Pasha, I., Sultan, M. T., Randhawa, M. A., Saeed, F., & Ahmed, W. (2013). Black Pepper and Health Claims: A Comprehensive Treatise. *Critical Reviews in Food Science and Nutrition*, 53(9), 875–886. https://doi.org/10.1080/10408398.2011.571799

[14] Trinidad, T.P., Sagum, R.S., de Leon, M.P., Mallillin, A.C., & Borlagdan, M.P. (2012). Zingiber Officinale and Curcuma Longa as Potential Functional Foods/Ingredients. *Food and Public Health*, 2(2), 1–4. https://doi.org/10.5923/j.fph.20120202.01

[15] Mao, Q. Q., Xu, X. Y., Cao, S. Y., Gan, R. Y., Corke, H., Beta, T., & Li, H. B. (2019). Bioactive Compounds and Bioactivities of Ginger (Zingiber officinale Roscoe). *Foods*, 8(6), 185. https://doi.org/10.3390/foods8060185

[16] Chang, R.B., Water, H., & Liman, E.R. (2010). A proton current drives action potentials in genetically identified sour taste cells. *PNAS*, 107(51): 22320-22325. https://doi.org/10.1073/pnas.1013664107

Utilization of Signal Global Navigation Satellite System (GNSS) for Meteorological

Syachrul Arief

Geospatial Information Agency Indonesia
Email: syachrul.arief@big.go.id

Abstract— In this study, I show that microwave signals emitted from satellites and received by the GNSS system can be utilized for meteorological purposes, such as heavy rain in Indonesia, using open-source software goGPS. Comparison of the gradient of the North and East atmospheric delays between goGPS, IGS, and UNR. The correlation of the results of goGPS with those from IGS and UNR is relatively high. The gradient of the late goGPS and IGS, and UNR products show good consistency. This means that the process of goGPS is good enough to get an accurate value. The standard deviation and RMS difference between goGPS and IGS are 16.99 and -1.32 (in mm), respectively. The ones for the goGPS-UNR pair are 18.33 and -1.08. goGPS software works very well at different times and stations. This means that the goGPS software package can be an alternative to commercial software systems for ZTD estimation. The correlation of PWV values from GPS and radiosonde for the dry and rainy seasons are 0.84 and 0.73, respectively. PWV values during this dry period show a downward trend and an upward trend during the wet season period.

Keywords— Tropospheric, PWV, Signal, GNSS.

I. INTRODUCTION

As is generally known, the primary function of GNSS is to determine the position, speed, and time information in almost all places on earth, at any time and in any weather conditions. GNSS satellites broadcast microwave signals to GNSS receivers near the earth's surface to obtain position information. Then from the microwave signal data, much information is obtained through the process and extraction stages following the needs and objectives of the study.

This study aims to show that microwave signals from GNSS satellites can be used for meteorological purposes. Examples of meteorological events in this study are the heavy rain conditions in several places in Indonesia.

From this microwave signal, I process and extract it to get tropospheric delay information. From this tropospheric data, I convert it into Precipitation water vapor (PWV). PWV represents the condensation conditions of water vapor in the column in a particular area. This study in Indonesia is the first report that the GNSS microwave signal process is used to obtain information related to heavy rain conditions by using open-source software. Therefore this study can be a value-added use of microwave signals from GNSS for meteorological events.

II. DATA DAN METHOD

PWV Retrieved from GPS

The GNSS data's primary format in this study is the Receiver Independent Exchange (RINEX) format. In the first station, I use the BAKO station managed by BIG / BAKOSURTANAL, and I use, as the second station, the JOG2 station, which is managed by GFZ (Geoforschungs Zentrum), the German Geo-research Center. As the period of this study, I use days of the year (DOY) 022-025, 22-25 January, and DOY 230-233, August 17-20, 2018. The

former represents the rainy season, and the latte represents the dry season. Next, I process RINEX files to estimate tropospheric parameters using goGPS, an advanced open-source GNSS software, version 1.0 Beta, from Geomatics Research and Development s.r.l. - Lomazzo, Italy [Realini, 2009].

The leading software for processing RINEX data, goGPS. [Realini, 2009] is an open-source software initially developed by Dr. E. Realini in 2007 at the Geomatics Laboratory of the Politecnico di Milano, Como Campus. It is specifically designed to improve the low-cost positioning accuracy of GNSS devices by relative positioning and the Kalman filtering technique. The goGPS code was distributed online as free and open-source software in 2009. The project has been open to collaboration since publication. It has received supports and code contributions from users working in both academy and business companies in different countries (including Italy, Japan, Switzerland, Spain, and Germany). Strategies for processing RINEX data with goGPS are as shown below,

1. Constellation: multi GNSS

2. Processing Technique: Precision Point Positioning (PPP)

3. Elevation cut-off angle: 7°

4. Processing rate: once in 5 minutes

5. Mapping functions: the Niell mapping functions (NMF)

6. Ionosphere modeling: ionosphere-free linear combination

7. Dynamic model: static

8. Frequency: L1, L2

9. Types of observation data: code and phase

Accurate point positioning is used as a post-processing method for determining absolute position using stand-alone (thus un-differenced) GNSS receivers. GNSS PPP processing uses the ionosphere-free combinations of dual-frequency pseudoranges and carrier-phase observations. The preference for PPP instead of double-difference (DD) processing arises from the benefit that (1) each GNSS station can be processed independently and that (2) Continuous observation of the same satellite at two stations is not required (Yuan et al. 2014). To compare and validate the ZTD values, I estimated with goGPS. I used two international tropospheric products from International GNSS Services (IGS) with commercial software version 5 of the Bernese software. The other products from the University of Nevada Reno (UNR) were obtained using the GIPSY / OASIS II software from

NASA Jet Propulsion Laboratory (JPL) [Blewitt et al., 2018].

III. RESULT AND DISCUSSION

In this section, the process and extraction of the GNSS microwave signal in the discussion below I compare with other parameters and a validation test of the accuracy of both the opensource software and the value of the process result obtained.

A. Comparison of the estimated tropospheric delay gradients with other products

Here I compare estimated atmospheric delay gradient vectors using the data from the JOG2 station on August 20, 2018, with those from other products, namely those from IGS and UNR. The correlation of the goGPS and IGS delay gradient values for the north is 0.47 and 0.41 for the east, while the correlation between the goGPS and UNR delay gradient values is 0.71 for the north and 0.75 for the east. I plot them in Figure 1.
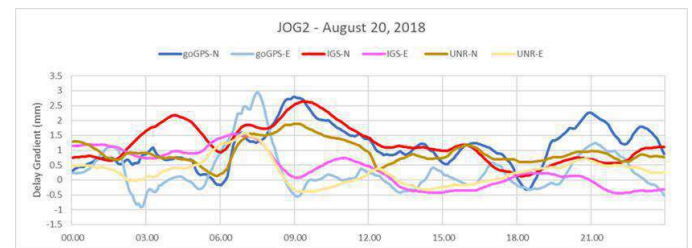
Fig.1: Comparison of North and East atmospheric delay gradient between goGPS, IGS, and UNR. The correlation of goGPS results with those from IGS and UNR is relatively high

The goGPS delay gradients, and the IGS and UNR products show good consistency. This means that the process on goGPS is good enough to get accurate values of not only the ZTD but also the delay gradients. Therefore, in this research, I estimate ZTD using goGPS software together with the tropospheric delay gradients

B. ZTD range value from goGPS

First, I process data from the BAKO and JOG2 stations from 22 to January 25, 2018. The rate of atmospheric parameter estimation is every 5 minutes, and so we get 288 values for one day (24 hours). From these 288 data, I get the smallest, the most significant, and the average values, as presented in Figure 2.

Next, I carried out the same process from 18 to August 21, 2018. The results in Figure 3. The ZTD value from 22 to January 25, 2018 (rainy season) at BAKO stations tends to be higher than 18 to August 21 (dry

season) 2018. Likewise, at the JOG2 station, ZTD in January 2018 is higher than those in August.
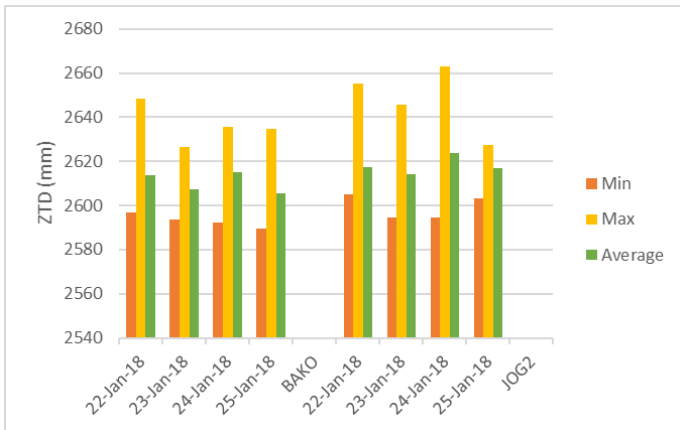
Fig.2: Minimum, maximum, and the average ZTD values for 22-25 Jan 2018 at BAKO and JOG2 stations
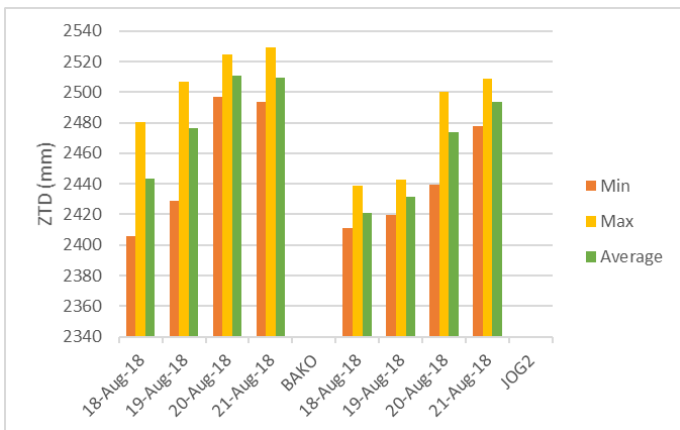
Fig.3: Minimum, maximum, and the average ZTD values for 18-21 Aug. 2018 at the BAKO and JOG2 stations. The difference of about -100 mm from those shown in Figure 2 reflects the smaller amount of water vapor during the dry season.

C.   Comparison of the ZTD values of goGPS, IGS and UNRs

Next, I compare the ZTD values for the three solutions, namely goGPS, IGS, and UNR, using the BAKO and JOG2 stations on January 24, 2018, and August 20, 2018. This is part of the validation process of determining ZTD values with goGPS.

The results obtained were small RMS values <2 mm. If the RMS is relatively tiny, the troposphere parameters estimated by goGPS show a good agreement with the products from UNR and IGS.
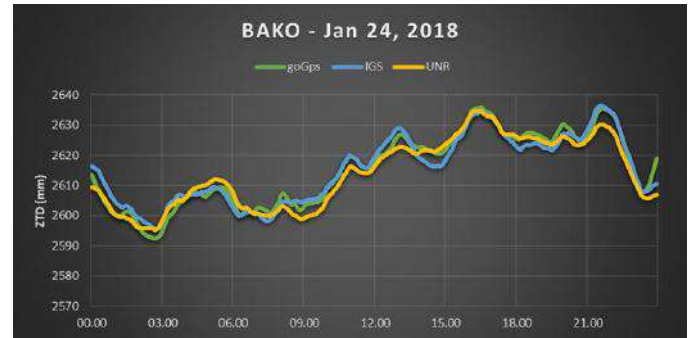
Fig.4: ZTD time series from goGPS, IGS, and UNR, on 24, Jan. 2018, at the BAKO station. The standard deviation and RMS of the difference between goGPS and IGS are 12.03, 0.81, respectively (in mm). Those for the goGPS-UNR pair are 11.22, 0.66
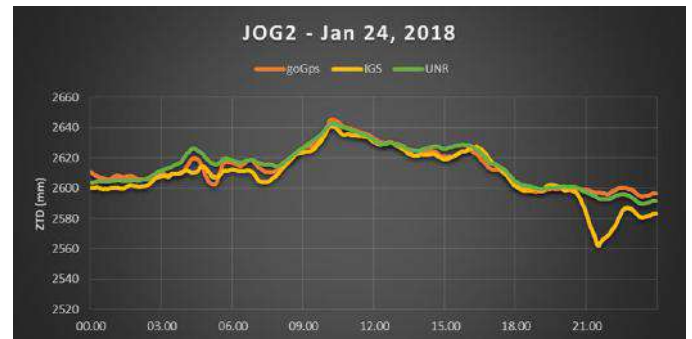
Fig.5: Same as in Figure 4, but the JOG2 station replaces the BAKO station. The standard deviation and RMS of the difference between goGPS and IGS are 16.11 and 1.11, respectively (in mm). Those for the goGPS-UNR pair are 15.00, 1.63

Fig.6: Same as Figure 4, but the date is August 20, 2018. The standard deviation and RMS of the difference between goGPS and IGS are 7.44 and -0.66, respectively (in mm). Those for the goGPS-UNR pair are 8.09 and 0.26
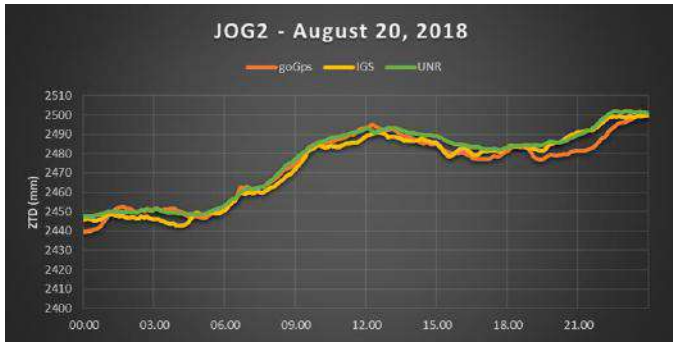
Fig.7: Same as in Figure 6, but the JOG2 station replaces the BAKO station. The standard deviation and RMS of the difference between goGPS and IGS are 16.99 and -1.32, respectively (in mm). Those for the goGPS-UNR pair are 18.33 and -1.08

The results of the ZTD values that I obtained using goGPS, both on January 24 and August 20 and the BAKO and JOG2 stations, are shown in Figures 4 - 7. The whole pictures show the consistency of the ZTD value from goGPS with other solutions. The GoGPS software works very well at different times and stations. Then the goGPS software package can be an alternative to commercial software systems for ZTD estimation.

D. Comparison of PWV and rainfall

The software package goGPS can estimate ZTD and convert the isolated ZWD to PWV values. Here I try to compare the PWV value of GPS at the CMAK station in Makassar, South Sulawesi, with the PWV value observed at the WAAA radiosonde station Makassar City. I perform the comparison in the dry season, April - September 2015, and in the rainy season, October 2015 - March 2016. The results are given in Figures 8 and 9.
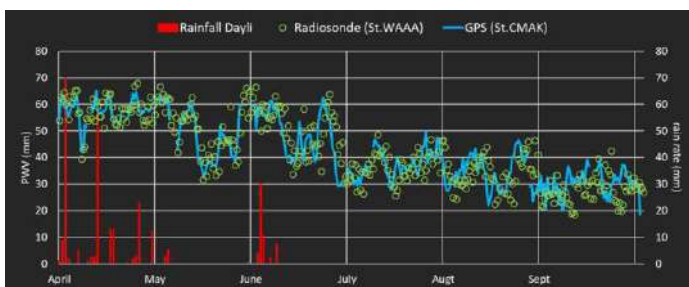
Fig.8: This is a comparative image of PWV from goGPS and PWV radiosonde, and daily rainfall data in the city of Makassar during the dry season in 2015

The correlation of PWV values from GPS and radiosonde for the dry season in Figure 8 and the rainy season in Figure 9 are 0.84 and 0.73, respectively.

The figures also show that the PWV value during this dry period has a downward trend (Figure 8) and an upward trend during this period of the rainy season (Figure 9). The PWV values from goGPS and radiosonde are primarily consistent with each other.
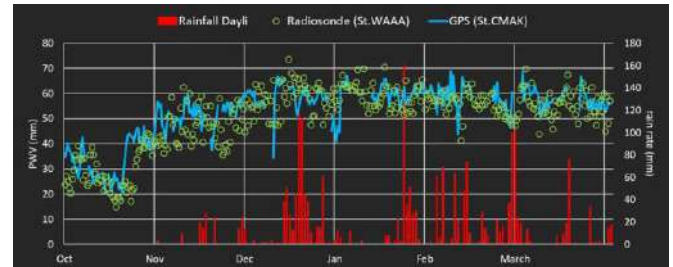
Fig.9: Comparison of PWV values estimated by the goGPS software and those measured by radiosonde, together with the daily rainfall data in Makassar. The period corresponds to the rainy season from 2015 to 2016

IV. CONCLUSION

The correlation of the results of goGPS with those from IGS and UNR is relatively high. The gradient of the late goGPS and IGS, and UNR products show good consistency. This means that the process of goGPS is good enough to get an accurate value.

Figures 4 through 7 show the consistency of ZTD values from goGPS with IGS and UNR solutions. GoGPS software works very well at different times and stations. This means that the goGPS software package can be an alternative to commercial software systems for ZTD estimation.

Comparison of PWV values from goGPS and radiosonde, together with daily rainfall data in the city of Makassar during the dry season and rainy season in 2015-2016, shows the correlation of PWV values from GPS and radiosonde is quite good. PWV values of goGPS and radiosonde are primarily consistent with each other.

REFERENCES

[1] Blewitt, G., W. C. Hammond, and C. Kreemer (2018), Harnessing the GPS data explosion for interdisciplinary science, Eos, 99, https://doi.org/10.1029/2018EO104623.

[2] Enge, P., & Misra, P. (2006). Global Positioning System (2 ed.). Ganga-Jamuna Press.

[3] Gabor, M. (1997, May 5). Remote Sensing of Water Vapor from GPS Receivers. Retrieved May 16, 2018, from http://www.csr.utexas.edu/texas_pwv/midterm/gabor/gabor.html#anchor561367.

[4] Iwabuchi, Tetsuya & Miyazaki, Shin'ichi & Heki, Kosuke & Naito, Isao & Hatanaka, Yuki. (2003). An impact of estimating tropospheric delay gradients on tropospheric delay estimations in the summer using the Japanese nationwide GPS array. Journal of Geophysical Research. 108. 10.1029/2002JD002214.

[5] Koning, A. (2016). Precipitable water vapor estimation using GPS in Uganda: A study on obtaining the Zenith Wet Delay. Delft, The Netherlands.

[6] K. Yedukondalu, A. S. (2011). Estimation and Mitigation of GPS Multipath Interference Using Adaptive Filtering. Progress in Electromagnetics Research M(21), 133-148.

[7] Nicholas Zinas. (2019, October 04). Satellite and receiver clock errors. Retrieved from Tekmon Geomatics: http://www.tekmon.eu/1-3-2-satellite-and-receiver-clock-errors.

[8] Realini E (2009) goGPS free and constrained relative kinematic positioning with low-cost receivers. Ph. D. thesis, http://www.researchgate.net/publication/237520116X. S. Li, et al., "Analysis and Simplification of Three-Dimensional Space Vector PWM for Three-Phase Four-Leg Inverters," IEEE Transactions on Industrial Electronics, vol. 58, pp. 450-464, Feb 2011.

[9] Yuan, Yubin, Kefei Zhang, Witold Rohm, Suelynn Choy, Robert Norman, and Chuan-Sheng Wang. 2014. "Real-Time Retrieval of Precipitable Water Vapor from GPS Precise Point Positioning." Journal of Geophysical Research: Atmospheres 119 (16): 10044–57. https://doi.org/10.1002/2014JD021486.

Article

Cancer Therapy with CRISPR/Cas9: Prospects and Challenges

Muhammad Fahad Chaudhary[1]*, Unsa Saleem[1], Samia Khan[1], Rabiya Khan[2], Muhammad Asim[1], Fakhra Azam[1], Mariam Sharif[3], Zainab[1], Ali Abbas Dilawar[1], Muhammad Jehanzaib[1]

[1]Department of Microbiology, Government College University Faisalabad, Pakistan
[2]Department of Medical Lab Technology, Government College University Faisalabad, Pakistan
[3]Institute of Microbiology, University of Agriculture Faisalabad, Pakistan
*Corresponding Author: Fahadchaudhary1999@gmail.com

Abstract— Globally, Cancer is believed to be second biggest reason of mortality and one of the significant social as well as economic liabilities. Despite our advance at molecular level in comprehension of cancer, more therapeutic tools and tactics are needed to exploit this advance. The CRISPER/Cas9 genome modifying approach has lately appeared as an effective cancer therapy method due to its high accuracy and efficiency. CRISPER/Cas9 has enormous clinical potential in discovering new targets for cancer treatment and also to dismember genetic-chemical interaction thus helping us to understand the response of tumor to the treatment by drugs. Additionally, Cas9/CRISPER can also be used in cancer immunotherapeutic applications by engineering immune cells and oncolytic viruses. Perhaps the most important therapeutic application of Cas9/CRISPER is its ability to edit genes with great precision both in animal models and humans. In this review, we will debate and explore some important concerns of using CRISPER/Cas9 in remedial settings and some vital hurdles that are needed to overcome before it is used for a clinical trial for a polygenic and complex ailment like cancer.

Keywords— Advance therapy; CRISPER; Cas9; Genome engineering; Cancer models.

I. BENEFITS AND TECHNIQUES OF CRISPER GENOME MODIFICATION

The discovery of clustered regularly interspaced short palindromic repeats (CRISPR) (Ishino, Shinagawa, Makino, Amemura, & Nakatura, 1987; Van Soolingen, De Haas, Hermans, Groenen, & Van Embden, 1993)and their role as an adaptive prokaryotic immune system in combination with CRISPR-associated (Cas) genes (Bolotin, Quinquis, Sorokin, & Dusko Ehrlich, 2005; van der Oost, Jore, Westra, Lundgren, & Brouns, 2009)helped open the way to be used as a potent tool for genome related engineering (Jinek et al., 2012; Mali, Esvelt, & Church, 2013). The Cas9/CRISPR is considered to be the major bio-tech breakthrough of the century for its

precision, efficiency, and ease, and has paved the way for more accurate genome modification and in vivo visualization. Generally, Cas9/CRISPR has appeared to have unparalleled therapeutic possibility for studying and targeting disorder, as well as opening up new drug development pathways. More precisely, it heralds the arrival of novel diagnostic and therapeutic approaches.

Gene editing techniques operate by generating double-strand breaks (DSBs) in particular genome sections, which are then repaired by cellular processes. RNA-guided DNA targeting CRISPR/Cas, in comparison to earlier genome editing methods such as zinc-finger nucleases (ZFNs) and transcription activator-effector nucleases (TALENs), was instantly and extensively embraced by clinicians due to its

cost-effectiveness, adaptibility, and convenient for use, thus innovating the work of genome related engineering. Cas9 technology is based on a bacterial and archaeal immune defence mechanism that protects the host from viruses and phages that target nucleic acids(Barrangou et al., 2007). According to their most popular classification, CRISPER/Cas system is classified into three main types, all with various sub classification(Makarova et al., 2011). The most widely used gene editing system is the type II CRISPR/Cas system, which consists of three components: an endonuclease (Cas9), a CRISPR RNA (crRNA), and a transactivatingcrRNA (tracrRNA) (Jinek et al., 2012). The guide RNA (gRNA) is a duplex structure formed by the crRNA and tracrRNA molecules which can be substituted using a fused synthetic chimeric single gRNA (sgRNA), making CRISPR/Cas9 easier to use in genome engineering(Jinek et al., 2012).The sgRNA carries a special sequence of approximately 20 base-pairs (bp) and is intended to complement the DNA site that is targeted, and it should be accompanied by a small length DNA sequence known as the "protospacer-adjacent motif" (PAM), that is required for affinity of Cas9 protien. The expression of sgRNA as well as Cas9 nuclease in the cell create a ribonucleoprotein (RNP) complex, which is directed to a target DNA site by the sgRNA. Cas9 specifically cleaves the DNA to generate a DSB after the sgRNA binds to the target sequence using Watson-Crick base-pairing. The cleavage takes place inside the protospacer, three nucleotides upstream of the PAM, resulting in blunt ends. Cas9 active-site motifs RuvC and HNH, which acts on the (-) and (+) strands respectively, are liable for splitting of opposite DNA strands (Gasiunas, Barrangou, Horvath, & Siksnys, 2012; Mojica, Díez-Villaseñor, García-Martínez, & Almendros, 2009). The machinery of cell mends the DSB using one out of two major mechanisms, depending on the cell state and the availability of a repair template: homology-directed repair (HDR) or non-homologous end joining (NHEJ)(Differ, Cobb, Fishman-lobell, & Habert, 1992; Liang, Han, Romanienko, & Jasin, 1998). The HDR pathway works at the DSB site by recombining a template donor DNA, ensuing repairment that must be accurate. Relevant sequences or mutations may be introduced into a target area of the genome using homology-directed repair. A more common NHEJ pathway is error-prone mechanism that generates frameshift mutations at the DSB siteby randomly inserting or deleting nucleotides (indels). It may thus be used to cause specific gene knockouts (Fig. 1).

Cas9/CRISPER techniques permit for accurate as well as effective splitting of desired targeted DNA sequences, and it has greatly enabled genome editing due to the relative ease along with clarity of constructing

sgRNAs. The use of diverse sgRNAs allows this technology to be multiplexed, which is an additional benefit. Only the CRISPR/Cas9 method, among genome editing nucleases, can edit several loci at the same time by adding sgRNAs to different locations(Jakočinas et al., 2015; Li, Teng, Li, & Zhou, 2013).Where two sgRNAs are used in the same cell, minor deletions(Wyman et al., 2013), complex rearrangements(P. S. Choi & Meyerson, 2014; Torres, Martin, et al., 2014), and even whole-chromosome suppression can occur(Adikusuma, Williams, Grutzner, Hughes, & Thomas, 2017). One more significant benefit of Cas9/CRISPR is its adaptability: amendments and personalization of Cas9/CRISPR modules and also interactors have upgraded the system's precision and efficacy while also broadening its scope of applications beyond editing (Dominguez, Lim, & Qi, 2016).

CRISPR technology's DNA precision has become a main focus in the work field and the existence of off-target activity is shown by many experiments(Tsai & Joung, 2016). As a result, a number of tactics have been devised to reduce the products that are off-target. Out of various methods, one method uses a cellular delivery of in vitro-assembled RNP complexes instead of plasmid delivery, which generates longer-lived Cas9 and sgRNA expression, as well as a higher ratio of on-target:off-target editing of genes in the cells of mammals and vastly effective editing(Kim, Kim, Cho, Kim, & Kim, 2014; Torres-Ruiz et al., 2017). Other approaches include using Cas9 variants that are light or small molecule inducible(Davis, Pattanayak, Thompson, Zuris, & Liu, 2015; Nihongaki, Kawano, Nakajima, & Sato, 2015), broken Cas9 variants (for safe rebuilding), and Cas9 which are regulated allosterically(Oakes et al., 2016).The Cas9 was modified to induce break just one strands of DNA, researchers were able to use pair of Cas9 nickases directed by two different gRNAs aiming at the same locus but on opposing DNA strands. This technique creates highly precise DNA splitting with efficacy similar to traditional Cas9/CRISPR but with less far-off incidents(Ran et al., 2013). A similar strategy uses two Cas9 which are catalytically inactivated mutants fused to (fCas9) FokI nuclease (directed by two opposing gRNAs projecting at the same position), in which the only dimer (fCas9) is functional. In human cells, Fokl nucleases were found to alter a specified location with >150-folds greater accuracy than Cas9 which are wild-type nucleases(Guilinger, Thompson, & Liu, 2014). Finally, study of Cas9 mutations to improve specificity revealed that 3-4 modified point variations can neutralize electrostatic interaction between its targeted DNA and Cas9, resulting in a considerable improvement in the specificity of its action(Kleinstiver et al., 2016; Slaymaker et al., 2016). Although CRISPR/Cas9

technology has a number of benefits over prior genome editing programmable nucleases, but it also has some disadvantages. CRISPR/Cas9 performance and sequence specificity requires to be developed beyond. Effects that are off-target must also be minimized, and developing a CRISPR/Cas9 delivery system that is reliable, stable, and cell-specific remains a major challenge.
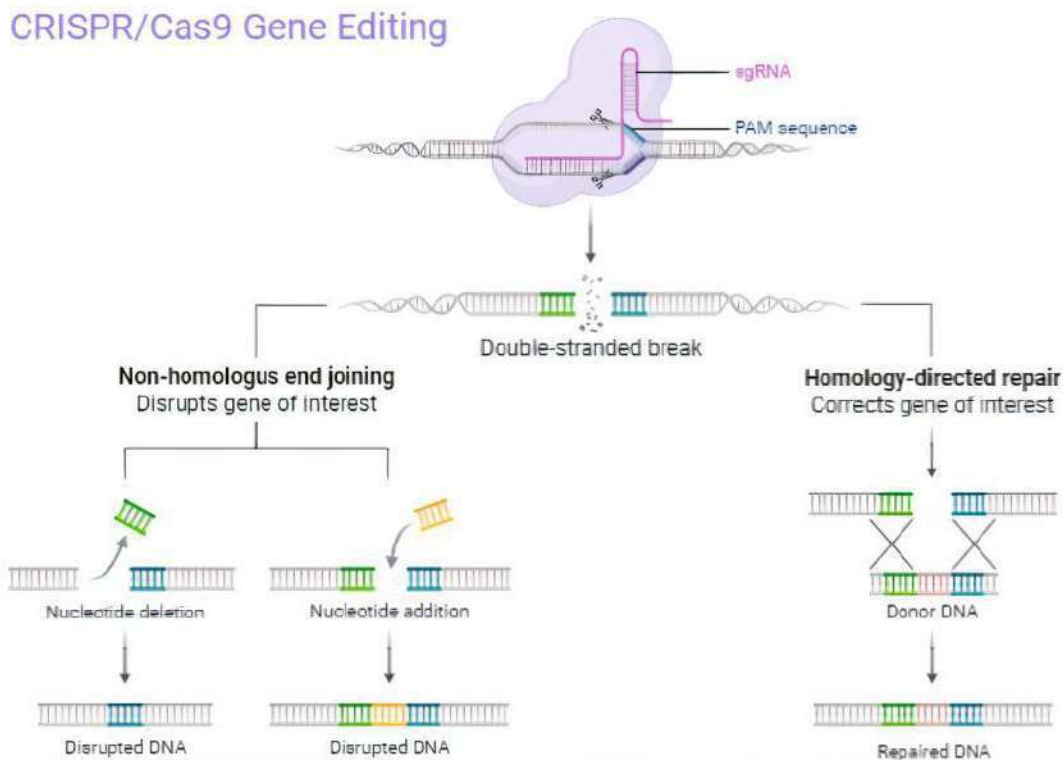
Fig.1: Mechanism of action of CRISPER/Cas9. When the protein Cas9 attaches to sgRNA, it forms a (RNP) ribonucleoprotein complex. After recognizing the protospacer-adjacent motif (PAM) sequence adjoining to the selected sequence, this complex fuse to the genomic target sequence with base-pairing complementarity and accurately splits double-stranded DNA. When breaks occur in double-strand, the NHEJ or HDR channels are triggered. In the absence of homologous repair template, NHEJ usually results in deletions or insertions of arbitrary base pairs disturbing the selected sequence. By supplying a donor DNA template and using the homology-driven repair pathway, specific genome edition can also be achieved.

II. DRUG EXPLORATION WITH CRISPER

Drug discovery and production is a lengthy and complicated procedure that involves recognizing novel products and presenting them in the market. Typically, the procedure starts with the proposition that disrupting certain biological target will result in one useful outcome that will alter disease progression. These targets should be confirmed in physiologically pertinent models of animals prior to clinical models whose pharmacological adjustment may lead to the required therapeutic impact.

In cancer studies, drug formulation aims to recognize molecules against genetic defects in tumor suppressor gene as well as oncogenes that lead to tumor formation. Various prominent examples contains vemurafenib, that directs at BRAF V600E mutant variations in melanoma; Osimertinib, for EGFR-mutated non-small cell lung cancer

treatment; or imatinib, that targets fusion of ABL1-BCR in long-term myeloid leukemia(Fleuren, Zhang, Wu, & Daly, 2016).

Drug discovery program can be aided with genome engineering techniques by identifying genes that are accountable for specific ailment. The process of identifying and confirming high-value targets is laborious and time-consuming, but the CRISPER/Cas9 method has the ability to speed it up.The quick and efficacious generation of accurate disease models, both animal as well as cellular, using Cas9/CRISPER modification has an approving effect on discovery of drug because it is an instant way for active drug testing by identifying selected molecules that are activated or inhibited to induce or prevent disease(Ahmad & Amiji, 2018).

III.   DRUG TARGET EXPLORATION USING CRISPER/CAS9 LIBRARY SCREENS

The discovery of genes that are not known and the finding out about their role are normally done using high-throughput genetic screening platforms. Screening of mutations has been utilized to recognize fundamental biotic procedures and pathways for signaling, and it can also be used to establish genes which are accountable for a specified phenotype. The major restraint of mutagenesis screening for targeted discovery of drug with the mutations that are not known is the propagation of heterozygous mutants. Targeted RNA interference (RNAi) is a way to overcome this constraint. High-production RNAi genomic library tests may provide vital details on connections among single genes and functional loss phenotypes, however there are yet some drawbacks, such as useless knockdown and significant far off targets(Ahmad & Amiji, 2018). CRISPER/Cas9 has some leads over RNAi including complete inactivation, whole genome targeting capability including introns, promoter, enhancer and intergenic regions, and high reproducibility(Wang, Wei, Sabatini, & Lander, 2014).The expertise acquired through

the creation of operative RNAi approaches has aided the rampant growth of CRISPER/Cas9 libraries in recent years. In 2013, these CRISPER libraries in the beginning were said to be more effective than RNAi libraries (Wang et al., 2014).

Three distinct kind of genome-wide libraries are lately in use: (1) To find out new biological mechanism encompassing cell survival signals and drug resistance, CRISPER-based loss-of-function (CRISPER knock-out)is used (Ribeiro, 2014); (2) CRISPER based new gene activation (CRISPERa) is vital in testing for gain of function (Joung et al., 2017); and (3) Screening for loss of function can be accomplished by using CRISPER based gene inhibition (CRISPERi) (Luo, 2016). CRISPERa and CRISPERi libraries, unlike CRISPER knock-out libraries, use catalytically ineffective Cas9 in conjunction with regulative cofactors like VP64 (activation)(Maeder et al., 2013)or Kruppel associated box (KRAB) repressin (inhibition) (Gilbert et al., 2014)or other factors like SAM or SunTag(Chavez et al., 2016; Konermann et al., 2015), developed to speed up CRISPERa activity. (Fig. 2).
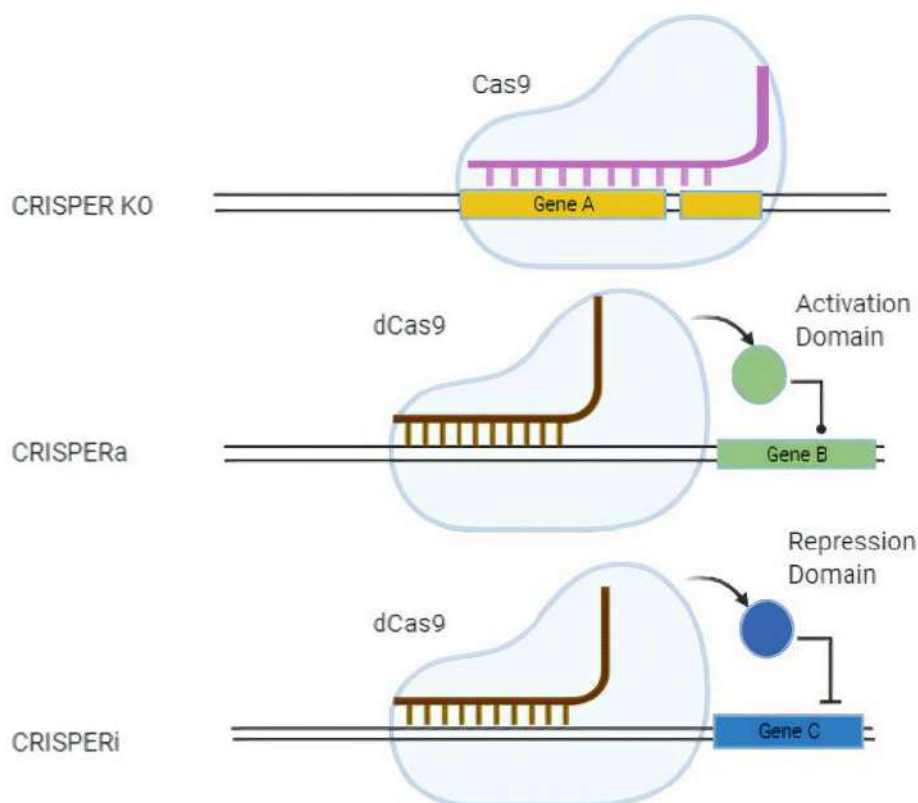
Fig. 2: Three main techniques for transcriptional modulation: (1) CRISPER Knock-out, for production of protiens that are non-functional or knock-out a specific gene by using wild type CRISPER system; (2) CRISPER activation, It uses a catalytically deactivated variant of Cas9 enzyme in conjunction with various activators domains to produce specific gene activation(SunTag, SAM, VPR); (3) CRISPER repression or deactivation, by fusing repressor domain (KRAB) with catalytically inactivated dCas9.

IV. DRUG RESISTANCE AND CRISPER/CAS9

Finding of genes that play a part in resistance of drugs is a crucial implementation of CRISPER/Cas9 in drug finding. Conventionally, worldwide mutagenesis across a cell population is used to evaluate the mechanism of resistance of anticancer agents. Only cells carrying the mutations that spoil the action of drug would survive in ensuing usage of drug to be tested. However, generation of significant number of false positives is the drawback of this approach (Guichard, 2017).

CRISPER/Cas9 screens are the most appropriate tactic for the detection of gene deletion that is linked with drug resistance. Cells resistant to the desired drug are revealed to a pool of CRISPER/Cas9 gRNAs that targets genome in a way that each cell has one guide that knocks out single gene. Subsequent examination of cells that becomes vulnerable by exposure of drugs, point out the genes that confer drug resistance. Resistant genes can then be targeted with other drugs to evade exposure of resistance (Scott, 2018)E.g., HPRT1 gene interference through CRISPER/Cas9 editing generates 6-thioguannie resistant cells (Smurnyy et al., 2014).

V. DRUG EFFECTIVENESS MODELS OF DISEASES

In drug development, cells and animal models are of substantial importance. Before clinical testing on humans, experiments must be performed in models to test drug effectiveness and toxicity. Most subjects, encompassing cell as well as animal models, are not able to, however, accurately represent the condition observed in patients. Moreover, to generate subjects that precisely summarize the variety and complexity of disease are very expensive and time consuming process. Cancer cell line can be modified to accurately mimic the deviations seen in patients by using CRISPER/Cas9 and it is cheap as compared to standard protocol. Model of ovarian cancer of mice ID8 was altered to hinder TP53 and BRCA2 which ultimately resulted in increased sensitivity to inhibit PARP, is a good example (Walton et al., 2016).

The example above demonstrates clearly that CRISPER/Cas9 platform has become vital element of drug discovery in oncology. This mechanization has enhanced the finding and authentication of novel drug targets, as well as providing more accurate models of human diseases for evaluating safety of drug in a more prognostic way as well as reducing and combating drug resistance.

VI. CRISPER AS A CANCER-FIGHTING TOOL

Although there is some advancement in past decade but significant number of people still die due to cancer which demonstrate the dire need for novel and more effective therapeutic options. CRISPER/Cas9 genome editing has a great potential in cancer therapeutic besides its use as a research tool. The regulation of endogenous gene expression is a probable application of CRISPER/Cas9 system in Cancer therapy. As discussed above, gRNAs can be used to recruit catalytically inactive dCas9 to specific target DNA sites(Friedland et al., 2013) and can also be utilized to activate or suppress particular target genes by fusing it with transcriptional activation or inhibition domain(Chen et al., 2013). Epigenome editing could be another therapeutic application based on linking dCas9 to histone modifiers and proteins involved in altering DNA methylation (Klann et al., 2017). Finally, by specifically targeting tumor markers in cancer cells, it allows for the elimination of genetic changes that can contribute to tumor proliferation and/or metastatic capacity (Shachaf et al., 2004). However, the effectual delivery of CRISPER component in all the cancer cells is still a challenge.

Elaborate interaction between tumor, host and environment is needed for effective immunity against cancer cells because cancer is a complex disease. Immunotherapy, which targets PD-1 or increases immune action to cancer cells that has chimeric antigen therapy (CAR) therapy, has recently emerged as a promising treatment choice for cancer (Shachaf et al., 2004). Unlike chemotherapy or radiotherapy, Cancer immunotherapy has many benefits like durable activity, favorable benefits, and low risk ratio. The development of new generation therapy techniques is needed for cancers that are untreatable with traditional chemo or radiotherapy.

Viruses capable of oncolysis are becoming increasingly relevant in cancer related therapy.These can be modified genetically to attack only the cancer cells with deficient antiviral defense leaving normal cells intact. Virally motivated disruption of tumor cells has many mechanism including direct cellular lysis in which tumor antigen is released from dying cells which causes further immune stimulation (A. H. Choi, O'Leary, Fong, & Chen, 2016). CRISPER/Cas9 mediated genome editing has enormous potential to be used in cancer therapeutics because it can be used to modify viruses capable of oncolysis for increased immune stimulation and optimized tumor selectivity. One example of genome modification for immunotherapy application is the generation of herpes simplex virus type 1 variants with improved lysis specifications by deleting of theICP34.5 neurovirulence and ICP6 (ribonucleotide reductase) genes(Goldsmith,

Chen, Johnson, & Hendricks, 1998). Furthermore, the ICP6 gene can be deleted to give selectivity which is replicative for cells with P16^{INK41}tumour repressor gene deactivation, which is one of the major usual cancer insufficiencies(Aghi, Visted, DePinho, & Chiocca, 2008). The wild type of adenovirus, in case of DNA tumor viruses, encodes protein (E1A) that can bind to pRb, and therefore capturing the cycle of cell by release of transcription factor E2F. The release of this transcriptional factor E2F also causes an orderly activation of genes which are viral, resulting in the propagation of new viruses, which then cause the infected cell to lyse and release novel virus. Because cancerous cells usually contain genetic changes in the pathway named Rb, the EIA gene has been knocked out of oncolytic adenoviruses to avoid replication.

Isolation and the in-vitro extension of tumor-specific T-cells, accompanied by their reintroduction into the individual, are part of some immunotherapy methods such as Adoptive cell therapy (ACT). Many form of ACT are still under development which include engineered T-cells to efficiently recognize and attack tumor cells. This can be done by deleting PD-1 gene in T-cells(Su et al., 2016). Interaction of PD-1 and its ligand PD-L1 halts T-Lymphocytes propagation, effector function and their survival(Tseng et al., 2001), induce resistance of tumor cells to cytolytic T-lymphocyte reaction(Dong et al., 2002), as well as induce death of tumor specific T-cells(Iwai et al., 2002; Tsushima et al., 2007). The whole idea of this approach is that when PD-1 gene is deleted from T-cells in-vitro by using CRISPER/Cas9 and then subsequently reintroduced into the patient, those genedeleted T-cells will home tumor and turn on immune system which may eliminate tumor. One powerful tool for anti-tumor treatment is immune checkpoint blockade which include deletion of gene on anti-PD-1/PD-L1 and anti-CTLA-4 antibodies. This method has led the way to the innovation in the treating of many kinds of modern tumor by avoiding exhaustion triggered through checkpoint molecules. With PD-1 knockout T-cells, this technique is being investigated in six clinical studies for prostate, stomach, bladder, lymphoma, renal cell carcinoma, and hepatocellular carcinoma(Fellmann, Gowen, Lin, Doudna, & Corn, 2017).

Production of the next generation CAR T-cells that are genetically altered to present tumor-targeting receptor is another impressive anti-cancer immune therapy that has a great potential for the treatment of hematological and solid cancers (Maus, Grupp, Porter, & June, 2014). Intracellular chimeric signaling domain that is capable of activating T-cells and an extracellular binding domain that recognize a highly specific antigen for and strongly expressed on tumor cells, together constitute a chimeric antigen receptor (CAR) and both domains work in combination to reprogram T-cell facilitated killing of Tumor cells. In 2016, an oncologist team led by LU You at Sichuan University in China became the first to inject T-cells modified by CRISPER/Cas9 to disable PD-1 into patients with aggressive lung cancer(Cyranoski, 2016). Although ACT therapies are of great potential in the treatment of leukemia and lymphoma, but some individuals died while conducting trial phases due to neurotoxicity and cytokine release syndrome (Gauthier & Turtle, 2018). At this moment, FDA has approved CAR T-cell therapy only for the treatment of relapsed and refractory B-cell acute lymphoblastic leukemia in paediatric and young adults (Kansagra & Litzow, 2017).

VII. GENE MODIFICATION BIOLOGICAL TECHNIQUES FOR IN-VIVO DELIVERY

Development of effective and safe method for delivering gene editing element to the tumor cells as well as metastatic sites is still a defiance for the upcoming application of gene modification techniques such as systems like CRISPER/Cas9. Hitherto, In-Vitro gene editing has been performed mainly in hematopoietic precursors or T-cells. Developing efficient and safe methods for In-Vivo delivery in somatic cells is essential to widen the scope of CRISPER-based therapy. These delivery hurdles can be overcome by developing novel viral and non-viral systems (Kay, 2011; Torres, Garcia, Jimenez, Rodriguez, & Ramirez, 2014).

Adeno-associated virus (AAV), lentivirus and adenovirus can be used as a viral delivery system for CRISPER/Cas9 components (Yin, Kauffman, & Anderson, 2017).Presently, the latest approach for in-vivo delivery of gene make use of Adeno-associated viruses (AAVs)(Yin et al., 2016). There are many reasons for which AAVs are considered to be an outstanding vehicle for gene therapy: (1) AAV incite little or no immune response; (2) extensive variety of serotypes for infection of different cell types are known; (3) these viruses do not cause any disease in humans (Daya & Berns, 2008). Furthermore, these viruses have been tested for their efficiency and safety in clinical trials (Kotterman & Schaffer, 2014); and they have been favorably put to use in models of mice(Gaj et al., 2017). However, the drawback of their use in delivery is their tiny packing which make it essential to utilize multiple virions to deliver different components of CRISPER/Cas9 components (sgRNAs, Cas9 or donor DNA) which cause further decrease in efficiency (Yin et al., 2017). However, the utilization of AAV causes the incessant articulation of CRISPER components in edited cells which may cause

unwanted off-target genome effects or may lead to increased immune response. Adenovirus and lentivirus can both infect dividing and non-dividing cells, but unlike lentiviruses, adenoviruses do not integrate into the genome of the recipient cell. Both lentiviruses and adenoviruses has their drawbacks as both induce strong immune response (Follenzi, Santambrogio, & Annoni, 2007).

To solve these difficulties, non-viral delivery vectors or preassembled and short-lived Cas9 RNP complexes can be used. Non-viral delivery approach uses liposomes, gold nanoparticle or inorganic nanoparticle and many others (Yin et al., 2014). Wide range of molecules are delivered for long through lipid nanoparticle and because they are devoid of any viral component so they lessen immunogenicity and security concerns and these nanoparticles can also be used both in vitro and in vivo. Additional benefits of using these nanoparticle for delivery of CRISPER/Cas9 components are their high loading capacity and the integration of genomic risk is not present and continuous articulation of CRISPER/Cas9 (Kaczmarek, Kowalski, & Anderson, 2017). Researchers have documented successful provision of Cas9-sgRNA RNP compounds through nanoparticles in xenografts of U2OS human osteosarcoma cells (Sun et al., 2015). Moreover, Cas9 RNP complexes with donor DNA was shown to be delivered by gold nanoparticle combined with DNA a well as further added with disruptive polymers that are endosomal and cationic, could induce homology DNA repair (HDR) to fix DNA mutant variations of Duchenne muscular dystrophy in the mice(Lee et al., 2017). Gold-nanoparticle is an excellent carrier that is not toxic for gene and drug delivery application because gold core of the particle provides solidity to the assemblage, meanwhile the monolayer permits surface tuning of properties like hydrophobicity and charge. There is still need for testing the safety and efficiency of this method, but it is expected CRISPER components delivery mechanism. As nanoparticles that are inorganic including bare mesoporus or dense silica nanoparticles and carbon nanotubes have already been used for various purposes, they are the natural and potential carriers of CRISPER components (Xu, Zeng, Lu, & Yu, 2006).Furthermore, inorganic nanoparticles have some other benefits including their reproducible composition, size and stability over time as well as simplicity to generate them.

VIII.   CONCLUSION

Although CRISPER/Cas9 based technology is still in development but it has already displayed its potential in research and hold great therapeutic promise but for favorable clinical application of this technology, secure and efficient transport into selected tissue is required. There are high expectations for this technology, which necessitated careful planning, such as allowing regulatory processes for its development. However, the technology still requires optimization mainly with respects to safety, specificity and efficacy before its widespread translation into clinics. Despite the many obstacles that must be met, we expect that the continued development of genetic science will significantly contribute to existing cancer therapies.

REFERENCES

[1] Adikusuma, F., Williams, N., Grutzner, F., Hughes, J., & Thomas, P. (2017). Targeted Deletion of an Entire Chromosome Using CRISPR/Cas9. *Molecular Therapy*, *25*(8), 1736–1738. https://doi.org/10.1016/j.ymthe.2017.05.021

[2] Aghi, M., Visted, T., DePinho, R. A., & Chiocca, E. A. (2008). Oncolytic herpes virus with defective ICP6 specifically replicates in quiescent cells with homozygous genetic mutations in p16. *Oncogene*, *27*(30), 4249–4254. https://doi.org/10.1038/onc.2008.53

[3] Ahmad, G., & Amiji, M. (2018). Use of CRISPR/Cas9 gene-editing tools for developing models in drug discovery. *Drug Discovery Today*, *23*(3), 519–533. https://doi.org/10.1016/j.drudis.2018.01.014

[4] Barrangou, R., Fremaux, C., Deveau, H., Richardss, M., Boyaval, P., Moineau, S., … Horvath, P. (2007). CRISPR Provides Against Viruses in Prokaryotes. *Science*, *315*(March), 1709–1712.

[5] Bolotin, A., Quinquis, B., Sorokin, A., & Dusko Ehrlich, S. (2005). Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. *Microbiology*, *151*(8), 2551–2561. https://doi.org/10.1099/mic.0.28048-0

[6] Chavez, A., Tuttle, M., Pruitt, B. W., Ewen-Campen, B., Chari, R., Ter-Ovanesyan, D., … Church, G. (2016). Comparison of Cas9 activators in multiple species. *Nature Methods*, *13*(7), 563–567. https://doi.org/10.1038/nmeth.3871

[7] Chen, B., Gilbert, L. A., Cimini, B. A., Schnitzbauer, J., Zhang, W., Li, G. W., … Huang, B. (2013). Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas system. *Cell*, *155*(7), 1479–1491. https://doi.org/10.1016/j.cell.2013.12.001

[8] Choi, A. H., O'Leary, M. P., Fong, Y., & Chen, N. G. (2016). From benchtop to bedside: A review of oncolytic virotherapy. *Biomedicines*, *4*(3), 1–20. https://doi.org/10.3390/biomedicines4030018

[9] Choi, P. S., & Meyerson, M. (2014). Targeted genomic rearrangements using CRISPR/Cas technology. *Nature Communications*, *5*, 1–6. https://doi.org/10.1038/ncomms4728

[10] Cyranoski, D. (2016). CRISPR gene editing tested in a person. *Nature*, *539*(7630), 479.

[11] Davis, K. M., Pattanayak, V., Thompson, D. B., Zuris, J.

A., & Liu, D. R. (2015). Small molecule-triggered Cas9 protein with improved genome-editing specificity. *Nature Chemical Biology*, *11*(5), 316–318. https://doi.org/10.1038/nchembio.1793

[12] Daya, S., & Berns, K. I. (2008). Gene therapy using adeno-associated virus vectors. *Clinical Microbiology Reviews*, *21*(4), 583–593. https://doi.org/10.1128/CMR.00008-08

[13] Differ, G., Cobb, M. H., Fishman-lobell, J., & Habert, J. E. (1992). *Rad1 Nhej. 42580*(September).

[14] Dominguez, A. A., Lim, W. A., & Qi, L. S. (2016). Beyond editing: Repurposing CRISPR-Cas9 for precision genome regulation and interrogation. *Nature Reviews Molecular Cell Biology*, *17*(1), 5–15. https://doi.org/10.1038/nrm.2015.2

[15] Dong, H., Strome, S. E., Salomao, D. R., Tamura, H., Hirano, F., Flies, D. B., … Chen, L. (2002). Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. *Nature Medicine*, *8*(8), 793–800. https://doi.org/10.1038/nm730

[16] Fellmann, C., Gowen, B. G., Lin, P. C., Doudna, J. A., & Corn, J. E. (2017). Cornerstones of CRISPR-Cas in drug discovery and therapy. *Nature Reviews Drug Discovery*, *16*(2), 89–100. https://doi.org/10.1038/nrd.2016.238

[17] Fleuren, E. D. G., Zhang, L., Wu, J., & Daly, R. J. (2016). The kinome "at large" in cancer. *Nature Reviews Cancer*, *16*(2), 83–98. https://doi.org/10.1038/nrc.2015.18

[18] Follenzi, A., Santambrogio, L., & Annoni, A. (2007). Immune Responses to Lentiviral Vectors. *Current Gene Therapy*, *7*(5), 306–315. https://doi.org/10.2174/156652307782151515

[19] Friedland, A. E., Tzur, Y. B., Esvelt, K. M., Colaiácovo, M. P., Church, G. M., & Calarco, J. A. (2013). Heritable genome editing in C. elegans via a CRISPR-Cas9 system. *Nature Methods*, *10*(8), 741–743. https://doi.org/10.1038/nmeth.2532

[20] Gaj, T., Ojala, D. S., Ekman, F. K., Byrne, L. C., Limsirichai, P., & Schaffer, D. V. (2017). In vivo genome editing improves motor function and extends survival in a mouse model of ALS. *Science Advances*, *3*(12), 1–11. https://doi.org/10.1126/sciadv.aar3952

[21] Gasiunas, G., Barrangou, R., Horvath, P., & Siksnys, V. (2012). Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(39), 2579–2586. https://doi.org/10.1073/pnas.1208507109

[22] Gauthier, J., & Turtle, C. J. (2018). Insights into cytokine release syndrome and neurotoxicity after CD19-specific CAR-T cell therapy. *Current Research in Translational Medicine*, *66*(2), 50–52. https://doi.org/10.1016/j.retram.2018.03.003

[23] Gilbert, L. A., Horlbeck, M. A., Adamson, B., Villalta, J. E., Chen, Y., Whitehead, E. H., … Weissman, J. S. (2014). Genome-Scale CRISPR-Mediated Control of Gene Repression and Activation. *Cell*, *159*(3), 647–661. https://doi.org/10.1016/j.cell.2014.09.029

[24] Goldsmith, B. K., Chen, W., Johnson, D. C., & Hendricks, R. L. (1998). Neurovirulence by Blocking the CD8+ T Cell Response. *The Journal of Experimental Medicine*, *187*(3), 0–7.

[25] Guichard, S. M. (2017). CRISPR–Cas9 for Drug Discovery in Oncology. In *Annual Reports in Medicinal Chemistry* (1st ed., Vol. 50). https://doi.org/10.1016/bs.armc.2017.08.006

[26] Guilinger, J. P., Thompson, D. B., & Liu, D. R. (2014). Fusion of catalytically inactive Cas9 to FokI nuclease improves the specificity of genome modification. *Nature Biotechnology*, *32*(6), 577–582. https://doi.org/10.1038/nbt.2909

[27] Ishino, Y., Shinagawa, H., Makino, K., Amemura, M., & Nakatura, A. (1987). Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isoenzyme conversion in Escherichia coli, and identification of the gene product. *Journal of Bacteriology*, *169*(12), 5429–5433. https://doi.org/10.1128/jb.169.12.5429-5433.1987

[28] Iwai, Y., Ishida, M., Tanaka, Y., Okazaki, T., Honjo, T., & Minato, N. (2002). Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(19), 12293–12297. https://doi.org/10.1073/pnas.192461099

[29] Jakočinas, T., Bonde, I., Herrgård, M., Harrison, S. J., Kristensen, M., Pedersen, L. E., … Keasling, J. D. (2015). Multiplex metabolic pathway engineering using CRISPR/Cas9 in Saccharomyces cerevisiae. *Metabolic Engineering*, *28*, 213–222. https://doi.org/10.1016/j.ymben.2015.01.008

[30] Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). *A Programmable Dual-RNA – Guided*. *337*(August), 816–822.

[31] Joung, J., Konermann, S., Gootenberg, J. S., Abudayyeh, O. O., Platt, R. J., Brigham, M. D., … Zhang, F. (2017). Genome-scale CRISPR-Cas9 knockout and transcriptional activation screening. *Nature Protocols*, *12*(4), 828–863. https://doi.org/10.1038/nprot.2017.016

[32] Kaczmarek, J. C., Kowalski, P. S., & Anderson, D. G. (2017). Advances in the delivery of RNA therapeutics: From concept to clinical reality. *Genome Medicine*, *9*(1), 1–16. https://doi.org/10.1186/s13073-017-0450-0

[33] Kansagra, A., & Litzow, M. (2017). Treatment of Young Adults with Acute Lymphoblastic Leukemia. *Current Hematologic Malignancy Reports*, *12*(3), 187–196. https://doi.org/10.1007/s11899-017-0377-y

[34] Kay, M. A. (2011). State-of-the-art gene-based therapies: The road ahead. *Nature Reviews Genetics*, *12*(5), 316–328. https://doi.org/10.1038/nrg2971

[35] Kim, S., Kim, D., Cho, S. W., Kim, J., & Kim, J. S. (2014). Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins. *Genome Research*, *24*(6), 1012–1019. https://doi.org/10.1101/gr.171322.113

[36] Klann, T. S., Black, J. B., Chellappan, M., Safi, A., Song, L., Hilton, I. B., … Gersbach, C. A. (2017). CRISPR-Cas9 epigenome editing enables high-throughput screening for functional regulatory elements in the human genome.

Nature Biotechnology, *35*(6), 561–568. https://doi.org/10.1038/nbt.3853

[37] Kleinstiver, B. P., Pattanayak, V., Prew, M. S., Tsai, S. Q., Nguyen, N. T., Zheng, Z., & Joung, J. K. (2016). High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. *Nature*, *529*(7587), 490–495. https://doi.org/10.1038/nature16526

[38] Konermann, S., Brigham, M. D., Trevino, A. E., Joung, J., Abudayyeh, O. O., Barcena, C., … Zhang, F. (2015). Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. *Nature*, *517*(7536), 583–588. https://doi.org/10.1038/nature14136

[39] Kotterman, M. A., & Schaffer, D. V. (2014). Engineering adeno-associated viruses for clinical gene therapy. *Nature Reviews Genetics*, *15*(7), 445–451. https://doi.org/10.1038/nrg3742

[40] Lee, K., Conboy, M., Park, H. M., Jiang, F., Kim, H. J., Dewitt, M. A., … Murthy, N. (2017). Nanoparticle delivery of Cas9 ribonucleoprotein and donor DNA in vivo induces homology-directed DNA repair. *Nature Biomedical Engineering*, *1*(11), 889–901. https://doi.org/10.1038/s41551-017-0137-2

[41] Li, W., Teng, F., Li, T., & Zhou, Q. (2013). Simultaneous generation and germline transmission of multiple gene mutations in rat using CRISPR-Cas systems. *Nature Biotechnology*, *31*(8), 684–686. https://doi.org/10.1038/nbt.2652

[42] Liang, F., Han, M., Romanienko, P. J., & Jasin, M. (1998). Homology-directed repair is a major double-strand break repair pathway in mammalian cells. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(9), 5172–5177. https://doi.org/10.1073/pnas.95.9.5172

[43] Luo, J. (2016). CRISPR/Cas9: From Genome Engineering to Cancer Drug Discovery. *Trends in Cancer*, *2*(6), 313–324. https://doi.org/10.1016/j.trecan.2016.05.001

[44] Maeder, M. L., Linder, S. J., Cascio, V. M., Fu, Y., Ho, Q. H., & Joung, J. K. (2013). CRISPR RNA-guided activation of endogenous human genes. *Nature Methods*, *10*(10), 977–979. https://doi.org/10.1038/nmeth.2598

[45] Makarova, K. S., Haft, D. H., Barrangou, R., Brouns, S. J. J., Charpentier, E., Horvath, P., … Koonin, E. V. (2011). Evolution and classification of the CRISPR-Cas systems. *Nature Reviews Microbiology*, *9*(6), 467–477. https://doi.org/10.1038/nrmicro2577

[46] Mali, P., Esvelt, K. M., & Church, G. M. (2013). Cas9 as a versatile tool for engineering biology. *Nature Methods*, *10*(10), 957–963. https://doi.org/10.1038/nmeth.2649

[47] Maus, M. V, Grupp, S. A., Porter, D. L., & June, C. H. (2014). *ANTIBODY DERIVATIVES AS NEW THERAPEUTICS FOR HEMATOLOGIC Antibody-modi fi ed T cells : CARs take the front seat for hematologic malignancies*. *123*(17), 2625–2635. https://doi.org/10.1182/blood-2013-11-492231.organs

[48] Mojica, F. J. M., Díez-Villaseñor, C., García-Martínez, J., & Almendros, C. (2009). Short motif sequences determine the targets of the prokaryotic CRISPR defence system. *Microbiology*, *155*(3), 733–740.

https://doi.org/10.1099/mic.0.023960-0

[49] Nihongaki, Y., Kawano, F., Nakajima, T., & Sato, M. (2015). Photoactivatable CRISPR-Cas9 for optogenetic genome editing. *Nature Biotechnology*, *33*(7), 755–760. https://doi.org/10.1038/nbt.3245

[50] Oakes, B. L., Nadler, D. C., Flamholz, A., Fellmann, C., Staahl, B. T., Doudna, J. A., & Savage, D. F. (2016). Profiling of engineering hotspots identifies an allosteric CRISPR-Cas9 switch. *Nature Biotechnology*, *34*(6), 646–651. https://doi.org/10.1038/nbt.3528

[51] Ran, F. A., Hsu, P. D., Lin, C. Y., Gootenberg, J. S., Konermann, S., Trevino, A. E., … Zhang, F. (2013). XDouble nicking by RNA-guided CRISPR cas9 for enhanced genome editing specificity. *Cell*, *154*(6), 1–10. https://doi.org/10.1016/j.cell.2013.08.021

[52] Ribeiro, N. (2014). *No 主観的健康感を中心とした在宅高齢者における健康関連指標に関する共分散構造分析Title*. *343*(January), 84–88.

[53] Scott, A. (2018). A CRISPR path to drug discovery. *Nature*, *555*(7695), S10–S11. https://doi.org/10.1038/d41586-018-02477-1

[54] Shachaf, C. M., Kopelman, A. M., Arvanitis, C., Karlsson, Å., Beer, S., Mandl, S., … Felsher, D. W. (2004). MYC inactivation uncovers pluripotent differentiation and tumour dormancy in hepatocellular cancer. *Nature*, *431*(7012), 1112–1117. https://doi.org/10.1038/nature03043

[55] Slaymaker, I. M., Gao, L., Zetsche, B., Scott, D. A., Yan, W. X., & Zhang, F. (2016). Rationally engineered Cas9 nucleases with improved specificity. *Science*, *351*(6268), 84–88. https://doi.org/10.1126/science.aad5227

[56] Smurnyy, Y., Cai, M., Wu, H., McWhinnie, E., Tallarico, J. A., Yang, Y., & Feng, Y. (2014). DNA sequencing and CRISPR-Cas9 gene editing for target validation in mammalian cells. *Nature Chemical Biology*, *10*(8), 623–625. https://doi.org/10.1038/nchembio.1550

[57] Su, S., Hu, B., Shao, J., Shen, B., Du, J., Du, Y., … Liu, B. (2016). CRISPR-Cas9 mediated efficient PD-1 disruption on human primary T cells from cancer patients. *Scientific Reports*, *6*, 1–13. https://doi.org/10.1038/srep20070

[58] Sun, W., Ji, W., Hall, J. M., Hu, Q., Wang, C., Beisel, C. L., & Gu, Z. (2015). Self-Assembled DNA Nanoclews for the Efficient Delivery of CRISPR-Cas9 for Genome Editing. *Angewandte Chemie - International Edition*, *54*(41), 12029–12033. https://doi.org/10.1002/anie.201506030

[59] Torres-Ruiz, R., Martinez-Lage, M., Martin, M. C., Garcia, A., Bueno, C., Castaño, J., … Rodriguez-Perales, S. (2017). Efficient Recreation of t(11;22) EWSR1-FLI1+ in Human Stem Cells Using CRISPR/Cas9. *Stem Cell Reports*, *8*(5), 1408–1420. https://doi.org/10.1016/j.stemcr.2017.04.014

[60] Torres, R., Garcia, A., Jimenez, M., Rodriguez, S., & Ramirez, J. C. (2014). An integration-defective lentivirus-based resource for site-specific targeting of an edited safe-harbour locus in the human genome. *Gene Therapy*, *21*(4), 343–352. https://doi.org/10.1038/gt.2014.1

[61] Torres, R., Martin, M. C., Garcia, A., Cigudosa, J. C.,

Ramirez, J. C., & Rodriguez-Perales, S. (2014). Engineering human tumour-associated chromosomal translocations with the RNA-guided CRISPR-Cas9 system. *Nature Communications*, *5*, 1–8. https://doi.org/10.1038/ncomms4964

[62] Tsai, S. Q., & Joung, J. K. (2016). Defining and improving the genome-wide specificities of CRISPR-Cas9 nucleases. *Nature Reviews Genetics*, *17*(5), 300–312. https://doi.org/10.1038/nrg.2016.28

[63] Tseng, S. Y., Otsuji, M., Gorski, K., Huang, X., Slansky, J. E., Pai, S. I., … Tsuchiya, H. (2001). B7-DC, a new dendritic cell molecule with potent costimulatory properties for T cells. *Journal of Experimental Medicine*, *193*(7), 839–845. https://doi.org/10.1084/jem.193.7.839

[64] Tsushima, F., Yao, S., Shin, T., Flies, A., Flies, S., Xu, H., … Chen, L. (2007). Interaction between B7-H1 and PD-1 determines initiation and reversal of T-cell anergy. *Blood*, *110*(1), 180–185. https://doi.org/10.1182/blood-2006-11-060087

[65] van der Oost, J., Jore, M. M., Westra, E. R., Lundgren, M., & Brouns, S. J. J. (2009). CRISPR-based adaptive and heritable immunity in prokaryotes. *Trends in Biochemical Sciences*, *34*(8), 401–407. https://doi.org/10.1016/j.tibs.2009.05.002

[66] Van Soolingen, D., De Haas, P. E. W., Hermans, P. W. M., Groenen, P. M. A., & Van Embden, J. D. A. (1993). Comparison of various repetitive DNA elements as genetic markers for strain differentiation and epidemiology of Mycobacterium tuberculosis. *Journal of Clinical Microbiology*, *31*(8), 1987–1995. https://doi.org/10.1128/jcm.31.8.1987-1995.1993

[67] Walton, J., Blagih, J., Ennis, D., Leung, E., Dowson, S., Tookman, L. A., … Vousden, K. (2016). *CRISPR/Cas9-mediated.*

[68] Wang, T., Wei, J. J., Sabatini, D. M., & Lander, E. S. (2014). Genetic screens in human cells using the CRISPR-Cas9 system. *Science*, *343*(6166), 80–84. https://doi.org/10.1126/science.1246981

[69] Wyman, J., Changeux, J. P., Filmer, D., Jovin, T. M., Baehr, W., Holbrook, J. J., … Bustamante, C. (2013). Multiplex Genome Engineering Using CRISPER/Cas System. *Science*, (February), 819–824.

[70] Xu, Z. P., Zeng, Q. H., Lu, G. Q., & Yu, A. B. (2006). Inorganic nanoparticles as carriers for efficient cellular delivery. *Chemical Engineering Science*, *61*(3), 1027–1040. https://doi.org/10.1016/j.ces.2005.06.019

[71] Yin, H., Kanasty, R. L., Eltoukhy, A. A., Vegas, A. J., Dorkin, J. R., & Anderson, D. G. (2014). Non-viral vectors for gene-based therapy. *Nature Reviews Genetics*, *15*(8), 541–555. https://doi.org/10.1038/nrg3763

[72] Yin, H., Kauffman, K. J., & Anderson, D. G. (2017). Delivery technologies for genome editing. *Nature Reviews Drug Discovery*, *16*(6), 387–399. https://doi.org/10.1038/nrd.2016.280

[73] Yin, H., Song, C. Q., Dorkin, J. R., Zhu, L. J., Li, Y., Wu, Q., … Anderson, D. G. (2016). Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components in vivo. *Nature Biotechnology*, *34*(3), 328–333. https://doi.org/10.1038/nbt.3471

Article

Efficacy of different fungicides against the in-vitro growth of *Pyricularia oryzae* causing Rice blast disease

Karuna Kafle[1*], Abichal Poudel[1], Shrinkhala Manandhar[2], Nirmal Adhikari[1]

[1]Purbanchal University, GP Koirala College of Agriculture and Research Center (GPCAR)
[2]Scientist (S1) - National Plant Pathology Research center, Nepal Agricultural Research Council
*Corresponding author– kaflay71@gmail.com

Abstract– Rice blast disease caused by Pyricularia oryzae is a major threat to the farmers as the disease severity may lead upto 80% crop loss in severe conditions. A laboratory experiment was conducted at National Plant Pathology Research center of NARC, Khumaltar,Lalitpur for testing the efficacy of different fungicides adopting poisoned food method against the pathogen Pyricularia oryzae causing rice blast disease. The experiment was carried out in a completely randomized design with 10 treatments including control following 3 replications of each. Two concentrations (50 ppm and 100 ppm) each of different fungicides viz. Dithane M-45 (Mancozeb 75%W.P), Bavistin (Carbendazim 50 % W.P), Antracol (Propineb 70%W.P) , Diamethmorph 50%WP, Sectin (Fenamidone 10%+ Mancozeb 50%W.G),Protector ZN (Chlorothalonil 75% W.P), Safaya (Carbendazim 12%+ mancozeb63% W.P) , Nativo 75% W.G (Tebuconazole 50%+ Trifloxystrobin25%W.G) and Topcare (Azoxystrobin 50% W.D.G) were evaluated against the growth of the pathogen. Results showed that lower concentration (50ppm) of Bavistin and Safaya fully inhibited the growth of the pathogen while Nativo at higher concentration (100 ppm) was shown to exhibit 100% inhibition of the pathogen. The fungicides significantly inhibited the mycelia growth with the increase in concentration of fungicide. However, the fungicides found effective for mycelia inhibition of P.oryzae should be further tested in field conditions to verify their efficacy as well the optimum dozes of fungicide should be re-evaluated.

Keywords— Efficacy, Fungicide, Inhibition, in-vitro, Poisoned food technique.

I. INTRODUCTION

Rice (*Oryza sativa* L.) is the main cereal crop of the world and is a staple food for more than a half population of the world(Ahmad et al., 2020, Singh et al., 2019).More than 2.7 billion people of the world have rice as a major source for their food (Kulmitra et al., 2017). Rice is also the major source of food for the people of Nepal. Rice, maize and wheat are the three major food crops grown in different parts of Nepal. However, rice has the highest productivity in Nepal. Rice is grown in all three ecological belts of Nepal ranging from the plain terai to the high Himalayan region (Joshi et al., 2011).The terai region of Nepal has high productivity of rice contributing to 70% total domestic production of the country.

The productivity of rice in the country is hindered by many factors among which disease is the prime one. The crop loss due to diseases and pest is recorded 37% annually in rice (Moktan et al., 2021). Blast is the major fungal disease attacking the rice plant and is caused by the fungus *Pyricularia oryzae*. This disease is prevalent in humid areas in all the rice growing region of the world as the conidia are not developed below 88% R.H (Neelakanth et al., 2017) .*P.oryzae*, the cause of rice blast disease was reported for the first time in China. In Nepal, it was reported for the first time from Thimi, Bhaktapur in

1964(Neupane & Kiran Bhusal, 2021).The fungus attacks all the above ground parts of rice plant at all growth stages viz., leaf, collar, node, internodes, base or neck and other parts of the panicle and sometimes the leaf sheath. A typical blast lesion on a rice leaf is spindle shaped, gray at the centre, and has a dark border (Kulmitra et al., 2017). Rice blast disease may cause crop loss ranging up to 80 per cent depending upon part of the plant infected (Neupane & Kiran Bhusal, 2021).

Depending on the host affected, it shows variable symptoms as leaf and leaf sheath blast, nodal blast, collar blast, neck blast and panicle blast. Planting of the resistant varieties, use of different fungicides, timely application of fertilizers are the most commonly used method for controlling the disease development. However, application of fungicide has been found quick and effective for mitigating the fungal development. Seed treatment and foliar application of different fungicides have been found effective in controlling the disease (Magar et al., 2015). Rice blast can be effectively controlled upto 40-84 % by application of different fungicides mostly in the temperate and subtropical rice growing regions of the world. (Pandey,2016). To find out the most effective fungicide against the disease, a laboratory experiment was conducted in National Plant Pathology Research Centre, NARC, Khumaltar.

II.   MATERIALS AND METHOD

A laboratory experiment was conducted at NARC, Khumaltar with 10 treatments (50 ppm and 100 ppm) including control and experiment was arranged in completely randomized Design (CRD) to test the efficacy of different fungicides against the in-vitro growth of pathogen *P. oryzae* causing rice blast disease.The experiment was carried out fromJuly 2020 to October 2020.

Culture of *P.oryzae*, previously isolated from rice, Mulpani was obtained from the National Plant Pathology Research Centre NARC, Khumaltar and pure culture was maintained on potato dextrose agar (PDA) under incubated condition at 25°C for 7 days.

Different fungicides (Table. 1) were evaluated in-vitro for testing their efficacy to inhibit the mycelium growth of fungal pathogen *P.oryzae*. Stock solution was prepared for each fungicide by diluting 1gm in 100 ml of distilled water and required amount was incorporated into the conical flask containing Oat meal agar to get 50ppm and 100ppm concentration and mixed thoroughly before autoclaving.

After autoclaving, 20 ml of the poisoned media was poured aseptically into the sterilized petri plates each of 9 cm diameter under laminar air flow chamber and allowed to solidify. As for control, only OMA media was used without addition of any fungicide. 5mm radial disc of the pathogen from the 7 days old culture plate was placed on the center of the petri plate containing OMA media and incubated at 24^{0}C.

The diameter of the pathogen was taken from two directions after 2 day after inoculation (DAI) and recorded for 10 days after. Percent inhibition of fungal growth was calculated using the formula (Vincent, 1947).

$$I\% = \frac{C - T}{C} x 100$$

Where,

C = growth of pathogen in control plate (without fungicide)

T = growth of pathogen in treated plate (with fungicide)

I%= Percentage growth inhibition

Data was entered in MS-Excel (2007) and subjected to ANOVA with the help of R-studio (R-version 3.5.3 statistical data analysis package). Mean comparison among significant variables was carried out by Fisher-LSD test at 5% level of significance. MS-Excel (2007) was used for construction of graph and tables.

Table. 1: Treatments with different fungicides used in the study

S.N	Treatment	Trade name	Composition
1	T1	Dithane M-45	Mancozeb 75%W.P
2	T2	Bavistin	Carbendazim 50% W.P
3	T3	Dimethomorph	Dimethomorph 50%W.P
4	T4	Protector ZN	Chlorothalonil 75% W.P
5	T5	Safaya	Carbendazim 12%+ Mancozeb63%W.P
6	T6	Nativo	Tebuconazole 50%+ Trifloxystrobin25%W.G
7	T7	Antracol	Propineb70% W.P
8	T8	Topcare	Azoxystrobin50%W.DG
9	T9	Sectin	Fenamidone 10%+ Mancozeb 50%W.G
10.	T10	Control	-

III.   RESULTS AND DISCUSSION

3.1 Effect of different fungicides in inhibiting the growth of *P.oryzae* at 50ppm and 100ppm

Different fungicides at 50 ppm and 100 ppm were tested against the pathogen *P.oryzae* following the poisoned food technique and their efficacy ispresented in Table 2. The table shows that the fungal mycelia gradually increased in both treated and control plate with the increase in incubation period of the pathogen. All the 9 fungicides of different concentrations (50 and 100 ppm) visibly inhibited mycelial growth over control during the study (Table 2).

Bavistin and Safaya were found most effective in inhibiting the mycelia growth among the tested 9 fungicides. Bavistin, Safaya and Nativo were individually effective against the pathogen as they showed maximum inhibition of the mycelia growth. At lower concentration (50ppm), Bavistin and Safaya showed complete inhibition of the mycelia growth whereas at higher concentration(100ppm), Bavistin, Safaya along with Nativo showed complete inhibition of the mycelia. They were significantly superior over other fungicides and on par with each other. These were followed by Azoxystrobin and Sectin (100ppm)with more than 70% mycelia inhibition.

Dithane M-45, Dimethomorph and Chlorothalonil showed low inhibition of the fungal mycelia as compared to above fungicides. The fungicide Protector ZN was ineffective against the pathogen with least inhibition percentage. It was noted that the mycelial growth was decreased with the increase in concentration of fungicides.

Table 2. Effect of different fungicides on inhibition of mycelial growth of Pyricularia after nine days of inoculation

Treatment	Inhibition percentage (%)	
	50 ppm	100ppm
Bavistin	$100.00^a \pm 0.00$	$100.00^a \pm 0.00$
Safaya	$100.00^a \pm 0.00$	$100.00^a \pm 0.00$
Nativo	$85.99^b \pm 0.64$	$100.00^a \pm 0.00$
Topcare	$70.42^c \pm 1.72$	$78.29b^b \pm 3.51$
Sectin	$45.44^d \pm 3.59$	$72.82^c \pm 3.41$
Antracol	$50.40^d \pm 5.98$	$47.66^d \pm 5.31$
Dimethomorph	$34.28^e \pm 3.05$	$37.19^e \pm 3.31$
Dithane M-45	$26.80^f \pm 4.81$	$26.38^f \pm 2.17$
Protector ZN	$13.93^g \pm 2.12$	$24.78^f \pm 3.10$
$LSD_{0.05}$	5.39	5.03
CV (%)	5.37	4.49

F-Test	**	**

CV: Coefficient of Variation; **: Significant at 1% level of significance; LSD: Least Significant Difference; Values with same letters in a column are not significantly different at 5% level of significance by Fisher-LSD test and figure ± indicate standard error values.

3.2 Comparison of growth of *P.oryzae* at 50 ppm and 100ppm

Pathogen at different concentration 50 and 100ppm showed different growth from 1st to 9th day after inoculation (Fig.1 and 2). At 50 ppm, Bavistin and Safaya exhibited no growth of the fungal pathogen till the 9th day after inoculation.

Slow growth of the pathogen was observed in the treated plate of Nativo during the early days and the growth of pathogen was not observed from 5th day of inoculation. Similar growth of mycelia was observed in case of Sectin and Antracol treated plates. Dithane M-45 and Dimethomorph also exhibited the similar growth pattern of the pathogen .The growth of pathogen in Protector ZN suddenly increased on the 9th day whereas the growth was similar in other days. Increase in growth of the pathogen was seen every day in Control plate until the plate was fully covered.
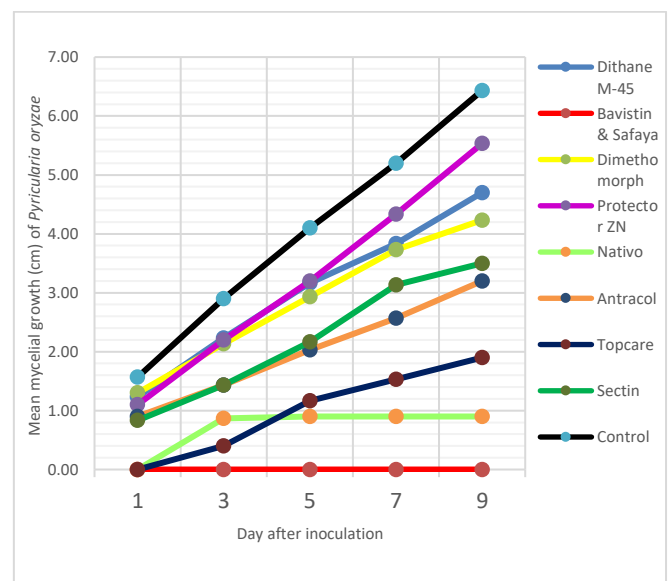
Fig. 1: Effect of different fungicides at 50ppm on mycelial growth of P. oryzae at different days after inoculation

At 100 ppm, Bavistin, Safaya and Nativo showed no growth of pathogen until the 9th day after inoculation. Sectin and Topcare at 100ppm showed similar trend of the pathogen growth along with Protector ZN and Dithane M-45. Antracol exhibited gradual increase in fungal mycelium from the 1st day of inoculation. Control visibly

exhibited the increasing growth of the pathogen every day until full growth on the plate.
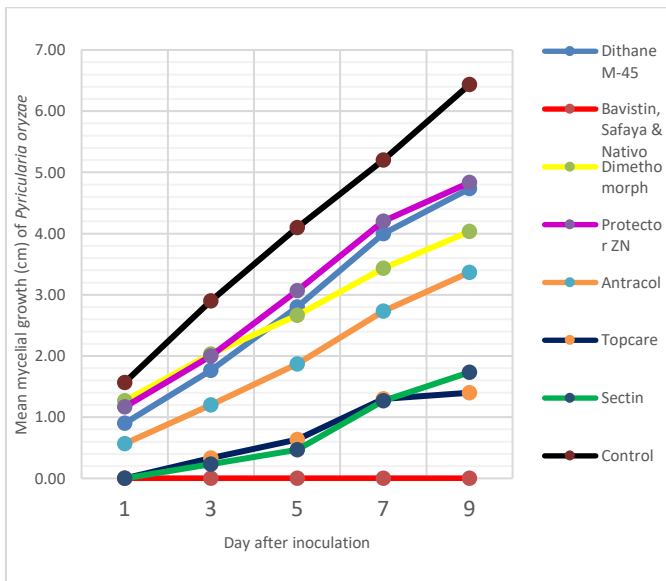
Fig.2: Effect of different fungicides at 100ppm on mycelial growth of P.oryzae at different days after inoculation
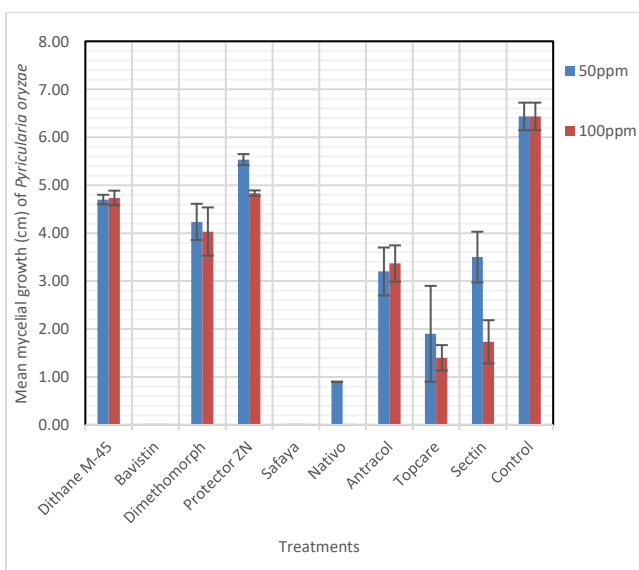
Fig.3: Mean mycelial growth (cm) of P. oryzae in 50 ppm and 100 ppm of different fungicides at 9th day after inoculation

Fungicide at different concentration 50ppm and 100ppm showed different growth of mycelium at 9th day of inoculation (Fig. 3). Growth of fungal mycelium in treated plate of Dithane M-45, Dimethomorph and Antracol were similar in both concentrations (50ppm and 100ppm). Bavistin and Safaya both showed no growth of pathogen even on the 9th day. Nativo at 50ppm concentration showed slight growth of pathogen and at 100ppm exhibited no growth at all. The growth of pathogen was

relatively lower in higher concentration (100ppm) of Protector ZN and Sectin. The growth of mycelia is similar in both concentrations of Antracol. Control on the 9th day of inoculation showed more growth of pathogen as compared to that of the treated plates.

Different concentration of the tested fungicides showed variation in the growth of fungal mycelium. Experiment shows that the highest inhibition of the mycelia was observed in the treated plate of Bavistin and Safaya. This result is in accordance with the finding of Konda et al., 2016 and Neelakanth et al., 2017who found 100% inhibition of the mycelia growth of *P. oryzae* at different concentrations of chemical composition present in Safaya and Bavistin, respectively. From the experiment, it is recorded that Nativo at higher concentration has greatly inhibited the growth of fungal mycelium. Kulmitra et al., 2017 and Singh et al., 2019also recorded that Tebuconazole50%+Trifloxystrobin 25% (WG) was found to be the most effective fungicide against the leaf blast disease.

The study shows that increasing the concentration of Azoxystrobin can increase the inhibition percentage of the pathogen. In our experiment the inhibition% of Azoxytrobin was found to be 70% at 50ppm which increased to 78% at 100ppm.This is in accordance with Neelakanth et al., 2017 who recorded that the inhibition percentage of pathogen was increased with increase in concentration of Azoxystrobin.

DithaneM-45 has been reported effective against *P.oryzae* for seed treatment Konda et al., 2016 and Hajano et al., 2012. However, in our in vitro experiment, it was not found effective. Dithane could be effective as a protectant rather than for control of the disease once the pathogen has invaded.

IV.   CONCLUSION

The use of different chemical fungicides have been found effective to control various plant diseases. The treatments used in our experiment are easily available in the market. Farmers apply same fungicide for various type of fungal diseases and our findings show that there is difference among the fungicides in controlling the fungal growth.Same concentration of these fungicides is not equally effective against the pathogen**.**Results of this study show that rice blast can be effectively controlled by application of different fungicides. From the experiment, it is concluded that Bavistin (Carbendazim 50% W.P), Safaya(Carbendazim12%+Mancozeb63%W.P) are the most effective ones among the tested fungicides with cent percent inhibition of mycelia growth. Nativo (Tebuconazole 50%+ Trifloxystrobin25%W.G) is also

effective against the pathogen *P. oryzae* at higher concentration. The percent of inhibition of pathogen is increased with increasing concentration of fungicides. Further, green house trials and application of these treatments in infected host plants should be carried out to find their effectiveness under field condition.

ACKNOWLEDGMENT

CONFLICT OF INTEREST

Authors declare no conflict of interest regarding the publication of manuscript.

REFERENCES

[1] Ahmad, H. M., Shahid, M. I., Ali, Q., Awais Anjum, N., Ayyub, M., Ikram, A., Faisal, M., Ali, A., & Palwasha, A. (2020). Efficacy of different fungicides against rice blast under field condition in rice crop. Journal of Global Innovations in Agricultural and Social Sciences, 8(1), 15–18. https://doi.org/10.22194/JGIASS/8.882

[2] Hajano, j.-u.-d., a. Mubeen lodhi, mumtaz a. Pathan, m. Ali khanzada, & g. Serwar shah. (2012). In-vitro evaluation of fungicides, plant extracts and bio-controlagents against rice blast pathogen *Magnaporthe oryzae* couch. Pak. J. Bot, 44(5), 4.

[3] Joshi, N. P., Maharjan, K., & Piya, L. (2011). Production economics of rice in different development regions of Nepal. J Int Dev Coop, 17(1), 103-112. Journal of International Development and Cooperation, 17(1), 11.

[4] Konda, S., Nagaraja, Gowdra Nagamma, P.S. Sangeetha, & Suresh Patil. (2016). In vitro Evaluation of Bio-agents and Fungicides Against Leaf Blast (*Pyricularia setariae*) in Foxtail Millet [Setaria italica (L.) Beauv.]. Journal of pure and applied microbiology, 10(1), 8.

[5] Kulmitra, A. K., Sahu, N., Kumar, V. B. S., G., T. A., Ghosh, A., & Gulnaz, Y. (2017a). In vitro evaluation of bio-agents against *Pyricularia oryzae* (Cav.) causing rice blast disease. Agricultural Science Digest - A Research Journal, 37(03). https://doi.org/10.18805/asd.v37i03.8989

[6] Magar, P. B., Acharya, B., & Pandey, B. (2015). Use of chemical fungicides for the management of rice blast (*Pyricularia grisea*) disease at jyotinagar, chitwan, nepal. *International Journal of Applied Sciences and Biotechnology*, *3*(3), 474–478. https://doi.org/10.3126/ijasbt.v3i3.13287

[7] Moktan, R., Aryal, A., Karki, S., Devkota, A. K., Acharya, B., Joshi, D., & Aryal, K. (2021). Evaluation of different chemical fungicides against rice blast in field conditions. *Journal of Agriculture and Natural Resources*, *4*(2), 295–302. https://doi.org/10.3126/janr.v4i2.33942

[8] Neelakanth , Sidde Gowda D. K, Chethana B. S., & Parasappa H. H. (2017). In vitro and In vivo Evaluation of Fungicides Against *Pyricularia oryzae* Causing Blast of Rice. *Int. J. Pure App. Biosci*, *5*(3).http://dx.doi.org/10.18782/2320-7051.3080

[9] Neupane, N. & Kiran Bhusal. (2021). A Review of Blast Disease of Rice in Nepal. Journal of Plant Pathology & Microbiology, 12(1). https://doi.org/10.35248/2157-7471.20.12.528

[10] Pandey, s. (2016). Effect of fungicides on leaf blast and grain yield of rice in kymore region of madhya pradesh in india . Bangladesh j. Bot., 45(2), 8.

[11] Singh, H. S., Kaushik, S. S., Chauhan, M. S., & Negi, R. S. (2019). Efficacy of different fungicides against rice blast caused by *Pyricularia oryzae* (Cav.) under field condition in satna district of madhya pradesh. International Journal of Current Microbiology and Applied Sciences, 8(06), 63–69. https://doi.org/10.20546/ijcmas.2019.806.009

[12] Vincent, J. M. (1947). Distortion of fungal hyphæ in the presence of certain inhibitors. Nature, 159(4051),850–850. https://doi.org/10.1038/159850b0

Article   *Peer-Reviewed Journal*

Dynamics of Dissolved Oxygen in Relation to pH and Survival of Fish Culture in Fiber Glass Tank

T. E. Adams[1,*], V. A. Okonji[2], R. O. Edokpia[3], A. F. Yakubu[1]

[1]Nigerian Institute for Oceanography and Marine Research. Sapele, Delta State. Nigeria.
[2]Department of Production Engineering. University of Benin, Benin City. Nigeria.
[3]Department of Fisheries. University of Benin, Benin City. Nigeria.
*Corresponding author

Abstract— The study aims at determining the dynamics of dissolved oxygen in relation to pH and survival of fish culture in fibre glass tank. Three feeds were used. They are dried chicken manure only (Treatment I), mixture of dried chicken manure with commercial diet (coppens) (Treatment II) and commercial diet only (coppens only) (Treatment III). The physicochemical characteristics of water for the treatments (T_I, T_{II} and T_{III}) in fibre glass tank were determined twice a month using standard methods. ANOVA was used to analyze the effects of the rate of changes between dissolved oxygen, pH and the fish survival.

The result from this study showed that the fish in treatment III had the highest weight gain of 272.10g followed by treatment II (172.07g) and 3 (143.47g) (P<0.05). There was no significance difference at probability level 0f (P<0.05) in survival rate. Variation exists in the water quality parameters examined, dissolved oxygen and pH falls within the range for fish survival. pH was correlated with dissolved oxygen. The findings also provide the useful information about the conditions of the three treatment which will ultimately help to manage the water body for sustainable production. The model showed that dissolved oxygen depends on pH.

Keywords— Dissolved Oxygen, dried chicken, commercial diet, Fiber Glass Tank.

I.   INTRODUCTION

Water quality is the totality of physical, biological and chemical parameters that affect the growth and welfare of cultured organisms. Water quality affects the general condition of cultured organism as it determines the health and growth conditions of cultured organism.

Water quality parameters vary with feeding frequency and have wider impacts on primary productivity and fish production. Good water quality refers to that with adequate oxygen, proper temperature, transparency, limited levels of metabolites, and optimum levels of other environmental factors affecting fish culture.

Water quality in tanks change continuously and are affected by each other along with the physical and biological characteristics.

The term pH refers to the hydrogen ion (H^+) concentration in water, pH refers to how acidic or basic a water is. pH is interdependent with a number of other water quality constituents, including carbon dioxide, alkalinity and hardness. It is known to influence the toxicity of hydrogen sulphide, cyanides and heavy metals, as well as having an indirect effect on ammonia levels; un-ionized NH_3 increases with pH (Klontz 1993).

Meade (1989) recommended that pH be maintained at between 6.5 and 8 for all aquaculture species. In fresh water, pH can change quickly due to the amount of carbon dioxide added or removed during plant growth.

Most estuarine and freshwater species are tolerant of a relatively wide range of environmental pH (Tomasso 1993). Swingle (1969) claims that the desirable range for warm-water pond fish is 6.5 to 9. A range of 5 to 9 was

considered safe by the European Inland Fisheries Advisory Commission (EIFAC 1969). ANZECC and ARMCANZ (2000) and (Begum et al, 2014) recommended guide lines for pH maintained between 5.5 and 9.0 for fresh water.

It should be noted that pH can change by the hour as a function of photosynthesis which removes carbon dioxide.

Dissolved oxygen (DO) is considered as one of the most important water quality parameters in aquaculture. It is needed by fish to respire and perform metabolic activities. Thus, low levels of dissolved oxygen in fish culture cause stress to cultivated fish (Boyd, 1982) resulting in reduced feed intake, poor feed conversion and growth, and are often linked to fish kill incidents.

Dissolved oxygen refers to the level of free, non-compound oxygen present in water or other liquids. It is an important parameter in assessing water quality because of its influence on the organisms living within a body of water (Wetzel, 2001). A dissolved oxygen level that is too high or too low can harm aquatic life and affect water quality (Kemker, 2013). UNEP (2007) pointed out that dissolved oxygen level was a good indicator for water pollution.

Dissolved oxygen is also produced as by product of photosynthesis from phytoplankton, algae, seaweed and other aquatic plants (Kemker, . 2013).

Dissolved oxygen and pH affects directly or indirectly other limnological parameters such as transparency, viscosity, total dissolved solids and conductivity (Whitney, 1942); all of which constitute the very important physical and chemical parameters that form the basis for an enlightened fisheries and water resources management (Araoye et al., 2007).

DO is measured by the azide modification of the Winkler method. The DO level in natural and wastewater depends on the physical, chemical and biochemical activities in the water bodies. Oygen is considered as poorly soluble in water. Its solubility is related to pressure and temperature. In fresh water, DO reaches 14.6mg/l at $0^{o}C$ and approximately 9.1, 8.3 and 7.0mg/l at 20, 25 and $35^{o}C$, the level of saturated DO is 9.0 – 7.0mg/l. for living organism, about 4mg/l of minimum DO should be in water.

Dissolved oxygen is critical for fish and other water inhabitants. Generally, waters with dissolved oxygen concentrations of 5.0 milligrams per liter (mg/L) (equivalent to 5 parts per million (ppm))*1* or higher can support a well balanced, healthy biological community. As dissolved oxygen drops below 5.0 mg/L, aquatic life is put under stress. (Hach Company 2001).

II.   MATERIALS AND METHODS

The study was conducted at the Nigerian Institute for Oceanography and Marine Research Sapele out station Sapele Local Government Area of Delta State, Nigeria (N50 54´.5´´E005°39´56.4´´). six circular fibre glass tanks were used in the experiment. All experimental tanks were identical in shape and size. Tanks capacities were $3.08m^3$ and depth of 60.5cm each and diameter 176.78cm. Sex reversed Nile tilapia (*Oreochromis niloticus)* of 0.80g average size was stocked.

The tanks were divided into three culture systems that is intensive, semi intensive culture and extensive culture in triplicates for each culture system. The treatments were dried chicken manure only, dried chicken manure plus commercial diet (coppens) and commercial diet (coppoens) only These treatments was used to determine the dissolved oxygen dynamics in each culture system and to predict the effect of pH and survival of fish culture in fibre glass tank. Three hundred (300) fish were stocked in each tank.

The fish used for this experiment were fingerlings of all male Nile tilapia (*O. niloticus*). Fish were fed at 800hr and 1600hr with dried chicken manure, chicken manure plus commercial feed (coppens) and commercial feed (coppens) only. The feeding rate was 5% of the total fish biomass presented in each tank and the feed amount was adjusted every two weeks for each tank separately according to the biomass available which was determined during sampling. Random samples of 75 fish were taken biweekly from each treated tank during the experimental period. Fish samples were obtained in the early morning (between 7.00hr to 9.00hr)

From the measurements, the following parameters were determined:

Initial weight of Fish in gram (g):

Final weight of fish harvested (g)

$$Bi-weekly\,mean\,weight\,gain(g) = \frac{Total\,bi-weekly\,weight\,(g)}{Total\,No\,of\,fish\,\,weight\,(g)} \tag{1}$$

$$Absolute\,growth\,rate\,\,\,(g/day) = \frac{Final\,weight - Initial\,weight\,of\,\,fish}{Culture\,peroid\,(days)} \tag{2}$$

$$Specific\,growth\,rate\,(SGR\%\ day) = 100 \times \frac{\left(\ln Finalbodyweight - \ln Initialbody\,weight\right)}{\mathrm{Re}\,aring\,peroid\,in\,days\,(t)} \quad (3)$$

where

ln = natural log

$$Feed\,Conversion\,Ratio = \frac{Total\,weight\,of\ feed\,consumed}{Total\,weight\,of\ fish\,produced} \quad (4)$$

$$Fish\,Survival\,Rate\,(SR\%) = \frac{Total\,fishnumber\,harvested}{Total\,fishnumber\,stocked} \times 100 \quad (5)$$

$$Fish\,Yield = \frac{Total\ weight\ of\ fish\,harvested\,over\,culture}{\left(g\,/180\,days\,/\,3.08m^3\right)} \times 100 \quad (6)$$

The water quality was monitored using the following water testing meters: At the end of the hand picking. Total weight of the fish was taken Random fish samples 50 from each treatment were taken to determine the final mean weight. Harvested fish were kept in plastic containers for marketing.

From the foregoing the primary data collected where used for modeling the dissolved oxygen in the fibre glass tanks with respect to the treatments administered to them vis-à-vis Chicken manure, Chicken manure plus coppens and Coppens only . The average (mean) for each parameter per two weeks was computed, considering the values from three treatments. their interrelations of twenty four weeks were determined by the analysis of variance (ANOVA) using the MiniTab 17 software. All test were carried out at 5% probability level (P <0.05).

III.   RESULT AND DISCUSSION

Treatment I

Table 1: Water quality parameter in time-weeks for Treatment l

Bi Weekly	pH	Yield	Dissolved oxygen
2	6.77	0.83	3.577
4	9.246	1.07	12.797
6	9.826	4.5	14.642
8	10.206	9.14	16.425
10	10.088	14.05	16.736
12	9.47	19.74	17.094
14	8.704	26.57	15.842
16	8.924	40.38	16.603
18	8.598	59.38	16.875
20	8.577	79.27	17.222
22	8.463	111.48	17.211
24	8.314	143.47	16.481
Total	**107.186**	**509.88**	

Table 2: Water quality parameter in time-weeks for Treatment lI

Bi-Weekly	pH	Yield	Dissolved oxygen
2	6.77	0.77	4.581
4	8.563	1.39	8.058
6	8.66	6.63	10.521
8	9.202	13.64	12.978
10	9.149	20.55	14.114
12	8.326	27.84	11.718
14	8.135	35.79	11.397
16	7.847	58.04	10.792
18	7.809	85.04	10.561
20	7.743	107.67	10.567
22	7.651	139.87	10.461
24	7.611	171.97	10.706
Total	**97.466**	**669.2**	

Table 3: Water quality parameter in time-weeks for Treatment lII

Bi-Weekly	pH	Yield	Dissolved oxygen
2	6.77	0.8	3.767
4	9.163	1.98	10.525
6	8.921	15.53	9.662
8	8.831	33.17	9.733
10	8.065	51.57	10.7
12	7.8	72.09	7.625
14	8.009	103.81	9.439
16	8.237	135.35	10.125
18	8.316	174.63	10.461
20	8.109	206.73	10.339
22	7.981	239.1	10.428
24	7.904	272.1	10.45
Total	**98.106**	**1306.86**	

The model intended to be developed is of the form

$$Y = b_o + b_1 y_1 + b_2 y_2 \qquad (1)$$

The Table below show the result of the experimental Do and model DO

For Treatment I

Table 4: Percentage error of Model and Experimental DO for Treatment I

WEEK	DOM	DO$_E$	%error
2	3.644	3.577	-0.0187
4	14.5444	12.797	-0.1365
6	15.1345	14.642	-0.0342
8	16.997	16.425	-0.0348
10	18.3992	16.736	-0.0994
12	18.7659	17.094	-0.0978
14	19.9305	15.842	-0.2581
16	17.8626	16.603	-0.0759
18	18.4584	16.875	-0.0938
20	18.6662	17.222	-0.0839
22	18.7688	17.211	-0.0905
24	19.3276	16.481	-0.1727

For Treatment II

Table 5: Percentage error of Model and Experimental DO for Treatment II

WEEK	DOM	DO$_E$	%error
2	4.58089	4.581	0.00002
4	9.11581	8.058	-0.1313
6	9.86828	10.521	0.0620
8	12.4121	12.978	0.0436
10	14.3228	14.114	-0.0147
12	11.674	11.718	0.0038
14	11.521	11.397	-0.0109
16	10.585	10.792	0.0192
18	10.5961	10.561	-0.0033
20	10.4627	10.567	0.0099
22	10.6665	10.461	-0.0199
24	10.6623	10.706	0.0041

For Treatment II

Table 6: Percentage error of Model and Experimental DO for Treatment III

WEEK	DO$_M$	DO$_E$	%error
2	3.80452	3.767	-0.01
4	10.5692	10.525	-0.0042
6	9.25225	9.662	0.0424
8	10.2043	9.733	-0.0484
10	10.1484	10.7	0.0516
12	8.05753	7.625	-0.0567
14	9.09966	9.439	0.0359

16	10.6125	10.125	-0.0481
18	10.2605	10.461	0.0192
20	10.7015	10.339	-0.0351
22	10.2144	10.428	0.0205
24	10.3366	10.45	0.0109

Table 7: ANOVA results for Treatment l

Source	Sum of Squares	Degree of freedom	Mean square	F value	P value
Model	159.770	7	22.8243	24.34	0.004
x_1	14.449	1	14.4491	15.41	0.017
x_2	22.032	1	22.0321	23.50	0.008
Error	3.750	4	0.9376		
Total	163.520	11			

Table 8: ANOVA results for Treatment l1

Source	Sum of Squares	Degree of freedom	Mean square	F value	P value
Model	60.6163	7	8.65947	17.39	0.008
x_1	6.4582	1	6.45815	12.97	0.023
x_2	0.1662	1	0.16622	0.33	0.594
Error	1.9919	4	0.49798		
Total	62.6082	11			

Table 9: ANOVA results for Treatment lll

Source	Sum of Squares	Degree of freedom	Mean square	F value	P value
Model	41.2256	7	5.8894	16.05	0.009
X1	0.3885	1	0.3885	1.06	0.362
X7	7.7167	1	7.7167	21.03	0.010
Error	1.4676	4	0.3669		
Total	42.6932	11			

The results presented in Tables 7, 8 and 9 shows that for the models representing all three treatments, the model p-value was less than 0.05. This suggests that the response models were significant and can be used for predictive purpose.

Table 10: Coefficient estimate for model representing Treatment 1

Source	Coefficient estimate	Standard error Coefficient	T value	VIF
Const	101.9	26.5	-3.84	-
X1	5.65	1.44	3.93	21.47
X7	0.04841	0.00999	4.85	2.57

Table 11: Coefficient estimate for model representing Treatment ll

Source	Coefficient estimate	Standard error Coefficient	T value	VIF
Constant	25.1	31.6	-0.79	-
x_1	4.42	1.23	3.60	16.28
x_7	0.010	0.0173	0.58	21.95

Table 12: Coefficient estimate for model representing Treatment lll

Source	Coefficient estimate	Standard error Coefficient	T value	VIF
Const	-67.4	17.8	-3.78	
x_1	-2.09	2.02	-1.03	47.91
x_7	0.0241	0.00525	4.59	7.55

The variance inflation factor in all the three treatments are very large since multicollinearity exist among the variables. The coefficient estimate show that there is positive effect on the model and the response for both Treatment I and Treatment II while Treatment III show both positive and negative values. The positive value indicate positive effect on the model while the negative value indicate antagonistic effect on the response.

Growth Performance Mean Weight Gain (MWG)

Using eq. .1, the mean weight gain in treatment I was found to be 42.49 while that of treatment II recorded was 55.77 and treatment III was 108.91. Fish in treatment III showed the highest mean weight gain of 108.91 which was significantly different from all the other treatments (Table 4.11). Fish in treatment I and II recorded 42.49 and 55.77 respectively, which were not significantly different from each other.

Table 13: Bi Weekly Mean Weight Gain of All Male

Tilapia for the three Treatment tanks for 24 weeks of culture

Weeks	Chicken Manure Only (g)	Chicken Manure With Coppens (g)	Coppens Only (g)
2	0.83	0.77	0.80
4	1.07	1.39	1.98
6	4.50	6.63	15.53
8	9.14	13.64	33.17
10	14.05	20.55	51.57
12	19.74	27.84	72.09
14	26.57	35.79	103.81
16	40.38	58.04	135.35
18	59.38	85.04	174.63
20	79.27	107.67	206.73
22	111.48	139.87	239.10
24	143.47	172.97	272.10
X	42.49b	55.77b	108.9a

Growth Performance

Survival Rate

Treatment III fed with coppens only perform better in terms of growth performance while Treatment I has the lowest performance. Survival rate exceeded 90% in all treatments. Treatment I recorded 77%; treatment II 99% and treatment III 99.33% which is the highest survival rate. Table 14 gives the values and this was estimated using eq. 5.

Table 14: Growth performance of all male Tilapia fed with chicken manure only, chicken manure with coppens and coppens only in a fibre glass tank.

Parameters	Treatments		
	I **(Chicken manure only)**	**II** **(Chicken manure with coppens)**	**III** **(Coppens only)**
Initial mean weight (g)	0.83	0.77	0.80
Mean weight gain (g)	42.49b	55.77b	108.91a
Final mean weight (g)	143.47c	171.97b	272.10a
Specific growth rate (%/day)	4.96a	5.14a	5.60a
Survival rate (%)	77a	99a	99.33a
Feed intake (g feed/fish)	584.50c	823.70b	1596.30a
Feed Conversion Ratio	1.95	2.75	5.32

Mean with different superscripts in the same row are significantly different

Water quality analysis

Table 15: Water quality parameters during the experimental period

Parameters	Treatments		
	Chicken manure only	**Chicken manure + coppens**	**Coppens only**
Dissolved Oxygen	15.164	10.539	9.417
pH	8.860	8.073	8.108
Temperature ^{0}C	28.517	28.191	29.044
Electrical Conductivity	293.10	314.30	313.40
Unionized ammonia (NH_3)	0.50	12.80	0.20
Total Dissolved Solid	147.09	157.19	156.55
Transparency (cm)	32.50	25.20	16.42

IV.   CONCLUSION

The water quality parameter table show suitable environmental conditions for rearing All Male Tilapia during the experimental period.

It was observed that dissolved oxygen interact with pH, the yield increase as the dissolved oxygen increase, also the lower the pH the better the dissolved oxygen for the survival of the fish.

It was observed that the response model is significant ($p < 0.05$).

REFERENCES

[1] Araoye PA, Adedeji RA, Dada JO, Adebiyi GO (2007). Relationship bet- ween the rainfall water levels flooding and fish supply from Asa dam Ilorin, Nigeria. In: Proceedings of the Fisheries Society of Nigeria. Eds. Araoye PA, Adikwu IA, Banke ROK. Publ. Fisheries Society of Nigeria. pp. 236-239.

[2] ANZECC & ARMCANZ (2000). (Australian and New Zealand Environment and Conservation Council & Agriculture and Resource Management Council of Australia and New Zealand): Australian and New Zealand guidelines for fresh and marine water quality National Water Quality Management Strategy Paper No4, Canberra.

[3] Beigum A., Mondal S., Ferdous Z., Zafar M. A. And Ali M. M. (2014). Impact of water quality parameters on monosex tilapia (*Oreochromis niloticus*) production under pond condition. IJAFS: ISSN 2308-4715, Volume 2 Issue 1.

[4] Boyd CE (1982). Water quality management for pond fish culture. Elsevier Scientific Publishing Co. New York. p. 318.

[5] EIFAC (1969). European Inland Fisheries Advisory Commission. Working Party on Water Quality Criteria for European Freshwater Fish. List of literature on the effect of water temperature on fish. EIFAC Tech.Pap., (8):8 p.

[6] Hach Company. (2001). Important water quality factors. H2O University. http://www.hach.com/h2ou/h2wtrqual.htm. (April 2001).

[7] Klontz, G. W. (1993): Producing a marketable fish. Part V. Inventory techniques. Northern Aquaculture 10, 21– 25

[8] Kemker, C. (2013). Dissolved Oxygen." *Fundamentals of Environmental asurements*. Fondriest Environmental, Inc. Web. http://www.fondriest.com/environmental-Measurements/parameters/water-quality/dissolved-oxygen.

[9] Meade, I. W. (1989): Aquaculture Management. Van Nostrand Reinhold, New York.

[10] Mitchell and Stapp, 1992. The effect of rain water on stream erosion and total suspended solid levels

[11] Swingle, H. S. (1969). Methods of Analysis for waters, organic matter and Pond Bottom Soils Used in Fisheries Research. Auburn Univ. Auburn, Ala, 119pp.

[12] Tomasso, J. R. (1993). Environmental requirements and diseases of freshwater and estuarine temperature fishes. Pages 240-246 in M stoskopf (Editor). Fish Medicine. WB Saunders, Philadelphia.

[13] UNEP (2007). Water Quality monitoring programme in the Iraq marsh lands. Report from United Nation Environment programme support from the environmental Management of The Iraqi Marsh lands, pp. 1-24.

[14] Wetzel, R. G. (2001): Limnology: Lake and River Ecosystems (3rd ed.). San Diego, CA: Academic Press.

[15] Whitney RJ (1942). Diurnal fluctuations of oxygen and pH in two small ponds and a stream. J. Expt. Biol. 19: 92-99.

Article   *Peer-Reviewed Journal*

Extent of Compliance and Perception of Mothers on Childhood Immunization in Barangay Ugac Sur, Tuguegarao City, Philippines: A Descriptive Study

Tito A. Addun, Jr.[*], Marilyn B. Martinez, Deniz Marie G. Felix, Marfelle Czarina H. Sayoc

School of Medicine, St. Paul University Philippines, Philippines
[*]Corresponding Author

Abstract— This study aimed to determine the extent of compliance of mothers to immunization of their children and the perception of mothers on Expanded Program for Immunization (EPI). Cross-sectional Study, Descriptive. A total of 50 mothers with child or children <5 years of age in Barangay Ugac Sur, Tuguegarao City, Philippines was included in the study. A self-administered semi-structured questionnaire was used. Descriptive statistical tools were utilized to characterize the study population. Fisher's exact test was employed to find out significant association between categorical variables. A p-value < 0.05 was considered statistically significant. The mean age of respondents was 32±7.19 years old. Ninety-four percent (94%) of the respondents were compliant and 6% were non-compliant. Non-compliance was attributed to conflict of schedule. Among the categorical variables, only the employment status and the place of vaccination were significantly associated with the extent of compliance of the mothers to childhood immunization. The proportion of compliant mothers who brought their children to Health center (82%) was significantly higher (p-value<0.05) than the proportion of compliant mothers who brought their children to private clinics (18%). Majority of the respondents had their children's vaccination at the health center, while only a few mothers opted to bring their children in private clinics for the routine vaccination of their children. The respondents gave the highest rating on the perception that giving immunization at the right time is more effective in strengthening the immune system of their children, and that there is little to no risk of an epidemic if children have been immunized. Majority of the mothers were compliant to their children's vaccination, and viewed immunization as an important preventive strategy for their children.

*Keywords— **EPI, public health, vaccine, vaccine-preventable disease.***

I. INTRODUCTION

Immunization is an established tool for the control and elimination of fatal infectious diseases. It is estimated to prevent about 2 to 3 million deaths each year (WHO, 2008). It is considered to be one of the greatest achievements of the 20th century because it has led to the eradication of smallpox in 1977, the dropped in the incidence of poliomyelitis by 99% since 1988, the

elimination of measles in the Western Hemisphere, and the decreased in the measles mortality by about 68% globally from 2000 to 2006 (WHO, 2008).

In the routine childhood immunization in the Philippines, several vaccines were included to target infectious diseases such as tuberculosis, hepatitis B infection, diphtheria, pertussis, tetanus, poliomyelitis, measles. These vaccines

protect children from serious illnesses and complications of vaccine-preventable diseases (WHO, 2008).

In the Philippines, 9 out of 10 children aged 12 to 23 months have received basic vaccinations, namely, BCG, measles, DPT, and polio vaccines (PSA 2013). The Philippine Statistics Authority (PSA) reported in 2013 that those children belonging in poor households are less likely to be vaccinated against the preventable childhood diseases compared to those who belong in non-poor households.

In 2019, the Philippines was in the midst of a measles crisis. According to UNICEF-WHO Philippines (2019), a total of 18553 measles cases, including 286 deaths, were reported from January 1 to March 7 2019. Out of this number, 248 were from Cagayan Valley Region. It was also reported that 60% of all measles cases were not vaccinated (UNICEF-WHO Philippines 2019).

Despite the availability and accessibility of vaccines, people often delay or refuse vaccines for their children. This matter is a growing challenge for countries that are seeking to close the immunization gap. Worldwide data show that 1 out of 5 children are not getting routine life-saving immunizations, and that about 1.5 million children still die each year due to vaccine-preventable diseases (WHO, 2015).

Parent's role in immunization is crucial. This is so because they act as proxy decision-makers for their children (Damnjanovic et al 2018). It is therefore important to deal with the mother's compliance to their children's vaccination. However, there are only limited studies about the compliance of mothers to childhood immunization. In the study of Alfred and Alfred (2012) in Nigeria, 56% of the respondents had a high participation to EPI. Majority of their respondents had favorable attitude towards EPI and perceived it as important. It was also noted that age, marital status, household size and religion had significant relationship with the respondents' perception towards EPI.

With the scarcity of local data, the researchers of this study delved on the determination of the compliance of the mothers and their perception towards childhood immunization. Additionally, this paper hoped to use the obtained data to address non-compliance to vaccination to further improve the health status of the community.

1.1 Objectives of the Study

In general, this study aimed to determine the perception of mothers in Barangay Ugac Sur, Tuguegarao City about the Expanded Program for Immunization (EPI).

Specifically, it sought to determine the following:

a) Profile of the mothers with regards to Marital Status, Educational attainment, Monthly income of the family, Number of Children, Occupation, and Type of Family

b) Significant correlation between the profile of the mothers and the compliance status of the respondents

c) Extent of compliance of mothers to immunization of their children;

d) Perception of mothers on the Expanded Program for Immunization (EPI) in terms of Health promotion, Disease prevention, and Reduction in Morbidity or Mortality.

II.   METHODOLOGY

2.1 Study Design

The researchers used a Cross-sectional Descriptive Type of Research Design. It is a self-reported data which was collected from the respondents to explore and describe the perception of mothers toward childhood immunization and the extent of compliance to immunization. In this study, convenience sampling was done to determine the respondents, and only those who satisfied the inclusion criteria were included in the study.

2.2 Research Locale

The study was conducted in Barangay Ugac Sur, Tuguegarao City. The researchers chose this barangay because, out of 49 barangays in Tuguegarao City, Cagayan, Philippines, this community caters the largest population.

2.3 Respondents of the Study

The respondents of the study were the mothers in Barangay Ugac Sur, Tuguegarao City, Cagayan, Philippines. A convenience sampling of 50 mothers was done in this study. Only those who satisfied the following inclusion criteria were included in the study:

a)   A mother

b)   A resident of Barangay Ugac Sur, Tuguegarao City

c)   Has a child or children <5 years of Age

2.4 Instrumentation

The data gathering instrument that the researchers used was a self-administered semi-structured questionnaire that is based on the study of Castillo and colleagues (2014) entitled "Extent of Compliance to Immunization: Reasons for Non-continuity and Its Consequences," which was administered in Barangay Darasa, Tanauan, Batangas.

The questionnaire was composed of two parts. The first part was about the profile of the respondents, which include marital status, number of children, educational attainment, monthly income, type of family, and occupation. Also included in the first part were the compliance of the respondents to vaccination and the place of vaccination. The second part of the questionnaire included questions regarding the perception of mother about the expanded program on immunization (EPI).

2.5 Data Gathering Procedure

Prior to the conduct of the study, request letters addressed to the City Health Office and the Barangay Council of Ugac Sur, Tuguegarao City were given to allow the researchers to gather preliminary data from the Barangay Health Center in Ugac Sur, Tuguegarao City and to acquire the list of mothers who have children in need of immunizations.

The researchers personally distributed the questionnaires, and interviewed and assisted the target respondents. After which, accomplished questionnaires were retrieved, and raw data were statistically evaluated.

2.6 Ethical Consideration

The study was approved by the review panel of the School of Medicine of St. Paul University Philippines, Tuguegarao City, Cagayan, Philippines. In addition, pertinent consent form and information sheet were integrated in the data collection sheet. All of the respondents were clearly informed about the objectives as well as the significance of the study. Finally, informed consent was obtained before the conduct of the interview.

2.7 Data Analysis

The statistical analysis of the data obtained in this study was done through Microsoft Excel and SPSS version 22. The researchers used appropriate statistical tools to ensure valid and reliable analysis and interpretation of data. Frequency distribution, Percentage distribution, and Measures of Central Tendency were utilized. Fisher's exact test was used to find out significant association between categorical variables. The "mean" was used to denote the perception of mothers to the EPI. A p-value of <0.05 was considered statistically significant.

III.   RESULTS AND DISCUSSION

3.1 Profile of the Respondents

In this study, a total of 50 respondents from Ugac Sur, Tuguegarao City, Philippines were included. The mean age of the respondents was 32 ± 7.19 years old.

Table 1 shows the characteristics of the respondents based on their compliance status. Among the categorical variables, only 2 variables showed significant correlation with the compliance status of the respondents. This significant association was determined using a Fisher's exact test. It showed that the employment status of the respondents, and the place of vaccination of their children were significantly associated with the compliance of the mothers to childhood immunization.

Table 1. The characteristics of the respondents according to their compliance status.

Variable	Compliant (N)	Not Compliant (N)	p-value
Marital Status			
Single	12	1	p=0.6036
Married	35	2	
Number of Children			p=0.03053
One or 2	31	3	
3 or more	16	0	
Educational Attainment			
College Undergraduate	30	1	p=0.3119
College graduate	17	2	
Monthly Income			
<PHP10,000	28	2	p=0.6510
≥PHP10,000	19	1	
Employment Status			p=0.0286*
Employed	13	3	
Unemployed	34	0	
Type of Family			
Nuclear	25	1	p=0.4694
Extended	22	2	
Place of Vaccination			p=0.0084*
Health Center	39	0	
Private Clinic	8	3	

*p-value<0.05 is considered statistically significant

In the present study, the number of compliant mothers who are married is greater than that of the single mothers. However, in this study, there was no significant correlation found between the marital status of the respondents and their compliance to childhood immunization. Nonetheless, various studies found significant association between marital status and vaccination coverage. In fact, in the study of Kim et al (2007), it was noted that single

motherhood was significantly associated with lower completion rates.

In this study, though majority of the compliant mothers have 1 or 2 children, there was no significant association noted between the number of children and compliance of mothers to childhood immunization. But various studies found inverse relationship between the number of children in the family and the immunization status of their children. In the study of Holipah et al (2018), they were able to observe that mothers with 5 children or more have a lesser likelihood to get their children immunized.

Several studies showed significant association between mother's literacy and vaccination status of their children. In the present study, majority of the mothers who were compliant to their children's immunization were college undergraduate. However, in the present study, there was no noted significant correlation between the educational attainment of the respondents and their compliance to immunization. On the other hand, in the study conducted by Singh et al (2018), in which it was found out that mothers who have completed 12 or more years of education are more likely to have their children fully vaccinated compared to those mothers who have low educational attainment.

Majority of the respondents in this study have a monthly income of less than PHP10,000. In the study conducted by Balogun et al (2017), it was mentioned that household economic status is a significant factor contributing to immunization uptake. Also, some studies revealed that children of parents in higher socioeconomic status are more likely to complete immunization. Conversely, the present study did not show any significant association between monthly income and the compliance of mothers to immunization.

Previous research has shown that unemployed mothers had less likelihood to immunize their children compared to the employed mothers (Subhani et al 2015). In contrast to this, the present study revealed that more than half of the compliant mothers are unemployed ($p < 0.05$). This finding of the present study was consistent with the finding of Sing et al (2018) and Verulava et al (2019). It was also noted in the present study that all of the mothers who were not compliant to childhood immunization were employed. The non-compliance to immunization of employed mothers may be attributed to the difficulty of getting the immunization done on a fixed date (Sing et al 2018).

A significantly strong association was found between place of vaccination and compliance of mothers to their children's immunization. Majority of the respondents had their children's vaccination at their health center. Only a few mothers opted to bring their children in private clinics for the routine vaccination of their children. Opposite result was observed by Pattnaik et al (2015). In their study, majority of the parents preferred private service providers due to convenient time and place. In the present study, the preference of mothers to health center could be attributed to the accessibility of the place and free vaccination.

3.2 Extent of Compliance

Majority (94%) of the respondents were compliant to their children's immunization. Nonetheless, 6% of them were found to be non-compliant to the vaccination of their children. These non-compliant mothers mentioned that the reason for their non-compliance to their children's immunization was conflict of schedule. All of the non-compliant mothers were employed (2 were private employees and 1 was a vendor), and had difficulty in finding available time for their children's immunization. As such, to be able to cope with this, all of the non-compliant mothers had their children vaccinated in private clinics.

3.3 Perception of Mothers to EPI
Table 2. Immunization as a Health Promotion

HEALTH PROMOTION	Mean	Interpretation
Children who receive complete vaccine is healthy and less likely to get sick	3.9	Strongly Agree
Children who received completed immunization has stronger immune system	3.8	Strongly Agree
Giving immunization at the right time is more effective in strengthening the immune system of the children	3.96	Strongly Agree
Average weighted Mean	3.88	Strongly Agree

Table 2 illustrates the perception of the respondents on Immunization as health promotion. It shows that the mothers strongly agree that giving immunization at the right time is more effective in strengthening the immune system of their children (3.96), that children who receive complete vaccine is healthy and less likely to get sick (3.9), and that children who received complete immunization has stronger immune system (3.8).

Table 3. Immunization as a Disease Prevention

DISEASE PREVENTION	Mean	Interpretation
Children who has completed vaccination prevents spread of communicable diseases	3.9	Strongly agree
The more the number of children who are completely vaccinated, the lesser the risk of contracting immunization preventable diseases.	3.9	Strongly agree
Immunization helps in protecting the child from serious illnesses.	3.9	Strongly agree
Average weighted mean	3.9	Strongly agree

Table 3 illustrates the perception of the respondents on Immunization as disease a prevention. It shows that the mothers strongly agree that children who has completed vaccination prevents spread of communicable diseases (3.9), that the more the number of children who are completely vaccinated, the lesser the risk of contracting immunization preventable diseases (3.9), and that immunization helps in protecting the child from serious illnesses (3.9).

Table 4. Immunization as a Means in Reducing Mortality/Morbidity

REDUCE COMPLICATION/DEATH	Mean	Interpretation
There is little to no risk of an epidemic if children have been immunized.	3.96	Strongly agree
Immunization provides an opportunity to deliver other lifesaving measures.	3.68	Strongly agree
Immunization can prevent death caused by communicable diseases.	3.76	Strongly agree
Average weighted mean	3.8	Strongly agree

Table 4 illustrates the perception of the respondents on Immunization as a means in reducing complication or death. It shows that the mothers strongly agree that there is little to no risk of an epidemic if children have been immunized (3.96), that Immunization can prevent death caused by communicable diseases (3.76), and that

immunization provides an opportunity to deliver other lifesaving measures (3.68).

IV. CONCLUSION

Childhood immunization is an important tool in the prevention of many vaccine-preventable diseases. To achieve a high rate of childhood immunization, there must be a concomitant high level of compliance of the mothers. Nonetheless, there are several factors which can influence compliance with immunization that should be considered.

In this study, majority of the mothers were found to be compliant to the immunization program of the Department of Health. This high compliance rate of the mothers may be attributed to the easy access to public health facility (such as health center) and free vaccines in these facilities. It was evident that employment status was significantly associated with the compliance of mothers to childhood immunization. All of the non-compliant mothers were employed and tend to have conflict of schedule with their children's immunization.

Majority of the mothers showed positive perception towards childhood immunization as a health promotion, as a component of disease prevention, and as a factor that alleviates morbidity and mortality from vaccine-preventable diseases. This study revealed that majority of the mothers perceive immunization as an important preventive strategy for their children.

V. RECOMMENDATION

This study showed a baseline data on the compliance of mothers to their children's vaccination. It showed that there is a high rate of compliance by the mothers. However, those who were not compliant were found to have difficulty looking for available time for their children to get vaccinated. The researchers therefore would like to recommend to the public health facilities to devise a plan to address this matter.

With the limited number of respondents in the study, the researchers would like to recommend a larger population size for future studies.

The study focused on the perception of the mothers toward immunization. Thus, for future studies, the researchers suggest to use a theory that can cover the practice of the respondents that lead to their behavior in the immunization program.

REFERENCES

[1] World Health Organization (WHO). 2008. WHO immunization work: 2006-07 highlights. Retrieved from https://apps.who.int/iris/handle/10665/43899. Accessed on

March 17, 2019

[2] Philippine Statistics Authority (PSA). 2013. Breastfeeding, Immunization and Child Mortality. Retrieved from https://psa.gov.ph/content/breastfeeding-immunization-and-child-mortality. Accessed on March 10, 2019.

[3] UNICEF-WHO Philippines. 2019. Measles outbreak Situation Report 5, 12 March 2019. Retrieved from https://reliefweb.int/report/philippines/unicef-who-philippines-measles-outbreak-situation-report-5-12-march-2019. Accessed on March 17, 2019.

[4] World Health Organization (WHO). 2015. Vaccine hesitancy: A growing challenge for immunization programs. Retrieved from http:// www. who.int/ mediacentre/ news/releases/ 2015/ vaccine-hesitancy/en/. Accessed on March 10, 2019.

[5] Damnjanović, K., Graeber, J., Ilić, S., Lam, W. Y., Lep, Ž., Morales, S., Pulkkinen, T., & Vingerhoets, L. 2018. Parental Decision-Making on Childhood Vaccination. Frontiers in psychology, 9, 735.

[6] Alfred, S. & Alfred, B.. (2012). Rural Households' Perception of the Expanded Programme on Children Immunization (Epi) in the Southwest of Nigeria.. Greener Journal of Medical Sciences. 2. 012-018. 10.15580/GJMS.2012.1.GJMS1203.

[7] Castillo et al. 2014. Extent of Compliance to Immunization: Reasons for Non-Continuity and Its Consequences. CAM Research Journal Vol 2 No. 1.

[8] Kim, S. S., Frimpong, J. A., Rivers, P. A., & Kronenfeld, J. J. 2007. Effects of maternal and provider characteristics on up-to-date immunization status of children aged 19 to 35 months. American journal of public health, 97(2), 259–266.

[9] Holipah, Maharani, A. & Kuroda, Y. 2018. Determinants of immunization status among 12- to 23-month-old children in Indonesia (2008–2013): a multilevel analysis. BMC Public Health 18, 288.

[10] Singh J, Deepti SS, Mahajan S, Lal M, Singh T, Neki NS. 2018. Assessment of Socio-demographic factors affecting Immunization status of children of age 0-2 years in Slums of Amritsar city. Int J of Current Research in Med Sci 4(3): 17-25.

[11] Balogun, S. A., Yusuff, H. A., Yusuf, K. Q., Al-Shenqiti, A. M., Balogun, M. T., & Tettey, P. 2017. Maternal education and child immunization: the mediating roles of maternal literacy and socioeconomic status. The Pan African medical journal, 26, 217.

[12] Verulava T, Jaiani M, Lordkipanidze A, Jorbenadze R, Dangadze B. 2019. Mothers' Knowledge and attitudes towards child immunization in georgia. Open Public Health J.12(1).

[13] Pattnaik, S., Selvaraj, K., Kumar, G. M., & Elango, R. 2015. Why do some parents prefer private vaccine providers in urban area?.Journal of Family Medicine and Primary Care, 4(4), 606.

Article

Mini review of parasitoids collected in the Cerrado Biome in Brazil

Carlos Henrique Marchiori

Department of Biological Sciences, CHM, Instituto Federal Goiano, Brazil

Abstract— The climate is predominantly hot with a rainy and a dry season. Second only to the Amazon, the Cerrado occupies an area of more than 2 million km, corresponding to about 25% of the national territory. It is not only the second largest ecosystem in Brazil, but also in all South America. It is known as such in our country, but in other parts of the world it occurs under the name of savannas, which are characterized by vegetation with low trees, spaced shrubs, and grasses. For the elaboration of this mini review, which consists of the construction of a bibliographic summary of the main groups of parasitoids of the Order Hymenoptera, with an emphasis on the hymenopterans parasitoids collected in the Cerrado Biome (savanna). A bibliographic search was carried out that contained papers published from 2001 to June 2021 on the quantitative aspects of Superfamilies, Families, Subfamilies, Genera and Species. The mini review was carried out from February to June 2021. Parasitoids are often called parasites, but the term parasitoid is more technically correct. By definition, insect parasitoids have a free-living adult stage and an immature life stage that develops on or within an insect host and ultimately kills it. After feeding on host body fluids and organs, most parasitoids leave their hosts to pupate or emerge as adults. Parasitoids have a free-living adult stage and an immature life stage that develops on or within an insect host and ultimately kills it. They are effective control agents in pest population.

Keywords— **Agriculture, Biological control, Biological Sciences, Insect pests.**

I. CERRADO

It is known as such in our country, but in other parts of the world, it occurs under the name of savannas, which are characterized by vegetation with low trees, spaced shrubs, and grasses. The climate is predominantly hot with a rainy and a dry season. Second only to the Amazon, the Cerrado occupies an area of more than 2 million km, corresponding to about 25% of the national territory. It is not only the second largest ecosystem in Brazil, but also in all South America. In the Cerrado domain, the rivers that form the main hydrographic basins in the country are born. They are: Parnaíba, Paraguay, Paraná, Tocantins-Araguaia, São Francisco, and Amazônia. Paraguay and Paraná join the Uruguay River and form the La Plata basin, which is of great economic importance for Brazil and the bordering countries. In its area there are also three large aquifers: Guarani, Bambuí and Urucuia. The Cerrado is considered

a global hotspot - an area that is home to great biological diversity and endemic species, that is, they are not found anywhere else in the world (Costa 2019).

It is estimated to have more than 6,000 species of trees and 800 of birds. Approximately 40% of woody vegetation species and about 50% of bees are endemic species. Regarding insects, there are more than 14 thousand species, representing 47% of the insect diversity in Brazil. Research claim that 20% of native and endemic species no longer occur in protected areas and that at least 137 species of fauna present in the Cerrado are threatened with extinction. After the Atlantic Forest, the Cerrado was the Brazilian biome that received the most exploitation by human activities. Agricultural and livestock expansion is largely responsible for this occupation, along with the use of firewood to produce charcoal (Costa 2019).

The Cerrado is one of the six Brazilian biomes, concentrated mainly in the Central Plateau, and is present in the states of Goiás, Tocantins, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Bahia, Maranhão, Piauí, Rondônia, Paraná, São Paulo, and Federal District (Costa 2019).

Despite the unique characteristics of biodiversity, the Cerrado, along with the Caatinga, is the least protected area by the government. This biome has 8.21% of its territory legally protected by conservation units; of this total, 2.85% are full protection conservation units, in which only indirect uses are allowed; and 5.36% of sustainable use conservation units, in which the use of natural resources is compatible with nature conservation (Costa 2019).

Fig.1: General aspect of the Cerrado.

II. PARASITOIDS

Parasitoids are often called parasites, but the term parasitoid is more technically correct. By definition, insect parasitoids have a free-living adult stage and an immature life stage that develops on or within an insect host and ultimately kills it. After feeding on host body fluids and organs, most parasitoids leave their hosts to pupate or emerge as adults.

Parasitoids have a free-living adult stage and an immature life stage that develops on or within an insect host and ultimately kills it. They are effective control agents in pest population (Gibb 2015).

Most beneficial insect parasitoids are wasps or flies, although other insects may have life stages that are parasitoids as well. Parasitoids usually complete their life cycles much more quickly and increase their numbers faster than predators, even though a parasitized host does not die as quickly as those eaten by predators (Gibb 2015).

Parasitoids are often the most effective natural enemy of pest insects, even though they may not be readily visible.

Sometimes pest populations increase after pesticides are applied. This can happen when a chemical insecticide poisons the parasitoids before they can kill the pest insect host. Once free of the natural parasitoids (Figure 2), the pest insects are then free to increase in number (Gibb 2015).

Fig.2: Parasitoids of Mechanitis menapis mantineus: A-B (Eulophidae), from huevos; C-D (Chalcididae), from larvae; E (Ichneumonidae) and F (Braconidae), from pupa. Parasitoids of Mechanitis menapis mantineus: A-B (Eulophidae), from eggs; C-D (Chalcididae), of larvae; E (Ichneumonidae) and F (Braconidae), from pupa. Source: Paola Santacruz, Science Museum · Research Master of Science. https://www.researchgate.net/profile/Paola-Santacruz-3

III. METHODS

For the elaboration of this mini review, which consists of the construction of a bibliographic summary of the main groups of parasitoids of the Order Hymenoptera, with an emphasis on the hymenopterans parasitoids collected in the Cerrado Biome. A bibliographic search was carried out that contained papers published from 2001 to June 2021 on the quantitative aspects of Superfamily, Family, Subfamily, Genera and Species. The mini review was carried out from February to July 2021 using the Online Electronic Scientific Library (Scielo) and Internet (Google) in Goiânia, Goiás, Midwest region.
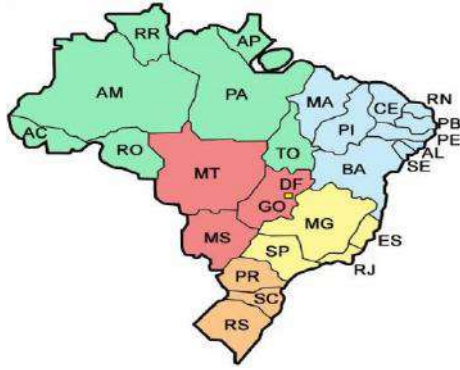
Fig.3: Map of Brazil, Goiás (GO), Midwest region (red color). Source: https://br.pinterest.com/pin/576320083551631769/

IV.   STUDIES PERFORMED

Study 1

The objective of this research work was to identify the genera of Braconidae collected in a remnant area of native forest located in Itumbiara County, State of Goiás, Brazil, using Malaise traps (Figure 4). Twenty-four samplings were performed from January to December 200. From these collections, 49 specimens from 19 different genera and 10 subfamilies of Braconidae were obtained. The subfamily Doryctinae had the highest diversity of genera. The number of specimens obtained was low considering the collection period. *Chelonus* was the most abundant genus comprising 34.7% of the specimens collected. Most specimens were obtained in the months of March and June, with 20.4% and 24.5% occurrence frequency, respectively. These results contribute to the knowledge of entomofauna occurring in the Itumbiara region, southern Goiás state, Brazil (Marchiori 2014).

Fig.4: Malaise traps

Study 2

The objective of this study was to investigate the families of the Hymenoptera parasitoids collected in areas of native forest, using Malaise traps and Moericke traps in western Minas Gerais and southern Goiás. The experiment was conducted at the Faculty of Agronomy Farm in southern of Goiás and the western of Minas Gerais in Alvorada Farm in the period February to October 2002. Sampling was weekly, with 10 Moericke traps and 3 Malaise. A total of 15827 specimens of parasitoids distributed in 8 superfamilies and 21 families were collected, of which 10435 in Malaise traps and 5400 in the Moericke traps (Figure 5).

A total of 9540 specimens were collected in western Minas Gerais with and 6287 in southern Goiás. The most abundant families were Ichneumonidae with 4562 and Braconidae with 2532. The Ichneumonidae was the most frequent family collected in the Moericke traps. Also, in the Malaise traps, the family Ichneumonidae was the most frequently found. The superfamily Chalcidoidea presented the highest diversity of families (10 families) in both trap types (Marchiori et al. 2002).

Fig.5: Moericke traps

Study 3.

With this work, it is intended to get acquainted with Eucoilinae collected in a remnant of thick wood by the trap "Malaise" in Itumbiara, Goiás. The trap "Malaise" captures the insects by interception. During the period from February to September 2000, 20 specimens of the subfamily Eucoilinae (Hymenoptera: Figitidae) were collected, of which 1 specimen of *Dicerataspis* sp., 3 specimens of *Dettmeria* sp., 1 specimen of *Odonteucoila* sp., 1 specimen of *Paraganaspis egeria* (Diaz, Gallardo & Wash), 10 specimens of *Steleucoela* sp., 1 specimen of *Trybliographa* sp. and 2 specimens of *Zaeucoila unicarinata* Ashmead. *Steleucoela* sp. was the most abundant species with 50.0%. The number of females (35.0%) was lower than that of males (65.0%) in an approximate proportion of 2:1. This work registers the first occurrence of *Dicerataspis* sp., *Trybliographa* sp., and

Zaeucoila unicarinata to the State of Goiás *and Steleucoela* sp. to Brazil. *Steleucoela* sp. (Marchiori et al. 2001)

Study 4.

794 specimens of hymenopterans were sampled parasitoids distributed in seven superfamilies and 27 families. The most predominant Family of individuals was Ichneumonidae with 21.9% followed of Ceraphronidae with 17.4% of the total samples. At Malaise traps were the ones that captured the biggest number of families and individuals, 26 and 541 respectively. The family that was most captured by these traps was Ceraphronidae, with 17.0% of the individuals collected. The Moericke and Sweep traps captured a close number of families, 13 and 14 respectively, despite the large difference in the number of individuals, 183 and 70, this difference can be justified by differences in the sampling effort of each method. At Moericke traps have not captured an exclusive family, on the other hand, sweeping the vegetation was the only method that collected the Pelicinidae family. In both methods, the family Ichneumonidae was the most sampled. Being a pioneering study involving parasitic collection in the southwest region of the state of Goias, and the results of the collections having been carried out in a short space of time (20 days), the number of copies and families collected can be considered quite representative, considering the small size of the sampled fragment (Morais et al. 2011).

Study 5

The aim of this study was to know the parasitoid hymenopteran fauna in an agricultural area. The survey was carried out between May 2003 and June 2004 in an area of 24.2 hectare located in the municipality of Rio Claro, SP, Brazil during this period, the cultivation of sorghum, corn, beans and wheat was carried out in a rotation system under no-tillage. Collections were carried out every two weeks, using 17 Moericke traps (Souza et al. 2006).

A total of 5,308 parasitoid hymenopteran were collected belonging to 22 families distributed in 8 superfamilies. The most collected superfamilies in this survey were Chalcidoidea and Platygastroidea with 60.08% and 21.65%, respectively, representing 81.73% of the total collected. The most abundant families were Mymaridae (30.88%), Encyrtidae (19.05%) (Chalcidoidea), Scelionidae (14.96%) and Platygastridae (6.69%) (Platygastroidea). The remaining eighteen families presented relative frequencies, in relation to the total of collected parasitoids, inferior to 5%. The other superfamilies found had a relative frequency lower than 5%, being Ceraphronoidea (4.52%), Proctotrupoidea

(4.45%), Ichneumonoidea (4.09%), Cynipoidea (3.01), Chrysidoidea (1.92 %) and Evanioidea (0.28%) (Souza et al. 2006).

Study 6.

This work presents a survey of the entomofauna associated with the fruits of the bacupari (*Salacia crassifolia*) (Ippocrateaceae family) in the cerrados of Goiás. The fruits were collected from September 1995 to December 1999, at the time of fruiting (Rocha et al. 2001).

A total of 1,090 insects were obtained from 2,898 fruits in 11 municipalities of the State of Goiás. The flies collected were from the families Tephritidae (54.22%), Lonchaeidae (6.60%), Muscidae (1.01%) and Oititidae (1.01%). Among the Tephrididae the species collected were *Anastrepha fraterculus* (Wiedemann), *Anastrepha obliqua* ((Macquart), *Anastrepha serpentina* (Wiedemann), *Anastrepha sororcula* Zucchi and *Anastrepha* sp. (new species). Insects of the Lonchaeidae family represented by Neosilba spp. Another species of lesser occurrence was *Atherigona orientalis* Schiner (Diptera: Muscidae). A total of 405 parasitoids were collected from 3,474 fruit fly pupae grouped in the Braconidae (390), Figitidae (12) and Pteromalidae (03) families. The most frequent species was *Doryctobracon* sp., with a parasitism rate of 8.78% (Rocha et al. 2001).

Study 7.

This work aimed to carry out a survey of winged aphids and parasitoids associated with wheat crop in a Cerrado area in the state of Minas Gerais. *Rhopalosiphum padi* (Linnaeus), *Sitobion avenae* (Fabricius), *Rhopalosiphum maidis* (Fitch) were the most collected aphid species during the three years of study. The largest aphid population observed in 2014 in Uberaba, when compared to 2013 and 2015, may be related to the lower rainfall that occurred that year, during wheat cultivation. Six species of parasitoids associated with wheat aphids were recorded, with *Aphidius colemani* Viereck (Hymenoptera: Braconidae, Aphidiinae) being the most collected species during the two years of study (Júnior et al. 2021).

Study 8.

This paper presents a survey of parasitic Hymenoptera obtained from fruits of *Ilex affinis* Gardner (Aquifoliaceae) collected in area of the Brazilian savannah (cerrado) in São Paulo State, Brazil. Green fruits of *I. affinis* were collected in a residual area of Brazilian savanna located at Fazenda Itaúna in Descalvado County, São Paulo State, Brazil, and maintained in plastic containers covered with synthetic gauze and daily observed until putrefaction. Eighty-eight seeds, from 50 fruits, were dissectedm under

stereomicroscope and data of the presence of insects in its interior recorded (Perioto et al. 2009).

From the fruits were reared 21 specimens of Eurytomidae (Hymenoptera): 20 females of *Prodecatoma juliae* sp. nov. and one male of *Sycophila* sp., 20 females and 10 males of *Galeopsomyia itauna* sp. nov. (Hymenoptera, Eulophidae) and two males of a not identified species of Doryctinae (Hymenoptera, Braconidae). In the 50 dissected seeds fruit were found 88 seeds (1.8 seed/fruit), 63 (71.6%) infested by chalcids from which 8 (12.7%) were attacked by *Galeopsomyia itauna* sp. nov., 55 by (87.3%) *Prodecatoma juliae* sp. nov. and one (1.6%) by *Sycophila* sp. Records in the literature show that immature stages of *Prodecatoma* and *Sycophila* are seed infesting chalcids; probably *Galeopsomyia* is a infesting parasitoid of the seed and the ecological function of the Doryctinae was not established. Records in the literature show that immature stages of *Prodecatoma* and *Sycophila* are seed infesting chalcids; probably *Galeopsomyia* is a infesting parasitoid of the seed and the ecological function of the Doryctinae was not established (Perioto et al. 2009).

Study 10.

Accordingly, aim of this study was to conduct a broad-scale assessment of the faunal composition of parasitoid Hymenoptera of the Parque Nacional Serra da Canastra. Sampling was carried out between September 2009 and October 2010 and totaled 45 samples sweepings from all four sites. Each monthly sample was a result of 15 minutes of simultaneous collection effort, by four people (total excluding time for stops to removal of collected material). The technique was applied randomly to vegetation from ground level to a height of approximately two meters. During data collection, the insects captured were transferred to killing bags containing cotton pads soaked in ethyl acetate, to fix the material collected (Pádua et al. 2014).

Eight superfamilies and 24 families. Three Pimplinae genera were also identified. *Neotheronia, Pimpla* and *Zonopimpla* were genera of pimplineds collected in this study, through the sweeping technique. Eulophidae, Braconidae, Platygastridae, Pteromalidae and Encyrtidae together were the most abundant families, giving 73% of the total sample (4,743 exemplars). The abundance of Braconidae was higher than the other families for six months of the collecting, while Eulophidae was highest for four months, already Platygastridae and Pteromalidae for one month each. Eulophidae, Braconidae, Platygastridae and Ichneumonidae occurred in all samples by month (Pádua et al. 2014).

Study 11.

The objective of this study was to verify whether fruit flies present seasonality in three contiguous orchards, relating these data to host phenology and to parasitoids. The study was conducted, during 2002 and 2003, in Monte Alegre do Sul, SP. The experiments were carried out in three orchards: a collection of guava progenies (*Psidium guajava* L.); a collection of loquat cultivars [*Eriobotrya japonica* (Thunb.) Lindl.]; and a collection of peach [*Prunus persica* (L.) Batsch] and nectarine (*P. persica* var. *nucipersica*) cultivars (Souza Filho et al. 2009).

No phytosanitary treatments were performed in the orchards during the assays. The fruits were taken to the laboratory and placed individually in 500 mL capacity plastic pots containing sand + vermiculite covered with voile fabric held in place with elastic tape. Each fruit remained in the plastic pot for approximately 20 days until the emergence of flies and/or parasitoids (Souza Filho et al. 2009).

Ten parasitoid species were collected. The braconids belonged to five species – *Asobara anastrephae* (Muesebeck), *Doryctobracon areolatus* (Szépligeti), *Doryctobracon brasiliensis* (Szépligeti), *Opius bellus* Gahan, and *Utetes anastrephae* (Viereck). *Doryctobracon areolatus*, *D. brasiliensis*, and *U. anastrephae* can be highlighted as the most important parasitoids in the State of São Paulo, parasitizing fly larvae on practically all known host plants. Figitids were retrieved from larvae attacking only guava – *Aganaspis pelleranoi* (Brèthes), *Dicerataspis grenadensis* Ashmead, *Lopheucoila anastrephae* (Rhower), *Leptopilina boulardi* (Barbotin, Carlton and Kelner-Pillaut), and *Trybliographa infuscata* Diaz, Gallardo and Uchôa. Most braconid species developed on tephritid larvae in peach in 2002, but only *Doryctobracon* species – *D. areolatus* and *D. brasiliensis* were also obtained in 2003 (Souza Filho et al. 2009).

Study 12.

The purpose of this paper is to report the parasitoids of dipterans in poultry farms in Brazil. The experiment was carried out in two poultry farms in the Midwest Region in Brazilian territory. Feces were removed from the farm sheds placed in 10 basins to be transported to the laboratory for removal of pupae. In the laboratory, pupae were obtained. The pupae were removed and placed on absorbent paper for drying and later individualized in glass capsules for the emergence of adult flies or parasitoids. The collections were carried out monthly from January to December 2007. From 10,608 pupae of dipteran collected 816 parasitoids emerged from 540 pupae (Marchiori 2021).

The total percentage of parasitism found in this study was 5.1% (540/10.608) and species: *Muscidifurax raptorellus* Kogan & Legner, with (5/504) 1.0%, *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) (10/504)2.0%, *Pachycrepoideus* vindemmiae (Rondani) (Hymenoptera: Pteromalidae) (367/504) 93.9%, *Spalangia cameroni* Perkins (Hymenoptera: Pteromalidae) (12/504) 2.4%, *Spalangia drosophilae* Ashmead (Hymenoptera: Pteromalidae) (2/504) 0.4%, *Spalangia endius* Walker (Hymenoptera: Pteromalidae) (77/504) 15.2%, *Spalangia nigra* Latrielle, (Hymenoptera: Pteromalidae) (17/504) 3.3%, *Spalangia nigroaenea* Curtis (Hymenoptera: Pteromalidae) (8/504) 1.6%, *Spalangia* sp. (Hymenoptera: Pteromalidae) (15/504) 3.0% and *Tachinaephagus zealandicus* (Ashmead, 1904) (10/504) 2.0% (Encyrtidae) (Marchiori 2021).

Study 13

The objective of this study was to verify the parasitoid species of *Musca domestica* L. (Diptera: Muscidae) and their biological characteristics collected in an urban area in Brazil. This experiment was conducted in the urban area near Botafogo River, Goiânia, Goiás, Brazil. Parasitoids were collected by exposing larvae of *M. domestica* and its breeding substrate (bovine beef liver) outdoors, in containers filled with sand that were placed next to garbage containers. The parasitoids were collected by using four traps. The following items were used as baits: human feces, cattle kidneys, cattle liver and fish which were placed inside the cans, over a layer of earth. To obtain the parasitoids, the contents of the traps were placed in plastic containers with a layer of sand for use as a substrate for transformation of the larvae into pupae (Marchiori 2019).

From March to September 2015, 35 specimens of parasitoids were collected. The species of Hymenoptera collected and their frequency were: *Hemencyrtus* sp. (Encyrtidae), with 1 specime, *Tachinobia* sp. (Eulophidae) with 1 specime, *Pachycrepoideus vindemmiae* (Rondani) with 26 specimens and *Spalangia* spp. (Pteromalidae) with 7 specimens (Marchiori 2019).

Study 14.

This work aimed to evaluate the frequency and distribution of natural enemies of *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) from artificial infestation with pest postures in maize crops, in order to associate the presence of biological control agents to the different stages of development of the host insect. The experiment was carried out in the summer crop, in a cerrado area, in Sete Lagoas, MG. At each collection, 20 plants from each plot were randomly covered with a plastic bag and then cut at the base, thus allowing the collection of all insects present on the leaves. Plants were collected in the morning, starting at eight o'clock, and then taken to the laboratory (Figueiredo et al. 2006).

Each plant had its leaves detached and evaluated for the presence of insects. The eggs and caterpillars of *S. frugiperda* were placed in 50 ml cups containing artificial diet; the other insects present in the plants were placed in a 70% alcohol solution. Natural enemies, coming from the different stages of pest development, were separated, and grouped according to their entomological classification (Figueiredo et al. 2006).

Among the parasitoids obtained from the parasitized caterpillars, there was a predominance of species of the order Hymenoptera. The occurrence of the egg/caterpillar parasitoid *Chelonus insularis* Cresson (Braconidae) and of the caterpillar parasitoids *Eiphosoma laphygmae* Costa Lima (Ichneumonidae), *Campoletis flavicincta* (Ashmead) (Ichneumonidae), *Cotesia marginusonidae* (Braconidae) (*Braspinus sonris* (Cres) Fabricius) (Ichneumonidae) and *Exasticolus fuscicornis* (Cameron) (Braconidae). *Archytas incertus* (Macquart) (Diptera: Tachinidae) was the only representative of this order. Among the parasitoids, *Chelonus insularis* (Cresson) (Hymenoptera: Braconidae), present in all collections, accounted for 91% of the parasitism (Figueiredo et al. 2006).

Study 15.

The present study aimed to analyze the parasitoid insects and their interactions with endophytic insects in *Stryphnodendron adstringens* (Family: Fabaceae) (Mart.) Coville fruits in an area of Cerrado in southwestern Minas Gerais, located in the vicinity of Serra Nacional Park of Canastra. This Park is the second largest conservation unit in the Southeast, located in the southwestern portion of the state of Minas Gerais, between the municipalities of São Roque de Minas, Delfinópolis, Sacramento, São João Batista do Glória, Capitólio and Vargem Bonita. The fruits were randomly collected from one or several plant specimens, at heights distinct from the crown and at different stages of development. Both immature but developed fruits, ripe and still closed fruits and ripe ones starting to dehiscence were collected, selecting those with traces of insect attack. To remove fruits from the upper regions of the crown, long-stemmed pruning shears and pruning shears were used (6 meters) (Silva and Zampieron 2016).

From the eight collections carried out, from March to October 2013, a total of 210 fruits of *S. adstringens* were obtained. The order Hymenoptera, second most abundant, was represented by 11 families, which are mostly parasitoids of Coleoptera or Lepidoptera, and some phytophagous specimens. The biology of these parasitoids

associated with seed predators is still poorly understood, with the main families being: Pteromalidae, Eulophidae (Chalcidoidea) and Braconidae (Ichneumonoidea). As for the families of the order Hymenoptera, the best represented was Braconidae (49.25%), with emphasis on the genus *Allorhogas* Gahan, included in Doryctinae. Of the Braconidae considered parasitoids, the following genera were associated with lepidopterans: *Pseudophanerotoma* Zetel, *Chelonus* Panzer, *Hormius* Nees, *Apanteles* Foerster and *Clinocentrus* Haliday (Silva and Zampieron 2016).

Study 16.

The aims of this study were to characterize galls of three species on this genus, *Copaifera depilis* Dwyer, *Copaifera luetzelburgii* Harms and *Copaifera sabulicola* Costa & Queiroz (Leguminosae), which occur sympatrically in Western Bahia and compare their abundance. This municipality is located in the Cerrado Biome and presents two well defined seasons, a dry and rainy. The collections were carried out from December 2012 to January 2013 (Santos et al. 2018).

In the three *Copaifera* species, a total of 23 gall morphotypes were obtained, 9 morphotypes found on *C. sabulicola*, 7 on *C. luetzelburgii* and 7 on *C. depilis*. In the present study, we observed that five morphotypes were induced by Cecidomyiids and the gallers of 18 morphotypes cannot be determined, because the gall samples were collected occupied by parasitoids or without dweller. Cecidomyiidae are the most important galler in all zoogeographic regions. Microhimenopterans identified represents possibly parasitoids, natural enemy of Cecidomyiidae (Hymenoptera, except for ants (Formicidae, predator), which were found on abandoned galls, being considered in this study as a successor for *Copaifera langsdorffii* Desf. (Fabaceae), Oliveira et al. (2013) pointed that the level of (Santos et al. 2018).

Study 17.

The main purposes hereof were: 1) to correlate climatic factors (specify rainfall, temperature, and relative humidity) with the incidence of fruit flies and their parasitoids under the conditions of the region Cariri, Ceará; 2) to estimate the fruit fly species infestation rates in guavas, and 3) to determine the natural parasitism rates and the frequency of native parasitoids. The guava sampling was performed every week, randomly, and according to the fruit development period, from September 2013, to August, 2014, collecting every fruit of the trees, totalizing 167 trees in the whole orchard

(Azevedo et al. 2018)

During the study period, 1184 fruit fly pupae were collected from 667 fruits gathered on the orchard located in the city of Barbalha (CE). Five fruit fly species were obtained: *Anastrepha zenildae* (Zucchi, 1979) (62.7%), *Anastrepha sororcula* (Zucchi, 1979) (27.3%), *Anastrepha obliqua* (Macquart, 1933) (19%), *Anastrepha antunesi* (Lima, 1938) (0.6%), and *Ceratitis capitata* (Wiedemann, 1824) (26.2%). Besides fruit flies, four parasitoids species were found: *Doryctobracon areolatus* (Szépligeti, 1911) (75%), *Asobara anastrephae* (Muesebeck, 1958) (18.4%), *Utetes anastrephae* (Viereck, 1913) (5.3%), and *Opius bellus* (Gahan, 1930) (0.6%), all belonging to the Family Braconidae (Azevedo et al. 2018).

Study 18.

This work aimed to determine the richness of fruit flies species, their respective host plants and their parasitoids in municipalities in the northern region of Mato Grosso. Fruits from 34 plant species were collected from July 2016 to November 2017. The collected fruits were placed in plastic containers to observe the emergence of fruit flies and parasitoids. Samples of 34 plant species were collected, grouped into 15 different botanical families (Silva et al. 2019).

Of these, 12 species were registered as host plants of fruit flies, belonging to the families Anacardiaceae, Malpighiaceae, Myrtaceae and Oxalidaceae. Fruits were collected according to field availability, which influenced the diversity of plant species sampled in each municipality. Myrtaceae was the family with the highest number of species sampled (9), followed by Anacardiaceae (4) and Rutaceae (4). From the collected samples, 2,709 specimens of fruit flies (five species) and 179 specimens of parasitoids (three species) were obtained (Silva et al. 2019).

Five tephritid species were recorded: *Anastrepha fraterculus* (Wiedemann), *A. obliqua* (Macquart), *A. sororcula* Zucchi, *A. striata* Schiner and *Ceratitis capitata* (Wiedemann). In this study three species of parasitoids were recorded: *D. areolatus, Utetes anastrephae* Viereck and *Asobara anastrephae* (Muesebeck), all obtained in the municipality of Nova Guarita (Silva et al. 2019).

In Sinop, only *D. areolatus* and *U. anastrephae* were obtained: *D. areolatus* was obtained from samples of *Averrhoa carambola* (Oxalidaceae), *Eugenia pyriformis* (Myrtaceae), *Plinia cauliflora* (Myrtaceae), *Psidium cattleianum* (Myrtaceae), *Psidium guajava* (Myrtaceae) and *Spondias purpurea* (Anacardiaceae). Association of the parasitoid *D. areolatus* with A. striata in *P. cattleianum* and *P. guajava* fruits, and with *C. capitata* in *A. carambola* fruits were verified. The parasitoid *U. anastrephae* was obtained by collecting fruits of *A.*

carambola, E. pyriformis, P. cauliflora and *S. purpurea.* Association of *U. anastrephae* with *A. obliqua* in *S. purpurea* fruits was verified (Silva et al. 2019).

Study 19.

The objective of this study was to records the parasitoid *Gnathopleura semirufa* (Brullé 1846) parasitizing parasitizing flies of the Family Sarcophagidae in Brasil. The study on pitfall traps was also conducted on the campus of the Agronomy School in Itumbiara, Goiás, between January and November 2005. Six traps were used, with separations of two meters between each other, placed randomly. The bait was replaced every 15 days. The pupae that were found in the bait were separated out by means of the floatation method. These were then individually packed in gelatin capsules until the parasitoids emerged (Marchiori 2014).

Collected from 305 pupae *Oxysarcodexia thornax* (Walker) (Diptera: Sarcophagidae), 143 *Peckia chrysostoma* (Wiedemann) (Diptera: Sarcophagidae) and 182 of *Sarcodexia lambens* (Wiedemann) (Diptera: Sarcophagidae) that emerged 75, 51 and 31 parasitoid species *Gnathopleura semirufa* (Brullé, 1846) (Hymenoptera: Braconidae) respectively. The total percentage parasitism observed was around 25.0% (Marchiori 2014).

The host showing the highest percentage parasitism was *P. chrysosotoma* in cattle liver, and this was probably due to the seasonality factor presented by this species. The percentage of parasitism observed in *O. thornax*, *P. chrysosotoma* and *S. lambens* was 24.6%, 35.7% and 17.0%, respectively. The total percentage parasitism observed was around 25.0%. The host showing the highest percentage parasitism was *P. chrysosotoma* in cattle liver and this was probably due to the seasonality factor presented by this species. The percentage of parasitism observed in *O. thornax*, *P. chrysosotoma* and *S. lambens* was 24.6%, 35.7% and 17.0%, respectively (Marchiori 2014).

Study 20

This study had the objective of determining the species of parasitoids of dipterous insects that were present in bovine feces collected in Goiânia Goiás, Brazil. Every fortnight, 10 plates of fecal cake produced from fresh bovine feces that were collected immediately after defecation in pastures and in corrals. The feces remained exposed (a total of 10 plastic basins, five in the pastures and five in the corrals) for 15 days. After this period, the feces were taken to the laboratory for extraction of pupae by means of the flotation method. The pupae were removed with the aid of a sieve; they were counted and individually stored in

gelatin capsules until the flies and/or parasitoids emerged (Marchiori et al. 2013).

We collected 359 pupae of Diptera, of which 23 parasitoids emerged. The most frequently observed species in Goiânia was *Aleochara notula* Erichson, 1839 (Coleoptera: Staphylinidae), accounting for 26.1%. The overall percentage of parasitism contacted was 6.4%. *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Pteromalidae) was the species in the central region that presented the highest percentage parasitism, with 9.8% (Marchiori et al. 2013).

In relation to the attraction of parasitoids towards dipterous insect in Goiânia, it was found that *A. notula* was attracted to *Sarcophagula occidua* Fabricius (Diptera: Sarcophagidae); *Neralsia splendens* (Borgmeier) (Hymenoptera: Figitidae) to *Cyrtoneurina pararescita* Couri (Muscidae) *N. splendens* to *C. pararescita* and *S.occidua*; *P. egeria* to *S occidua*; *S. cameroni* to *Brontaea debilis* Williston (Diptera: Muscidae); *Spalangia drosophilae* (Ashmead) (Hymenoptera: Pteromalidae) to *B. debilis*, *Brontaea quadristigma* (Thomson) (Diptera: Muscidae) and *Palaeosepsis* spp. (Diptera: Sepsidae) and *Spalangia nigra* (Hymenoptera: Pteromalidae) (Curtis) to *B. debilis* (X2 = 47.28; GL = 8; P < 0.05) (Marchiori et al. 2013).

Study 21

The objective of this study was to understand the main species of parasitoids of frugivorous flies in southern Goiás and west Minas Gerais, Brazil. The study was conducted at the farm in Santa Therese in this region west of Minas Gerais (MG). In southern Goiás (GO) Brazil. The experiment was conducted at the farm of the Faculty of Agronomy. Each week the carrabolla and cherry fruits collected were deposited on a 5cm layer of fine sand, in plastic containers, cylindrical, transparent, and open at the top. The openings of the containers were sealed after placing the fruit with organza secured with elastic to prevent the entry of other insects (Marchiori 219).

Weekly substrate pupae were separated by flotation bucket with water removed with the aid of a sieve, dried, counted and packaged in glass bottles with fine sand kept at room temperature until the emergence of the flies and/or their parasites. The collections were made in southern Goiás of September 1998 to January 1999 and west of Minas Gerais of November 2001 to January 2002 (Marchiori 2019).

A total of 304 *Anastrepha* spp. obtained 45 specimens of parasitoids in western Minas Gerais and 870 Anastrepha spp. were obtained. 48 specimens of parasitoids in southern Goiás. *Trichopria Anastrepha* Costa Lima (Hymenoptera: Diapriidae) was the species most collected in western Minas Gerais with 44.0%. *Doryctobracon*

areolatus (Szépligeti,) (Hymenoptera: Braconidae) was the most collected species in southern Goiás with 89.6%. Regarding the total percentage of parasitism west region of Minas Gerais was 14.8% and southern Goiás 5.5%. Probably related to the low synanthropy of the species of dipterans and parasitoids collected in the southern region of Goiás studied the locality is now surrounded by human populations on all sides. *Trichopria anastrephae* Lima showed most percentage of parasitism in western Minas Gerais of 6.6 % in southern Goiás was *D. areolatus* with 5.0%. *T. anastrephae* was obtained from *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) in guavas, with a parasitism rate of 5.8% (Marchiori 2019).

Study 22.

The objective was to identify species of Chalcididae (Hymenoptera: Chalcidoidea) collected in the savanna forests and pastures in Itumbiara County, State of Goias, Central Brazil using yellow pan traps. The experiment was carried out at the Agronomy College Farm, located near the Paranaiba River shore, 5 km from downtown Itumbiara County, State of Goias, Central of Brazil. The farm has approximately 12 alqueires. The sampling area had 1.5 alqueires constituted of ciliary forest along the Paranaiba River, gradating to mesophytic semi-deciduous forest and savanna. The area had a background of selective deforestation and forest burning and was circled by sugar cane cropping and pastures. The samplings were conducted Moericke traps10 traps randomly placed at the soil level, totaling five traps in the pasture and five traps in the woods for each sampling (Marchiori et al. 2003).

In the 520 samplings performed during the period of January to December 1998, 121 specimens of Chalcididae from five different genera and 25 different species were collected. From these individuals 73.6% were collected in the pastures and 26.4% in the forests. The Chalcididae fauna in the pastures and forest were similar between each other at the 92.0% level. Among the Chalcididae specimens collected, the species most frequently found was *Haltichella* sp.4 (15,7%), followed by *Conura* sp.1 (13,2%) (Marchiori et al. 2003).

V. CONCLUSION

Currently, Brazil has many laboratories that work with biological control contributing to several programs of great success, mainly in the control of insect pests. Several recent works have questioned the safety of classical biological control and, specifically, the use of generalist natural enemies. There are many examples of introductions that have resulted in severe impacts on untargeted organisms, extinctions, biodiversity loss and imbalance of native communities

REFERENCES

[1] Costa A (2019) Instituto Jurumi. www.institutojurumi.org.br/2019/05/5-curiosidades-que-voce-deveria-saber.html.

[2] Gibb T (2015) In Contemporary Insect Diagnostics.1st ed. Oxford: Academic Press is an imprint of Elsevier.

[3] Marchiori CH (2014) Hymenoptera parasitoids collected in southern of Goiás and western of Minas Gerais, Brazil. *Journal Pensee* 76: 357-361.

[4] Marchiori CH, Penteado-Dias, AM (2002) Braconidae (Hymenoptera: Ichneumonoidea) Collected in a Native Forest Area in Itumbiara, Goiás, Brazil. *Neotropical Entomology* 31(4): 647-649.

[5] Marchiori CH, Silva CG, Caldas ER, Almeida KGS, Carvalho AS, Penteado-Dias AM Diaz NB, Gallardo FA (2001) Parasitóides da subfamily Eucoilinae (Hymenoptera: Cynipoidea: Figitidae) coletados em um remanescente de mata de cerrado em Itumbiara, GO. *Arquivos do Instituto Biológico* 68(1): 65-67.

[6] Morais AB, Perre P, Sobzaq JF (2011) Fauna de vespas parasitoides Insecta, Hymenoptera) coletadas em um fragmento de cerrado, Jataí, Goiás. X Congresso de Ecologia do Brasil. São Lourenco, MG.

[7] Souza L, Braga SMP, Campos MJO, Campos (2006) Himenópteros parasitóides (Insecta: Hymenoptera) em área agrícola de Rio Claro, SP, Brasil. *Arquivos do Instituto Biológico* 73(4): 465-469.

[8] Rocha JB, Veloso R, Naves RVN, Ferreira GA (2001) Características e entomofauna associada aos frutos do bacupari. *Pesquisa Agropecuária Tropical* 31(1): 47-54.

[9] Júnior ALM, Júnior AIC, Raizel CBE, Lira NLTA, Pereira PRV, Lau D, Moresco ER, Sobrinho SJ (2021) *Levantamento de espécies de afídeos e parasitoides associadas à cultura do trigo no cerrado mineiro.* http://www.alice.cnptia.embrapa.br/alice/handle/doc/1064568.

[10] Perioto NW, Lara RIR, Costa VA (2009) Parasitic wasps (Hymenoptera) associated with fruits of *Ilex affinis* Gardner (Aquifoliacea) in Brazil with description of two new Chalcidoidea. *Arquivo do Instituto Biológico* 76(1): 85-90.

[11] Pádua DG, Zampieron SLM (2012) Inventário da Fauna de Hymenoptera Parasitóides Coletados com Redes de Varredura em um Fragmento da Serra da Babilônia, no Sudoeste do Estado de Minas Gerais. *EntomoBrasilis* 5(3): 211-216.

[12] Pádua DG, Zampieron SLM, Nunes JF (2014) Composition of the Families of Parasitoids Wasp in the Parque Nacional da Serra da Canastra, Minas Gerais, Brazil. *EntomoBrasilis* 7(3): 199-206.

[13] Souza-Filho, MFA Raga, AA, Azevedo-Filho JAB, Strikis PCC, Guimarães JAD, Zucchi RA (2009) Diversity and seasonality of fruit flies (Diptera: Tephritidae and Lonchaeidae) and their parasitoids (Hymenoptera: Braconidae and Figitidae) in orchards of guava, loquat and peach. *Brazilian Journal of Biology* 69(1): 31-40.

[14] Marchiori CH (2021) Parasitoids Collected in Poultry Farms in Brazil. *European Journal of Biology and Biotechnology* 2(3): 39-40.

[15] Marchiori CH (2019) Parasitoids of Fly collected in urban area in Brazil. *Annals of Marine Science* 3(1): 11-12.

[16] Figueiredo MLC, Martins-Dias, AMP, Cruz I (2006) Associação entre inimigos naturais e *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) na cultura do milho. *Revista Brasileira de Milho e Sorgo* 5(3): 340-350.

[17] Silva TM, Zampieron SLM (2016) Interações entre parasitoides e insetos endófagos em frutos de *Stryphnodendron adstringens* (Mart.) Coville (Fabaceae) no Cerrado Mineiro. *Revista Agrogeoambiental* 8(2): 53-63.

[18] Santos IM, Lima VP, Soares EKS, Paula M, Calado DC (2018) Insect galls in three species of *Copaifera L.* (Leguminosae, Caesalpinioideae) occurring sympatrically in a Cerrado area (Bahia, Brazil) parasitoidism is very high, and sampling for cecidomyiids adults has been unsuccessful. *Biota Neotropica* 18(1): e20170356.

[19] Azevedo FR, Araújo EL, Santos IS, Moreno NBC, Pereira MLL, Azevedo R A, Alves ACL (2018) Fruit flies and parasitoids associated with guava in Barbalha, Ceará, Brazil. *Revista Brasileira de Ciências Agrária* 3(4): 55-86.

[20] Silva MES, Wochner MA, Sousa MSM, Barreto MR, Adaime R (2019) Moscas-das-frutas (Diptera: Tephritidae), suas plantas hospedeiras e parasitoides (HYMENOPTERA: Braconidae). Nativa Sinope 7(5): 513-519.

[21] Marchiori CH (2014) *Gnathopleura semirufa* (Hymenoptera: Braconidae (Brullé, 1846) parasitoid of dipterous synanthropic in Southern Goiás, Brazil. *Annals of West University of Timisoara, ser. Biology* 17: 9 – 12.

[22] Marchiori CH, Borges LMF, Ferreira LL. (2013) Dipterous parasitoids collected from cattle dung in Goiânia, central Goiás, Brazil. *Pensee Journal* 75(9): 418-426.

[23] Marchiori CH (2019) Parasitoids of fruit flies collected in Brazil. *Qeios* 12: 1-8. [24] Marchiori CH, Penteado-Dias AM, Tavares MT (2003) Parasitoids of the family Chalcididae collected in pastures and forests using yellow traps, in Itumbiara, Goiás, Brazil. *Brazilian Journal of Biology* 63: 357 – 360.

Article

Utilization of E-learning by Agricultural Students of Public Higher Institutions in Southwest of Nigeria

Sola Joshua Oluwasusi[1], Oluwatoyin Olagunju[2*], Adeyose Emmanuel Akinbola[3], Oyedeji Taofeeq Yekinni[4]

[1,4]Department of Agricultural Extension and Rural Development, University of Ibadan, Ibadan, Nigeria
[2]Department of Agricultural Extension and Rural Development, Adekunle Ajasin University, Akungba-Akoko, Nigeria
[3]Department of Agricultural Economics, Adekunle Ajasin University, Akungba-Akoko, Nigeria
*Corresponding Author

Abstract— This study aimed at determining the utilization of e-learning by agricultural students' of public higher institutions in the Southwest of Nigeria. A multistage sampling procedure was used to select 300 respondents from the higher institutions. Students were mostly female with a mean age of 22.4 years and with majority enrolled in undergraduate programmes. Respondents had a low usage of e-learning due to low awareness and knowledge level, complexity of technology and inadequate e-learning infrastructures in their schools. A significant relationship existed between constraints, awareness, knowledge, and utilization of e-learning. The regression analysis carried out in this study resulted in R-square of .957. These findings indicate that about 95.7% of variance in the level of usage of e-learning is explained by awareness, knowledge and different constraints. The study recommends that efforts should be geared towards encouraging students to integrate e-learning usage in their academic activities by providing e-learning infrastructures and easy access through competent e-learning personnel.

Keywords— Agricultural students, E-Learning, Higher institution, Utilization

I. INTRODUCTION

Technology has inevitably become the most powerful tool in almost every aspect of human's daily life as is regarded as the precursor of major revolutions in various aspects of human endeavours, including education (Lee *et al.,* 2018). The use of Information Technology (IT) is the new paradigm of learning in the 21st century that allows people to easily access, gather data, analyse and transfer of knowledge (Darling-Hammond *et al.,* 2020). This makes it possible to function as teachers, study mates and more importantly, as tools to improve the entire teaching and learning process. This current development which relates to the role of IT and the Internet shows that the phase of the whole educational system has changed. Information and communication technologies (ICTs) hold immense capability to gradually transform and remould education (Assar, 2015). The ultimate impact of ICTs may be seen on the structure, content and outcomes of learning, both inside and outside of school. Bader and Kottstorfer (2013) reported trends in technology influence on education and knowledge management and that E-learning is gradually becoming important in higher institutions of learning by providing E-learning resources through allowing profile registrations on the internet by more students.

E-learning is an aspect and/or manifestation of e-readiness, which is the general term for using computer and other electronic technologies to promote teaching and learning that may include the use of the technologies as part of the conventional or traditional teaching where learners and teachers may never meet face to face (Khvilon & Patru, 2018; Lakshmi *et al.,* 2020). The technology includes not just computers and networks that connect them but also software such as email, online databases, CD-ROMs and the peripherals such as video cameras and interactive white

boards (Anderson, 2010). E-learning is also the use of ICT to promote the acquisition of more efficient and effective learning materials and results, facilitate the accessibility of research findings and educative write-ups, allow greater students' access to information and make researchers more accountable to students and the general public (Eze *et al.,* 2018). The concept of e-learning has brought about changes in knowledge management and human resources development (Subramanian, 2016). Increasing capacity of ICT has further been empowered by the growth of a global network of computer networks known as internet (Teng *et al.,* 2020). It has impacted in the way business is conducted, facilitated learning and knowledge sharing, generated global information flows, empowered citizens and communities in ways that have re-defined governance and have created significant wealth and economic growth resulting in a global information society (Cascio & Montealegre, 2016).

Agricultural education in tertiary institutions is one of the ways of preparing for sustainable agricultural development through the training of farmers and allied professionals as well as agricultural extension practitioners (Kozicka, 2018). Agricultural education implies training people in a way to be futuristic in their exploitation of nature with adequate consideration for the environmental, societal and economic factors in a balanced way, in the pursuit of development and improved quality of life (Smith & Rayfield, 2016). Many of today's major challenges include; energy security, national security, human health, and climate change that are closely tied to the global food and agriculture enterprise (Islam & Kieu, 2020). Academic institutions with programmes in agriculture are in a perfect position to foster the next generation of leaders and professionals needed to address these challenges (Ikehi *et al.,* 2014). However, to keep pace with changing times, undergraduate agricultural education needs a new focus. Agriculture is affected by so many factors and its participants must always be prepared to react, adapt, and think ahead (Alawa *et al.,* 2014).

Tertiary institutions with undergraduate programmes in agriculture must undergo a significant transformation to foster the agricultural workforce of tomorrow. Such institutions must position themselves at the cutting-edge and offer students the opportunity to learn about the complexities of agriculture, grapple with its emerging challenges, and find their opportunity to contribute as leaders and participants (Carlisle *et al.,* 2019). Keeping up with the evolving nature of the agricultural enterprise is not a simple task. It requires a much more dynamic approach to the curriculum and teaching methods than most academic institutions have developed (Njura *et al.,* 2020).

Increased awareness of agriculture's important role in addressing major societal problems can help to raise the profile of the field and attract more students (FAO, 2014). Transforming and sustaining education in agriculture requires an ongoing commitment with strong leadership and interest in agriculture (Ameyaw *et al.,* 2019). The investments in undergraduate education will play an important role in shaping the future of agriculture in meeting the challenges of the 21st century and beyond. In achieving sustainable agriculture, e-learning is one of the ways to improving Agricultural education in the world (Ra *et al.,* 2019).

According to Apuke and Iyendo (2018), the educational sector is yet to tap into this technology to deliver services especially in our tertiary institutions due to high cost of internet access and lack of reliable and permanent sources of power, causing loss of interest from students who believed in e-learning as a good way of impacting knowledge on them. Poor telecommunication penetration hinders access to e-learning facilities in many towns and cities in the country and also, the cost of owing personal computers is a major hurdle for the most of the Nigerian students (Atanda, 2014).

ICTs hold great potential for supporting and augmenting existing education as well as national development efforts (Idowu & Esere, 2013). The functionality of ICTs in agricultural education delivery is faced with enormous challenges in the nation's higher institutions, among which include inadequate ICT infrastructure, including high cost of band width access, lack of skilled manpower, to manage available systems and inadequate training facilities for ICT education at the tertiary level (Yushau & Nannim, 2018). Moreover, resistance to change from traditional pedagogical methods to innovative, technology based teaching and learning methods from students and academic staff is yet an important issue (Howard & Mozejko, 2015). The overall educational system is grossly under-funded and therefore, available funds are used to solve more seemingly urgent and basic needs by the institutions (Adeniran *et al.,* 2019). In addition, the overdependence of educational institutions on government has limited tertiary institutions ability to collaborate with the private sector or seek alternative funding sources for ICT educational initiatives and more so, ineffective coordination of various ICT for educational initiatives, give room to mushroom education across the nation's educational institutions (Gana, 2017). These challenges throw many tertiary institutions to operate beyond capacity.

Addressing the agricultural students' usage of e-learning in tertiary institutions across the country is paramount, in order to gauge progress and pro-actively address stumbling blocks. It is important to conduct a study on the utilization

of e-learning among agricultural students as it is crucial for the country's participation in the information society and a promising effective strategy academic performance and agricultural transformation in the country. Thus, this study aimed at examining the utilization of e-learning by agricultural students of public higher institutions in Southwest of Nigeria. Specifically, the objectives to be achieved are as follows:

1. describe the personal characteristics of agricultural students;

2. determine the level of awareness of e-learning facilities available to agricultural students;

3. examine the knowledge level of the agricultural students on the use of e-learning;

4. determine the level of usage of e-learning by agricultural students;

5. identify constraints to the use of e-learning by agricultural students.

1.1. Hypotheses of the study

1. H_o: There is no significant relationship between personal characteristics of agricultural students and their usage of e-learning.

2. H_o: There is no significant relationship between agricultural students' levels of awareness, knowledge, constraints and their level of usage of e-learning.

II. MATERIAL AND METHODS

2.1. Study area

The study was carried out in Southwest zone of Nigeria. Southwest lies between latitude 60 30'N to 9'N and longitude 30 0'E to 50 30'E. It is majorly a Yoruba speaking area, although there are different dialects even within the same state. The weather conditions vary between the two distinct seasons in Nigeria; the rainy season (March-November) and the dry season (November-February). The dry season also brings Harmattan dust and cold dry winds from the northern deserts blow into the southern regions around this time.

2.2. Study population

The research population comprises of all agricultural student in public tertiary institutions in Southwestern Nigeria.

2.3. Sampling procedure and sample size

Multistage sampling procedure was used to select students for the study. The first stage involved the simple random selection of three out of the six states in the zone; the selected states are Osun, Ondo and Ekiti. The second stage

involved the stratification of the institutions in the selected states into University, Polytechnic and College of education. Osun State had two Universities, two Polytechnic and two Colleges of Education; Ondo State had four Universities, two Polytechnics and one College of Education, while Ekiti State had two Universities, one College of Education and one Polytechnic. In the third stage simple random sampling was adopted to select 50% of the selected Universities, Polytechnics and Colleges of Education in the selected States respectively. Hence, three, four and three institutions were selected in Osun State (one University, one Polytechnic and one College of Education; Ondo State (two Universities, one Polytechnic and one College of Education and Ekiti State (one University, one Polytechnic and one College of Education) respectively. The fourth stage involved simple random selection of one department in the Faculty of Agriculture in the selected institutions. The fifth stage involved simple random selection of (a quota) thirty (30) students using the final year list of 2018/2019 from the selected Departments of the sampled fifteen tertiary institutions, which gave a sample size of 300 respondents.

2.4. Data collection and analysis

Data collection was conducted using structured questionnaire. The information was gathered by using an interview schedule made up of well-structured open and close-ended questions. To test the stated hypotheses, the data were analyzed using descriptive and inferential statistics like Pearson Product Moment Correlation (PPMC) and Regression analysis. The personal characteristics of the respondents were presented using frequency counts, percentages and means.

III. RESULTS AND DISCUSSION

3.1 Personal characteristics of the respondents

The findings presented in Table 1 shows that more than half (55.3%) of the respondents were female, while 44.7% of them were male. This implies that the females were more involved in agricultural education in the study area. Obayelu & Fadele (2019) supported this finding that across tertiary institutions, the ratio of female to male respondents studying Agricultural Science is most times skewed towards the females emphasizing that they are more willing to study agriculture in tertiary institution than their male counterparts.

The mean age of the students was 22.4, with more than half (63.3%) of them falls between 20 and 25 years of age. This implies that they were young and very active in learning by having a high tendency of learning innovations in various e-learning facilities. This makes it easy for them

to change from analogue age to the digital age and the departure philosophy to do something new will not be frustrating (Eze *et al.,* 2018).

Likewise, the findings showed that 70.3% students are Christians, while 29.3% are Muslims and only 0.3% practice traditional religion. Hence, students of the selected public higher institutions are predominantly Christians. This is manifested in the existence of churches in the areas. This shows that education is accepted by the two prevailing religions. The implication is that there is no religious taboo against the acquisition of tertiary education in the study area. Majority (92.0%) of the respondents were single while 8.0% of the respondents were married. This indicates that the single people could get more focused, boost their capacity to seek, utilize information and become excellent in their academic pursuits than the married people as they are limited to little family responsibilities (Moses *et al.,* 2020).

On the category of students programme, majority (82.0%) were undergraduate students, 11.0% were preliminary students, while 7.0% of them were postgraduate students. This implies that undergraduate programme is the foundation and mainstay of tertiary education in the study area with the attraction of lesser fees compared to post graduate study which is an afterthought degree, carried out by fewer respondents because it requires more money, dedication and vast knowledge of ICT application to pursue satisfactorily.

Table 1 further revealed 53.3% of students were University students, 26.7% were in the Polytechnic and 20.0% were Colleges of Education students. This implies that many students were studying agriculture in the university compared to other higher institutions because university education is more popular, recognized and better professionalized than other tertiary education in the study area. This result is consistent with the study of Yang *et al.* (2015) and Chankseliani *et al.* (2021); they validated that Universities play major roles not only in national but also, increasingly in regional economic development in the delivery of life-long learning and development of civic culture.

Also, as revealed on Table 1, 87.6% of the respondents were between 100 and 500 levels, while few of them were between 600 and 800 levels (Postgraduate). This implies that the higher the students grow in academics, the tougher the agricultural programmes become. Fewer students survive it to higher levels and could only pursue higher studies in agricultural based courses through the University alone.

Table 1 Personal characteristics of the respondents (n=300)

Variable	Category	Freq.	Percentage	
Gender	Male	134	44.7	
	Female	166	55.3	
Age	14-19	60	20	
	20-25	190	63.3	Mean
	26-31	48	16	Age =
	32-37	1	0.3	22.4
	38-43	1	0.3	
Religion	Christianity	211	70.3	
	Islam	88	29.3	
	Traditional	1	0.3	
Marital status	Married	24	8.0	
	Single	276	92.0	
Category of student programme	Preliminary	33	11.0	
	Undergraduate	246	82.0	
	Postgraduate	21	7.0	
Type of institution	University	160	53.3	
	Polytechnic	80	26.7	
	College of Education	60	20.0	
Level of programme	1-5	263	87.6	
	6-8	37	12.3	
Monthly stipend per semester (₦)	< ₦10000 ($21)	104	34.7	
	₦10001-20000 ($21-42)	108	35.9	Mean Income = 20109
	₦20001-30000 ($42-63)	39	13.0	
	₦30001-40000 ($63-83)	16	5.3	
	₦40001-50000 ($83-104)	12	3.9	
	₦50001-60000 ($105-125)	13	4.3	
	₦60001-70000 ($126-145)	4	1.3	
	₦70001-80000 ($146-167)	2	0.6	
	₦80001 ($168) and Above	2	0.7	

Table 1 further shows the mean income of students per semester was ₦20,109 ($42). The majority (70.6%) of them earn between ₦10,000 ($21) and ₦20,000 ($42) as monthly stipends per semester, while the remain students (29.1%) earn above ₦20,000 ($42) as monthly stipends per

semester. This indicated that almost all the respondents earn lesser income as monthly stipends from parents, guardians or self-labour to cope effectively with their academic demands and that could negatively affect their information seeking and utilization behaviour to e-learning facilities. This finding was consistent with Okoro (2021) as she confirmed that inadequate funding is the major constraints of using e-learning facilities during teaching and learning process.

3.2. Level of awareness of e-learning facilities available to agricultural students

In accordance with the rating of low and high, as indication of level of awareness of available e-learning facilities, the respondents' scores in level of awareness as shown in Table 2, range 1-22 with a mean of 10.9. To find the level of awareness of available e-learning facilities, the scores were grouped into two categories; low (0-11) and high (12-22). The analysis revealed that level of awareness of available e-learning facilities by the respondents, with a majority (71.0%) claiming to be as low, while 29.0% indicated that the level of awareness of available e-learning facilities is high. The result shows that the respondents' low level of awareness was due to inadequate provision of the needed e-learning facilities on campuses by various institutions which will not be the best way for students to be aware of new technology, willing and interested to study in various concepts in searching and upgrading their knowledge (Suresh *et al.,* 2018).

Table 2 Distribution of respondents' on level of awareness of available e-learning facilities

Level of awareness	Frequency	Percentage (%)
Low (0 – 11)	213	71.0
High (12 – 22)	87	29.0
Total	300	100.0

3.3. Knowledge level of the agricultural students on the use of e-learning

As shown in Table 3, the students' scores of knowledge level on the use of e-learning range from (1-14) with a mean score of 5.4. To determine the level, the respondents' scores were grouped into two categories. It described (0-7) as low, and (8-14) as high. The results of the level of knowledge according to the respondents', with more than half (53.0%) stating as low, while 47.0% of the total respondents indicated high level of knowledge towards the use of e-learning facilities. The implication is that many of the respondents did not have good knowledge of the use of e-learning facilities and this might hinder their readiness

for the usage and invariably lead to the experience of low readiness for e-learning. Eze *et al.* (2018) validated this finding by attributing some of the reasons for low e-learning adoption in higher institutions ranges from lack of specialized and social aptitudes required for the execution of e-learning and teachers to lack of teachers and students knowledge and the know-how to use e-learning platforms.

Table 3 Distribution of respondents' on level of knowledge on the use of e-learning

Level of knowledge	Frequency	Percentage (%)
Low (0-7)	159	53.0
High (8-14)	141	47.0
Total	300	100.0

3.4. Level of usage of e-learning facilities by agricultural students

The respondents' score in Table 4 reveals the mean is 8.8. To determine the level of usage of e-learning, the respondents' scores were grouped into two categories. It described (0-9) as low, and (10-18) as high. The results on the usage of e-learning facilities indicated that more than half (56.0%) stating as low. While 44.0% of the total respondents indicated high level on the usage of e-learning facilities which implies that the extent at which the students use e-learning facilities are still very low and this can be as result of not being knowledgeable enough about the usage. In line with this, Almaiah *et al.* (2020) found that academic staff knowledge of learning technologies, student knowledge and technical infrastructure, were significant factors in facilitating the successful acceptance and usage of e-learning in universities.

Table 4 Distribution of respondents' on level of usage of e-learning facilities

Level of usage	Frequency	Percentage (%)
Low (0 – 9)	168	56.0
High (10 – 18)	132	44.0
Total	300	100.0

3.5. Constraints encountered in the use of e-learning facilities by agricultural students

Table 2 shows the distribution of respondents by weighted scores in which poor electricity supply (WS=148.3), complexity of technology (WS=131.1), insufficiency of financial resources for technology integration (WS=128.7) and inadequate e-learning infrastructures in my school (WS=128.3) as a serious constraints to the use of e-

learning facilities. While inadequate access to e-learning facilities (WS=118.9), poor ICT literacy (WS=109.9), lack of interest of teachers and students (WS=109.3) indicate mild constraints to the used of e-learning. This indicates that irregular supply of electricity, lack of fund to integrate the technology, inadequate infrastructure and complexity of the technology serves as serious constraints towards the use of e-learning facilities in public higher institutions in Nigeria. The result of the study corroborate the assertion of Eze *et al.* (2020) that decrease in the annual budget for the education sector and current electricity supply in Nigeria is very low, unstable and not available in many part of the country where most of the higher institutions of learning are located.

Table 5 Distribution of respondents according to constraints to use of e-learning

Constraints	Serious constraint	Mild constraint	Not a constraint	Weighted Score
Poor electricity and power supply	60.0	28.3	11.7	148.3
Complexity of Technology	50.7	29.7	19.7	131.1
Insufficiency of financial resources for technology integration	43.7	41.3	15.0	128.7
Inadequate e-learning infrastructures in my school	46.3	35.7	18.0	128.3
Inadequate access to e-learning facilities	38.3	42.3	19.3	118.9
Inefficiency of guidance and support by administration	38.3	38.3	23.3	114.8
Lack of relevance of courses to e-learning offered to students	37.7	36.3	26.0	111.7
Incompetent e-learning personnel to put me through	39.7	31.7	28.7	111.1
Poor ICT literacy	36.3	37.3	26.3	109.9
Lack of interest of teachers and students in IT usage	36.0	37.3	26.7	109.3

3.6. Hypotheses testing

The result obtained in Table 6 clearly reveals that there had been no significant relationship between selected personal characteristics of the students; gender (t = 0.857, p>0.05), age (t = -0.007, p>0.05), category of student programme (t = 1.136, p>0.05), level of programme (t = -1.600, p>0.05), monthly stipend per semester (t = 0.609, p>0.05) and their usage of e-learning. This result implies that the students' usage of e-learning is irrespective of their personal characteristics but on their level of awareness and knowledge to the use of e-learning. In a related study, Fleming *et al.* (2017) and Bączek *et al.* (2021) reiterated that age, gender and level of study are not a significant factor impacting the use of e-learning among the students.

Furthermore, the result from Table 6 shows that a significant relationship existed between students' levels of awareness (t = 14.672, p<0.05), knowledge (t = 31.903, p<0.05), constraints (t = 2.555, p<0.05) and the level of e-learning usage. The predicted relationship has been established; meaning that for student to use e-learning facilities effectively, their level of awareness and knowledge need to be increased as well as reduce certain constraints to its usage such as poor electricity and power supply which serves as a serious constraint in the study (Oyediran *et al.,* 2020). This result might stem from the fact that the students were not previously exposed to e-learning facilities due to inadequate awareness and knowledge (Olayemi *et al.,* 2021). Similarly, result obtained by Ngampornchai and Adams (2016) on students' acceptance and readiness for e-learning in Northeastern Thailand and Nigeria. They revealed the reason why usages of e-learning were low among students was as a result of low awareness and knowledge level, complexity of technology and inadequate e-learning infrastructures in the school. Almaiah *et al.* (2020) also reported similar results that lack of awareness and knowledge lead to students not taken responsibility for their e-learning utilization.

The regression analysis carried out in this study resulted in R-square of .957. These findings indicate that about 95.7% of variance in the level of usage of e-learning is explained by awareness, knowledge and constraints. The significant variables are shown in Table 6 below. Since the p value is less than 0.05, it indicates that there is a significant relationship between level of awareness, knowledge, constraints and utilization of e-learning.

Table 6 Determinants of the use of e-learning among agricultural students

Variables	Coefficient	t-value	Significance
Constant		-25.018	0.000
Gender	0.011	0.857	0.857
Age	-0.007	-0.007	-0.404
Category of Student Programme	0.018	1.136	0.257
Level of Programme	-0.030	-1.600	0.111
Monthly Stipend Per Semester	0.008	0.609	0.543
Awareness of e-learning	0.221	14.672	0.000
Knowledge of e-learning	0.411	31.903	0.000
Constraints to the use of e-learning	0.333	2.555	0.011

R=0.978, R-square=0.957, Adjusted R-square=0.955, df=11, F=577.761

IV. CONCLUSION

The study investigated the e-learning utilization among agricultural students in public higher institutions in Southwest of Nigeria. It can be concluded that there is low utilization of e-learning in the study area. The study found significant association between constraints faced by students in the utilization of e-learning, students' awareness of the use and knowledge of e-learning. The major constraints affecting respondents' utilization of e-learning include poor electricity and power supply, complexity of technology, insufficiency of financial resources for technology integration, and inadequate e-learning infrastructures in their various higher institutions.

V. RECOMMENDATIONS

Based on the findings of the study, it is recommended that:

1. Tertiary institutions should concentrate on sustainable and internal power generation through usage of solar panel or independent power projects by converting dams in schools where they have them to power generation centres. This could be achieved by sensitizing and lobbying the public through mass media on the importance of stable electric supply for concrete and quality research to attain good and reliable policy formulations for government to accede to this lofty demand.

2. Higher Institutions should enhance institutional rewards for high quality teaching and research development among lecturers.

3. Adequate attention should be given to up-to-date and make e-learning facilities accessible in public higher institutions, to broaden the horizon of agricultural students on relevant information and exchange of ideas that will change their knowledge, skills and attitudes to proper and good usage of e-learning facilities for effective learning.

4. Agricultural students should be more encouraged to integrate e-learning usage in their daily academic and non-academic endeavours in order to internalize and get more acquainted to its usage every time.

5. e-learning facilities should be made more available, accessible and affordable to students with less bureaucracy so that students would enjoy a more viable, robust, reliable, efficient, effective and cost beneficial educational acquisition.

6. Tertiary Institutions should increase student opportunities to participate in the outreach and extension activities during the farm practical training year programmes.

7. Staff and students of the university need to be well informed about the content and provisions in available ICT policies as a means of making all stakeholders adequately informed.

8. There is need for higher institutions to seek private sector collaboration as alternative means of funding ICT and e-learning educational initiatives, as it availability in schools is pertinent for effective teaching and learning.

REFERENCES

[1] Adeniran, A., Onyekwena, C., Onubedo, G., Ishaku, J., & Ekeruche, A. (2019). Is Nigeria on track to achieving quality education for all? Drivers and implications.

[2] Alawa, D. A., Asogwa, V. C. & Ikelusi, C. O. (2014). "Measures for mitigating the effects of climate change on crop production in Nigeria." *American Journal of Climate Change, 2014*(3), 161-168.

[3] Almaiah, M. A., Al-Khasawneh, A., & Althunibat, A. (2020). Exploring the critical challenges and factors influencing the E-learning system usage during COVID-19 pandemic. *Education and Information Technologies*, 25, 5261-5280.

[4] Ameyaw, J., Turnhout, E., Arts, B., & Wals, A. (2019). Creating a responsive curriculum for postgraduates: lessons from a case in Ghana. *Journal of Further and Higher Education*, *43*(4), 573-588.

[5] Anderson, J. (2010). ICT transforming education: A regional guide. *Published by UNESKO Bangkok*, *120*.

[6] Apuke, O. D., & Iyendo, T. O. (2018). University students' usage of the internet resources for research and learning: forms of access and perceptions of utility. *Heliyon*, *4*(12), e01052.

[7] Assar, S. (2015). Information and Communications Technology (ICT) and Education. International Encyclopedia of the Social & Behavioral Sciences, Elsevier, pp.66 - 71, 2015, 978-0-08-097087-5.10.1016/B978-0-08-097086-8.92104-4.   hal-02386986

[8] Atanda, B. N. (2014). Issues of e-learning in developing countries: a Nigerian perspective. In *The 5th International Conference on Information and Communication Technology for the Muslim World (ICT4M)* (pp. 1-4). IEEE.

[9] Bączek, M., Zagańczyk-Bączek, M., Szpringer, M., Jaroszyński, A., & Wożakowska-Kapłon, B. (2021). Students' perception of online learning during the COVID-19 pandemic: a survey study of Polish medical students. *Medicine*, *100*(7), 1-6.

[10] Bader, L., & Köttstorfer, M. (2013). E-learning from a student's view with focus on Global Studies. *Multicultural Education & Technology Journal*, 7(2/3), 176-191.

[11] Carlisle, L., De Wit, M. M., DeLonge, M. S., Calo, A., Getz, C., Ory, J., ... & Iles, A. (2019). Securing the future of US agriculture: the case for investing in new entry sustainable farmers. *Elementa: Science of the Anthropocene, 7*(17), 1-20.

[12] Cascio, W. F., & Montealegre, R. (2016). How technology is changing work and organizations. *Annual Review of Organizational Psychology and Organizational Behavior*, *2016*(3), 349-375.

[13] Chankseliani, M., Qoraboyev, I., & Gimranova, D. (2021). Higher education contributing to local, national, and global development: new empirical and conceptual insights. *Higher Education*, *81*(1), 109-127.

[14] Darling-Hammond, L., Flook, L., Cook-Harvey, C., Barron, B., & Osher, D. (2020). Implications for educational practice of the science of learning and development. *Applied Developmental Science*, *24*(2), 97-140.

[15] Eze, S. C., Chinedu-Eze, V. C., & Bello, A. O. (2018). The utilisation of e-learning facilities in the educational delivery system of Nigeria: a study of M-University. *International Journal of Educational Technology in Higher Education*, *15*(1), 1-20.

[16] Eze, S. C., Chinedu-Eze, V. C., Okike, C. K., & Bello, A. O. (2020). Factors influencing the use of e-learning facilities by students in a private Higher Education Institution (HEI) in a developing economy. *Humanities and Social Sciences Communications*, *7*(1), 1-15.

[17] Fleming, J., Becker, K., & Newton, C. (2017). Factors for successful e-learning: does age matter?. *Education+ Training*, 59(1), 76-89.

[18] Food and Agricultural Organization of the United Nations (FAO). (2014). Youth and agriculture: Key challenges and concrete solutions.

[19] Gana, E. (2017). Innovative Approaches to Teaching and Learning of Christian Religious Studies in Nigerian Tertiary Institutions: A Case Study of Ahmadu Bello University, Zaria, Nigeria. *International Journal Of Education, 2*(2), 166-180.

[20] Howard, S. K., & Mozejko, A. (2015). Teachers: technology, change and resistance. *Teaching and digital technologies: Big issues and critical questions*, 307-317.

[21] Idowu, A. I., & Esere, M. (2013). ICT and higher educational system in Nigeria. *Educational Research and Reviews*, *8*(21), 2021-2025.

[22] Ikehi, M. E., Ifeanyieze, F. O. & Ugwuoke, C. U. (2014). Integration of Climate Change into the Senior Secondary School Agricultural Science Curriculum in Nigeria. *Atmospheric and Climate Sciences, 2014*(4), 614-621.

[23] Islam, M. S., & Kieu, E. (2020). Tackling Regional Climate Change Impacts and Food Security Issues: A Critical Analysis across ASEAN, PIF and SAARC. *Sustainability*, *12*(3), 1-21.

[24] Khvilon, E., & Patru, M. (2018). Information and communication technologies in teacher education: A planning guide.

[25] Kozicka, O. K. (2018). Vocational Education and Training for Farmers and Other Actors in the Agri-Food Value Chain in Africa. Bonn: Center for Development Research, University of Bonn.

[26] Lakshmi, Y. V., Das, J., & Majid, I. (2020). Assessment of e-Learning Readiness of Academic Staff & Students of Higher Education Institutions in

Gujarat, India. *Indian Journal of Educational Technology*, *2*(1), 31-45.

[27] Lee, M. H, Yun, J. J., Pyka, A., Won, D., Kodama, F., Schiuma, G., Park, H., Jeon, J., Park, K., Jung, K., Yan, M. R., Lee, S., & Zhao X. (2018). How to respond to the fourth industrial revolution, or the second information technology revolution? Dynamic new combinations between technology, market, and society through open innovation. *Journal of Open Innovation: Technology, Market, and Complexity*, *4*(21), 1-24.

[28] Moses, J. B., Akporehwe, N. J., & Agah, R. (2020). Effect of Electronic-Learning on the Academic Achievement of Basic Science Students in Bayelsa State, Nigeria. *African Research Review*, *14*(1), 210-216.

[29] Ngampornchai, A., & Adams, J. (2016). Students' acceptance and readiness for E-learning in Northeastern Thailand. *International Journal of Educational Technology in Higher Education*, *13*(1), 1-13.

[30] Njura, H. J., Kaberia, I. K., & Taaliu, S. T. (2020). Effect of agricultural teaching approaches on skills development for food security: a case of secondary schools in Embu County, Kenya. *The Journal of Agricultural Education and Extension*, *26*(3), 239-252.

[31] Obayelu, O. A., & Fadele, I. O. (2019). Choosing a career path in Agriculture: A tough calling for youths in Ibadan metropolis, Nigeria. *Agricultura Tropica Et SubTropica*, *52*(1), 27-37.

[32] Okoro, P. E. (2021). Constraints and Prospects of E-Learning in Universities as Perceived by Business Education Lecturers in South-South Nigeria. *Library Philosophy and Practice*, 1-15.

[33] Olayemi, O. M., Adamu, H., & Olayemi, K. J. (2021). Perception and Readiness of Students' Towards Online Learning in Nigeria During Covid-19 Pandemic. *Perception*, *3*, 4-2021.

[34] Oyediran, W. O., Omoare, A. M., Owoyemi, M. A., Adejobi, A. O., & Fasasi, R. B. (2020). Prospects and limitations of e-learning application in private tertiary institutions amidst COVID-19 lockdown in Nigeria. *Heliyon*, *6*(11), e05457.

[35] Ra, S., Ahmed, M., & Teng, P. S. (2019). Creating high-tech 'agropreneurs' through education and skills development. *International Journal of Training Research*, *17*(sup1), 41-53.

[36] Smith, K. L., & Rayfield, J. (2016). An Early Historical Examination of the Educational Intent of Supervised Agricultural Experiences (SAEs) and Project-Based Learning in Agricultural Education. *Journal of Agricultural Education*, *57*(2), 146-160.

[37] Subramanian, R. M. (2016) "The role of e-learning, the advantages and disadvantages of its adoption in Higher Education, *Tamilnadu, 2*(12), 397–410.

[38] Suresh, M., Vishnu Priya, V., & Gayathri, R. (2018). Effect of e-learning on academic performance of undergraduate students. *Drug Invention Today*, *10*(9), 1797-1800.

[39] Teng, M. F., Putra, Y. K., Ilham, M., & Pratama, W. A. Y. (2020). How much does distance learning affect social life and psychology of growing adolescent? *Bulletin of Social Informatics Theory and Application*, *4*(2), 52-58.

[40] Yang, J., Schneller, C., & Roche, S. (Eds.). (2015). *The role of higher education in promoting lifelong learning*. Hamburg: UNESCO Institute for Lifelong Learning.

[41] Yushau, B., & Nannim, F. A. (2018). ICT facilities and their utilization for educational purposes in Nigerian universities: A review of literatures from 2004 to 2018. *ATBU Journal of Science, Technology and Education*, *6*(1), 237-263.

Article   *Peer-Reviewed Journal*

Effects of land management practices on soil physicochemical properties of selected sites in the Migina catchment, Kansi sector, Gisagara District, southern province of Rwanda

C.Kasanziki[1, 2], F.X. Naramabuye[3], E. Tuwamine[3], R. Miiro[4], J.G.M. Majaliwa[5]

[1]Makerere University, P.O. Box 7062, Kampala, Uganda., College of Agricultural and Environmental Sciences, Department of Extension and Innovation studies, PhD Program in Agricultural and Rural Innovation,
[2]University of Rwanda, College of agriculture, Animal Sciences and Veterinary Medicine, School of Agriculture engineering and Environmental Management (SAEEM) , Department of Irrigation and Drainage,
[3]University of Rwanda, College of Agriculture, Animal science, and Veterinary Medicine (CAVM) School of Agriculture and Food Science, Department of Soil Science,
[4]Makerere University, P.O. Box 7062, Kampala, Uganda., College of Agricultural and Environmental Sciences, Department of Extension and Innovation studies,
[5]Makerere University, P.O. Box 7062, Kampala, Uganda., College of Agricultural and Environmental Sciences, Department of Geography, Geoinformatics& Climatic Sciences,
Corresponding author: Email:kasanziki123@gmail.com

Abstract— Land use changes from natural ecosystems into managed ecosystems may have impacts on soil structure and quality. Land management practices contribute to the change of soil properties and agriculture production. Rwanda's livelihood and social economy is strongly dependent on agriculture. Moreover, the exponential Rwandan population growth is making a severe pressure on natural resources including forests. In order to contribute to sustainable and environmentally friendly agriculture, the present research focused on the impact of land management on soil quality. Many scholars have proved that even the downhill surface water quality is mainly affected by unsustainable land management practices. This research was carried out to determine the impact of land management on soil quality in order to contribute to its protection and a sustainable agricultural production for food security, soil and water conservation. This study was conducted at Akaboti cell, Kansi Sector, Gisagara District of the Rwandan Southern Province, exactly in the Migina catchment watershed with different agricultural activities for comparison. The following physicochemical characteristics were evaluated: soil reaction (pH), organic carbon, Nitrogen, Phosphorus, Exchangeable basis (Calcium, Sodium, Magnesium, Potassium), exchangeable acidity, soil texture, moisture content, bulk density, and electrical conductivity. The laboratory results showed that soils across the different land management practices were dominated by the soil texture classified as sandy loam textural class. The soil bulk density varied between 0.96 and 1.44 g /cm^{-3}, the soil porosity was 50.2 to 52.08 % in terraced land; 47.2 to 58.16 % in trenches; 53.8 to 63.87 % in agroforestry and 53.08 to 53.8 % in forested land. In general values for this parameter ranged from 56.39 to 58.9 % in managed lands and 45.9 to 51.84% in non-managed land (the control). The soil pH ranged from 4.73 to 5.14 for the radical terraced land, 5.79 to 5.29 for trenches, 6.29 to 5.79 for agroforestry land, 4.49 to 4.93 for forested land, 5.11 to 6.34 for amended soil and 4.69 to 5.27 for the

---

control. The electrical conductivity was low in all treatments ranging from 0.076 to 0.39. The values of mineral nitrogen measured in managed lands were higher compared to those measured in the control. Ammonium values ranged from 2.55 to 7.24 mg kg^{-1} while those of nitrate ranged from 4.36 to 28 mg kg^{-1}. Available P values were higher in the managed land than in the control. The values of available P ranged from 8.55 to 17.10 mg kg^{-1}. The values of exchangeable bases were slightly higher in managed land than in control; however, those values were generally low. Generally, the agroforestry land showed high nutrients values compared to the control and other treatments. From the results of this study, it is clear that the land management practices have generally a positive impact on soil properties. Farmers are therefore advised to adopt those practices, especially agroforestry.

Keywords*— *Soil quality parameters, land use changes, soil degradation, Migina catchment.

I. INTRODUCTION

Land use/land cover (LULC) changes influence the biogeochemistry, hydrology, and climate of the earth (Tellen&Yerima, 2018). The land management has great importance on land productivity, ecosystem function, soil health, environmental protection and social-economic activities (Ruslanjari&Taufan, 2017). Land is one of the natural resources that provides profits and food through agricultural activities. Most people living in Sub- Saharan African depend upon natural resources especially land and water for their livelihoods. Agriculture production supplies a big contribution to the most gross domestic product of Africa (Majule, 2010). Across sub-Saharan Africa, natural resources remain central to rural people's livelihoods (Roe et al., 2009).

In Rwanda, the majority of people rely on agriculture production, which employs over 80% of the population and generates over 40% of the country's GDP (Mbarushimana, J. D. D, 2019). The land management sustainability depends on a combination of different approaches from different perspectives including technologies and policies that integrate socio-economic values for enhancing production. Land management works out issues of both population increasing and natural resources degradation while available agricultural land decreases. Even if land management practice is very important, it is challenged by several factors including climate variation, erosion, overexploitation, overgrazing, population pressure, deforestation, improper land management and other different human activities (Mongi, H., Majule, A. E., &Lyimo, J. G, 2010). The land degradation is influenced by composite interaction among human activities and the environment, wherever it is the permanent or temporary decreasing of the possible productive capability of land resources and for that case the utilization and management of natural resources could be a central issue (Tesfahunegn, G. B, 2019).

As results of land degradations, are manifested by soil erosion, compaction of soil, animal and plant species, soil biota losses, and nutrient depletion, with associated risks to the production sustainability of ecological and food commodities and services (GEF, 2005).

Furthermore, recently few studies have made attention of the interrelation among land management practices and water quality then the lack of information has impaired the sustainability of agricultural productivity, ecosystem functioning and welfare of livelihood of farmers (Paul & Rattan, 2014). Watershed management is being implemented by public and private institutions to improve land use sustainability but unfortunately many rural farmers are not participating in local community activities since they are not involved in practices' decision making as well as insufficient research, lack of skills and financial constraints (Kuria et al., 2018). The poor land management and overexploitation of soil in Migina catchment leads to the soil and water degradation then agricultural productivity decline, removal of vegetation cover, soil erosion acceleration, nutrient depletion and decrease in arable land. To feed the rapidly growing population, it is necessary to improve agricultural productivity in the country; this can be achieved by a change in agricultural practices (Wali, 2014).

The population cultivates even the marginal areas in trying to satisfy their needs in food security and they are growing but the arable land decreases (Olson & Berry, 2003).

Therefore, this study aims to assess the impact of land management practices such as the radical terrace, forest, agroforestry, trenches and soil amendment on soil quality in Migina catchment and we were based on physic-chemical elements that are improving soil fertility for increasing crop productivity.

There are few land management practices that are established in this area but some of them are degraded due to the population's poor management and others are not well established. Then to achieve sustainable agricultural development, the soil restitution of land management activities is needed for soil quality and land productivity improvement. For achieving the increase of agricultural productivity, it is necessary to take care on land

degradation issues for preventing loss of soil nutrients and sustainability of agricultural productivity and then it is very important to reinforce the level and awareness of citizen's participation in the land use management process. This study was released in order to find the equivalent solution to the cited above problem and to enhance land management practice improvement for managing soil quality in Migina Catchment. Specifically, it was based on the result of the difference.

The main purpose of this study was to evaluate the impact of land management on soil quality and the specific objectives being: (1) To characterize the physic-chemical properties of soil under different land use systems which are radical terrace, trenches, forest, agroforestry, soil amendment and a control; (2) To determine the ability of different land management practice for improving soil fertility status compared to fertility standards; (3) Assess the impact of land use systems on properties such as the soil texture, electrical conductivity, bulk density and moisture content improvement. These objectives are established based on the hypothesis that a good land management practice may improve soil quality including chemical nutrients and soil physical properties. The effects of land management practices on physicochemical properties variation is based on the elements that may influence soil fertility status. More researches have proved that there is direct interrelationship between water quality and land use management activities such as deforestation, clustered settlements releasing wastes, erosion due to non-protected slopes, and non-protected river banks. Particularly the study took place in Gisagara District, Kansi Sector at Akaboti Cell where we found our proposed different land management practices in farmer's land. This study was released in order to find the equivalent solution to the cited above problem and to enhance land management practice improvement for managing soil quality in Migina Catchment. The effects of land management practices on physic-chemical properties variation is based on the elements that may influence soil fertility status.

II. MATERIAL AND METHODS

2.1. Description of the study sites

The latest study was performed in the Migina catchment, which is found in Rwanda's Southern Province and passes through Huye, Gisagara and Nyaruguru districts. Our analysis was done within five purposely selected land management practices that are agroforestry, forestry, radical terraces, trenches and soil amendment.

Geographically, Migina catchment is situated between latitudes 2°32' to 2°48' South and with longitudes of 29°40' to 29°48' East, in the southern part of Rwanda. Its mean annual temperature is about 20°C and its mean annual rainfall is 1200 mm/a and then 917 mm/a is estimated to be its mean annual actual evaporation (Munyaneza, O., Mukubwa, A., Maskey, S., Uhlenbrook, S., &Wenninger, J, 2014).

The average altitude of Migina Catchment is 1681 m. Migina catchment is among the Akagera river sub basin and is subdivided into 5 sub-catchments referring to the main draining rivers area, in upstream. Two sub-catchments are located upriver; Munyazi-Rwabuye with about 38.6 km^2 and Mukura with 41.6 km^2; two in the center which are Akagera with 32.2 km^2 and Cyihene-Kansi with 69.6 km^2; and another one also is located in downstream area contains the outlet of the whole catchment: Migina with 61.1 km^2 (Manzi, A., Munyaneza, F., Mujawase, F., Banamwana, L., Sayinzoga, F., Thomson, 2014).

The studied area has a pronounced slope due to hilly and mountainous relief alternated to marshland. The western part has a higher elevation than the eastern part. Towards the south, the hills and elevation becomes lower, with maximum elevations up to 1,900 m. The eastern side of the catchment is relatively low. The river valleys have an elevation of approximately 1,650 m. (Harmen et al. 2010). The topographic conditions are extremely variable, with valley slopes value from 5 to 10% upstream and 1 up to 15% downstream (the average slope of the sub-catchments is between 2 and 3 percent (Kayiranga, A., Ndayisaba, F., Nahayo, L., Karamage, F., Nsengiyumva, J. B., Mupenzi, C., &Nyesheja, E. M, 2017).
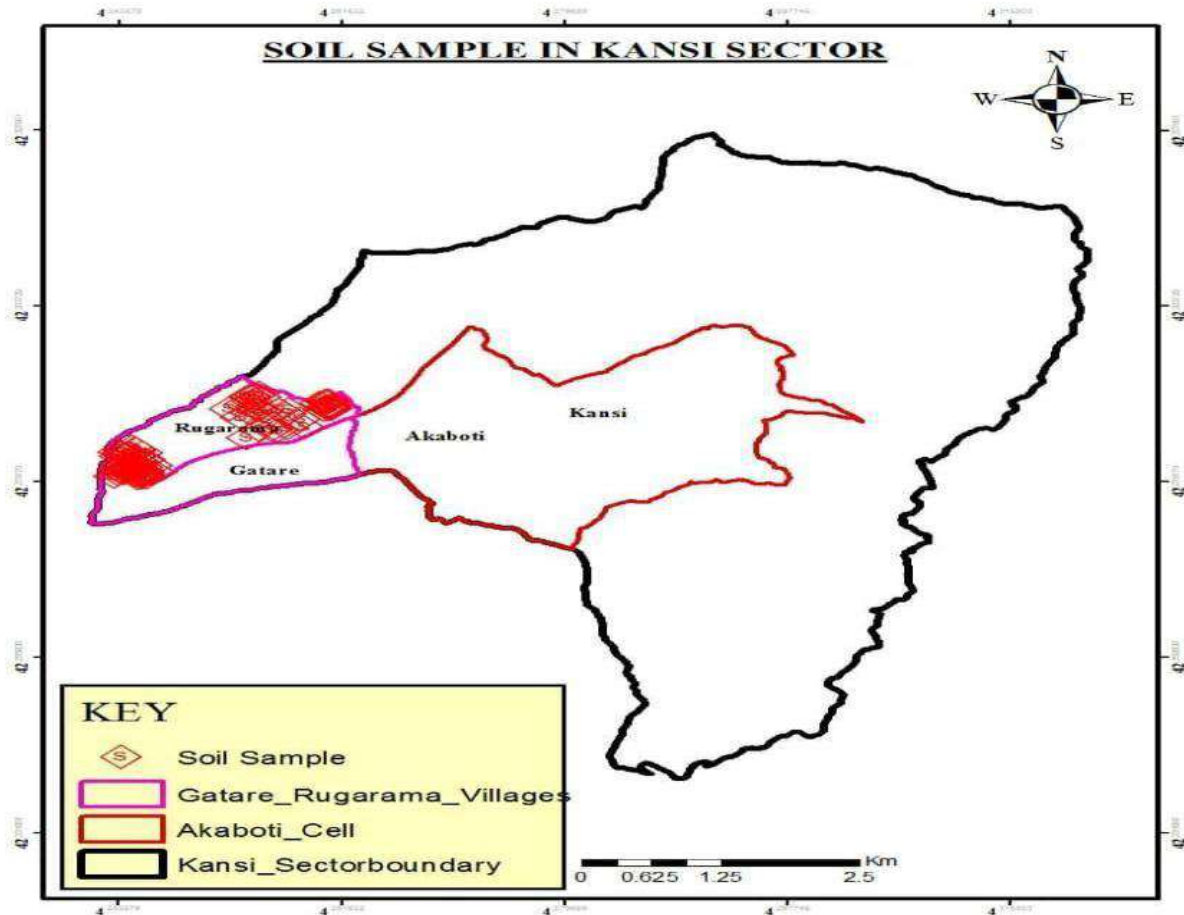
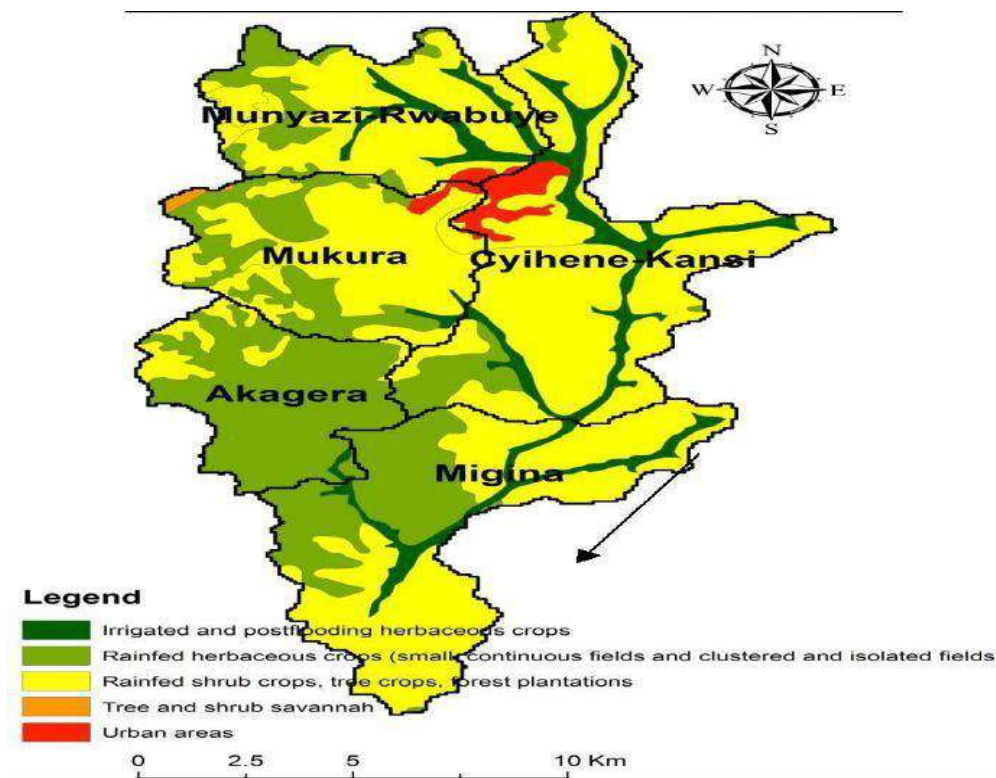*Fig.1: Soil sampling sites inKansi sector, Akaboti cell, Rugarama village*

Fig.2: *Land use, sub-catchments and main rivers in the Migina catchment, Source: MINAGRI, 2016*

2.2. Materials

Field and Laboratory equipment

Different materials were used on the field in order to gather soil samples for laboratory analysis comprising Auger, Basket, Cooler box, Coiling, Coiling box, Knife, Hoe, Decameter, Timber and GPS.

- Auger was used for taking a soil sample from different locations of the field (on the Top slope, middle slope, and bottom and Cooler box used to keep cold and fresh soil for available Nitrogen Analysis.

- Coiling and Coiling box was used to take flesh soil and to keep them fresh for soil moisture and bulk density analysis and Basket used to make mixture of composite soil sample for better laboratory analysis

- GPS is an electronic field material that was used to take Geographical Coordinates.

We used appropriate equipment such as pH -meter, stirrer, spectrophotometer, digester, distiller flasks, funnels, pipettes, precision balance, cylinders, filter papers, Erlenmeyer, sieves, watch glass, crucibles, sand bath, spoons, auger, mortar, pestle, Electronic Balance, Volumetric flask, Conical flask, Steam distillation, Micro burette, Mechanical shaker, plastic bottles with stoppers, Magnetic stirrer, Beakers, Hydrometer, Thermometer, etc. and many laboratory chemical product to analyze soil constituent elements. Some more levels of elements existing in soil were determined using AAA (Atomic Absorption Spectrophotometer) which provides an advantage of saving time giving results quickly than other materials. It has been used to analyze Ca, Na, Al and K elements.

2.3. Research methodology

Soil sampling

Stratified soil sampling method using auger was used to collect samples in different land management practices (terraced land, trenches, agroforestry, forestry and control).

The sampling was done along a topography sequence (Upland, middle and low land) and simple sites were located by international coordinates using the worldwide positioning system GPS (model GARMIN etrex 20). From each sampling unit, disturbed (bulk) and undisturbed (core samples) the physicochemical analysis in the laboratory used those taken samples.

Gathered samples were 18 composite soil samples collected to a depth of 30 cm, labeled and prepared for analysis.

Laboratory analysis

The laboratory work was done in the soil and plant analysis Laboratory at University of Rwanda. The collected soil samples mean disturbed one were air-dried and ground to pass through a 2 mm and 0.5 mm sieve for laboratory analyses. Without interruption core samples were used for calculating the soil bulk density, moisture content, particle density, porosity and texture.

The core methods were used to determine the bulk density (Black and Hartge, 1986). Particle density of the soil was calculated by determining the mass and volume those particles solid occupy.

The solid particles mass was also determined by measuring the solid particle and the same at the volume was obtained from the weight and water density displacement by soil sample (Blake and Hartge, 1989). Total soil porosity was determined using a formula outlined by NSS (1990) as follows: Soil porosity (Particle density - Bulk density) / Particle density) *100 for every sample.

Those samples of soil that are disturbed were used for analyzing the remaining physical and all chemical properties of soil except available nitrogen ($N-NO_3^-$ and $N-NH_4^+$) which used flesh soil. Hydrometer method after dispersion with sodium hexametaphosphate 5% (Mbaga, H., &Makoi, J. H, 2018). The textural classes were calculated using the USDA textural class triangle (USDA, 1975) which is illustrated below:
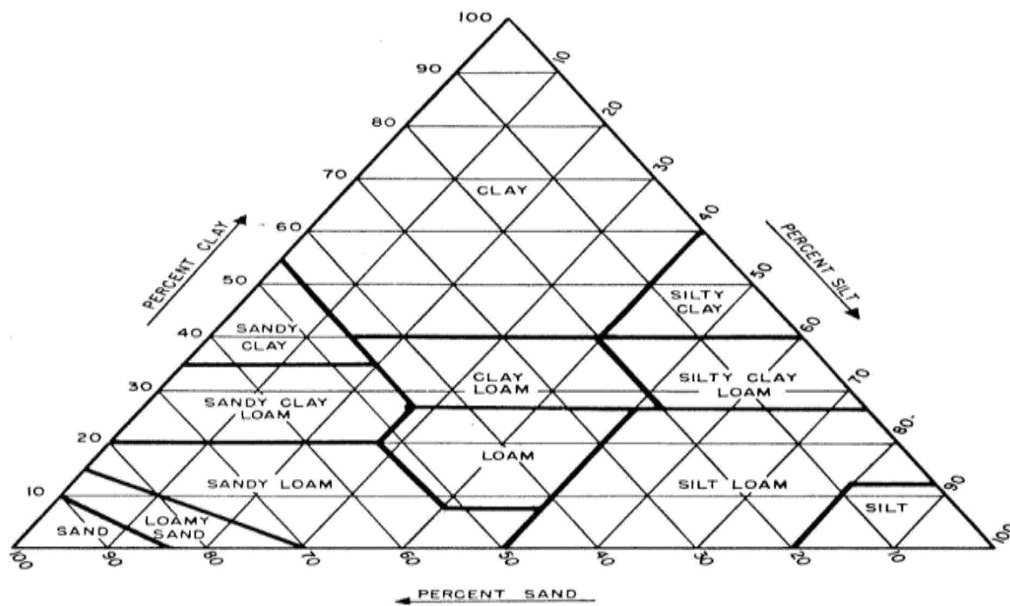
Fig.3: Textural triangle

An EC meter was used to calculate the electrical conductivity of a 1:2.5 ratio extract (Okalebo, 2002). The soil reaction (pH) was determined through potentiometric methods in water and 1N KCl at a 1:2.5 ratio (soil: water and KCl) (Okalebo, 2002). Total exchangeable acidity was determined by 1M KCl extraction solution and the soil extract titrated with sodium hydroxide. A second titration with 1M HCl after addition of sodium fluoride was used to obtain the exchangeable aluminum ((NSS, 1990). Aluminum saturation as a measure of toxicity was calculated by dividing exchangeable aluminum by adding interchangeable bases and interchangeable aluminum.

The organic carbon was measured using the Walkley and Black wet oxidation process (Nelson, D. W., & Sommers, L. E, 1996), and organic matter was detected by multiplying organic carbon by 1.724. (Duursma and Dawson, 1981). The extracting, Bray II solution was used to extract available phosphorus from the soil. The extracted phosphorus is calorimetrically determined using the ammonium molybdate reaction and the formation of molybdate blue color.

In a spectrophotometer, the compound's absorbance is assessed at 882nm and is proportionate to the amount of phosphorus derived from the soil (Okalebo, 2002).

The extraction of soil bases was determined using 1M NH_4OAc (ammonium acetate) and the absorbed NH_4^+ displaced by K^+ using 1M KCl (Thomas, G. W., & Hargrove, W. L, 1984). The Atomic Absorption Spectrophotometer was being used to test the bases Ca^{2+}, Mg^{2+}, K^+, and Na^+ displaced by NH_4^+ (Thomas, 1982). The ECEC was computed as the sum of four exchangeable

bases (Ca^{2+}, Mg^{2+}, Na^+, and K^+) plus exchangeable acidity (Al^{3+} and H^+) for a given soil sample.

III.   DATA ANALYSIS

Genstat software was used to test differences between treatments in the Migina catchment area. Least significance was measured to determine a significant difference between treatments for each measured parameter and differences were declared significant at α: 0.05 levels. Excel software for data entry, calculations and management.

IV.   RESULTS PRESENTATION ON ANALYZED PARAMETERS

Tables of results presentation and interpretation

pH (Water), pH(KCl) in different land management practices

This section presents the results obtained in physicochemical properties and statistical soil sample analysis of Migina Catchment at Akaboti cell, Kansi Sector, Gisagara District in Southern Province of Rwanda. Those results are presented in the form of figures and tables for facilitating their interpretation. The soil nutrients and chemical parameters that assed, are soil pH (H_2O), pH (KCl), Available Nitrogen (N-NH_4^+ and N-NO_3^-), Available Phosphorus, exchangeable basis (K, Ca, Na, Mg and base saturation), pH (Al^{2+},H^+) and organic carbon, we was analyzing also soil physical properties like moisture content, bulk density, electrical conductivity , particle density and soil texture all of those physicochemical

properties analyzed for observing the parameters improving soil fertility under different land management practices in Migina catchment.
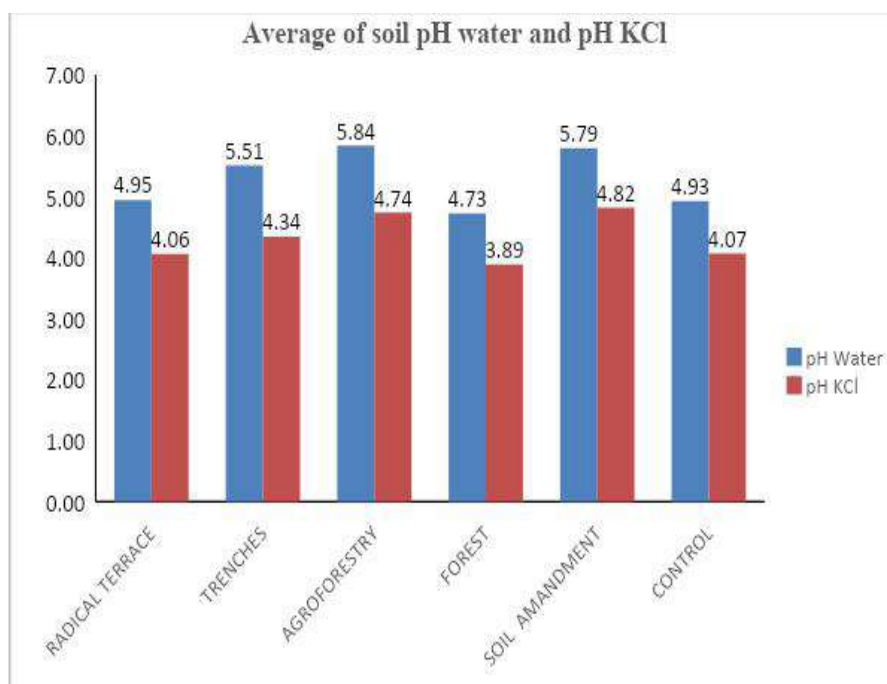

Fig.4: pH Water, pHKCl in different land management practice in Migina catchment

Table I: The results of pH*, electrical conductivity, organic carbon, mineral nitrogen, (N-NH4-N-NO3), available phosphorus*

							Mineral Nitrogen		
Land Management Practice	Soil Sample Stream	$P^H H_2O$	P^H_{KCl}	EC (dS/m)	% OC	% OM	N-NH$_4$ ppm	N-NO$_3^-$ ppm	ppm (Av. P)
Radical terrace	**Upper**	4.98	4.08	0.080	1.53	2.63	2.554	6.89	9.24
	Middle	5.14	4.18	0.076	3.33	5.73	3.359	18.61	10.21
	Bottom	4.73	3.93	0.145	1.71	2.94	3.019	4.36	8.55
Trenches	**Upper**	5.46	4.27	0.138	2.61	4.49	4.104	18.89	17.10
	Middle	5.29	4.17	0.087	2.88	4.96	4.852	11.04	9.49
	Bottom	5.79	4.59	0.366	3.24	5.58	3.574	16.18	12.11
Agroforestry	**Upper**	6.29	5.32	0.188	4.04	6.97	6.315	24.00	16.79
	Middle	5.45	4.3	0.112	3.77	6.51	5.658	18.75	14.78
	Bottom	5.79	4.61	0.390	3.24	5.58	4.466	28.00	12.58
Forest	**Upper**	4.93	3.9	0.102	2.61	4.49	2.982	9.64	10.02
	Middle	4.49	3.91	0.098	2.88	4.96	3.383	18.57	9.98
	Bottom	4.77	3.85	0.129	3.59	6.20	5.304	12.46	14.75
Soil amendment	**Upper**	6.34	5.37	0.141	3.33	5.73	5.727	17.75	14.65
	Middle	5.94	4.96	0.105	3.41	5.89	4.554	20.82	11.99
	Bottom	5.11	4.13	0.155	2.70	4.65	7.239	19.54	12.40
Control	**Upper**	4.69	3.95	0.098	2.34	4.03	3.322	13.39	9.12

	Middle	4.83	4.03	0.080	2.88	4.96	3.767	12.82	9.17
	Bottom	5.27	4.24	0.198	3.95	6.82	3.063	14.29	9.42

The ANOVA table shows that there is a highly significant difference between pH values measured in samples collected from different treatments. The values presented in the figure above are pH water and pH KCl of different land management in Migina Catchment, the pH water and pH KCl of the studied area varies slightly along a topo sequence in general, (Table 1).

The figure shows that P^H water and pH KCl of agroforestry, trenches and soil amendment practices are greater than the P^H of radical terrace and forest management practices. Especially for agro forestry pH is higher than the other land management practices (Figure1).

Generally, the pH of this area is acidic in nature (Table 1). The soils irrespective of the physiographic position along the topography sequence were varying from strongly to slightly acidic with the pH (H_2O) values of 4.73 – 5.14 for radical terraced land; 5.79 – 5.29 for trenches; 6.29 – 5.79 for agroforestry; 4.93 – 4.49 for forest and 5.27 – 4.69 for control (Uwingabire, 2016). The pH in amended land increased with altitude with the values of 6.34, 5.94 and 5.11 for the upper, middle and bottom land respectively; there are no trends of pH decreasing or increasing except in soil amendment practice where pH is decreasing downward.

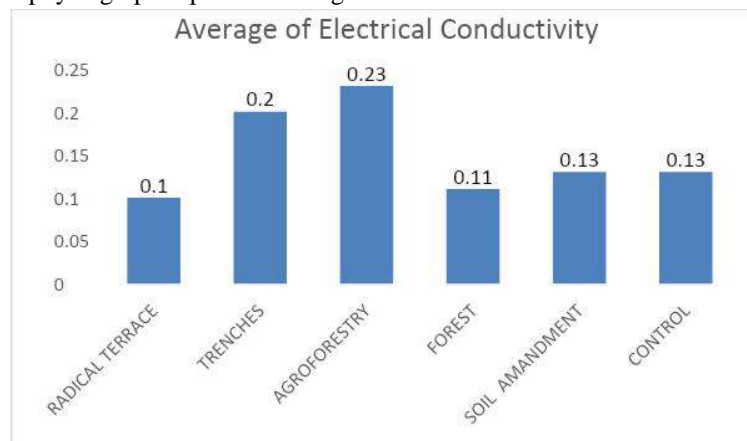**Electrical Conductivity in different land management practice**

Fig.5: Electrical Conductivity in different land management practice

The ANOVA table shows a highly significant difference between land management ($P < 0.01$). The value presented in the figure above is Electrical conductivity of different land management in Migina Catchment, the Electrical conductivity of our studied area varies slightly along a topo sequence in general, (Table 1). According to (E.S. Marx, J. Hart, 1999), The figure shows that the Electrical conductivity is low for all treatments means there is not any salinity effect in my study area and specifically electrical conductivity of agro forestry land management practice is higher compared to the other land management practices while in radical terrace its electrical conductivity is smaller than the other land management practice in our study area (Figure 2).

The Electrical Conductivity in this study area is low in general (Table 1.). Without based on topo sequence electrical conductivity variation, normally were varying as this follow, the electrical conductivity values of 0.076 – 0.146 ds/m for radical terraced land; 0.087 – 0.366 ds/m for trenches; 0.112 – 0.390 ds/m for agroforestry; 0.98 – 0.102 ds/m for forest; 0.15-0.141ds/m for amended land and 0.080 – 0.198 ds/m for control. There are no trends of electrical conductivity decreasing or increasing in topo sequence except in radical terrace practice where electrical conductivity decreases downward.

Carbon and organic matter Percentage in different land management practice
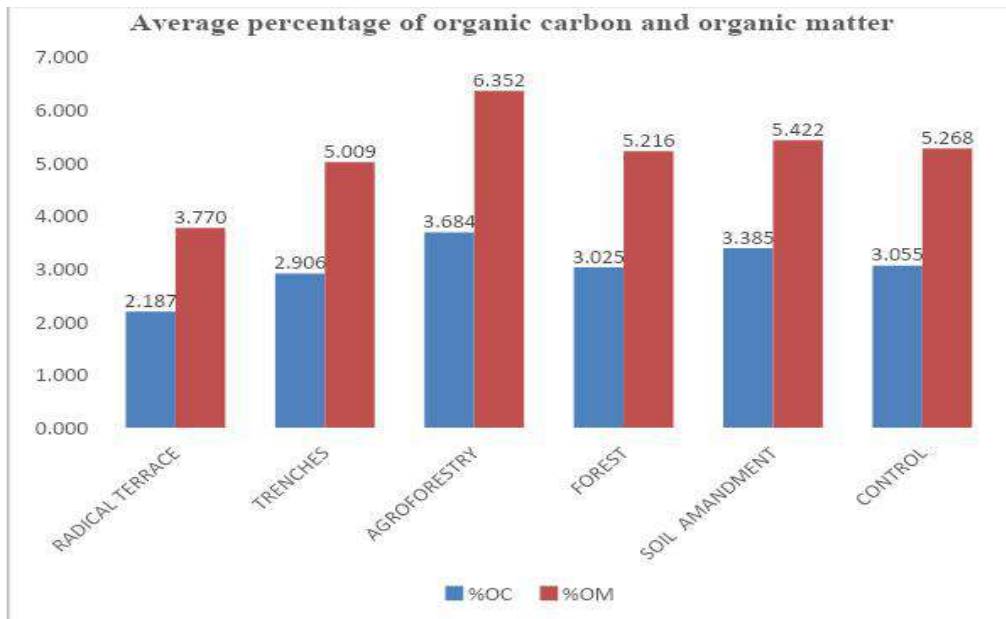
Fig.6: Percentage organic Matter and Carbon Percentage in different land management practice

The ANOVA table shows a significant difference between land management (P < 0.02).

The values presented in the figure above are organic carbon and percentage of organic matter in different land management practices in the Migina catchment area. In this different land management practice organic carbon varies slightly along a topo sequence in general, (Table 1).

The figure shows that the agroforestry practice contains higher organic matter and organic carbon than other land management practices while radical terrace has lower organic matter and carbon percentage compared to the other selected land management in this study area (Figure 3). According to Kileo (2000) and EUROCONSULT (1989), generally the organic carbon and organic matter percentage of the study area varies from medium to very high (Table 1.).

The percentage of soil carbon irrespective of the physiographic position along the topography sequence were varying from Medium to high with the % C values from 1.53 – 3.33 for radical terraced land; 2.61– 3.24 for trenches; 3.24 – 4.04 for agroforestry; 2.61 – 3.59 for forest; 2.70-3.33 for amended soil and 2.34 – 3.95 for control as observed from the laboratory analysis.

Soil organic carbon varies from the upper, middle and bottom land respectively There is a specific trend of % OC in treatment as it is increasing downward except in soil amendment practice and in radical terraces where there is no specific trend of % OC variation, and in Trenches increase downward.

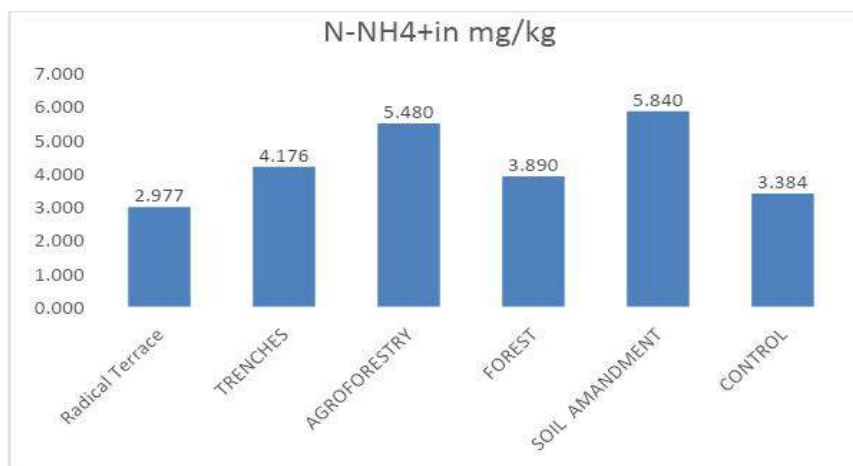**Ammonium (NH₄⁺) of different land management practice in Migina Catchment**

Fig.7: Ammonium (NH4⁺) percentage in different land management practice

The ANOVA table shows that there is no significant difference between land management practices (P < 0.042). The values presented in the figure above are Ammonium (NH_4^+) concentration measured between different land management practices in Migina Catchment, Ammonium (NH_4^+) concentration of the studied area varies slightly along a topo sequence in general, (Table 1). The figure shows that Ammonium (NH_4^+) of amended soil are greater than the Ammonium (NH_4^+) of other practices in this area while radical terrace has the smallest Ammonium (NH_4^+) compared to the other land management in Migina catchment (Figure 4). The Ammonium (NH_4^+) of the study area is generally enough (Table 1) as the effective value in soil crop production is from 2-10 ppm (E.S. Marx, J. Hart, 1999). The value of ammonium is presented in (Table 1).

According (Murphy, B, 2014), the upper, middle and lower slopes showed medium to high levels of nitrate ranging from of 2.554 – 3.353 ppm for radical terraced land; 3.574 – 4.852 ppm for trenches; 4.466 – 6.315ppm for agroforestry; 2.982 – 5.304 ppm for forest, 4.554 – 7.239 ppm for amended soil and 3.063-3.767 ppm for control. Referring to topo sequence there are no specific trends of Ammonium (NH_4^+) variation except in forest land management practice where Ammonium (NH_4^+) is increasing downward (Table1).

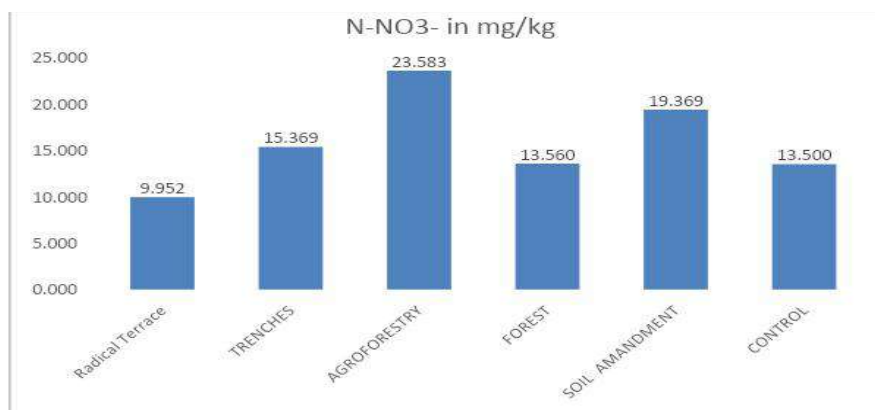**Nitrate (NO_3^-) of different land management practice in Migina Catchment**

Fig.8: Nitrate (NO_3^-) percentage in different land management practice

The ANOVA table shows that there is a significant different between land management (P<0.006). The value presented in the figure above is Nitrate (NO_3^-) of different land management in Migina Catchment, Nitrate (NO_3^-) of the studied area varies slightly along a topo sequence in general, (Table 1). The figure shows that Nitrate (NO_3^-) of Agroforestry are greater than the Nitrate (NO_3^-) of other practices in this area while radical terrace has the smallest Nitrate (NO_3^-) compared to the other land management in Migina catchment (Figure 5).

Generally, the Nitrate (NO_3^-) of the study area is high (table 1.). The value of Nitrate is presented in (Table 1).

According to (Murphy and Hazalton, 2007), the upper, middle and lower slopes showed medium to high levels of nitrate ranging from of 4.36 – 18.61ppm for radical terraced land; 11.04 – 18.89 ppm for trenches; 18.75 – 28 ppm for agroforestry; 9.64 – 18.57 ppm for forest,17.75 – 20.85 ppm for amended soil and 12.82-14.29 ppm for control. Referring to topo sequence, there are no specific trends of Nitrate (NO_3^-) variation (Table1).

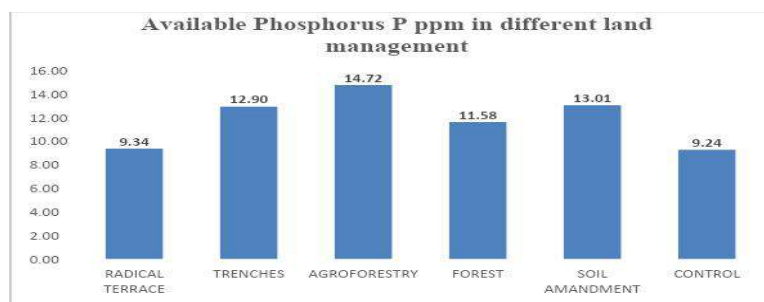**Available phosphorus in different land management practice**

Fig.9: Available phosphorus percentage under different land management practice

The ANOVA table shows a significant difference between land management (P<0.02).

The available phosphorus P varies from 9.24 ppm to 14.47 ppm based on the average available phosphorus of different selected land management in Migina catchment. Generally, all land management Av. P varies in medium but are different from their topo sequence values. According to EUROCONSULT (1989), all practices of our study area classified in mediums differ in values of phosphorus availability in their treatment, where agro forestry, soil amendment, trenches and forest have great value respectively compared to the radical terrace and control.

The most competitive to soil available phosphorus is agroforestry and control is less compared to the other treatments (Figure 6). The soil phosphorus is classified as medium according to (ILACO, 1993; Landon, 1991; Baize, 1993; Msanya*et al.,* 2001). But referring to the topo sequence there significant variation of their availability as this follow for radical terrace varies from 8.55-10.21 ppm, for trenches is 9.24-17.10 ppm, for is Agroforestry 12.58-16.79 ppm, for forest is 9.98-10.02 ppm, for soil amendment is 11.99-14.75ppm and Control is varies from 9.42-9.12 ppm, and there is no specific trends on treatment topo sequence except at control where values varies by increasing downward and in agro forestry varies by decreasing down ward (Table 1).

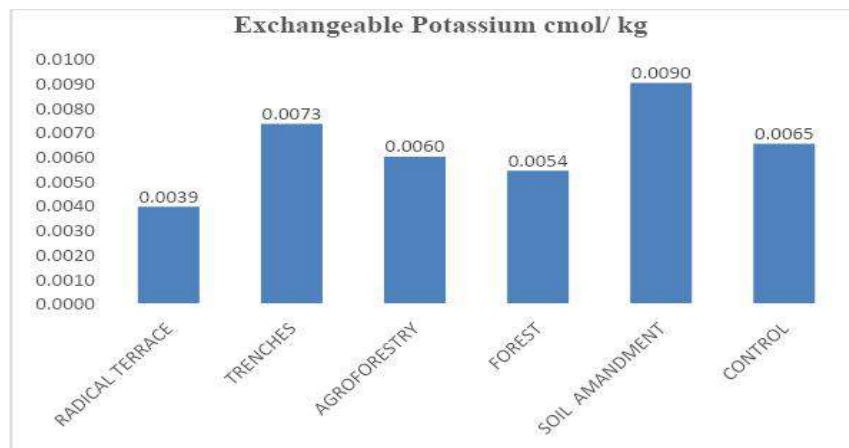**Exchangeable Bases, Exchangeable potassium**

Fig.10: Potassium (K) of different land management practices in Migina Catchment,

The ANOVA table shows a significance between different land management (P < 0.04). The value presented in the figure above is Potassium (K) of different land management in Migina Catchment, Potassium (K) of the studied area varies slightly along a topo sequence in general, (Table 1). The figure shows that Potassium (K) of Agroforestry are greater than the Potassium (K) of other practices in this area while radical terrace has the smallest Potassium (K) compared to the other land management in Migina catchment (Figure 7).

Generally, the Potassium (K) of the study area is very low (table 2). The soils vary based on their physiographic place along the topo sequence as this follow values of 0.0028 –

0.0056 cmol/kg for radical terraced land; 0.0035 – 0.0144 cmol/kg for trenches; 0.0036 – 0.0145 cmol/kg for agroforestry; 0.0043 – 0.0066 cmol/kg for forest, 0.0043 – 0.0142 cmol/kg for amended soil and 0.0052-0.0084 cmol/kg for control (ILACO,1993; Landon, 1991; Baize, 1993; Msanya*et al.,* 2001). Referring to topo sequence there is a trends of Potassium (K) in radical terrace, trenches increase downward also in forest and control (undisturbed land) is decreasing downward except in Agroforestry and soil amendment land management practices where there is no trends of Potassium (K) variation (Table 2).
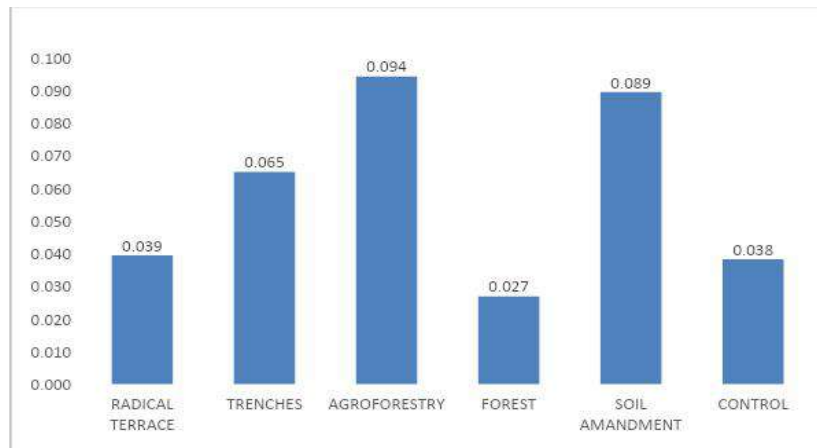
Exchangeable Magnesium

Fig.11: Exchangeable Magnesium under different land management practices

The ANOVA table shows that there is a less significant difference between land management (P<0.042). The value presented in the figure above is Magnesium (Mg) of different land management in Migina Catchment, Magnesium (Mg) of the studied area varies slightly along a topo sequence in general, (Table 1). The figure shows that Magnesium (Mg) of Agroforestry is greater than the Magnesium (Mg) of other practices in this area while forest has smallest Magnesium (Mg) compared to the other land management in Migina catchment (Figure 8). The Magnesium (Mg) of the study area is in general very low to low (Table 2). The value of Magnesium (Mg) is presented in (Table 1). According (Murphy and Hazalton, 2007), the upper, middle and lower slopes showed the levels of Magnesium (Mg) ranging from 0.03 – 0.44cmol/kg for radical terraced land; 0.05 – 0.9 cmol/kg for trenches; 0.08 – 0.11 cmol/kg for agroforestry; 0.02 – 0.09 cmol/kg for forest, 0.03 – 0.11 cmol/kg for amended soil and 0.03-0.06 cmol /kg for control (ILACO, 1993; Landon, 1991; Baize, 1993; Msanya*et al.,* 2001). Referring to the topo sequence there are no trends of Magnesium (Mg) values (Table 2).

Table 2: The results of exchangeable bases and acidity Calcium (Ca), Magnesium (Mg), Potassium (K), Sodium (Na), ECEC and Base Saturation

Land mgt practice	Soil Sample Stream	Cmol/kg (Ca)	Cmol/kg (Mg)	Cmol/kg (K)	Cmol/kg Na	(TEB)CEC (cmol(+)/kg	ECEC Cmol(+) /kg	% BS
Radical Terrace	Upper	0.1	0.04	0.00287	0.021	0.16	4	11.34
	Middle	0.14	0.04	0.00328	0.027	0.21	2.29	9.3
	Bottom	0.13	0.03	0.00564	0.016	0.18	1.3	14.12
Trenches	Upper	0.14	0.06	0.00354	0.012	0.21	1.81	11.81
	Middle	0.18	0.05	0.00395	0.062	0.29	1.01	28.83
	Bottom	0.24	0.09	0.01446	0.106	0.45	0.93	48.27
Agroforestry	Upper	0.14	0.11	0.01421	0.058	0.32	0.4	79.82
	Middle	0.13	0.08	0.00303	0.101	0.31	0.95	32.65
	Bottom	0.2	0.09	0.01456	0.117	0.42	4.02	10.48
Forest	Upper	0.12	0.03	0.00667	0.019	0.18	3.22	5.46
	Middle	0.13	0.02	0.00518	0.03	0.19	2.91	6.43
	Bottom	0.16	0.03	0.00436	0.017	0.21	1.01	20.96
Soil amendment	Upper	0.14	0.11	0.01421	0.058	0.32	0.4	79.82

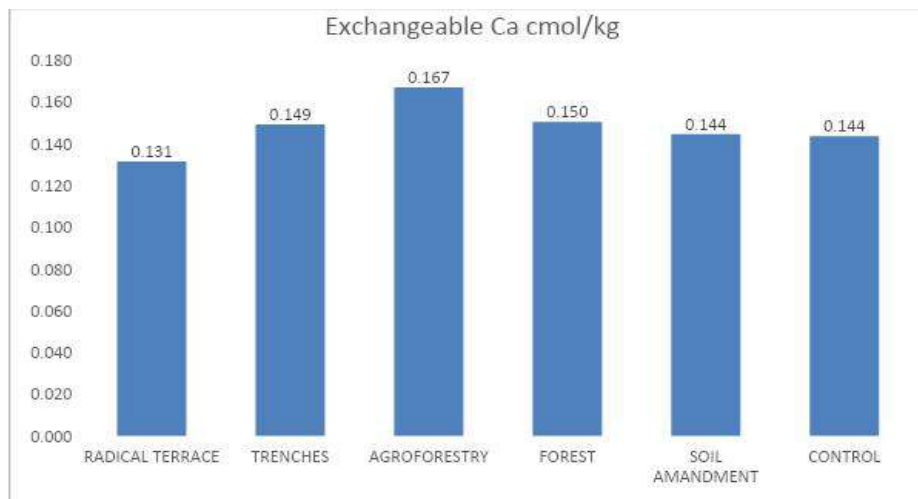|	Middle	0.14	0.1	0.00692	0.238	0.48	1.84	25.98
	Bottom	0.15	0.06	0.00585	0.091	0.31	0.95	32.52
Control	Upper	0.13	0.03	0.00841	0.051	0.22	3.1	7.06
	Middle	0.16	0.03	0.0059	0.075	0.27	3.31	8.02
	Bottom	0.18	0.05	0.00523	0.021	0.26	1.38	18.7

Exchangeable Calcium

Fig.12: Exchangeable Calcium under different land management practices

The ANOVA table shows less significance between land management (P<0.046).

The values presented in the figure above is Calcium (Ca) of different land management in Migina Catchment, Calcium (Ca) of the studied area varies slightly along a topo sequence in general, (Table 2). The figure shows that Calcium (Ca) of Agroforestry are greater than the Calcium (Ca) of other practices in this area while radical terrace has smallest Calcium (Ca) compared to the other land management in Migina catchment (Figure 9). Generally, the Calcium (Ca) of the study area is low (table 2). The soils irrespective of the physiographic position along the topo sequence were varying as this follow values of 0.10 – 0.14 cmol/kg for radical terraced land; 0.14 – 0.24 cmol/kg for trenches; 0.13 – 0.20 cmol/kg for agroforestry; 0.12 – 0.16 cmol/kg for forest, 0.14 – 0.16 cmol/kg for amended soil and 0.11-0.14 cmol/kg for control (ILACO, 1993; Landon, 1991; Baize, 1993; Msanya*et al.,* 2001). Referring to topo sequence there are no trends of Calcium (Ca) except in trenches and control (undisturbed land) land management practices where Calcium (Ca) is increased downward (Table 2).
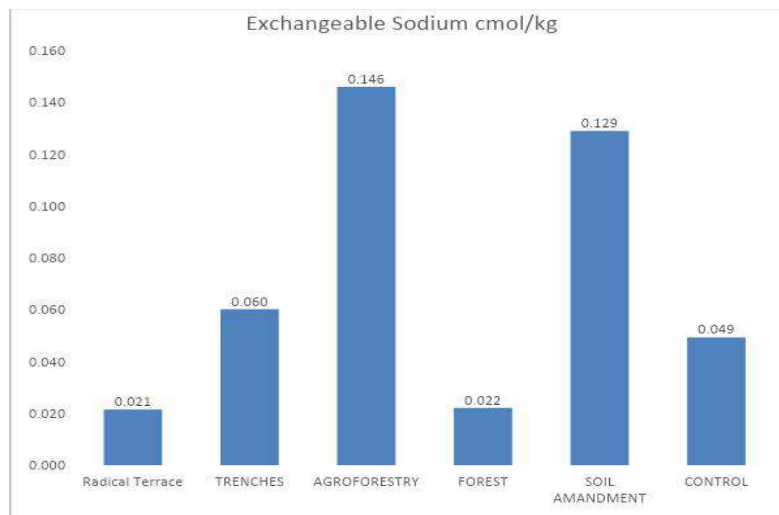
Exchangeable sodium

Fig.13: Exchangeable sodium under different land management practices

The ANOVA table shows that there is a significant difference between land management (P < 0.002). The value presented in the figure above is Sodium (Na) of different land management in Migina catchment, Sodium (Na) of the studied area varies slightly along a topo sequence in general, (Table 2). The figure shows that the Sodium (Na) of Agroforestry is greater than other practices in this area while radical terrace and forested land have smallest Sodium (Na) compared to the other land management in Migina catchment (Figure10).

Generally, the Sodium (Na) of the place of study is very low (Table 2). The value of Sodium (Na) is presented in

(Table 1). According (Hazelton, P., & Murphy, B, 2007), the upper, middle and lower slopes showed the levels of Sodium (Na) ranging from 0.016 – 0.021 cmol/kg for radical terraced land; 0.012 – 0.106 cmol/kg for trenches; 0.101 – 0.230 cmol/kg for agroforestry; 0.017 – 0.030 cmol/kg for forest, 0.058 – 0.238 cmol/kg for amended soil and 0.021-0.075 cmol/kg for control (ILACO, 1993; Landon, 1991; Baize, 1993; Msanya*et al.,* 2001). Referring to topo sequence there are no trends of Sodium (Na) except in trenches management practice where Sodium (Na) is increasing downward (Table 2).

ECEC (Effective Cation Exchange Capacity)
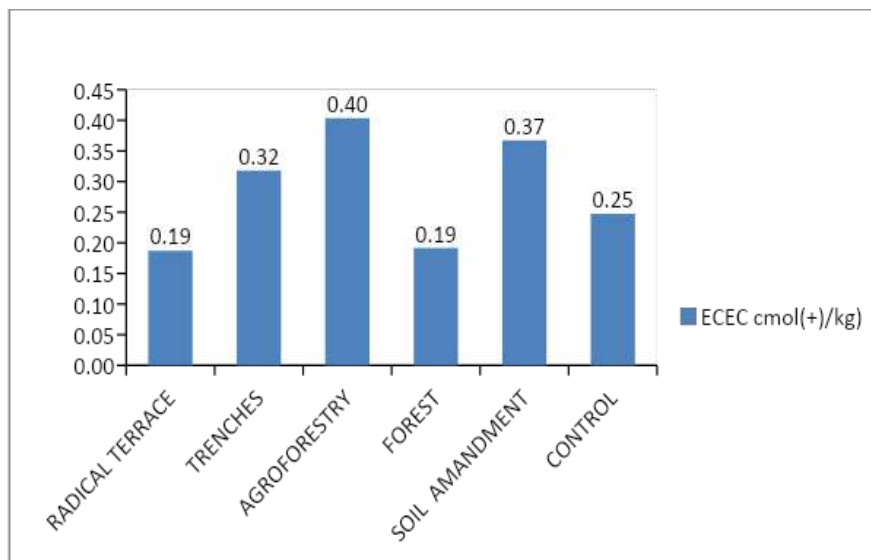
Fig.14: Effective Cation Exchange Capacity (ECEC) under different land management practices in Migina catchment

The ANOVA table shows that there is a less significant difference in different land management (P < 0.046). The value presented in the figure above is Effective Effect of Cation Exchange capacity (ECEC) of different land

management in Migina Catchment, Effective Cation Exchange Capacity of the studied area varies slightly along a topo sequence in general, (Table 2). The figure shows that the Effective Cation Exchange Capacity of

Agroforestry is more than other practices in this area while Forestry and radical Terrace have the smallest Effective Cation Exchange Capacity compared to the other land management in Migina catchment (Figure11). Generally, the Effective Cation Exchange Capacity of the study area is low (table 2.). The soils irrespective of the physiographic position along the topo sequence were varying as this follow values of 0.16 – 0.21 cmol/kg for radical terraced land; 0.21 – 0.45 cmol/kg for trenches; 0.31 – 0.48 cmol/kg for agroforestry; 0.18 – 0.21 cmol/kg for forest, 0.31 – 0.48 cmol/kg for amended soil and 0.22-0.27 cmol/kg for control (ILACO, 1993; Landon, 1991; Baize, 1993; Msanyaet $al.,$ 2001). Referring to topo sequence there are no trends of Effective Cation Exchangeable Capacity (ECEC) except in trenches land management practice where Effective Cation Exchangeable Capacity (ECEC) is increased downward (Table 2).

Base Saturation

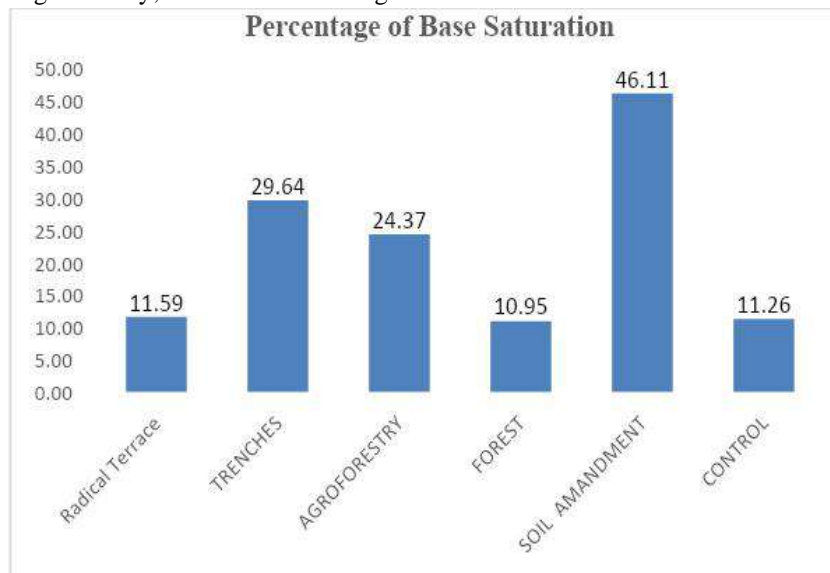*Fig.15: Percentage of base saturation under different land management in Migina catchment*

The ANOVA table shows there is a less significant difference in land management (P < 0.04).

The value presented in the figure above is percentage Base Saturation of different land management in Migina catchment, Base Saturation of the studied area varies slightly along a topo sequence in general, (Table 2).

The figure shows that the Base Saturation of Agroforestry is greater than other practices in this area while radical terrace and forested land have smallest Base Saturation compared to the other land management in Migina catchment (figure12). The Base Saturation of the study area is in general, low (table 2). The soils irrespective of the physiographic position along the topo sequence were varying as this follow values of 9.30 – 14.12 % for radical terraced land; 11.81 – 48.27 % for trenches; 10.48 – 29.99 % for agroforestry; 5.46 – 20.96 % for forest, 25.98 – 79.82 % for amended soil and 7.06-18.70 % for control (ILACO, 1993; Landon, 1991; Baize, 1993; Msanyaet $al.,$ 2001). Referring to topo sequence there are no trends of Base Saturation amended, terraced and agro forested land but in trenches, forest and control land management practices, base saturation is increasing downward (Table 2).

Table 3: The results of exchangeable acidity Aluminium (Al^{3+}), hydrogen (H^+), Aluminum Saturation and Total Exchangeable Acidity

Land Management Practice	Sample Stream	TEA (meq/100g)	Al^{3+} (meq/100g)	H^+ (me/100)	Al. Saturation
Radical Terrace	Upper	1.28	1.12	2.4	0.887
	Middle	2.08	0.96	1.12	0.907
	Bottom	1.12	1.36	2.48	0.859
Trenches	Upper	1.6	0.88	0.72	0.882

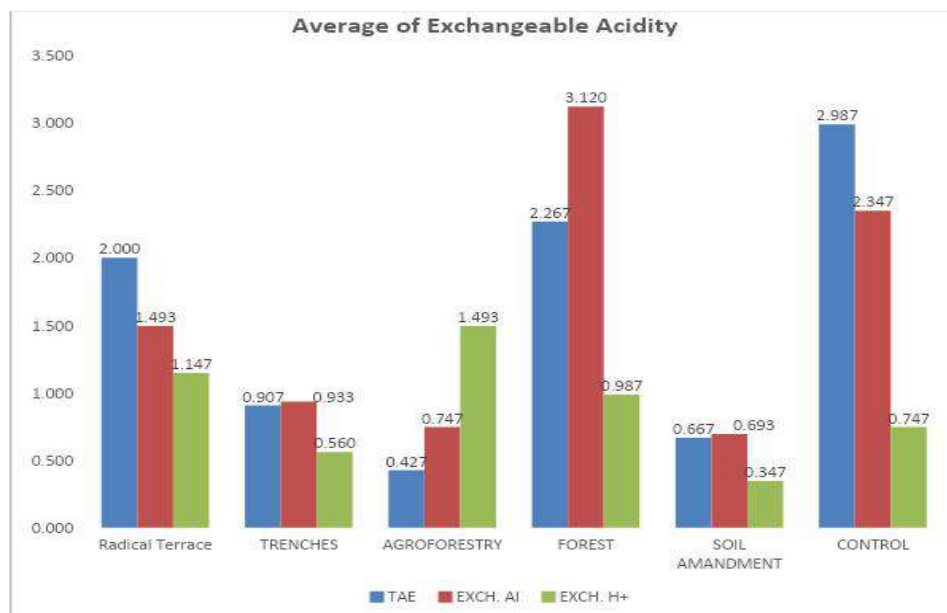|					
	Middle	0.72	0.8	1.52	0.712
	Bottom	0.48	0	0.48	0.517
Agroforestry	Upper	1.12	1.12	0	0.7
	Middle	0.64	0.16	0.8	0.673
	Bottom	3.6	3.2	0.4	0.895
Forest	Upper	3.04	0.16	3.2	0.945
	Middle	2.72	0.48	3.2	0.936
	Bottom	0.8	2.32	3.12	0.79
Soil Amendment	Upper	0.08	0.08	0	0.202
	Middle	1.36	0.48	1.84	0.74
	Bottom	0.64	0.48	0.16	0.675
Control	Upper	2.88	0.64	3.52	0.929
	Middle	3.04	0.16	2.88	0.92
	Bottom	1.12	1.44	2.56	0.813

Exchangeable Acidity

Fig.16: Exchangeable acidity of different land management practices in Migina Catchment

The ANOVA table shows that there is a significant difference in land management (P < 0.006), (P < 0.04) for Aluminum and Hydrogen respectively. The value presented in the figure above is Total Exchangeable Acidity, exchangeable aluminum and   of different land management in Migina Catchment, the exchangeable acidity of the studied area varies slightly along a topo sequence in general, (Table 2). The figure shows that the acidity is very dominant in control, forest and radical terrace compared to the other land management in Migina catchment, the exchangeable Aluminum is more than exchangeable hydrogen in the study area (Figure13). Generally, the Total Exchangeable Acidity of the study area is high (Table 3.). The soils irrespective of the physiographic position along the topo sequence were varying as this follow respectively exchangeable aluminum and hydrogen values are 1.12 – 2.08,0.96-1.36 meq /100g for radical terraced land; 0.48 – 1.16, 0-1.12meq /100g for trenches; 0.48 – 1.12 ,0.16-3.2meq /100g for agroforestry; 0.8 – 3.04, 0.16 – 2. 32 meq /100g

for forest, 0.008 – 1.36, 0.8-0.48 meq /100g for amended soil and 1.12-2.88, 0.16-1.44 meq /100g for control (ILACO, 1993; Landon, 1991; Baize, 1993; Msanya*et al.,* 2001). Referring to topo sequence there are no trends of

Exchangeable acidity except in control or undisturbed land where Exchangeable acidity is decreasing downward (Table1).

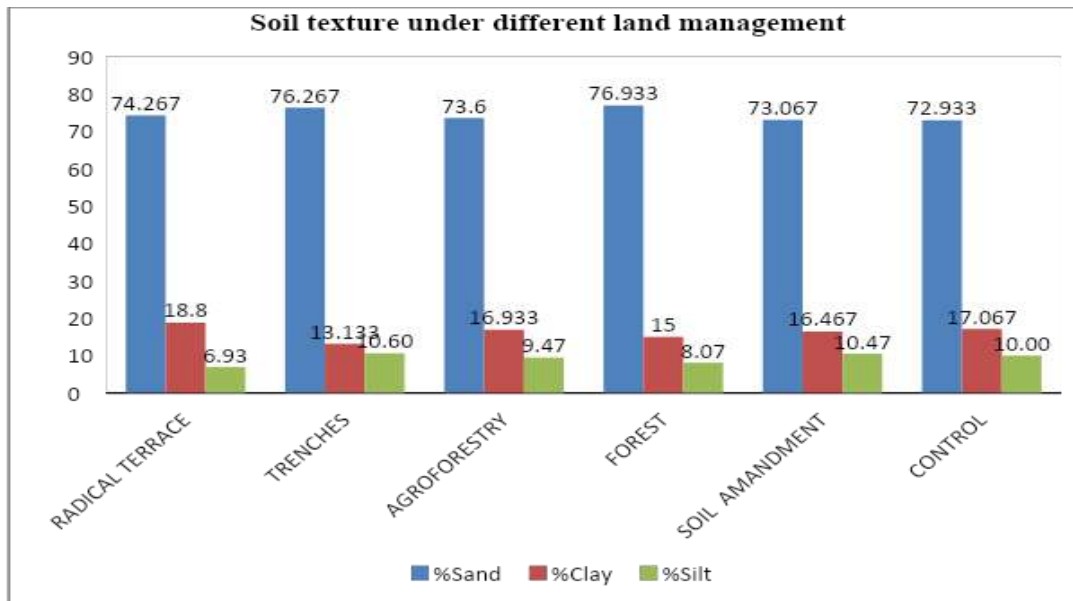**Soil texture under different land management**

Fig.17: Soil Texture under different land management in Migina catchment

The study site is dominated by sandy loam (Table 4) except bottom of terraced land and upper land of agroforestry land use. All samples had a higher content of sand (79.6-69.6 %) than that of silt and clay content. The result (Table 3) of the soil texture showed that percent

sand decreased down the slope in terraced and controlled lands ranging from 75.6 and 73.6 % and 75.6 and 71.6 % respectively (Figure 14).

Percentage of porosity and moisture content under different land management
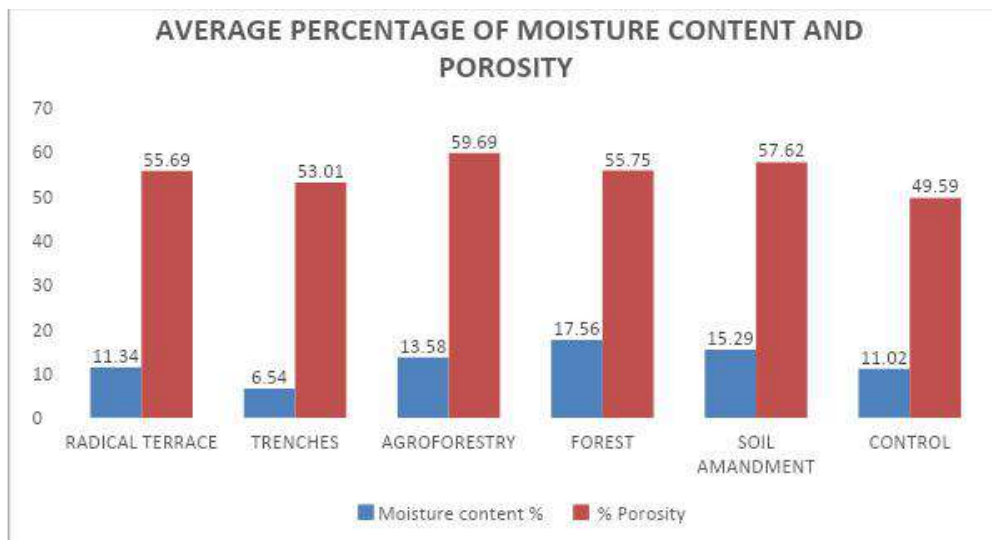
Fig.18: Percentage of porosity and moisture content under different land management

The ANOVA table shows a highly significant difference management on moisture content ($P < 0.001$). The values of total porosity ranged between 50.2 and 52.08 in terraced land; 47.2 and 58.16 in trenches; 53.8 and 63.87 % in

agroforestry; 53.08 and 53.8 % in forestry; 56.39 and 58.9 % in amended soil and 45.9 to 51.84 in the control 0r undisturbed land (Table 4). There is no trend of increasing or decreasing slope position in terraced, agroforestry,

amended and controlled land uses. The increases with slope have been found in forest land whereas the increase with slope was in the trenched farm. High porosity was found in agroforestry land use (Figure 15), since agroforestry systems increase soil organic matter hence high porosity.

Percentage of moisture content

The values presented in the previous figure shows that in general moisture content varies in range of 6.54 % to 17.56 % in our study area where forest land has the highest moisture content which is (17.56 %) and the smallest one is trenched land with 6.54% (Figure 15). The values of moisture content ranged between 9.93 and 16.18 % in terraced land; 3.09 and 10.09 % in trenches; 10.23 and 17.276 % in agroforestry; 15.27 and 19.37 % in forestry; 13.237 and 17.032 % in amended soil and 7.23 to 13.077 % in the control (undisturbed land) (Table 3). There are no

specific trends of moisture content values variation in our study area except in agroforestry and forestry where they increase down ward. The ANOVA Table shows a significant difference between land management (P< 0.01) on bulk density. The values presented on figures are Bulk density and particle size distribution and the figure shows that the bulk density is low in terraced, trenches, agroforestry, forestry, amended soil and in control ranging from 0.96 to 1.44 g/cm^3 this is a good range for plant growth. The values presented on the previous figure shows that agro forestry has small value of bulk density (1.07 g/cm^3) compared to the other land management practices while control (undisturbed soil) has a highest value of bulk density (1.34 g/cm^3) but all of them has favorable bulk density for root growth penetration and crop growth in general (Figure 16).
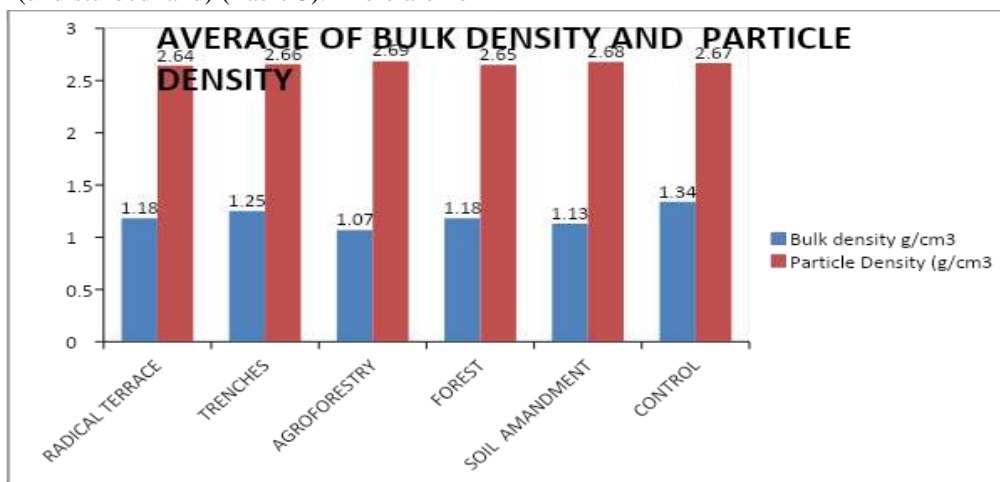
Fig.19: *Bulk Density and particle density under different land management in Migina Catchment.*

Bulk density and particle density under different land management practices in Migina Catchment

Table 4: The results on Soil texture, Moisture content, Bulk Density, Particle Density and Porosity

| Land management practices | Sample Stream | Particle size distribution | | | Textural classes | Moisture content % | Bulk Density g/cm³ | Particle Density g/cm³ | % Porosity |
		% Sand	% Clay	% Silt					
Radical Terrace	Upper	75.6	16.2	8.2	Sandy Loam	9.936	1.276	2.61	52.03
	Middle	73.6	17.8	8.6	Sandy Loam	7.917	1.11	2.65	58.27
	Bottom	73.6	22.4	4	Sandy Clay Loam	16.18	1.15	2.68	56.77
Trenches	Upper	75.6	13.8	10.6	Sandy Loam	6.434	1.404	2.52	47.22
	Middle	77.6	14.6	7.8	Sandy Loam	3.09	1.233	2.7	53.65
	Bottom	75.6	11	13.4	Sandy Loam	10.09	1.113	2.62	58.16

Agroforestry	Upper	69.6	20.8	9.6	Sand Clay Loam	10.213	1.027	2.71	61.39
	Middle	73.6	15.2	11.2	Sandy Loam	13.241	1.229	2.68	53.8
	Bottom	77.6	14.8	7.6	Sandy Loam	17.276	0.961	2.67	63.87
Forest	Upper	75.6	17.4	7	Sandy Loam	19.3713	1.04	2.72	60.9
	Middle	79.6	10.8	9.6	Sandy Loam	18.031	1.243	2.62	53.27
	Bottom	75.6	16.8	7.6	Sandy Loam	15.2737	1.248	2.61	53.08
Soil Amendment	Upper	75.6	17.4	7	Sandy Loam	19.3713	1.04	2.72	60.9
	Middle	79.6	10.8	9.6	Sandy Loam	18.031	1.243	2.62	53.27
	Bottom	75.6	16.8	7.6	Sandy Loam	15.2737	1.248	2.61	53.08
Control	Upper	75.6	14.4	10	Sandy Loam	7.2309	1.439	2.69	45.9
	Middle	71.6	18.4	10	Sandy Loam	13.077	1.281	2.64	51.84
	Bottom	71.6	18.4	10	Sandy Loam	12.743	1.303	2.67	51.02

V.   DISCUSSION

5.1 pH Water, pH KCl of different land management practices

The soil reaction level is an indicator of soil acidity. In our study area, especially in agroforestry and amended soil practices, there is high concentration of population settlement on the top and middle who used to amend their land with organic and household waste fertilizers, the heavy rainfall (1200mm/year) could be to reason for the high pH rate because of the gritty hardness of the soil caused by high weathering, the research region suffers extensive leaching of basic cations. Low pH in the study area is probably induced by acidifying nitrogen fertilizer, nitrate leaching, exclusion of the bases through crop harvests and the farming practices especially in amended soil, trenches, agroforestry and radical terrace (Mackenzie, B., & Erickson, J. D, 2004). Agroforestry systems, the bases deposition above numerous years by growing can influence the arising of pH of soil, the buffering effects of nutrients leaching (Sharma, R., Chauhan, S. K., &Tripathi, A. M, 2016). Particularly in radical terraces the source of their acidity becomes from their poor establishment and poor management of those terraces where in their establishment used to remove top soil which contain all nutrient elements and put them away, the farmers who used to cultivate this radical terrace, they do not use mulching, liming or other pH restoring strategies for increasing soil pH in soil except in middle area where the farmers are tried to use cattle fertilizers. There is low pH also in forest, the leaves and litters increase soil organic carbon in soil after their decomposition but in this process of decomposition release organic acid in soil which decreases soil pH.

The strongly acid reaction amounts propose potential low accessibility of both the macro and micro plant nutrients for being taken by plants. pH values in the soil below 5.5 have the ability to cause toxicity issues. It can also result in the breakdown of aluminum and iron minerals, which precipitate with phosphorus, effectively fixing it and lowering the soil pH (Brady and Weil, 2008). The majority of plants thrive in soils with a pH of 6.5 to 7.5. (Baize, 1993). Thus, soils studied may present limitations to crop growth because of the low P^H values of less than 5.5 which may limit availability of various plant nutrients such as phosphorus and bases (Marschnr, 1995). Application of liming materials may be considered necessary to raise the pH to favorable levels of around pH 6.5 and 7.5. Alternatively, crops that tolerate acidity are recommended for, because plant species and varieties differ in the degree to which they tolerate pH values outside the range, application of more organic fertilizers and application of agroforestry systems are recommended here.

5.2 Electrical Conductivity of different land management practice

Electrical conductivity testing is a consistent way to evaluate how salts are impacting the growth of plants. The EC of soil and water is improved by decomposition and the concentration of salt which is dissolved. Salts arise from the ability of solution to conduct an electrical current, High salinity level is indicated by higher values of EC (Apal, 2014). Therefore, our study area does not face the problem of salinity. Excessive salt in soil may hinder germination of seed. Excessive fertilization and poor water quality used in irrigation are source of salt (Marx, E. S., Hart, J. M., & Stevens, R. G, 1996)

5.3 Percentage of organic carbon and organic matter of different land management practice

The high amount of OC observed may be due to the fallow period. The area under study has been kept as fallow like our control which is not used for tillage activities Johnsons (2002) observed the higher concentration of OM in the surface soil under no-tillage systems this important reason of %OC sufficient in our control. Greater accumulation of surface carbon is resulted by less disruption because the fodder crops roots and the slower decomposition rate of OM might have contributed to the increase of OM in soil with no tillage. It also has a high water holding capacity and penetration rate, which can help to mitigate soil erosion caused by runoff surface water during rainstorms (Njuguna, J.W, 2019).

In agro forestry the high values of OM in topsoil than subsoil may be attributed by decomposition of large quantities of plant residues into the soil every season and high population density in top and middle land. Such good climatic conditions are favorable for vegetation. Low cropping activity and harvesting lead to the losses of nitrogen and carbon (Dowuona*et al*., 2012 and Kebeney*et al*.,2015). And also while soil has low soil P^H lead to the low organic carbon in soil as that low P^H inhibits the microbial activity for decomposing organic matter (Sultana, 2011). While the ground is covered really generate a great potential and generate soil organic carbon and influencing biological activity and favors soil protection, the high concentration of population settlement in agroforestry and soil amendment is one of proposed reason of OC % increasing in this areas where they used to apply more organic fertilizers, agroforestry species and mulching (Sharma et al., 2009).

5.4 General discussion of Nitrate and Ammonium in study area

Nitrate and Ammonium are chemical parameters which show the nitrogen form that are taken up by plan, the level of nitrate and ammonium is affected by rainfall, level of stored water at sowing, time of sampling, depth of taken sample. The high values of nitrate in the study area may be due to fertilizer application containing nitrogen. Ammonium nitrogen concentration values are within the range (2-10 ppm) of agricultural soil (Marx E.S., Hart J., 1999). Ammonium-nitrogen generally does not accumulate in the soil, as soil temperature and moisture condition which is appropriate for the growing of the plant and are ideal for conversation of NH_4-N to NO_3-N. Soil NH4-N level above 10 ppm may take place in cold or extreme soil which is wet, while soil hold fertilizers from a later application, while there is high or low soil pH, and while there is a high soluble salts (measured by electrical conductivity). (D.A. Horneck, D.M. Sullivan, J.S. Owen, and J.M. Hart, 2011). The nitrate level in soil changes widely, basing on the types of soil, rainfall and condition of climate and practices of fertilizing. Any level of nitrate ranging from or more than 25 to 30 ppm is enough to the growth of plants in a vegetable garden (Camberato and Nielsen, 2017).

Due to the leaching and heavy rain the sand soils loses its nitrate, then by denitrification also the coarsely textured soil loses nitrates and this might be the cause of low nitrate in the studied area because the soils have sand presence in its soil texture (Brouder*et al*., 2003). Another reason for high amount is that nitrate accumulates more in the soil because in the period of rain season, the temperature of soil is low and the tension of oxygen is also low referring to the saturation with water and this situation decelerates denitrification (Nielsen, 2017). Agro forestry trees, especially leguminous trees, enrich soil by biological nitrogen fixation, organic matter addition, and nutrient recycling. The fixed nitrogen can provide a symbiotic advantage to the crops that grow alongside it, as well as aid in soil fertility improvement. The amount of nitrogen taken up by the first harvest from legumes or forest trees replanting is stated to be very limited, with a significant portion remaining in the soil organic matter, suggesting a long-term nitrogen advantage rather than an immediate gain. Different tree elements, such as leaves, twigs, berries, and wood, decompose at different speeds, which helps to spread nutrient release over time.

5.5 Available phosphorus of different land management practices

The medium values of P may probably be due to continuous cultivation without replenishment of P from different P fertilizers, for this case the application of chemical fertilizer increase availability of phosphorus probably can be the reason for having high values of available phosphorus in some amended soil. The relatively high values of P found in some topo sequences may be caused by anthropogenic effects including addition of manure, crop residue and inorganic P fertilizers and low potential for phosphorus fixation. Low available phosphorus may be attributed to low soil P^H (<5.8) that could react with iron (Fe) and Aluminum (A_l) to make insoluble Fe and A_l phosphates that are not readily available to be taken by plant (Hodges, 2007, Raymond and Roy, 1990). By binding Al, Fe, and Ca and creating soluble P that may be accessible to plants, raising the amount of organic matter in the soil may help to reduce any P 'fixation' reactions that may be present. (Hodges, 2007). Then the insufficient soil organic matter in some treatment is another issue of decreasing availability of

phosphorus in some treatment of our study area. The mulching is also in order for reasons which hinder the leaching of nutrient elements and keeping good physical characteristics of soil, this also other issues of great value of AV. P in trenches, amended soil and agroforestry because in the same areas of these practice areas, farmers used to apply mulching. Therefore, by Defoer et al. (2000) this means that very limited amount of phosphorus is available as pH decreases, and unavailability of phosphorus leads to the low productivity of crops and trees as an essential element for crop growth.

5.6 General discussion of exchangeable basis

The values of Exchangeable bases show that it is on Low level, this low level of exchangeable bases is probably due to poor practice of cultivation, soil which is poor and conservation of water and insufficient supply of fertilizer to refill nutrients removed with crops. Another reason could be that the parent material on which soil has developed is poor in bases.

Msanya*et al*. (2001) reported that the desired saturation level of exchangeable Mg is between 10 to 15%. Consequently, for crop processing, the soils of the study areas lack sufficient amounts of exchangeable magnesium, calcium, and potassium. This low exchangeable caution value has direct consequences for cation exchange potential (CEC), soil P^H, and, consequently, plant nutrient imbalances, unavailability, and nutrient induced deficiency.

5.7 Effective cation exchange capacity of different land management practices

The values of ECEC of the soils studied show that it is low; these values are contributed by the kaolinite and sesquioxide or oxidic clays which are dominant clay minerals in highly weathered soils, lacking negative charges. Consequently, they don't retain adsorbed cations and end up with insufficiency CEC due to the nutrient retention capacity which is low (Landon, 1991).

The land management which has more ECEC values in our study area can be credited to higher content soil organic matter (Tomašić*et al*., 2013). Due to the observation in the field, the low ECEC levels in control or undisturbed land observed could also be attributed to low leaching instead of strong runoff due to high erosion rate as these sites are steeply sloping areas. Erosion causes sediment loss from the upper part (soil truncation) and deposition of new material in the lower part (soil aggradation), this resulted in loss of nutrients in the upper part of the mountains.

The ECEC values indicate possible negative influence on the soil buffering capacity and reduced retention of base cations by the soils studied. Consequently, it doesn't have

the ability to protect soluble cations from leaching out the root zone of plants and helps soils to resist variation in change P^H (Barker *et al*., 2007; Brady and Weil, 2008). The rainfall of our studied land is high; this is another reason for ECEC decreasing because many cations are leaching into the soil (Paul & Rattan, 2014).

5.8 Base Saturation of different land management practices

The values of base saturation of the soils studied are presented in table 3. The findings suggest that there are low base saturation levels in this sample region, which may be due to the pH being very acidic and potentially toxic cations such as aluminum and manganese in the soil (Hodges, 2007). Low levels of bases in most soils are due to improper agricultural methods, poor soil and water management, and an insufficient availability of fertilizer to replenish nutrients removed by crops, among other factors (Ellenkamp, 2004; Jones et al., 2013)., suggesting strong soil productivity for crop development. It also means that there is little to no extensive leaching of bases from the topsoil to the subsoil (Msanya*et al*., 2016).

5.9 Exchangeable Acidity of different land management practices

These results indicate that the acidity of these soils is mostly contributed by exchangeable Al to a large extent and by hydrogen to a moderate extent. Aluminum ions are released from clay lattices at P^H values of about below 5.5 and become exchangeable in the clay complex (Landon, 1991).

5.10 Soil texture under different land management

According to Mc Donald *et al*. (1994), the sand content is very high compared to clay and silt in the study area. The sand nature may probably be attributed by parent material and climate as earlier reported by (Onweremadu et al., 2011; Osujieke et al., 2016).

Soils of high altitude cold desert which have come from rock weathered; they are not mature and have higher amounts of sand gravels and stones in them showing the presence of sand forming minerals in parent material. Sand is a present particle in the hilly soil and they are coming from parent material of sandstones. Clay and silt content have been rated as low. This low value indicates that a soil doesn't have enough ability for retaining available water for growth of plants due to the unique combination of surface area and sizes of pores (McDonald *et al*.,1994).

5.11 Percentage of porosity and moisture content of different land management practices

The moisture content referring to the water content in brief is an indicator of water present in the soil (ASTM, 2014). Our study is moisture content varies from 6.54 to 17.56%

then according to Bandyopadhyay& Reza (2014), The studied soil result show that there is moderate water retention and referring to Mbaga, Msanya, &Mrema (2017), Tennga et al. (2018) and Uwingabire et al. (2016), the moisture content depends on the soil organic matter, particle size distribution, bulk density and structure of the soil influence the variation of available moisture content in the soil. In fact, the result of soil particle size distribution shows that it has a good bulk density which implies the high holding capacity of water. And we have an organic matter in general which is medium to high (table1) even particle size distribution and bulk density determine the distribution of macro pores and micro pores density are good in our study area (Table 3), all those factors influence our study are influencing our soil study area to retain water.No-tillage systems seemed to be more suitable for sustaining favorable soil porosity by preserving the elongated transmission pores that aid root growth. Since the porosity in both treatments was greater than 40%, they are unlikely to limit crop growth since they show no soil compaction, easy root penetration, proper aeration, and water preservation within the soil (Gachene*et al*., 2003).

5.12 Bulk density and particle density of different land management

The values of particle density of the soils studied are presented in table 3. The results show that the texture class of the studied soils was dominated by sandy loam and a little sandy clay loam. According to Hazelton & Murphy, (2007), the sand content is very high in the studied soil and there are no trends changes along topo sequence of studied area and the figure below shows that in general the value of particle density varies between 2.58 and 2.72 g/cm^3 while normal particle density for plant growth is 2.66 g/cm^3 (E.S. Marx, J. Hart, 1999). The possible causes of decrease of BD in the study area are organic matter addition in the field, if soils are wetter than field capacity, bulk density may increase. Growth of root, in general, starts to be restricted when the bulk density reaches 1.55 to 1.6 g/cm^3 and is forbidden at about 1.8 g/cm^3. Bulk density had a specific trend by increasing soil depth in the crest and mid-slope but recorded no specific trend in foot-slope. However, bulk density decreased from the crest to the foot-slope and had no significance among the physiographic positions. This is in concurrence with the works of (Aweto and Iyamah, 1993) in the soils of southern Nigeria. Also, some researchers (Gafar et al., 2004; Abrams et al., 1997) have reported similar findings on soil along topo sequence. The bulk density was found to be below the critical limit (1.75 – 1.85 g kg^{-1}) as recommended by SSS, (2006) for root penetration.

VI.   CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The assessment on impact of different land management on soil quality in Migina catchment using soil laboratory analytical methods was one direction of achieving this research.   The physicochemical parameters already analyzed have shown that the land management practices have a positive impact on soil nutrient availability and also have positive effects in social-economic development of population and agricultural sustainability. Good establishment, well monitoring and evaluation of different land management practices are essential factors for land management sustainability and crop productivity.

The main objective of this research work was to analyze the impact of land management practices on soil quality in Migina Catchment at Akaboti Cell, Kansi Sector, Gisagara district, in southern province of Rwanda, with focus on physicochemical properties of soil, this work was done under different land management practices that are radical terrace, trenches, agroforestry, forestry, soil amendment and control or undisturbed land in order to know the best to be used for conserving our soil fertility status.

 The laboratory results show that the soil texture was dominated by a sandy loam class, the soil bulk density varies from 0.96 to 1.44 g/cm^3 in general, the soil porosity ranges from 50.2 to 52.08 % in terraced land; 47.2 to 58.16 % in trenches; 53.8 to 63.87 % in agroforestry; 53.08 to 53.8 % in forestry; 56.39 to 58.9 % in amended soil and 45.9 to 51.84 % in the control (undisturbed land). The soil pH measured in water ranges from 4.73 to 5.14 for radical terraces land, from 5.79 to 5.29 for trenches; 6.29 to 5.79 for agroforestry land, 4.93 to 4.49 for forested land, 5.11to 6.34 for amended soil and 4.69 to 527 for control. The electrical conductivity is low in treatments ranging from 0.076 to 0.390%, The values of mineral Nitrogen measured in treatments were higher compared to control. Ammonium values range from2.55 to 7.24 mg/Kg while Nitrate values range from 4.36 to 28 mg/kg. Available P values were high in treatments compared to control.

The values of available P range from 8.55 to 17.10 ppm. The values of exchangeable bases were slightly high in treatments compared to control. Those values were generally low. Generally, the agro forestry land showed high nutrients values compared to the control and other treatments. From the results of this study, it is clear that the land management practices have generally a positive impact on soil properties.

6.2 Recommendations

- Considering the output of our research project we put forward the following

recommendations to be used for reinforcing land management practice and to mitigate the negative impact on the land degradation and soil fertility deterioration, so as to guarantee sustainability of land management in this study area.

● The government, agricultural institutions and other institutions involved in land management activities should invest more in research across the whole country in order to enable farmers to adopt appropriate soil fertility management practices in relation to both soil and water quality improvement.

● The government should provide qualified technicians who are able to establish sustainable land management practices especially terraces and trenches, to select improved seeds of trees species for agroforestry and forestry which is generating not only more organic material for producing sufficient organic matter but also retain material that may compromise downhill surface water.

● Farmers should be more sensitized by extension workers about new agricultural technologies such as agroforestry system which increase in soil organic matter in order to replenish the lost plant nutrient and to manage their land fertility sustainably as well as environment and also as source of multiple benefits,

● Farmers also must apply lime in order to reduce soil reaction acidity of this area, apply sufficient organic manure for improving soil nutrients needed by plant, microbial activity reinforcement and soil physicochemical parameters availability,

● Farmers should learn how to use rationally and efficiently both organic and chemical fertilizers and so that they increase soil productivity potential of their land.

● For the soil quality; waste management must be carefully considered because they are considered as a source of more toxic elements which may be harmful and so act for decreasing the soil fertility status. The acidic tolerant plants should be adopted to be grown in the studied area, if there is no other possibility to improve soil trend to be acidic such as lime application as usually done.

● It would be better if we could cover the whole country, but we didn't get enough means and time for that purpose, so our study has been small scale oriented and just limited to Migina catchment. For that we invite other researchers to conduct deep research on the impact of land management practice by extending their research to all aspects of the complex issues on soil and Environments.

ACKNOWLEDGEMENTS

REFERENCES

[1] Aburas, M. M. (2009). Assessment of Soil Erodibility in Relation to Soil Degradation and Land Use in Mediterranean Libya (Doctoral dissertation, University of Newcastle upon Tyne).

[2] Hazelton, P., & Murphy, B. (2007). Interpreting Soil Test Results: What do all the numbers Mean 2nd. Australia: Csiro Publishing.

[3] Kayiranga, A., Ndayisaba, F., Nahayo, L., Karamage, F., Nsengiyumva, J. B., Mupenzi, C., &Nyesheja, E. M. (2017). Analysis of climate and topography impacts on the spatial distribution of vegetation in the Virunga Volcanoes massif of east-central Africa. Geosciences, 7 (1), 17.

[4] Mackenzie, B., & Erickson, J. D. (2004). Sodium-coupled neutral amino acid (System N/A) transporters of the SLC 38 gene family. P Archive, 447(5), 784-795.

[5] Manzi, A., Munyaneza, F., Mujawase, F., Banamwana, L., Sayinzoga, F., Thomson, D. R., ... &Hedt-Gauthier, B. L. (2014). Assessing predictors of delayed antenatal care visits in Rwanda: a secondary analysis of Rwanda demographic and health survey 2010. BMC pregnancy and childbirth, 14(1), 1-8.

[6] Mbarushimana, J. D. D. (2019). Effect of Inorganic Fertilizers and Planting Density on Growth and Yield of Selected Maize Varieties in Eastern Rwanda (Doctoral dissertation, University of Nairobi).

[7] Mmbaga, H., &Makoi, J. H. (2018). Soil Chemical Characterization and Fertility Status in Selected Irrigation Schemes in Unguja and Pemba Isles, Zanzibar. International Journal of Applied Agricultural Sciences, 4(3), 60.

[8] Mongi, H., Majule, A. E., &Lyimo, J. G. (2010). Vulnerability and adaptation of rain fed agriculture to climate change and variability in semi-arid Tanzania 4(6). African Journal of Environmental Science and Technology.

[9] Mongi, H., Majule, A. E., &Lyimo, J. G. (2010). Vulnerability and adaptation of rain fed agriculture to climate change and variability in semi-arid Tanzania 4(6). African Journal of Environmental Science and Technology.

[10] Munyaneza, O., Mukubwa, A., Maskey, S., Uhlenbrook, S., &Wenninger, J. (2014). Assessment of surface water resources availability using catchment modelling and the results of tracer studies in the mesoscale Migina Catchment, Rwanda. Hydrology and Earth System Sciences, 18(12), 5289-5301.

[11] Murphy, B. (2014). Soil Organic Matter and Soil Function– Review of the Literature and Underlying Data. Department of the Environment, Canberra, Australia.

[12] Ndah, H. T. (2020). Improving farmers' livelihoods through conservation agriculture: options for change promotion in Laikipia, Kenya. International Journal of Agriculture.

[13] Nelson, D. W., & Sommers, L. E. (1996). Total carbon, organic carbon, and organic matter. Methods of soil analysis: Part 3 Chemical methods, 5, 961-1010.

[14] Paul Obade, V., &Lal, R. (2014). Soil quality evaluation under different land management practices. Environmental earth sciences, 72(11), 4531-4549.

[15] Ruslanjari, D., &Alam, T. (2017). Determination of soil quality as a foundation of sustainable land management for chilli in the agroforestry system based on coconuts in sandy soil of bugel beach 7(2), 115-120.JurnalKawistara.

[16] Shah, F., & Wu, W. (2019). Soil and crop management strategies to ensure higher crop productivity within sustainable environments. Sustainability, 11(5), 1485.

[17] Sharma, R., Chauhan, S. K., &Tripathi, A. M. (2016). Carbon sequestration potential in agroforestry system in India: an analysis for carbon projects. Agroforestry systems, 90(4), 631-644.

[18] Tellen, V. A. (2018). Effects of land use change on soil physicochemical properties in selected areas in the North West region of Cameroon P 7(1), 1-29. P 7(1). Environmental Systems Research.

[19] Tesfahunegn, G. B. (2019). Farmers' perception on land degradation in northern Ethiopia: Implication for developing sustainable land management 56 (2), 268-287. The Social Science Journal.

[20] Tesfahunegn, G. B. (2019). Farmers' perception on land degradation in northern Ethiopia: Implication for developing sustainable land management. The Social Science Journal, 56 (2), 268-287.

[21] Thomas, G. W., & Hargrove, W. L. (1984). The chemistry of soil acidity. Soil acidity and liming, 12, 3-56.

[22] Uwingabire, S. (2016). Pomological characterization of soils developed on gneissic-granites in Congo Nile watershed divide and central Plateau zones of Rwanda Doctoral dissertation, Sokoine University of Agriculture).

Article

Detection of mycotoxins from some micro and macro endophytic ascomycetes fungi

Aditi Saini[1], Vipin Parkash[*1], Vineet Kumar[2], Akshita Gaur[1], Harsh Chirag[1]

[1]Forest Pathology Discipline, Forest Protection Division, Forest Research Institute (Indian Council Forestry Research & Education, Autonomous Council under Ministry of Environment & Forests, Government of India), Dehradun-248006, Uttarakhand, India.
[2]Chemistry and Bio-prospecting Division, Forest Research Institute, Dehradun, India
*Corresponding author: bhardwajvpnpark@rediffmail.com

Abstract— The immense importance of endophytic fungi in industries has attracted the attention of researchers for their chemical and biochemical properties. Some of ascomycetes fungal species like Penicillium, Aspergillus, Neurospora are the novel source of secondary metabolites such as mycotoxins, tannins, alkaloids, terpenoids, etc. Some of the fungal secondary metabolites are useful in pharmaceutical industries for drugs production whereas some mycotoxins are screened to be harmful in most of the cases; hence, it becomes imperative to know the biochemical aspects of wild fungi regarding their usage. This study involves screening of mycotoxins from some endophytic micro ascomycetes fungi e.g. Fusarium solani, Penicillium chrysogenum, Geotrichum candidum (micro fungi) and macro ascomycetes fungi e.g. Xylaria hypoxylon and Daldinia concentrica (macro fungi) through Thin Layer Chromatography (TLC) technique which is a simple physicochemical laboratory procedure and conventional analytical method for quantifying the actual amounts of toxins. In TLC, different solvent systems were used for extrication of compounds/mycotoxins based on their colours. The identification of mycotoxins was done by toning the colours on the TLC plates as per available standard methods and protocols in laboratory. The probable detected and tentatively identified mycotoxins on Thin Layer Chromatographic plates that appeared in different colours were Zearlenone (faint blue), Citrinin (yellow or bright yellow), Aspertoxin (pink), Nivalenon acetate (orange) and Diacetoxiscipenol & Beauvericin (light brown to brown). This kind of studies can be helpful in categorizing any wild fungal species as toxigenic or non-toxigenic based on their natural occurrence and either edible or inedible in nature. There are also some mycotoxins that possess medicinal properties and some are harmful, thus, this detection can open new outlooks and prospects of new wild fungal source/s of medicinally important compounds/mycotoxins. The future proposal of this study is to further identify the particular mycotoxin/s available in target fungal species by standardizing the isolation and identification through some advance techniques.

Keywords— Daldinia concentrica, Fusarium solani, Geotrichum candidum, Penicillium chrysogenum, Thin Layer Chromatography, Xylaria hypoxylon.

I. INTRODUCTION

Some ascomycetes fungi are unicellular whilst some are organized to multi-cellular ascocarps that produce spores. Ascomycetes are cosmopolitan in nature generally terrestrial, often found in fresh and tidal zones of marine waters and some are endophytic in nature while some are parasitic on insects and animals, saprophytic and rests are responsible for causing destructive plant diseases (wilts and powdery mildews) [1-2]. Organized fungal hyphae of some ascomycetes fungi produce secondary metabolites such as mycotoxins, tannins, terpenoids, alkaloids, etc. Some of these secondary metabolites are commercially

utilized in pharmaceutical industry for drugs production and in bio-refineries. For instance, *Fusarium*, *Penicillium* and *Neurospora* are being using as a source of natural pigments with the functionality for food feed and cosmetic application [3]. Goswami *et al.* [4] reported 'Echinocandins' produced by *Aspergillus* species which is a new novel class of drugs for fungal infections. Sharmila *et al.* [5] investigated that *Fusarium oxysporum* produces Cyclosporin-A which is an immunosuppressant and currently used in the treatment of cancer, organ transplant patients and in the treatment of auto-immune diseases including Acquired Immunodeficiency Syndrome (AIDS). *Penicillum* and *Aspergillus* both fungi produce mycotoxins which are used as the wonder drug [6]. However, mycotoxins are screened to be harmful in most cases. Several hundred different mycotoxins have been identified but most commonly observed mycotoxins that present a concern to human health and livestock include aflatoxins, ochratoxin A, patulin, fumonisins, zearalenone and nivalenol/deoxynivalenol [7-9]. Many species of *Penicillium* can also produce a wide range of toxic compounds such as citrin and citreoviridin [9-11]. Awuchi *et al.* [12] reported Aflatoxin B1 and B2 (AFB) and Aflatoxin G1 and G2 (AFG); their metabolites include Aflatoxin M1 (AFM1), Aflatoxin M2 (AFM2), Aflatoxicol (AFL) and Aflatoxin Q1 (AFQ1). Aflatoxin B1 is regarded as the most toxic and detected in scantily stored agricultural produce including maize, rice, cassava, cottonseed, millet, sorghum, sesame seeds, sweet corn, chilli peppers, tree nuts, sunflower seeds, spices and wheat. Nielsen *et al.* [13] found that the mycotoxins ochratoxin A (*Aspergillus carbonarius* and less frequently *A. niger*) and fumonisin B2 (*A. niger*) are the most problematic compounds for human and animal safety point of view. Therefore, it is important to identify the mycotoxins that may help us to categories these fungal species as toxigenic or non-toxigenic and/or either edible or non-edible because some mycotoxins have medicinal uses and some are harmful in nature. There are some following reports in the literature on extraction methods of mycotoxins. Zheng *et al.* [14] gave an overview for analysis of mycotoxins by rapid methods and conventional analytical methods while Turner *et al.* [15] also described the analytical methods for determination of mycotoxins. Betina [16] discussed on general technical aspects of thin-layer chromatography of mycotoxins including extraction and clean-up procedures, adsorbents and solvent systems, detection methods, two-dimensional TLC, high-performance TLC (HPTLC), quantization and preparative TLC (PLC). Scott *et al.* [17] detected mycotoxins by using thin-layer chromatography technique with suitable general solvent systems and only with one initial spraying reagent.

They detected the toxins namely aflatoxins B1, B2, G1, and G2, ochratoxin A, aspertoxin, luteoskyrin, zearalenone, 4-acetamido-4- hydroxy-2-butenoic acid y-lactone, diacetoxyscirpenol, and its 8-(3-methylbutyryloxy) derivative [T-2 toxin (5)] and nivalenol and its acetate, in addition to several antibiotics now regarded as mycotoxins, namely gliotoxin, citrinin, patulin, penicillic acid and sterigmatocystin. Visible or ultraviolet light was used to visualize the toxins, before and after spraying reagent. These mycotoxins are produced mainly by species of *Aspergillus*, *Penicillium* and *Fusarium*. Hassan *et al.* [18] worked on *Aspergillus* species to extract mycotoxins. Similarly, Azliza *et al.* [19] examined 30 fungal strains which represent 12 *Fusarium* species *i.e. F. oxysporum, F. solani, F. semitectum, F. nelsonii, F. compactum, F. equiseti, F. chlamydosporum, F. proliferatum, F. subglutinans, F. sacchari, F. lateritium* and *F. incarnatum-equiseti* species. These complex isolated from wild grasses in Peninsular Malaysia identified four major mycotoxins *i.e.* moniliformin (MON), fumonisin B1 (FB1), zearalenone (ZEN) and beauvericin (BEA) using TLC and HPLC techniques. Gerding *et al.* [20] studied on human urinary mycotoxin excretion patterns in Bangladesh, Germany and Haiti using a rapid and sensitive LC-MS/MS approach. Rodríguez-Carrasco *et al.* [21] determined a new analytical method for the rapid detection of ten mycotoxins including patulin, zearalenone and eight trichothecenes (nivalenol, fusarenon-X, diacetoxyscirpenol, 3-acetyl-deoxynivalenol, neosolaniol, deoxynivalenol, T-2 and HT-2) in wheat semolina by using an acetonitrile-based extraction procedure and gas chromatography–tandem mass spectrometry. Although, there are reports available on mycotoxins in food stuffs contaminated by different fungi but there are very few reports on mycotoxins of endophytic micro and wild macro fungi in literature.

Keeping in view all literature review, the present study was aimed to detect the mycotoxins from some endophytic micro ascomycetes fungi *e.g. Fusarium solani*, *Penicillium chrysogenum*, *Geotrichum candidum* and wild macro ascomycetes fungi *e.g. Xylaria hypoxylon*, *Daldinia concentrica* and to find whether if there is any difference in mycotoxins occurrence and probable mycotoxins were detected and perceived based on colours obtained in plates through Thin Layer Chromatography.

II.   MATERIALS AND METHODS

Fresh samples of macro fungi *e.g. Xylaria hypoxylon* and *Daldinia concentrica* were collected from adjoining/forest area of Asan River which is located in between 30°21'05" N 77°59'39" E and 30°21'06" N

77°59'37"E and kept in poly bags and refrigerated whereas the micro endophytic fungal samples of *Fusarium solani, Geotrichum candidum*, *Penicillium chrysogenum* were taken and procured from National Type Culture Collection, Forest Research Institute, Dehradun, Uttarakhand, India which were already isolated in the laboratory from the roots of host plant/s under a project (P. no. R&D/UK-02/2018-19/NMPB-IVA). Culturing of different micro fungal samples *e.g. Fusarium solani* (AG/C3/1), *Geotrichum candidum* (AG/C3/2), *Penicillium chrysogenum* (AG/C3/3) and macro fungal samples *e.g. Xylaria hypoxylon* (AS/F1), *Daldinia concentrica* (AS/F2) (endophytic in nature as per review of literature) [29-30], were accomplished again in the laboratory of Pathology Discipline, Forest Research Institute, Dehradun, under aseptic conditions. Sub culturing was done from different cultures to get the pure colony of all micro and macro fungal samples. Mass culturing was facilitated using PDB (Potato Dextrose Broth) as the appropriate growth medium. Pure fungal colonies from prepared sub cultures were inoculated in broth under sterile conditions. Flasks were then incubated for the desired growth of fungi. After incubation of 1-2 weeks, cultures of the preferred fungal samples were acquired.

The identification was facilitated by standard methods and microscopy. The identification of all collected macro fungal samples was done by standard microscopic methods and considering various morphological and anatomical features into account [22-26]. Besides these identification keys, some following authentic websites were also browsed for identification.

(http://www.mushroomobserver.org,http://www.mushroomexpert.com,http://www.rogersmushrooms.com,http://www.americanmushrooms.com,http://www.fungiphoto.com)

The glassware used were cleaned with distilled water and ethanol followed by oven drying. Fractionation and Soxhlet methods were used for the extraction of fungal samples. Media was filtered in vacuum distillation unit by which the two components of the media were separated. Separation was carried in two parts of the media *i.e.* Culture and liquid part. All the fungal samples except *Daldinia concentrica* got separated out in vacuum filtration but centrifugation was done for 6 minutes at 4250 rpm to separate out the fungal sample of *D. concentrica.* The following Figure 1 shows the map and site of collection along with sporophores of endophytic macro fungi.

Fig.1: (A) Map of collection site, (B) Satellite image of Tons river flowing through reserve forest, (C) Site of collection, (D) Fruiting bodies of Daldinia concentrica attached to forest tree snag and (E) Fruiting body of Xylaria hypoxylon.

Fractionation-The filtered part was treated with three solvents namely petroleum ether, chloroform and butanol to fractionate the compound present in complex mixture. The media was treated with chemicals according to their polarity. After fractionation the fraction of Petroleum

ether, chloroform and butanol were collected in conical flask; 2 tablespoons of anhydrous sodium sulphate were added to remove any trace of water. The samples were then filtered by using fluted filter paper rinsed with solvent before distillation. Distillation was carried out by heating

and reflux with petroleum ether and chloroform at 75°C for 5 hours. The solvent was removed with the help of rotary evaporator to get a concentrated sample [27].

Soxhlet-Fungal mycelium was extracted with chloroform and methanol in 1:1 ratio using a Soxhlet extractor at 60°C for 6-8 hours. Extracted solvent washed and then removed by rotary evaporator to get a subsequent amount of

extracts [28]. Processed extracts were then used for detecting mycotoxins employing TLC [16].

III.   RESULTS AND DISCUSSION

The Thin Layer Chromatographic plates were run in different mobile phase/solvents (Table 1) followed by heating at 120°C for 30 minutes in hot air oven.

Table 1: Different solvents and spraying reagents used for Thin Layer Chromatography

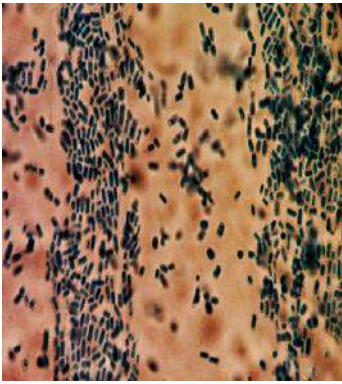| Fungal specimens	TLC Plates	Solvents	Spraying Reagents
Endophytic micro fungi			
Fusarium solani	(A)	1.5% Methanol in Chloroform	Freshly preprared p-anisaldehyde reagent*
	(B)	Benzene Methanol Acetic Acid (12:1:0.5)	Freshly preprared p-anisaldehyde reagent*
	(C)	Toluene Ethyl Acetate with Formic Acid (6:3:1)	Freshly preprared p-anisaldehyde reagent*
Penicillium chrysogenum	(D)	4% Methanol in Chloroform	5% Sulphuric Acid
	(E)	3% Methanol in Chloroform	Freshly preprared p-anisaldehyde reagent*
	(F)	Toluene Ethyl Acetate with Formic Acid (6:3:1)	Freshly preprared p-anisaldehyde reagent*
Geotrichum candidum	(G)	Chloroform Petroleum Ether (1:1)	5% Sulphuric Acid
	(H)	Toluene Ethyl Acetate with Formic Acid (6:3:1)	Freshly preprared p-anisaldehyde reagent*
	(I)	First, dip in 10% Oxalic Acid and run 3% Methanol in Chloroform	Freshly preprared p-anisaldehyde reagent*
Endophytic macro fungi			
Daldinia concentrica	(J)	1.5% Methanol in Chloroform	Freshly preprared p-anisaldehyde reagent*
	(K)	Toulene Ethyl Acetate with Formic Acid (6:3:1)	Freshly preprared p-anisaldehyde reagent*
	(L)	First, dip in 10% Oxalic acid and run in 1% Methanol in Chloroform	Freshly preprared p-anisaldehyde reagent*
Xylaria hypoxylon	(M)	Toluene Ethyl Acetate with Formic Acid (6:3:1)	Freshly preprared p-anisaldehyde reagent*
	(N)	Benzene Methanol Acetic Acid (24:2:1)	Freshly preprared p-anisaldehyde reagent*

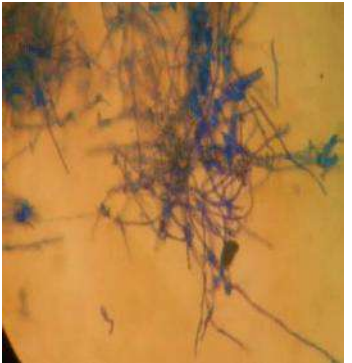|	(O)	1.5% Methanol in Chloroform	Freshly preprared p-anisaldehyde reagent*
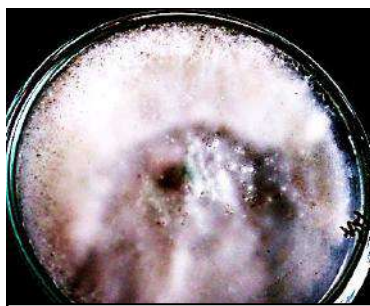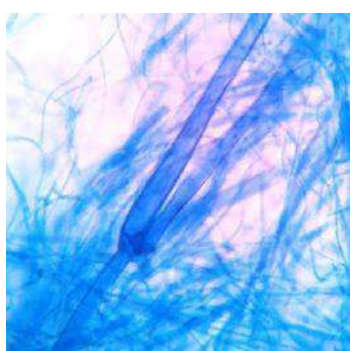
*p- anisaldehyde reagent (glacial acetic acid, conc. H$_2$SO$_4$ and p- anisaldehyde)

Culturing of different endophytic micro fungal species namely *Fusarium solani*, *Geotrichum candidum*, *Penicillium chrysogenum* and endophytic macro fungal species *e.g. Xylaria hypoxylon*, *Daldinia concentrica* were carried out under aseptic conditions. Sub-culturing was done from different fungal cultures to get the pure colony of all tested fungi. The fungal extracts from the various fungal samples after mass culture were used for the detection of mycotoxins using different methods like fractionation and Soxhlet. The solvents obtained after fractionation and Soxhlet extraction were then concentrated in water bath and rotary distillation resulting in various extracts of different solvents. Thin Layer Chromatography (TLC) was done for detecting mycotoxins. Silica gel plates were used for running of solvents as already stated above (Table-1), the plates were run in different mobile phase/solvents using various spraying reagents. The following Table 2 shows the microscopic characteristics of all micro and macro endophytic fungi.

Table 2: Cultured fungal colony and microscopic characteristic of ascomycetes fungal samples

S. No.	Cultures of Ascomycetes fungi	Microscopic view	Characteristics of fungal Colony
Endophytic micro fungi			
A	*Penicillium chrysogenum*		Growth was umbonate, dark green with pale yellow at the margins and highly branched network of multinucleate cells located on septum lacking hyphae, brush like appearance.
B	*Geotrichum candidum*		Colony was thin, spreading, soft creamy and white with acutely branching hyphae, disintegrated entirely into brick- shaped conidia.

C	*Fusarium solani*		Cottony colony owing to dense growth of white hyphae with septate curved to crescent or sickle shaped conidia.
Endophytic macro fungi after culture and subculture			
D	*Daldinia concentrica*		White felty colony and black grey coloration on reverse side with septate hyphae, branching conidiophores, large apical conidiogenous cells.
E	*Xylaria hypoxylon*		Mycelial mat white at first, later black, mostly submersed with irregular margins. Asci-8 spored, uniseraite, stipitate, cylindrical hyphae, branched.

In endophytic micro fungi *e.g. Fusarium solani* TLC plates (A, B & C), 1.5% methanol in chloroform, benzene methanol acetic acid and toluene ethyl acetate with formic acid respectively were used as solvent whereas freshly preprared p-anisaldehyde was taken as visualising reagent. Yellow, faint blue, orange and pink were spotted in TLC plate (A), yellow and faint blue in TLC plate (B), while pink, bright yellow, faint blue, orange and brown were clearly observed in TLC plate (C). In *Penicillium chrysogenum* TLC plates (D, E & F), 4% methanol in chloroform, 3% methanol in chloroform and toluene ethyl acetate with formic acid were taken as solvents for plates respectively, for visualisation 5% sulphuric acid was used

for TLC plate (D) and freshly preprared p-anisaldehyde was used for TLC plates (E & F). Prominent colours observed were yellow and pink for TLC plate (D) while yellow and faint blue in TLC plates (E & F). In *Geotrichum candidum,* chloroform petroleum ether, toluene ethyl acetate with formic acid were used in TLC plates (G & H) respectively, while plate (I) was pre-treated with oxalic acid (10%) in methanol was used as solvent to prevent peak from tailing by acidic mycotoxins such as cyclopiazonic acid, penicillic acid, citrinin and ochratoxin A [16]. For visualisation 5% sulphuric acid was used for plate (G) and freshly preprared p-anisaldehyde reagent was selected for plates (H&I). Faint blue, yellow and brown

were the prominent colours spotted in TLC plate (H) whereas no clear separations of colours were seen in TLC plates (G & I) due to low polarity of solvents the spots could not run on the TLC plates. While in case of endophytic macro fungi *e.g. Daldinia concentrica,* 1.5% methanol in chloroform, toulene ethyl acetate with formic acid as solvents were used in plates (J & K), while plate (L) was pre-treated with oxalic acid by submersion in 10% solution of oxalic acid in methanol. Whereas freshly preprared p-anisaldehyde was the spraying reaget. Major colours observed in plates were yellow and faint blue in (J

& K) whereas pink, yellow and orange were dominant in TLC plate (L). In case of *Xylaria hypoxylon,* toluene ethyl acetate with formic acid, benzene methanol acetic acid and methanol in chloroform were taken as solvents in TLC plates (M, N & O) respectively. For visualisation freshly preprared p-anisaldehyde was used. Yellow, pink and faint blue were the prominent colours observed in TLC plates (M) whereas faint blue and yellow were spotted in TLC plates (N & O). The following Figure 2 shows the diffrence of mycotoxin occrrence between endophytic micro and macro fungi.

Illumination of mycotoxins of endophytic micro fungi in TLC plates -
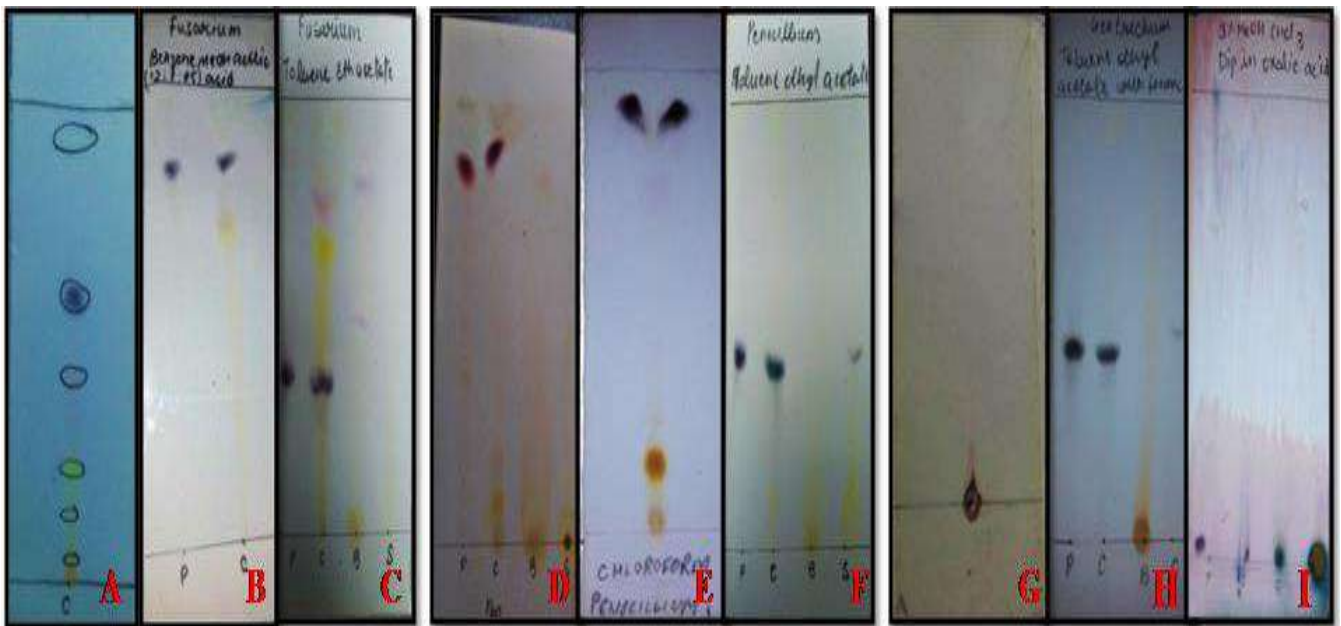
Illumination of mycotoxins of endophytic macro fungi in TLC plates -
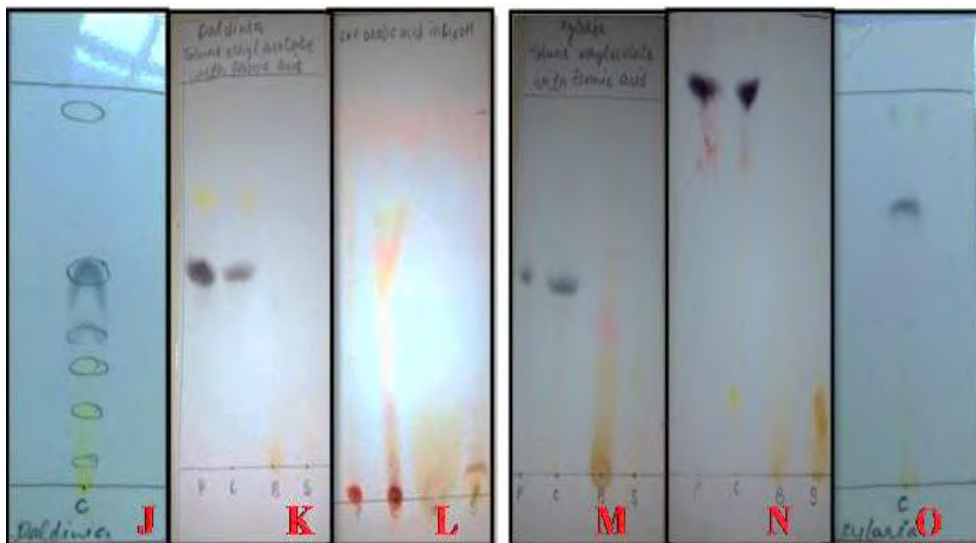

Fig.2: Mycotoxins in different endophytic micro (A-I) and macro (J-O) fungal extracts using Thin Layer Chromatography (TLC)

All plates were run in different solvent systems, some plates reflected colour whereas some systems were found unsuited for the extracts and thus, no colour was observed. Based on the colour appeared on the TLC plates, detection and tentative identification of all illuminated mycotoxins was done cautiously as per standard key and note available in laboratory [17]. Beauvericin (BEA) was found to be the highest frequency of mycotoxins in *Fusarium* species,

followed by MON, ZEN and FB1 mycotoxins [19]. This study also revealed the presence report of BEA production by *F. solani*. Screening procedure for the detection of mycotoxins used by Scott *et al.* [17] was referred for further detection and perceived identification of mycotoxins on the basis of colours obtained on the TLC plates in Table 3.

Table 3: Probable mycotoxins based on colours using Thin Layer Chromatography; Scott et al. [17]

Fungal specimens	TLC Plates	Colours (under visible light)	Probable Mycotoxins in fungal samples
Fusarium solani	(A)	Yellow brown, Faint blue, Orange and Pink	Zearalenone, Citrinin or Luteoskyrin, Beauvericin, Diacetoxiscirpenol, Aspertoxin and Nivalenol acetate
	(B)	Yellow, Faint blue	
	(C)	Yellow, Pink, Bright yellow, Faint blue, Orange and Brown	
Penicillium chrysogenum	(D)	Yellow and Pink	Zearalenone, Diacetoxiscirpenol and Citrinin
	(E)	Yellow and Faint blue	
	(F)	Faint blue and Yellow	
Geotrichum candidum	(G)	No separation of colours	Zearalenone, Nivalenol acetate and Citrinin
	(H)	Faint blue, Yellow and Brown	
	(I)	No separation of colours	
Daldinia concentrica	(J)	Bright yellow and Faint blue	Zearalenone, Aspertoxin, Diacetoxiscirpenol and Luteoskyrin
	(K)	Faint blue and Yellow	
	(L)	Pink, Yellow and Orange	
Xylaria hypoxylon	(M)	Yellow, Pink and Faint blue	Zearalenone, Citrinin or Luteoskyrin and Diacetoxiscirpenol
	(N)	Faint blue and Yellow	
	(O)	Faint blue and Yellow	

For the confirmation of these mycotoxins some more specific tests have to be done. The colour of mycotoxins may vary for different solvent systems and spraying reagents under ultraviolet light. The further biochemical analysis is under progress.

IV.   CONCLUSION

The wide importance of ascomycetes fungi in medicines and industries attracted our attention towards its chemical and biochemical properties. This study provides an idea about the essential and non-essential biochemical compounds present in different species of the fungi. Mycotoxins are very important metabolites produced by the fungus in their defence against insects and plants. The present study focuses on mycotoxins produced by micro and macro endophytic fungi naturally and for which three micro endophytic fungal species *e.g. Fusarium solani*, *Geotrichum candidum, Penicillium chrysogenum* and two

macro endophytic fungal species *e.g. Xylaria hypoxylon, Daldinia concentrica* were selected and tested for the presence of mycotoxins and differences (if any) in them. For the confirmation of mycocompounds/toxins in these chosen wild endophytic fungal species which has never been done before thus, it was the first attempt of its kind to extract and identify mycocompounds/toxins from wild endophytic micro and macro fungi. A basic approach *i.e.* Thin Layer Chromatography was used for the screening of mycotoxins on the basis of colour obtained on the TLC plates as the purpose was to check the presence or absence of unidentified mycotoxins in the selected wild fungal

species. Various extracts of the species were taken in different solvents, as mycotoxins were soluble in discrete solvents. Probable mycotoxins were identified on the basis of colours obtained in thin layer chromatography which may help us to categories these fungal species as either toxigenic or non-toxigenic or edible or non-edible (macro fungi) as some mycotoxins have medicinal importance and some are detrimental in nature. The future prospect of this study is to identify the particular mycotoxins available in the target fungal species by standardizing isolation and identification techniques with the help of advanced and sophisticated modern techniques.

V.   ACKNOWLEDGEMENTS

REFERENCES

[1] Carlile MJ, Watkinson SC, Gooday GW. The fungi (2nd ed.), San Diego. CA: Academic. 2001.

[2] Reitz EJ, Shackley M. Viruses, Bacteria, Archaea, Protists, and Fungi. In: Environmental Archaeology. Manuals in Archaeological Method, Theory and Technique, Springer, Boston, MA.2012; 161-189. doi:10.1007/978-1-4614-3339-2_6

[3] Gmoser R, Ferreira JA, Lennartsson PR, Taherzadeh MJ. Filamentous ascomycetes fungi as a source of natural pigments, Fungal Biology and Biotechnology, 2017; 4:(1), 1-25.

[4] Goswami S, Rani A, Priyadarshini R, Bhunia B, Mandal T. A Review on production of echinocandins by *Aspergillus* sp., Journal of Biochemical Technology, 2013; 4 : (1), 568-575.

[5] Sharmila K, Thillaimaharani KA, Logesh AR. Production of cyclosporin A by saprophytic filamentous fungus *Fusarium oxysporum*, Int. J. Pharm. Pharm. Sci.,2012; 4, 149-153.

[6] Egbuta MA, Mwanza M, Babalola OO. A review of the ubiquity of ascomycetes filamentous fungi in relation to their economic and medical importance, Advances in Microbiology, 2016; 6: (14), 1140-1158.

[7] Pitt JI. Toxigenic fungi and mycotoxins, British Medical Bulletin. 2000; 56: (1), 184-192.

[8] Frisvad JC, Thrane U, Samson RA, and Pitt, JI. Important mycotoxins and the fungi which produce them, Advances in Food Mycology, 2006; 3-31.

[9] Richard JL. Some major mycotoxins and their mycotoxicoses—An overview, International Journal of Food Microbiology, 2007; 119 :(1-2),3-10.

[10] Adeyeye SA. Fungal mycotoxins in foods: A review, Cogent Food & Agriculture, 2016; 2: (1), 1213127.

[11] Barbosa TS, Pereyra CM, Soleiro CA, Dias EO, Oliveira AA, Keller KM, Rosa CA. Mycobiota and mycotoxins present in finished fish feeds from farms in the Rio de Janeiro State, Brazil, International Aquatic Research, 2013; 5:(1),1-9.

[12] Awuchi CG, Amagwula IO, Priya P, Kumar R, Yezdani U, and Khan MG. Aflatoxins in foods and feeds: A review on health implications, detection, and control, Bull. Env. Pharmacol. Life Sci., 2020; **9**: 149-155.

[13] Nielsen KF, Mogensen JM, Johansen M, Larsen TO, and Frisvad JC. Review of secondary metabolites and mycotoxins from the *Aspergillus niger* group. Bio-analytical Chemistry, 2009; 395:(5), 1225-1242.

[14] Zheng MZ, Richard JL, Binder J. A review of rapid methods for the analysis of mycotoxins, Mycopathologia, 2006; 161: (5), 261-273.

[15] Turner NW, Subrahmanyam S, Piletsky SA. Analytical methods for determination of mycotoxins: a review, Analytica Chimica Acta., 2009; 632: (2), 168-180.

[16] Betina, V. Thin-layer chromatography of mycotoxins, Journal of Chromatography A, 1985; 334, 211-276. https://doi.org/10.1016/S0021-9673 (00)80272-1.

[17] Scott PM, Lawrence JW, Van Walbeek W. Detection of mycotoxins by thin-layer chromatography: application to screening of fungal extracts, Applied Microbiology, 1970; 20: (5), 839p.

[18] Hassan G, Bullerman LB. *Aspergillus flavus* and *Aspergillus parasiticus*: Aflatoxigenic fungi of concern in foods and feeds: A review, Journal of Food protection, 1995; 58: (12), 1395-1404. https://doi.org/10.4315/0362-028X-58.12.1395

[19] Azliza IN, Hafizi R, Nurhazrati M, Salleh B. Production of major mycotoxins by *Fusarium* species isolated from wild grasses in Peninsular Malaysia, SainsMalays, 2014; 43, 89-94.

[20] Gerding J, Ali N, Schwartzbord J, Cramer B, Brown DL, Degen GH, Humpf HU. A comparative study of the human urinary mycotoxin excretion patterns in Bangladesh, Germany, and Haiti using a rapid and sensitive LC-MS/MS approach, Mycotoxin Research, 2015; 31:(3), 127-136.

[21] Rodríguez-Carrasco Y, Berrada H, Font G, Mañes J. Multi-mycotoxin analysis in wheat semolina using an acetonitrile-based extraction procedure and gas chromatography–tandem mass spectrometry, Journal of Chromatography A, 2012;1270, 28-40. https://doi.org/10.1016/j.chroma.2012.10.061

[22] Smith AH. The mushroom hunter's field guide, University of Michigan Press, Annarbor. 1963; 67pp.

[23] Miller OK. Mushrooms of North America. 1997 New York, E.P. Dutton.

[24] Kaul TN. Introduction to Mushroom Science (systematic), Oxford and B.H. Publ. Co. Pvt. Ltd., New Delhi,1997; 198p.

[25] Smith AH, Smith HV, Weber NS. How to know the gilled mushrooms, Wm. C. Brown Co. Dubuque. 1979; 334pp.

[26] Coetzee JC. Taxonomic notes on the Clathraceae (Phallales: Phallomycetidae) *sensu* Bottomley and a new key to the species in Southern Africa, Bothalia. 2010; 40: (2), 155-159.

[27] Pudziuvelyte L, Jakštas V, Ivanauskas, L. LaukevičienėA, IbeCFD, Kursvietiene L, Bernatoniene J. Different extraction methods for phenolic and volatile compounds recovery from *Elsholtziaciliata* fresh and dried herbal materials, Industrial Crops and Products, 2018; 120: 286-294.

[28] Yang L, Jiang JG, Li WF, Chen J, Wang DY, Zhu L. Optimum extraction process of polyphenols from the bark of *Phyllanthus emblica* L. based on the response surface methodology, Journal of Separation Science, 2009; 32:(9), 1437-1444.

[29] Liarzi O, Bar E, Lewinsohn E, Ezra D. Use of the endophytic fungus *Daldinia* cf. *concentrica* and its volatiles as bio-control agents, PLoS One. 2016; 11; (12), e0168242. doi:10.1371/journal.pone.0168242

[30] Petrini L, Petrini O. Xylariaceous fungi as endophytes, Sydowia. 1985; 38: 216-234.

Websites consulted and retrieved during the study period-

[31] www.mushroomobserver.org

[32] www.mushroomexpert.com

[33] www.rogersmushrooms.com

[34] http://americanmushrooms.com

[35] http://www.fungiphoto.com

Peer-Reviewed Journal

Effect of scarification methods on different forest seeds

R. Priyadharshini[1], Dr. K. Lekha[2]

M. Sc. Botany, Department of Botany, PSG college of arts and science, Coimbatore, Tamilnadu, India.
Assistant professor, Department of Botany, PSG college of arts and science, Coimbatore, Tamilnadu, India.

Abstract— A study describes various pre-sowing treatment attributed to the seed germination and seedling growth of seeds such as Terminalia chebula (Gaertn.) Retz., Terminalia bellirica (Gaertn.) Roxb., Pongamia pinnata (L.) Pierre, Santalum album L., Tamarindus indica L by different methods of scarification to determine the germination percentage, germination rate and germination period of seeds. These seeds were collected from IFGTB, Coimbatore in the month of January 2021 respectively. The effects of depulping and soaking period on seed germination and seedling growth performance were explored. The fastest seed germination and highest germination percentage was observed in acid scarification(T4) followed by coconutwater treatment(T3) and rice water treatment(T2) and delayed germination with lowest percentage was observed in mechanical scarification(T4). Growth parameters including shoot length and root length were also maximum and significantly higher in the same treatment compared to others. Considering the practicability of the nursery raising technique for the species, the best treatment option obtained in this study was soaking the five types of seeds in concentrated acid such as 10% of con sulphuric acid for one hour. Since, the acid treatment for a particular period of time is easily applicable and cost effective, the treatment may be recommended for large scale seedling productions in the nurseries.

Keywords— Pre-sowing treatment, Germination percentage, germination rate, germination period, depulping and soaking.

I. INTRODUCTION

Germination is the process by which an organism grows from a seed or similar structure. The term is applied to the sprouting of a seedling from a seed of an angiosperm or gymnosperm, the growth of a sporeling from a spore, such as the spores of fungi, ferns, bacteria, and the growth of the pollen tube from the pollen grain of a seed plant. Seed germination depends on both internal and external conditions. The most important factors include right temperature, water, oxygen, or air and sometimes light or darkness. Various plants require different variables for successful seed germination. Often this depends on the individual seed variety and is closely linked to the ecological conditions of a plant's natural habitat. For some seeds, their future germination response is affected by environmental conditions during seed formation; most often these responses are types of seed dormancy. The

seed of many species are often impervious to water and gases, thus preventing on delaying germination. Any process designed to make the seed coat more permeable to water and gases, thus help to germination of seed is called as scarification. Scarification is a process that weakening, opening or otherwise altering the seed coat to developing the germination. Scarification is often done by **mechanical, rice water, coconut water** and **acid treatment**. The seed of plants which are otherwise difficult to grow from seed may be made viable through scarification. In horticulture, scarification is often used to facilitate the controlled and uniform germination of seed.

***Terminalia chebula* (Gaertn.) Retz.** is an important medicinal tree species in the Asian countries. It is routinely used as traditional medicine by tribes of tamilnadu to cure several ailments such as fever, cough, diarrhea, gastroenteritis, skin diseases, candidiasis, urinary tract

infection and wound infections. Unripe fruits are useful medicine for diarrhea, dysentery an leprosy(Singh, *et al*., 1965). Dry fruits posses a potential broad spectrum of antihelminthic, antimicrobial activity against both Gram-positive and Gram-negative bacteria. Aqueous extract of *T. chebula* fruits exhibits antifungal activity against a number of dermatophytes and yeasts (Dutta, *et al*., 1998). It possesses antiviral activity against Herpes Simplex Virus type-1 (HSV-1), Human Immune Deficiency Virus-1 (HIV-1) and cytomegalovirus (Yuka, *et al*., 1996). Seventy percent methanol extract and phenolic compound of *T. chebula f*ruits decrease cancer cell viability, cell proliferation, and induced cell death in a dose dependent manner (Saleem, *et al*., 2002). However, the poor germination capacity (Shankar, 2001) and longer germination period (up to 2-3 months) are the major constraints of raising seedling and establishing plantation of the species (Luna 1996).

Terminalia bellirica* (Gaertn.) Roxb.** (Combretaceae) is an ecologically and medicinally important deciduous canopy tree species having wide distribution throughout india, but with limited distribution and density in evergreen forests of the Western Ghats and is often associated with teak. Seed is edible; exocarp is one of the important components of a medicine commonly known as "Triphala churan" (used in bronchitis, cough problems, indyspepsia, constipation, impurity of blood and stomach problem) (CSIR 1985). The fruit of *T.bellirica* contain very high level of phenolic compounds (TPC) (Bajpai *et al*., 2005) including Glucoside (bellaricanin), Gallo-tannic acid, resins, oil, Ellargic acid, gallic acid, lignans 7-hydroxy 3'4' flavones and anolignan B, tannins, ethyl gallate, phenyllemblin, mannitol, glucose, fructose and rhamnose. The delay and irregularity in germination of seeds is believed to be due to the hard seed coat and thick fleshy pericarp of the fruits of the species (Shivanna *et al.*, 2001). Seed germination may be reduced by the presence of impermeable seed coats that restricts the availability of water, which can penetrate into seeds at their optimum concentration (Kumar *et al*., 2015a,b).Seeds were given a pretreatment to overcome the problem of hard seed-coat. They were soaked in boiled water for a period of 24 h (water was boiled, seeds were dipped into it and then the vessel was removed from the hot plate and kept at room temperature for 24 h) (Todaria & Negi 1992). Indian sandalwood (Santalum album* L.**) is a commercially and culturally important plant species, known for its fragrant heartwood and oil. The sandalwood oil, known as santalol is highly demanded by the perfume industry due to its sweet fragnance, persistent aroma and the fixative property (Jain *et al*., 2003).

***Pongamia pinnata* (L.) Pierre** is one of the plant species, which yield oil as a source of energy in the form of bio-diesel, with high oil recovery and the quality of oil (Shrinivasa, 2001). ***Pongamia pinnata* (L.) Pierre** also called as *Derriss indica*(Lam.) Bennet is a tree species belonging to the family Fabaceae. This species is commonly known as Pongam or karanj. The seed oil is used as a lubricant, water paint binder, pesticide, in soap making and tanning industries. The oil is used for the treatment of rheumatism and scabies. All parts of the plant have been used as crude drug for the treatment of tumors, piles, skin diseases, wounds and ulcers (Tanaka *et al*., 1992). The press cake is used as poultry feed and also as organic fertilizer. It controls soil erosion and binds sand dumes (Wani, *et al*., 2005). The *Pongamia pinnata* seeds contain about 40٪ oil, which can be converted to biodiesel by transesterification method (Meher *et al*., 2006). Seed germination, one of the most important phases in the life cycle of a plant, is highly responsive to existing environment (Kuriakose *et al*., 2008). Water stress is one of the abiotic stresses, which affect seed germination, delaying its beginning or decreasing the final germinability (Silva *et al*., 2005). Its participation is crucial in the enzymatic reactions, solubilisation and transportation of metabolites, as well as reagent in the hydrolysis of proteins, carbohydrates and lipids in the storage tissues of the seed (Mayer *et al.,* 1989) According to Hadas(Hadas, *et al*., 1976), the critical soil water potential for seed germination, is typical for each plant species. Therefore, solutions with different osmotic potentials have been used in several studies in order to establish gradient of water stress.

***Tamarindus indica* L.** is a leguminous tree in the family fabaceae native to tropical Africa. *T. indica* is used as traditional medicine in India, Pakistan, Bangladesh, Nigeria, and most of the tropical countries. Temperature requirements for germination vary with species. Many seeds germinate at temperatures slightly above 60–75°F (16–24°C), though there are certain seeds that require exposure to cold temperature (vernalization) in order to break dormancy, for example, *Helleborus niger* of the family Ranunculaceae (Gama-Arachchige,2013). (Nwoboshi,1982) stated that low temperature prolongs the length of seed viability; hence creating artificially low temperatures has been found useful in seed storage. High temperatures affect germination of seeds by influencing the water holding capacity of the soil which, thus, determines to a large extent the survival of germinating seeds.

II.   MATERIALS AND METHOD

The seeds of ***Terminalia chebula* (Gaertn.) Retz.,** ***Terminalia bellirica* (Gaertn.) Roxb.,** ***Pongamia pinnata*** **(L.) Pierre,** *Santalum album* **L.,** *Tamarindus indica* **L,** were collected from IFGTB, Coimbatore in the month of January 2021. Scarification is a process of weakening, opening or otherwise altering the seed coat to enhance the germination. Scarification is often done by **mechanical, rice water, coconut water** and **acid treatment**. T denotes test has been listed below.

T1-Mechanical scarification

T2-Ricewater treatment

T3-Coconutwater treatment

T4-Acid treatment

Mechanical scarification (T1): In mechanical scarification, factors for breaking hard seed coat was applied in this study includes emery sheet, nailed hammer. Nailed hammer was used to breaking the seed coat and emery sheet used to scarped the testa of seed. In this five types of seeds (*Terminalia chebula* **(Gaertn.) Retz.,** *Terminalia bellirica* **(Gaertn.) Roxb.,** *Pongamia pinnata* **(L.) Pierre,** *Santalum album* **L.,** *Tamarindus indica* **L.)** were scarified using nail hammer.

Ricewater treatment (T2): Scarification of seeds in rice water, it contains triglycerides and lipids each makeup 10 percent of the rice water composition, while starch is present at 9 percent. Carbohydrates, inositol, phytic acid, and inorganic substances components helps to scarify the seed coat are other components in rice water. The five types of seeds were soaked in rice water overnight, to promote the germination of seed.

Coconutwater treatment (T3): Scarification of seeds in coconut water, it contains physicochemical properties such as volume of water, titratable acidity, pH, Turbidity, sugar content such as fructose, glucose, sucrose, mineral content such as potassium, sodium, magnesium, calcium, iron, protein, total phenolic content. These properties helps to scarify the seed coat. The five types of seeds were soaked in coconut water overnight, promote the germination of seed.

Acid treatment (T4): In this five types of seeds (*Terminalia chebula* **(Gaertn.) Retz.,** *Terminalia bellirica* **(Gaertn.) Roxb.,** *Pongamia pinnata* **(L.) Pierre,** *Santalum album* **L.,** *Tamarindus indica* **L.)** can be soaked in concentrated acid such as 10% of con sulphuric acid. This can be soaked for one hour.

Pre-sowing treatment and growing media: The extracted seeds were cleaned and thoroughly hand mixed to improve the homogeneity during the sampling. Seeds were subjected to pre-sowing treatments. The pre-treated seeds were sown in polythene bags containing the rooting medium soil mixed with decomposed cow-dung, sand and vermicompost contains water-soluble nutrients and is an excellent, nutrient-rich organic fertilizer and soil conditioner, The soil was moderately coarse to fine textured sandy loams with pH 4.5. Before filling with the prepared soil mixture, few holes were made in the polybag by punching to facilitate aeration and proper drainage. Randomized complete block design was adopted for the study with five types of seeds for each treatment. Thus each treatment consisted of 5 seeds and a total of 20 seeds were subjected to 4 different pre-sowing treatments.

Seed sowing and aftercare: Only one seed was sown in each polybag filled with growing media directly. Seeds were dibbed to 0.5 cm beneath the soil surface by pressing them with thumb and covered with thin layer of soil. After sowing the seeds, protective measures were adopted against the hot sun, intensive rains, birds, rodents and pests. Proper care was taken since the sowing of seeds till the harvesting of seedlings for assessment. Watering and weeding were done regularly to obtain maximum growth of seedlings. Loosening of topsoil was also done whenever necessary to prevent the growth of green mold on the soil surface.

Data recording and growth assessment: The effects of pre-sowing treatments on germination of seeds and seedling growth were explored periodically through counting germinated seeds and assessing initial growth performance of seedlings. Germination phase like imbibition period was determined by counting the number of days required for the commencement of germination from the day of sowing and germination period was the number of days required for completion of germination from sowing the seeds.

For assessing the growth performance, all seedlings were measured for total length (root length, shoot length). Five seedlings from each replication treatment were randomly uprooted and measured for total length. The seedlings were then separated into root and shoot were also recorded to assess the growth performance of the seedlings.

Assesment variables:

Germination percentage: Seed germination percentage was calculated using the following formula: **Germination percentage %=** Number of seeds germinated / total number of seeds sown ×100.

III. RESULTS AND DISCUSSION

Terminalia bellirica (Gaertn.) Roxb.

Germination period: Seeds of *T. bellirica* **(Gaertn.) Roxb.** started to germinate 21 days after sowing and continued up to 65 days. Different pre-sowing treatments significantly affected the germination period for the species. The fastest seed germination (least imbibition period; 21 days) was observed in **acid scarification(T4)** (using 10% of con.H2SO4) followed by **rice water treatment(T2)** in 27 days, **coconutwater treatment(T3)**

in 25 days and delayed (highest imbibition period) was observed in **mechanical scarification(T1)** (nail punched method). However, the fastest completion of seed germination (35 days) was noticed in **acid scarification(T4)** and slowest completion (65 days) was in **mechanical scarification(T1)**. The germination percentage of the seeds, shoot length and root length has been listed in table.

Terminalia chebula (Gaertn.) Retz.

Germination period: Seeds of *T.chebula (Gaertn.) Retz.* started to germinate 25 days after sowing and continued up to 70 days. Different pre-sowing treatments significantly affected the germination period for the species. The fastest seed germination (least imbibition period; 25 days) was observed in **acid scarification(T4)** (using 10% of con.H2SO4) followed by **rice water treatment(T2)** in 30 days, **coconut water treatment(T3)** in 33 days and

delayed (highest imbibition period, 70 days) was observed in **mechanical scarification(T1)** (nail punched method). However, the fastest completion of seed germination (40 days) was noticed in **acid scarification(T4)** and slowest completion (70 days) was in **mechanical scarification(T1)**. The germination percentage of the seeds, shoot length and root length has been listed in the table.

Tamarindus indica L.

Germination period: Seeds of *T.indica* **L.** started to germinate 8 days after sowing and continued up to 51 days. Different pre-sowing treatments significantly affected the germination period for the species. The fastest seed germination (least imbibition period; 8 days) was observed in **acid scarification(T4)** (using 10% of con.H2SO4) followed by **rice water treatment(T2) in** 12 days, **coconut water treatment(T3)** in 19 days and

delayed (highest imbibition period, 25 days) was observed in **mechanical scarification(T1)** (nail punched method). However, the fastest completion of seed germination (30 days) was noticed in **acid scarification(T4)** and slowest completion (51 days) was in **mechanical scarification(T1)**. The germination percentage of the seeds, shoot length and root length has been listed in the table.

Santalum album **L.**

Germination period: Seeds of *Santalum album* **L.** started to germinate 15 days after sowing and continued up to 60 days. Different pre-sowing treatments significantly affected the germination period for the species. The fastest seed germination (least imbibition period; 15 days) was observed in **acid scarification(T4)** (using 10% of con.H2SO4) followed by **rice water treatment(T2)** in 20 days then **coconut water treatment(T3)** in 22 days and delayed (highest imbibition period, 26 days) was observed in **mechanical scarification(T1)** (nail punched method). However, the fastest completion of seed germination (35 days) was noticed in **acid scarification(T4)** and slowest completion (60 days) was in **mechanical scarification(T1)**. The germination percentage of the seeds, shoot length and root length has been listed in the table.

Pongamia pinnata (**L.**) **Pierre**

Germination period: Seeds of *Pongamia pinnata* (**L.) Pierre** started to germinate 12 days after sowing and continued up to 56 days. Different pre-sowing treatments significantly affected the germination period for the species. The fastest seed germination (least imbibition period; 12 days) was observed in **acid scarification(T4)** (using 10% of con.H2SO4) followed by **rice water treatment(T2)** in 20 days, **coconut water treatment(T3)** in 25 days and delayed (highest imbibition period, 30 days) was observed in **mechanical scarification(T1)** (nail punched method). However, the fastest completion of seed germination (32 days) was noticed in **acid scarification(T4)** and slowest completion (56 days) was in **mechanical scarification(T1)**. The germination percentage of the seeds, shoot and root length has been listed in the table.

Tabulation:

GERMINATION PERCENTAGE OF SEED:

Germination percentage%= Ng/Ns ×100 where, Ng= number of seeds germinated, Ns=total number of seeds.

Table: Germination percentage of seeds as influenced by different pretreatments

NAME OF SEEDS	NAME OF SCARIFICATION	TOTAL NO OF SEEDS	DAYS			GERMINATION PERCENTAGE%	STANDARD DEVIATION	
			0-25	25-50	50-75		SHOOT LENGTH	ROOT LENGTH
Terminalia bellirica (Gaertn.) Roxb.	Mechanical	4	-	2	2	50%	7.36±3.15	3.28±0.79
	Ricewater treatment	4	1	3	3	75%	9.24±4.24	4.19±1.32
	Coconutwater treatment	4	2	3	3	75%	8.54±3.83	4.54±1.48
	Acid treatment	4	2	3	4	100%	10.36±4.88	5.24±1.88
Terminalia chebula (Gaertn.) Retz.	Mechanical	4	-	1	2	50%	6.66±1.97	3.22±0.04
	Ricewater treatment	4	2	3	4	100%	7.29±2.34	3.86±0.58
	Coconutwater treatment	4	-	1	2	50%	8.59±3.09	4.02±0.67
	Acid treatment	4	2	4	4	100%	9.32±3.51	4.67±1.04
Tamarindus indica L.	Mechanial	4	1	3	3	75%	9.87±3.83	4.45±0.70
	Ricewater treatment	4	-	2	2	50%	10.21±4.02	5.46±1.28
	Coconutwater treatment	4	2	2	3	75%	7.32±2.35	3.97±0.42
	Acid treatment	4	3	3	4	100%	12.17±5.15	6.78±2.04

Santalum album **L.**	Mechanical	4	-	-	1	25%	5.16±1.79	2.22±0.24
	Ricewater treatment	4	-	1	2	50%	7.19±2.96	3.53±0.87
	Coconutwater treatment	4	2	2	3	75%	6.18±2.38	3.18±0.67
	Acid treatment	4	3	3	4	100%	8.14±3.50	4.78±1.57
Pongamia pinnata **(L.) Pierre**	Mechanical	4	-	3	3	75%	7.78±2.62	3.98±0.43
	Ricewater treatment	4	1	3	3	75%	10.45±4.16	5.87±1.52
	Coconutwater treatment	4	2	2	2	50%	8.54±3.06	4.87±0.94
	Acid scarification	4	2	4	4	100%	11.02±4.49	6.02±1.60
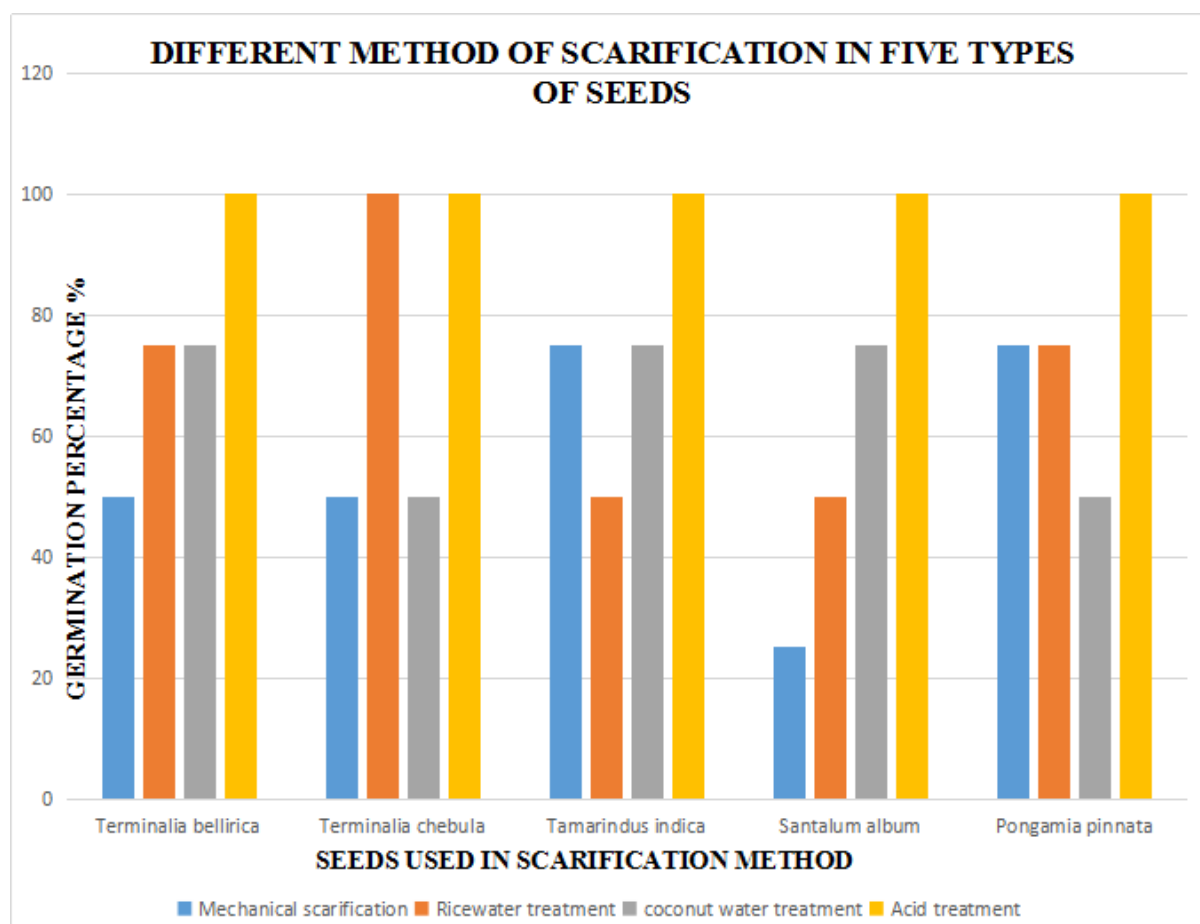
The highest germination percentage were observed in acid treatment(T4) in all five types of seeds. Sulphuric acid treatments were effective in softening and increase in permeability of the hard endocarp of seeds and increases the seed germination significantly. This treatment leads to easy penetration of water and activation of embryos.

Coconut water (CW) is the colorless liquid endosperm of green coconuts (Cocos nucifera) which its liquid endosperm contains a number of amino acids, organic acids, nucleic acids, several vitamins, sugars and sugar alcohols, plant hormones (auxins, cytokinins), minerals and other unidentified substances, none of which alone is totally responsible for promoting the growth of seeds in coconut water treatment (T3). Ricewater contains Triglycerides and lipids each make up 10 percent of the rice water composition is present at 9 percent. Carbohydrates, inositol, phytic acid and inorganic substances are other components in rice water. These helps to promote the growth of seeds in rice water treatment (T2).

IV. CONCLUSION

The study material *Terminalia chebula* **(Gaertn.) Retz.,** *Terminalia bellirica* **(Gaertn.) Roxb.,** *Pongamia pinnata* **(L.) Pierre ,** *Santalum album* **L.,** *Tamarindus indica* **L. seeds** were collected from IFGTB, Coimbatore.The five types of seeds are pre-soaked in different treatment of scarification for different period of time. The result showed positive for most of the seeds germination. For establishing nursery of a particular species for producing maximum number of quality seedlings with minimum cost, time and labour, the seed pretreatments are required.Since the seed coat of *Terminalia chebula* **(Gaertn.) Retz.,** *Terminalia bellirica* **(Gaertn.) Roxb.,** *Pongamia pinnata* **(L.) Pierre ,** *Santalum album* **L.,** *Tamarindus indica* **L.** are hard, it takes more time to germinate with lower germination percentage in nursery establishment.**t**he present study of pre-sowing treatments of seeds would prove itself potential in the practical fields. Among the treatments applied in the experiment for *Terminalia chebula* **(Gaertn.) Retz.,** *Terminalia bellirica* **(Gaertn.) Roxb.,** *Pongamia pinnata* **(L.) Pierre ,** *Santalum album* **L.,** acid treatment (10% of con H2SO4) was found more effective in respect to faster germination, higher germination percentage, seedling growth compare to other treatment. Therefore, the different methods of scarification may be an important aspect of future study to avoid the difficulties of hard coat seeds for better germination.

REFERENCES

[1] Singh, U., Wadhwani, A. M. and Johir, B. M. 1965. *Dictionary of Economic Plants in India. Indian Council of Agricultural Research*, New Delhi, 288 p.

[2] Dutta, B. K., Rahman, I., & Das, T. (1998). Antifungal activity of Indian plant extracts Antimyzetische Aktivitat indischer Pflanzenextrakte. *Mycoses*, *41*(11/12), 535-536.

[3] Yukawa, T. A., Kurokawa, M., Sato, H., Yoshida, Y., Kageyama, S., Hasegawa, T., Namba, T., Imakita,M., Hozumi, T. and Shiraki, K.(1996). Prophylactic treatment of cytomegalovirus infection with traditional herbs. *Antiviral research*, *32*(2), 63-70.

[4] Saleem, A., Husheem, M., Härkönen, P., & Pihlaja, K. (2002). Inhibition of cancer cell growth by crude extract and the phenolics of Terminalia chebula retz. fruit. *Journal of Ethnopharmacology*, *81*(3), 327-336.

[5] Shankar, U. (2001). A case of high tree diversity in a sal (Shorea robusta)-dominated lowland forest of Eastern Himalaya: Floristic composition, regeneration and conservation. *Current Science*, 776-786.

[6] Luna, R. K. (1996). Plantation Trees. Dehradun. *International Book Distributors. 975p*.

[7] CSIR. 1985. The Wealth of India. (*A Dictionary of India Raw Material & Industrial Products*). Publication & Information Directorate CSIR, New Delhi. Vol. I A.

[8] Todaria, N. P., & Negim, A. K. (1992). Pretreatment of some Indian Cassia seeds to improve their germination. *Seed science and technology*, *20*(3), 583-588.

[9] Bajpai, M., Pande, A., Tewari, S. K., & Prakash, D. (2005). Phenolic contents and antioxidant activity of some food and medicinal plants. *International journal of food sciences and nutrition*, *56*(4), 287-291.

[10] Shivanna, H., Balachandra, H. C., & Suresh, N. L. (2010). Effect of pre-sowing treatment on germination of Terminalia bellerica (Ber). *Karnataka Journal of Agricultural Sciences*, *20*(2).

[11] Kumar, R., Bohra, J. S., Kumava, N., & Singh, A. K. (2015). Fodder yield, nutrient uptake and quality of baby corn (Zea mays L.) as influenced by NPKS and Zn fertilization. *Research on Crops*, *16*(2).

[12] Kumar, R., Bohra, J. S., Singh, A. K., & Kumawat, N. (2015). Productivity, profitability and nutrient-use efficiency of baby corn (Zea mays) as influenced of varying fertility levels. *Indian J. Agron*, *60*(2), 285-290.

[13] Jain, S. H., Angandi, V. G., Shankaranarayana, K. H., & Ravikumar, G. (2003). Relationship between girth and percentage of oil in sandal provenances. *Sandalwood Research Newsletter*, *18*, 4-5.

[14] Shrinivasa, U. (2001). A viable substitute for diesel in rural India. *Current Science*, *80*(12), 1483-1484.

[15] Tanaka, T., Iinuma, M., Yuki, K., Fujii, Y., & Mizuno, M. (1992). Flavonoids in root bark of Pongamia pinnata. *Phytochemistry*, *31*(3), 993-998.

[16] Wani, S. P., & Sreedevi, T. K. (2005). Pongamia's journey from forest to micro-enterprise for improving livelihoods. *Pongamia as a source of Biodiesel in India*, *189*.

[17] Meher, L. C., Dharmagadda, V. S., & Naik, S. N. (2006). Optimization of alkali-catalyzed transesterification of Pongamia pinnata oil for production of biodiesel. *Bioresource technology*, *97*(12), 1392-1397.

[18] Kuriakose, S. V., & Prasad, M. N. V. (2008). Cadmium stress affects seed germination and seedling growth in Sorghum bicolor (L.) Moench by changing the activities of

hydrolyzing enzymes. *Plant growth regulation*, *54*(2), 143-156.

[19] Silva, L. M. D. M., Aguiar, I. B. D., & Rodrigues, T. D. J. (2001). Seed germination of Bowdichia virgilioides Kunth, under water stress. *Revista Brasileira de Engenharia Agrícola e Ambiental*, *5*(1), 115-118.

[20] Mayer, A. M., & Poljakoff-Mayber, A. (1963). The germination of seeds. *The germination of seeds.*

[21] Hadas A., (1976).Water uptake and germination of leguminous seeds under changing external water potential in osmoticum solution*, Journal of Experimental Botany* 27(98), 480-489.

[22] Gama-Arachchige, N. S., Baskin, J. M., Geneve, R. L., & Baskin, C. C. (2013). Quantitative analysis of the thermal requirements for stepwise physical dormancy-break in seeds of the winter annual Geranium carolinianum (Geraniaceae). *Annals of Botany*, 111(5), 849-858..

[23] L. C. Nwoboshi,(1982). *Tropical Silviculture: Principles and Techniques*, Ibadan University Press.

Article   *Peer-Reviewed Journal*

SmartAgriChain: A Blockchain Based Solution for Agri-food Certification and Supply Chain Management

Rocha T.[1], Costa P.[1], Sousa V.[2], Coelho P.[2], Sousa F.[1], Cardoso N.[1]

[1]Fraunhofer Portugal Research, Research and Development, Porto, Portugal
[2]Zenithwings, Aveiro, Portugal

Abstract— *Management of certification issuance and product counterfeit verifications in the Agri-food supply chain are very serious and reaching problems nowadays. The currently existing management systems for this process are either outdated or have significant issues when it comes to security, trust, traceability, management or product certification. The introduction of Blockchain technology, due to its intrinsic properties, has the potential to solve identity, ownership, data temper, traceability and certification issues. This is possible due to the unique identity of each actor and signing verification at each transaction/action. The decentralized nature and constant verification of the chain state also contribute to this security and trust in the system. The proposed solution does not compromise currently existing features, but it will, however, allow all the actors to take part in the Agri-food supply chain system and constantly monitor its actions. The SmartAgriChain project intends to implement a supply chain and certification system based on Hyperledger Sawtooth that will be capable of identity management, hierarchical users/organizations, significant scalability, low costs, low energy consumption and compatibility with legacy systems. In this paper, we will explore and explain the system design and architecture in detail as well as a cost projection based on the number of nodes of the distributed system.*

Keywords— *Agri-Food, Certification, Food Traceability, Sawtooth, SupplyChain*

I. INTRODUCTION

Nowadays, supply chain systems are expanding in volume while becoming increasingly complex and global (Unno et al. 2020; Gonczol et al. 2020). However, little information about the product life cycle is available to the end-user. More importantly, actors in the middle of the supply chain may not have detailed information regarding the product's certification and origins of the raw material they use. This may allow greedy companies or individuals to hide some less correct behaviours for the sake of profit. These behaviours can range from low-quality control, product contamination, false quality certificates, unskilled or illegal workforce, among several other possibilities (Koegh et al. 2020; Tse et al. 2017). Another crucial point here is the product(s) certification reflected in the price and brand image/status. For these reasons, the need for a more transparent and traceable system in supply chain businesses is of the most importance (Unno et al. 2020; Koegh et al. 2020; Tse et al. 2017). Typically, supply chain systems are managed by a single central organization, having complete

control over what and how information is shared. Not only does this raises trust issues, as it also provides leverage to big organizations due to possession and authority over valuable information (Koegh et al. 2020). Nonetheless, this approach also represents a single point of failure for the organization, leaving the whole system vulnerable if compromised (Gonczol et al. 2020).

The field of agri-food represents one of many where supply chain management is of utmost importance. From the production of an alimentary good to its processing, distribution, retail, and finally, the end consumer, a lot can happen. It is challenging to assure the end-user of the process's full validity to ensure all quality control and guidelines were followed. This issue is crucial for premium products such as, for example, biologically grown products certification (Casado-Vara et al. 2018; Kamilaris et al. 2019). However, traceability is essential not only to the end-user but to all participating actors within the supply chain. This happens because, to produce a good product, the producer will need to make sure its raw materials are

consistent with the expected quality patterns and certification guidelines. Otherwise, the final product may have lower quality and should not be considered verified (Unno et al. 2020; Tse et al. 2017). All these issues may be traced to some complicated yet straightforward features of the system. Features such as transparency, security, trust, and accountability should not rely on a single entity.

SmartAgriChain is a project that intends to address the short falling mentioned above and focus on certification by adopting Distributed Ledger Technology (DLT), more specifically, blockchain. Blockchain is, in a very shallow description, a decentralized database for storing transaction information. It provides a complete, immutable history of transactions where entities do not have to trust each other to exchange information securely. Since it operates on a decentralized network, there is no single entity that controls the flow of information in the network, nor does it have a single point of failure. Every actor can have a stake in the network process, improving system decentralization, and requiring most of the network to validate transactions. Since each transaction must be digitally signed by the sender and verified by the network trust, traceability and transparency are naturally increased (Unno et al. 2020; Casado-Vara et al. 2018; Ghode et al. 2020). It becomes evident that blockchain technology usage in the supply chain could favour both producers and end-users theoretically. This article will lay the groundwork for SmartAgriChain with a study of the technologies available to understand the available solutions' vantages and disadvantages. We will also conduct a comprehensive cost estimation for the best candidates and proposes a solution addressing SmartAgriChain requirements.

The rest of this paper is organized as follows. Section II presents an overview of agri-food supply chains, a description of the SmartAgrichain objectives, how blockchain can be used. and DLT platforms available. The related work is specified in Section III. Next, Section IV describes the SmartAgrichain solution and architecture. Section V draws the main conclusions and points out future work.

II.   OVERVIEW

1.1. Agri-food supply chain

To better understand how DLT can solve the currently existing problems in the agri-food supply chain, it is necessary to describe how several actors should ideally interact and how the system works. Figure 1 allows us to have a high-level overview of the generic process, where several actors, processes, and interactions take part in the agri-food supply chain.
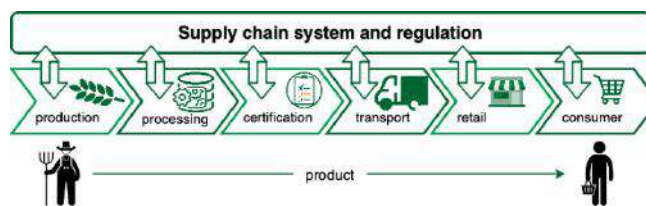

Fig.1: Generic representation of the Agri-food supply chain

Fig.1 represents a fairly complete product life cycle, however, most products and goods can have only a part of this cycle. We can briefly identify the most common actors in a complete supply chain and understand how they interact with each other and with the supply chain system and regulation. Ideally, the entities participating in the supply chain would have access to the relevant history of the products they process. This would allow them to be sure of the product origin and lifecycle. Nevertheless, most of the time, this is not the case. The actors receiving products from producers or other actors down the chain trust that the quality and type of the product given is what they expect. The supply chain information systems are not interconnected and do not work with the full process in mind. In the rare cases where we have a full-fledged supply chain system from the start of the process to the end, it will, in most cases, depend on a centralized infrastructure with all associated downsides of this architecture.

To summarize, this process needs an infrastructure where all the actors can trust the system, perform identity management, trace actions, and verify that records' immutability is assured.

1.2. SmartAgriChain proposed solution

Farmers, producers, and sellers are challenged to identify the best solution to safely and reliably interconnect all product management stages. The main goal is to identify opportunities for improvement in its operation and obtain certifications and quality guarantees in a more comfortable and faster way. SmartAgriChain aims to research and develop a solution to support the various entities related to the production and sale of agri-food products (farmers, sellers, certifying entities, etc.) in improving their supply chain processes and the respective certification of their products. The main objective of the SmartAgriChain is to investigate and develop a web-based technological solution that will combine blockchain technology with the entire process necessary for the certification of agri-food products (for example, certification of organic products), thus eliminating the excessive bureaucracy generally associated with this step and making the process faster, more transparent, safer and easily verifiable. Product certification is also an instrument that allows producers to 1) demonstrate impartially and credibly the quality, reliability,

and performance of its products, insofar as it reinforces customer confidence; 2) differentiate from the competitors; 3) increase competitiveness by reducing non-quality costs; 4) reinforce the company's image; and 5) facilitate access to new markets, showing compliance with regulatory requirements. Digital certification through blockchain technology (traceable, decentralized, and reliable) has the potential not only to facilitate everyday processes in the agri-food value chain but also to include small producers in the value chain, stimulated by the reduction of process bureaucracy, ease of access and language, in addition to reducing the transaction cost related to the other certification models currently in force. With all the mistrust in the agri-food sector regarding the origin, quality, and veracity of products, this digital tool can benefit all parties involved, valuing certified goods/foods. SmartAgriChain should include and act on all phases associated with supply chain management to transform the current chain into a more modern one, with guarantees of security, transparency, traceability, and tracking of products in all its phases. In this way, the consumer will be able to check all the information related to the product he is consuming, such as certifications and licenses acquired, information on the purchase of the seed, production methods, transport, sale, among other aspects. Therefore, the implementation of the SmartAgriChain project is based on meeting the following objectives:

- Simplify and improve the certification process for agri-food products, making it easier, faster, automated, and less paper-based
- Democratize access to certification services by small producers in the agri-food sector
- Provide a meeting point between producers and sellers seeking certification services, certifying entities, their experts, and consultancy entities in the area of certification
- Scan the certification processes
- Connect, safely and reliably, all stages of product management to identify opportunities for improvement in its operation
- Supporting producers and sellers of agri-food products in improving their supply chain management processes
- Provide the final consumer with information on certified products, giving greater confidence at the time of purchase.

To allow all existing features in legacy systems while reaching these objectives we must select a blockchain platform that provides the following requirements: (1) High transaction throughput and scalability - The network capacity to cope with an increasing demand for transactions and interactions. The supply chain management system should deal with products at least at the lot/stack level. Given this, the Supply chain management system based on blockchain technology needs to cope with many transactions, least close to the thousands per day; (2) Open-access - For the SCM ecosystem participants, this point is not relevant, but we want to allow partial access to the end-user/consumer. By doing so, we can provide any consumer with verifiable information regarding the products they buy; (3) Secure and traceable - We do not want the system to be exposed or compromised. This relates to up-time-keep, system stability, external attacks, nefarious information temper, and role-controlled access; (4) Decentralized - Not only geographical decentralization is needed. The system should also offer subjective decentralization, meaning that each actor interested should deploy a node and be part of the network's validation process. By having several copies of the system, we can be sure that there is no single point of failure; (5) Competitive cost - The cost of the infrastructure must be competitive. If the system cost is not competitive, there is no practical advantage, so the system must be cost-competitive with existing SCM solutions. Also, the cost evolution over time must be predictable and ideally constant with the system volume.

1.3. Why Blockchain

This section will dive into a high-level overview of the technical details that allow blockchain to offer a significant set of advantages needed for scenarios such as this one. First, it is essential to address the differences and similarities between DLT and Blockchain. It is often common for these two terms to be confused or regarded as one. Despite their similarities and relation, blockchain and distributed ledger are different things. A blockchain can be considered a DLT, but there are several DLTs other than blockchain.

So, what is a Distributed Ledger Technology? A DLT is a decentralized database operated and managed by multiple actors across multiple validation nodes. On the other hand, a Blockchain is a type of DLT capable of recording transactions with an immutable cryptography signature called a hash. All "transactions" (meaning changes on the database) are then grouped into blocks. Each block contains a hash of the previous block, creating a connection between them, hence the term "blockchain".
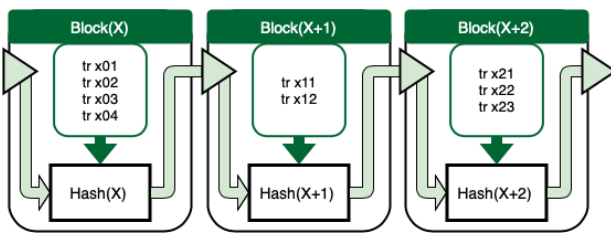
Fig.2: How blockchain achieves data immutability and integrity

For the cryptography concept details on how a blockchain works, we will analyze Fig.2. As the name indicated, a blockchain is defined by a group of blocks connected, resulting in a chain. These blocks are connected in a specific order, and once connected, they can no longer be disconnected or changed. This is the basic concept that allows for security and immutability in the blockchain. As we can see in Fig.2, for each block, two core elements exist, the transactions also referred to as payload and the hash of the block. The basic concept to assure the immutability of the chain is directly related to how each block's hash is generated:

$$hash(x) = hash((hash(x-1) + payload(x))$$

Each block hash depends on its content and the hash of the previous block. The by-product of these designs is the immutability of record, precisely what we wanted to achieve. The logic dictates that it is impossible to keep the cryptography puzzle intact for every change of record of any block. This will break the connection between the tempered block and the next one, resulting in a compromised chain and invalid records if any malicious attempt to alter past data takes place (Unno et al. 2020; Kamilaris et al. 2019; Ferrag et al. 2018; Wang et al. 2018). However, the system's malicious actor could somehow alter the target block and all the forward block on the chain to make it valid. This takes us to the second security layer of the blockchain model, decentralization. There is no central entity in a Blockchain to process the network and validate the transactions, yet every transaction within the system is considered secured and verified. This can only be possible with the usage of a consensus protocol, a core part of any blockchain network. The consensus protocol acts as a mechanism through which all peers of the network or "nodes" reach a common agreement about the current state of the distributed ledger. By doing so, the protocol can achieve reliability and trust between unknown actors within the network. At its core, the algorithm assures that every new block added to the chain is the truth and agreed by all participants. However, according to the use-case and application field, several implementations are currently available with pros and cons.

1.4. Viable blockchain platforms

Once we established the advantages of Blockchain, the following step is to study the existing platforms and solutions that will allow us to implement a solution tailored to our needs. This section studies some of the existing platforms and verifies their pros and cons to implement our proposed solution.

1.4.1. Ethereum

One of the most well-known platforms when it comes to programable blockchain solutions is Ethereum, arguably the most widespread option. Ethereum has a virtual machine, called the Ethereum Virtual Machine (EVM). The EVM allows code to be verified and executed on the blockchain, providing guarantees it will be run the same way on everyone's machine. This code is contained in Smart Contracts, chunks of logic that can have autonomous behaviour according to its programmable logic. They can interact with other Smart Contracts or with other actors in the blockchain. The state of the network, memory, and logic of the Smart Contracts is processed and confirmed in a decentralized manner. The consensus in the network is achieved currently with PoW based algorithm called Ethash. Ethereum is a proven solution from a technical standpoint with several working decentralized applications. However, at the time of writing, the currently available Ethereum network has several scaling problems and a high cost. Nevertheless, it is also the biggest public blockchain in existence.

1.4.2. Hyperledger Sawtooth and Fabric

Hyperledger is an umbrella project of open-source frameworks, tools, and libraries for enterprise-grade blockchains. It was started by the Linux Foundation and has received contributions from Intel, IBM, J.P. Morgan, Airbus, and many others. The main objective is to develop reliable and efficient blockchains to support global businesses. The several subprojects integrate different protocol and consensus algorithms as well as different ways of managing smart contracts. For this case, we will check Hyperledger Sawtooth and Hyperledger Fabric.

Sawtooth is designed for versatility and scalability. It supports both permissioned and permissionless deployments, meaning the access or participation of all users to the blockchain is optional. With its distribution, a novel consensus algorithm is included, Proof of Elapsed Time (PoET). However, in this implementation, the consensus algorithm is interchangeable. PoET, however, targets mostly distributed validator populations with minimal resources. Transactions business logic is decoupled from the consensus layer and abstracted into Transaction Families. Due to this architectural characteristic it currently offers various programming

languages for development such as Python, Go and JavaScript, Java, C++, and Swift. Currently, Hyperledger Burrow integration allows for Ethereum Smart Contract support. Sawtooth currently has several deployed enterprise applications with a wide range of fields.

On the other hand, also from Hyperledger, we have Fabric. The main differences between Sawtooth and Fabric are that Fabric only supports permissioned blockchains and that there is no concept of transaction families. Smart Contracts are quite similar to Ethereum ones. Fabric presents a new architecture in smart contracts execution. Most existing smart-contract blockchain platforms follow an order-execute architecture: each transaction is validated, ordered, and sent to all nodes; only after does each node execute the transaction. This means that smart contracts must be deterministic. Otherwise, a consensus might not be reached. Usually, this ends up being forced with a DSL.

1.4.3.   Other considered platforms

Other platforms could be used for the proposed scenario. However, some of the other considered candidates are either in early development phases and are not yet ready to be used, such as Cardano, or offer no significant advantages over the Hyperledger solutions as the case of R3 Corda.

III.   RELATED WORK

Before explaining our solution, it is essential to explore and understand previous studies and the work conducted in the same field. This section will study and evaluate the similarities and differences that SmartAgriChain is meant to have compared to some other implementations in the same field, or at least with a similar purpose and the same technology.

The first case study (Shahid et al, 2020) is relatively recent and involves a solution with the Ethereum blockchain. This solution uses the InterPlanetary File System (IPFS) as a base for raw data storage. However, the Ethereum blockchain is used as a confirmation method for the IPFS content using data hashes from the stored content. By doing so, the validity of the data that is off-chain can be confirmed. Since Ethereum is a public blockchain, to control user access, a smart contract was developed to manage new user registrations and allow these new users to take part in the process according to their role. The 3 SmartContracts employed in this solution will assure user identity when interacting with the system, store the hashes of each action in the process, and interact with the IPFS. This system works as intended and can scale to a degree. It seems to be an excellent approach to offload work from the main chain. However, it is necessary to address the fact that even though the transactions and interaction with the Ethereum

blockchain are minimal and the cost estimations in this case study are realistic, the price of Ethereum GAS and the price of the Ethereum token are largely outdated. If the same study were conducted at the date of writing this article, the costs would not be practical.

Kamilaris (Kamilaris et al. 2019) study is based on the same agri-food field as the one previously mentioned. However, it has a less practical approach and instead studied existing projects in agriculture, food, and supply chain to employ blockchain solutions. This study reached 49 different projects/initiatives where the used blockchain implementations ranged from Ethereum, Hyperledger Fabric, Hyperledger Sawtooth, and some proprietary implementations. They concluded that the usage of this type of solution has real advantages and increasing potential. The technology is still in its infancy, but with new developments, tools, and solutions, the implementation process will be improved. The most used technology in this case study was Ethereum.

Another related but slightly different scenarios are the ones with a focus on IoT devices (Caro et al. 2018; Ferrag et al. 2018). These studies, while still applied to agri-food goods' supply chain management, this scenario does not focus on the chain actors as entities. Instead, it mainly intends to deploy a solution that allows tracking product information with IoT devices along the supply chain. Information such as temperature, humidity and light. Caro et al specify a practical scenario where the authors deployed two proof of concept solutions using Hyperledger Sawtooth and Ethereum. In conclusion, the authors point out the different advantages and disadvantages of each implementation. For Ethereum, in some cases, it may be convenient to trade off the high latency of Ethereum with its scalability and reliability since it enables larger numbers of participants and the platform at the time was significantly more mature than Sawtooth. On the other hand, Sawtooth offered a significant range of development languages when compared to Ethereum. It also offers significantly faster transaction times, higher scalability and significantly lower costs to operate. It also does not require as much computational power since it offers a novel consensus algorithm, more suitable for low-end devices. Nevertheless, the level of decentralization is not even close to what Ethereum achieves.

Baralla (Baralla et al. 2019) also applied a case study to the food supply chain scenario developed with Hyperledger Sawtooth. This paper intends to create a solution from-farm-to-fork capable of being integrated into existing chains and legacy processes while allowing full traceability of goods. It points out the separation between the application level and the core system as a significant sawtooth advantage that focuses exclusively on defining the rules and

application logic. The paper itself is the initial development steps for an ongoing project, and as such, it still does not offer details regarding transaction throughput, scalability, and costs. Furthermore, it has a high level of system specification, architecture, workflow, and requirements for this use case and shows that Hyperledger Sawtooth is highly flexible to this end.

A very interesting article regarding sawtooth scalability and performance specifics by Ambel (Ampel et al. 2019) reveals very interesting and promising results. With a maximum of 2300 transactions per second achieved using a cost-effective setup composed of a Ryzen 1600x 6 core CPU and 16Gb Ram. Other interesting metrics are the memory usage versus the transaction numbers and transaction execution times averaging less than a second. Nevertheless, the results achieved are pretty encouraging and show that the Sawtooth platform is completely able to handle the throughput needed for our scenario.

IV. COST AND VIABILITY STUDY

Based on the existing platforms and previous case studies, we selected the solution that best fits our requirements and is currently mature enough to start the implementation. Due to its maturity and proven technical record we decided to use Ethereum as the public blockchain platform since it checks all the technical requirements and is perfectly capable of executing the use cases for the agri-food certification and supply chain scenarios. From the private/enterprise blockchain side, Fabric and Corda are not public. Given this, we will decide on Hyperledger Sawtooth.

Once established the technical capability for these 2 platforms it is extremely important to verify the business scalability and cost viability. Both overtime and by volume. For the purpose of this study, we will assume a base scenario and then linearly extrapolate the costs for the Ethereum and Sawtooth hypothesis.

Note that due to the differences in the proposed platforms Ethereum has a "pay to use" based policy where every non-read interaction with the blockchain is charged as a transaction fee. On the other hand, Sawtooth, as a deployable network does not have a token or transaction associated costs. However, it has the associated costs of deploying the network nodes by the interested participating agents. These costs range from hardware costs, network costs and maintenance.

The test scenario does not need to be very specific to have an idea of the costs for each platform. So, we will use a basic scenario with a simple smart contract for Ethereum with 1

interaction per user per day and a network of up to 100 nodes for Hyperledger sawtooth.

1.5. Ethereum

Since this is a best-case scenario, we will use a very simple Smart Contract deployment:

```
contract Storage {
    uint32 number;
    function store(uint32 num) public {
        number = num;
    }
    function get() public view returns (uint32){
        return number;
    }
}
```

To overview the process's cost in the Ethereum network, we first need to know how it works. For the eth blockchain, some concepts need to be addressed:

- Gas: Refers to the fee, or pricing value, required to conduct a transaction or execute a contract successfully.
- Gas price/gwei: Is a denomination of the cryptocurrency ether (ETH) used on the Ethereum network to buy and sell goods and services. Gwei is the most used unit of ether because gwei can specify Ethereum gas prices easily.
- Gas limit: The maximum amount of Gas that a user is willing to pay for a given action.

Other than these base concepts, we need to understand how the Ethereum Virtual Machine works and processes costs. Without going into specific calculations, some base costs are fixed, and others depend on the contacts' deployment and execution. We can divide them into two categories:

- Base transaction costs: are based on the cost of sending data to the blockchain. Four items make up the full transaction cost: 1) Base cost of a transaction 21000 gas; 2) Base cost of a contract deployment 32000 gas; 3) Cost for every zero bytes of data or code for a transaction; 4) Cost of every non-zero byte of data or code for a transaction.
- Execution costs: are based on the cost of computational operations executed as a result of the transaction.

Based on this we can verify the current gwei and Ehtereum's token price to estimate the price of a given transaction or SmartContract deployment. The gwei is not static. It will depend on the network usage. The Ethereum token price is also variable according to the Market. We will use the first eth/gwei prices we have tested and the current ones to have 2 distinct scenarios in Table 1.

Table 1: Ethereum gas and token prices for September 16 th, 2020 and Match 18th, 2021

Date	high priority gwei	avg priority gwei	low priority gwei	Ethereum $
September 2020	139	146	160	365
March 2021	79	91	99	3410

The priority represents how fast the transaction can be processed when compared to the other network request. For simplicity, we will use the avg priority values in our calculations. Based on an IDE such as remix[1], we can check the cost in Gas of each smart contract deployment or call. For this cost evaluation purpose, we will use the smart contract mentioned above.

Table 2: Transaction costs for sample SmartContract deployment and interaction

	SC deployment	SC interaction
base cost	71632 gas	21464 gas
execution cost	51705 gas	21101 gas
total cost	123337 gas	42565 gas

Table 2 represents the costs associated with the sample SmartContact. With this data, we can calculate the actual cost of interaction in USD. To do so we can use the following formulas:

$$EthCost = GasPrice * GasConsumed * 10^9$$

$$Cost\ \$ = EthCost * EthPrice$$

With this information, we can create a table with the overall costs for this SmartContract deployment and interaction on both dates.

Table 3: Cost per interaction for the deployment and Interaction scenarios at 2 distinct dates

Date	Scenario	Cost
September 2020	Deployment	6.57 $
	Interaction	2.26 $
March 2021	Deployment	38.27 $
	Interaction	13.2$

At this point, with the data available in Table 3 we can already see the costs of Ethereum network usage are at a non-competitive level. However, if we extrapolate a single

smart contract deployment and an increasing number of users with 1 interaction/day we have the following results:

Table 4: Daily cost estimation for N users with SmartContract deployment included

users	September 2020	March 2021
1	7.83 $	51.47 $
10	28.17 $	170.27 $
100	231.57 $	1358.27 $

In Table 4 we can verify that the daily costs in September 2020 were already noncompetitive. However, with the current situation, an Ethereum Mainnet based solution is completely unpractical from the cost standpoint.

1.6. Hyperledger Sawtooth

Unlike Ethereum, Hyperledger sawtooth does not have a direct token cost associated with a transaction. Sawtooth doesn't even have a token. This is because unlike Ethereum there is not a publicly available Mainnet. The stakeholders of the system must do the hardware deployment with multiple nodes/servers to construct the network. So, instead of counting the cost of an estimated number of transactions, we will evaluate the cost of deployment per month of N nodes. Note that the greater N is, greater the cost, but also more decentralized the network will become. Later on, we will also need to check the scalability of the solution based on the number of users. Since running a sawtooth node using Proof of elapsed time is not computationally intensive, we can assume that all the actors participating in the validation process will not need expensive hardware. Some research articles even managed to have a Sawtooth node running in Raspberry Pi's (Kromes et al. 2019).

For the sake of simplicity, we will assume DigitalOcen's listing of 2 CPU cores, 4GB of memory, and 80GB of storage at 20$ monthly. This system is more than enough to run a Sawtooth Node. Tests executed in-house in a Virtual Machine system with half of those requirements allowed for 200+ transactions per second.

Table 5: Cost estimation for Hyperledger Sawtooth based on the number of nodes

Deployed nodes	Daily cost	Monthly cost
3	2 $	60 $
10	6.6 $	200 $
100	66.6 $	2000 $

---

[1] https://ethgasstation.info/

Table 5 shows a cost estimation for hyperledger sawtooth based on the number of nodes. When comparing to Ethereum, the costs on a Sawtooth network are significantly more competitive. The minimum number of nodes needed to run a Poet consensus algorithm is 3. So, we cannot deploy the network with less than 3 Nodes. Also, it is important to mention that, unlike Ethereum, the cost only increases with the number of deployed nodes. Not the number of users. Nevertheless, there are several implementations of sawtooth capable of 1000 transactions per second (TPS) (Ampel et al. 2019). This would easily be able to handle 10000+ active users. In our specific case, due to the possible complexity of the transactions, however, there are no guarantees we would achieve the same.

Another interesting aspect of this network is that due to the volume capabilities we could even enable IoT devices integration to track aspects such as humidity, temperature, location or even light. It is currently not in the scope of this project, but it is a nice future development.

V. PROPOSED SOLUTION

In this section, we will discuss details of the sawtooth implementation for SmartAgriChain. In Hyperledger Sawtooth the network nodes deployment can be progressive. This means that as soon as the network has the required 3 nodes it will be operational and extra nodes can be added over time.

Fig.3 shows a basic representation of a sawtooth network deployment. There we can clearly see the current node and the connections with other nodes representing the entire distributed network. A node can also contain a REST API to connect with clients and serve as a gateway to the blockchain contents. The Transaction Processor is the core part where we have the SmartAgriChain field-specific logic. It contains the rules of our system and processes the logic needed for our application. The Sawtooth design allows several transactions processors in the same network, allowing several applications to be used. For instance, our network deployment could also accommodate other application running in the same blockchain but utilizing a different Transaction Processor.
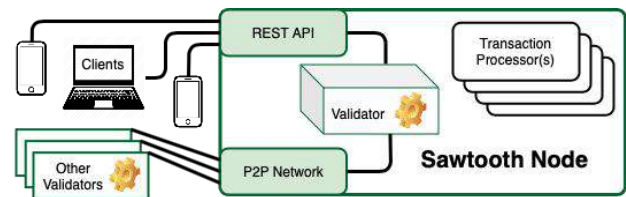
Fig.3: Hyperledger Sawtooth network layout and interactions

Given this network morphology now we need to specify the transaction workflow and structure to be able to map it to the logic we need for the supply chain and certification use-cases.
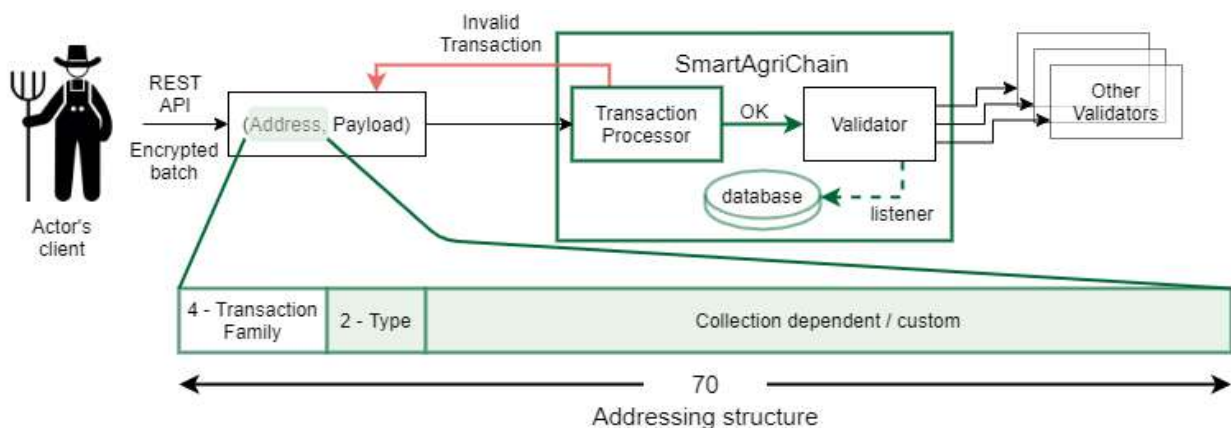
Fig.4: High-level overview of the proposed sawtooth implementation

Sawtooth stores the transactional data in a Merkle-Radix tree – using LMDB[2] as the underlying database – where each node can be accessed by an address of 70 hex characters. So, for this purpose, we need to map all existing records in this address space. **Error! Reference source not found.** represents a high-level overview of the proposed sawtooth implementation.

Each address then contains data in a format specified by the system. There is no specific method for building these addresses in Sawtooth. However, as recommended in their documentation, some structure is desirable since it can provide an intuitive way of uniquely mapping an object to an address. Typically, this is done using either the hex-encoded hash values of each stored object's main fields or

---

[2] http://www.lmdb.tech/doc/

a unique pre-defined hexstring for each type. Independent of the chosen method, all addresses must contain a unique prefix of 6 hex-characters corresponding to the transaction family. This will define which Transaction Processor is used to process the transaction. One way of doing this is to hex-encode the name of the developed transaction family.

Table 6: Addressing model for SmartAgriChain collections

Collection	Transaction Family	Collection ID	Dependent on the collection	
Organization		ae #2	orgID #62	
Agent	4c0aba #6	ed #2	orgID #31	agentID #31
Certification		ac #2	orgID #20	agentID #20 · certID #22
Product		ec #2	agentID #31	productID #31

To start explaining how the addresses are generated we will define the collections needed for our application. We defined 4 core collection types: Organizations, Agents, Certifications and Products. These collections represent the core logic and information needed by the system to hierarchically control access to the collections and allow actors to execute certain actions according to their role. For instance, a certification process can only be validated by a certification entity. To be able to access data based on the addressing structure of Sawtooth we defined addressing model available in **Error! Reference source not found.**.

We can easily explain this table with the following product example:

Table 7: Address generation example for a product

4c0aba	ec	sha(agent,31)	sha(product,31)
4c0abaeccf9009bd1cfaa91b4e87ca81746bfb3994a70d6a1bd 9f087414804c914694d			

As seen in Table 7 we use a cryptographic hash function with the agentID and productID and use the first 31 characters to generate a given part of the address. Note that the total length of the address is always 70! If we wanted, for instance, to query all products of a specific agent we would use a partial address without the final product part, and the Sawtooth API would return a list of available products in that partial address.

Since queries for sawtooth use the first variable number of elements of an address this structure easily allows us to have access to the following queries:

- All records from an agent
- All certifications assigned to an organization
- All agents of an organization
- All certifications of a specific agent

Other complex queries can also be executed but not directly with Sawtooth addressing. If in the future the queries needed become too complex or unpractical a local database listening to ongoing accepted transactions can be implemented at each accessing node and replicate the blockchain state. This database can be verified with the blockchain at any point in time to assure coherence.

The addressing part of the implementation is then covered. However, we still need to explain exactly how these addresses will map to collections and data. To do so, in our implementation we used Protobuf[3] and created a ProtoFile for each collection we use. Protobuf is an open-source, platform-independent tool used to serialize data structures like JSON. However, Protobuf offers several advantages when it comes to processing time and data volume. To prevent the unlikely scenario where we may have to change the same address for 2 different collection instances, we implemented a hash collision failsafe system that will store a list of collections on each address.

Currently, the system is being tested for deployment, so we can only provide preliminary results when it comes to the network capabilities regarding transactions per seconds (TPS) and response time. Tests revealed an average transaction confirmation time of less than 1 second and a maximum of 3 seconds. Also, in tests, the transactions throughput achieved was well above 200 TPS but note that this number may have significant variation based on the

---

[3] https://developers.google.com/protocol-buffers

verifications, access and queries needed by the Transaction Processor for each specific request.

VI. CONCLUSION AND FUTUTRE WORK

With the study and architecture presented along with this article for SmartAgriChain, we believe blockchain technology should be part of the future of agri-food supply chain management and certification. It can provide all the features needed while adding value to the solution itself and the actors. It can also be done with concrete and acceptable costs for system usage and implementation. The current tools and platforms are not yet fully matured but are evolving rapidly and already allow for a complete implementation. Public blockchains do not provide the scalability and cost prediction needed for a no-compromise solution such as this one. This article represents an ongoing effort of development for SmartAgriChain, with a partially implemented solution that allows legacy systems to interact with our system via a rest API. The logic on the transaction processor is also not yet fully implemented but already allow for most operations needed in Agri-food management and certification of the use-cases. SmartAgriChain combines blockchain technology with the management of producers' supply chains, transforming the current chain into a more modern one with guarantees of security, transparency, traceability, and tracking of products in all its phases. In turn, consumers will have at their disposal a platform where they can confirm, quickly and easily (for example, using a smartphone), whether the products they buy respect the principles of sustainable agriculture and conscious consumption, principles that can be attested via a certification. It is increasingly proven that bringing consumers and producers closer together through technology will allow new food consumption forms/products. Besides, the platform will have mechanisms to simplify and improve the agricultural-based products' certification process, not being tied to a single type of certification. In the following phase, SmartAgriChain also intends to be a meeting point between producers and points of sale looking for certification services, the certifying entities responsible for all the management of the certification process in force, the respective certification experts and/or consulting entities in the area of certification or production. A kind of marketplace that aims to provide innovative components and blocks to serve as a basis for producers and sellers in the agri-food sector to improve their supply chain and the chances of certifying their products.

From a more technical point of view, it should be noted that this blockchain-based network provides high scalability to the system and guarantees security, transparency,

decentralization and traceability. Likewise, another focal point of this project is simplifying and digitalizing the procedural mechanisms of certifying an agri-food product. Since we provide a rest API based solution this can be achieved without dealing directly with blockchain logic, using a layer of abstraction instead. However, this mechanism still needs more research to find out exactly how information is shared between all stakeholders of the SmartAgriChain platform to guarantee its operation in a production environment. Blockchain technology already provides a mechanism to ensure that all stakeholders have access to information. Though, it is necessary to define mechanisms for access to information and associated knowledge. To this end, auxiliary mechanisms to the network based on blockchain will be investigated to provide controlled access to data, thus ensuring more transparency and security in the certification process.

REFERENCES

[1] Unnu, K., Pazour, J. A., Megahed, A., Narayanaswami, C. Blockchain Enabled Supply Chains: Classification, Decision Framework (2020). IIE Annual Conference. Proceedings , 483-488

[2] Gonczol, P., Katsikouli, P., Herskind, L., Dragoni, N. (2020). Blockchain implementations and use cases for supply chains-a survey. Ieee Access, 8, 11856-11871.

[3] Keogh, J. G., Rejeb, A., Khan, N., Dean, K., Hand, K. J. (2020). Data and food supply chain: Blockchain and GS1 standards in the food chain: a review of the possibilities and challenges. Building the Future of Food Safety Technology, 145.

[4] Tse, D., Zhang, B., Yang, Y., Cheng, C., Mu, H. (2017, December). Blockchain application in food supply information security. In 2017 IEEE International Conference on Industrial Engineering and Engineering Management (IEEM) (pp. 1357-1361). IEEE.

[5] Casado-Vara, R., Prieto, J., De la Prieta, F., Corchado, J. M. (2018). How blockchain improves the supply chain: Case study alimentary supply chain. Procedia computer science, 134, 393-398.

[6] Kamilaris, A., Fonts, A., Prenafeta-Boldú, F. X. (2019). The rise of blockchain technology in agriculture and food supply chains. Trends in Food Science & Technology, 91, 640-652.

[7] Ghode, D., Yadav, V., Jain, R., & Soni, G. (2020). Adoption of blockchain in supply chain: an analysis of influencing factors. Journal of Enterprise Information Management.

[8] Ferrag, M. A., Derdour, M., Mukherjee, M., Derhab, A., Maglaras, L., Janicke, H. (2018). Blockchain technologies for the internet of things: Research issues and challenges. IEEE Internet of Things Journal, 6(2), 2188-2204.

[9] Wang, W., Hoang, D. T., Xiong, Z., Niyato, D., Wang, P., Hu, P., Wen, Y. (2018). A survey on consensus mechanisms and mining management in blockchain networks. arXiv preprint arXiv:1805.02707, 1-33.

[10] Shahid, A., Almogren, A., Javaid, N., Al-Zahrani, F. A., Zuair, M., Alam, M. (2020). Blockchain-Based Agri-Food Supply Chain: A Complete Solution. IEEE Access, 8, 69230-69243.

[11] Caro, M. P., Ali, M. S., Vecchio, M., Giaffreda, R. (2018, May). Blockchain-based traceability in Agri-Food supply chain management: A practical implementation. In 2018 IoT Vertical and Topical Summit on Agriculture-Tuscany (IOT Tuscany) (pp. 1-4). IEEE.

[12] Baralla, G., Pinna, A., Corrias, G. (2019, May). Ensure traceability in European food supply chain by using a blockchain system. In 2019 IEEE/ACM 2nd International Workshop on Emerging Trends in Software Engineering for Blockchain (WETSEB) (pp. 40-47). IEEE.

[13] R. Kromes, L. Gerrits and F. Verdier, "Adaptation of an embedded architecture to run Hyperledger Sawtooth Application," 2019 IEEE 10th Annual Information Technology, Electronics and Mobile Communication Conference (IEMCON), 2019, pp. 0409-0415, doi: 10.1109/IEMCON.2019.8936264.

[14] B. Ampel, M. Patton and H. Chen, "Performance Modeling of Hyperledger Sawtooth Blockchain," 2019 IEEE International Conference on Intelligence and Security Informatics (ISI), 2019, pp. 59-61, doi: 10.1109/ISI.2019.8823238.

OJS Editorial and Publishing Process

Author	Editor and Section Editor	Journal Manager, Reviewer, Copyeditor, Layout Editor, and Proofreader

Submission
Author uploads file to journal Web site, and enters metadata for OAI indexing

1. Submission Queue
Editor assigns submission to Section Editor to see through the editorial process

Journal Management
Setup and configure journal; enroll editors, reviewers, copyeditors, proofreaders.

Submission Review
Author can track process, see files, reviews, revise and resubmit, at editor's request

2. Submission Review
a) Check submission
b) Conduct peer review
c) Reach editorial decision

Select Reviewers
Editor invites reviewers from database with interests, load

Review Conducted
Reviewer submits review and recommendation (which may be rated by editor)

Submission Editing
Authors reviews copyedits and proofreads galleys

3. Submission Editing
a) Copyedit submission
b) Layout of formatted galleys
c) Proofread galleys

Copyediting

Layout
Layout Editor prepares galleys in HTML,PDF. PS, etc.

4. Issue Management
a) Create issues
b) Schedule submissions
c) Organize Table of Contents

Proofreading

Key

Journal Manager	
Editor	
Section Editor	
Reviewer	
Copyeditor	
Layout Editor	
Proofreader	
Author	
Reader	

Open Journal Systems is an open source development of the Public Knowledge Project.

http://pkp.sfu.ca

5. Publication
Offering immediate open access or delayed open access, with subscriptions

Submission Archive
Complete records kept of submission process for published and declined items

Current Issue
Items appear with Reading Tools linking to related internal and external resources

Journal Archive
Browsing issues, with indexing by OAI search engines, as well as Google, etc.

~OJS Workflow~

Important links:

Paper Submission Link:

OJS:

https://ijeab.com/ojs/index.php/ijeab/about/submissions

https://ijeab.com/submit-paper/

Editorial Team:

https://ijeab.com/editorial-board/

Peer Review Process:

https://ijeab.com/peer-review-process/

Publication Ethics:

https://ijeab.com/publication-policies-and-ethics/

Author Guidelines:

https://ijeab.com/author-guidelines/

Join Us a Reviewer:

https://ijeab.com/join-us/

Journal Indexed and Abstracted in:

- Qualis-CAPES -Brazil
- Normatiza (Under Review)
- Bielefeld Academic Search Engine(BASE)
- Aalborg University Library (Denmark)
- WorldCat: The World's Largest Library Catalog
- Semantic Scholar
- J-Gate
- Open J-Gate
- CORE-The world's largest collection of open access research papers
- JURN
- Microsoft Academic Search
- Google Scholar
- Kopernio - powered by Web of Science
- Pol-Index
- PBN(Polish Scholarly Bibliography)Nauka Polaska
- Scilit, MDPI AG (Basel, Switzerland)
- Tyndale University College & Seminary

- indiana Library WorldCat
- CrossRef DOI-10.22161/ijeab
- Neliti - Indonesia's Research Repository
- Journal TOC
- Dimensions.ai: Re-imagining discovery and access to research
- Citeseerx
- Massachusetts Institute of Technology (USA)
- Simpson University (USA)
- University of Louisville (USA)
- Biola University (USA)
- IE Library (Spain)
- Mount Saint Vincent University Library (Halifax, Nova Scotia Canada)
- University Of Arizona (USA)
- INDIANA UNIVERSITY-PURDUE UNIVERSITY INDIANAPOLIS (USA)
- Roderic Bowen Library and Archives (United Kingdom)
- University Library of Skövde (Sweden)

- Indiana University East (campuslibrary (USA))
- Tilburg University (The Netherlands)
- Williams College (USA)
- University of Connecticut (USA)
- Brandeis University (USA)
- Tufts University (USA)
- Boston University (USA)
- McGill University (Canada)
- Northeastern University (USA)
- BibSonomy-The blue social bookmark and publication sharing system
- Slide Share
- Academia
- Archive
- Scribd
- SJIF-InnoSpace
- ISSUU
- Research Bib
- DRJI
- journal-repository

Infogain Publication

International Journal of English, Literature and Social Science (IJELS)