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FOREWORD

I am pleased to put into the hands of readers Volume-8; Issue-5, September-October 2023 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
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Studies on chemical, physical and microbiological quality characteristics of Barki sheep burger fed on medicinal plants with yeast

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Abstract— *The current study aimed to evaluate the effect of dietary supplementation of medicinal plants with yeast on the fatty acid profile, chemical, physical and microbiological quality of Barki sheep burger during frozen storage at -20 °C for 90 days. Fifteen Barki lambs (13.46 ± 3.47 kg body weight, 4.5 months of age) were randomly divided into three groups (5 lamb/group). T1: control group fed on berseem hay and concentrate feed mixture. T2: fed on control diet + air dried powder of medicinal plants mixture which consisted of (garlic, cumin, ginger and turmeric). T3: fed as group two with (Saccharomyces cerevisiae). Results indicated that addition of yeast to medicinal plants significantly decreased ($p<0.05$) palmitic acid, myristic acid and increased the content of oleic acid and decatrienoic acid with non-significant effect on linoleic acid and α linolenic acid. Burger of medicinal plants with yeast group had the lowest index of atherogenicity (IA) which is healthier for human consumption. Feeding type had a slight significant effect on pH values of fresh sheep burger. No significant differences were found in shear force values of sheep burger. Cooking loss of sheep burger processed from sheep fed on medicinal plants recorded the lowest cooking loss, followed by sheep burger of medicinal plants with yeast. Burger of medicinal plants group showed the lowest TBA value. Feeding types and storage conditions significantly ($p<0.05$) affected on color parameters (L*, a* and b*). No significant changes in microbiological quality of sheep burger during frozen storage. In conclusion, sheep burger processed from lambs fed on diet supplemented with medicinal plants and yeast improved the composition of fatty acids, delayed the lipid oxidation and decreased the cooking loss without any negative effects on color parameters, shear force and microbial analysis which is subsequently affected on sheep burger quality and human health.*

Keywords— ***sheep burger; medicinal plants; yeast; quality characteristics***

I. INTRODUCTION

Sheep is Known as excellent source of protein, fatty acids, amino acids, vitamins and minerals, beside its unique flavor. Barki sheep breed is also commonly known for its tolerance to arid and semi-arid environmental conditions. For such advantages, sheep production is becoming increasingly important in Egypt because of sheep meat can be contributed in solving the problem of animal protein deficiency for human consumption [1].

Sheep meat quality is affected by many factors including, age, breed, sex, slaughter weight and feeding type [2].

Diet is the most important factor that influence on the fatty acids profile of animals' meat. In this regard, it is possible to enrich lamb meat and provide high quality products for consumers by modification sheep diet [3]. Recently, one of the feeding strategies to improve animal production is phytogenic additives, using of natural substances has a beneficial impact on animal health, animal products (milk and meat) quality and subsequently human health [4].

Medicinal plants can be used as natural feed additives to improve feed efficiency and growth rate [5]. Probiotics could enhance growth performance by improving rumen fermentation and increasing the uptake of nutrients which is resulting in increased yield of livestock products.

Yeast (*Saccharomyces cerevisiae*) is a commonly used probiotic in ruminant production to improve feed efficiency by increasing nutrients availability and utilization resulting in increased animal products' yield such as wool, milk and meat [6].

Despite these advantages, there is a lack of information available regarding the efficacy of feeding animals on medicinal plants alone or combined with yeast on the quality of meat products processing. Thus, this filed of research needs more investigations on how to modify and enrich meat to achieve meat products that meet consumer's desire.

Therefore, the objectives of the current study were to evaluate the effect of addition medicinal plants and yeast to sheep diets on fatty acid profile, chemical, physical and microbiological quality of sheep burger during frozen storage.

II. MATERIALS AND METHODS

The experimental procedures were approved by Animal and Poultry Nutrition Department and Animal Breeding Department, Poultry Production Division, Desert Research Center.

2.1 Location

The current study was conducted at Maryout Research Station belonging Desert Research Center, located 35km south west of Alexandria (Latitude 31.02 °N and Longitude 29.80 °E), Egypt.

2.2 Experimental design

Fifteen Barki lambs (4.5 months age, 13.46 ±3.47 kg body weight) were used. Lambs were randomly divided into three feeding groups (5 lamb/group). T1: the control group fed on berseem hay (*Trifolium alexandrinum*) and concentrate feed mixture (CFM) without additives. T2: fed on control diet + 1% air dried powder of medicinal plants mixture which consisted of garlic (*Allium sativum*), cumin (*Cuminum cyminum*), ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) with equal ratio (1:1:1:1). T3: fed as group T2 + yeast (*Saccharomyces cerevisiae*) 5 gm /head/ day. All animals were fed on 1.5 % of LBW hay + 2.5% of LBW concentrate according to NRC [7] requirements. At the end of the experiment period (224 days), all lambs were fasting for 12h and slaughtered at average final body weight 36.72 ± 5.70 kg. The hot carcass weighted and kept at 4 °C overnight, then weighed again.

2.3 Preparation of sheep burger

Carcasses of each feeding treatment were deboned with a sharp knife. Lean meat and fat were separately minced and weighted. Three batches of each feeding treatment were used in burger processing. Each batch was mixed with salt (1%, w/w) and black pepper (0.1%, w/w) and handily mixed and formed by using manual burger press machine (Metaltex No.25.17.25). Sheep burgers (1cm thickness, 10cm diameter and 65±2g weight) were placed in plastic foam trays packed in polyethylene bags and frozen at -20 ºC±1until further analysis.

2.4 Chemical analysis

2.4.1 Fatty acid profile

Fatty acid profile of raw sheep burger was determined as described by Folsch et al [8]. The fatty acids are methylated with boron trifluoride in methanol, extracted with heptane and determined by using a gas chromatograph (GC/MS/MS Agilent 7000, Germany) with FID detector (PE Auto System XL) with an auto-sampler and Ezchrom integration system. Carrier gas (He); ca. 25Psi- air 450ml/min- Hydrogen 45ml- split100 ml/min.

2.4.2 TBA value

Assessment of lipid oxidation in raw sheep burger samples was determined as described in Oil Chemists' Society AOCS [9] by measuring 2- thiobarbituric acid reactive substances (TBARS). Results were expressed as (µmol TBARS/g).

2.5 Physical analysis

2.5.1 pH value

Estimation of pH values in raw sheep burger were assessed according to Khalil [10] by using a digital pH-meter (Jenway 3320 conductivity and pH meter, England).

2.5.2 Cooking measurements

Sheep burger were cooked in a preheated oven for 15 min each side. Cooking loss was determined by using the following equation according to Naveena et al. [11] as follows:

$$\text{Cooking loss (\%)} = \frac{(\text{Uncooked sample weight}) - (\text{Cooked sample weight})}{(\text{Uncooked sample weight})} \times 100$$

2.5.3 Shear force value

Cooked sheep burger shear force was determined by using Instron Universal Testing Machine (Model 2519-105, USA) for three times at different positions. Results were expressed as (Kg/f).

2.5.4 Color measurements

Color measurements (*L**, *a** and *b**) of raw sheep burger were evaluated according to CIE [12] by using Chroma meter (Konica Minolta, model CR 410, Japan). Lightness (*L** value), redness (*a** value) and yellowness (*b** value). Color measurement data for the samples was under standard illuminant D65 and 10º observer. The average was obtained of three spectral readings at different locations for burgers of each treatment.

2.6 Microbial analysis

The microbiological quality of raw sheep burger was evaluated at 0, 30, 60 and 90 days of storage at -20 °C. Total bacterial and psychrophilic bacteria counts were estimated as described by Ercolini et al. [13]. Molds and yeasts were estimated according to NMKL [14]. Results were expressed as log of Colony Forming Unit (log CFU/g).

2.7 Statistical analysis

All data were analyzed using statistical analysis system SAS [15]. Two- way ANOVA was applied for physical analysis (pH, shear force, cooking loss and color measurements), TBA and microbiological analysis. One – way ANOVA was applied for fatty acid.

III. RESULTS AND DISCUSSIONS

3.1 Fatty acid profile

Fatty acids profile of sheep burger processed from sheep fed on different types of medicinal plants and yeast is presented in Table 1. It was observed that feeding type had no significant effect on capric acid (C10:0), lauric acid (C12:0), pentadecanoic acid (C15:0), heptadecanoic acid (C17:0), stearic acid (C18:0). However, supplemented diets with yeast significantly decreased ($p<0.05$) palmitic acid (C16:0) and myristic acid (C14:0). Contrariwise, addition of yeast to diet increased the content of oleic acid (C18:1ω9) and decatrienoic acid (C16:3ω4). On the other hand, feeding types had no significant effect on linoleic acid (C18:2ω6) and α linolenic acid (C18:3ω3). However, burger of sheep fed on diet containing yeast had the higher ∑UFA, MUFA/SFA and UFA/SFA than the other feeding groups. On the other hand, burger of yeast group had the lowest index of atherogenicity (IA) which is healthier for human consumption. These results are constant with Moreno-camarena et al. [16] result, where they found that dietary supplement with different levels of chromium-yeast decreased the palmitic (C16:0) and stearic (C18:0), while palmitoleic (C16:1n-7), vaccenic (C18:1n-7), linoleic (C18:2n-6) and arachidic (C20:4) fatty acids increased in lamb meat. Addition of yeast in sheep diets significantly decreased ($p<0.05$) the content of saturated, increased the content of monounsaturated and total of unsaturated fatty acids. Milewski et al. [17] found that meat of sheep fed on *Saccharomyces cerevisiae* (Inter Yeast® S brewer's yeast) had the lower palmitic acid (C16:0) and higher linoleic acid (C18:2ω6). Similar results were found by Milewski and Zaleska [18]. On the other hand, Rodríguez-Gaxiola et al. [19] found that supplemented diet with enriched-chromium yeast increased palmitic acid and decrease vaccenic acid in Rambouillet lamb meat. In addition, enriched-chromium yeast had no significant effect on the total content of saturated, unsaturated and unsaturated/saturated fatty acid ratio. Regards to feeding on medicinal plants, the results of the current study contradict the findings of Redoy et al. [20] they found that sheep meat of herbal-supplemented feeding groups had lower saturated fatty acids and higher unsaturated fatty acids content compared with the control diet group. Data of atherogenic index of sheep burger came in accordance with the results of [16] they found that significant decrease was found in the index of atherogenicity in sheep *Longissimus dorsi* as the level of yeast in the diet increased. Similar results were found by [17].

Table 1 Fatty acids profile of sheep burger

Fatty acids		T1	T2	T3	SEM
Capric acid	C10:0	0.71	0.71	0.11	0.20
Lauric acid	C12:0	0.94	0.89	0.12	0.27
Myristic acid	C14:0	4.41	4.79	2.41	0.66
Pentadecanoic acid	C15:0	1.13	1.14	1.22	0.13
Palmitic acid	C16:0	25.60^{ab}	29.37^{a}	22.96^{b}	1.13
Heptadecanoic acid	C17:0	2.83	2.38	2.22	0.27
Stearic acid	C18:0	21.81	20.92	23.26	0.92
Arachidic acid	C20:0	0.14	0.14	-	0.02
∑SFA		57.60^{ab}	60.37^{a}	52.33^{b}	1.47

Palmitoleic acid	C16:1ω7	1.77	1.75	2.42	0.17
	C16:1ω5	0.20	0.25	0.25	0.01
Oleic acid	C18:1ω9	32.38	30.94	37.17	1.36
Vaccinic acid	C18:1ω7	2.94	1.95	2.65	0.26
6 Octadecosanic acid	C18:1ω5	0.89	0.74	0.84	0.07
∑MUFA		38.79^{ab}	36.15^{b}	44.07^{a}	1.45
Linoleic acid	C18:2ω6	2.28	2.26	2.63	0.18
	C18:2ω5	0.28	0.29	0.24	0.01
Decatrienoic acid	C16:3ω4	0.59^{ab}	0.50^{b}	0.73^{a}	0.04
γ Linolenic acid	C18:3ω6	0.14	0.13	0.14	0.01
α Linolenic acid	C18:3ω3	0.21	0.20	0.19	0.01
α Octadectetraenoic	C18:4ω3	0.28	0.24	0.35	0.03
∑PUFA		3.22	3.14	3.57	0.20
∑UFA		42.01^{ab}	39.29^{b}	47.64^{a}	1.56
UFA/SFA		0.73^{ab}	0.65^{b}	0.91^{a}	0.04
MUFA/ SFA		0.68^{ab}	0.60^{b}	0.84^{a}	0.04
PUFA/ SFA		0.05	0.05	0.07	0.04
∑ω6		2.43	2.39	2.78	0.18
∑ω3		0.50	0.45	0.54	0.04
n-6: n-3		4.84	5.75	5.23	0.44
Index of atherogenicity (IA)		1.08	1.27	0.68	0.13

$^{a-b}$ means within the same row with different superscripts letters are different ($p<0.05$). T1: control, T2: contains medicinal plants, T3: contains medicinal pants with yeast. SEM: standard error of means. Index of atherogenicity (IA) = [C12:0 + (4 × C14:0) + C16:0]/ ∑UFA.

3.2 Physical properties

3.2.1 pH values

Data of the physical properties of sheep burgers processed from sheep fed on different types of medicinal plants and yeast during frozen storage are illustrated in Table 2. It can be noticed that feeding type had a slight significant effect on pH values of fresh sheep burger. Regards to storage time, significant decrease ($p<0.05$) was found in all burger samples after 60 days of storage and surprisingly significant increase was found at the end of storage time 90 days. These results are consistency with data of Tekce et al. [21] they indicated that slight significant differences were found in pH values of lambs fed on rations supplemented with different doses of probiotics and yeast. Similarly, Gloria-Trujillo et al. [22] postulated that lambs fed diets supplemented with different levels of *Saccharomyces cerevisiae* had no significant effect on pH values of meat. On the other hand, Smeti et al. [23] they stated that feeding lambs on aromatic plants not affected on pH values of lamb meat. Similarly, Lozano-Sánchez et al. [24] they reported that lambs fed on diet supplemented with different levels of polyherbal had no significant effect on pH values of lamb meat. However, the decrease or the increase in pH values during frozen storage is due to the microbial activity. The decrease in pH values is probably due to the increase in psychrophilic bacteria activity leading to producing lactic acid which is responsible for the decrease in the pH values. On the other hand, the increase in decomposition bacteria and enzymes activity resulting in producing alkali compounds which is responsible for increase the pH values [25].

Table 2 Physical properties of sheep burger during frozen storage at -20 °C for 90 days

Storage periods (days)					
Treatments	0	30	60	90	SEM
pH value					
T1	6.39^{aAB}	6.03^{abB}	5.81^{bA}	6.04^{abA}	0.06
T2	6.23^{aB}	6.33^{aA}	5.86^{bA}	6.17^{aA}	0.06
T3	6.56^{aA}	5.96^{bB}	5.99^{bA}	6.20^{abA}	0.06
Tenderness (Shear force value kg/f)					
T1	1.50^{aA}	1.87^{aA}	1.31^{aA}	1.61^{aA}	0.20
T2	1.75^{aA}	1.40^{aA}	1.23^{aA}	1.81^{aA}	0.20
T3	1.28^{aA}	1.59^{aA}	1.04^{aA}	1.51^{aA}	0.20
Cooking loss (%)					
T1	45.96^{bA}	51.51^{aA}	48.34^{aA}	42.07^{bA}	1.38
T2	39.31^{bB}	49.44^{aA}	48.19^{aA}	45.07^{aA}	1.38
T3	40.91^{bAB}	48.49^{aA}	45.90^{aA}	38.41^{bB}	1.38

$^{a-b}$ means within the same row with different superscripts letters are different ($p<0.05$).

$^{A-B}$ means within the same column with different superscripts letters are different ($p<0.05$).

T1: control, T2: contains medicinal plants, T3: contains medicinal pants with yeast.

SEM: standard error of means.

3.2.2 Shear force

No significant differences were found in shear force values among sheep burger of feeding groups as shown in Table 2. Regards to storage condition, although slight increase was found in shear force values during frozen storage, but the differences were not significant. These findings came in accordance with the results of Orzuna-Orzuna et al. [26] they documented that feeding lambs with diet supplemented with polyherbal mixture had a slight difference on shear force of meat. Similar results were found by [24] they reported that shear force values not affected by feeding lambs on polyherbal diets. On the same line, Marcon et al. [27] indicated that diet supplemented with curcumin had no significant effect on shear force of lamb meat. On the other hand, Guimarães et al. [28] found that diets containing yeast (*Saccharomyces cerevisiae*) not significantly affected on shear force value of lamb meat.

3.2.3 Cooking loss

Cooking loss of sheep burger processed from sheep fed on different types of medicinal plants recorded the lowest cooking loss, followed by sheep burger of medicinal plants with yeast (Table 2). During frozen storage, significant increase ($p<0.05$) in cooking loss was found in all burger samples after 30 days, followed by significant decrease till the end of frozen period 90 days. However, at any time of frozen storage burger processed from medicinal plant with yeast recorded the lowest cooking loss. The results of the current study contradict the findings of [26] they stated that cooking loss significantly increased in lamb meat fed on polyherbal mixture and cooking loss increased as the doses of polyherbal mixture increased. Similarly, [20] they found that cooking loss of sheep meat feeding on diets supplemented with herbals was higher than control feeding group. Regards to storage conditions, the changes in cooking loss are consistency with results of Sadallah and Khalil [29] they found that cooking loss of all the lamb samples significantly increased after 30 days of frozen storage after that the reduction in cooking loss was small and not significant till the end of storage period.

3.2.4 Color measurements

Data of color measurements of sheep burger from sheep fed on different types of medicinal plants and yeast are showed in Table 3. Feeding types and storage conditions slightly affected in L^* values of sheep burger. Contrarily, feeding types and storage conditions significantly affected in a^* values. Burger of control group had the highest a^* value followed by burger of medicinal plants, while the lowest a^* value found in burger of medicinal plants with yeast. Regards to storage time, a^* values significantly decreased after 30 days of storage in all burger samples, followed by

slight increase after 60 days and the decreasing continued till the end of storage time 90 days. Conversely, *b** values not affected by types of feeding. Regarding to storage time, significantly increased after 30 days of storage and slight decreased after 60 days and the increased continued to the end of storage 90 day. The changes in color measurements of sheep burger are close to results that obtained by [26] they found that supplemented lamb's diet with polyherbal mixture had no significant changes on meat color parameters (*L**, *a**, *b**). In the same line, [24] indicated that addition of polyherbal as supplementation not significantly affected on meat color measurements. On the other hand, [28] found that addition of yeast (*Saccharomyces cerevisiae*) to lambs' diet not affected on meat color. The changes in color parameters during frozen storage are concordant with the results of Fernandes et al. [30] they found that (*L** values) of lamb meat did not significantly change during frozen storage time. On the other hand, the changes in *a** values were not significant. Contrarily, *b** values showed significant variation during storage, which can be explained by a quadratic model of decreased followed by increase till the end of storage period.

Table 3 Color measurements of sheep burger during frozen storage at -20 °C for 90 days

	Storage periods (days)				
Treatments	0	30	60	90	SEM
		*L**			
T1	55.35^{bA}	58.44^{aA}	57.72^{bA}	57.18^{bA}	0.67
T2	56.26^{aA}	58.58^{aA}	57.10^{aA}	57.83^{aA}	0.67
T3	56.37^{aA}	56.47^{aA}	56.73^{aA}	58.52^{bA}	0.67
		*a**			
T1	8.94^{aA}	3.78^{bA}	4.51^{bA}	3.67^{bA}	0.28
T2	7.53^{aB}	3.88^{bA}	4.47^{bA}	4.04^{bA}	0.28
T3	5.71^{aC}	4.33^{bA}	4.24^{bA}	2.89^{cB}	0.28
		*b**			
T1	9.40^{cA}	11.72^{abA}	11.06^{bB}	12.45^{aA}	0.27
T2	9.69^{bA}	12.05^{aA}	12.04^{aA}	12.89^{aA}	0.27
T3	9.86^{bA}	12.58^{aA}	12.21^{aA}	12.38^{aA}	0.27

$^{a-c}$ means within the same row with different superscripts letters are different ($p<0.05$).
$^{A-C}$ means within the same column with different superscripts letters are different ($p<0.05$).
T1: control, T2: contains medicinal plants, T3: contains medicinal pants with yeast.
SEM: standard error of means.

3.3 TBA value

Table 4. showed the effect of feeding and storage time on TBA values of raw sheep burger. It can be noticed that at zero time, no significant differences were found in sheep burger samples. During frozen storage, significant decreased ($p<0.05$) were found in all burger samples after 30 days of storage and the decreasing continued till 60 days of storage which recorded the lowest TBA values in all burger treatments. Significant increase was found at the end of frozen period 90 days. Regards to feeding type, burger processed from sheep fed on medicinal plants showed the lowest TBA value during storage time followed by burger processed from sheep fed on medicinal plants with yeast while, burger of control group showed the highest TBA values at the end of storage time. These results are close to that obtained by Leal et al. [31] they found that TBA values of lamb meat significantly increased as the time of storage increased. Also, they found that meat from lambs supplemented with vitamin E and rosemary extracts exhibited the lower TBA than meat of control feeding group. In the same line, Afele et al. [32] they found that Malondialdehyde (MDA) significantly decreased in meat of sheep fed on diet supplemented with garlic and ginger powder than meat of control group. Generally, the decrease in TBA values in meat of medicinal plants feeding groups may be due to the bioactive compounds such as Gingerol in ginger and allicin in garlic which are responsible for the high antioxidant activity. TBA value of lamb meat fed on yeast was higher while, the lower TBA value found in meat fed on yeast with minerals [28].

Table 4 TBA value of sheep burger during frozen storage at -20 °C for 90 days

Storage periods (days)				
Treatments	0	30	60	90
TBA value (µmol TBARS/g)				
T1	26.51^{aA}	10.50^{bA}	6.58^{bA}	25.42^{aA}
T2	30.38^{aA}	13.34^{bA}	9.21^{bA}	14.35^{bB}
T3	33.12^{aA}	14.66^{bcA}	10.55^{cA}	18.08^{bB}
SEM	1.71	1.71	1.71	1.71

$^{a-c}$ means within the same row with different superscripts letters are different ($p<0.05$).

$^{A-B}$ means within the same column with different superscripts letters are different ($p<0.05$).

T1: control, T2: contains medicinal plants, T3: contains medicinal pants with yeast.

SEM: standard error of means.

3.4 Microbiological quality

Table 5. showed the microbiological quality of sheep burger during frozen storage. It can be noticed that feeding types had slight effect on total bacterial count of sheep burger samples. During frozen storage, burger of medicinal plants group exhibited lower total bacterial count than burger of yeast group. On the other hand, feeding types and storage condition had no significant effects on psychrophilic bacteria, mold and yeast counts. These results are close to that obtained by [30] they found that microbiological analysis showed good stability during storage period. On the other hand, Al-Rubeii and Hussen [33] found that feeding lambs on diet supplemented with medicinal plants resulting in significant decreased in total plate count and psychrophilic count in lamb meat during cold storage periods.

Table 5 Microbiological quality of sheep burger during frozen storage at -20 °C for 90 days

Treatments	**Storage periods (days)**				
	0	**30**	**60**	**90**	**SEM**
Total bacterial count (log CFU/g)					
T1	4.24^{aB}	4.56^{aA}	4.08^{aAB}	4.08^{aA}	0.33
T2	4.60^{aB}	4.51^{aA}	3.47^{bB}	4.46^{aA}	0.33
T3	5.85^{aA}	4.45^{bA}	4.43^{bA}	4.78^{abA}	0.33
Psychrophilic (log CFU/g)					
T1	3.84^{aA}	3.28^{aA}	3.70^{aA}	3.57^{aA}	0.37
T2	3.82^{aA}	3.86^{aA}	3.50^{aA}	3.74^{aA}	0.37
T3	4.64^{aA}	3.46^{aA}	3.88^{aA}	4.23^{aA}	0.37
Mold and yeast (log CFU/g)					
T1	4.23^{aA}	5.54^{aA}	3.27^{aA}	2.95^{aA}	0.57
T2	3.56^{aA}	4.00^{aA}	3.79^{aA}	2.41^{aA}	0.57
T3	5.11^{aA}	4.39^{aA}	4.14^{aA}	3.44^{aA}	0.57

$^{a-b}$ means within the same row with different superscripts letters are different ($p<0.05$).

$^{A-B}$ means within the same column with different superscripts letters are different ($p<0.05$).

T1: control, T2: contains medicinal plants, T3: contains medicinal pants with yeast.

SEM: standard error of means.

IV. CONCLUSION

The purpose of the current study was to evaluate the quality characteristics of sheep burger processed from sheep fed on diets supplemented with medicinal plants as feed additives and yeast as probiotic during frozen storage. The addition of medicinal plants combined with yeast improved the fatty acid profile, delayed the lipid oxidation and decreased the cooking loss without negative effects on color parameters, shear force and microbial analysis during frozen storage which subsequently affected on sheep burger quality and human health.

AUTHORS CONTRIBUTION

E.F.Z. and M.M.G. Formal analysis: E.F.Z. and M.M.G. Investigation: E.F.Z. and M.M.G. Methodology: E.F.Z. and M.M.G. Writing—original draft preparation: E.F.Z. Writing—review, and editing: E.F.Z. Validation and supervision: E.F.Z. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Animal care and experimental protocol were reviewed and approved by the Local Experimental Animals Care and Welfare Committee in Desert Research Center.

FUNDING

No funding.

DATA AVAILABILITY

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

CODE AVAILABILITY

Not applicable.

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Analysis of Factors Affecting the Growth and Development of Maize Crops (Zea Mays L) in Adiankoting Sipoholon and Paranginan Districts

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Abstract— *This study aims to determine the factors that affect the growth and development of corn plants (zea mays l.) in the sub-districts of Adiankoting, Sipoholon and Paranginan. This study uses the Statistical Package Social Sciences (SPSS) stastika formula consisting of each sub-district using 50 questionnaires from Adiankoting sub-district 50 questionnaires, from Sipoholon sub-district 50 questionnaires and from Paranginan sub-district 50 questionnaires, and the total amount to 150 questionnaires. This research was conducted by a direct field survey system and interviews with respondents or corn farmers in each of the 150 farmers, the results of this study indicate that in each sub-district there are different and some are the same in terms of altitude, land area, planting distance, age of farmers and others. The three sub-districts are dominated by male farmers, mostly only diparanginan more female farmers but maybe in other areas or in other villages there may also be many female farmers. I conducted this research during October to November 2021 where there is still strict health protocol supervision because corona has spread everywhere, so caution is needed. In the Adiankoting, Sipoholon and Paranginan sub-districts, the various types of fertilizers used and the way they are applied and pests and diseases are too many to attack in these areas.*

Keywords— ***Growth, Development, Zea Mays L***

I. INTRODUCTION

Maize is one of the food crops that is also an important class of cereals in the world economic sector. Apart from being a staple food, corn products are usually processed into animal feed, processed food ingredients and so on. In the historical record, the corn plant originated from southern Mexico and Latin America and then spread to Europe, India and finally spread throughout the world including Indonesia (Riwandi, et al., 2014).

The need for corn (Zea mays) consumption from year to year is increasing (Muis, et al. 2015). Corn is one of the important food crops in Indonesia and has a strategic role in the national economy, given its multipurpose function, as a source of food, feed, and industrial raw materials (Khairiyah et al., 2017).

In an effort to meet national maize demand and zero imports, since 2007 the government has implemented a program to increase maize productivity through Integrated Crop Management of maize (PTT maize). Currently, the government (Decree of the Minister of Agriculture Number: 1243/Kpts/OT.160/12/2014) continues the program with a special effort program to increase maize production through the Maize Integrated Crop Management Improvement Movement (Maize GP-PTT) and the Expansion of Planted Areas with Increased Maize Planting Index (PAT-PIP), which aims to increase production, farmers' income, and preserve the environment (MOA, 2015). The purpose of this study is to determine the factors that influence the growth and development of corn plants in Adiankoting sub-district, to determine the factors that influence the growth and development of corn plants in Sipoholon sub-district, to determine the factors that influence the growth and development of corn plants in Paranginan sub-district.

II. REVIEW OF LITERATURE

Sweet corn plants belong to the grass family with the species Zea mays saccharata Strurt. The classification of sweet corn plants is as follows:

Kingdom : Plantae

Division : Spermatophyta

Subdivision : Angiosperms

Class : Monocotyledonae

Order : Poales

Fanili : Poaceae

Genus : Zea

Species : Zea mays saccharata Sturt (Rukmana, 2008).

Corn is a seasonal plant that has a tall, sturdy stem and usually a dominant single stem, although there may be some that contain buds (tillers). This plant has a stem height of between 60 and 300 cm. The position of the leaves is dystopic (two rows of single leaves that come out in an alternating position) with leaf midribs overlapping and the leaves are wide and relatively long.

Maize is a fibrous-rooted plant that consists of three types of roots: seminal roots, adventitious roots, and aerial roots. Seminal roots grow from the radicle and embryo. Adventitious roots are called supporting roots, which grow from the lowest book while aerial roots are roots that grow from books above the soil surface.

a. Roots

Corn plant roots consist of 3 kinds of roots, namely seminal roots, adventitious roots and prop roots. The root system functions as a tool for sucking water and mineral salts contained in the soil, removing organic substances and compounds that are not needed and respiratory equipment. Prop roots function to keep the plant upright. Corn roots are included in fibrous roots that can reach a depth of 8 m although most are in the 2 m range. (Subekti, et al., 2007).

b. Stem

Maize stems are erect and easily visible like sorghum and sugarcane, but not like rice or wheat. The stems of the maize plant are branched with the number of internodes varying between 10-40 internodes. Maize plants are generally unbranched. The length of maize stalks generally ranges from 60-300 cm, depending on the type of maize. Maize stems are quite sturdy but do not contain much lignin. Corn stems have three main components: epidermis, vascular tissue and stem center (Subekti et al., 2007).

c. Leaves

Corn leaves are perfect leaves. The shape is elongated, between the midrib and leaf blade there is a ligula. The leaf bone is parallel to the mother leaf bone. The leaf surface is smooth and some are hairy. Each stoma is surrounded by fan-shaped epidermal cells. This structure plays an important role in the plant's response to water deficit in leaf cells (Wirawan and Wahab, 2007).

d. Fruit

Maize fruit consists of cobs, seeds and wrapping leaves. Maize seeds vary in shape, color and endosperm content, depending on the species. In general, maize has straight or winding rows of seeds that number between 8-20 rows of seeds. Corn kernels consist of three main parts: seed coat, endosperm and embryo (Rukmana, 2008). Corn seeds are located regularly, according to the location of the embryo flower consisting of plumula, radicle and acutelina. Seeds are round, tooth-shaped or flat according to the variety. Seed color also varies, including yellow, white, red, orange and red almost black. Contains starch protein and fat.

Corn plants are monocious plants. In a plant there are male flowers and female flowers that are located separately. Male flowers are located at the tip of the plant, while female flowers are along the middle of the corn stem and are in one of the leaf axils. Maize has separate male and female flowers (diklin) in one plant (monoecious). Each flower has a structure typical of flowers from the Poaceae tribe, called a floret. Male flowers grow at the top of the plant, in the form of a wreath (inflorescence). The pollen is yellow and has a distinctive aroma. Female flowers are arranged in cobs that grow between the stem and leaf midrib. In general, one plant can only produce one productive cob despite having a number of flowers. Female flowers grow on the side cob end of the stem originating from the leaf axils, usually at about mid-length of the main stem. The lateral (side) stems are very short because of their short internodes. On each book of the lateral stem, a leaf grows. Due to the close proximity of the books, the leaves close together, forming the kelobot that wraps around the developing cob. In certain cultivars, the development of tassels seems to affect the development of the cob stem (Rukmana, 2008).

e. Climate

Maize plants are widely adaptable to the environment. In general, maize plants can grow in lowlands to highlands (+ 1300 m above sea level), with a temperature range between 130°C-380°C and full sunlight. In Indonesia, corn plants grow

and produce optimally in the lowlands up to 750 m above sea level. The ideal air temperature for seed germination is 300°C-320°C with soil water capacity between 25-60%. During growth, corn requires an optimum temperature between 230°C-270°C (Budiman, 2009). Maize plants are native to the tropics and can adapt to environments outside the region. Maize does not demand too strict environmental requirements. Corn plants require rainfall of around 100-140 ml/month. For this reason, it is necessary to observe rainfall in order to determine the planting time appropriately (Murni, et al., 2008). Corn plants should get full sunlight with an optimum temperature of 210-340 C. In older plants, especially towards the ripening of seeds, hot conditions and sufficient sunlight intensity are needed, and if high humidity will result in poor corn plant production, especially seed quality. Because during its growth period, sweet corn plants need sufficient sunlight to be able to produce seeds and form fruit properly (Nasution, 2019).

f. Soil

Soil fertility is often linked to the state of the top soil. In this layer, the root system of plants usually develops well. For this reason, tillage before planting and tillage during maintenance play an important role in plant fertility. In tillage, the ratio of solid, liquid and air in the tillage layer makes the soil loose and favorable for plant root growth. Corn plants can grow well on all types of soil. But it will grow better on loose, humus-rich soil. Soil that is dense and can retain water is not good for planting corn because it grows poorly or will rot (Budiman, 2009). Soil acidity (pH) required for optimal growth of corn plants is between 5.6 - 6.2 but 6.8 is best. Soil acidity below 5.5 is not good for corn plant growth, the soil already needs to be limed (Hardiyanto, 2020). Soil slope has to do with water movement on the soil surface. It is also one of the requirements for plant life including corn plants. Soils with a slope of less than 8% can be planted with corn at this level of slope, which is very likely to cause soil erosion (Budiman, 2009).

g. Maize Pests and Diseases

Plant-disrupting organisms or pests are a problem in the cultivation of maize. Armyworms are one of the pests that often plague agriculture in Indonesia, including corn crops. Currently, there is a new type of armyworm that is currently endemic in the world, namely Fall Armyworm (FAW) or Spodoptera frugiperda. The pest belongs to the order Lepidoptera, family Noctuidae. Spodoptera frugiperda attacks food crops such as corn, rice and wheat. This pest is one that is difficult to control, because its imitators spread quickly, even including strong fliers that can reach a considerable distance in one week. If assisted by the wind, it can reach 100 km. The pest has spread rapidly from the Americas in 2016, to the African continent and spread in Asia to Thailand in 2018 (Harahap, 2018). Armyworms (Spodoptera litura) belong to the order Lepidoptera. This pest is polyphagous, making it rather difficult to control. Symptoms of armyworm (Spodoptera litura) attack begin at the larval stage, where young larvae damage the leaves and leave traces of the upper epidermis (transparent) and leaf bones. Advanced larvae damage the leaf bones, and cause the plant to run out of leaves. This rapid and uncontrollable attack has led to the need for intensive control (Marwoto and Suharsono, 2008).Litura larvae attacks can cause significant losses to farmers. Symptoms of attack are usually caused by the presence of caterpillars or larvae of this pest. The larvae will damage the leaves and attack simultaneously and in groups. The larvae/caterpillar attack will leave remnants of the upper epidermis of the leaves, so that the leaves become transparent and only the leaf bones are left behind. To overcome the pest is still using chemicals where we know the adverse effects on the environment and the phenomenon of resistance in insect pests due to the use of insecticides has increased the attention of experts to research on the utilization of pathogens to control plant pests. Insect pathogens are relatively specific and their effects are much smaller than those caused by chemicals on the environment or non-target organisms (Christina, 2013). Corn stalk borer (Ostrinia furnacalis) is one of the pests of corn plants that can be found in almost all parts of Asia including in Indonesia where this insect spreads in Papua, Sulawesi, Nusatenggara and Sumatra. Female insects lay their eggs under the surface of the main leaves of corn plants when the plants are two weeks old. The female insect is capable of laying 300-500 eggs. The larvae of this insect can damage almost all parts of the corn plant with the characteristics of the attack, namely small holes in the leaves, slit holes in the stems, the base of the cob to damage the corn cob. This pest can be controlled by spraying insecticides and releasing

natural enemies such as larval predators, ants and cocopeat (Adnan, 2009). The corn cob borer (Helicoverpa armigera Hbn.) consists of a female H. armigera imago that lays its eggs on the corn hairs when the cob has started to come out. The female imago can produce up to 730 eggs. The female imago will lay eggs on the corn silk and will enter the corn cob and eat the seeds that are undergoing development so that it can reduce the quality and quantity of corn cobs. Signs of cob borer attack on maize are characterized by transverse holes in the leaves of vegetative stadia plants. The hair of the corn cob is cut off, the tip of the cob has graze marks and often has larvae. Control that can be done is by technical culture such as perfect tillage which will damage pupae formed in the soil and can reduce subsequent pest populations. The use of insecticides made from active ingredients such as dimehipo, monocrotophos, karbofuran, effectively suppresses the attack of corn cob borers. Insecticide application is recommended when one egg cluster per 30 plants has been found. Liquid or spray insecticides are only effective in the egg and larval phase of instrars I-III, before the larvae enter the cob. (Adnan, 2009). Rust diseases in corn can be grouped into southern corn rust, common corn rust, and tropical corn rust, this fungal disease affects corn plants after milk ripening. When weather conditions are significant, it has an impact on the development and spread of the disease. The symptoms of this disease are small elongated circles on the leaf surface. The spread of this disease is caused by spores that are blown by the wind and then spread and infect the leaf surface. Control for this type of disease can be done by spraying fungicides containing mankozeb and pyraclostrobin, in addition to sanitation can also help prevent the development of disease (Puspawati and Sudarma, 2016). Downy mildew is a disease of corn caused by several species of fungi such as Peronosclerospora maydis, P. phillipinensis, and P. sorgi which are distributed in Java, Sulawesi and North Sumatra. Transmission of this fungus is aided by wind that occurs in the afternoon where the fungus will fall on the leaf surface and in the growing point area and then infect the corn plant until it dies. Symptoms of this disease can be seen from the presence of chlorotic color extending parallel to the leaf bone and on the lower surface of the leaf there is a white color like flour (Talanca, 2013).

III. RESEARCH METHODOLOGY

This research was conducted with a field survey system and interviews with respondents or corn farmers guided by a list of questions that have been prepared according to the research objectives in North Tapanuli and Humbang Hasundutan Districts. This research was conducted in three sub-districts in North Tapanuli and Humbang Hasundutan, including Adiankoting, Sipoholon and Paranginan sub-districts. This research was conducted from OCTOBER 2021 to NOVEMBER 2021. With the altitude of the place in Adiankoting District 400-1300 meters above sea level, in Sipoholon District 400-1300 meters above sea level and in Paranginan District 600-1700 above sea level.

IV. RESULT AND DISCUSSION

Based on the statistical results that have been carried out on the research questionnaire on corn farmers who are the identity of the respondents, namely gender, and age. Respondents in this study were 50 maize farmers. In this study, the characteristics of farmers who were respondents were the gender and age of farmers in Adiankoting, Sipoholon and Paranginan sub-districts. These characteristics can indirectly affect land cultivation, land area, pests found, maintenance and control carried out in Adiankoting, Sipoholon and Paranginan sub-districts. Respondents in this study were 50 farmers organized by maize farm ownership status. Gender is the difference between women and men biologically since a person is born. Farmer gender can indirectly affect maize farming. Where in this study showed the number of male farmers as many as 27 people and 23 women in Adiankoting sub-district. The number of male farmers was 32 people and 18 women in Sipoholon sub-district, while the number of male farmers was 20 people and 30 women in Paranginan sub-district. In fact, it is often found that field labor is generally dominated by men. According to Lionberger (1960), age in the productive category tends to be easier to accept new innovations and more open to technological advances. Age is information about a person's date, month and year of birth. Age information contains a measure of the length of a person's life in years. Age can influence a person in making a decision. Age can also be one of the benchmarks for the success of farming activities. Farmers who have a productive age will usually work better and more optimally than farmers who are no longer productive. The number and percentage of farmer respondents in the age group of 15 - 64 years are classified as a productive group of people to work because in this age range they are considered capable of producing goods and services. Productive age is one of

the success factors in farming activities. According to Hasyim (2006) and Ryan et al. (2018), farmers with productive age will work better and more optimally than non-productive farmers. However, older farmers can understand field conditions better. This is in accordance with the opinion of Novia (2011) who states that older farmers usually have relatively less understanding, but have advantages in recognizing the condition of the farmland. The processing of maize fields in Adiankoting, Sipoholon and Paranginan subdistricts is divided into two parts, namely processing and not processing. Those who do the processing by means of tractors and bulldozing the soil. Land cultivation greatly affects the growth and development of the corn plant in each farmer's land. Where land processing is very useful as loosening the soil so as to create spaces and pores that allow the soil to get air aeration. Helps to mix plant residues, soil organic matter, and nutrients more evenly.Kill weeds mechanically. Drains the soil before planting seeds. This is a positive effect in wet climates. When done in the fall, tillage helps to weaken the soil throughout the winter through freezing and thawing mechanisms that can occur many times throughout the winter. This helps prepare the planting for spring. However, tillage can also dry out the soil before seed planting. This is a negative impact in arid climates as the soil loses nutrients such as nitrogen and its ability to store water. Reducing the rate of water absorption increases soil erosion. Ploughing reduces the cohesion between soil particles, thus accelerating erosion. With reduced water absorption rates, there is a risk of surface water runoff carrying residues of fertilizers and pesticides used in the previous planting period. Reduced soil organic content. Reduces the number of beneficial soil organisms such as microbes, earthworms, ants, and so on. Destroys soil aggregates. Risk of soil compaction in unplowed areas. Crop residues that are destroyed and left in the soil can attract unwanted organisms and insects and potentially disrupt production, as well as attract diseases. The agricultural sector is a sector that supports the lives of most people. The agricultural sector needs to be developed along with the increase in population and the development of technology that can increase agricultural production. In addition, the agricultural sector plays a role in meeting the needs of the population, increasing farmers' income, providing industrial raw materials, providing business opportunities and employment opportunities, and supporting national food security. The goal of farmers in farming is to obtain high production at low cost. Agricultural development needs better attention, even though the priority on industrialization policy has been dropped, but the agricultural sector can have the ability to generate increased income (Sudarman, 2001). In agriculture, the production factor of land has the most important position, as evidenced by the amount of services received by land compared to other factors. The area of land planted will affect the number of crops that can be planted which in turn can affect the amount of vegetable production produced. If the farmer's land area is large enough, then the economic opportunity to increase production and income will be greater (Soekartawi et al., 2002). Land area for wetland farmers is one of the factors that influence the increase in yield income. Villagers whose main activity is farming depend on their land. Thus, the area of land owned is one of the clues to the amount of income received. If the land area increases, the income of farmers will also increase and vice versa if the land area used is small or narrow, then the income earned by farmers will also decrease because the rice planted is small. So, the relationship between land area and farmer income has a positive relationship (Isfrizal & Rahman, 2018). In addition to land area, working capital is essentially an amount that continues to exist in sustaining a business that bridges the time between expenditures to obtain materials or services and the time of receipt of sales. In addition, it is the most important aspect in the activities of a business. Without having capital, a business will not be able to run even though other conditions for setting up a business are already owned. Capital is a factor that determines the amount of production and income. Lack of capital in farming will cause the use of production facilities to be very limited which in turn will affect production and income (Karyanto, 2008). Capital in farming can be classified as a form of wealth in the form of money or goods used to produce something either directly or indirectly in a production process (Soekartawi, 2006). In addition to land area and capital there is the amount of production. According to Soekartawi (2006) agricultural production is influenced by various factors including commodity types, land area, labor, management capital, climate and socio-economic factors of producers. For more details, Soekartawi (2006) states that the factors that affect production can be divided into two groups, namely: (1) biological factors, such as agricultural land with various levels of fertility, seeds, varieties, fertilizers, medicines and others. (2) Socio-economic factors such as production costs, prices, labor, education level, income and others. Suratiyah (2006) states that if the demand for production is high, the price at the farm level will also be high, so that with the same costs' farmers will get higher income. Conversely, if farmers have succeeded in increasing production, but prices fall, farmers' income will also fall. Planting distance can affect yield, because different plant populations will produce different plant growth. Increasing the planting distance to a certain level, the yield per unit area can increase while the yield per plant can decrease. Plant disturbing organisms found in the corn

fields of farmers in Adiankoting, Sipoholon and Paranginan subdistricts are crickets, fleas, crickets, walangsangit, aphids, Grayak caterpillars, crickets, dwarf, yellow stems, burnt leaves, not growing perfectly yellowed leaves, skinny stems2 and yellowed leaves. Maintenance carried out by farmers using various types of chemical and organic fertilizers such as NPK, UREA, Biogrent Organic Fertilizer, KCL, TSP. the types of insecticides used to control pests and diseases of corn crops are decis, antracol, Fosthin, Antracol, Mantap, Fosthin, Dithane, Tronton, Trivia, Mantap, Koge, Fierg EC, Synergy, Fenite. The obstacles in the field that I encountered were that the road access was still damaged a lot, the corona was still there at that time, not all the farmers we wanted to research accepted our interviews to get the research results. This research was conducted in three sub-districts of Adiankoting, Sipoholon, Paranginan, the reason I chose this place was because the place was quite close and I didn't want to get covid for sure if it was far away, and also in the three sub-districts it was pretty much planting corn.

V. CONCLUSION

In research conducted in three districts, namely in Adiankoting, Sipoholon and Paranginan, there are factors that affect the growth and development of corn plants, namely:

1. Land cultivation is not done
2. Lack of land clearing
3. The presence of pests and diseases in corn plants
4. The use of many types of insecticides
5. Planting distance is too tight

So, it can be concluded according to the data in the field that there are still many diseases of corn plants in Sipoholon District compared to Adiankoting District, Paranginan District. Where the productivity of corn plants is more in Paranginan District even though the land area is more in the sub-district of Sipoholon, this is because the knowledge of growing corn is more advanced in the sub-district of Paranginan.

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Development of *Dendrobium officinale* pulpy drink

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Abstract— *D. officinale is a renowned botanical species in China with a rich historical background associated with its extensive use in traditional medicine. This research aims to develop a pulpy drink using the Stem of the D. officinale plant and enhance its thermal stability. In order to enhance the thermal stability of the beverage, multiple amounts of xanthan gum were used in the formulation. The physical stability of the D. officinale pulpy drink was assessed by measuring particle size, Zeta potential, particle sedimentation, viscosity, and color. The current research findings indicated that the beverage with a composition of 3% D. officinale pulp and 0.4% xanthan gum exhibited the highest level of physical stability. This beverage exhibited the smallest particle size, negative zeta potential, viscosity, and no sedimentation.*

Keywords— ***Xanthine gum, D. officinale, herbal drink, Drink development, Drink stability.***

I. INTRODUCTION

Dendrobium is enriched in carbohydrates, which is used as oral medicine. It is also a main component of various food items and drinks [1]. In China, this is used as medicine for increasing the secretion of saliva and helping moisten the lungs. This polysaccharide is very prominent in medicine; various genera of *D. officinale* are used [2]. These plants from *the Orchidaceae* family are generally believed to help improve eyesight, relieve fever, and protect from CVDs if we use them as eatable or drink them. This is why people of China consider this plant one of the best herbs to cure all diseases and use it to increase their lifespan [3]. Various species present in the world of this plant include *D. aphyllum, D. candidum, D. chrysanthum, D. densiflorum, D. crystallinum, D. fimbriatum, etc.* [4].

Making drinks from plant extract as medicine is the traditional way to treat diseases in China. In the case of immunity, there is a drink known as National Herbal Drink (NHD). This drink has ingredients like *D. officinale, Atractylodes macrocephala Koidz., Paeonia lactiflora Pall., Lycium barbarum L., and Glycyrrhiza uralensis Fisch.* This drink is mostly consumed by individuals who have low immunity levels. In this drink, the prominent constituent is *D. officinale*, which plays an important role as an antioxidant, lowers blood pressure, and helps to fight liver diseases. Many studies have demonstrated that this compound also improves the mechanisms of phagocytosis by activating the macrophages; hence, their function is improved. In the gut of mice, there is a diversity of various microbes or microbiota. Their variety is also increased due to this compound [5].

The structure of *D. officinale* consists of various polysaccharide sub-units that may include glucose, pyranose, *etc*. The beta-glycosidic bond is present in the sugar sub-units of this compound. It has also been evaluated that the intestine and stomach secretions cannot

digest this compound. This compound is converted through digestion in various products like lactic acid, propanoic acid, lactic acid, etc. [1]. The *D. officinale* flower is also a food additive, sweetener, and drink. Its scent makes an individual stress-free. However, to give more color taste and improve the shelf-life of the drink, various additives like honey and alcohol are also used. These can then be used for therapeutic applications [6]. Due to the higher molecular weight and viscosity of *D. officinale*, it is used in the food industry to give the food gelling property [7]. Many Asians believe that *D. officinale*, when used as soup or juice, is more effective and used as a functional food [8]. *D. officinale* flower is also used as raw material in soft candy preparation. This soft candy is like gellies. In the Republic of China, another variety of Dendrobium is used in the food items and barrages known as *D. nobile LindI*. This plant consists of alkaloid compounds that protect the body from dementia abnormalities in the natural cell death process and protect it from neural diseases [9].

II. MATERIAL AND METHODS

2.1 Sample preparation

In this step, the plant stems were selected based on certain criteria such as ripeness, no signs of deterioration, and no foreign ingredients. Stems were washed twice to decontaminate. After washing, the Stem was weighed with analytical balance (Shanghai Yousheng Co., Ltd., Shanghai, China) and blended with water in a high-speed blender of 0.3:10 (w/v) provided by Shenzhen Kangjia Electronics, Shenzhen, China. This blended mixture was ground for 20 min using a collide mill to make a fine pulp. After that, hydrocolloids were added to the drink and mixed using a high-speed mixer (Shanghai Lichen Bangxi Instrument Technology Co. Ltd., Shanghai, China) at 1100 rpm for 10 min. Finally, the *D. officinale* drink was sterilized in an autoclave at 121 ℃. Different ratios of xanthan gum were used to investigate the physical stability of the drink. Formulations with xanthan gum were applied as follows: T1 0.1%, T2 0.2%, T3 0.3% and T4 0.4%. *D. officinale* pulpy drink was transferred to 50 ml glass bottles and sterilized in an autoclave (Bosun Medical Biological instrument, Shanghai, China). After sterilization, they were kept at room temperature to cool down and used for further analysis.

2.2 Particle Size Analysis

The particle size measurement was conducted using a methodology previously outlined by Ni et al. [10]. In this study, the *D. officinale* beverage samples' particle size was assessed using a laser diffraction technique, employing a Laser particle size analyzer (S3500, Microtrac Corporation of America). The sample was diluted 10 times with deionized water, and 5 ml was injected into the machine.

2.3 Zeta potential

The Zeta potential is an important parameter that significantly impacts the stability of colloidal systems. It was measured with a methodology previously outlined by Ni et al. [10]. It was calculated by using a Nano-size and zeta potentiometer. The samples underwent a dilution process of 50-fold using deionized water. Subsequently, these diluted samples were utilized to assess the zeta potential under ambient conditions. The final measurements of zeta potential values were obtained by doing a triplicate analysis of the beverage samples.

2.4 Particle Sedimentation

Natural sedimentation of *D. officinale* drink was measured by the volumetric method. In this method, the *D. officinale* pulpy drink was put in measuring cylinders and afterward kept at a temperature of 4 °C for 24 hours. The volumetric determination of separation was conducted in percentage after the storage process.

2.5 Drink Viscosity

A touchscreen digital viscometer was used to determine the viscosity of the *D. officinale* pulpy drink. A 15 ml sample was measured with spindle number 1 at 1.24/s share rate for 2 min.

2.6 Drink Color Analysis

In order to analyze the color properties and variations, a precision spectrophotometer (Ultra scan Pro, Hunter lab) was used. The values were measured in L, a*, and b*, where L, a*, and b* are referred to as brightness, green/red, and blue/ yellow, respectively. The spectrophotometer was calibrated as per company guidelines. After that, the sample was inserted into the apparatus, and color readings were evaluated.

2.7 Statistical Analysis

All the data is arranged and calculated by MS Excel 2019, Minitab, and Origin. ANOVA is one-way with LSD, used to calculate the statistical data. Data is presented in charts with standard deviation (±SD) and significant value $p > 0.05$.

III. RESULTS AND DISCUSSION

2.8 Size of particles in pulpy drink

The use of a high-speed blender and a colloidal mill reduced particle size. The sample was collected at different stages to determine the particle size of blended and ground drinks. Blending for 7.5 minutes reduced the particle size to 127 micrometers, and grinding for 20 minutes reduced it to 45 micrometers at optimal levels (Fig. 1A). The thermal treatment of beverages resulted in a significant rise in particle size (Fig.1 B). After the sterilization process, the

control sample showed a significant rise in particle size (100 um). A variation of 55 micrometers indicated the presence of large aggregated particles. The particle size difference was decreased to the initial particle size of the pulpy drink (45 micrometers) by increasing the ratio of xanthan gum.

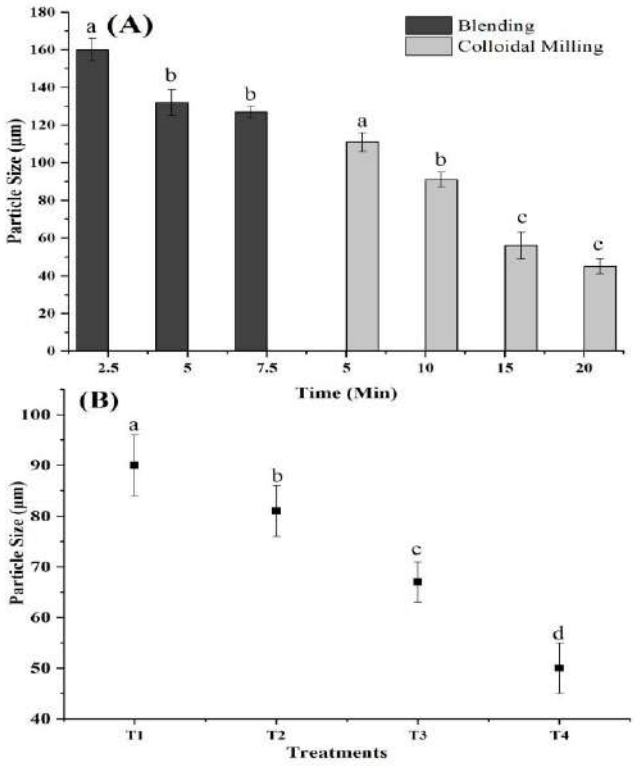

Fig.1: Particle size reduction in response to increasing the time of blender and collide mill (A) and Particle size of the formulations in response to Autoclave sterilization (B). Error bars present ±SD, and small letters present significant differences.

The observed increase in particle size could be because of the coagulation and denaturation of proteins and the aggregation of carbohydrates caused by the elevated temperature during sterilizing. The results of this research align with the conclusions drawn in a prior study conducted by Ni *et al.* [10]. According to a study conducted by Liu, Sun, Xue, and Gao *et al.* [11], the process of sterilization, specifically at a temperature of 121 ◦C for over 25 minutes, resulted in decreased physical stability in walnut beverage emulsions because of the denaturation and coagulation of walnut protein. While the observed increase in particle size of T4 was within the acceptable limit in this study, the drink remained stable. However, adding xanthan gum in combination with *D. officinale* polysaccharide showed advantageous effects in reducing the aggregation of suspended particles and enhancing the stability of the drink.

2.9 Zeta Potential of pulpy drink

The zeta potential values observed for all samples had a significant negative polarity, suggesting an abundance of negatively charged particles relative to positively charged particles in the turbid drink. This could be attributed to two factors: the isoelectric point (pI) of the *D. officinale* polysaccharide and the characteristics of the introduced stabilizers. The polysaccharide derived from *D. officinale* has a net negative charge, primarily due to the presence of uronic acid [12]. It was observed in this study that the value of the zeta potential in the beverage samples, which were supplemented with xanthan gum and subjected to the autoclave sterilization method, exhibited significantly negatively higher zeta values (Fig. 2). However, the anionic characteristics of xanthan gum and *D. officinale* polysaccharide resulted in the presence of negatively charged particles. After sterilization, it was observed that the zeta potential values of all drinks exhibited a rise. It may be attributed to a probable decrease in the effective surface charge of dispersed particles, which the aggregation of these particles may have generated. Their effective surface charge influenced suspended particles' dispersion and aggregation [13]. It suggests that the dispersed particles present in the drink exhibited limited aggregation tendencies as a result of the significant electrostatic repulsion forces.

*2.10*Particle Sedimentation in a pulpy drink.

Xanthan gum possesses several hydroxyl groups that actively bind to water molecules, facilitating the development of gel and viscous solutions [14]. Being an extracellular heteropolysaccharide, Xanthan gum is widely utilized as a thickening agent and emulsifier to improve the stability of food products [15]. Xanthan gum was also found stable in high temperatures, making the *D. officinale* drink stable [16]. As shown in Fig. 3 (A), the control sample had 35% sedimentation, but adding 0.4% xanthan gum reduced it to 0% (Fig. 3E). It could be because of the characteristics mentioned above of xanthan gum that it bounded the water and made a viscous solution to hold up the suspended particles.

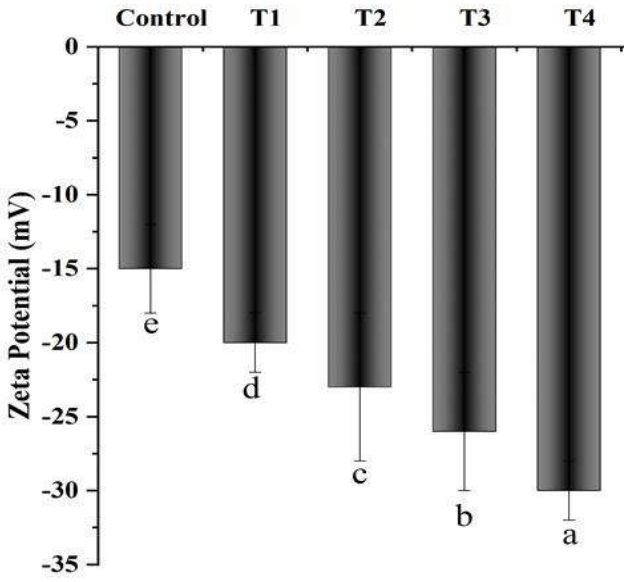

Fig.2: Zeta potential of the formulations. Error bars are presenting ±SD and small letters are presenting significant difference.

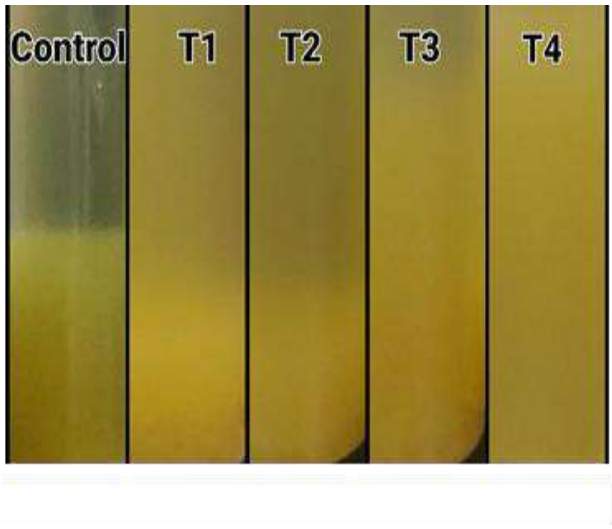

Fig.3: Particle sedimentation photograph of all the formulations

2.11 Viscosity of pulpy drink.

As presented in Table 1, the control sample indicated that the polysaccharide of *D. officinale* was not thermally stable [17]. However, after sterilization, the control sample significantly reduced the viscosity, and adding xanthan gum helped improve the viscosity of the drink to make it stable. The results suggested that xanthan gum improved efficiency in making a stable *D. officinale* pulpy drink by increasing the viscosity and protecting it from high heat impact. It has shown that incorporating hydrocolloids, typically at concentrations ranging from 0.1% to 3.0%, may effectively enhance the stability of cloudiness in drinks [16, 18]. Significant improvements may be attained with the incorporation of xanthan gum because of its exceptional capacity to exhibit higher viscosity at low shear rates. It can be attributed to the higher molecular weight and distinctive stiff rod-like structure of xanthan gum, which may also account for the pronounced shear thinning behavior seen in drinks thickened with the xanthan gum [19].

Table 1: Viscosity of the D. officinale pulpy drink with different xanthan gum ratios. Small letters in every column show a significant difference.

Formulations	Prior to sterilization	After sterilization
Control	$15.62 \pm 0.06_e$	$2.19 \pm 0.03_e$
T1	$29.12 \pm 0.03_d$	$12.35 \pm 0.05_d$
T2	$56.9 \pm 0.02_c$	$38.75 \pm 0.07_c$
T3	$86.43 \pm 0.05_b$	$62.15 \pm 0.08_b$
T4	$123.65 \pm 0.09_a$	$75.83 \pm 0.02_a$

2.12 Color Analysis of pulpy drinks

The acceptability of a food product may be greatly influenced by its color, with the proportional significance of this factor fluctuating across different food systems [20]. One of the drawbacks associated with thermal treatments is the alteration of pigments due to high heat, leading to modifications in the original hue of the food substance. Chlorophyll (the pigment responsible for the green color) degrades during high-heat processing, resulting in pheophytins (the pigment responsible for the gray-brown color), which is strongly correlated with a drop in product quality [21].

This study observed a significant alteration in the hue of all beverages compared to the control sample. All autoclaved sterilized drinks turned green to yellowish, as presented in Table 2. It could also be because of the degradation of chlorophyll under high heat. There was also a correlation between temperature and the polymerization reaction between pigments and polyphenols, which increased the possibility of degradation and destruction of pigments in drinks as the temperature increased [22].

Table 2: Color Analysis of sterilized D. officinale pulpy drink. Small letters in every column show a significant difference.

Formulation	L	a*	b*
Control	$44.65 \pm 0.3_c$	-1.56_c	67.01_a
T1	$58.72 \pm 0.1_b$	-0.17_b	43.36_b

T2	58.80 ± 0.1$_b$	-0.16$_b$	43.32$_b$
T3	60.64 ± 0.4$_a$	-.01$_a$	45.32$_c$
T4	60.61± 0.2$_a$	-.02$_a$	45.39$_c$

IV. CONCLUSION

The addition of hydrocolloids and their precise amounts has significantly influenced the physical stability of liquid beverages. Xanthan gum is an additive that enhances the stability of beverages due to its hydroxyl groups, which have the potential to bind water molecules and elevate the viscosity of the drink. This research indicates that enhancing the beverage's viscosity by optimizing the gum ratio and reducing the particle size might improve its stability. The physical stability of the Dendrobium officinal pulpy drink containing 0.4% Xanthan gum and 3% pulp was the highest after sterilization.

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Assessment of Ecological Environment in Zhanjiang Based on RSEI and PCA

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Abstract— *This study uses Landsat-8 remote sensing images as the data source and selects four indicators that directly reflect the quality of the ecological environment, such as greenness (NDVI), wetness (WET), dryness (NDBSI), and heat (LST). Meanwhile, we use principal component analysis (PCA) to construct a remote sensing ecological index (RSEI) model for exploring the changes in the ecological environment quality of Zhanjiang from 2013 to 2021. The results indicate that RSEI can better reflect the ecological environment of the region. The study area is mainly affected by dryness, followed by greenness and humidity, with heat having the smallest impact. The average RSEI values in Zhanjiang City in 2013 and 2021 were 0.5339 and 0.5576, respectively, indicating a slight improvement in overall ecological environment quality. Among them, NVDI showed an increasing trend, NDBSI and WET decreased, but LST increased.*

Keywords— ***Remote Sensing Ecological Index (RSEI); Principal Components Analysis (PCA); Ecological Environment Quality; Normalized Difference Vegetation Index (NDVI), Normalized Difference Bare Soil Index (NDBSI), Land Surface Temperature (LST)***

I. INTRODUCTION

The quality of the ecological environment is a comprehensive characteristic of the elements, structure, and function exhibited by an ecosystem (Kong et al., 2019; Li et al., 2018). Which reflect the degree of superiority or inferiority of the ecological environment and speculate on the impact of human activities on the environment. The research on monitoring and evaluation of ecological environment quality is receiving increasing attention, and there are many methods and models for ecological quality evaluation, including Analytic Hierarchy Process (AHP) (Li et al., 2006), Ecological Environment Status Index (EI) (Yao et al., 2012), "Pressure State Response" PSR model (Wang et al., 2017), and the use of the InVEST model (Lu et al., 2018) and landscape pattern index (Liu et al., 2011) for ecological environment quality evaluation and analysis.

The factors adopted by the above model indicators are related to vegetation and human activities. There are differences in the suitability of human activity indicators in different regions, which has limitations for some areas

where it is difficult to obtain human activity indicators. The Remote Sensing Ecological Index (RSEI) (Xu, 2013; Gao and Wang, 2023), which includes factors such as greenness, humidity, dryness, heat, etc., provides a more objective and comprehensive evaluation of the ecological environment of a region than traditional ecological quality index calculations. Moreover, this index is also applicable to soil erosion (Xu, 2013; Zhang et al., 2015) and urban areas (Yang et al., 2019).

In addition, some studies have added principal component analysis (PCA) to the RSEI model to evaluate and classify ecological indices. The method is a mathematical dimensionality reduction method that can convert multiple indicators into a few principal components, which are linear combinations of original variables and have no interrelationships with each other. They can reflect most of the information in the original data (Han et al., 2012), which is reasonable and feasible for evaluation. It has been applied in many practical fields and has the ability to classify and visualize a large amount of data.

In recent years, the regional economy in Zhanjiang has developed rapidly, with the industrial scale and total economic output continuously increasing (Liu et al., 2021; Yang and Li, 2022). Along with the advancement of urbanization and the expansion of urban land use, green development and ecological civilization construction are also important aspects of sustainable social and economic development. The use of RSEI science to monitor and evaluate the impact of human activities on cities has important theoretical and practical significance for protecting and promoting harmony between humans and nature and promoting sustainable social development. Thus, this study is based on the combination of RSEI and PCA to conduct a multi-indicator, large-scale, and multi-temporal ecological environment assessment. Analyze the changes in the ecological environment in the region from 2013 to 2021 and explore the key factors affecting urban environmental changes. The aim is to serve the ecological environment of Zhanjiang City and promote sustainable ecological development. Therefore, the main objectives of this paper include the following:

(1) By combining remote sensing technology with the current ecological environment situation in Zhanjiang, suitable ecological environment quality evaluation indicators are selected based on natural conditions, economic development, and other aspects. A suitable RSEI ecological environment quality evaluation system is selected, and relevant data and materials are searched based on the system.

(2) Utilize the ENVI software to extract information on greenness, humidity, dryness, and heat indicators required for experiments in remote sensing images.

(3) Assign the weight values of evaluation indicators through PCA, select the principal components with more information based on the results of PCA, and extract the RSEI index. Use ArcGIS software to comprehensively analyze the quality of the ecological environment.

(4) Based on the results of the comprehensive evaluation of the ecological environment quality in Zhanjiang, propose countermeasures and suggestions for the problems faced by the city's ecological environment.

II. STUDY AREA AND DATA SOURCES

2.1 Study Area

Zhanjiang City is located in the southernmost part of the Chinese Mainland, southwest of Guangdong Province, between 109 ° 40 ′ -110 ° 58 ′ E and 20 ° 13 ′ -21 ° 57 ′ N (Figure 1). Belonging to the tropical northern monsoon climate, it is year-round regulated by the marine climate, with no severe cold in winter and no scorching heat in summer. Subtropical crops and marine resources are abundant. The land of Zhanjiang is mostly composed of peninsulas and islands, with a terrain roughly high on the central axis, low on the east and west sides, high in the north and south, and low in the middle, with gentle undulations and mostly plains and plateaus. In the total land area of the city, plains account for 66%, hills account for 30.6%, and mountainous areas account for 3.4%. As of 2022, the permanent population of Zhanjiang City is 7.0354 million, with an urban permanent population of 3.3284 million. The proportion of the total population (urbanization rate) is 47.31%, and the total GPD ranks ninth in Guangdong Province. Zhanjiang mainly relies on

the natural advantages of the port and plays a pivotal role in the economic development and foreign trade of its hinterland.

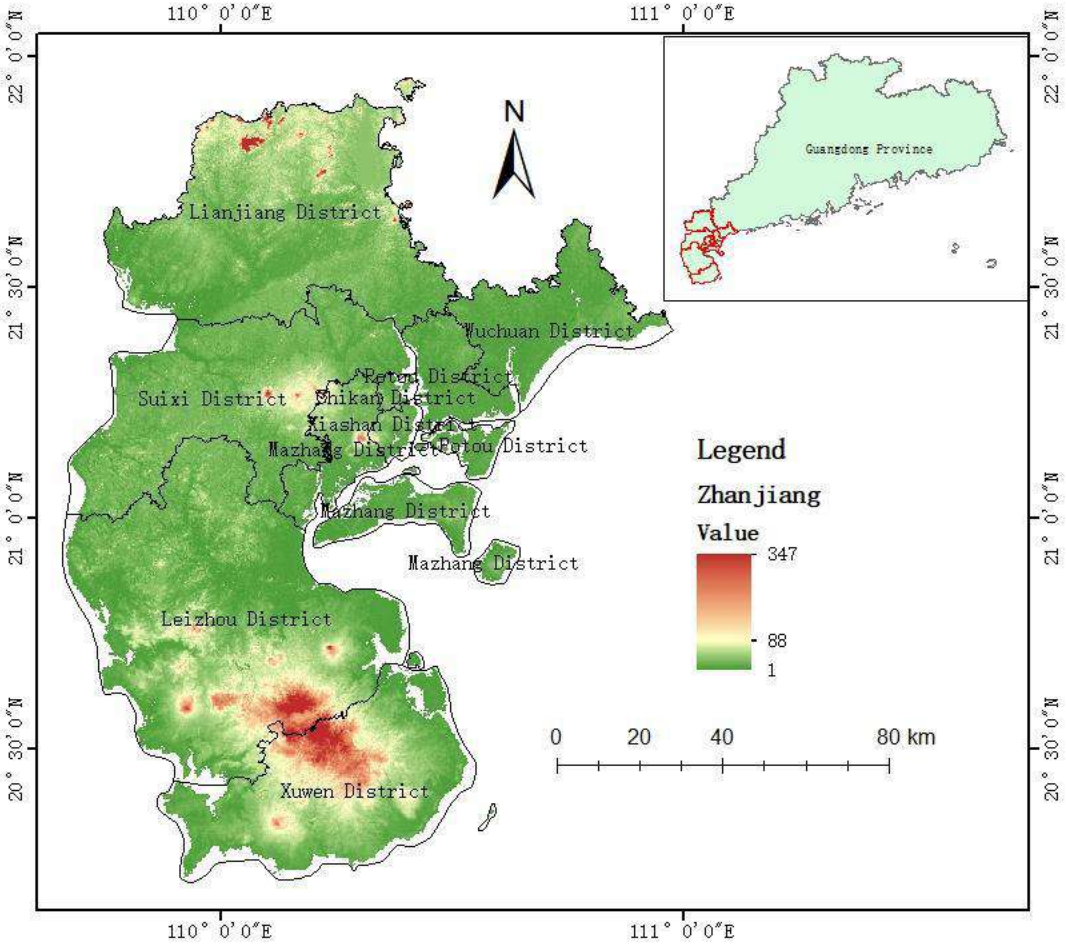

Fig.1 Map of Zhanjiang, Guangdong

2.2 Data Sources

The basic data used in this study is mainly Landsat-8 OLI remote sensing data (sourced from https://www.gscloud.cn/ ） . This includes two remote sensing images in 2013 and 2021, with the months concentrated in December, resulting in better image quality and data cloud coverage of less than 1% (Table 1). Vector data used in the research area (sourced from https://www.webmap.cn/) is mainly used for clipping and the production of remote sensing images. Terrain layer (from https://www.gscloud.cn/): the downloaded data is 30m DEM, which is used for the terrain's overview of the study area.

Table 1 Information of Remote Sensing Images

Satellite	Track Number	Resolution	Cloud cover	Imaging date
Landsat OLI	124/045	30m	0.01	2013/12/29
	124/046		0.09	2013/12/29
	124/046		0.08	2021/12/3
	124/045		0.03	2021/12/3

III. METHOD

The process of obtaining the remote sensing ecological index (RSEI) is shown in Figure 2. The technical route can be roughly divided into the following steps: remote sensing image preprocessing, RSEI index extraction, evaluation system construction and analysis, and production of thematic maps. Firstly, the selected images for the experiment were subjected to radiometric correction and mosaicism, and the study area images were clipped based on the vector data of the study area. Then, use relevant formulas to calculate four ecological indicators for each image, synthesize their images, and perform principal component transformation on the newly synthesized images; Next, calculate the numerical values based on the obtained ecological environment evaluation indicators, and conduct evaluation and analysis based on relevant data from the research area; Finally, create relevant thematic maps.

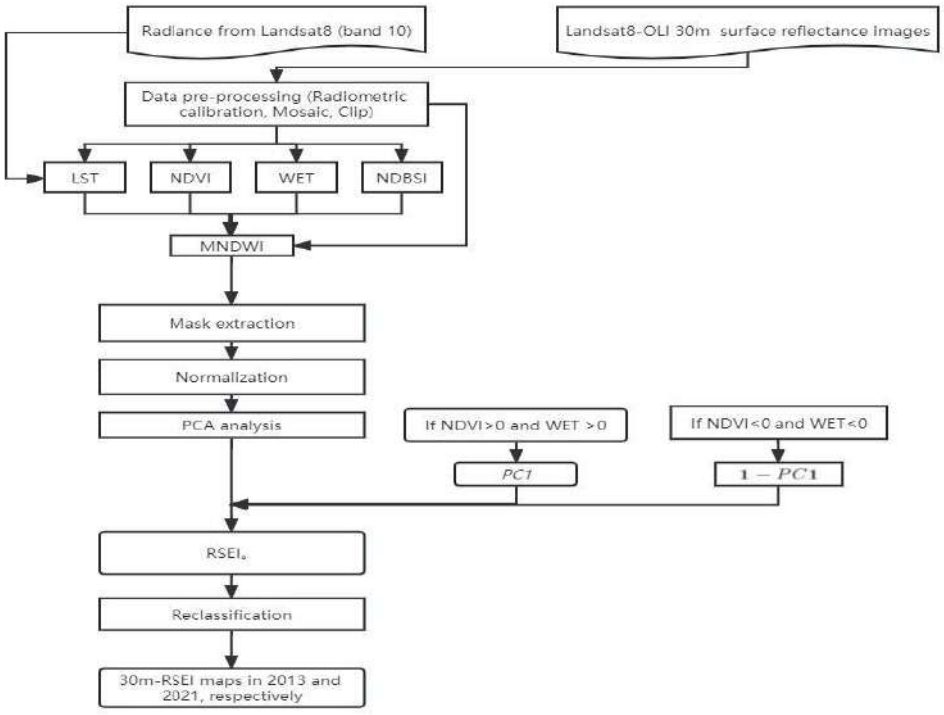

Fig.2 The Schema Flowchart of This Study

3.1 The Single Indicator of RSEI Model

（1）Greenness Index (NDVI): The Normalized Difference Vegetation Index (NDVI) is the most widely used vegetation index, which can accurately reflect vegetation greenness, regional land cover classification, and its changes (Song et al., 2011). Thus, NDVI is used to represent the greenness indicator:

$$NDVI = (\rho_{NIR} - \rho_{Red}) - (\rho_{NIR} + \rho_{Red}) \quad (1)$$

In equation (1), ρ_{NIR} is in the near-infrared band; ρ_{Red} is the red band.

（2）Humidity Index (WET): Based on remote sensing data inversion, humidity is commonly used to reflect changes in soil moisture. If soil moisture is too low, it can cause plants to absorb less water, resulting in a decrease in plant moisture content. Thus, studying the humidity of the ecological environment is particularly important. The tassel-hat transformation is an empirical forward transformation of images based on the spatial information structure of soil, vegetation, etc. in multiple spectra (Zhou et al., 2006). One component is humidity, and the wavelength range of Landsat OLI data in the red band is 0.63-0.68 μm. The humidity indicators are represented as follows:

$$WET_{OLI} = 0.1511\rho_1 + 0.19732\rho_2 + 0.32833\rho_3 + 0.34074\rho_4 - 0.71175\rho_5 - 0.45596\rho_6 \quad (2)$$

Equation (2) WET_{OLI} represents the humidity component of Landsat OLI remote sensing images, $\rho_1 - \rho_6$ represents the spectral reflectance of the blue, green, red, near-infrared, shortwave infrared 1, and shortwave infrared 2 bands, respectively.

（3）Dryness Index (NDBSI): With the acceleration of urbanization, land use types have shifted from agricultural land and forest land to impermeable construction land, while exposed soil and impermeable surfaces such as construction land will increase the degree of dryness. Thus, the study synthesized the soil index (SI) and intelligent building index (IBI) to obtain the dryness index (NDBSI) to reflect the changes in dryness:

$$SI = [(\rho_5 + \rho_3) - (\rho_4 + \rho_1)]/[(\rho_5 + \rho_3) + (\rho_4 + \rho_1)] \quad (3)$$

$$IBI = \{2\rho_5 + \rho_4) - [(\rho_4/\rho_4 + \rho_3) + \rho_2/(\rho_2 + \rho_5)]\}/\{2\rho_5/(\rho_5 + \rho_4) + [(\rho_4/\rho_4 + \rho_3) + \rho_2/(\rho_2 + \rho_5)]\} \quad (4)$$

$$NDBSI = (SI + IBI)/2 \quad (5)$$

In equations (3), (4), and (5), SI, IBI, and NDBSI represent soil index, building index, and dryness index, respectively, $\rho_1 - \rho_5$ represents the spectral reflectance of blue, green, red, near-infrared, and shortwave infrared bands.

（4）Heat Index (LST): The transformation of land cover leads to changes in thermal radiation and urban heat island effects, which affect ecological environment changes. For example, studying land surface temperature (LST) as one of the indicators to quantitatively evaluate urban land use and land use types (Yue et al., 2006). Thus, as an indicator of heat, LST has strong reliability. The variation of LST is inverted using atmospheric correction, and the specific formula is as follows:

$$L_\lambda = [\varepsilon B(LST) + (1-\varepsilon)L\downarrow]\tau + L\uparrow \quad (6)$$

$$B(LST) = [L_\lambda - L\uparrow - \tau(1-\varepsilon)L\downarrow]/\tau\varepsilon \quad (7)$$

$$LST = K_2 2/\ln(K_1/B(LST) + 1) \quad (8)$$

In equations (6), (7), and (8), L_λ is the brightness value of thermal infrared radiation, ε is the surface emissivity, LST is the true surface temperature, and B (LST) is the blackbody thermal radiance, τ for transmittance, L↑ and L↓represent the upward and downward radiance of the atmosphere, respectively. $K_1 = 774.84\,W/(m^2 \cdot \mu m \cdot sr)$，$K_2 = 1321.08K$。

3.2 Principal Components Analysis (PCA)

Let F1 be the PCA formed by the first linear combination of the original variable, $F_1=a_{11}X_1+a_{21}X_2+...+a_{p1}X_p$. According to mathematical knowledge, the amount of information extracted by each principal component can be measured by its variance. The larger the variance Var (F1), the more information F1 contains. It is often the first principal component (F1) that contains the largest amount of information, so F1 is selected as X1, X2,... in XP, the linear combination with the largest variance is called F1 as the first principal component (Xu and Deng, 2022).

3.2.1 Mathematical model of PCA method

From a geometric perspective, PCA is the process of rotating and transforming the original coordinates to obtain intersecting coordinate axes. The direction where all data points are scattered is the direction of the coordinate axes, and this new set of coordinate axes is arranged based on the size of the obtained eigenvalues. For n samples, X1，X2，...， the dataset X of Xp variables has a data matrix of:

$$\begin{bmatrix} x_{11} & \cdots & x_{1p} \\ \vdots & \ddots & \vdots \\ x_{n1} & \cdots & x_{np} \end{bmatrix} = [x_1, x_2, \ldots, x_p] \quad (9)$$

wherein： $x_i = (x_{1i}, x_{2i}, .., x_i)T$, i=1,2,...,p

PCA is the transformation of the original P observation variables X1，X2， Xp, forming P new variables (comprehensive variables), Namely:

$$\begin{cases} F_1 = a_{11}x_p + a_{12}x_p + \cdots + a_{1p}x_p \\ F_2 = a_{21}x_p + a_{22}x_p + \cdots + a_{2p}x_p \\ F_3 = a_{31}x_p + a_{32}x_p + \cdots + a_{3p}x_p \\ \cdots \\ F_p = a_{p1}x_p + a_{p2}x_p + \cdots + a_{pp}x_p \end{cases} \quad (10)$$

In equations (9) and (10), x_i is an n-dimensional vector, F_i is also an n-dimensional vector. The above model needs to meet the following conditions:

F_i, F_j Uncorrelated（i≠j,I,j=1,2,3,…,p）

The variance of F_1 is greater than F_2. The variance of F_2 is greater than F_3, and so on.

Simultaneously satisfying the above three conditions, the transformed new random variables are independent of each other, and the variance gradually decreases, and F_1 is the first principal component, F_2 is the second principal component, and so on. Represent the above model in matrix form as: F=AX

$$F = \begin{bmatrix} F_1 \\ F_2 \\ \cdots \\ F_P \end{bmatrix} \quad X = \begin{bmatrix} x_1 \\ x_2 \\ \cdots \\ x_P \end{bmatrix} \quad A = \begin{bmatrix} a_{11} & \cdots & a_{1p} \\ \vdots & \ddots & \vdots \\ a_{p1} & \cdots & a_{pp} \end{bmatrix} = \begin{bmatrix} a_1 \\ a_2 \\ \cdots \\ a_p \end{bmatrix} \quad (11)$$

Where, in equation (11), A is the main component coefficient matrix.

PCA can obtain multiple principal components, but their variance is decreasing. Therefore, in practical operations, it is generally determined based on the size of the contribution rate. The contribution rate reflects the amount of information in each principal component, and the first principal component accounts for the majority of the information in the principal component. The contribution rate calculation formula is:

$$F_1 = \lambda_1 / \sum_{i=1}^{p} \lambda_1 \quad (12)$$

In equation (12), F_1 is the number of main components with a contribution rate of p, λ is the characteristic values of the main components.

3.2.1 MRSEI model

MRSEI is the weighted value of the principal components in RSEI, which is:

$$MRSEI = \omega_1 PC1 + \omega_2 + PC2 + \omega_3 PC3 \quad (13)$$

The MRSEI index is a weighted sum calculation that adds the second and third principal components without ecological significance to the first principal component with clear ecological significance. When the characteristic value of PC1 accounts for a high proportion (contribution rate>80%), although the results of MRSEI are lower than those of RSEI, the difference is very small, so using MRSEI will only increase the computational workload and is completely unnecessary. When the characteristic value of PC1, i.e. contribution rate, is less than 80%, MRSEI will significantly reduce the value of RSEI, so it cannot be used (Xu and Deng, 2022). Therefore, in practical operation, PC1 is used as the initial value of RSEI.

3.3 Comprehensive Index Construction of RSEI Model

The RSEI model, composed of greenness, humidity, dryness, and heat, can be used to qualitatively and quantitatively evaluate the quality of the ecological environment, and the weight of the indicators can be determined through the principal components. This model is easily affected by water bodies, so when there is a large amount of water in the study area, such as reservoirs and lakes, the water body should be masked. The water body index model used here is WNDWI (Xu, 2005) to reflect

the ecological status of the study area. In order to unify each single indicator, it is necessary to normalize the individual indicators (Equation 14), then perform principal component analysis and retain the first component with the highest variance, namely the initial value of RSEI (equation 15).

According to the research results of RSEI (normalized initial value RSEI) (equation 16), it is divided into five levels using the equal interval method: Poor (0-0.2), Inferior (0.2–0.4), Moderate (0.4–0.6), Good (0.6–0.8), and Excellent (0.8–1) to analyze the changes in ecological environment quality in Zhanjiang.

$$NI_i = (I_i - I_{min})/(I_{max} - I_{min}) \quad (14)$$

In equation (14) NI_i is the normalized value for the i-th year: taking NDVI as an example I_i is the i-th year NDVI, I_{min} is the minimum value of NDVI, I_{max} is the maximum NDVI value in the i-th year.

$$RSEI_0 = 1 - \{PC1[f(NDVI, WET, VDBSI, LST)]\} \quad (15)$$

$$\mathrm{RSEI} = (\mathrm{RSEI}_0 - \mathrm{RSEI}_{0\_\min})/(\mathrm{RSEI}_{0\_\max} - \mathrm{RSEI}_{0\_\min}) \quad (16)$$

In equations (15) and (16), $RSEI_0$ is the initial value of the RSEI; PC1 is the first principal component obtained by principal component transformation of NDVI, WET, NDBSI, and LST; $\mathrm{RSEI}_{0\_\max}$、$\mathrm{RSEI}_{0\_\min}$ are the maximum and minimum values of the initial RSEI values, respectively. The range of RSEI is between [0-1]. The larger the RSEI, the better the local ecological environment quality. Conversely, the worse the local environmental quality.

IV. ANALYSIS AND RESULTS

The results of the four principal component analyses in Zhanjiang in 2013 and 2021 are shown in Table 2, which shows that the proportion of PC1 is very large, indicating that most of the characteristic values of the four indicators have been concentrated. In PC1, NDVI which represents greenness, and WET, which represents humidity, exhibit positive values, indicating their positive impact on ecology. If it is negative, the "1-PC1" operation (equation 10) needs to be performed. If it is positive, this operation is not required.

Table 2 Principal component analysis of each index

Year	Index	PC1	PC2	PC3	PC4
2013	NDVI	0.65	0.47	-0.58	-0.14
	WET	0.08	0.04	-0.1	0.99
	NDBSI	-0.72	-0.64	0.28	-0.01
	LST	-0.25	0.61	0.75	0.03
	Eigenvalue	0.1556	0.0559	0.0292	0.0013
	Contribution rate /%	64.3	23.11	12.05	0.54
2021	NDVI	-0.63	0.01	0.74	0.24
	WET	-0.27	-0.06	0.08	-0.96
	NDBSI	0.70	0.23	0.65	-0.16
	LST	0.18	-0.97	0.16	0.02
	Eigenvalue	0.1616	0.0381	0.0091	0.003
	Contribution rate /%	76.29	18.01	4.3	1.4

4.1 RSEI Graded Evaluation

Analysis shows that the RSEI in 2013 and 2021 were 0.5339 and 0.5576, respectively (Table 3), showing a slight upward trend, indicating an improvement in the ecological environment of the study area.

Further, analyzing the changes in environmental quality and spatial pattern in Zhanjiang from 2013 to 2021 (Figure 3), the annual RSEI was divided into five levels:

Poor (0-0.2), Inferior (0.2–0.4), Moderate (0.4–0.6), Good (0.6–0.8), and Excellent (0.8–1). Table 4 shows the ecological grade area and proportion of Zhanjiang in different periods. In 2013, the total area of areas with excellent and good ecological environments in Zhanjiang was about 43.48%, distributed in the eastern part of Xuwen County, the southwestern part of Leizhou City, and the northern part of Lianjiang City; the total area of areas with poor and inferior is about 24.89%, distributed in urban areas such as the eastern part of Leizhou and Chikan District, as well as towns around the city; and the area with a moderate ecological grade is about 41.63%, with a relatively scattered distribution. The Suixi area has a relatively large range with a moderate ecological grade, indicating that the ecological quality and environment in Zhanjiang are relatively stable and good. By 2021, the RSEI level difference in Zhanjiang had increased by 3.79%, while the ecological environment quality level had increased to 14.18% and 28.07%, respectively. In areas such as Suixi and Lianjiang, the RSEI level has shifted from medium to excellent, indicating that Zhanjiang's ecology practices the concept of "environmental protection". Ecological issues have been taken seriously, and environmental protection work has been implemented.

Table 3 Changes of Mean Values of Four Indicators and RSEI

Year	Item	NDVI	WET	NDBSI	LST	RSEI
2013	Mean	0.5837	0.5904	0.5428	0.4913	0.5339
2021		0.6152	0.3804	0.4512	0.5410	0.5576

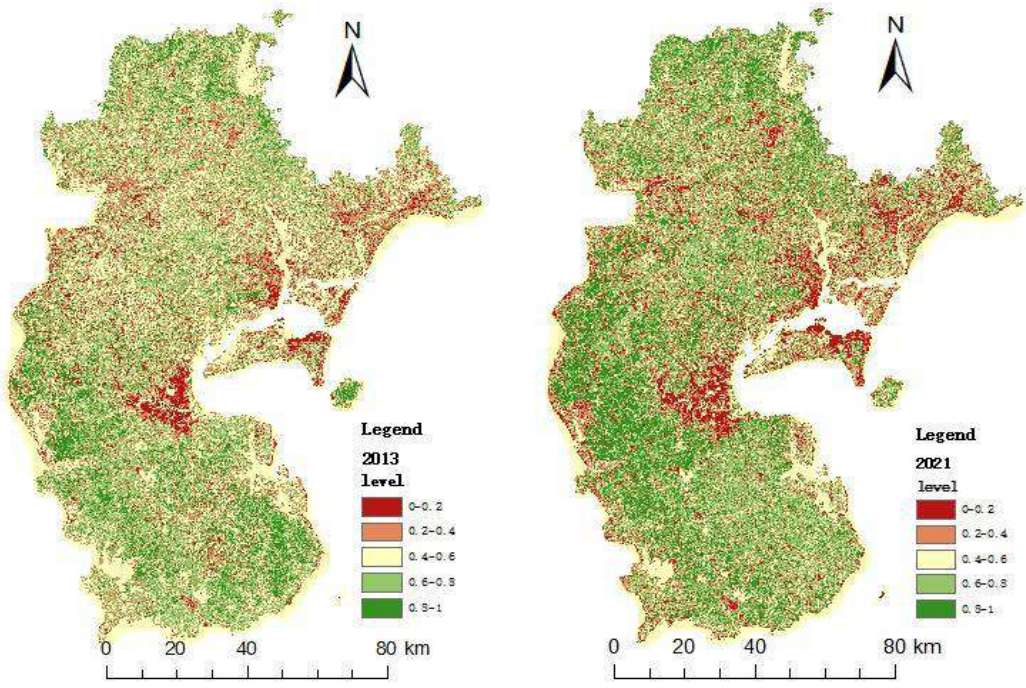

Fig.3 Changes of RSEI in Zhanjiang from 2013 to 2021

Table 4 Area and proportion of ecological grade in different periods of Zhanjiang

RSEI grade	2013 年		2021 年	
	Area/㎢	Rate/%	Area /㎢	Rate/%
Poor	858.19	6.71	1342.51	10.50
Inferior	2325.09	18.18	2139.22	16.73
Moderate	5324.34	41.63	3903.88	30.52
Good	2858.78	22.35	3590.63	28.07
Excellent	1423.64	11.13	1813.79	14.18
Total	12790.03	100.00	12790.03	100.00

4.2 Characteristics of Ecological Environment Changes

In Figure 4, the legends 1, 2, 3, 4, and 5, respectively, represent a significant change in RSEI level, with an obvious decrease, slight decrease, no change, slight improve, and significant improve. The statistical results of RSEI ecological level changes in Zhanjiang from 2013 to 2021 are shown in Table 5. Through ArcGIS and Excel statistical analysis, the area where the ecological level of Zhanjiang remains unchanged is 5769.85 km^3, accounting for approximately 42.77%. Mainly distributed in river and lake areas, the quality of the ecological environment in this area is relatively stable. About 26.29% of them have slightly or obvious decreased ecological levels, distributed in the eastern part of Xuwen County and Donghai Island. The proportion of slightly improved and significantly improved areas is about 30.94%, with the most significantly improvement being in the western part of Suixi County, indicating a gradual improvement in the environmental quality of the region.

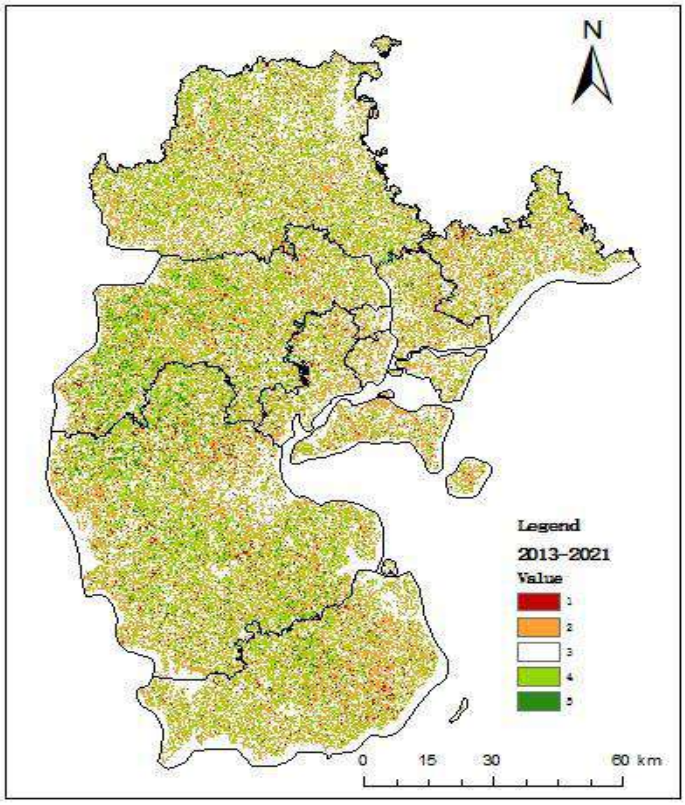

Fig.4 Change of RSEI in Zhanjiang between 2013 and 2021

Table 5 Area and Percentage Change of RSEI in Zhanjiang

Class	RSEI level transfer area/km^3	Change rate /%
Obvious decrease	250.19	1.85
Slight decrease	3296.78	24.44
No change	5769.85	42.77
Slight improve	3787.62	28.08
Significant improve	385.89	2.86
Total	13490.33	100.00

V. CONCLUSIONS

(1) Among the green index, humidity index, dryness index, and heat index, the dryness index has the highest contribution rate to RSEI, indicating that land use methods are closely related to the urban ecological environment. The increase in building land area caused by urban planning and layout will have a negative impact on the urban environment, while greening projects such as afforestation and the construction of landscape economic belts will improve the ecological environment of the area. Therefore, people should enhance their awareness of this aspect, effectively adapt to local conditions, and plan reasonably. The government should not only focus on urban economic development but also vigorously promote ecological environment construction, increase urban green area, and optimize urban and rural land use structures.

(2) Between 2013 and 2021, the RSEI value of Zhanjiang increased from 0.5339 to 0.5576, an increase of 2.37%, indicating that the ecological quality of Zhanjiang is developing towards a positive trend. According to the RSEI index grading table, the ecological environment quality of Zhanjiang is mostly in the "medium" and "good" stages, with a decrease in the proportion of "poor" and "Inferior", and an increase in the proportion of "excellent" and "good". This indicates that people's awareness of ecological protection has been continuously strengthened in recent years, and in recent years, Zhanjiang has vigorously developed the tourism industry, promoting the protection of tourism resources, including natural resources, which has to some extent protected the local ecological environment.

(3) The areas where the ecological environment level of Zhanjiang has decreased are mainly on Donghai Island and around towns. The towns may have been affected by comprehensive poverty alleviation policies in recent years, increased investment in infrastructure by the government, expansion of construction land, and the construction of hardened roads in villages, resulting in a certain degree of ecological damage along the way. Donghai Island may be due to the ecological environment decline caused by the large industrial group Baosteel. Thus, the expansion of urban land use, the increase in bare soil area, and environmental pollution will all be the main tasks of governance in Zhanjiang in the future. Therefore, in future development, Zhanjiang should focus on rational planning of land resources, increasing vegetation coverage in the urban area, reducing industrial pollution, encouraging green consumption among citizens, and promoting green living.

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Air pollution in Bamako: Modeling, Pollution-Population Index

La pollution de l'air à Bamako: Modélisation, Indice Pollution-Population

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Abstract— *This study on air pollution in Bamako carried out in 2019, aims to characterize the different sources of emissions, model the health impact of ambient air quality for the different scenarios, the proposal of an action plan to limit emissions, and the simulation of the evolution of emissions and ambient air quality by 2015 and 2020 with and without an action plan. The results of the two air quality measurement campaigns show concentration levels lower or substantially equal between those of July 2019 and those of the 2009 data. The PM10 concentration recorded indicates a significant excess area compared to the values concentration guides set by the WHO. Analysis of the evaluation of emissions and their health impact by 2015 and 2020 shows that pollution by volatile organic compounds and dust will become unacceptable if nothing is done. The number of cancers linked to benzene will increase to 686 cases and the increase in mortality due to dust to an average figure of 38.7%.*

Keywords— ***Pollution, modeling, health impacts, Bamako, Mali.***

Résumé— *Cette étude sur la pollution de l'air à Bamako réalisée en 2019, a pour objectif, la caractérisation des différentes sources d'émissions, la modélisation de l'impact sanitaire de la qualité de l'air ambiant pour les différents scenarios, la proposition d'un plan d'actions pour limiter les émissions, et la simulation de l'évolution des émissions et de la qualité de l'air ambiant à l'horizon 2015 et 2020 avec et sans plan d'actions. Les résultats des deux campagnes de mesures de la qualité de l'air montrent des niveaux de concentration inférieurs ou sensiblement égaux entre ceux de Juillet 2019 à ceux des données de 2009. La concentration en PM10 relevée indique une zone de dépassement importante par rapport aux valeurs guides de concentrations fixées par l'OMS. L'analyse de l'évaluation des émissions et de leur impact sanitaire aux horizons 2015 et 2020 montre que la pollution par les composés organiques volatils et les poussières deviendra inacceptable si rien n'est fait. Le nombre de cancers liés au benzène passera à 686 cas et l'augmentation de la mortalité due aux poussières à un chiffre moyen de 38,7%.*

Mots clés— ***Pollution, modélisation, impacts sanitaires, Bamako, Mali.***

I. INTRODUCTION

La principale source de pollution atmosphérique domestique est la combustion à l'intérieur des logements de combustibles fossiles, de bois et d'autres combustibles à base de biomasse pour cuisiner, chauffer et éclairer les habitations. Dans de nombreux pays, la production d'énergie est l'une des principales sources de pollution 400 000 décès prématurés. Près de la moitié des décès dus à la pollution de l'air imputable aux transports sont imputables aux émissions de diesel, tandis que les personnes vivant à proximité des principales artères de la circulation ont 12% plus de risques de souffrir de démence. La réduction des émissions des véhicules est une intervention importante pour améliorer la qualité de l'air, en particulier dans les zones urbaines. Les politiques et les normes qui exigent l'utilisation de carburants plus propres et de normes avancées en matière d'émission des véhicules peuvent réduire les émissions de ces véhicules de 90% ou plus.

L'agriculture consiste en deux sources principales de pollution atmosphérique : le bétail, qui produit du méthane et de l'ammoniac, et la combustion des déchets agricoles. Les émissions de méthane contribuent à l'ozone troposphérique, qui provoque l'asthme et d'autres maladies respiratoires. Le méthane est également un gaz plus puissant que le dioxyde de carbone pour le réchauffement de la planète. Son impact est 34 fois plus important sur une période de 100 ans. Environ 24% de tous les gaz à effet de serre émis dans le monde proviennent de l'agriculture, de la foresterie et des autres utilisations des terres.

La combustion des déchets à ciel ouvert ainsi que les déchets organiques dans les décharges rejettent dans l'atmosphère des dioxines, des furannes, du méthane et du carbone noir nocifs. À l'échelle mondiale, environ 40% des déchets sont brûlés à ciel ouvert. Le problème est particulièrement grave dans les régions en urbanisation et les pays en développement. La combustion à ciel ouvert de déchets agricoles et / ou municipaux est pratiquée dans 166 pays sur 193. L'amélioration de la collecte, de la séparation et de l'élimination des déchets solides réduit la quantité de déchets brûlés ou enfouis. La séparation des déchets organiques et leur transformation en compost ou en bioénergie améliorent la fertilité du sol et constituent une source d'énergie alternative. Réduire environ un tiers de tous les aliments perdus ou gaspillés conduit à améliorer la qualité de l'air.

L'ensemble de la pollution atmosphérique ne provient pas de l'activité humaine. Les éruptions volcaniques, les tempêtes de poussière et autres processus naturels posent également problème. Les tempêtes de sable et de poussière sont particulièrement préoccupantes. De fines particules de poussière peuvent parcourir des milliers de kilomètres à la suite de ces tempêtes, qui peuvent également véhiculer des agents pathogènes et des substances nocives, provoquant des problèmes respiratoires aigus et chroniques. La pollution atmosphérique tue 5,5 millions de personnes par an dans le monde (dont 2,6 millons de décès indirects) selon des chiffres 2016 de la Banque mondiale: elle est devenue le quatrième facteur de décès prématuré sur Terre. Maladies cardiovasculaires, cancers des poumons, maladies pulmonaires chroniques, infections respiratoires... La pollution de l'air est coupable d'un décès sur dix dans le monde, six fois plus que le paludisme. La cause est l'Homme et son activité, par les industries, le trafic routier, les incinérateurs de déchets, le chauffage individuel et les centrales électriques aux combustibles fossiles. La pollution dans les villes provoque souvent un brouillard de polluants ou *smog*, souvent révélateurs de la densité de micro-particules et de l'impact du CO2 et autres polluants sur l'environnement.

atmosphérique. Les centrales électriques au charbon y contribuent largement, tandis que les générateurs diesel sont une préoccupation croissante dans les zones hors réseau. Sur le plan mondial, le secteur des transports génère près d'un quart des émissions de dioxyde de carbone liées à l'énergie, et cette proportion augmente. Les émissions générées par les transports sont associées à près de

A Bamako, une prise de conscience des pouvoirs publics sur la pollution de l'air est apparue à la suite de quelques mesures de concentration en poussières réalisées en décembre 2008. La croissance rapide de la population et du trafic automobile de l'agglomération entraîne une croissance significative de cette pollution. Cette étude passe par un état des lieux de la pollution atmosphérique avec des mesures de qualité de l'air ambiant en un certain nombre de point, puis l'étude comprend un calcul des émissions réalisé à partir d'un modèle spécifique, enfin des propositions d'actions sont faites dans le but de diminuer les émissions et l'impact de ces actions est évalué par le biais d'un modèle sanitaire adapté. Les hypothèses du plan d'actions ont été testées sous la forme de scénarios. Les principales actions envisagées porteront sur le parc automobile et les transports, la qualité des carburants et enfin l'aménagement urbain.

II. OBJECTIF GENERAL

Caractériser de la qualité de l'air ambiant de Bamako et son impacte sur la santé.

Objectifs spécifiques

- Analyser la situation actuelle, de la qualité de l'air ambiant et les caractéristiques des émissions.

- Simuler l'évolution des émissions et de la qualité de l'air ambiant à l'horizon 2015 et 2020 avec et sans le plan d'actions
- Modéliser l'impact sanitaire de la qualité de l'air ambiant pour les différents scénarios

III. MATERIEL ET METHODE

3.1. Matériel

Les mesures réalisées :

- Par tubes passifs pour SO2, NO2 et BTX (marque Passam);

Les appareils de mesures utilisés étaient les suivants :

- Thermo Andersen ADR-1200S pour les particules :

PM10 en temps réel,

Précision de +/- 5% par rapport à méthode standard (gravimétrique);

- Drager Pac III E :

Mesure de CO en temps réel,

Enregistrement de 8000 valeurs,

Homologation Demko 02 ATEX 0135331 - EExia IIC T4.

Photos: 1.Thermo Andersen ADR-1200S, 2. Capteurs passifs, 3. Drager Pac

Milieu d'étude et contexte sanitaire de la ville de Bamako

Les données sanitaires de la ville ont été extraites des annuaires sanitaires réalisés par les hôpitaux et centres de santé de la ville. Pour chaque commune, il existe un inventaire du nombre d'admissions et de décès annuels selon la pathologie et l'âge du patient. Dans le contexte de cette étude, les pathologies liées au système respiratoire (toux et infections respiratoires aigües) ont été regroupées sous un même chapeau «pathologies respiratoires ».

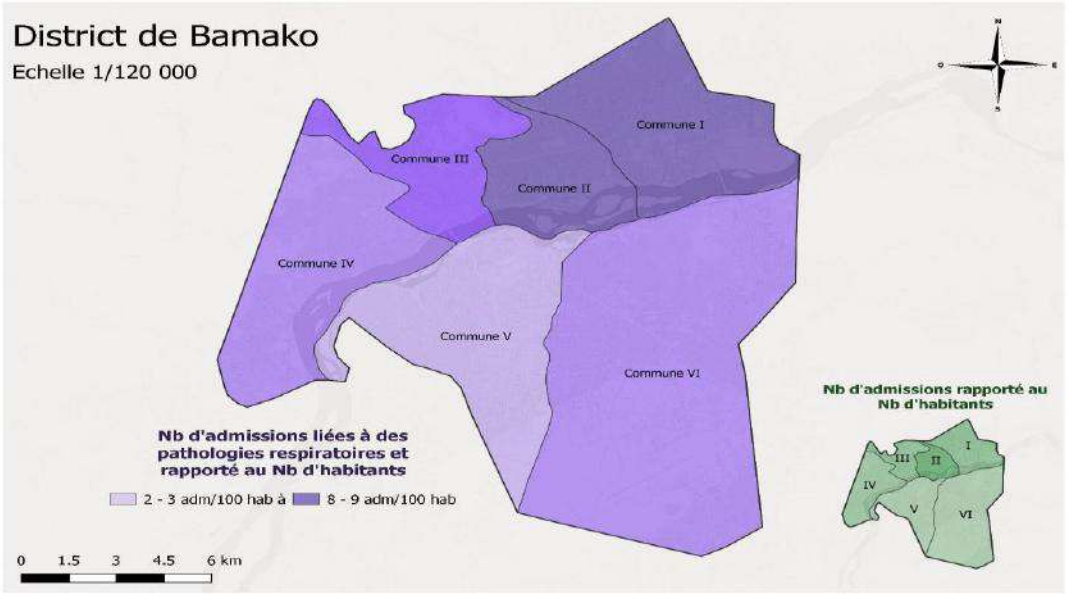

Fig.1: Admissions à l'hôpital liées à des pathologies respiratoires et admissions totales rapportées au nombre d'habitants par commune.

3.2. Méthode.

Outil numérique pour la modélisation atmosphérique

Dans le cadre de cette étude, le modèle ADMS est particulièrement bien adapté pour répondre aux enjeux de la pollution de l'air à l'échelle de la ville de Bamako. ADMS est capable de prendre en compte : La surélévation des panaches liée aux paramètres d'émissions des sources canalisées; Une historique représentative des conditions météorologiques caractérisées par les paramètres pouvant avoir une influence sur la dispersion des panaches : vitesses et direction du vent, températures, de la nébulosité et des précipitations; Les conditions de vents calmes; L'occupation des sols pouvant selon le type de sol modifier la dispersion des panaches; La prise en compte de la recirculation de la pollution dans les rues dites canyon.

Paramétrisation générale du modèle

La Paramétrisation générale de la modélisation intègre les caractéristiques suivantes :

Une zone d'étude de 730 km^2 qui contient la totalité du district de Bamako; Un maillage régulier de cette zone de 260 m x 260 m; Une hauteur de rugosité de 1 m, typique d'une zone urbaine; Les conditions météorologiques de 2018. Dans un souci d'optimisation des temps de calcul, les données ont été moyennées sur un pas de temps tri-horaire; Activation du module ADMS « vents calmes » qui tient compte de la composante de dispersion radiale caractéristique de ces vents calmes;

L'hypothèse majorante que les NOx sont convertis intégralement en NO_2. En sortie, des concentrations moyennes annuelles (moyenne de concentrations horaires). A noter que l'ensemble des cartographies présentées dans cette thèse sont des concentrations moyennes annuelles. Les centrales thermiques sont assimilées à des sources ponctuelles sur la base des informations fournies lors des enquêtes auprès des personnels concernés

Validation du modèle avec les résultats des mesures

Le modèle numérique a été calibré en utilisant les données d'observation réalisées lors de la campagne de mesure réalisée au mois de juillet 2019. Pour rappel, cette campagne a été l'occasion de caractériser les niveaux de pollution observés et définis comme le fond urbain dans la ville de Bamako.

IV. RESULTATS

Caractéristiques des sources aéroportuaires et industrielles

Les dépôts sont assimilés à des sources volumiques. En considérant la hauteur H du volume comme la hauteur des tanks. Les émissions CPU, GSE, et Taxiing de la plateforme aéroportuaire sont également assimilées à des sources volumiques. Les émissions par le cycle LTO n'ont pas été intégrées à la modélisation. La hauteur (principalement > 500 m) de ce type de rejet ne leur permet pas d'influencer de façon significative les sorties du modèle.

Les émissions sont recalculées en $g/m^2/s$ ou $g/m^3/s$ selon le type de la source et sur la base des budgets estimés lors de l'inventaire d'émissions.

Emissions par le trafic routier

La dispersion des émissions par le trafic routier a été modélisée via la suite d'ADMS, *ADMS Roads*, qui prend en compte l'effet canyon. Le réseau routier modélisé comprend les axes sondés pendant la campagne de sondage (axes structurants et secondaires) et le restant du réseau secondaire. Les axes tertiaires n'ont pas été pris en compte. Sur les axes sondés, ont été renseignés les vitesses moyennes, le nombre de véhicule, et la typologie des tronçons. Sur la base des données du réseau secondaire sondé, ont ensuite été calculées et utilisés dans le modèle, un nombre moyen de véhicules et une vitesse de circulation moyenne.

Emissions de la combustion résidentielle et de l'incinération des déchets

Les émissions de la combustion résidentielle et de l'incinération des déchets ont été traitées de façon analogue, en les considérants comme de type surfacique. Le processus de spatialisation présenté ici est tiré de la méthode développée par le (LCSQA, 2012). La zone d'étude a initialement été découpée en un maillage régulier de 260 m x 260 m. Pour chacune des cellules, le nombre d'habitant a été calculé en tenant compte de la population totale dans chaque commune du district et du taux d'occupation des bâtiments spécifique à chacune des cellules du maillage. Le processus de croisement des données de bâtiments et de population est présenté sur la figure suivante. Les émissions résidentielles et d'incinération des déchets ont été rapportées par habitant (spécifiées en partie). Ainsi, la spatialisation des émissions de ces deux secteurs sur le maillage régulier de 260 m x 260 m a été réalisée à partir du produit des émissions unitaires avec le nombre d'habitants résidents dans chaque cellule.

Le modèle de dispersion a été configuré pour reproduire au mieux les conditions de la campagne de mesures. Plus précisément :

Les conditions météorologiques observées durant cette période ont été appliquées;

Les points de prélèvement ont été modélisés à 1,5 m au-dessus du sol.

A noter que les émissions n'ont, quant à elles, pas été modulées sur la période de la campagne par manque de données consolidées et homogènes sur l'ensemble des sources caractérisées. Ces émissions sont des estimations annuelles prises en compte dans la modélisation. Par conséquent, la modélisation proposée ici n'est pas en mesure de reproduire la contribution d'une source locale ayant lieu durant la campagne de prélèvement.

Les résultats des comparaisons modèle / mesures sont présentés sous forme d'histogrammes dans la figure 27 pour chaque polluant. A titre indicatif, des barres d'incertitudes de 30% pour les mesures de CO, de benzène et de NO_2, 50% pour les poussières fines (PM10) et 25% pour le SO_2 ont été reportées conformément aux objectifs de qualité prévus dans la directive européenne CE/50/2008. Selon la même directive, une incertitude tolérée de 50% est acceptée pour les résultats obtenus avec le modèle de dispersion.

Cette comparaison polluant par polluant peut être résumée comme suit :

Pour les poussières (PM10) : les niveaux modélisés sont du même ordre de grandeur que les valeurs mesurées dans les gammes d'incertitudes établies. Les tendances sur les niveaux entre les deux points de mesures sont bien respectées avec modélisation.

Pour le SO_2, le NO_2 et le benzène : les concentrations modélisées sont relativement proches des mesures. De façon analogue, les tendances sont globalement respectées entre les points de mesures ;

Pour le CO : On observe une large sous-estimation du modèle par rapport aux mesures d'un facteur 10. Ceci peut s'expliquer par une sous-estimation des émissions caractérisées dans cette étude notamment en raison des facteurs d'émission appliqués. Ces derniers sont généralement utilisés dans un contexte européen et peuvent par conséquent être peu représentatifs des émissions de ce polluant au niveau de la ville de Bamako. Par conséquent, un facteur de correction a été appliqué sur l'ensemble du domaine aux concentrations modélisées de CO dans cette étude. Ce facteur correctif, une fois appliqué permet d'obtenir des niveaux de concentrations des polluants proches de ceux observés (écart de 10% en moyenne).

L'analyse comparative modèle / mesures montre que, pour l'ensemble des polluants, à l'exception du CO, les niveaux modélisés sont représentatifs des valeurs mesurées durant la campagne, et ceci dans la gamme d'incertitude tolérée. Concernant le CO, un facteur correctif a été appliqué afin de reproduire la signature des concentrations mesurées. Un point de vigilance sur ce polluant sera maintenu, dans cette étude, notamment pour comprendre l'origine de ces écarts, avec une attention particulière sur les sources d'émissions et les facteurs d'émission sectoriels employés.

Cartographie de la pollution atmosphérique

Les cartographies de la pollution atmosphérique ont été construites en intégrant les standards internationaux pour chacun des polluants investigués. Ainsi, les valeurs guide de l'OMS ont été utilisées par défaut pour établir ce diagnostic. Dans le cadre de cette recherche, seules les concentrations moyennes annuelles ont été évaluées à partir des standards applicables.

Enfin, l'OMS ne renseigne pas de valeur annuelle pour le monoxyde de carbone. La valeur règlementaire disponible aux Etats-Unis et en Europe a été utilisée dans le cadre de cette étude. Le tableau suivant compile l'ensemble des valeurs applicables remontées depuis les trois organismes références dans le domaine (OMS, US-EPA et EU). Les valeurs en gras correspondent aux valeurs retenues pour évaluer l'impact des émissions atmosphériques sur la qualité de l'air de la ville de Bamako.

Les résultats sont comparés aux valeurs de concentration de référence. Les cartographies de concentration des émissions sont présentées dans les figures suivantes :

La Figure 2 permet d'apprécier la répartition spatiale des panaches de pollution pour voir les zones d'émissions les plus impactantes. La Figure 29 permet d'évaluer les zones de la ville dont les niveaux de pollution présentes un impact sanitaire significatif. Dans ce cas de figure, les niveaux de pollution ont été mis en regard des valeurs règlementaires applicables discutées dans le paragraphe précédent. L'analyse de ces cartographies montre : Pour le NO_2, l'influence principale sur les niveaux de pollution calculés est d'origine routière. La valeur guide est dépassée uniquement au niveau des axes structurants dans la ville. Les valeurs tendent à diminuer rapidement avec l'éloignement des routes pour retomber sur des niveaux de l'ordre de quelques $\mu g/m^3$ au maximum.

Concernant les COV et plus particulièrement le benzène, les niveaux de pollution dépassent la valeur règlementaire sur une large partie de la ville de Bamako. Ces zones sont principalement localisées à proximité du réseau routier structurant de la ville et des lieux de fortes densités de population (notamment la partie sud-ouest de la ville). Ces tendances traduisent l'impact important des véhicules fonctionnant à l'essence, en particulier les véhicules 2-roues largement représentées dans le parc roulant de la ville. En parallèle, la contribution du secteur résidentiel, en particulier la combustion de cuisson a également une influence notable sur les niveaux de pollution en benzène.

Au niveau des concentrations de SO_2 : globalement les valeurs règlementaires sont respectées sur grande majorité

du domaine. Les zones de dépassement sont localisées aux niveaux des axes routiers les plus fréquentés. Les centrales thermiques ont également une influence significative mais localisée sur une petite partie de la ville au niveau de la zone nord-est.

Pour les poussières (PM10) et le CO : les niveaux de pollutions de ces deux traceurs montrent des signatures sur leurs impacts analogues, c'est-à-dire majoritairement influencés par le secteur résidentiel (combustion de bois). Au regard des valeurs règlementaires, des niveaux alarmants ont été calculés pour les PM10 sur la quasi-totalité de la ville. Cet impact significatif est un peu moins marqué sur les cartes de pollution du CO. La valeur règlementaire utilisée pour ce polluant est une valeur applicable sur une journée (valeurs maximale journalière des valeurs moyennes sur 8h glissantes), or les concentrations modélisées sont exprimées en valeur moyennes annuelles. Par conséquent, il est probable que l'impact sur une journée soit sous-estimé par les moyennes annuelles représentées sur cette cartographie. Nous attirons l'attention ici, que les cartes de concentrations du CO sont présentées dans ce rapport à titre indicatif. En conclusion, ces cartographies de la qualité de l'air sur la ville de Bamako indiquent des niveaux sanitaires potentiellement alarmants pour deux polluants : les poussières (PM10) et le benzène. Ces niveaux importants s'expliquent par les émissions résultantes du secteur résidentiel (combustion de bois) et du secteur routier. L'influence forte de ces deux secteurs révèle également des niveaux importants de monoxyde de carbone (CO). Ce polluant est présenté à titre indicatif dans ce document, une analyse plus fine sur ce dernier sera présentée dans le cadre de l'atelier de restitution. Il faut noter, finalement, l'influence du secteur industriel, marqué par des niveaux importants localisé sous les vents des installations pour le dioxyde de soufre (SO_2).

Impacts Sanitaires de la pollution atmosphérique.

Selon (Dockery et *al.*, 1993), plusieurs études ont mis en évidence l'impact de la mauvaise qualité de l'air sur la santé, en particulier sur le système respiratoire. Les particules fines entre autres sont susceptibles de pénétrer au plus profond du système pulmonaire et causant des inflammations. La pollution de l'air contribue ainsi au développement de pathologies tels que l'asthme, les cancers du poumon, les infections aigües, et dans les cas les plus graves conduire au décès des patients.

Diagnostic sur les populations exposées – indicateur IPP

L'indice de Pollution – Population (IPP) représente l'exposition potentielle des personnes à la pollution atmosphérique. Plus précisément, il s'agit de croiser les concentrations des polluants (le NO_2 et les PM10 qui sont les indicateurs les plus pertinents de la pollution urbaine) avec les populations exposées. A l'échelle d'une maille, on effectue le calcul suivant :

$$IPP = Ci \times Pi$$

Où *Ci* est la concentration du polluant considéré,

Pi est la population présente sur la maille considérée.

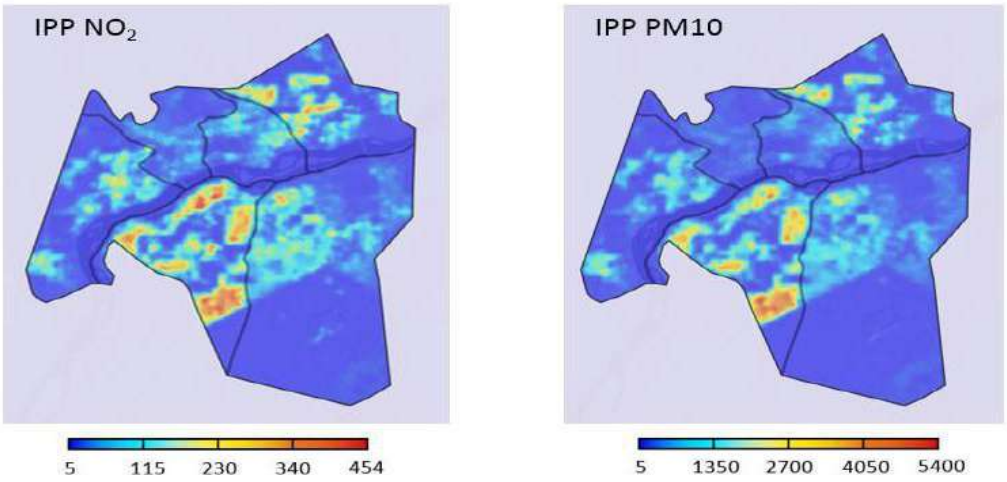

Fig.2 Indicateur IPP pour deux traceurs de la pollution en milieu urbain (2019)

Les cartographies des indices IPP PM10 et NO_2 sont présentées dans la figure ci-dessus (figure 2). Cette représentation spatiale permet de mettre en avant les zones les plus particulièrement touchées par la pollution atmosphérique. Les communes II, IV, et V en particulier sont les plus exposées à la pollution. L'indice IPP, en ce cas, ne reflète pas la réalité géographique des admissions à l'hôpital. Notamment, le nombre

d'admissions pour la commune V rapporté au nombre d'habitants y est plus faible malgré une exposition, selon

l'indice IPP, plus importante. Une première explication est dans l'approche même de l'interprétation des admissions, où il est implicitement fait l'hypothèse que les habitants ne sortent pas de leur commune, et que leur commune de travail et celle où ils sont admis sont les mêmes.

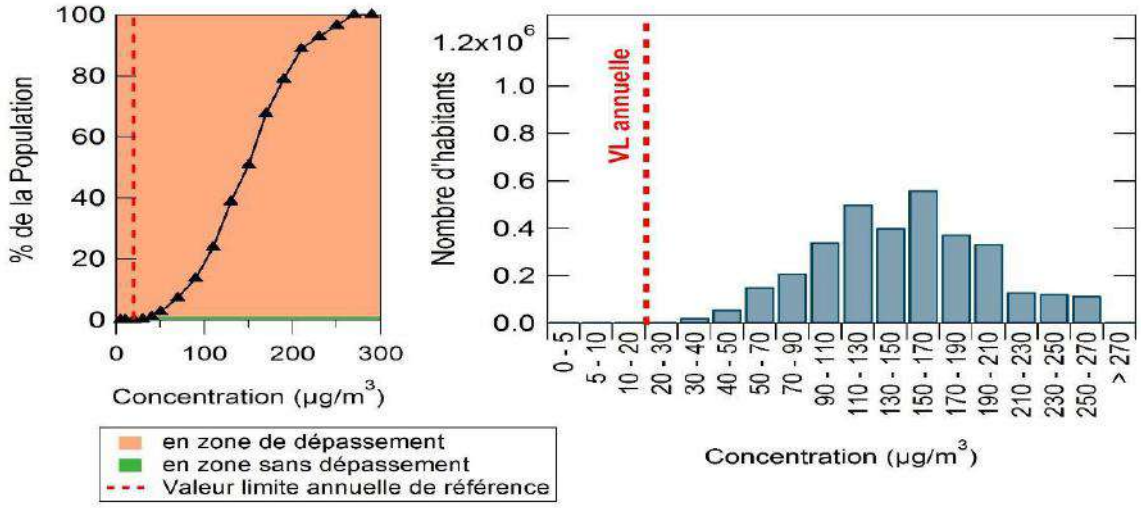

Fig.3 : Répartition cumulée du niveau d'exposition du benzène dans la population de la ville de Bamako (2019)

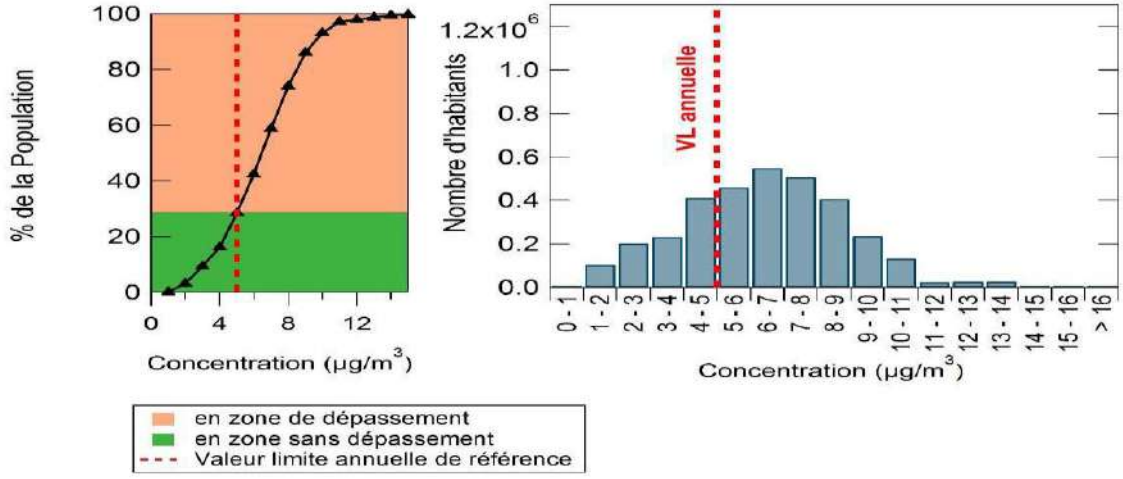

Fig.4 : Répartition cumulée du niveau d'exposition du benzène dans la population de la ville de Bamako (2019)

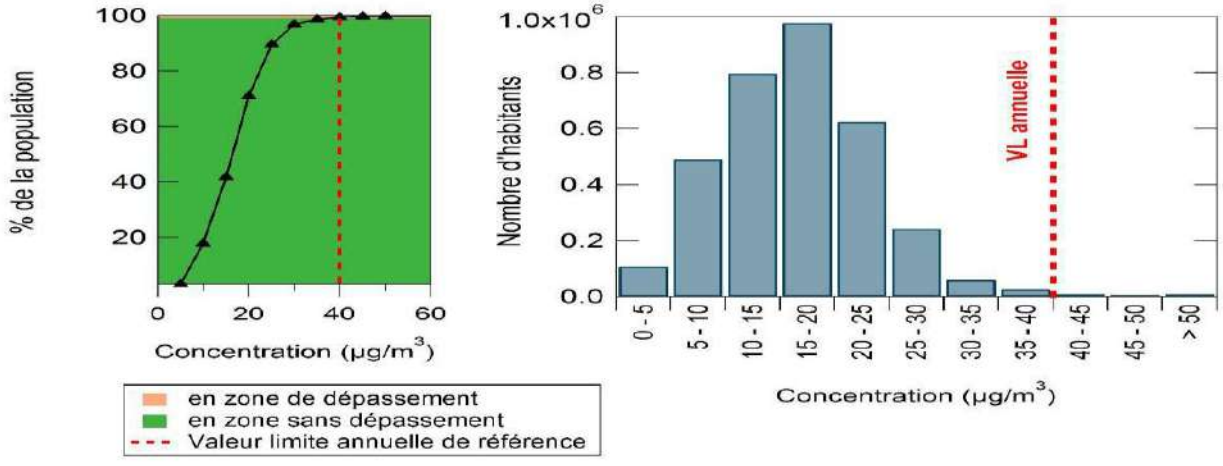

Fig.5 : Répartition cumulée du niveau d'exposition du NO2 dans la population de la ville de Bamako. (2019)

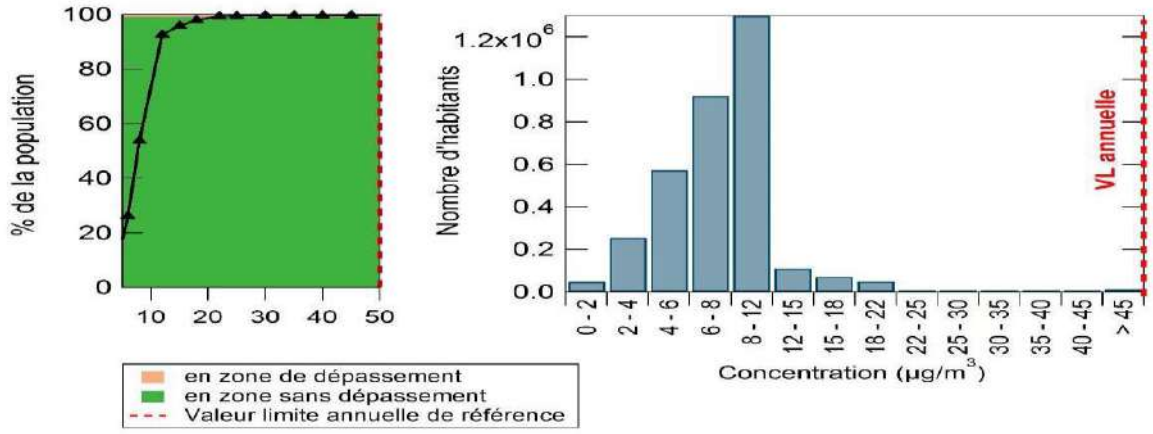

Fig.6 : Répartition cumulée du niveau d'exposition du SO2 dans la population de la ville de Bamako (2019)

V. CONCLUSION

La revue documentaire des concentrations dans plusieurs grandes villes Africaines a montré que les concentrations relevées dans le district de Bamako se situent au niveau de la médiane pour l'ensemble des polluants concernés. Fort de l'ensemble de ces éléments, un principe de plan de surveillance est proposé qui inclut une proposition techno-financière adaptée aux enjeux de la ville de Bamako. Le plan de surveillance doit pouvoir ainsi permettre de cibler au mieux les secteurs responsables et les zones à protéger afin de mettre en place les actions de mitigation et de protection les plus pertinentes. A long terme, aboutir à l'amélioration de la qualité de l'air et à la réduction de l'impact sanitaire sur les populations.

VI. DISCUSSION

La modélisation effectuée et les cartes de concentrations obtenues indiquent des zones de dépassement des valeurs retenues comme valeurs limites pour les PM10 (sur la quasi-totalité du territoire au nord de l'aéroport), pour le SO_2 (à proximité immédiate des centrales thermiques), pour le CO (sur certaines axes routiers des communes II, IV, V)

Les concentrations en PM10 à Bamako en 2019 ont atteint 69µg/m^{3}alors qu'elles étaient de 503.6 µg/m^3 en 2010 et 331 µg/m^3 sur site de fond entre décembre 2009 et janvier 2010 (BURGEAP, 2010). A Dakar à la même période les concentrations en PM10 étaient de 155 µg/m^3 (Doumbia et *al.*, 2013). En Afrique du sud elles sont de 72.4 µg/m^3 pour la ville de Secunda et de 42 µg/m^3 pour la ville de Witbank (Kuik et *al.*, 2015). Au Kenya à Nairobi 25 µg/m^3 (Gaita et *al.*, 2014), au Caire en Egypte 48 µg/m^3 (Boman et al., 2013) et 86 µg/m^3 à Ouagadougou au Burkina Fasso (Boman *et al.*, 2009).

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Assessment of the Nutritional Content and Hedonic Test on Pameling Avocado from Three Different Altitudes

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Abstract— *Malang Regency has superior avocado commodity which is so-called Pameling. Superiorities of the fruit are indicated by the large size of the fruit and quality of the fruit flesh which is liked by the consumers. Today, the development of Pameling avocado has expanded from the lowlands to the highlands in diverse regions. In order to obtain optimal harvest yields, both quantity and quality, the research affirmed the quality of Pameling avocado, which is grown at the lowlands and the highlands. The research was conducted by testing quality (nutritional content) and organoleptic. Results of the research showed that the avocados grown at the highlands were more preferred by the panelists based on the organoleptic test from the assessment aspects of appearance, texture, aroma, and taste. The lowlands produced preference of the color flesh which is mostly preferred by the panelists. Results of the nutritional test (quality) on fruits grown at the lowlands showed the highest level of sugar and protein. The middle plains are superior in fat content. Moreover, the highlands produce fruits with the highest coarse fibers. However, the differences in cultivation methods on three different altitudes will be the subject of further studies that need to be considered.*

Keywords— ***altitude, nutritional content, fruit nutrition, organoleptic***

I. INTRODUCTION

Avocado (*Persea americana* Mill.) is known as fruit that having soft texture as butter. Avocados originally came from Meso America and eventually spread to around the world including Indonesia. Avocado is mostly consumed as a source of healthy fats along with various processing, for instance, as dessert such as *es campur* (mixed ice) from Indonesia, as sauce such as in Mexican foods or protein companions such as in sushi from Japan. Malang Regency has superior avocado commodity which is so-called Pameling. Superiorities of the fruit are indicated by the large size of the fruit and quality of the pulp (mesocarp) which is liked by the consumers. Today, the development of Pameling avocado has expanded from the lowlands to the highlands in diverse regions. In order to obtain optimal harvests, both quantity and quality, the research affirmed the quality of Pameling avocado, which is grown at the lowlands and the highlands. It is expected that results of the study will be able to provide information about altitude categories which can produce the best quality of Pameling avocado. So that the grading process of the avocados will be conformed to the processing goal post-harvest in accordance with the area characteristics where the avocados grown particularly the altitude.

II. MATERIAL AND METHODS

2.1 Material

Fresh Pameling avocados were collected from several regions in Malang Regency with variation in altitudes. Each avocado represented the highlands, middle plains, lowlands, and the avocado that derived from the parent trees. The harvests of avocado were stored at room temperature (26-28^{o}C) in the Laboratory of Plant Physiology, Faculty of Agriculture, Brawijaya University.

2.2 Fruit Nutritional Variable

The nutritional variables of Pameling avocado are protein, fat, carbohydrate, coarse fiber, and total sugar. The nutritional content test was conducted at the Central Laboratory of Biology in Brawijaya University and Food Laboratory in University of Muhammadiyah Malang.

2.3 Organoleptic Test

Pameling avocados were evaluated in accordance with the hedonic organoleptic test with testing parameters for appearance, texture, aroma, taste, and color. The hedonic test criteria are scores 1-7, whereas 1 represents the opinion of really like and 7 represents really dislike. The hedonic test was conducted toward 30 panelists with the ages range 18-24 years old. The test was conducted at the Laboratory of Plant Physiology, Faculty of Agriculture, Brawijaya University.

III. RESULTS

3.1. Fruit morphological characteristics

Based on results of the observation on morphological characters of the Pameling avocado with the guidance of UPOV (2005) the seed shape in the elongated part (lateral) from 3 plains belong to the group 4, the elliptic.

Based on results of the observation on morphological characters of the Pameling avocado with the guidance of UPOV (2005) diameter of the base part of the peduncle from 3 plains belong to the group 5, the medium.

3.2. Fruit Nutrition

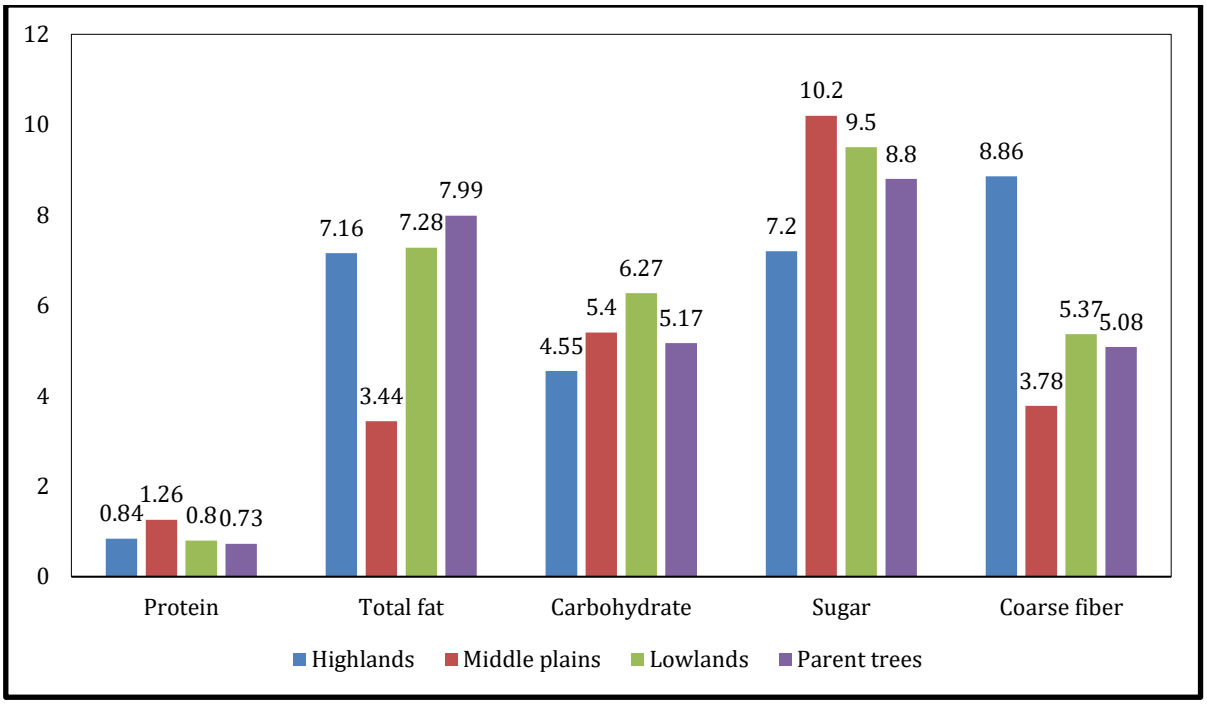

Fig.1. Nutritional Content of the Pameling Avocado that Derived from Three Different Altitudes

The nutritional test on avocado used proximate analysis that include protein, fat, carbohydrate, coarse fiber, and total sugar. Figure 1 shows the results of nutritional content in Pameling avocado. Results of the research showed that avocados grown in the lowlands have the highest content of protein and sugar. The middle plains are superior in fat content. Moreover, the highlands have the highest content of coarse fibers.

3.3. Result of the Hedonic Test

The hedonic test was conducted to find out preference of the consumers on parameters of appearance, texture, aroma, taste, and color. Results of the hedonic test are presented in Figure 2. Avocados from the highlands were more preferred by the panelists in accordance with the organoleptic test from the assessment aspects of appearance, texture, aroma, and taste of the fruits. Meanwhile, avocados from the lowlands were more preferred by the panelists based on the color of the pulp (mesocarp).

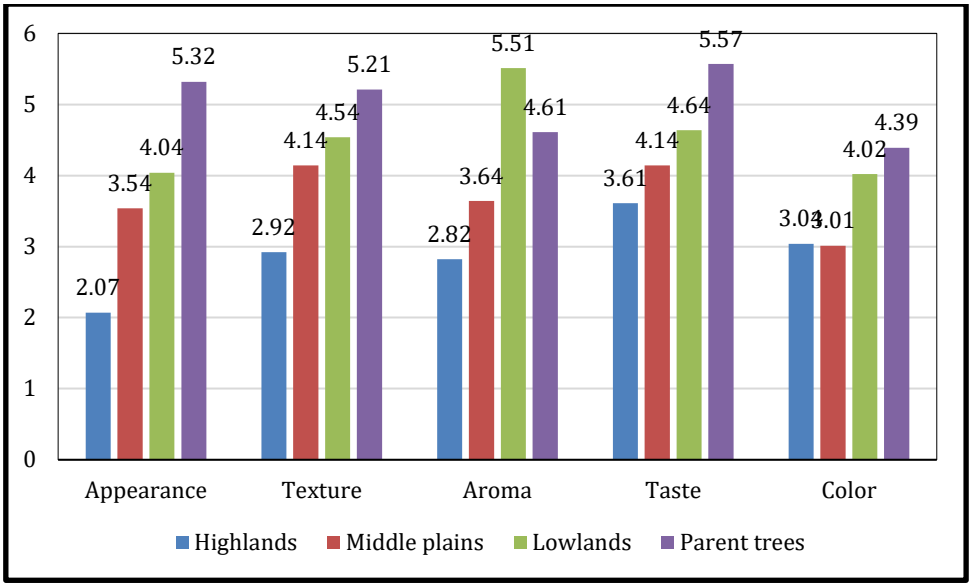

Fig.2. Preference Values of the Consumers toward Quality of the Pameling Avocado from Three Regions with Different Altitudes

IV. DISCUSSION

The highest contents of protein and sugar were found in avocados grown at the middle plains, but they have the lowest content of fat and coarse fibers compared to other avocados. Moreover, the carbohydrate content was 1.8 g lower than in avocados grown at the lowlands.

According to the health attribute, the consumers give higher assessment on the higher fat than lower calories (Ballen *et al*., 2022). Based on the analysis results, the highest fat contained in fruits which derived from the parent tree at the middle plains. According to NHANES analysis (Dreher and Davenport, 2013) the average consumption is a half of the avocado (68 g), which provide high nutritional and phytochemical foods, such as: fibers (4.6 g), total sugar (0.2 g), high-acid monounsaturated fats (6.7 g) and 114 kcal or 1.7 kcal/g, which help to promote a healthy blood lipid profile and to increase bioavailability of fat soluble vitamins and phytochemicals of the avocado or other fruits and vegetables, naturally low in fat, consumed with avocados.

In relation to the quality attribute of the avocado. The consumers are affected by quality of the fruits, both external and internal. The external qualities include weight (commercial size), shape (oval or piriformis), color of the rind (green or black), rind texture (smooth or rough), no blemish (for example, sunburn); and the internal factors include taste, pulp (flesh) texture, pulp color, and seed size.

Avocados grown at the highlands have lower scores, and it indicates that the avocados are preferred by the consumers. However, avocados grown at the lowlands are only superior in color parameters.

On data analysis using ANOVA and SPSS, it showed that F values for appearance, texture, aroma, taste, and color were 17.3, 7.5, 7.8, 3.5, and 4.8, respectively. These values were compared with F table values with df panelist 108 and df sample 3. F table value was 1.7. Based on the value, it showed that F values for appearance, texture, aroma, taste, and color were higher than F table. F table value is F value when α 5%. When F count values were higher than F table, it means that the error is below 5% so that H0 is rejected, H1 is accepted. H1 in ANOVA test is data of the sample is significantly different. It means, there is a significant difference between the appearance sensory attributes.

The consumers' choices depend on quality character of the products and consumers' preferences. Such consumers' preferences are varying in accordance with subjectivity of the consumers. Moreover, the quality character can be classified into intrinsic and extrinsic. The intrinsic quality refers to the attribute of the product appearances, such as color, shape, and size, while the experience attributes are taste, aroma, and ripeness. The extrinsic quality refers to food safety, sustainable production process and where the avocado comes from. Information on extrinsic quality will build the consumer trust over the related producer. Knowledge about the origin of the product is a potential factor to change evaluation and perception of the consumers toward the product. However, the consumers assumed that local products are synonymous with high quality, even though it could change in relation to products that being considered and the geographical context (Migliore *et al*., 2017). Avocado tends to thicken its epidermis rind to reduce water loss in warm climate with low humidity such as at the lowlands (Henao-Rojas *et al*., 2019).

Based on results of the organoleptic test, avocados grown at the highlands are preferred by the panelists in relation to the attributes of appearance, texture, aroma, taste, and color. It conformed to the literature about the avocado consumption, in which the ripeness level of the fruit consumed can affect the quality of eating, especially through the firmness of the pulp and "soft" texture. The consumers tasted the avocados that fall into "hard" category are significantly less likely to purchase compared to the fruits that fall into the "medium" and "soft" categories. (Migliore *et al*., 2017)

Taste is considered as an important element of quality that has positive and significant influence on intention of the consumers to purchase. Taste is twice as important as the price when it determines the consumers desire to purchase the fruit. It is expected that taste is an attribute of important experience quality which has significant influence on possibility of repeated purchase (Ballen *et al*., 2022).

Pameling avocado from Malang has become a major player in developing high-value products, given the superiority of the brand identity that is made or grown in Malang. Also, there is a changing trend for food consumers to show great interest in knowing the origin of their foods, with a preference for locally grown foods.

V. CONCLUSION

Avocados grown at the highlands were more preferred by the consumers based on the organoleptic test. Based on results of the research, it could not be concluded which altitudes could produce the best quality avocados. This is due to differences in the implementation of the SOP for farmers in the three areas with different altitudes.

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Analysis of the Potential Horticultural Products in Blitar Regency on the Agribusiness Market in Blitar Regency

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Abstract— *Blitar Regency has a wholesale market managed by the Wlingi Market Association, but some horticultural products from the farmers are still absorbed by the wholesale market outside Blitar Regency and reabsorbed by the retailers from Blitar Regency. Objectives of the research were to obtain information about the superior products, supply chain, and the inhibiting factors on the development of the horticultural products. Method of the research used descriptive analysis on each member of the links. Location of the research is the wholesale market in Blitar Regency, Wlingi. Variables of the research include types, volumes, and circulation of the horticultural products. Results of the research showed 3 major horticultural commodities that include chili, red pepper, and watermelon. The potential agricultural products in Blitar Regency have inefficient and complex supply chain because they involve many actors from farmers to consumers. The inhibiting factors are as follow: 1. The farmers do not have a collective system for marketing their crops so that information about the products as well as the prices are not integrated, 2. The supply chain patterns are inefficient and complex, so that the prices are costly, and 3. Inadequate location of the wholesale market. It is expected that the approach of supply chain on horticultural commodities in Blitar Regency will provide an overview of potential commodity supply availability as a consideration for supply chain management.*

Keywords— ***wholesale market, supply chain***

I. INTRODUCTION

Subsector of horticulture has a strategic position in the development of agricultural sector. Contribution of the horticultural subsector on the development of the agricultural sector tends to increase year by year marked by an increase in several macro indicators such as gross domestic product (GDP), export volume, employment absorption, and farmer exchange rate (FER) (Central Bureau of Statistics, 2014). The economic development of a region can be measured through the economic growth, which is at the same time an indicator that provides an overview of the extent to which regional economic activity in a certain period has resulted in increased income for the community as indicated by an increase of income per capita (Saragih, 2015). Basically, the regional economic growth is affected by the comparative advantage of a region, regional specialization, as well as the economic potential owned by the region (Wulandari, 2010). Therefore, utilization and development of the whole economic potentials become the major priority that must be explored and developed in carrying out the sustainable regional economic development (Syahab, 2013).

Superior commodity is a commodity which contributes income to the related region (Setiawan, 2010). Some criteria that can describe the superiority of a commodity in a region is because the commodity has been well known by local community, managed, and widely developed by local community (Asriani, 2003), as well as make significant contribution to the economy of the local community, and compete with other commodities. Competitiveness of the commodity was known in accordance with indicator of the community income from the business (Agus and Budiyanto, 2005). The commodity has a conformity based on agroecological aspect,

particularly that relates to location of the development. The agroecological condition can be identified using productivity indicator that describes the production efficiencies. The commodity has potential and market-oriented both domestic and export as well as support from the government policies, especially market support and the availability of the supporting factors such as: institutions, technology, capital, means and infrastructures, as well as human resources (Widayanto, 2000; Juarsyah, 2015).

At present, a wholesale market in Blitar Regency is managed by Wlingi market association. There are 120 sellers who have been registered in the association and 80 merchants have not registered. The merchants in the market take the agricultural yields from the farmer groups that are scattered in Blitar Regency as well as from outside the Blitar Regency for resale in large quantities (wholesale) to the market sellers or greengrocers. The horticultural commodities potential can be developed in varying ways besides knowing the potential horticultural products. Objectives of the study were to get information about the superior horticultural products in Blitar Regency and to analyze the supporting and the inhibiting factors that affect the potential development of the superior horticultural products in Blitar Regency.

II. MATERIALS AND METHODS

Location of the research is the wholesale market in Blitar Regency that lies on Jalan Bromo Babadan, Wlingi. The market conditions are considered unrepresentative because it is an impromptu market that locates at the Wlingi terminal and operates from 04.00 p.m. to 04.00 a.m. Such conditions have triggered the development of the sub terminal agribusiness and location for the horticultural commodity development is in the Animal Market area of Blitar Regency. An area of 13 hectares in the eastern part of the area is potential to be developed to become a horticultural product development of Blitar Regency. Methods of the research were data collection and direct observation that involved the market sellers, market association, association of farmer groups in the potential area for horticultural commodities, and the related agencies.

III. RESULTS

Analysis on the horticultural products in Blitar Regency was conducted on the horticultural commodities with the highest productivity. Central Bureau of Statistics in Blitar Regency (2021) reported that among 8 superior horticultural commodities in Blitar, the commodities of chilies, red peppers, and watermelons, have the highest productivities compared to 5 other commodities (onions, potatoes, cabbages, tomatoes, and honeydews). Results of the analysis on the potential horticultural products on 3 major commodities are described below.

1. Chili (*Capsicum frutescens*)

There are three mechanisms in SCM (Supply Chain Management) of chili commodity in Blitar Regency. Analysis on mechanism of the supply chain flow was conducted to describe the product flow pattern, information flow, and financial flow that relate to the agricultural commodities. Those three mechanisms are depicted in the form of SCM structure for chili commodity in Blitar Regency below.

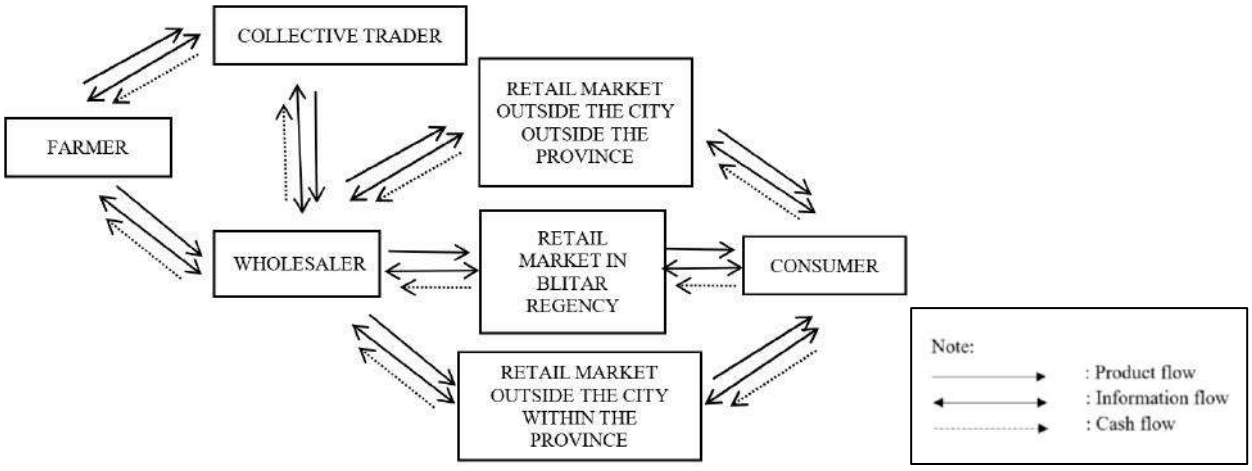

Fig.1. Supply Chain Management for Chili Commodity in Blitar Regency

2. Red Pepper (*Capsicum annuum* L.)

Blitar Regency is a producer of red pepper in Indonesia, especially in East Java. The supply chain analysis was applied to find out the mechanism of flows, distribution channels, and activities of the supply chain members on red pepper. As in the chili commodity, these

three mechanisms of the supply chain flows are illustrated in SCM structure for red pepper in Blitar Regency in Figure 2.

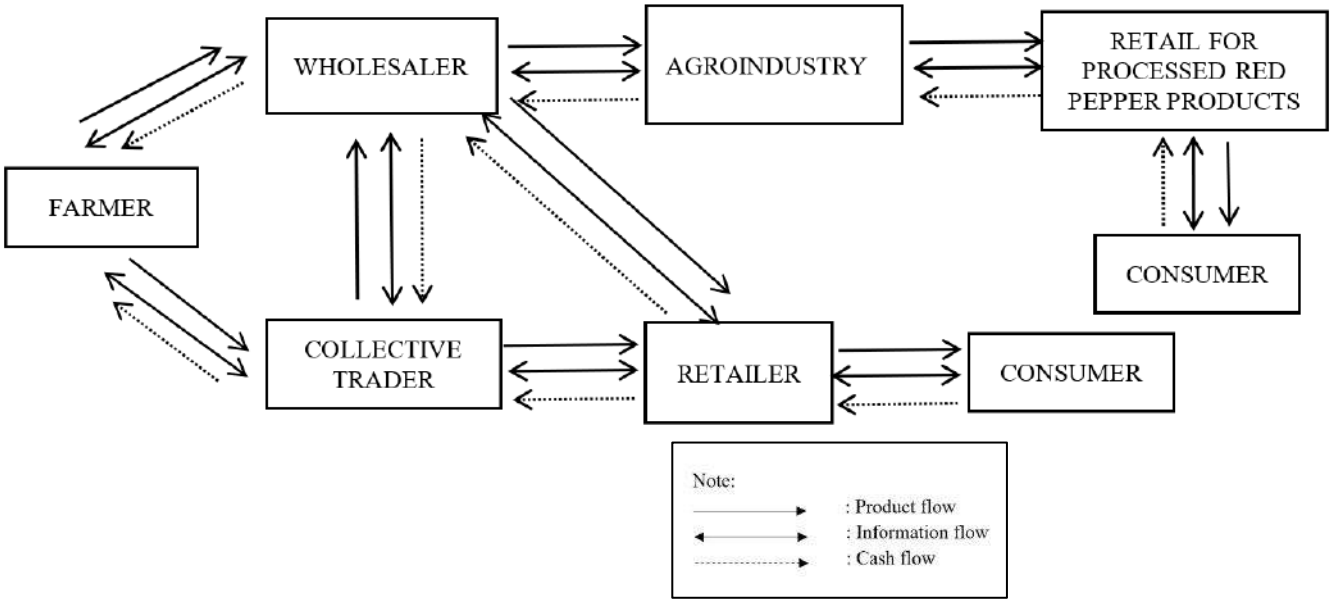

Fig.2. Supply Chain Management for Red Pepper Commodity in Blitar Regency

3. Watermelon (*Citrullus lanatus*)

Watermelon is one of fruits produced by the farmers in Blitar Regency, East Java of Indonesia. Therefore, it is important to perform an analysis of supply chain on watermelon in Blitar Regency to find out the mechanism of flows, distribution channels, and activities of the supply chain members on watermelon. SCM structure for the watermelon commodity in Blitar Regency is presented in Figure 3.

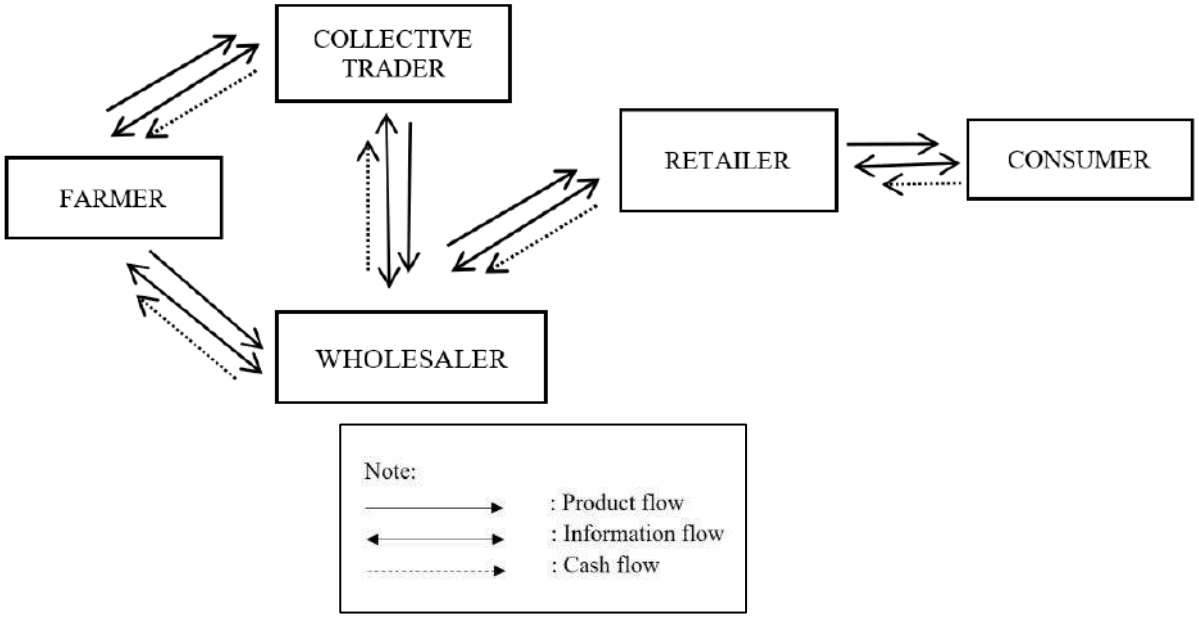

Fig.3. SCM for watermelon commodity in Blitar Regency

IV. DISCUSSIONS

Horticultural products are the basic needs in people's lives in Blitar Regency. The harvest area for chili in Blitar Regency reached 11,024 ha in 2019 and 10,745 ha in 2020 (Central Bureau of Statistics in Blitar Regency, 2021). The chili productions in Blitar Regency were very large that reached 1,503,782 quintals in 2019 and 1,881,377 quintals in 2020. The land area and the production value of the chili have made Blitar Regency to be the largest supplier of chili in East Java Province. Therefore, it is important to carry out a supply chain analysis on chili in Blitar Regency.

It is expected that the supply chain approach of chili commodity in Blitar Regency will be able to describe the availability of chili supply as consideration of the supply chain management for the consumers and the processing industries (Kurniawan, 2014). The supply chain analysis was carried out to determine the flow mechanism, distribution channel, and activities of the supply chain members on chilies. Based on Figure 1, the flow patterns in SCM of chili commodity in Blitar Regency are classified into 3 types, namely product flow, information flow, and financial flow. The product flow describes how the chilies are distributed from the first link (chain), the farmers, to the collective trader or the wholesalers, and subsequently distributed to the next link, and finally to the last link, the consumers. The information flow is existed when an information exchange has taken place between the farmers and the collective traders, the farmers and the wholesalers, as well as the information exchange in other links (chains) in accordance with the Figure 1. The exchange of information can be in the form of availability and supply demand, as well as information that relates to the price of red pepper. The financial flow is not only a transaction of red pepper, but also an example of a case in which the wholesalers and the collective traders could become the capital suppliers (stakeholders) for the red pepper farmers in Blitar Regency.

The links (chains) in Figure 1 are the chili farmers, the collective traders, the wholesalers, retailers, and the consumers. These five links (chains) are described below:

1). The chili farmers: the chili farmers play as the main producers in the supply chain of chili commodity in Blitar Regency.

2). Collective trader: in distributing the chili in Blitar Regency, the collective traders play as the first marketing intermediary.

3). Wholesaler: Wholesaler is a marketing agency that distributes the chili in bulk. The role of the wholesaler is as the collector of the production yields from the farmer as the producer in the scattered production areas and distribute the capital needed by the producers.

4). Retailer: Retailer is a marketing agency that deals directly with the consumers. Retailers become important connectors as because of them the consumers could enjoy the chili. The quantities taken by the retailers ranged 150-350 kg. It is due to the retailers only control the surrounding markets. Moreover, the retailers avoid to take high risks of losing if what they purchased were rotten or were not sold out.

5). Consumer: Consumer is an individual or groups who consume or utilize the chili for their own or group needs. Consumer is the last link of the supply chain. In this link, the products end up to be consumed as raw materials. The entire financing processes come from the consumer payments for the products ofchili purchased. So that the information about the needs and desires of the consumers is a directional determinant of the chili farming process.

Furthermore, the harvest area for red pepper in Blitar Regency reached 1,387 ha in 2019 and 1038 ha in 2020 (Central Bureau of Statistics in Blitar Regency, 2021). The red pepper productions in Blitar Regency were very large that reached 177,549 quintals in 2019 and 114, 923 quintals in 2020. Red pepper is a potential vegetable commodity that has high economic value and potential to be developed. Red pepper has important position in the dietary menu. Even though it is needed in small quantity, but it is consumed everyday by almost all of the Indonesian peoples. Red pepper is the superior vegetable commodity in the national and regional levels. Superior commodity is a commodity that is deserved to be cultivated to provide profits for the farmers, biophysically, socially, and economically. A commodity is worth developed if the commodity is cultivated in accordance with its agroecology, provide a business opportunity, as well as applicable and acceptable by the local community that could absorb workforces and economically profitable (Susanto and Sirappa, 2007).

Based on the Figure 2, the flow patterns in SCM of red pepper commodity in Blitar Regency are divided into 3 types, namely product, information, and financial flows. The product flow describes the distribution of red pepper from the first link (chain), the farmers to the collective traders or the wholesalers, and then distributed to the next link, and ended up to the last link (last chain), the consumers. The information flow occurred as a result of information exchange between the farmers and the collective traders, the farmers and the wholesalers, as well as information exchange in the other links (chains) in accordance with the figure above. The exchange of information can be in the form of availability and supply demand, as well as information that relates to the price of red pepper. The financial flow is not only a transaction of red pepper, but also an example of a case in which the wholesalers and the collective traders could become the

capital suppliers (stakeholders) for the red pepper farmers in Blitar Regency.

The links (chains) in Figure 2 are the farmers of red pepper, the collective traders, the wholesalers, agroindustry, the retailers, and the consumers. These six links (chains) are described below:

1). Farmer: The farmers of red pepper play as the main producers in the supply chain of red pepper commodity in Blitar Regency.

2). Collective trader: in distributing the red pepper in Blitar Regency, the collective traders play as the first marketing intermediary.

3). Wholesaler: Wholesaler is a marketing agency that distributes red peppers in bulk. The role of the wholesaler is as the collector of the production yields from the farmer as the producer in the scattered production areas and distribute the capital needed by the producers.

4). Retailer: Retailer is a marketing agency that deals directly with the consumers. Retailers become important connectors as because of them the consumers could enjoy the red pepper. The quantities taken by the retailers are only 300 kg. It is due to the retailers only control the surrounding markets. Moreover, the retailers avoid to take high risks of losing if what they purchased were rotten or were not sold out.

5). Agroindustry: Agroindustry is an activity that utilizes crops as raw materials, designs, and provides tools and services for the activity.

6). Consumer: Consumer is an individual or groups who consume or utilize the red pepper for their own or group needs. Consumer is the last link of the supply chain. In this link, the products end up to be consumed as raw materials. It must be remembered that the entire financing processes come from the consumer payments for the products of red pepper purchased. So that the information about the needs and desires of the consumers is a directional determinant of the red pepper farming process.

Furthermore, the harvest area of watermelon in Blitar Regency reached 151 ha in 2019 and 55 ha in 2020 (Central Bureau of Statistics in Blitar Regency, 2021). The watermelon productions in Blitar Regency reached 48,960 quintals in 2019 and 24,375 quintals in 2020. Empirically, an information was obtained that the marketing systems of horticultural commodities in the various SCM- Supply Chain Management have not been efficient as indicated by long marketing channels, the market structure tended to be oligopsonistic, uneven distribution of remuneration for the marketing functions, and price fluctuations in he short-term.

Based on the Figure 3, the flow patterns in SCM for the watermelon commodity in Blitar Regency can be divided into 3 types, namely the product, information, and financial flows. The product flow describes how the watermelons are distributed from the first link, the farmer to the collective trader or the wholesaler, and then distributed to the subsequent link up to the last link, the consumer. The information flow occurs when there is an exchange of information between the farmer and the collective trader, the farmer and the wholesaler, as well as the information exchange in other links that conform to the Figure above. The information exchange can be in the form of availability and supply demand as well as information that relates to the watermelon price. The financial flow can be in the form of buying and selling transaction of watermelon.

The links in Figure 3 are the watermelon farmers, collective traders, wholesalers, retailers, and consumers. These five links are defined as follow:

1). Watermelon Farmer: Watermelon farmer is the link who plays as the major producer in the watermelon commodity supply chain in Blitar Regency.

2). Collective Trader: in distributing the watermelon in Blitar Regency, the collective trader plays as the first marketing intermediary to local wholesaler and the retailer.

3). Wholesaler: Wholesaler is a marketing agency that distributes watermelons in bulk. The role of the wholesaler is as the collector of the production yields from the farmer as the producer in the scattered production areas.

4). Retailer: Retailer is a marketing agency that deals directly with the consumers. Retailers are an important link due to the consumers can enjoy the watermelons because of them. The quantity taken by the retailer is just around 150 kg. It is due to the retailers only control the market in their vicinities. Moreover, the retailers do not want to experience high risk of loss if the fruits they buy were rotten or unsold.

5). Consumer: Consumer is individual or group who consume or utilize watermelon for his/her own or group needs. Consumer becomes the last chain in the supply chain. In this chain, the product ends up for consumption as a raw material. It must be remembered that the entire financing process comes from consumer payments for the watermelon that has been purchased.

Based on results of the research on the supply chain management for 3 horticultural commodities in Blitar Regency, there were differences in the number of links in each commodity. The commodity which has many links is the red pepper. The application of Supply Chain Management (SCM) concept in the company will give indirect benefits, such as customer satisfaction, increase income, decrease the cost, the increase asset utilization, increase in profits, and enlarge the company (Jebarus, 2001). SCM does not only have indirect benefits, but also

has direct benefits such as physically converting raw materials into finished products and delivering them to the final consumers, identifying the products with characteristics as what the consumers want (Sucahyowati, 2011). Number of the supply chain links also affects the physical costs, for instance, material costs, storage cost, production cost, transportation costs, and etc.

V. CONCLUSION

Results of the research showed that 3 major horticultural commodities are chili, red pepper, and watermelon. The potential agricultural products in Blitar Regency have inefficient and complex supply chain due to involving many actors from farmers to the consumers. The inhibiting factors are as follow: 1. The farmers do not have collective system in marketing their harvests, so that information on products and prices was not integrated, 2. Ineffecient and complex supply chain pattern so that the price is costly, and 3. Inadequate wholesale market locations. It is expected that the approach of supply chain on horticultural commodities in Blitar Regency will provide an overview of potential commodity supply availability as a consideration for supply chain management.

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Dissecting Cultural Transition: A Deep Dive into Chinua Achebe's *Things Fall Apart*

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Abstract— *This study explores the profound narrative layers within Chinua Achebe's seminal work, Things Fall Apart, shedding light on the intricate dynamics of pre- and post-colonial Igbo society. Through a meticulous examination of the vibrant setting of Umuofia, the analysis uncovers the rich cultural tapestry depicted in the novel and its subsequent transformation under colonial influence. The paper seeks to foster a nuanced understanding of African history, urging readers to acknowledge the complex interplay of traditions and values that define the African heritage, thus challenging single narratives and reshaping public perception of a historically vibrant and multifaceted continent.*

Keywords— ***Chinua Achebe, Colonial influence, Pre-colonial Igbo society, Things Fall Apart, Umuofia***

I. INTRODUCTION

Things Fall Apart, authored by the venerable Nigerian writer, Chinua Achebe, stands as a pillar in the landscape of post-colonial African literature. First published in 1958, the novel delineates the life of Okonkwo, a revered leader and wrestling champion in the Umuofia clan, a Nigerian ethnic group. In meticulously crafting the narrative, Achebe revives the vibrant tapestry of the Igbo culture, unadulterated and untouched by European influence, before maneuvering into a vivid portrayal of the abrasive alterations brought about by colonialism.

In the pursuit to capture the authentic essence of pre-colonial Africa, Achebe veers away from the stereotypical representation of African culture often perceived through a Western lens. This discerning approach not only resurrects a fragment of history but also endeavors to reshape public perception, fostering a richer, multifaceted understanding of a civilization on the cusp of an epochal shift. Through the lens of *Things Fall Apart*, readers traverse the delicate boundary between a vibrant past and a turbulent, colonially molded present, offering profound insights into the societal and cultural upheavals that marked the transitional phase of the African continent.

As we delve deeper into the intricacies woven into the narrative of *Things Fall Apart*, this paper aims to analyze how Achebe utilizes the setting to enhance the thematic depth, fostering a vivid tableau of pre- and post-colonial African societies. The paper endeavors to explore how the detailed portrayal of the Igbo society prior to colonial intervention helps in reshaping public perception, promoting a nuanced view of a culture rich in traditions, values, and complexities.

II. HISTORICAL BACKGROUND

When we fully comprehend the nuances of *Things Fall Apart*, we can understand that the timeline of the novel spans across a critical period in Nigerian history, straddling the boundary between the pre-colonial era, characterized by rich, indigenous cultures and traditions, and the onset of colonialism, marked by the arrival of European settlers.

The Igbo society, as depicted in the early parts of the novel, is one that thrives on communal values, complex social hierarchies, and deep-rooted traditions. It is a society where titles are earned through personal achievements and where ancestral spirits hold significant sway over the living. Yet, this intricately structured society undergoes a tumultuous transformation with the arrival of British colonizers in the latter part of the nineteenth century, a period that saw the carving and division of Africa among European powers.

In *Things Fall Apart*, Achebe masterfully illustrates this paradigm shift, where the collision of two distinctly different worlds gives rise to a period of unrest and transformation. Through the experiences of Okonkwo and his community, the readers witness firsthand the systematic dismantling of a rich cultural tapestry by forces wielding a foreign, arguably oppressive, set of ideals and beliefs. The setting of the Umuofia village thus becomes a microcosm of the larger societal upheavals that engulfed numerous African societies during this transformative age.

In this paper, we venture further into the depths of this setting, dissecting its elements to unearth the potent narrative strategies employed by Achebe to convey the seismic shifts that characterized post-colonial Africa.

III. SHAPING PUBLIC PERCEPTION

In the rich literary tapestry of *Things Fall Apart*, Achebe sets forth a calculated endeavor to reshape the public perception of pre- and post-colonial African societies, especially emphasizing the Igbo community. The novel serves not merely as a window into the lives and traditions of the Igbo people, but as a tool of introspection, urging readers to navigate the complex layers of history that envelop the continent.

Before the advent of colonial forces, the Igbo society was a dynamic entity, operating on the principles of communal living, a keen sense of justice, and an intricate system of religious beliefs and practices. Achebe paints a vivid picture of a society rich in cultural traditions and rituals, a society where every individual, every deity, and even the natural elements held a distinctive place and significance. This vibrant depiction counters earlier representations that often-rendered African societies as simplistic or primitive in Western literature. Achebe, through his narrative, essentially reverses the gaze, offering a deeper, more nuanced perception that celebrates the complexities of African cultures.

As the narrative transitions into the colonial era, Achebe captures the jarring shift that comes with the imposition of European ideologies and the resulting fracturing of Igbo society. The character of Okonkwo stands as a representation of the traditional values being threatened by the encroaching colonial influence. His staunch adherence to the old ways and his eventual tragic downfall embodies the profound societal disruption that came with colonialism.

The novel highlights the abrasive alterations brought about by colonialism: the introduction of a new religion, the imposition of a foreign government, and the undermining of the traditional Igbo justice system. These shifts are not merely changes but are depicted as a form of cultural erosion, where the very fabric of society is being pulled at its seams. This critical portrayal fosters a revised understanding that encourages readers to view the colonial period not as a time of 'civilizing' a 'savage' land but as a time of significant loss and transformation for African societies.

Moreover, *Things Fall Apart* amplifies voices that have often been silenced in the historical narrative - the voices of the colonized, who bear the brunt of these transformations. Through the lens of the novel, the audience is urged to perceive history not as a one-sided tale but as a complex narrative woven from multiple perspectives, each bearing its truths and realities.

In unearthing the intricate dynamics of the Igbo society before the colonial encounter, Achebe gifts readers with a richer, more nuanced historical perspective. It serves as a testament to the resilience and depth of African cultures, urging a shift in public perception to acknowledge the vibrant tapestry that is African history, marked not by simplicity but by a complex interplay of traditions, values, and an enduring spirit of community and harmony.

IV. LITERARY ANALYSIS: SETTING

At the epicenter of *Things Fall Apart* lies the vibrant and intricately portrayed setting, Umuofia, which transcends beyond a mere backdrop to embody the life, customs, and traditions of the Igbo society. This section delves deep into the textual landscapes crafted by Achebe, highlighting how the setting functions as a potent narrative tool that enhances the thematic depth and facilitates a vivid portrayal of a society grappling with transformative forces.

In the early segments of the novel, Umuofia emerges as a living entity, resonating with the rhythms of nature and the pulsating energies of its inhabitants. This portrayal transcends stereotypical narratives about African societies, illustrating instead a society steeped in traditions, rituals, and an organized socio-political structure. The lush forests, the vibrant festivals, and the marketplace bustling with activity—all paint a vibrant tableau of a society that thrives in harmony with nature and its deeply rooted customs. Through detailed descriptions, Achebe fosters an intimate connection between the readers and the setting, immersing them into a world where the natural and the supernatural coalesce in everyday life.

As the novel progresses, the setting undergoes a metamorphosis parallel to the societal transformations catalyzed by the advent of colonial forces. The once vibrant festivals lose their luster, and the sacred spaces of the Igbo people, like the Oracle of the Hills and Caves, are overshadowed by the imposing

structures of colonial establishments. Achebe intricately captures this shift in the societal fabric, leveraging the setting to underline the pervasive impact of colonization, which seeps into every nook and cranny of Umuofia, altering the very essence of the community.

Furthermore, the setting serves as a silent witness to the cultural clash that unfolds within its boundaries, echoing the tensions, apprehensions, and resistance that mark this historical period. The infiltration of Christian doctrines, the establishment of government structures alien to the Igbo society, and the introduction of a foreign language—each of these elements contributes to the evolving setting, marking the gradual transition from the familiar to the unfamiliar, from harmony to discord.

Through a meticulous portrayal of setting, Achebe facilitates a profound engagement with the narrative, enabling readers to visualize and empathize with the changing realities of the Igbo people. The setting in *Things Fall Apart* stands as a testament to a community's vibrant past, its turbulent present, and an uncertain future, offering a rich canvas upon which the complexities of cultural interaction, adaptation, and resistance are vividly portrayed.

In conclusion, the setting in *Things Fall Apart* is not merely a passive backdrop but a dynamic entity, mirroring the cultural, social, and political upheavals that characterize the post-colonial period. It serves as a canvas where the vibrant hues of a rich cultural heritage meet the somber tones of change, crafting a compelling narrative that resonates with historical depth and contemporary relevance.

V. CONCLUSION

In *Things Fall Apart*, Chinua Achebe embarks on a nuanced literary expedition that resurrects the vibrant and rich tapestry of pre-colonial Igbo society, while simultaneously laying bare the disruptive forces ushered in by colonialism. Through the life and trials of Okonkwo, readers traverse a historical journey, witnessing the meticulous dismantling of a culture deeply entrenched in tradition, community values, and harmony with nature.

Achebe's portrayal of the setting, Umuofia, serves not merely as a canvas to illustrate the narrative but as an essential character that evolves, embodying the transformations and frictions of a society in flux. The novel stands as a testament to the resilient spirit of the African continent, showcasing a complex, multifaceted, and vibrant past that refuses to be overshadowed by the encroachments of colonial rule.

As we close this analytical journey, it becomes evident that *Things Fall Apart* plays a pivotal role in reshaping public perception, fostering a more inclusive and nuanced understanding of African history and culture. It beckons readers to look beyond the single narrative often propagated, urging them to embrace a diverse, rich, and complex tapestry that forms the African historical landscape.

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Fake Eggs on the Ghanaian Market: An Emperical Evidence - Shukura Market, Greater Accra Region, Ghana

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Abstract— *Background and objectives: In recent times, the Ghanaian media space has news of the proliferation of fake eggs in the market especially in the capital, Accra. The Food and Drugs Authority (FDA) of Ghana, the agency responsible for regulating wholesomeness of food has refuted this assertion after thorough investigations. The issue could affect the contribution of poultry to the economy. The aim of the study therefore is to determine the authenticity of eggs sold in the Ablekuma central sub-metro in the Greater Accra region of Ghana. The results shall contribute to the baseline data on eggs, and to restore consumer confidence in eggs sold in Ghana. Methods: The spectral bands of the shells, albumen and yolk of the egg samples were determined using FTIR analysis, and knowledge about fake eggs through survey. Results: The spectra of the shells, albumin and yolk of the samples corresponded to the standard spectra of authentic egg. The survey revealed lack of knowledge about fake eggs. Conclusion: In spite of the lack of knowledge about fake eggs, the eggs were authentic. This confirms the assertion of FDA of Ghana.*

Keywords— ***Albumin, Fourier Transform infrared (FTIR), poultry, fake eggs, yolk,***

I. INTRODUCTION

Eggs are great source of easily digestible proteins. It is essential nutrient for healthy nutrition (FAO/UN, 2006) [1]. With the advent of more sophisticated and improved poultry management techniques, egg production has risen quickly globally in recent years (FAO 2010) [2]. For instance in Ghana between 2004 and 2012, there was an increase of about 15,000 tons (FAOSTAT 2013) [3]. Ghana has a low per capita consumption rate of 12 eggs annually, which is far lower than the global average (Kirtchevsky, S. & Kritchevsky, D, 2000) [4]. Eggs, however, is linked to higher serum cholesterol levels and cardiovascular illnesses (CVDs) (Song, W.O & Kerver, J.M 2000) [5]. This perception has reduced consumption despite yearly output increases (FAOSTAT 2013, Yilma, K. T, et al., 2022) [3,6].

Another perception that could further reduce consumption is the proliferation of fake eggs in the market. The Food and Drugs Authority (FDA), of Ghana has however refuted this and emphatically stated that there are no such eggs sold on the market (GNA/FDA, 2019) [7]. In Bangladesh, such news was also condemned, and emphatic that no fake eggs on the market (https://www.thepoultrysite.com/news/2017/08/suspected-fake-eggs-sent-for-examination-govt-says-eggs-not-fake) [8].

Fake eggs are real, but used to encourage chickens to lay their eggs in a particular place. They can also be used under

broody chickens to encourage them to sit and incubate other eggs or even to raise day old chicks (ozfarmer.com https://www.ozfarmer.com › ceramic-china-fake-brooder) [9], they are not meant for consumption, because they contain no nutritional values compared to the real eggs (Dibyajyoti Saha S.M., et al., 2013) [10]. The aim of the study therefore is to determine the authenticity of eggs sold in the Ablekuma Central Sub-Metro in the capital of Ghana, Accra. The findings shall contribute to the existing data in informing policy making about poultry.

II. MATERIAL AND METHODS

2.1 Sampling Location

The sampling site has the coordinates at latitude $5^{O}33'01.5''N$ and longitude $0^{O}15'04.7''W$ and 5.55042622508, -0.251316335998 (www.shukura.com retrieved on 17/08/2023). It is part of Ablekuma Central in the capital Accra.

2.2 Sample Collection

Eggs from domestic fowls were from vendors in Shukura market in Accra. Shukura is chosen, because of the vast number of communities it serves, coupled with its economic importance to the sub- metro (Ghana Statistical Service, 2021). Two hundred crates of eggs of equal quantities of brown and white shells were collected in six months.

2.3. Sample Preparation and Analysis

Eggshells dried at 150° C for 6 hours, was pulverized. The powder sprayed, into a drying chamber at 150°C was collected for the analysis.

A drop of the yoke and the albumin, separately put on the surface of a highly polished KBr plate is scanned to generate the spectrum using the FTIR (Bruker Alpha-P ATR FTIR : standard operating procedure) [11] .

2.4. Survey

Questionnaire, administered to vendors and customers to solicit information. The questionnaire is structured into demographic status of vendors, data about eggs, capacity to test eggs, and complaints. Eighty volunteers were involved. The data obtained were analysed using SPSS version 16.

III. RESULTS

This section describes the various results obtained.

3.1. Questionnaire

The questionnaire is shown in Table 1.

Table 1: Template of the Questionnaire

Number	Parameter
	Demographic status
1.	Age: (< 18yrs, 18 – 50years)
2.	Educational background (literate/illiterate)
	Data about bird and eggs
3.	Source of eggs (local/imported)
5.	Cost of eggs: (affordable/expensive)
	knowledge to test fake eggs
6.	Any knowledge about authenticity of the eggs
	Knowledge about fake eggs
	Any education about fake eggs
	Complaint from customers
7.	Complaints about authenticity of eggs (none/often)

Table 2: Responses (%) from Questionnaire

Parameter	Age	Education	Eggs		Knowledge to test authenticity of eggs	Knowledge about fake eggs	Complaint
	18-50	literate	source	cost per crate	no	yes	none
responses	90	60	100 (poultry)	80 (expensive)	100	10	100

Source: Bartels/ Hussein/ Gadzekpo, statistical analysis, 2023

GC denotes Ghana cedis, the currency of Ghana.

Table 3: Possible Functional Groups in the Samples.

Peak (cm^{-1})	Possible functional group
712-876	C-H bending vibration
1426	Carbonate groups of stretching
1645	Carbonyl group stretching
1799	C=O stretching
2516	Hydrogen group stretching
2875-2926	C-H bending vibration
3419	Hydroxyl group stretching

Figures 1-4 depict the spectra of the brown shell, white shell, the yolk and the albumin of the samples.

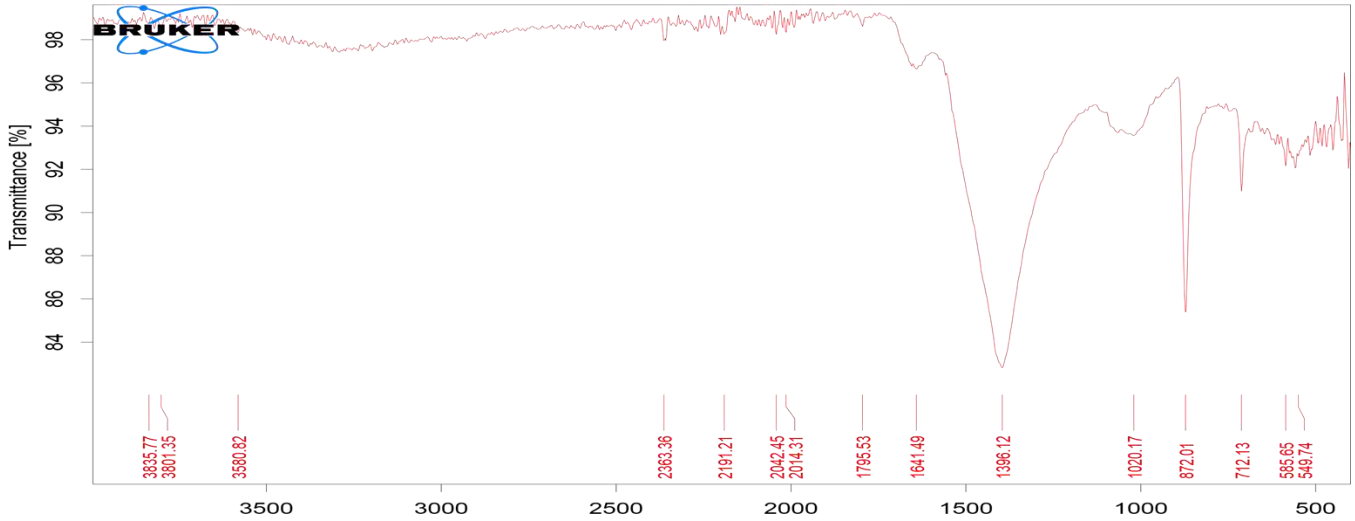

Fig.1: Spectra of Brown Shell

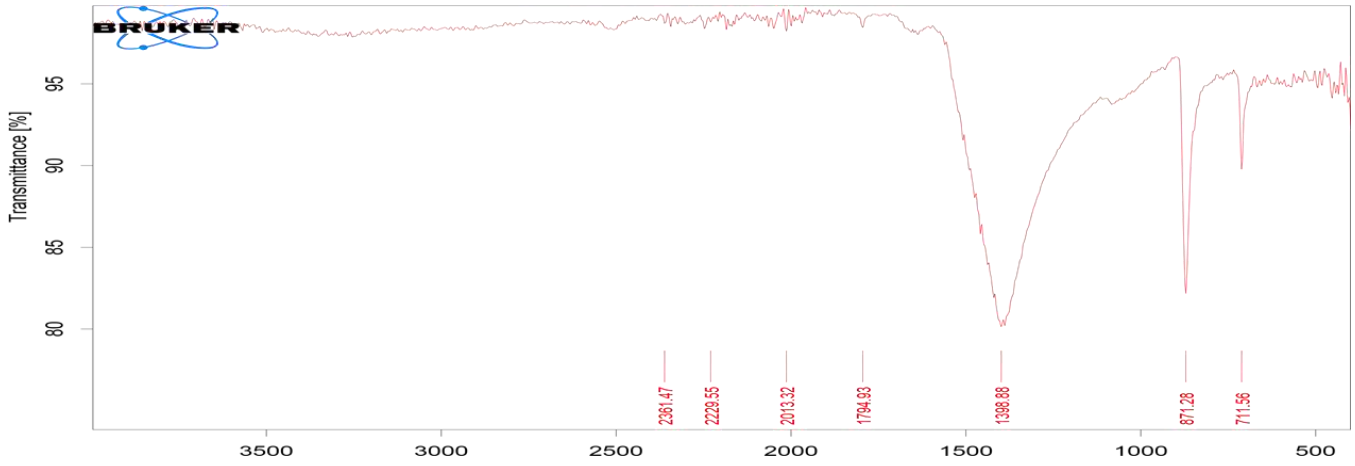

Fig.2: Spectra of White Shell

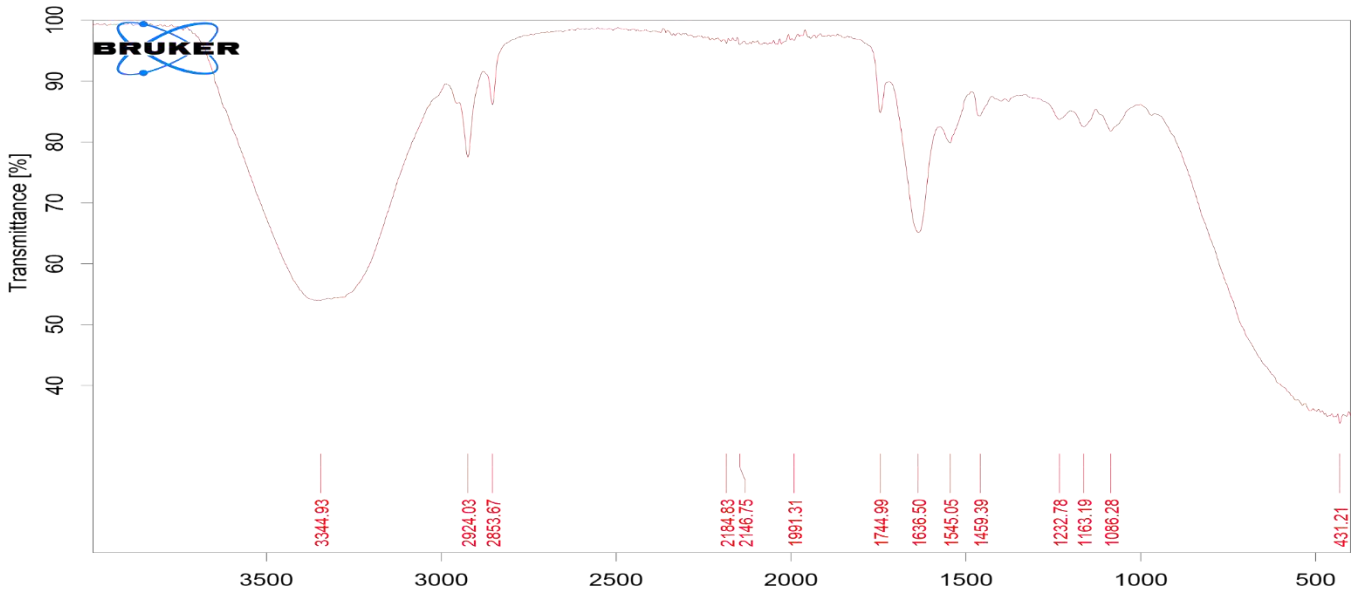

Fig.3: Spectra of Yoke

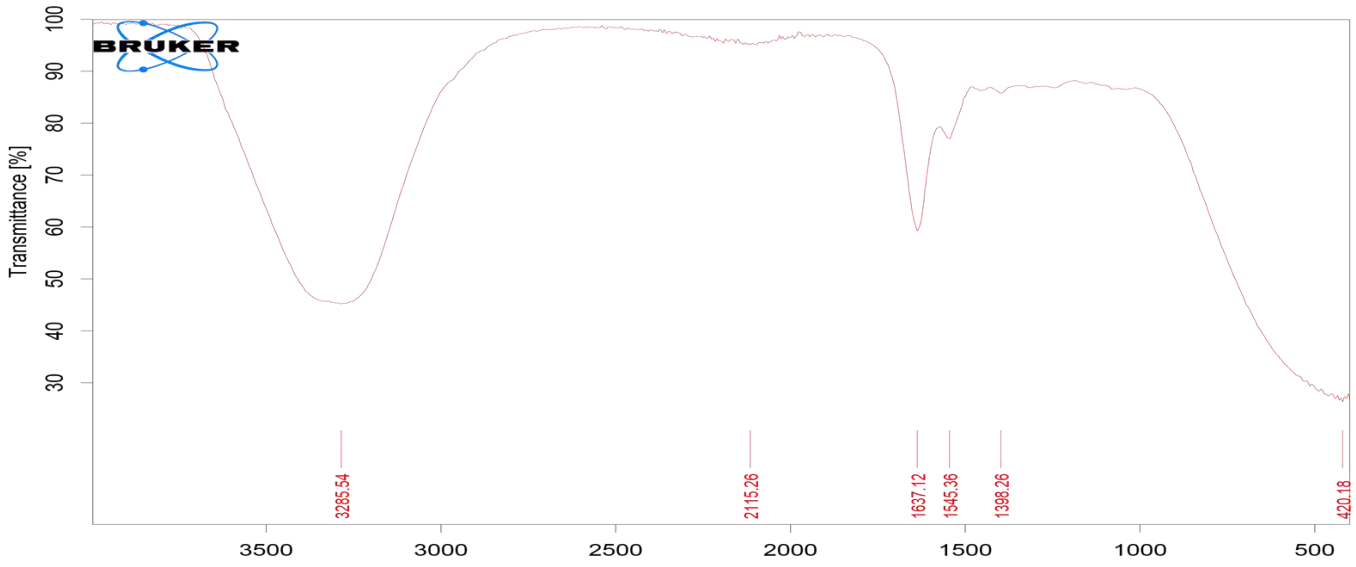

Fig.4: Spectra of Albumin

IV. DISCUSSION

4.1. Response from Survey

The survey instrument is shown in Table 1. As shown in Table 2, most (60%) of the vendors are literate suggesting the ability to acquire information and communicate if education about fake eggs is available. Concerning the supply, the eggs were sourced from local poultry farmers, indicating the inability of the farmers to produce fake eggs. Majority of the respondents (80%) found the eggs expensive at the current cost of 45 – 55 GC (4.1-5.0 USD) per crate; such could compel them to patronize fake eggs if available. The vendors (100%) had no knowledge about testing the authenticity of the eggs, because such information is not available to them through any of the media outlets including television, radio, information vans, and billboards. Few (10%) had knowledge about fake eggs, which came through rumors. About complaints, none received about authenticity of the eggs sold out; this could confirm the integrity of the eggs.

4.2. Peaks and Possible Functional groups

Figures 1-4 depict the spectra of the brown shell, white shell, the yolk and the albumin of the samples respectively. The various peaks are shown in Table 3. The functional groups in the samples thus include C-H at 712-876 and 2875-2926 cm^{-1}, carbonyl C=O at 1645 and 1799 cm^{-1}, carbonate C=O at 1426 cm^{-1}, and H at 2516 cm^{-1} whilst OH at 3419 cm^{-1}.

4.2.1. The eggshells

The *brown shell* showed peaks at 872.1 and 712.13 cm^{-1}. Such indicate the presence of carbonate and C-H functional groups, whilst 1795.53 and 1641.49 cm^{-1} correspond to carbonyl functional group as shown in Fig. 1 and Table 3 (Yilma, K. T et al., 2022, Brudnicki, P. A. P et al., 2022)[6,12]. Similar results for white shell is shown in Fig. 2 and Table 3 with peaks at 871.28 and 711.56cm^{-1}. This implies the shells are identical (Brudnicki, P. A. P et al., 2022) [12]; the difference could be the length of time it stays in the uterus, and the speed at which calcium deposits as the shell forms[13]. As shown in Figs. 1, 2, and Table 3, the intense peak observed around 1398.88 is due to the matrix's carbonate mineral content; this confirms the eggshell is entirely $CaCO_3$. Moreover, there is no peak around 1200 cm^{-1} indicating the presence of SO_4 (Plaster of Paris, CaSO4), or $CaCl_2$, which form shell of fake eggs (Brudnicki, P. A. P et al., 2022) [12]. The structure for the eggshells is authentic.

4.2.2. The yoke and albumin

As shown in Fig.3, the peaks at 1744.99 and 1615.39cm^{-1} which indicate C= O functional group for homogenized yoke of the eggs were identical (Brudnicki, P. A. P et al., 2022) [12]. However, concerning albumin, the distinctive peaks at 1636.72 cm^{-1} and 1545.03 cm^{-1} in Fig. 4 correspond to C=O stretching (due to the amide I band) and C-N stretch (with N-H bending mode due to the amide II band), and O-H stretch at 3285 cm^{-1}. This structure indicates the presence of protein (Tizo, M. S., et al., 2018a) [13]. The structure is –NHCHRCONHCHRCONHCHRCO or the amino acid moiety, n (NH2CHRCOOH) (Brudnicki, P. A. P et al., 2022) [12] .

V. CONCLUSION

The study investigated the speculation that the Ghanaian market is flooded with fake eggs, which created anxiety among the populace in view of its health hazards. This news is denied by the Food and Drugs Authority of Ghana, the body responsible for the authentication and approval of foods and drugs for consumption. The study thus aimed at providing an empirical evidence about the issue, and delved into some of the possible reasons for patronizing fake eggs.

1.The study revealed that there are no fake eggs on the Ghanaian market. The spectra obtained are consistent with that of authentic domestic birds' eggs. The eggs on sale are therefore good for consumption.

2.Among the possible reasons for patronizing fake eggs if available were high cost of eggs, lack of information and knowledge of testing fake eggs.

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RECOMMENDATION

Though the samples were not fake, there is the need for the relevant agencies to be vigilant in avoiding the sale of fake eggs for consumption. This could be educating the public and the stakeholders in the poultry industry about fake eggs.

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The Influence of Maize Cluster Development Interventions on the Performance of Actors in Morogoro Region, Tanzania

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Abstract— *This study aimed to investigate the influence of maize cluster development interventions on the performance of actors in Tanzania. Specifically, the study aimed at identifying types of interventions employed by actors of maize clusters, the influence of intervention on the performance of actors, and the challenges facing actors of maize clusters in the study area. Descriptive, linear regression and word clouds analysis were used to analyze the findings. Results show the common types of interventions employed by actors of maize clusters are; proper seed spacing, capacity building, control of aflatoxin, and marketing. Linear regression results show, that technological support, networking, and marketing were reported as interventions of high influences on actors of maize clusters with p-values of (0.01, 0.02, and 0.02) respectively. However, the key challenges reported to face actors of maize clusters in the study area were: lack of funds to buy agricultural inputs, limited access to financial resources, lack of transparency among cluster leaders, shortage of market, lack of coordination among respective research institutions and poor communication among actors. Regardless of different interventions to support actors of the maize cluster still challenges exist and some of them are among of the employed interventions. Thus, different transformative participatory strategies are required to be vested among actors of maize clusters in Morogoro regions and other areas of the country for proper performance of maize cluster actors and economic development of the country.*

Keywords— ***Actors, Maize cluster, Intervention, Morogoro, Performance***

I. INTRODUCTION

Cluster development interventions have been widely recognized for their role in stimulating economic development (Rwekaza, et al., 2020; Mwamila, 2014). The approach has been potential to transform government economies in developed and developing countries such as Europe, Asia, Latin America, and partly Africa (Rwekaza and Anania 2020; Rawat et al., 2017). In the process of promoting socio-economic development, countries tend to opt for different development approaches and employ various interventions to achieve goals. Among the initiatives being taken includes the adoption of cluster development initiatives (Adam et al., 2017; Stadenberg, 2016; Mwamila, 2014). The cluster comprises geographically concentrated firms, companies, and service providers that are interconnected in a particular field (URT, 2011; Ketel et al., 2013). Over the years, the cluster development approach has grown swiftly and has the potential to guide economic development policy in many countries Francis et al., (2020). The focal part of the cluster approach is the conception that policy action can change the collective behaviour of groups of firms hence encouraging the rise of self-sustaining structures of innovation and commercial regeneration (Stadenberg, 2016).

Through cluster development interventions, micro, small, and medium enterprises (MSMEs) have played a leading role in promoting equitable regional development and economic growth (UNIDO, 2017). Such enterprises have managed to employ at least 45 percent of the workforce in half of the high-income economies worldwide (Rawat et al., 2017; Kobersy, et al., 2015; Lai et al., 2014). In India, the cluster development approach has enabled the MSMEs to contribute beyond doubt to the Indian economy by generating employment opportunities, promoting exports and innovations, and by developing entrepreneurial skills (Elvir et al., 2017; Das et al., 2007). As a result, the MSME sector has emerged as a highly energetic and dynamic sector of the Indian economy and enabled the country to achieve industrial growth and development (Vasu and Jayachandra, 2014). Several countries in Asia, have utilized the cluster development approach as an engine for economic development these include China, India, Singapore, Malaysia Myanmar, and Sir-Lanka. These countries have established Special Economic Zones (SEZs) and supported them with the necessary infrastructures to build capacities of micro, small, and medium enterprises (MSMEs) to produce high-quality products and manage the competition (Vasu et., 2014).

Africa has great opportunities to invest in enterprises based on economic zones although clusters have not fully been integrated into the country's economic development (African Union Commission, 2015). The Africa Development Agenda 2063 focuses on building confidence that Africa can attain the capacity to utilize its full potential to promote development, culture, and peace accompanied by the creation of flourishing, inclusive, and prosperous societies. Based on cluster promotion, these ideas can be practicable. The promotion of micro, small, and medium enterprises (MSMEs) in Africa is expected to increase intra-African trade growth which is expected to be about 50% by 2045 (Maziku, 2019). The efforts are also expected to increase Africa's share of global trade (Doronina et al., 2016; AUC, 2015; Lei and Huang, 2014; Aquere et al., 2013). Efforts such as establishing the Pan-African Competitive Forum (PACF), Cluster Initiatives (CIs), and the Innovation Systems and Clusters Programme in East Africa (ISCP-EA) among others indicate the intention of African countries to promote the cluster development approach for its economic development (Mwamila, 2014).

In East Africa, the cluster organizations were introduced following the Regional Conference on Innovation Systems and Innovative Clusters in Africa which was held, in Jinja and hosted by the Faculty of Technology of Makerere University (Mwamila, 2006). Cluster initiatives were focused on basic industries like agriculture, food and basic manufacturing, while few of them are capital – intensive aimed at first tracking social–economic development in the region with an idea that through clusters even a small firm can gain the necessary critical mass to the service world market (Francis et al., 2020). Although the establishment of cluster initiative development, as a strategy to boost the basic industries like agriculture, food, and basic manufacturing aimed at tracking social–economic development in the region, their performance still raises questions (Roghgang and Lageman, 2016). Conflicting objectives and coordination between members may often result in inferior performance of the cluster (Adam et al., 2017). Also, active regional policies are debated to be an important factor in enabling sustainable clusters which will ensure the social economic development of the region (Francis et al., 2020).

In Tanzania, cluster organizations were formed following the first conference on Innovation Systems and Innovative Clusters in Africa held in Tanzania in 2004 and organized by the College of Engineering and Technology (CoET) of the University of Dar es Salaam (Diyamett & Komba, 2008). The establishment of the cluster was an initiative of a long-term National Development Vision 2025 which is aiming at transforming Tanzania to develop towards good governance, high-quality livelihoods, peace, stability, and unity. Commission for Science and Technology (COSTECH) is among of stakeholders supporting the initiative through various interventions like doing research, innovation, and capacity building (Stadenberg, 2016). Basically, cluster initiatives aimed at standardization of farms to increase production per unit area through modifying farming techniques (Msuya, 2006).

Despite the importance of cluster initiatives development to individuals, regions, and countries, still there are some farmers not engaging in cluster groups. However this paper aimed at investigating the influence of maize cluster development interventions on the performance of actors in Tanzania taking Morogoro region as a Case study.

II. METHODOLOGY

2.1 Study Area

The study area was conducted in three Districts: Kilosa, Gairo, and Morogoro town in Morogoro Region. The coordinates of the Morogoro region range at the Latitude – 6.82102 and Longitude of 37.66122. The region is boarded to the north by the Tanga Region, to the east by the Coastal region and Lindi regions, to the south by Ruvuma Region, and to the west by Iringa and Dodoma regions.

The study area had been purposely selected with the fact that these areas have received many project interventions based on improving maize cluster development initiatives compared to other places in the Region (Kimario, 2017).

2.2 Research Design

This study used descriptive research design because it enables the researcher to provide more insight into cluster development initiatives (Akhtar, 2016).

2.3 Research Approach

This study used a mixed approach where both qualitative and quantitative data were collected and analyzed (Creswell 2014). This is due to the nature of the study which requires both qualitative and quantitative data to gain a broad thought on the influence of maize cluster development interventions on the performance of actors in the study area.

2.4 Population Study

The study population was drawn from small-scale maize farmers, processors and exporters working in maize clusters in the Morogoro region. The study targeted the small-scale maize farmers, small-scale maize processors, and maize exporters in clusters in the study area. The total number of populations for the study was 988 small scale maize farmers.

2.5 Sample Size of the Study

The sample size for this study was determined by using Yamane (1967) as it provides an effective method of determining sample size. Hence, the following formula was applied.

$$\text{n} = \frac{N}{1 + Ne^2}$$

$$\text{n} = \frac{N}{1 + N(0.02)^2}$$

$$n = \frac{988}{1 + 988(0.0004)}$$

$$\text{n} = 708$$

Where;

n = required sample size

N = the population size

e = Marginal of error

Source: Yamane (1967)

The sample distribution for respondents by using the formula of Krejcie and Morgan (1970) is shown below.

Table 2.1 Sample Distribution Table for the Population

Location	Description	Cluster Members	
		Population	**Sample Size**
Kilosa District	Farmers	250	152
	Processors	80	66
	Exporters	50	44
Gairo District	Farmers	240	148
	Processors	85	70
	Exporters	60	52
Morogoro Town	Processors	113	90
	Exporters	110	86
	Total	**988**	**708**

Source: Chaokromthong, and Sintao, (2021)

2.6 Unit of analysis

The study adopted linear regression analysis. This technique attempted to investigate the strength of the relationship (dependent variable) and independent variables namely types and level of interventions used.

$$\boldsymbol{Y = \beta o + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + e}$$

Where by

$\boldsymbol{Y}$= Performance of actors

$\boldsymbol{\beta o}$ = Constant factor

$\boldsymbol{X_1}$ = Technology usage

$\boldsymbol{X_2}$= Participation

$\boldsymbol{X_3}$= Training

III. RESULTS AND DISCUSSION

3.1 Demographic Characteristics of Respondents

3.1.1 Sex of the respondents.

The findings as summarized in Table 3.1 show that the majority (59%) of respondents were male while (41%) were female. Therefore, study findings imply that there are more males than females who participate on the employed maize cluster interventions in the study area. Findings are supported by COSTECH report of (2016) and Rwekaza, (2020), where both coined that gender imbalances have become an issue in cluster development where men are commonly reported to predominately occupy both sunflower and rice clusters in Tanzania.

Table 3.1: Sex of the Respondents

Status	Frequency	Percentage
Male	428	59%
Female	280	41%
Total	708	100%

Source: Survey data (2022)

3.1.2 Education Level of the respondents

Findings as shown in Table 3.2 show that 33% of the respondents had primary education followed by 25% of the respondents who had secondary education, while 21% of them had Basic Technician certificate level of education and 16% attained diploma level of education and only 5% had Bachelor degree of education. Results imply that the majority of respondents who engage in maize clusters have low level of education which cannot help them adopt high and advanced technologies to run their business. Findings comply with the study by Kalumanga et al., (2023) who reported that most smallholder farmers who engage in self-help groups do lack enough education which can help them to transform their business portfolios. Lacking enough education it makes difficult for farmers to adopt advanced technology systems which can improve the performance of agriculture sectors.

Table 3.2: Educational Level of Respondents

Respondents education level	Frequency	Percentage
Primary education	236	33%
Secondary education	175	25%
Basic Technician Certificate	146	21%
Diploma	113	16%
Bachelor's degree and above	38	5%
Total	**708**	**100%**

Sources: Survey data (2022)

3.1.3 Age of respondents

Findings summarized in Table 3.3 indicate that the majority (62%) of the respondents were aged from 26-41 years. This implies that majority of respondents who engage on clusters are at the work force age, that means they can easier work successfully and improve clusters most sustainably if all important requirements like agricultural inputs, markets etc. are improved. Results are supported by Obua (2020) who argued that age determines the work-ability of a certain institution.

Table 3.3: Characteristics of Respondents by Age.

Respondent's age	Frequency	Percentage
18-25	43	6%
26-33	217	31%
34-41	219	31%
Above 42	229	32%
Total	**708**	**100%**

Sources: Survey data (2022)

3.2 Types of Interventions Employed on Maize Cluster in Morogoro Region

Results as shown in Table 3.4 revealed that 74% of the respondents mentioned control of aflatoxin as one of the common interventions conducted on maize cluster performance, and 80% of the respondents mentioned capacity building as another common intervention employed on maize cluster performance in the study area. Moreover, 87% of the respondents mentioned proper seed spacing as a common intervention practiced to enable cluster performance while 81% of the respondents also mentioned marketing as the common intervention conducted on maize cluster performance in the study area. This implies that proper seed spacing and marketing are the most frequent interventions adopted in enhancing the performance of actors in maize clusters. Findings correspond to those posed by Francis et al., (2020) who conducted his research on clusters development initiatives in sunflower and rice and find that proper seed spacing, networking, and marketing are among of the key interventions for cluster development initiatives.

Table 3.4: Common Types of Interventions Conducted on Maize Cluster Performance

Variables	1		2		3		4	
	F	**%**	**F**	**%**	**F**	**%**	**F**	**%**
Control of aflatoxin	262	37	262	37	113	16	71	10
Capacity building	276	39	290	41	92	13	50	7
Proper seed spacing	276	39	340	48	35	5	57	8
Marketing	248	35	326	46	85	12	50	7

Key: 1 Means Most frequent, 2 means Frequent, 3 means rarely, 4 means Not at all

Sources: Survey data (2022)

3.3: Linear Regression Analysis to Analyze the Influence of Intervention on Performance of Maize Clusters

Regression analysis results as shown in Table 3.5 highlighted several interventions used to support the performance of maize clusters in the study area. However, findings show that technology support, networking, and marketing are statistically significant to the performance of maize clusters in the study area with P-values of (0.001, 0.002, and 0.002 respectively. Findings correspond to those argued by Ketels and Memedovic (2008) who emphasize three approaches to be used by cluster organizations. The first one focuses on creating a platform for interaction between the actors, the second emphasizes the importance of collaboration between Public and Private Institutions and the third emphasizes the importance of research institutions. These three aspects if well interlinked will help the performance of clusters as they both recognize the role of networking, marketing, and technology transfer. Similar findings by Rothagang et al., (2016), and Kumari (2020) asserted that in order for clusters to develop, they need productivity increase which is forced by availability of improved market linkages, networking and financial linkages.

In connection to the importance of technology, other scholars including Kapange, (2010); Sanyanga et al., (2012), and Porter et al., (2019) both have shown that technology especially the advancement of information and communication technology (ICT) has the potential to connect actors in networks through the facilitation of communication and the exchange of information. They further argued that the development of ICTs brings farmers close to market actors and gives them the potential to bargain as well as use the information to make informed choices about marketing. Likewise, a study by Asenso-Okyere and Mekonnen (2012), found that ICTs enabled farmers to have strong interactions with market actors in many African countries including Ethiopia, Kenya, Malawi, Mozambique, Uganda, and Nigeria.

However, findings also show statistical significance with proper seed spacing and capacity-building interventions on the performance of maize clusters among actors in the study area. This implies that the common interventions to be employed for the performance of maize clusters in the study area requires seed spacing and capacity building among farmers which is provided through training. Nevertheless, findings correspond to those by Okeke et al. (2019); and Panetto et al., (2020) who propounded that training gives participants real-life skills in using clusters as powerful tools to promote local industry and enhance business growth and local prosperity. Findings resemble that of Ahmad et al. (2007) who also connote that agricultural training has benefited villagers in managing village associations and utilizing efficiently available natural resources for their organization's success. In the same vein, Ndombi and Kisimbii (2017) observed that training is an important component in transferring agricultural best practices including seed spacing and it gives alternatives to utilize effective technology for increasing yields.

..Networking and training enhance innovation which creates improvements in cluster performance. They went further and concluded that networking helps to build professional relationships, opens doors to new opportunities, and facilitates the exchange of ideas and best practices. It also aids in career development, personal growth, and business success…

Results from regression also show a positive correlation between the control of aflatoxin as an intervention and the performance of clusters in the study area. This implies that farmers have been well equipped with training on how to control aflatoxin which has contributed to producing quality maize products that increase sales and improve the market for processors and exporters selling quality maize products. The study is in line with Savic et al. (2020) who claimed that the use of biological control was useful in controlling aflatoxin levels in maize fields in Serbia and showed a reduction of aflatoxin biosynthesis in maize clusters.

Table 3.5: Results of the Regression Analysis on the Employed Interventions for Maize Clusters Performance

Model	Unstandardized Coefficients		Standardized Coefficients	T	Sig.
	B	Std. Error	Beta		
(Constant)	6.412	.366	.789	17.500	.000
Marketing	.113	.066	.065	1.706	.002
Capacity Building/Training	.113	.068	.062	1.660	.040
Control of Aflatoxin	.199	.069	.119	2.877	.044
Networking	.291	.076	.236	3.65	.002

Proper Seed Spacing	.376	.062	.241	2.043	.031
Technology support	.298	.067	.167	1.97	.001

a. Dependent Variable: Performance of actors

Significant = P-value < 0.05

Sources: Survey data (2022)

3.4: Challenges Facing Maize Cluster Performance in Morogoro Region

Findings, as shown in Table 3.6 indicate that major challenges facing Cluster performance in the Morogoro region include: lack of funds to purchase agricultural inputs such as improved seeds fertilizers, etc, limited access to financial resources eg. loans, and subsidies, lack of stable market, lack of transparency among leaders of clusters, absence of relevant researches on clusters, and poor weather conditions which results inadequate rainfall. Other challenges limiting the performance of clusters include; limited participation in cluster decision-making and lack of agriculture impact evaluation reports. Also, lack of funds has been a major challenge for maize cluster development for a couple of years despite government interventions. There are several items to be purchased when dealing with maize clusters such as fertilizers which cost around TZS 60-75 per 50kg bag and this cannot be afforded by the majority of farmers who have low income and who also still use traditional methods of farming. In addition to that, there is no regular visitation made by researchers or farm extension officers this has caused production process being complicated and poor to some farmers as they lack knowledge on how to deal with challenges which face them immediate. However, during Focus Group Discussion at Kilosa Districts, participants reported that.

There is a challenge concerning variations in the prices of seeds at local markets which has affected their performance. The prices of certified seeds ranged from (2000–2500 Tanzania Shillings per kg), and fertilizers (Tshs 52,000–63,000 per bag of 50 kgs). This price is very high because most of us don't have enough capital to purchase.

Findings are in line with the view of the government report (URT 2009b) which reported that farmers had limited access to agricultural credit due to not being creditworthy. Furthermore, commercial banks which are the biggest lenders were reluctant to approve investments in the agriculture sector owing to high risk. Similarly, the findings are consistent with those of Neef et al. (2006); Abate et al. (2011); Klerkx, van Mierlo, and Leeuwis (2012); and Bayissa (2015) who denoted that poor farmers have little opportunity to interact with credit institutions due to bureaucracies and lack of awareness on enabling opportunities including availability of marketing, advanced technologies etc. During key informant with Cluster Manager at Morogoro town, said that.

> *The government has been allocating inadequate budget to support clusters and sometimes fails to release the allocated amount instead it releases little amount which cannot help any plans including the construction of storage facilities.*

Table 3.6: Challenges Facing Maize Cluster Performance in Morogoro Region

4=Major challenge, 3= Moderate, 2=, Low and 1= not at all

Variables	4		3		2		1	
	F	%	F	%	F	%	F	%
Poor weather	262	37	262	37	113	16	71	10
Lack of timely receiving agricultural inputs such as seeds, fertilizers	276	39	290	41	92	13	50	7
Lack of transparency	276	39	340	48	35	5	57	8
Poor communication	248	35	326	46	85	12	50	7
Shortage of marketing capability	255	36	241	34	127	18	85	12
Lack of funds to buy agricultural input such as seeds, fertilizers	368	52	312	44	21	3	7	1
Gender inequalities in receiving agricultural inputs	7	1	14	2	255	36	432	61
Limited participation in cluster decision making	248	35	227	32	205	29	28	4
Limited access to financial resources eg. Loans and subsidies	340	48	205	29	106	15	57	8

Lack of coordination among respective research institutions.	368	52	219	31	42	6	78	11
Absence of agriculture impact evaluation reports	149	21	340	48	191	27	21	3
Limited land for cultivation	21	3	42	6	375	53	269	38

Sources: Survey data (2022)

Further Findings were obtained by using NVivo software version 12 where word clouds were generated to visualize the word frequencies on the challenges facing maize cluster performance in Morogoro Region. Results as shown in Figure 3.1 show that the common challenges mentioned were; lack of stable market, price, and enough funds to support actors, and lack of agricultural inputs including fertilizers, and seeds. Others were; lack of enough rainfall to support maize production, poor planting spacing, and lack of enough research on clusters especially on how these researches have supported maize actors.

Inadequate access to markets has caused many clusters not to perform properly because maize actors have been reported to lack sufficient market information for their products. The challenge is increased by limited access to finance among actors, lack of capacity of agricultural marketing institutions including cooperatives working with clusters, and lack of entrepreneurial skills among actors.

Fig.3.1 Word Clouds on the Challenges Facing Maize Clusters in the Study Area

3.5 Conclusion and Recommendation

Cluster development interventions in Tanzania are important in developing the micro, small and medium enterprises (MSMEs) which foster economic development of the country. There are several interventions which have been deployed among Actors of maize clusters in Morogoro region in Tanzania. These interventions have helped actors of maize clusters to perform with little success as expected. Providentially, these interventions are the ones required to uplift the development and performance of clusters in the region because most of them are still mentioned among the challenges facing cluster performance in the region. However, it is still demonstrated that regardless of the employed interventions still the actors of maize clusters underperform. The study recommend transformative strategies which embrace participation approaches of both actors and implementers for the development and positive performance of maize clusters in the region and country at large. Moreover, the existing interventions should be re-designed basing on scientific researches so that they can perform efficiently and bring the intended results.

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Performance of Exotic Cucumber Varieties under Local Cultivation Practices in Kapilvastu District of Nepal

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Abstract— *A field experiment was carried out in the Western Terai of Nepal, Buddhabhumi municipality of Kapilvastu district to screen out the best-performing cucumber variety among the five most cultivated exotic varieties; LHC-1395, LHC-Maria, Dynasty, NS-408, and Rehmat-1080 during February to July of 2019. The package of cultivation practiced in this study replicates that of the local farmers. The experimental setup was laid on Randomized Complete Block Design having five replications and five treatments in an area of the 750-meter square. The crop geometry was maintained at 25 cm×15 cm and the size of each plot was 1.4 m^2. Data was collected on the morphological and yield parameters such as plant height/vine length, number of leaves, number of branches, days to flowering and fruit development, number of fruits, fruit weight, and fruit yield per plant. The study revealed that the performance of Dynasty was best in terms of plant height with an average increase in height of 23.9 cm per week. LHC-1395 was demonstrated to be the variety with the highest increase in leaf number with an average of 2.08 leaves per week. Similarly, the highest number of branches was found to develop in NS-408 (3.2), the highest number of fruits per plant in LHC-1395 (11.36), the highest single fruit weight in LHC-Maria (208.44 grams), and the highest fruit yield per plant in LHC-1395 (2137.9 grams). Thus, the study concluded that LHC-1395 is a high-yielding variety suited for the study area.*

Keywords— ***Cucumber, field experiment, high-yielding variety, Nepal, screen out***

I. INTRODUCTION

Cucumber (*Cucumis sativus* L.) is a fast-growing major vegetable crop grown worldwide (Wehner & Guner, 2004). It is the fourth most important vegetable crop after tomato, cabbage, and onion in Asia (Kalloo & Bergh, 2012); the second most important vegetable crop after tomato in Western Europe (Phu, 1998); and is the fourth most cultivated vegetable in the world after tomatoes, brassicas and onions (Ene et al., 2016). Cucumber is a year-round outdoor vegetable in the tropics and an important greenhouse vegetable, in Northern Europe and North America (Adesina & Benjamin, 2016). In greenhouses, it is cultivated using artificial heating in the winter season (Phu, 1998). At present, cucumber is cultivated as a field crop in most areas of the world under cool climates and as a greenhouse crop (Mallick, 2022). Cucumber can be cultivated when the soil temperature is 60°F or higher, under extremely high temperatures, the fruits may turn green and bitter in many varieties (Brandenberger et al., 2021).

Cucumber belongs to the family Cucurbitaceae (Bist et al., 2020) purposively cultivated for its tender fruits which are eaten raw as salad or cooked as a vegetable. It is reported that the oil extracted from seeds is good for the brain and body. But consuming cucumbers may lead to digestive problems like bloating and flatulence in some people (Garg, 2022). Cucumbers, grown to be eaten fresh (called slices) and those intended for pickling (called picklers) are similar. Cucumbers are mainly eaten in the unripe green

form. The ripened yellow form normally becomes too bitter and sour. Cucumbers usually contain 90% water.

For Nepal, cucumber (*Cucumis sativus* L.) is an important summer vegetable crop commonly grown throughout the country. Cucumber ranks fifth behind cauliflower, tomato, cabbage, and pumpkin in terms of total cultivated area. In the year 2012/13, the total production of cucumber in Nepal was 127,918 tons with a productivity of 14.3 tons/ha (Khanal & Dhakal, 2020) which reached 172,566 Mt. with a productivity of 15.96 Mt./ha in 2018/19 (Bist et al., 2020). During the rainy season, the crop is grown under rain-fed conditions, and during the dry season using irrigation facilities; as a result, the crop can be seen in most vegetable markets in Nepal throughout the year. Though cucumber is one of the most potent commodities from an export point of view, the farmers are not getting reasonable prices due to the failure of the recommended varieties and lack of coordination among the commodity chain actors. Kapilvastu is one of the districts of Nepal lying in Province No. 5, covering an area of 1,738 square km (671 square meters). According to the census 2011, the total population of the area is 571,936. Most of the population of the district is dependent on agriculture. Dharampur, belonging to Buddhabhumi Municipality is one of the major vegetable-growing areas in the district which indicates agriculture, especially vegetable farming being a basis of life for most of the people of the district. Among varieties of crops, cucurbits have been able to establish themselves as the most preferred summer crop by farmers of the district. Farmers in this area import cucumber seeds from the neighboring country like India and follow the local cultivation practices. Thus, this experiment is carried out to identify the most suited varieties of cucumber in the study area by following the package of production that the local farmers have been adopting for many generations.

II. METHOD

2.1 Description of the experimental site

The research was carried out in Dharampur of Buddhabhumi municipality ward no. 10 of Kapilvastu district ranging from an altitude of 93 to 1491 meters above sea level. The geographical coordinates are 27°41' North and 83° 0.02' East (*Buddhabhumi, Kapilvastu, Province #5, 32809, Nepal*, n.d.) and are located in the tropical belt with the summer temperature above 27 °C and winter temperature remains below 15 °C and average rainfall is 1500mm. The physio-chemical condition of the soil of the study site is sandy loam as it is located by the riverside.

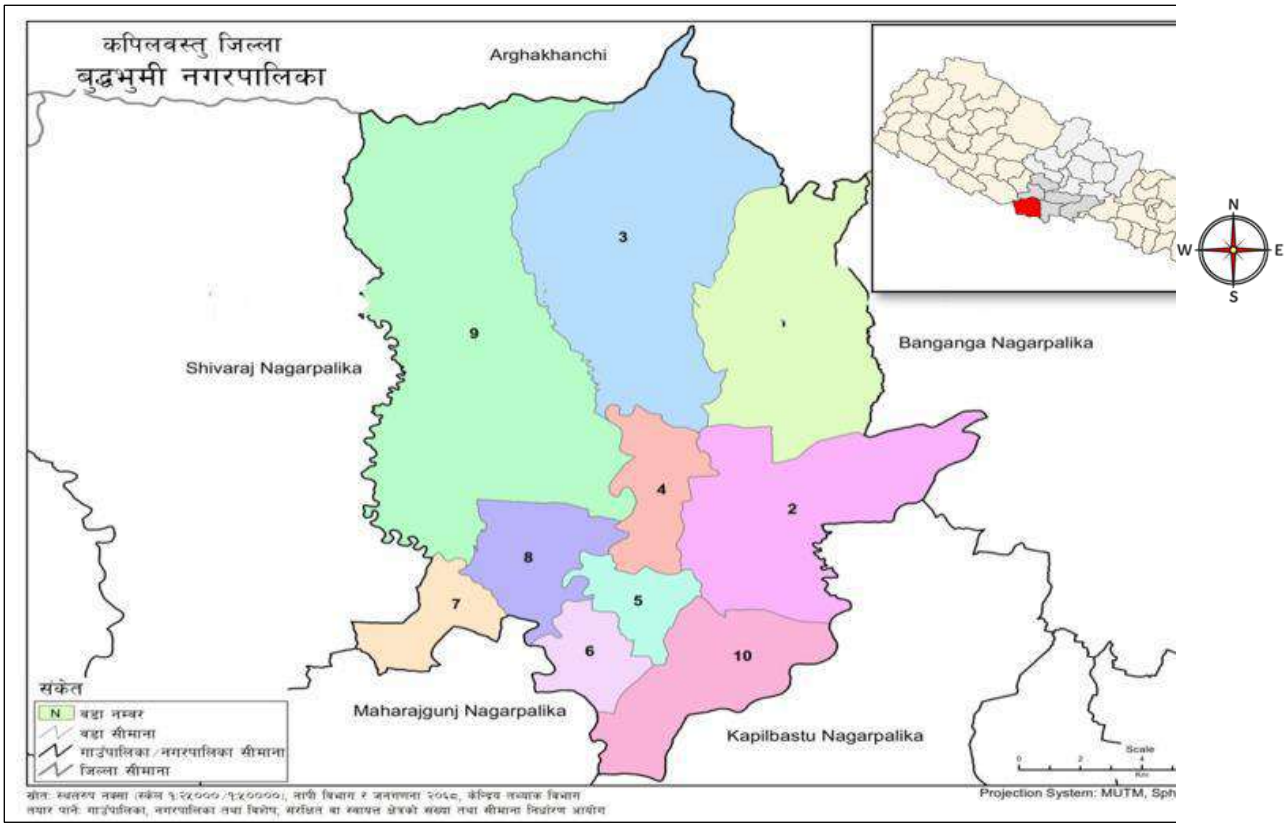

Fig.1. Map of Buddhabhumi Municipality showing wards

2.2 Experimental treatments

The farmers of the study site grow cucumber imported from neighboring countries like India. The varieties studied here are the hybrid varieties of cucumber preferred by the local farmers of the study site.

Table 1 Varieties of cucumber under study in Buddhabhumi Municipality of Kapilvastu district

Notation	Name of Variety	Country of Origin
T1	Rehmat-1080	Thailand
T2	LHC-Maria	India
T3	Dynasty	Korea
T4	Namdhari-NS-408	India
T5	LHC-1395	India

2.3 Design and layout of the experimental plot

The experiment was laid out in an area of 750m^2 with a Randomized Complete Block Design (RCBD) with five replications. The experimental plot was divided into five blocks, each block consisting of five units of plots; thus, composing a total of 25 plots. The individual net plot area was 1.4 m^2 (25*15cm^2) with a net experimental area of 35.16 m^2. Row-to-row spacing was maintained at 25cm and the space between plants was 15 cm thus, comprising 25 plants in each plot. A furrow of 10 cm space was maintained in between the plots for easy intercultural operations. The crop geometry here replicates the ones practiced by the local cucumber growers of the study site.

2.4 Field operations

2.4.1 Land preparation and fertilization

Pre-sowing irrigation and deep plowing followed by 2-3 secondary tillage using harrowing and leveling was carried out 15 days before the planting of cucumber. All the weed and plant residue was removed to make the field clean. Farm Yard Manure (FYM) was well mixed into the soil and the recommended dose of fertilizer in cucumber i.e., NPK@20:40:30 kg/ha was used. Farmyard manure along with the recommended a full dose of P2O5 and K2O, and ½ of the recommended N dose was used as basal dose. The remaining ½ N was used in two split doses of ¼ N just after the first harvest followed by second.

2.4.2 Seed sowing

Seeds were soaked in water for 20 hours and were sown directly in the main field as practiced by farmers on January 15, 2019. Seed sowing followed line sowing with two seeds sown on each hill at a distance of 15 cm from one hill to another. Similarly, the line-to-line distance was maintained at 25 cm. The sowing depth for smaller seeds was 3 cm under moist conditions, and that for larger seeds was 5 cm.

2.4.3 Intercultural operations

2.4.3.1 Weed Management and earthing up

Manual weeding was performed at 10, 20, and 30 days after seed sowing. The earthing up was practiced during the second weeding.

2.4.3.2 Irrigation and Drainage Management

Irrigation was carried out firstly after 2 days of sowing and then, in every 2 day intervals. The irrigation was scheduled according to the critical period of the crop i.e. after sowing, flowering, and fruiting as per requirement. Cucumber being susceptible to water-logging conditions was facilitated by proper drainage systems.

2.4.4 Protection of experimental site and plants

The experimental site was protected with fencing done by iron wire and cemented poles. Similarly, insect pests encountered were controlled by using cattle urine and water solution (1:3-4). Fruit fly was controlled using a pheromone trap and thrips with the yellow sticky trap.

2.4.5 Harvesting

Harvesting time is very crucial for a crop. Earlier harvest as well as delay in harvest may cause yield loss. Thus, harvesting at the physiological maturity stage is very important. Cucumber was harvested multiple times whenever fruits attained a handful size.

2.4.6 Collection of Experimental Data

The morphological and reproductive behaviors of the plants were recorded in every 7 days interval. 5 plants were selected from each plot as sample plants to record for different observations. The height of the main stem (cm), number of primary branches per vine, number of leaves, days to flower initiation, and days to fruit initiation were observed; and the average was calculated. Height was measured by using measuring tape. Yield-related data collection included the number of fruits per plant, average fruit weight per plant, and total yield of fruits.

2.4.7 Statistical analysis

The data was carefully refined and then entered into an MS Excel sheet. The data was analyzed to draw meaningful interferences by using Minitab version 19, and MS Excel 2016 software. Analysis for the variance for all parameters was done by using the Minitab statistical analysis system.

III. RESULTS

3.1 Morphological observations

3.1.1 Germination percentage

Germination percentage was 97% in all five varieties of cucumber. Germination of the plant was recorded by visual analysis.

3.1.2 Plant height/Vine length

The average increase in plant height in a one-week interval of the five varieties demonstrated significant differences ($P \leq 0.05$). The plant vines were found to increase by 18.42 cm on average to 23.9 cm. Among the five varieties, the tallest variety with the highest increase in vine length in 7-day intervals was found to be Dynasty. The average increase in the vine length of this variety was recorded to be 23.9 cm per week. Meanwhile, the shortest variety with the least increase in vine length in one-week intervals was observed to be LHC-Maria. This variety was recorded to grow an average of 18.42cm. The mean increase in plant height of the Dynasty variety was significantly higher than that of varieties NS-408, Rehmat-1080, and LHC-1395 respectively (Figure 2). The result suggests that NS-408 followed the variety Dynasty with an average increase of plant height by 22.76 cm. Similarly, the varieties LHC-1395, LHC-Maria, and Rehmat-1080 were insignificant concerning the increase in plant height with an average increment of 19.17cm, 18.42cm, and 17.82cm respectively.

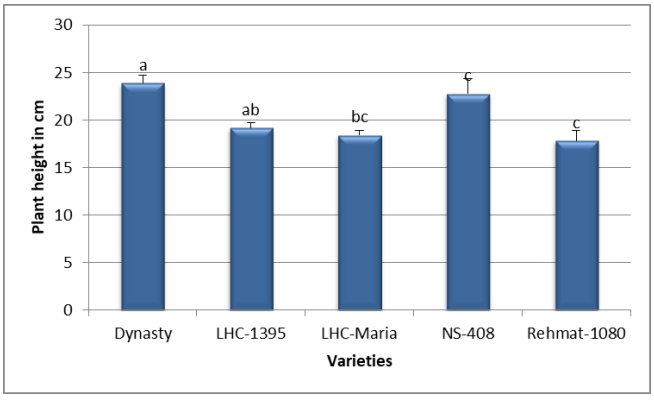

Fig.2 Average increases in plant height per week in different cucumber varieties

3.1.3 Number of leaves

The average increase in plant height in a one-week interval of the five varieties demonstrated significant differences ($P \leq 0.05$). The plant vines were found to increase by 18.42 cm on average to 23.9 cm. Among the five varieties, the tallest variety with the highest increase in vine length in 7-day intervals was found to be Dynasty. The average increase in the vine length of this variety was recorded to be 23.9 cm per week. Meanwhile, the shortest variety with the least increase in vine length in one-week intervals was observed to be LHC-Maria. This variety was recorded to grow an average of 18.42cm. The mean increase in plant height of the Dynasty variety was significantly higher than that of varieties NS-408, Rehmat-1080, and LHC-1395 respectively (Figure 2). The result suggests that NS-408 followed the variety Dynasty with an average increase of plant height by 22.76 cm. Similarly, the varieties LHC-1395, LHC-Maria, and Rehmat-1080 were insignificant concerning the increase in plant height with an average increment of 19.17cm, 18.42cm, and 17.82cm respectively.

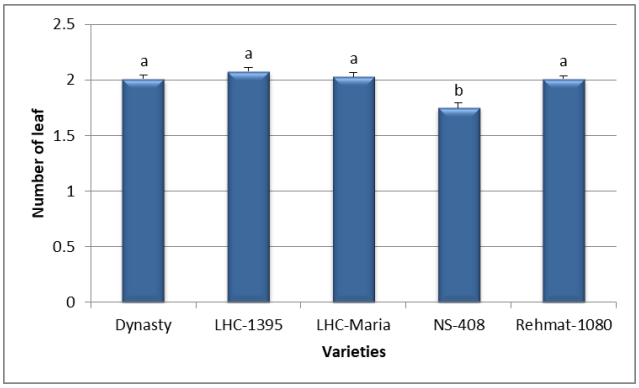

Fig.3 Average increases in leaf number per plant in one-week interval in different cucumber varieties

3.1.4 Number of branches

The increment in branch number of the varieties ranged from 2.72 to 3.2. The statistical analysis revealed that there was no significant difference among the varieties for an increase in the number of branches per plant. All five varieties of cucumber formed almost the same number of branches in the one-week interval. However, the highest number of branches was observed to form in NS-408 i.e., 3.2 whereas the least number of branches was observed in Dynasty and LHC-Maria i.e., 2.72 branches per week (Figure 4). LHC1395 was found to develop 3 branches per week, and Rehmat-1080 developed 2.76 on average. This indicates that NS-408 had vigorous growth in terms of branch number compared to other varieties.

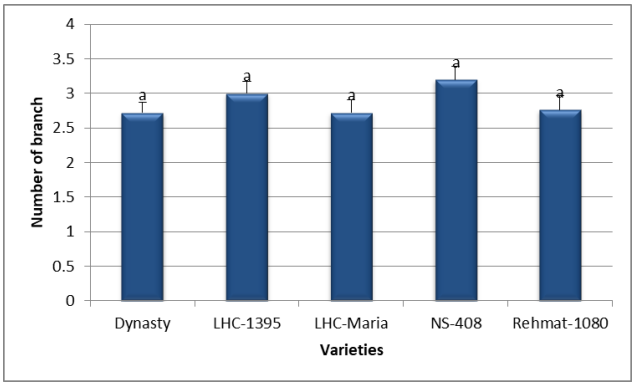

Fig.4 Average increases in branch number per plant in one-week interval in different cucumber varieties

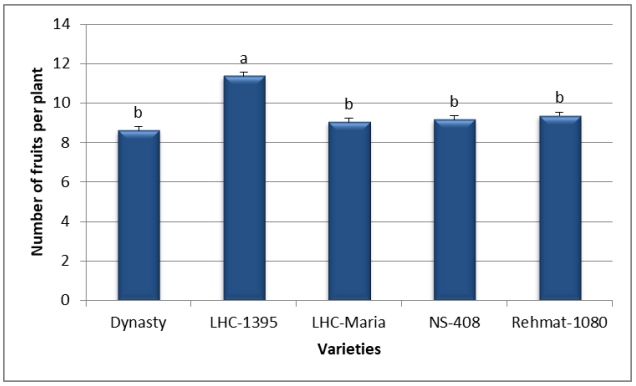

Fig.5 Average numbers of fruits per plant in different cucumber varieties

3.1.5 Days of Flower Initiation

The study showed no significant difference for the days to flower initiation. All five varieties; Dynasty, LHC-1395, LHC-Maria, NS-408, and Rehmat-1080 started flowering at an average of 30 days after planting.

3.1.6 Days of Fruit Development

The five varieties were investigated for the days to fruit development. No significant difference was observed among the varieties LHC-1395, LHC-Maria, Dynasty, NS-408, and Rehmat-1080. All of them were found to start fruit development in an average of 30 days after flower initiation.

3.2 Yield and yield attributes

3.2.1 Number of fruit per plant

The average number of fruits per plant of the varieties ranged from 8.6 to 11.36. Analysis of variance (ANOVA) revealed that there existed significant differences ($P \leq 0.05$) among the varieties for the number of fruits per plant. The highest number of fruits was observed in LHC-1395 with 11.36 per plant. In contrast, the least number of fruits were developed in Dynasty with 8.6 fruits per plant on average. The average number of fruits developed by LHC-1395 is significantly higher than those of LHC-Maria, NS-408, and Rehmat-1080 with fruit yield per plant of 9.04, 9.16, and 9.36 respectively. However, varieties Dynasty, LHC-Maria, NS-408, and Rehmat-1080 did not show much difference among each other. The fruit yield was somewhat equal in them as shown in Figure 5.

3.2.2 Individual fruit weight

The varieties were investigated for individual fruit weight. The Analysis of variance revealed highly significant differences in the individual fruit weight among the five varieties as shown in Figure 6. The highest weight of individual fruit was observed in LHC-Maria with an average fruit weight of 208.44 grams. In contrast, the lowest individual fruit weight was recorded in Rehmat-1080 with an average fruit weight of 133.3 grams. The individual fruit weight of LHC-Maria was significantly higher than that of Rehmat-1080, NS-408, LHC-1395, and Dynasty. The average individual fruit weight of Dynasty was 197.73 grams, LHC-1395 was 190.06 grams, and NS-408 was 174.87 grams. For this trait, the results for LHC-1395 are similar to that of (Choubey et al., 2023). The results indicated that LHC-Maria is a potential variety for higher yield among the five varieties under the study.

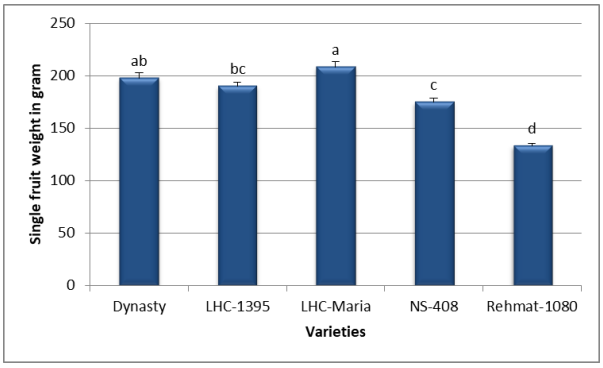

Fig.6 Average individual fruit weight of different cucumber varieties

3.2.3 Total fruit yield per plant

The average fruit yield in the five varieties ranged from 1239.7 grams to 2137.9 grams. The statistical analysis revealed that there was a significant difference among the varieties for the total fruit yield per plant. Among the five

varieties, the highest yield was recorded in LHC-1395 with 2137.9 grams per plant whereas, the least yield was observed in Rehmat-1080 with 1239.7 grams per plant. The LHC-1395 yielded significantly higher in comparison to other varieties as shown in Figure 7. The yield of the LHC-1395 was followed by LHC-Maria with an average total yield of 1859.8 grams. This yield is more than that of Dynasty which yielded 1674.5 grams, and NS-408 which yielded 1580.9 grams. The yield for Rehmat-1080 is unsatisfactory as compared to the 2-3 kg average fruit weight per plant mentioned by (*Rehmat 1080*, n.d.). The results suggested that LHC-1395 is a high-yielding variety of cucumber with the highest total fruit yield per plant among the five varieties under the study. (Choubey et al., 2023) also mentioned LHC-1395 as a high-yielding hybrid variety of cucumber in their study.

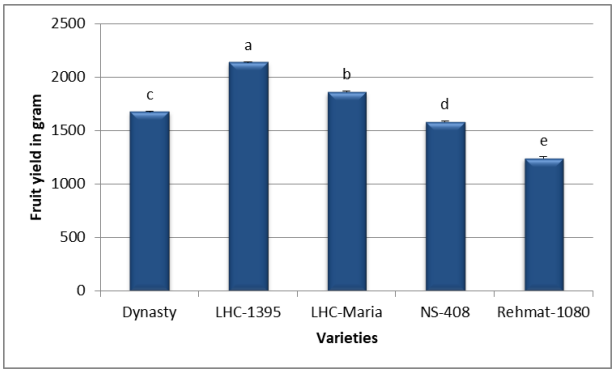

Fig.7 Yield per plant of different cucumber varieties

IV. CONCLUSION

Varietal screening is a foremost step during the cultivation of crops in a specific area as it directly affects the yield. The fact cannot be ignored that the farmers favor the cultural practices that they have been practicing for many generations. Thus, this study has been focused on selecting the best exotic variety for the study site using the same package of production that the local farmers have been using. During the study, among the five varieties selected, none were seen as equal in results in the context of the yield. The variety LHC-1395 yielded highest, followed by LHC-Maria, Dynasty, NS-408, and Rehmat-1080 respectively, and thus, LHC1395 is the best-suited variety for the area, followed by LHC-Maria. The overall performance of LHC-1395 is good compared to other varieties in terms of both vegetative and reproductive attributes. This variety is found to be competitive with other varieties in terms of the number of leaf and branch development per week. Moreover, it exhibits a good capacity for fruiting as the number of fruits per plant was found highest; also, the individual fruit weight was higher in comparison to other varieties i.e., NS-408, and Rehmat-1080. Thus, it can be concluded from the investigation that LHC-1395 is a superior variety for the study area under the local cultivation practices.

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Appendix 1: Change in different morphological and yield attributes

Varieties	Average plant height (cm)	Number of leaves	Number of branches	Number of fruit	Average fruit weight (kg)	Total yield (kg)
Dynasty	23.900±0.823a	2.0100±0.0306a	2.720± 0.147a	8.600± 0.208b	197.73 ±5.28ab	1674.5 ±5.72c
LHC-1395	19.165±0.576ab	2.0800±0.0345a	3.000± 0.173a	11.360 ±0.230a	190.06 ±3.88bc	2137.9 ±6.10a
LHC-Maria	18.420±0.479bc	2.0300±0.0363a	2.720± 0.187a	9.040 ±0.212b	208.44 ±5.09a	1859.8 ±10.5b
NS-408	22.76±1.58c	1.7500 ±0.0408b	3.200±0.191a	9.160 ±0.221b	174.87 ±4.03c	1580.9± 8.07d
Rehmat-1080	17.82±1.09c	2.0100±0.0227a	2.760 ±0.202a	9.360 ±0.181b	133.30 ±2.26d	1239.7 ±13.8e

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Nutritional Potential of Two Lactogenic Plants after Cooking in the Prevention of Hypogalactia: the Case of *Euphorbia hirta L.* and *Secamone afzelii (Shult) K. Shum*.

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Abstract— *To combat hypogalactia, breastfeeding women in rural areas use certain plants in their cooking to induce lactation. However, the nutritional properties of these local products after cooking are not well known. Thus, the aim of this study is to evaluate the nutritional potential of the leafy stems of two plants such as Euphorbia hirta and Secamone afzelii used in Côte d'Ivoire after cooking. The results obtained indicate the presence of proteins (7.55±0.02% ; 8.486±0.04%), total sugars (16.77±0.13 ; 17.93±0.09), reducing sugars (0.619±0.02% ; 0.872±0.03%) and mineral elements such as calcium (1.12±0.08% ; 1.20±0.10%), iron (0.20±0.03%; 0.32±0.02%) and magnesium (0.15±0.01%; 0.54±0.04%) in aqueous extracts from Euphorbia hirta and Secamone afzelii plants after cooking. These results could justify the culinary use of these food plants, which are potential sources of essential nutrients for improving the quality and quantity of breast milk for young children, through the treatment of breastfeeding disorders in women in general. Both plants deserve be valorized. However, further studies on the toxicity of the Secamone afzelii plant are needed.*

Keywords— ***Euphorbia hirta, Secamone afzelii, nutritional potential, nutrients*.**

I. INTRODUCTION

Since prehistoric times, man has always found natural ways to treat himself. The pathologies for which natural means are sought include public health diseases such as malaria, anemia, hypogalactia and malnutrition in nursing mothers. Among these methods, the use of plants plays a major role. In Africa, a great deal of research has been carried out on galactogenic plants, proving their importance for rural populations in particular. The results of work by **Bourobou-Bourobou *et al* (1996)** in Gabon, **Betti and Van Esche (1998)** and **Betti (2002)** in Cameroon, for example, demonstrate the importance of galactogenic plants for populations in rural and urban areas, where breast-feeding should be the main mode of infant nutrition. In rural areas of most developing countries, malnutrition remains one of the major nutritional problems for infants **(Jelliffe, 1989)**, due to the inadequacy of breast milk. In rural areas, indigenous populations often use treatments based on plant extracts to stimulate or induce milk secretion in some women who are unable to breastfeed their offspring after childbirth. The reasons given for this inability to breastfeed seem to be essentially linked to a partial or total absence of milk production in all failing mothers. And yet, as we all know, breast milk plays a vital role not only in the physiology (nutrition and growth) of newborn babies, but also in protecting them against certain illnesses such as diarrhoea

and other deficiencies that can affect them during the first months of life. The lack of this noble foodstuff among breastfeeding women is therefore a very serious threat to the health of infants. One of the main causes of insufficient milk production is severe maternal malnutrition **(Butte and Stuebe, 2020)**. In a bid to provide answers to this unfortunate situation, several authors have carried out scientific studies on African plants reputed to stimulate lactation **(Sawadogo, 1987)**. These have demonstrated that extracts of some of these plants, when administered orally, are indeed capable of inducing milk synthesis in test animals **(Sawadogo, 1987)**.

In Côte d'Ivoire's southern zone, ethnobotanical surveys of lactogenic plants have been carried out. As well as in Benin, a country in the sub-region **(Salifou *et al.*, 2017).**

The survey study in the aforementioned country enabled an inventory to be made of the plants used by rural populations to provide solutions to the breastfeeding problems of Beninese infants but no similar work has been carried out on the knowledge of lactogenic plants used in the joint fight in southern Côte d'Ivoire.

According to research findings, extracts of the plants *Euphorbia hirta* L and *Secamone afzelii* administered orally have been reputed to produce breast milk from traditional recipes, hence the need to conduct a nutritional analysis of *Euphorbia hirta* L and *Secamone afzelii* plants to help assess their nutritional value, the impact of which could influence the quality and quantity of milk produced in women's udders during lactation.

The aim of this work is to carry out a quantitative analysis of the macronutrients (proteins, total sugars and reducing sugars) and micronutrients (calcium, iron and magnesium) in the extracts obtained from the two wild plants, with a view to assessing their nutritional potential in combating breastfeeding disorders in nursing mothers.

II. MATERIAL AND METHODS

MATERIAL

Plant material

The plant material, identified at the Floristic National Centre of Félix Houphouët-Boigny Université of Abidjan, consisted of the plants *Euphorbia hirta* L. (Euphorbiaceae) with number P 00575846 according to the Paris national herbarium and *Sécamone afzelii* (Schult) K. Schum (Asclepiadaceae) with specimen number 120801 (Gabon herbarium).

METHODS

Site selection and data collection

Two localities were chosen; Akoupé on the one hand, because it's in the interior of Côte d'Ivoire. On the other hand, Abidjan, the economic capital and major metropolis, is a cosmopolitan city where many cultures meet. The preliminary surveys carried out enabled us to list the recipes indicated by the female population of these localities with regard to breastfeeding problems. This choice made it possible to study the recipes of these populations in their cultural diversity with regard to breastfeeding problems. Thus, the choice of the two towns enabled us to assess the place occupied by recipes in the dietary habits of the populations of the chosen localities.

Data collection was carried out in southern Côte d'Ivoire. These data are: locality, plant species, harvest, lactogenic plants, endogenous recipes for stimulating lactation for milk production, plants used for recipes, whole plant part, and harvest period.

A sample of 100 women was surveyed, 50 from the town of Akoupé and 50 from the town of Abidjan, during the short dry season from August to September, after the long rainy season from April to July **(SODEXAM/DMN,2008)**.

The plants were harvested during this period for two reasons: firstly, the period indicated will enable us to obtain an undoubtedly high nutrient content, and secondly, easy access to the plants after the renewal cycle in the main rainy season. The people surveyed (n = 100) are in the 25-55 age bracket, the age of the working population.

Sampling

The plant species studied were harvested in the two localities mentioned above (Abidjan and Akoupé). During each month of the short dry season, 3 batches of plants were harvested. Each harvested batch was split into 3 samples of identical mass. A single sample was selected for experimentation. A sample dried in a dry place was placed in a MEMMERT 845 type B 40 oven at 60°C, for quantitative mineral analysis and partially purified extraction for nutrient quality analysis. With two plants, two months and two locations selected, the number of samples is 24.

Preparation of plant extracts for analysis.

Selected plants were harvested, dried and stored in a dry place until use. Extracts were prepared according to the protocol described by **Sawadogo *et al* in 1988**.

Whole plants were ground using a ball mill. One hundred (100) g of the powder obtained was placed in water (5 mL per gram of plant powder). The mixture was boiled for 15 minutes. The mixture was centrifuged at 3000 rpm for 15 minutes.

Once the pellet had been removed, partial purification was carried out using the resulting supernatant. The crude

extract obtained after centrifugation was stirred in the presence of the same volume of chloroform for 5 minutes. The mixture was centrifuged under the same conditions as before, and the supernatant aqueous phase obtained was taken up in 2 volumes of ethanol, followed by the addition of Nacl (0.1). The mixture was stirred and centrifuged again. The resulting pellet was recovered and redissolved in a small volume of water, then freeze-dried to form the partially purified extract for nutritional analysis.

The nutrient analysis of said extract concerns nutrients such as proteins and sugars, with the exception of lipids, as the search for the nutrients mentioned should constitute the nutritional contribution listed as a cofactor **(Houdebine *et al.*, 1990)** to reinforce milk stimulation does not contain lipids. What's more, when a plant product is to be subjected to a partially purified extraction, it is de-oiled beforehand, since fat reduces polysaccharide-water solubility during extraction **(Boni *et al.*, 1990)**. The presence of lipids increases the molecular weight of polysaccharides and blocks the binding of water molecules, preventing swelling of polysaccharide grains and diffusion of polysaccharides out of them.

Determination of protein content

Protein determination **(B.I.P.E.A, 1976)** was carried out using the kjeldhal method and according to the standard **(A.O.A.C, 1975)** using a TECATOR DIGESTOR /Spain mineralizer and a FOSS TECATOR KJELTEC SYSTEM 1002 /Sweden distiller.

Determination of soluble sugar content

Determination of soluble sugar content was carried out using the phenol-sulfuric method, with extraction, defecation and sugar assay stages. Determination was carried out using the **Dubois *et al*. method (1956)**.

Determination of mineral content

Dry mineralization of plants

The mineralization process used is that described by **Biégo *et al.* (2004)**. The dosing method used is Spectr AA-5 flame atomic absorption spectrophotometry of the Varian type, based on the principle that the solution to be analyzed is drawn through a capillary into the nebulizer.

Statistical analysis

The experiments carried out enabled us to collect quantitative data. These data concern proteins, sugars and minerals. An analysis of variance with two classification criteria was used to compare the mean macronutrient and mineral contents of two plants from two harvesting sites. A NEWMAN KEULS test at the 5% threshold was performed to assess the significant difference between the means of macronutrient content in aqueous extracts and mineral content in dried plants, using Statisticat version 7.1 software. For all tables, the means ± standard deviation assigned to various alphabetical letters are significantly different according to the NEWMAN- KEULS test at p less than 5%. Values are means ± standard deviation of 12 trials.

III. RESULTS AND DISCUSSION

Table 1: Yields of partially purified extracts from the Abidjan and Akoupé sites, by plant species.

LOCALITIES	**ABIDJAN**		**AKOUPE**	
Dry plant / Fraction	***Euphorbia hirta***	***Secamone afzelii***	***Euphorbia hirta***	***Secamone afzelii***
Partially purified fraction (%)	1.81±0.09**a**	1.41±0.10**a**	1.79±0.08**a**	1.71±0.07**a**

Table 2: Content of biochemical parameters in extracts

LOCALITIES	**ABIDJAN**		**AKOUPE**	
Extracts / Nutrients	***Euphorbia hirta***	***Secamone afzelii***	***Euphorbia hirta***	***Secamone afzelii***
Total sugars	17.33±0.10**a**	17.93±0.09**a**	17.43±0.12**a**	16.77±0.13**a**
Reducing sugars	0.872±0.03**a**	0.861±0.01**a**	0.619±0.02**a**	0.623±0.04**a**
Proteins	7.74±0.06**a**	8.27±0.07**a**	7.55±0.02**a**	8.486±0.04**a**

Table 3: Mineral composition (mg/l) of plant species harvested at various sites

LOCALITIES	ABIDJAN		AKOUPE	
Plants / Minerals	***Euphorbia hirta***	***Secamone afzelii***	***Euphorbia hirta***	***Secamone afzelii***
Calcium	1.20±0.10**b**	1.12±0.08**b**	1.19±0.07**b**	1.15±0.08**b**
Iron	0.24±0.01**a**	0.20±0.03**a**	0.32±0.02**a**	0.26±0.05**a**
Magnesium	0.35±0.02**a**	0.15±0.01**a**	0.27±0.03**a**	0.54±0.04**a**

According to the recipes, the organs used in Abidjan and Akoupé localities are, on the whole, leafy stems, the decoction of which is said to promote the rise of breast milk in the udder **(Adepo, 2013)**. The decoction preparation method is identical for the *Euphorbia hirta* and *Secamone afzelii* plants. Yields of extracts collected were generally similar, irrespective of harvesting site and plant type (Table 1).

The contents of reducing sugars, total sugars and proteins were equal from one plant to another, whatever the collection site (Table 2). The levels of reducing sugars, total sugars and proteins reported for the *Euphorbia hirta* plant are in line with the results of the qualitative test by **Lanheurs, (2005)**.

The presence of reducing sugars in the *Secamone afzelii* plant is confirmed by the results of the author's qualitative test **Kemeuzé, (2010)**. Also, the experimental protein, iron and magnesium contents of the *Secamone afzelii* plant are lower than those of **Rita *et al*, (2014)** (*Secamone afzelii*; Iron 0.01%, magnesium 12.03%, protein 3.94%). The mean contents of minerals such as calcium, magnesium and iron in plants harvested in the Abidjan and Akoupé regions also show no significant differences (Table 3). This concordance of results could be explained by the proximity of the harvesting areas of the plants listed, as the two regions cited are geographically in the same locality in southern Côte d'Ivoire.

The high levels of calcium in both *Euphorbia hirta* (1.20%) and *Secamone afzelii* (1.15%) compared to magnesium and iron would be justified by the potential use of calcium for plant growth. In addition, the availability of mineral elements according to soil type could justify the variability of mineral contents in the plants studied.

Experimental mineral analysis shows conformity with the qualitative test results of **Lanheurs, 2005**. However, the experimental results for minerals in *Euphorbia hirta* (calcium 1.79%, iron 0.4% and magnesium 0.485%) are generally lower than those of **Tonganga 2014** (calcium 1.04%, magnesium 2.18% and iron 4.19%). This difference in results may be explained by the fact that leaves are the appropriate site for several metabolic reactions in the synthesis of organic compounds involving the use of a high concentration of minerals. Unlike the mineral content of *Euphorbia hirta* plants, for which data are available, there are virtually no studies on the mineral content of the *Secamone afzelii* plant studied.

In general, minerals are said to be the basis for stimulating the action of enzymes involved in the phenomenon of lactation secretion, as minerals constitute the enzymes' metal cofactors. Moreover, according to the experimental study by author **Ndiaye 2018**, calcium phosphate was used.

The *Euphorbia hirta* plant is used as a dietary supplement and lactogen **(Lanhers *et al*., 2005)**. According to the author **Tonganga 2014**, the *Euphorbia hirta* plant would be considered a wild plant but also a food plant due to its moderate levels of anti-nutritional factors such as cyanide, which is found in moderate quantities, nitrate, recorded in trace form, and the absence of oxalate. In addition, the *Secamone afzelii* plant is also considered a food supplement **(Vidal, 2003; Rita *et al*., 2014)**.

A study of the ways in which different plants are used by different populations reveals that these plants could also be used as alicaments. Indeed, many recipes involve incorporating the plant parts studied into everyday dishes.

In view of all the above, the search for minerals in the plants analysed has enabled us to verify that their use could be justified for the treatment of breastfeeding disorders, as they can undoubtedly constitute an important mineral and nutritional source for the lactation stimulation essential for breastfeeding women.

IV. CONLUSION

The results obtained indicate the simultaneous presence of minerals such as calcium, iron and magnesium and nutrients such as reducing and total sugars in the two plants studied. The existence of nutrients that can act in concert with the active ingredient to improve milk production is also noteworthy. Oral administration of the recipes involving the plants mentioned would contribute to the synthesis of breast milk in general, and of antibodies

specifically, by providing some of the macronutrients and micronutrients needed for milk secretion. In fact, these two plants containing proteins, total sugars, reducing sugars and mineral elements such as iron, calcium and magnesium could improve the quantity and quality of breast milk for the infant with the nutritional contribution relative to the nutritional value in plants. As a result, the health of the child and the nursing mother could be significantly improved.

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Yield Modeling of Okra (*Abelmoschus esculentus L. moench*) in Bituminous Soils of Southern Ondo State, Nigeria

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Abstract— *This study investigated the growth and yield response of okra (Abelmoschus esculentus L. Moench) in bituminous soils of selected communities (Loda, Lofo and Legbogbo) in Irele, southern Ondo State for two consecutive growing seasons (2020 and 2021). The Legbogbo site, which of very low bitumen, serves as the control. The textural class is sandy loam and the soil is acidic. Some samples of soil were collected from three dug soil profile pit at depths 0-100 cm at an interval of 10 cm and moved to the soil laboratory for the analysis of the chemical concentrations of macro and trace elements. The highest value of the heavy metal was recorded at Loda. More so, the least amount of the bitumen concentration was recorded at Legbogbo site (control) and in some cases at Lofo site. Result from the study showed that there is significant difference ($P < 0.05$) in the values of the heavy metals among the location at all depths. The results from various locations also showed that the soil depth and location were significant ($P < 0.05$) in their main effects on the soil heavy metals. It was observed that the heavy metal concentration of soil was lower than the permissible limit, an indication that the concentration of heavy metals of the study areas may not have negatively influenced the growth and yield of okra. All agronomic parameters in all locations during the two seasons of experiment were in the way of Legbogbo>Lofo>Loda. Okra had 0 kg.ha^{-1} pod yield at the Loda site at both 2020 and 2021 growing seasons, while Legbogbo had the greatest fruit yield for both seasons. Okra yield prediction model was developed using the stepwise regression model and it was observed that Okra yield significantly correlated to potassium ($r^2 = 0.95$) at $P < 0.05$ and phosphorus ($r^2 = 0.99$) ($P < 0.01$).*

Keyword— ***Bitumen deposit, bituminous soil, Heavy metal, Okra, Yield***

I. INTRODUCTION

Bitumen is a generic term used to cover a wide range of high molecular weight hydrocarbons (Ojovan and Lee, 2014), it can act as a hydrophobic water shield. Three main types of hydrocarbons occur in bituminous materials and they are asphaltenes, resins and oils (alphatic hydrocarbons), (Ojovan and Lee, 2005). One of the properties of bitumen is that, it is visco-elastic. Bitumen is primarily composed of polycyclic aromatic hydrocarbons (New World encyclopedia, 2016). Examples of composition of Bitumen in terms of minerals are nickel, vanadium, lead, chromium, mercury etc.

Bitumen can be described as thick, sticky, tar-like form of petroleum which cannot flow unless it is heated or diluted. At room temperature, bitumen is looks like cold molasses (Government of Alberta, 2008). Bitumen can be described as any hydrocarbon deposit that possess a gas- free viscosity above 10,000 centipoises (cp) measured to original

reservoir condition. Bitumen is around 95% hydrogen, and up to 5% Sulphur, 1% oxygen and 200ppm metals (Tom, 2009). Bitumen possesses some properties like, waterproof, adhesive, durability and resistance to heavy loads which make it an ideal material that can be used in all environments (Muritala and Adewole, 2016). Nigeria has about the third largest deposits of bitumen all over the world. This bitumen deposits in Nigeria is mainly located within the southern axis of Ondo State, Nigeria, where inhabitants are predominantly farmers. Surface and sub-surface intrusions of this very important economic resource has made it almost impossible for inhabitants to freely practice their farming operations due to low productivity and/or poor yield.

The heavy metals that infiltrate or through seepage enters the ecosystem may lead to varieties of accumulation in the soil and plant thereby creating negative influence on plant growth. The accumulated toxic heavy metals in soils and plants brings negative influence on the activities of plants such as photosynthesis, gaseous exchange and nutrient absorption in the plants and these helps in determining the reductions in plant growth, accumulated dry matter and crop yield (Suciu *et al*., 2008). Bitumen deposits normally have negative effects on water and soil used for agricultural practices especially in areas that are covered with bitumen deposits, and the deposits will have adverse effect on agricultural soils in such areas. Bitumen deposits are generally harmful to the soil due to its toxicity (Agarry and Oghenejoboh, 2014). There is always occurrence of environmental effects that can bring threat to any area where there are bitumen deposits (Akinmosin *et al*., 2009).

Atojunere and Ogedengbe (2019) reported that lead was found in leaf vegetables and some fruits samples collected from some markets in Lagos, Nigeria. Heavy metal gets into plants through adsorption which refers to binding of materials onto the surface or absorption which implies penetration of metals into the inner matrix where both adsorption and absorption can also take place (Lokeshwary and Chandrappa, 2006). Bitumen deposit in small concentration in a soil cannot confirm the toxicity of the heavy metals in the plants or animals but there are some heavy metals like lead, cadmium and mercury which are very toxic even when their concentration is low (De Vries *et al*., 2007).

Elements which are of different forms and composition can be deposited in the soil in different proportions. Akintola *et al.* (2011) reported that the heavy metals in the soil are associated with biological and chemical properties and the influence by anthropogenic activities (Ubah *et al*., 2009). The degree of pollution or contamination in the soil can be attributed to the effect of the heavy metals embedded in bitumen deposit soil, the environmental impact and their origin (Ramirez et al., 2005, Akintola et al., 2011). Large numbers of toxic heavy metals which are dangerous and have certain effect on man and his environment are released into the environment through various means (Dembitsky, 2003).

Okra (*Abelmoschus esculentus* L. *moench*) or Lady's finger is one of the important vegetables grown throughout the tropics and subtropics. Okra is one of those crops that is largely produced and consumed in the study area due to its nutritional and health benefits (Habtamu *et al*., 2014). Okra when consumed is very rich in vitamins, folic acid, carbohydrates, phosphorus, magnesium, calcium, potassium and other minerals. Despite this importance, okra production is either rendered impossible or marginally possible due to various reasons, which until this present research cannot be confirmed. This research therefore was aimed at investigating the yield response of Okra to soils of different communities around bituminous areas of southern Ondo State, Nigeria.

II. METHODOLOGY

1.1 Site Description

The research was conducted during the cropping seasons of 2020 and 2021 in three different communities (Loda, Lofo and Legbogbo), all located within the bituminous areas of Southern Ondo State, Nigeria. The site was located within latitudes $6^0 16^1$N to $6^0 47^1$ N and longitudes $4^0 45^1$ E to $5^0 10^1$ E, and at elevation of about 405 m above the mean sea level. The climate of the study site is humid subtropical, with an annual rainfall of about 1800 mm and the mean temperature is 25°C (Imoukhuede *et al.*, 2023). The economic mainstay of the inhabitants of the three communities is farming and fishing, but the deposits of bitumen (an important economic resource) that was explored but not exploited has turned a huge disadvantage to farming and fishing operations of the people living in the area. More often, there are free surface flow of molten bitumen on soils, which eventually flow further to nearby streams and rivers and the impact of the adverse environmental impact of it had brought untold hardship on the human, plant, animals, water and soil of communities with overlay and underlay of bitumen in Irele, Ondo State, Nigeria (Ogedengbe and Akinbile, 2009; Fagbote and Olanipelun, 2010; Imoukhuede *et al.*, 2023).

2.2 Treatments and Experimental Design

The experimental site consisted of three locations. The first experimental site was at Loda, the second site was at Lofo and the third site, which served as the control experiment was located at Legbogbo, a non-bituminous soil, all in Irele local government area of Ondo State, Nigeria. Land area of about 15 m x 15 m was cleared manually in each of the three

selected communities for the experiment. The cleared land were thereafter ploughed using Tractor (Marsey Fegusson) (3.82 tons) attached with a disc plough implement. The ploughed plots were harrowed to further pulverize the soil and loosen all clods before the formation of ridges using local technique that is widely adopted in the three selected communities. Okra seeds were planted in 15 rows of local ridges with ten stands per row (replicates) following a split plot design and at a spacing of 0.5 m by 0.5 m to make a total of 150 plants per experimental sites. The total number of okra stands was 450 in the three different sites (Legbogbo, Loda, and Lofo). This experiment was replicated the second year (2021) to give a clear confirmation how the severity/concentration of the concentration of heavy metal in a bituminous soil can affect crop yield in the study areas.

2.3 Statistical Analysis

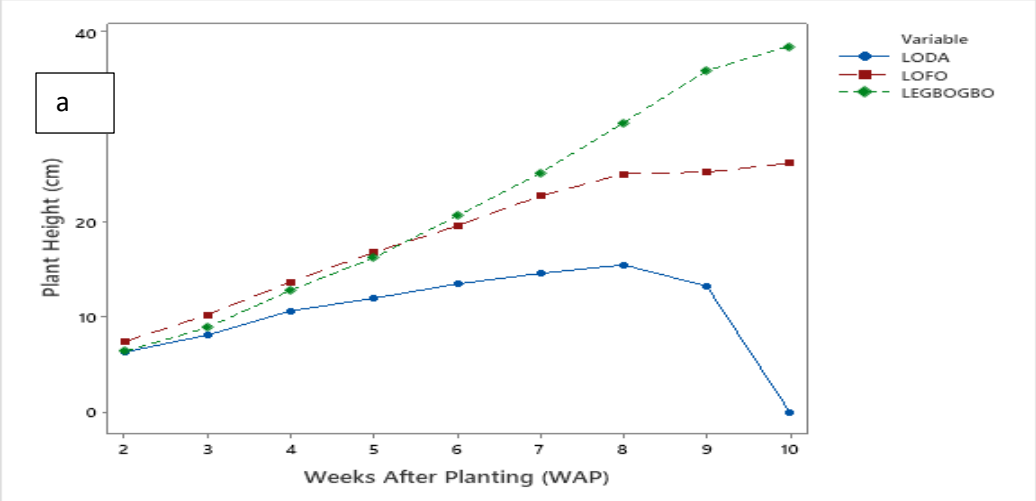
Yield components of okra were subjected to statistical analysis such as mean and standard deviation. Analysis of variance (ANOVA) was conducted to determine the difference in means of the yield parameters at the 5% and 1% level of significance. Linear and non-linear regression analysis was used to develop yield models for okra based on soil and crop data obtained from the field. Models predicting the okra yield were developed using the four important soil factor: organic matter content (OMC), percentage nitrogen (N), moisture content (MC), and three important crop factors: fresh pod yield (FPY), pod length (PL) and biomass yield (BY). Minitab uses normalization to enable data to conform more to ideal random or Gaussian distribution.

III. RESULTS AND DISCUSSION

3.1 Okra Growth Parameters on function of Weeks After Planting (WAP)

The mean of the plant height, number of leaves and stem girth from all the treatment (locations) taken up to 10 weeks after planting (WAP) are presented in Figures 1 - 2. Mean okra height was highest (38.53 ± 11.14)) at Legbogbo and least (0.00 ± 0.00)) in okra planted at Loda. The relationship between the mean heights with respect to days after planting among the locations showed that there were no rapid increase in the mean height of the leaf during the emergence and there was rapid increase at about 3 WAP. At 7 Weeks After Panting and beyond, the plant height reduced sharply in okra planted at LODA until it reached the zero level. However, the height of the crop, number of leaves and stem girth in Legbogbo site increased steadily till the crop reached maturity, before it started to decline.

There was pronounced variability in the plant heights among the locations. At 2 WAP, significant difference ($P < 0.05$) in the average plant heights was recorded among the locations. There was also significant difference ($P < 0.05$) in plant heights at 6 and 10 WAP. The number of leaves increased up to 8 WAP before it started experiencing decline in the number of count. Similar observation was recorded in okra planted during the second season. The decrease in the number of leaves may be due to the dropping of the plant leaves to the ground, which characterized the late season (Allen *et al.*, 1998). Similar trend was observed for the stem girth at both growing seasons. The increasing trend for the agronomic measurement in all locations and seasons were in the trend of LEGBOGBO > LOFO > LODA in most cases with respect to weeks after planting (WAP).



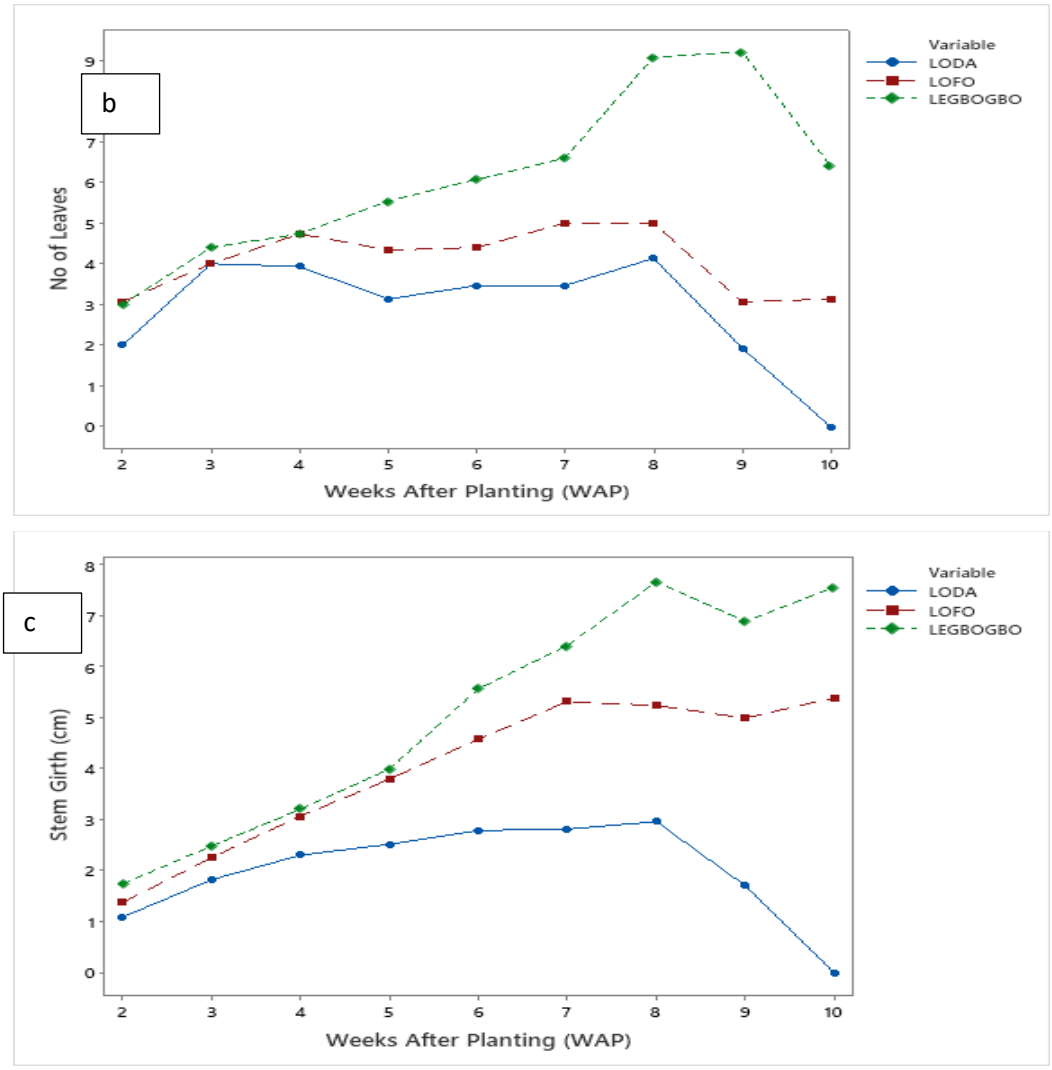
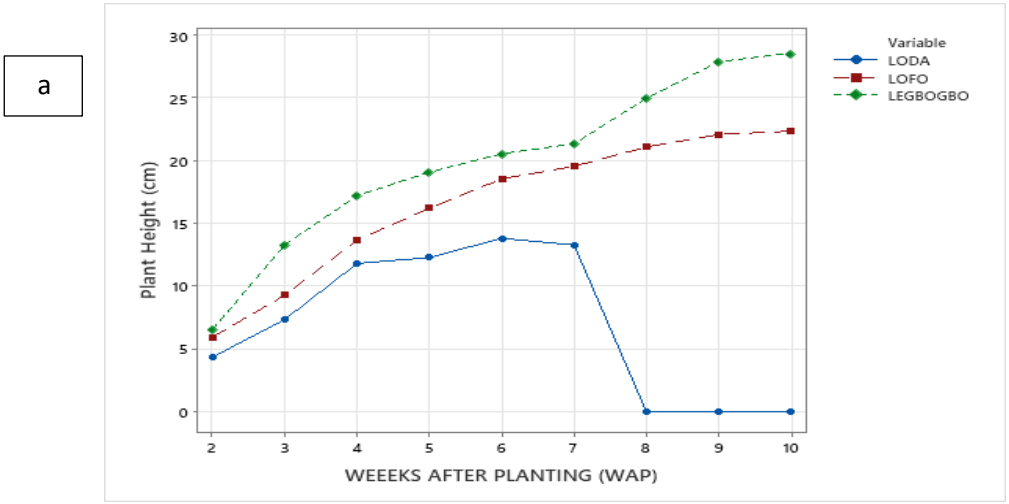


Fig.1: (a) Plant height (b) Number of leaves (c) Stem girth of Okra, as influenced by bitumen content in each of the locations (Loda, Lofo and Legbogbo) in the first year of planting.



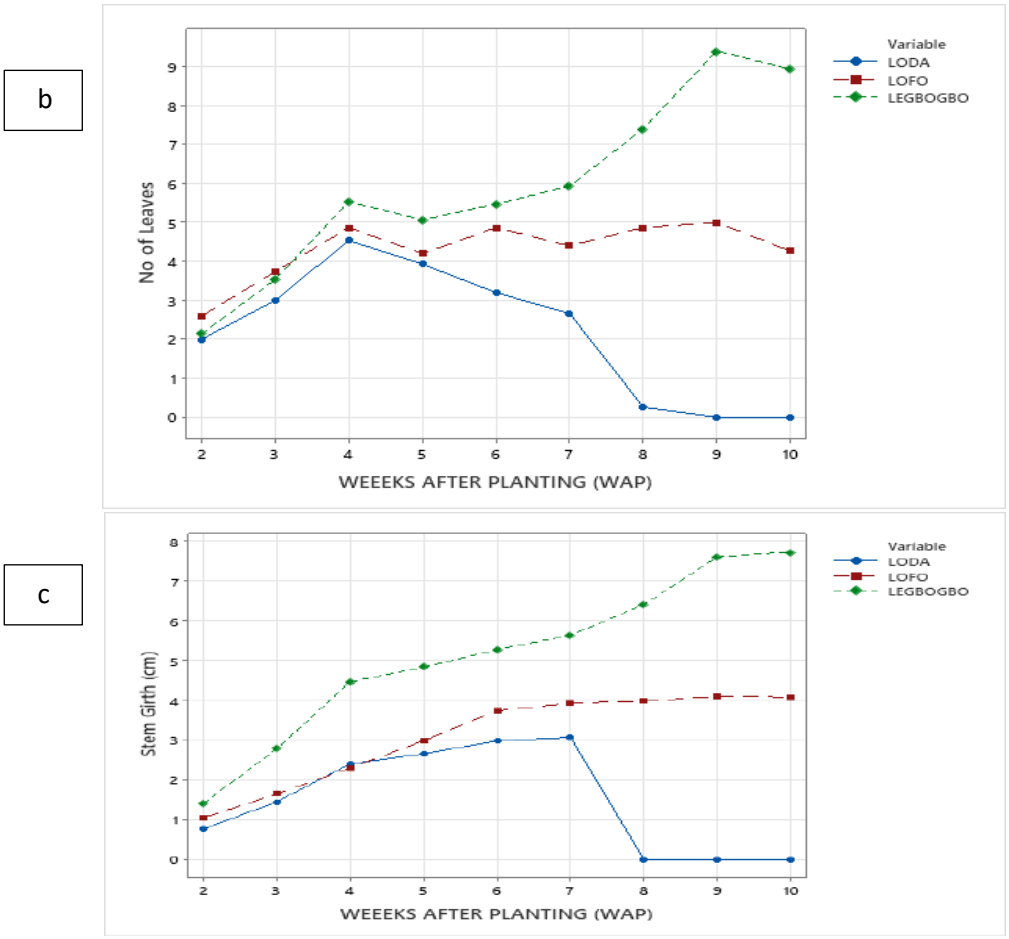


Fig.2: (a) Plant height (b) Number of leaves (c) Stem girth of Okra, as influenced by bitumen content in each of the locations (Loda, Lofo and Legbogbo) in the second year of planting.

3.2 Effects of Bitumen Deposits on Growth of Crops

The growth parameter of okra as affected by the bitumen content in soil at the different locations (Loda, Lofo and Legbogbo) is presented in Tables 1. The Tables showed the average plant heights of the three crops at different location with varying bitumen contents at 2, 6 and 10 weeks after planting (WAP). There was pronounced variability in the heights of the plants at the locations. At 2 WAP, significant difference ($P < 0.05$) in the average plant heights was recorded among the locations. There was also significant difference ($P < 0.05$) in plant heights at 6 and 10 WAP. Tables 4.9 and 4.10 also showed the average stem girth of the three crops (Maize, Groundnut and Okra) grown at different locations (Loda, Lofo and Legbogbo) at 2, 6 and 10 weeks after planting. At 2, 6 and 10 WAP, there was significant difference ($P < 0.05$) in the average stem girth in both seasons of growth. At 2, 6 and 10 WAP, there was significant difference ($P < 0.05$) in number of leaves among the locations (LODA, LOFO and LEGBOGBO) where the crops were planted in both seasons. Highest values of plant height, stem girth and number of leaves was recorded in most cases at the Legbogbo location, which is the control site, while the lowest value was recorded at the LODA site, which is characterized by some large amount of bitumen contents. Despite the lowest rainfall values of 592 mm recorded at LEGBOGBO during the first growing season, highest values of growth parameters were measured. The LOFO site rainfall value of 618 mm with corresponding values of growth parameters is lower, when it is compared with the LEGBOGBO site. LODA site was a bit lower in rainfall value (616 mm) comparatively with the LOFO site but had the lowest plant growth parameters. The growth parameters obtained at the LEGBOGBO site had the highest value which can be explained by moisture availability to crop due to free percolation of water into soil. However, lower values of values of growth parameters recorded at the LOFO and LODA sites may have been caused by the surface seal caused by bitumen deposits, and may also be due to enhanced runoff of surface flow after rainfall, thus affecting water availability at the tap root in the soil for metabolism and cell division (Ndakidemi and Dakora, 2007).

Table 1: Effects of Bitumen Deposits on Okra Growth Parameters of Crops (Season 1)

Location	WAP	LODA	LOFO	LEGBOGBO
Plant height	2	6.33b (± 1.13)	7.43a (± 0.84)	6.40b (± 0.91)
	6	13.50a (± 1.59)	19.60a (± 2.71)	20.67a (± 3.32)
	10	0.00c (± 0.00)	26.20b (± 5.39)	38.53a (± 11.14)
Stem girth	2	1.09b (± 0.16)	1.40ab (± 0.43)	1.74a (± 0.55)
	6	2.84c (± 0.44)	4.58b (± 0.88)	5.56a (± 1.58)
	10	0.00c (± 0.00)	5.39b (± 1.16)	7.55a (± 2.80)
Number of leaves	2	2.00b (± 0.00)	3.07a (± 0.26)	3.00a (± 0.00)
	6	3.47b (± 0.83)	4.40b (± 0.82)	6.07a (± 1.58)
	10	0.00c (± 0.00)	3.13b (± 0.52)	6.40a (± 2.95)

Means that do not share same letter are significantly different (p = 0.05)

3.3 Effects of Bitumen Deposits on the Yield of Crops

The yield response of okra planted at the three sites is shown in Table 2. Planting the crops at the different locations had significant effect ($p < 0.05$) on the pods, length of pod, weight of pod and pod breadth per plants. Okra has no fruit yield (pod) at LODA site during the two growing seasons. The higher values of the yield component recorded at the LEGBOGBO site can be explained by the free inflow of water and air into soil for plant use and uptake of nutrients. Water that would have been available for crop use at the LODA and LOFO sites were intercepted by bituminous materials, thus disallowing percolation to the root zone. This affected largely moisture uptake by plant for nutrient translocation into shoots, which ultimately led to poor growth and zero yield of okra at the sites.

The enhanced nutrient transportation at the LEGBOGBO site could have resulted to the significant ($P < 0.05$) increase in crop yield parameters (number of pods, length of pod, breadth of pod, and pod weight). The above explained the mechanism for the higher values of yield components at the LEGBOGBO, in accordance with the findings of Abd El Lateef *et al*. (2018) and Chemutai (2018). The zero (0) pod weight reported for the vegetable crop (Okra) at the LODA site can be explained by inability of the Okra crop to take up soil nutrient. Macro-nutrients like Nitrogen, Phosphorus and Potassium are essential elements and important determinant of the growth, yield and development of vegetables. Likewise, adequate supply of phosphorous early in plant life is important in laying down the primordial for plant growth (Chemutai, 2018; Chemutai *et al*., 2018). The fact that the yields from the LOFO and LEGBOGBO were generally much greater than from LODA on its own indicates that the nutrients satisfied the growth and yield parameters of okra. Presumably, this can be related to the functionality of soil and contents of the organic matter and macro nutrients (Abd El Lateef *et al*., 2018). The presence of organic matter in soils is very important in the tropical region for sustainable yield of crops (Sangakkara, 1993). Organic matter improves soil tilth, infiltration rate and soil water holding capacity; contributes nutrient to the crop, and it is an important source of raw or partially decomposed organic matter (Bill, 2001).

Table 4.11: Effects of Bitumen Deposits on Yield of Okra

Location	LODA	LOFO	LEGBOGBO
	Season 1		
Number of pod per stand	0.00c (± 0.00)	2.27b (± 1.44)	6.07a (± 1.53)
Weight of pod (g)	0.00b (± 0.00)	19.80b (± 13.91)	123.20a (± 65.10)
Length of pod (mm)	0.00c (± 0.00)	41.04b (± 16.74)	76.68a (± 11.81)
Pod breadth (mm)	0.00c (± 0.00)	18.97b (± 6.23)	26.26a (± 3.11)
	Season 2		
Number of pod per stand	0.00b (± 0.00)	1.00b (± 0.83)	9.00a (± 3.83)

Weight of pod (g)	0.00b (± 0.00)	16.40b (± 9.58)	104.40a (± 40.20)
Length of pod (mm)	0.00b (± 0.00)	23.11b (± 10.72)	68.59a (± 12.54)
Pod breadth (mm)	0.00c (± 0.00)	17.28b (± 3.62)	28.50a (± 5.44)

Regression models between Soil Properties and Okra yield

Finally, for the Okra yield prediction, the stepwise regression model was developed as shown in equations 4.5 and 4.6 below. Equation 4.5 and 4.6 represent yield models for seasons 1 and 2, respectively.

Yield = 3169 - 16995 K * r^2 = 95.5 (1)

Yield = -1978.9 + 246.08 P** r^2 = 99.9 (2)

Note: * significance at 0.05, ** significance at 0.01

The result indicated that Okra yield significantly correlated to potassium and phosphorus ($P < 0.05$). The r^2 values of 0.95 and 0.99 obtained from the model indicated that about 95.5 and 99.9 % variability in the response could be explained by the model, which contained potassium and phosphorus as the predictors. The model validation of the model is presented in Figure 5, which showed a strong relationship between the predicted yield and measured yield for Okra, with r^2 value equal to 0.99.

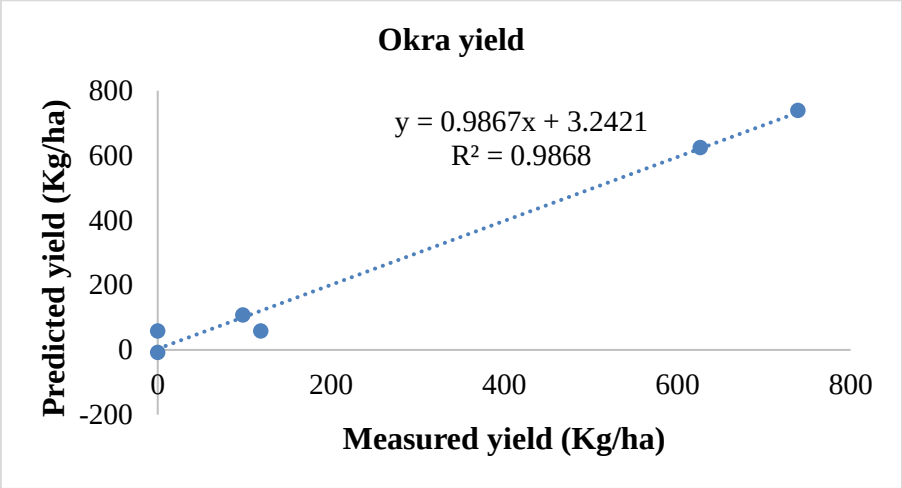


Fig.5. Predicted versus Measured yield of okra (Abelmoschus esculentus, L. moench)

IV. CONCLUSION

The growth and yield response of okra planted in three different locations around the bituminous area of Irele, Southern Ondo State, Nigeria has been investigated in this research. It was observed that the LEGBOGBO site (control site) without bitumen deposit had the highest growth and yield parameters, followed by the LOFO, which had traces of bitumen deposits that gave rise to yield reduction of okra. However, the LODA site that was characterized with surface and underground layers of bitumen had the least growth parameters and okra yield. Soil amendments and remediation would have to be carried out to reclaim the usefulness of LODA soils for agricultural purposes.

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Analysis of Urban Thermal Environment Effect by TIRS and GIS: A Case Study of Zhuhai, Guangdong

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Abstract— *The rapid development of urbanization in China is not only reflected in the tight land area and rapid population growth but also causes changes in the local urban climate, such as the increasingly obvious urban heat island effect (UHIE). This study explores the impact of urban surface cover types on the urban thermal environment. Taking Zhuhai City, Guangdong Province as an example, based on Landsat-8 thermal infrared remote sensing (TIRS) data, the atmospheric correction method (also known as Radiation Transfer Equation, RTE) and spilt-window inversion algorithm are used to invert the land surface temperature (LST) of the study area and compare their accuracy. After applying ArcGIS to normalize the data, the standard deviation method was used to classify the LST and obtain the distribution map of surface temperature levels in the urban area. In addition, the urban heat island proportion index was used to evaluate the UHIE in the study area, and the distribution of UHIE intensity was obtained. Based on geographical and national data, a combination of mathematical and spatial statistics was used to establish a correlation between the proportion of underlying surface coverage and LST in three different types of water bodies: vegetation and impermeable water surfaces. The results of the effect of urban underlying surface layout on the thermal environment were obtained, and the overall thermal environment effect of the city was obtained.*

Keywords— ***Thermal Infrared Sensor (TIRS); Land Surface Temperature (LST); Radiative Transfer Equation (RTE); Underlying Surface; Urban Heat Island Effect (UHIE).***

I. INTRODUCTION

Land surface temperature (LST) is of great research significance in the fields of urban thermal environment changes, landscape pattern analysis, and ecological characteristics' analysis, and is an important parameter for studying the exchange of matter and energy between land and atmosphere. In recent years, scholars have conducted research on LST based on remote sensing (RS) technology (Price, 1990; Yue et al., 2006), ranging from LST inversion to results analysis and application. One of the hotspots is the quantitative analysis of the relationship between urban surface temperature changes and the underlying surface (which is an important factor in climate formation and refers to the Earth's surface interacting with the atmosphere during heat, momentum, and water vapor exchange).

For example, Atsuko et al. (2009) pointed out after studying the impact of land use and land cover (LULC) on ambient temperature in Takamatsu City, Japan, that the growth of urban impermeable underlying surface area is one of the most important factors leading to temperature rise. Xiao et al. (2007) further found that there is a positive correlation between the impermeable underlying surface and LST in Beijing. Streutker (2002) and Roth et al. (1989) used RS data to invert LST and its spatial distribution in several cities along the western coast of North America. They believe that there is a clear correlation between the thermal characteristics inside cities during the day and land use, while the correlation between nighttime heat island intensity and land use is relatively small. Chen et al. (2006) and Li et al. (2008) analyzed the correlation between NDVI (Normalized Difference Vegetation Index), MNDWI (Modified Normalized Difference Water Index), NDBI (Normalized Difference Building Index), and NDBSI (Normalized Difference Bare Soil Index) and LST, respectively. They found a clear correlation between them, and there were significant differences in LST among different LULC types. The above study obtained the correlation between underlying surface and temperature in different types of cities.

This article takes Zhuhai City, Guangdong Province, as an example and uses Landsat-8 thermal infrared (band) image data as the basis to invert its LST. Combined with the geographical and national data of the research area, a quantitative analysis is conducted on the relationship between surface temperature and underlying surface in order to provide scientific reference for the evaluation of natural resource ecological environment and urban planning in domestic cities.

II. STUDY AREA AND DATA SOURCE

2.1 Study Area

Zhuhai City has three administrative regions under its jurisdiction: Xiangzhou, Doumen, and Jinwan. The location is superior, bordering the South China Sea, with a distance of 36 nautical miles from Hong Kong's waterway to the east and connected to Macau's land to the south. After the completion of the Hong Kong-Zhuhai-Macau Bridge, Zhuhai has become the only city on mainland China that is connected to both Hong Kong and Macau by land. Zhuhai is an important port city in China, with five land transportation ports, including Gongbei, Hengqin, Qingmao, the Hong Kong- Zhuhai-Macao Bridge, the Zhuhai Highway, and the Zhuhai-Macao Cross-Border Industrial Zone. There are 5 water transportation ports, including Jiuzhou Port, Wanzai Port for ferry passenger transportation, Zhuhai Port, Doumen Port, and Wanshan Port. There are a total of 10 national first-class ports, making it the second-largest port city in China after Shenzhen. It has rich marine resources, vast sea areas, and numerous islands.

The climate of Zhuhai is pleasant, with an obvious alternation of winter and summer winds. The temperature is relatively high all year, with occasional cold showers. The annual and daily temperature differences are small, and they belong to the transitional marine climate between the South Asian tropics and the tropics. The city is rich in solar energy and abundant in heat. It is the only city in China that has been selected as one of the "Top 40 National Tourist Attractions" for its overall urban landscape, with mountains and rivers alternating and land islands facing each other (Figure 1 and Figure 2).

with land use status maps.

(4) The climate pattern of Guangdong Province has been characterized by a continuous increase in temperature since June, with a high temperature period from July to September and a significant UHIE. Therefore, when selecting data sources, priority should be given to image data from June to September. However, due to the large amount of cloud cover in the image data during this time span, it will have a serious impact on temperature inversion. Thus, after further evaluation, February images were selected for inversion analysis.

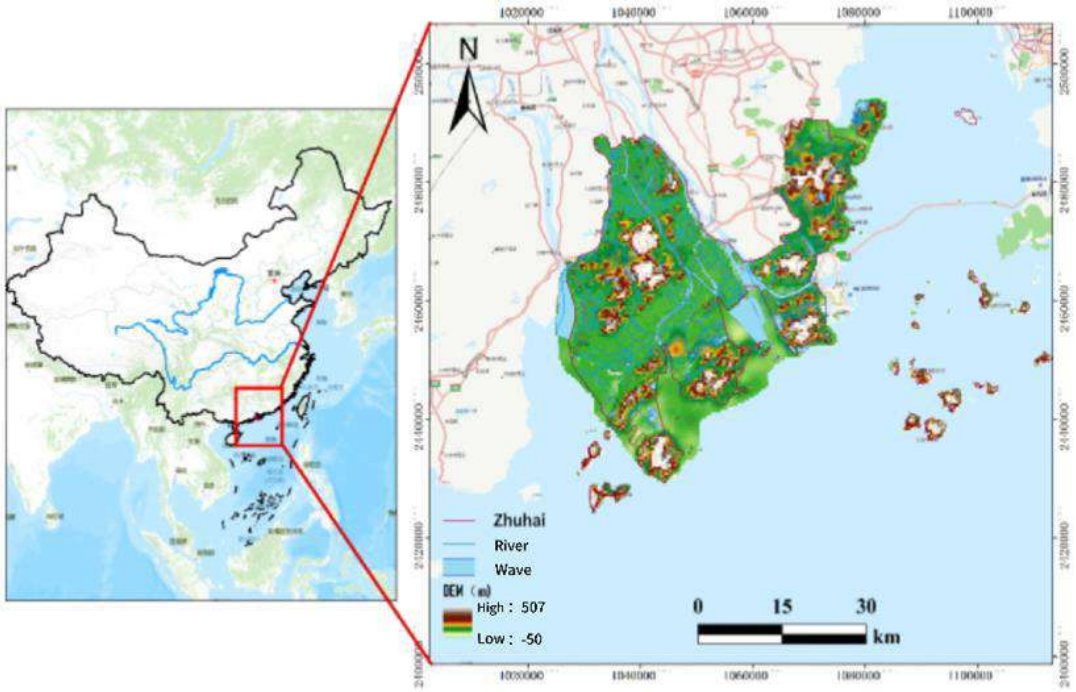

Fig.1 Geographical Area Map of Zhuhai City

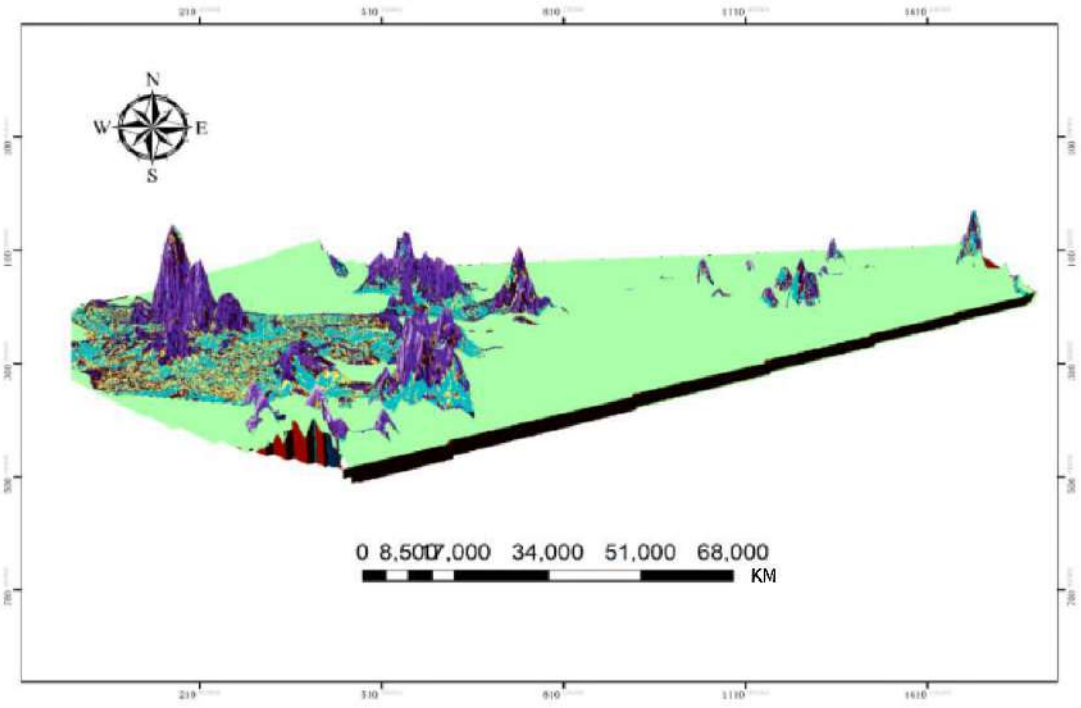

Fig.2 Topographic Profile of Zhuhai

2.2 Data Source and Preprocessing

The data sources used in this study chiefly include geographic national condition vectors and Landsat-8 satellite images.

(1) Geographic national vector data includes surface coverage data from the 2017 Geographic National Survey and the 2020 Geographic National Monitoring results.

(2) Landsat-8 satellite images, including three thermal infrared image data from February 7, 2016, February 17, 2016, and February 20, 2021.

(3) To compare the different temperature changes of the underlying surface in Zhuhai in different years, two

images were selected for analysis and comparison. When selecting images from the source database, it was found that the number of available images was relatively small. Thus, an image mosaic method was adopted to obtain the full image of February 2016. Among them, the 10th and 11th bands of the Landsat-8 thermal infrared sensor (TIRS) are used to estimate brightness temperature; the operational land imager (OLI) data is used to calculate the NDVI, MNDWI, and NDBI. Next, the data is subjected to radiometric calibration processing and FLAAS atmospheric correction. In addition, land use types are based on NDVI, MNDWI, and NDBI, using normalized density segmentation methods for classification, and calibrated in conjunction

III. METHODOLOGY

This study selected Zhuhai City as the research area, comprehensively utilizing various methods such as geographic information systems (GIS), RS technology, and spatial modeling. Based on multi-temporal Landsat image data, a supervised classification method is used to classify land use/cover. The RTE method and split-window algorithm are used to invert LST in order to study the characteristics of underlying surface changes and the spatio-temporal changes of LST during urbanization. Analysis of the relationship between underlying surface changes and LST uses the quantitative method. The specific research route is as follows (Figure 3):

(1) Analysis of the spatio-temporal dynamics of underlying surface changes: Using land use/cover change (LUCC) as the characteristic, the spatio-temporal dynamics of underlying surface changes are analyzed, as well as the characteristics of landscape pattern changes in Zhuhai's underlying surface from the perspective of patch types and landscape levels.

(2) Analysis of spatio-temporal characteristics of LST changes based on RS inversion data in Zhuhai City from 2016 to 2021. And using methods such as urban heat island proportion index analysis, normalize the retrieved temperature for hierarchical classification, analyze the spatial agglomeration effect of LST, and explore the differences in the contribution of land use/cover types in different regions and underlying surfaces to the urban thermal environment.

(3) Analysis of the relationship between the underlying surface and surface temperature based on statistical methods to clarify the characteristics of LST changes on the underlying surface and explore the relationship between changes in the underlying surface and LST.

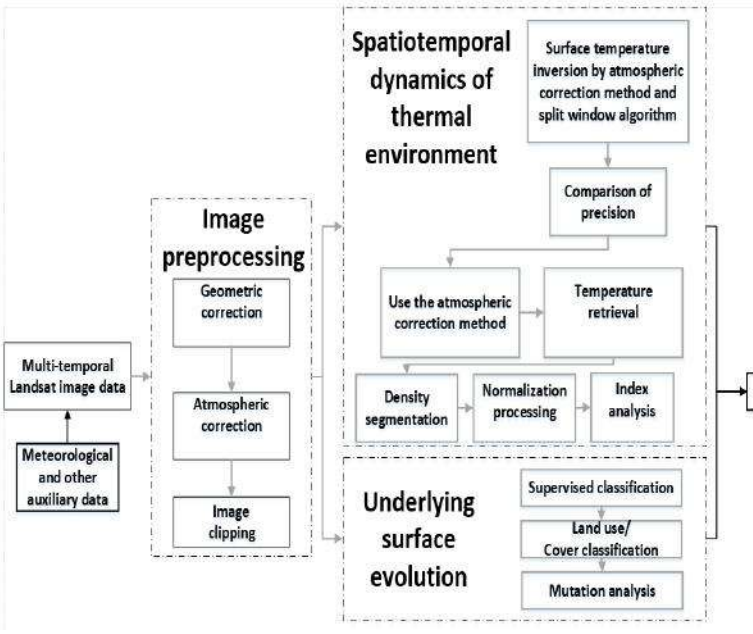

Fig.3 the Schema Flowchart of the Study

3.1 LST Inversion

3.1.1 Radiative Transfer Equation (RTE) Method

■Basic principle:

There are three main types of LST inversion based on RS: the RTE, the single channel algorithm, and the split-window algorithm. Among them, retrieving LST requires three parameters, namely: average atmospheric temperature, atmospheric transmittance, and surface emissivity.

The main approach is to first estimate the impact of the atmosphere on surface thermal radiation, and then subtract the atmospheric impact from the total amount of thermal radiation observed by satellite sensors to obtain the intensity of surface thermal radiation, and then convert the intensity of thermal radiation into the corresponding surface temperature.

■Basic steps:

1. Data Preprocessing

(1) Calculate radiation brightness temperature (T6): After radiation correction and atmospheric correction are

applied to the multispectral and thermal infrared bands, calculate radiation brightness temperature (b1 selects the thermal infrared band after radiation correction).

T6=k2/ln (k1/B (TS)+1) (1)

In the formula (1), B (TS) is the thermal radiation brightness of the blackbody in TS derived from Planck's law, and T is the transmittance of the atmosphere in the thermal infrared band. The radiation brightness B (TS) of a blackbody at temperature T in the thermal infrared band is as formula (2):

$$B(TS) = [L\lambda - L\uparrow - \tau\cdot(1-\varepsilon)L\downarrow]/(\tau\cdot\varepsilon) \quad (2)$$

λ is the thermal radiation brightness of the blackbody at TS, which is derived from Planck's law.

k2=1321.08, k1=774.89 are preset constants for Landsat-8 band10 before transmission, and after substitution, formula (3) is obtained.

T6=1321.08/log (774.89/b1+1) (3)

(2) NDVI calculation such as formula (4): b3 and b4, respectively, select the red and near-infrared bands after atmospheric correction.

NDVI= (float (b4 b3))/(b4+b3) (4)

(3) Vegetation coverage FVC calculation: NDVIS and NDVIV generally use the minimum edge value of the vegetation index and the maximum vegetation index. This article uses a 5% confidence interval, and the calculation formula is as follows (formula 5):

FVC= (NDVI - NDVIS)/(NDVIV NDVIS)
= (b1 gt 0.506826) * 1+(b1 lt 0.156625) * 0+(b1 ge 0.156625 and b1 le 0.506826) * (b1-0.156625)/(0.506826-0.156625)) (5)

(4) Surface emissivity (Surf) is a basic parameter of LST that mainly depends on the geological structure of the surface. This study uses the same surface emissivity calculation method as TM/ETM+6. Calculate surface emissivity using the NDVI threshold method proposed by Sobrino (2006). In band math, the formula is converted to:

Surf=0.004 * b1+0.986 (6)

In the formula, b1 is vegetation coverage (VFC)

2. LST Inversion

(1) Calculate variables C and D, as shown in formulas (7) and (8):

C = 0.34 * b1 (7)

D= (1-t) * (1+(1-b1) * t) (8)

(b1 represents the surface emissivity (Surf), 0.34 is the atmospheric transmittance of the day; the atmospheric transmittance is obtained by inputting the photography time and central latitude and longitude through NASA's official website.)

(2) Calculate the surface temperature in degrees Celsius using the formulas (9), (10), and (11):

Ts=[a * (1-C-D)+(b * (1-C-D)+C+D) * T6+D * Ta]/C (9)

(Ts is the true surface temperature; a and b are constants; a = -67.355351, b = 0.458606.) C and D are intermediate variables, as shown in formulas (7) and (8). The radiant brightness temperature T6 can be obtained using the inverse function of the Planck formula (as shown in formula 3), where Ta is the average atmospheric temperature (in K). In addition, there is a linear relationship between the average atmospheric temperature Ta and the near surface temperature T0 (usually 2m) as follows:

Ta=17.9769+0.91715 * T0 (tropical average atmosphere)

Ta=16.0110+0.92621 * T0 (mid-latitude summer average atmosphere)

Ta=19.2704+0.91118 * T0 (mid-latitude winter average atmosphere)

Among them, Ta is the average atmospheric temperature, and T0 is the local temperature at the time of remote sensing image acquisition (T0's temperature needs to be converted into Kelvin temperature).

Ta=17.97669+0.91715 * (273.15+19)=285.992 (10)

LST=Ts-273.15 (11)

In the above equation, the conversion formula between Kelvin (K) and Celsius (℃) is: K=℃+273.15, ℃=K -273.15. Among them, K represents Kelvin, and ℃

represents Celsius. Thus, subtracting 273.15 from Kelvin K is the value of degrees Celsius.

3.1.2 Double-Channel Nonlinear Split-Window Algorithm

(1) LST Inversion Algorithm

Using the dual channel nonlinear split-window algorithm to invert surface temperature (Chen et al., 2004) (as shown in formula 12):

$$T = b_0 + \left(b_1 + b_2\frac{1-\varepsilon}{\delta} + b_3\frac{\Delta\varepsilon}{\delta^2}\right)\frac{T_i+T_j}{2} + \left(b_4 + b_5\frac{1-\varepsilon}{\delta} + b_4\frac{\Delta\varepsilon}{\delta^2}\right)\frac{T_I-T_J}{2} + b_7(T_I - T_J)^2 \quad (12)$$

Among them, e and Δε Represent the mean and difference of emissivity for two channels, depending on surface classification and coverage: T_I and T_J are the observed brightness temperatures of two channels, b_i (i=0, 1... 7) represents various coefficients that can be obtained from simulated datasets of laboratory data, atmospheric parameter data, and atmospheric radiation transfer equations. To improve inversion accuracy, coefficient b_i depends on the water vapor content in the atmospheric column.

(2) Atmospheric Water Vapor Content

To reduce dependence on external atmospheric conditions, a new algorithm has been developed to estimate water vapor from thermal infrared images themselves. Firstly, an empirical relationship between the atmospheric transmittance ratio T_i/T_J of two split window channels and the atmospheric water vapor content wv is established using MODTRAN and TIGR atmospheric profiles. Then, the transmittance ratio is estimated using the ratio of covariance to variance between the brightness temperatures of the two channels within a certain size sliding window (as shown in formula 13)..

$$wv = a + b\left(\frac{T_I}{T_J}\right) + c.(T_I/T_J)^2 \quad (13)$$

(3) Pixel Emissivity Inversion

The vegetation coverage weighting method uses Landsat-8 visible and near-infrared data to invert NDVI and vegetation coverage f to estimate pixel emissivity.

$$\varepsilon_p = \varepsilon_v.f + \varepsilon_g(1-f) + 4 < d\varepsilon > f.(1-f) \quad (14)$$

f= (NDVI-NDVIs)/ NDVIv-NDVIs)^2 (15)

Among them, the emissivity data of vegetation components ε and the emissivity data of background components ε_g come from the spectral database. The <dε> represents the cavity effect parameter formed by multiple scattering of components within a pixel, which is determined by the red structure of the pixel canopy and surface roughness. NDVIs and NDVIv are NDVI values for bare soil and dense vegetation, respectively. To maintain consistency between different images of NDVIs and NDVIv, fixed values are taken here, namely NDVIs=0.2 and NDViv=0.86. When the NDVI of a pixel is greater than NDVIv, the plate coverage of the pixel is 10, and the emissivity of the pixel is ε_v. When the NDVI of a pixel is less than NDVIs, the vegetation cover of the pixel is 0,0; Pixel reflectance is ε_g .

3.2 Quantitative Analysis of LST Change and Underlying Surface

This study is based on the results of surface coverage in geographical and national conditions. Within the study area, quantitative analysis is conducted on the relationship between LST changes and underlying surfaces, including the urban heat island proportion index and the analysis of the relationship between underlying surfaces and LST changes. Among them, the urban heat island proportion index is a new viewpoint proposed to address the difficulty of quantitative comparison of UHIE in different time periods. That is, the comparison of UHIE between different time periods cannot only consider the temperature itself but also the different temperature levels that make up the urban heat island, the proportion of the area in the urban built-up area (Li, 2020), and the contribution of temperature intensity to the formation of the heat island effect to analyze the urban heat island proportion index (Sun, 2020).

The analysis of urban heat island proportion index is based on the inversion of LST, obtaining information such as the highest temperature, lowest temperature, and average temperature in the study area, and analyzing the spatial distribution characteristics of LST in each study area. And density classification technology is used to classify, calculate, and analyze the absolute value of LST. In addition, analysis of the relationship between underlying surface and LST changes: analyze the LST of

the study area in 2016 and 2021, and calculate the area and proportion of LST at all levels; Based on geographical and national land cover data, calculate the area and proportion of different types of underlying surfaces within different levels of temperature; Based on statistical results, quantitatively analyze the relationship between LST changes and underlying surfaces.

IV. ANALYSIS AND RESULTS

The underlying surfaces of this study are divided into three categories: vegetation, water bodies, and impermeable surfaces, where impermeable surfaces refer to surfaces covered by various impermeable building materials, such as buildings, roads, and parking lots composed of materials such as tiles, asphalt, cement concrete, etc.

4.1 Vegetation Information Extraction

The most commonly used vegetation information extraction is the normalized difference vegetation index (NDVI), which is between -1 and 1. The vegetation index is positive, and the larger the value, the more obvious the vegetation features. The calculation formula is shown in (11), and the vegetation cover map is shown in Figure 4:

$$NDVI=\frac{NIR-RED}{NIR+RED} \quad (11)$$

where RED and NIR, respectively, select the red and near-infrared bands after atmospheric correction.

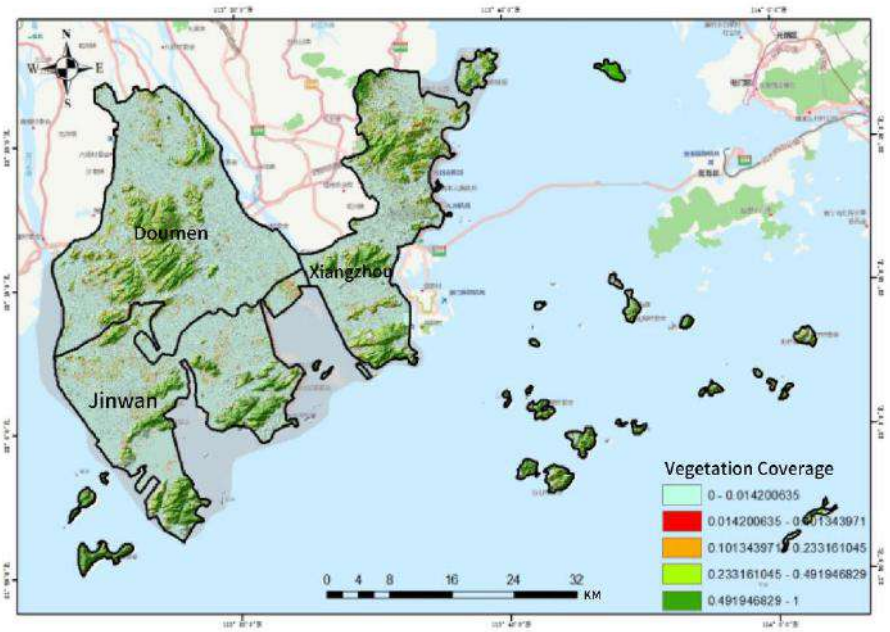

Fig.4 Vegetation Cover Map of Zhuhai City

4.2 Water Body Information Extraction

Mcfeeters (Vitousek, et al., 2008) proposed the Normalized Difference Water Index, abbreviated as the NDWI. Its formula is as follows (12):

$$NDWI=\frac{BGreen-BNIR}{BGreen+BNIR} \quad (12)$$

where BGreen represents the green light band; BNIR represents the near-infrared band.

The basic principle of the model is that as the reflection of water gradually weakens from visible light to the mid-infrared wavelength range, it has the strongest absorption in the near-infrared and mid-infrared wavelength ranges and almost no reflection. Therefore, the NDWI composed of the contrast between the visible and near-infrared bands can highlight the water body information in the image (the NDWI value of the water body is the largest). In addition, due to the generally strongest reflectance of vegetation in the near-infrared band, the ratio of the green light band to the near-infrared band can be used to suppress vegetation information to the greatest extent, thereby achieving the goal of highlighting water information (Figure 5).

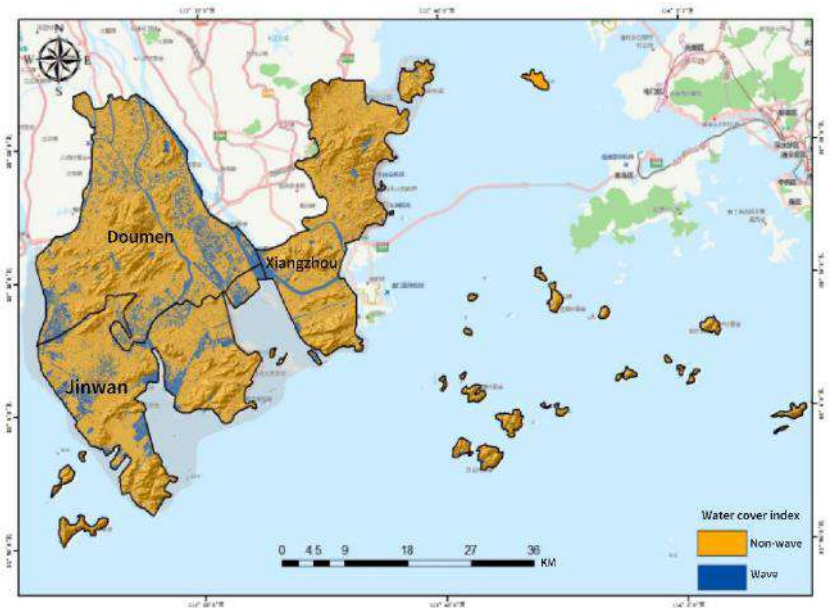

Fig.5 Water Cover Map of Zhuhai City

4.3 Urban Construction Land Information Extraction

NDBI is proposed based on the pseudo-normalized difference vegetation index proposed by Yang (Zhuang et al., 2019). It can accurately reflect information about building land. A larger value indicates a higher proportion of building land and a higher building density. Through visual interpretation, the NDBI threshold is continuously adjusted until a suitable threshold is found, and construction land is extracted. After adjusting the threshold, the impermeable water surface distribution is obtained. The calculation formula is as follows (13):

$$\text{NDBI}=\frac{RMIR-\text{BNIR}}{BMIR+\text{BNIR}} \quad (13)$$

where RNIR and RMIR are the reflected radiation values of the near-infrared and mid-infrared images, corresponding to the band 5 and band 6 OLI data. The darker the color of the NDBI image, the higher the representative value, indicating a higher proportion of building land and a higher density of buildings (Figure 6).

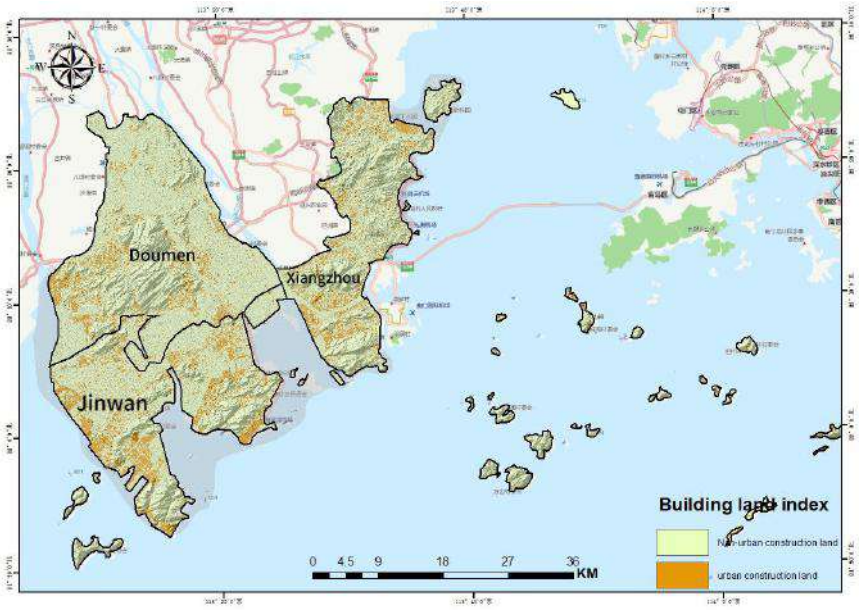

Fig.6 Construction Land Coverage Map of Zhuhai City

4.4 Analysis of UHI Ratio Index

The proportion of vegetation area in the urban area of Zhuhai remained unchanged from 2016 to 2021, while the proportion of water body area decreased slightly and the proportion of impermeable water surface area increased. According to the geographical and national survey and monitoring data statistics of Guangdong Province (Figure 7 and Table 1).

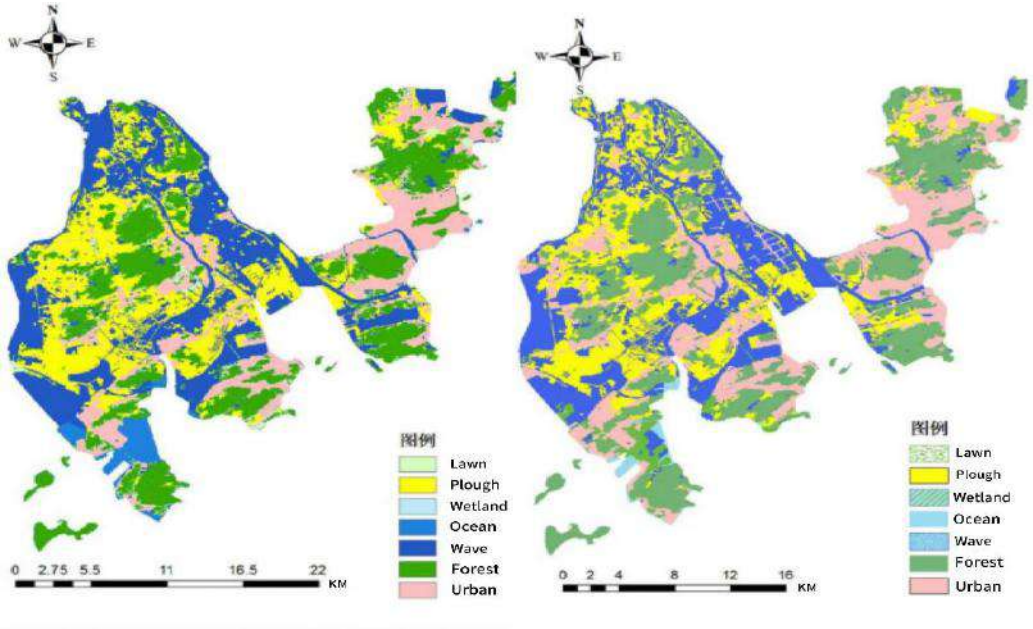

Fig.7 Remote Sensing Images of Land Use in Zhuhai in 2016 and 2021

(Image Source: 2022 GlobeLand30 Surface Cover Data from the Ministry of Natural Resources of China)

Table 1 Proportion of Different Underlying Surface Areas in Zhuhai in 2016 and 2021

Study area	Category	Year of 2016		Year of 2021	
		Area /km^2	Ratio （%）	Area /km2	Ratio （%）
Zhuhai City District	Water bodies	468.84	27	416.75	24
	Vegetation	573.03	33	573.03	33
	Impervious surface	260.47	15	382.02	22

4.5 Inverted LST Situation

The surface temperature situation of Landsat-8 remote sensing image data for temperature inversion is shown in Figure 8 and Table 2. The atmospheric correction method, also known as the RTE method, is based on the Planck equation to invert surface temperature. This method is simple and clear and has higher inversion accuracy when obtaining or simulating more accurate atmospheric parameters. Compared with the dual-channel nonlinear split-window algorithm, it reduces the impact of atmospheric water vapor content on temperature data with a smaller standard deviation. The data is relatively stable and the error is small, so this article uses the atmospheric correction method for the temperature inversion of Zhuhai City in relevant years.

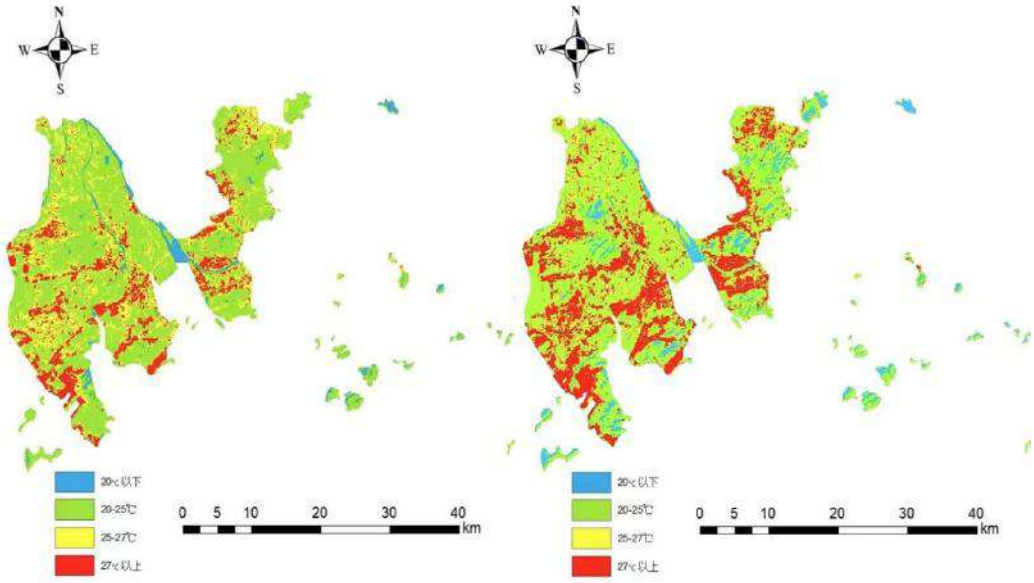

Fig.8 Temperature Inversion Image in 2021
[Split Window Algorithm (Left), Atmospheric Correction Method (Right)]

Table 2 Statistical Characteristics of Temperature Inversion

Study area	Method	Highest	Minimum	Average temperature	Standard deviation
Zhuhai	Split-window	42.499	10.958	24.174	2.411
	RTE	37.777	11.407	22.222	1.884

The surface temperature situation of the reverse performance (Figure 9, Table 3) shows that the daily highest and lowest temperatures in the urban area of Zhuhai in 2021 were higher than those in the same month of 2016. The standard deviation of temperature decreased, indicating a smaller dispersion of temperature distribution and gradually stabilizing.

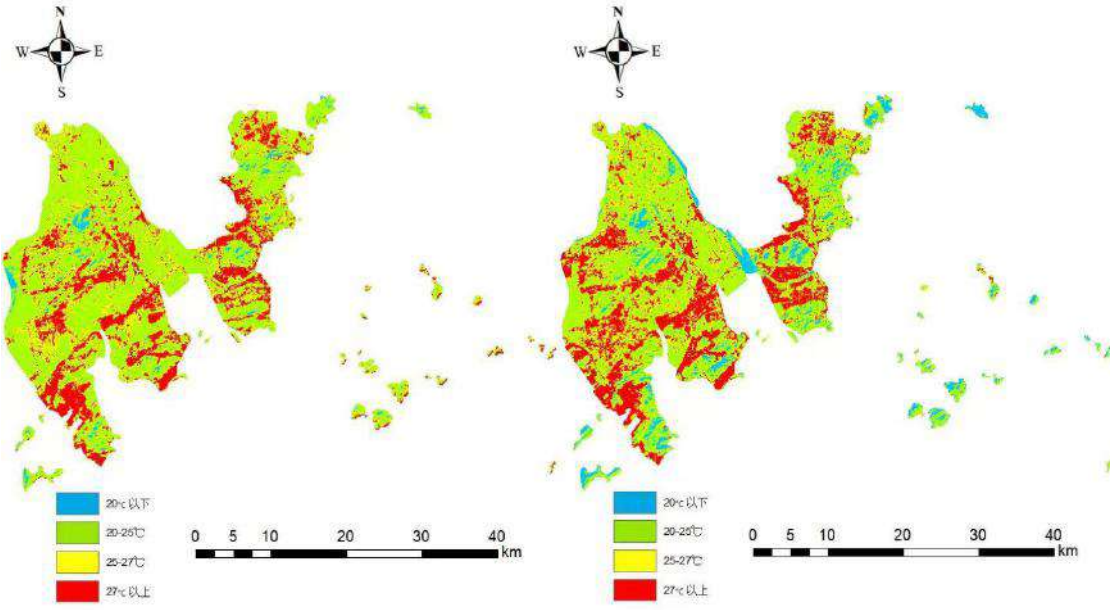

Fig.9 Temperature Inversion Image [2016 (Left) and 2021 (Right)]

Table 3 Statistical Characteristics of Surface Temperature in Zhuhai in 2016 and 2021

Study area	year	Highest	Minimum	Average temperature	Standard deviation
Zhuhai	2016	35.256	8.109	17.096	2.144
	2021	37.777	11.407	22.222	1.884

Due to the significant differences in surface temperatures among different time periods, it is not feasible to directly compare the surface temperatures of each time period. Thus, normalization and density segmentation techniques are used to classify the surface temperatures over two time periods. By calculating the urban heat island ratio index, the trend of surface temperature changes in different regions and different time periods is studied.

The specific steps are as follows: first, normalize the inverted LST according to formula (14), and unify the surface temperature between 0 and 1. Then, using density segmentation technology, the normalized s LST is divided into seven levels using an even distribution method, including extremely high temperature (EHT), high temperature (HT), relatively high temperature (RHT), medium temperature (MT), relatively low temperature (RLT), low temperature (LT), and extremely low temperature (ELT), with corresponding level values ranging from 7 to 1 (Pan and Han, 2011; Li and Xv, 2014). Finally, calculate the proportion of these levels in the built-up area and calculate the URI based on the formula (15).

$$LST_{norm}=\frac{LST-LSTmin}{LSTmax-LSTmin} \qquad (14)$$

$$URI=\frac{1}{100m}\sum_{i=1}^{n} wipi \qquad (15)$$

In the formula (14), LSTnorm is the normalized surface temperature value; LST that has not been normalized; and LSTmax is the maximum value of surface temperature. LSTmin is the minimum value of surface temperature;

In the formula (15), URI refers to the proportion of urban heat islands; m is the number of surface temperature levels; i is the temperature level in the urban area that is higher than the moderate temperature zone; n is the number of temperature levels in urban areas that are higher than those in moderate-temperature areas; W is the weight value, selecting the level value of the i-th level; and P is the percentage of level i. In this study, the natural breakpoint classification method was used to classify the LST into 7 levels, so m is 7. Areas above moderate temperatures represent the development range of urban heat islands; therefore, n is 3.

Based on the above method, the calculated values and distribution maps of Table 4 and Figure 10 were obtained. Analysis shows that in 2016, the temperate zone in the urban area of Zhuhai accounted for the largest proportion of the total area (23.6%). The sub-high temperature zone (46%) accounted for the largest proportion of the total area in 2021, with the URI increasing from 0.307 in 2016 to 0.393. Compared with 2016, the total proportion of EHT, HT, and RHT areas increased by 10.804%, resulting in an increase of 0.086 in the urban heat island proportion index of Zhuhai City. From 2016 to 2021, the surface of the urban area showed an increase in temperature, with a small increase in the range of EHT, HT, and RHT.

Table 4 Heat Island Ratio Index and Area Proportion of Temperature Class

Study area	Year	Proportion of area occupied by temperature zones, Pi/(%)							
		EHT	HT	RHT	MT	RLT	LT	ELT	URI
Zhuhai	2016	0.111	0.175	0.233	0.236	0.146	0.079	0.019	0.307
	2021	0.189	0.2	0.46	0.396	0.07	0.004	0.001	0.393

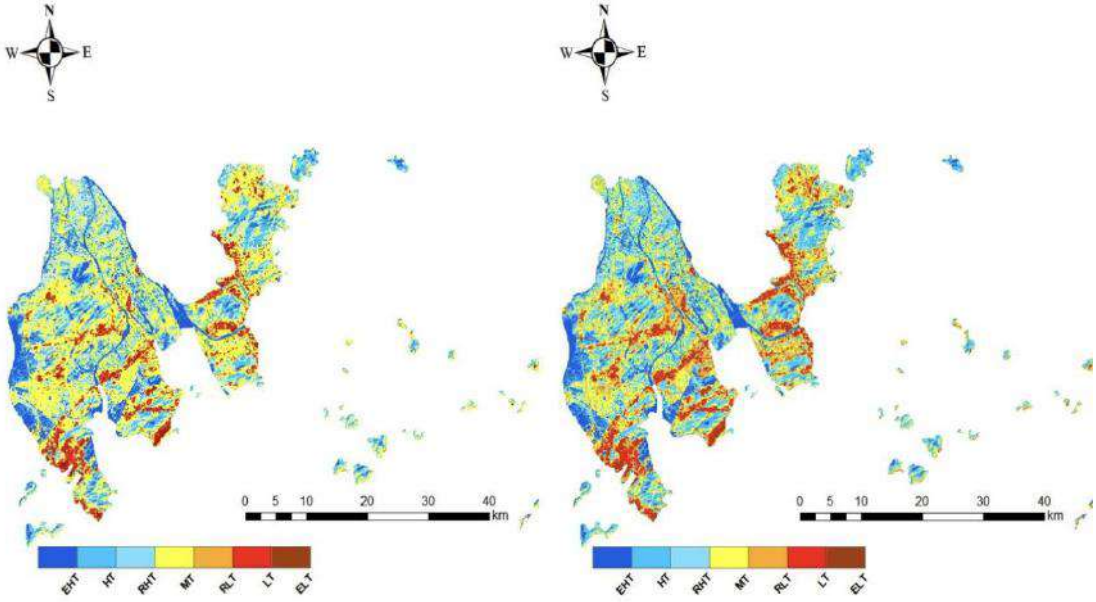

Fig.10 Temperature Inversion Normalization Processing Images
[2016 (Left) and 2021 (Right)]

4.6 Analysis of Underlying Surface and LST Change

From the perspective of underlying surfaces, compared to 2021, the proportion of underlying surfaces and impermeable surfaces in extremely high-temperature and high-temperature areas in Zhuhai City remained relatively stable at over 95% in 2016. This indicates that the high-temperature areas of the city are mainly concentrated in buildings, highways, railways, and other impermeable areas without vegetation or water cover. From 2016 to 2021, the proportion of impermeable surfaces in urban areas increased by 7%, while the proportion of water bodies decreased by 3%, with little change in the proportion of vegetation.

V. CONCLUSIONS

The study analysis shows that from 2016 to 2021, the proportion of impermeable water surface in the urban area of Zhuhai City has slightly increased, the proportion of water body area has slightly decreased, and the vegetation area remains unchanged. After careful comparison and analysis of the proportion of underlying surface area, it was found that the vegetation area on the underlying surface of Zhuhai City remained relatively stable at 33%. The proportion of water body area is about 30% (27% in 2016 and 24% in 2021). From this, it can be concluded that for small and medium-sized cities, vegetation plays a greater role in alleviating the UHIE than water bodies. That is, for small and medium-sized cities, in the process of urban development, if the high proportion of vegetation coverage in the area can be maintained, even if the proportion of impervious surface area decreases and human activity areas increase (15% in 2016 and 22% in 2021), it may not lead to a sharp intensification of the urban heat island phenomenon.

In this study, only two images of February were selected for the inversion of urban temperature. Although the months studied do not belong to the annual high temperature period, the patterns show that there are changes (increases) in urban temperature between years. However, the temperature changes throughout the year cannot be fully demonstrated, so it is insufficient to highlight the trend of heat island changes in the city. In the future, we plan to make full use of geographical and national data, refine the underlying land types, and conduct quantitative analysis of the relationship between LST changes and underlying surfaces in the study area during the four seasons in order to provide a useful reference for urban development planning.

Technically, this article uses the traditional atmospheric correction (RTE) method for LST inversion,

which has room for improvement in accuracy. In the future, we plan to optimize the inverse algorithm from the perspectives of physical simulation and mathematical statistical analysis and then compare the accuracy differences with traditional calculation results. In addition, it is not possible to verify the inversion results of surface temperature due to the lack of synchronous meteorological observation data on satellite transit time and surface temperature, and the estimation of emissivity has not addressed the issue of pixel mixing. In summary, the above analysis elements are the focus of further experiments and discussions in this study in order to improve the reliability and accuracy of LST inversion.

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The genetic stability of date palm shoots regenerated from leaves explant

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Abstract— *The purpose of this research was to develop a micro-propagation method for the date palm Zaghlol cv. using juvenile leaves. To produce the necessary results, different plant growth regulator combinations were used. The leaves were grown on MS medium supplemented with PVP to prevent the explants from browning. The results showed that adding PVP at a concentration of 1.0 g/l considerably reduced browning. On the induction medium, callus formation occurred during the fourth week of culture; however, callus formation (87.5%) was more prevalent on the ¾ MS medium containing with 10.0 mg/l NAA, 1.0 mg/l BA and 2.0 mg/l 2ip. The greatest development of embryogenic callus (94.50%) occurred on a ¾ MS medium supplemented with 5.0 mg/l NAA and 2.0 mg/l BA. The largest fresh callus weight (3.53 g) was produced by this treatment after four months in culture. On MS medium supplemented with 2.0 TDZ, 1.5 BA and 0.5 NAA, which was regarded as the optimum medium for increasing the number of embryos to 32.10 embryos/culture, the best results (65.67%) were obtained. Further investigation into the stimulation and development of somatic embryogenesis involved using MS basal medium supplemented with BA at 0.5 mg/l, kin at 0.1 mg/l and NAA at 0.05 mg/l; this treatment formed the most leaves (20.11 leaf/cluster). The cluster of shoots grown on MS basal medium supplemented with BA at 2.0 mg/l and NAA at 0.5 mg/l had the highest leaf number (34.25 leaf/cluster) and leaves length values after three sub-cultures (4.75 cm). The DNA-based fingerprinting technology ISSR was used to confirm the genetic stability of this protocol. The mother tree and tissue culture-derived shoots evaluated exhibited no differences in the ISSR banding patterns. The micro-propagation method could be used to produce genetically stable date palm plants.*

Keywords— ***Date palm, juvenile leaf explants, plant growth regulators, embryogenic callus induction, somatic embryos formation and maturation and Polymorphism ISSR.***

I. Abbreviation:

II. MS: Murashige and Skoog medium
III. PVP: Polyvinyl Pyrrolidone
IV. NAA: Naphthalene acetic acid
V. BA: 6-Benzyl amino purine
VI. 2ip: 6-dimethylallyl amino purine
VII. TDZ: Thidiazuron
VIII. Kin: Kinetin
IX. DNA: Deoxyribonucleic acid
X. ISSR: Inter simple sequence repeat

I. INTRODUCTION

Phoenix dactylfera L. (Family: Palmaceae) is a dioecious monocotyledonous plant. It is regarded as the most significant fruit tree in a number of Arab nations, including Saudi Arabia and Iraq **(Mirani, 2018)**. Date palm is a resistant fruit tree that grows in a variety of climates, but is most prevalent in hot, arid regions of the Middle East and North Africa. The date fruit has a large number of chemical components with nutritive and medicinal properties **(Al-Khayri and Naik, 2017)**. The yearly worldwide market value of date crops (including imports and exports) reached about 1.9 billion USD **(FAOSTAT, 2013)**, providing a solid important basis upon which a country may develop a successful economy.

Due to a shortage of suitable planting materials, expansion of date palm plantation in various locations of Egypt and replanting trees to compensate for loss due to diseases or human causes are limited. Additionally, owing to the extended generation time and heterozygosity of date palm cultivars, development of Egyptian date palm cultivars using conventional breeding programs has encountered challenges. In recent years, significant advancements in plant biotechnology have complemented traditional approaches for date palm multiplication, conservation, and genetic improvement **(Bekheet, 2013)**. The date palm may have a significant ecological impact on a variety of desert and semi–arid environments **(Badawy *et al.*, 2005)**.

Worldwide, an estimated 150 million date palm trees have been developed utilizing a variety of propagation techniques, including seed germination and offshoot transplanting as natural and traditional means of propagation, as well as small-scale tissue culture technologies **(Al-Khayri *et al.*, 2015)**. Due to the heterozygosis of the seed propagated date palm, it cannot be used for commercial production of selected cultivars **(Tisserat, 1982)**, due to the significant differences in fruit maturation, fruit quality, harvesting time, and production potential between plants propagated vegetative and seedlings **(Zaid *et al.*, 2011)**. Whereas true to type date palm propagation by offshoots is a sluggish process that is often impeded by the restricted number of offshoots that a tree produces during its life, a poor survival rate, and the potential of disease spread such as Bayoud and Red Palm Weevil infection **(Al-Khalifah and Askari, 2011)**.

The micro-propagation is a potential method for producing vast quantities of disease-free and pest-free plants for plant material exchange, as well as genetically identical and high-quality planting material. Due to the rising demand for unusual and superior quality date palm cultivars, micro-propagation is an inescapable means of propagation **(Al-Mayahi, 2020)**. The effectiveness of plant tissue culture as a technique of plant multiplication is highly dependent on the composition of the growing medium **(Al-Mayahi *et al.*, 2020)**.

The tissue culture method of propagation is the most potential tool for the efficient production of high-quality plant materials **(Sane *et al.*, 2006)**. Due of the inherent limits of traditional date palm growth by offshoots **(Al-Khayri, 2011)**. For date palm micro-propagation, somatic embryogenesis is regarded the most effective regeneration mechanism **(Fki *et al.*, 2003)**. It is considered to be a rapid and effective approach for large-scale date palm propagation and may also be very beneficial for breeding operations.

The induction of embryogenic callus in date palm is controlled by a variety of factors, including genotype, explant type, induction duration, and plant growth regulators (PGRs). In the case of either shoot tips or leaf primordial, large amounts of auxins have been employed to produce embryogenic callus. The most efficacious auxin is 2,4-dichlorphenoxyacetic acid (2,4-D), which is used to induce embryonic callus in date palm **(Behnas *et al.*, 2014)**. However, **Fki *et al.* (2011)** indicated that large dosages of 2,4-D may generate somaclonal variation. As a result, several researchers induced somatic embryogenesis using lower 2,4-D doses or different auxins.

To begin the process of *in vitro* date palm formation by indirect organogenesis or somatic embryogenesis, relatively large doses of 2,4-D or NAA must be applied. However, it is well established that these auxins are related with genetic instability in plants **(Behnas *et al.*, 2014)**. Numerous research have been conducted on date palm micro-propagation using a variety of explants, among which shoot tips of young offshoots **(Al-Khayri and Naik, 2017; Tisserat, 1984)** and juvenile spathes **(Fki *et al.*, 2003; Jatoi *et al.*, 2019)** have been proven to be sustainable and prolific. However, *in vitro* conditions may result in the development of multiple genetic and epigenetic variations in date palm plantlets generated by tissue culture owing to the stress caused by plant growth regulators and the prolonged production or multiplication cycle **(Mirani *et al.*, 2020)**.

Date palm cultivars fall into three groups: soft, semi-dry, and dry, based on the texture of the fruit when it is ripe. There are a lot of countries that produce dates. Egypt is the first country that produces the most dates. Saudi Arabia, Iran, the United Arab Emirates, Pakistan, Algeria, Sudan, Oman, Libya and Tunisia are the 10 countries that produce the most of these dates **(Kader and Hussein, 2009)**. The ISSR approach is one of the efficient methods for determining date palm genetic diversity **(Haider *et al.*,**

2012). Numerous research has examined the use of ISSR markers to measure date palm genetic variation **(Adawy *et al.*, 2011; Zehdi-Aziuzi *et al.*, 2009)**. It has been demonstrated by numerous researchers that ISSR can detect genetic variety in date palms and has the capability to amplify polymorphism fragments, which is consistent with our findings. For example, **Zehdi *et al.* (2004)** used seven ISSR primers to analyze the genetic diversity of 12 cultivars of Tunisian dates and were able to achieve a significant polymorphism rate. They found that there were enough polymorphic bands in their study to distinguish between all cultivars. **Cullis (2011)** successfully distinguished date palm cultivars in a different study utilizing RAPD, AFLP and ISSR markers. Each of the aforementioned indicators can be used effectively both individually and in combination.

II. MATERIAL AND METHODS

This investigation was conducted in the Central Laboratory of Date Palm Researches and Development - Agricultural Research Center, Egypt during 2022-2023.

2.1- Plant materials:

The date palm Zaghloul cv. offshoots had a height of 80-100 cm, a diameter of 30 cm, a weight of 25-35 kg, and was two years old. Offshoots were carefully separated from field-grown mother trees in Natron Valley and immediately transferred to the laboratory for use as mother plant material, as shown in **Fig (1)**.

Fig.1: The date palm Zaghloul cv. Offshoots

2.2- Leaf primordial explant separation and sterilization:

To remove the leaf primordial explants, the leaves and fiber sheath were cut acropetally from the offshoot using a hatchet and a sharp knife. Separation of mature leaves began at the base and continued until the shoot tip material attained a width of 18 cm and a length of 30 cm.

To prevent browning of the explants, they were placed in beakers containing antioxidant solutions (100 mg/l citric acid and 150 mg/l ascorbic acid) for 20 minutes, then thoroughly washed in running tap water for 30 minutes. The outermost leaves were then removed and the palm heart was reduced to approximately 10 cm in length (measured from the meristem base to the leaf apex of the central cylinder) using a sterile blade. It was then surface sterilized in a laminar flow chamber by flaming once. The explants (shoot tips and leaf primordial) were surface sterilized under aseptic conditions by immersion in a 50% Clorox solution (5.25% sodium hypochlorite) (NaOCl) containing two drops of tween 20 for 20 minutes with continuous stirring, followed by three sequential one-minute rinses in sterile distilled water. Following that, remove two more leaves around the shoot tip and immerse in 30% NaOCl for 30 minutes then thoroughly rinsing with sterile distilled water. Finally, the explants were soaked for 10 minutes in a 0.1% mercuric chloride ($HgCl_2$) solution and washed three times with sterile distilled water. Following surface disinfestation, shoot tips ranging in length from 3-5 cm and inner and primary leaflets generally ranging from 1-3 cm were separated and sliced into three to four pieces. The explants were split into pieces of 0.5 cm in length. On the culture medium, leaf segments were put with the abaxial surface. The trials were divided into four stages: callus induction, callus proliferation, somatic embryo differentiation and plant regeneration.

2.3- Culture Medium

The basal nutrient medium used was MS medium **(Murashige and Skoog, 1962)** supplemented with (mg/l): 0.5 nicotinic acid, 0.5 pyridoxine-HCL, 2.0 glycine, 1.0 thiamine-HCL, 100 myo-inositol, 2.0 biotin, 40 mg/l adenine sulphate, 200 glutamine, 170 mg/l $NaH_2PO_4.2H_2O$, Ca-pantothenate 0.2 g/l, 1.0 activated charcoal (AC), 35.0 g/l sucrose and solidified with 6.0 g/l agar in addition different plant growth regulators according to each growth stage. The pH of all media was adjusted to 5.7 with NaOH or HCl then the medium was distributed in 40 ml aliquots in 250 ml jars. The culture medium was autoclaved for 25 min at 121 °C and 1.4 Kg cm^{-2}.

2.4- Embryogenic callus induction

- To avoid browning of explants, all surface sterilized explants were grown for 3 weeks on MS medium devoid of growth regulators and supplemented with Polyvinyl Pyrrolidone (PVP) at doses of 0.0, 0.1, 0.5 and 1.0 g/l.
- The juvenile leaves were grown in ¾ MS medium supplemented with varied doses of Naphthalene acetic acid

(NAA) (0.0, 2.0, 5.0 and 10.0 mg/l), 1.0 mg/l 6-Benzyl amino purine (BAP), and 2.0 mg/l 6-dimethylallyl amino purine (2ip). All cultures were incubated at 26°C in the dark and re-cultured every six weeks on the same fresh medium. On the juvenile leaves, white nodular embryonic callus was noticed after three subcultures.

- After four months, juvenile leaves with initiation callus were grown on ¾ MS medium supplemented with NAA at a concentration of 5.0 mg/l and BA at 0.5, 1.0 and 2.0 mg/l. All cultures were incubated at 26°C in the dark and re-cultured every six weeks in the same fresh medium for three subcultures.

2.5- Somatic embryos proliferation

After the induction period (8 months), 0.5 g of embryogenic callus was separated from the original explants and transferred to MS medium containing 40.0 g/l sucrose, 6.0 g/l agar and 1.0 g/l activated charcoal, as well as TDZ at various concentrations (0.0, 0.5, 1.0 and 2.0 mg/l), BA at 1.5 mg/l and NAA at 0.5 mg/l (differentiation medium), in order to stimulate differentiation of embryogenic callus into somatic embryos. For three months, the cultures were kept in the dark at 27°C and sub-cultured every four weeks under the same culture conditions. After three subcultures, the proportion of somatic embryos that proliferated (%), the number of somatic embryos (embryo/culture), and the length of embryos (cm) were determined.

2.6- Somatic embryos maturation and germination

From the previous stage, the somatic embryo clusters were divided into small clusters containing around 8-13 embryos. The embryo clusters were cultivated in jars (150 ml) containing 45 ml modified MS media supplemented with BA at 0.5, N^6-furfuryladenine (Kin) at 0.1 and NAA at 0.05 (mg/l) (germination medium), as well as the control treatment. The cultures were maintained and proliferated by sub-culturing at intervals of 6 weeks for three sub-cultures, nine similar jars were utilized. The cultures were incubated at a temperature of 27±1°C and a photoperiod of 16 hours with a light intensity of 1500 lux. After three months, the germination (%), the number of shoots per jar, the number of leaves per jar and the length of the leaves (cm) were all recorded.

2.7- Regeneration and shoots multiplication

After germinating the somatic embryos, the cluster of shoots was moved to MS medium supplemented with 200 mg/l NaH_2PO_4, 40 g/l sucrose, 200 mg/l casein hydrolysate, 1.5 g/l charcoal, 2.0 mg/l BA and 0.5 mg/l NAA. The cultures were incubated in a culture room at 28±2°C with a 16-hour photoperiod provided by fluorescent lights and three subcultures were conducted at a 6-week interval.

2.8- The genetic stability among regenerated date palm shoots in comparison with their mother plant

The several regenerated shoots were studied at the molecular level using ISSR analysis in order to study the genetic similarities among the regenerated date palm shoots induced from the leaves explants of Zaghloul cultivar.

2.8-1 PCR reaction using ISSR primers

The following ingredients were used to construct the ISSR amplification reactions: 25 ng of template DNA, 0.2 µM dNTPs, 0.7 mol of primer, 1.0 µl x 10 PCR buffer, 1.5 µM of $MgCl_2$, and one unit of Taq polymerase (Cinnagen, Iran) in a final volume of 10 µl. The reactions were designed for 40 cycles of pre-denaturation at 94 °C for 4 min, followed by denaturation at 94 °C for 30 s, annealing for each primer for 45 s and extension at 72 °C for 2 min. The amplifications were carried out using a PEQStar 96 Universal Gradient 96 wells thermal cycler. After 40 cycles, there was a final 7 min extension at 72 °C. The polymorphism of DNA fragments was assessed using six ISSR primers. The DNA fragments were amplified and then separated by electrophoresis in 1.5% agarose gel. The fluoroDye dyed the DNA. Using the Uvitec Geldoc technology, DNA fragments were seen and recorded.

2.9- Statistical Analysis

The design of the experiments was completely random, with six replicates of each treatment. MSTAT, a computer program, was used to look at the best three results from each treatment. Ducan's Multiple Range Test was used to compare the means of different treatments to see if they were different.

III. RESULTS AND DISCUSSIONS

3.1- Embryogenic callus induction

- Regarding the impact of PVP on explant browning, the findings indicated that adding PVP at a concentration of 1.0 g/l greatly decreased the degree of browning when compared to the other concentrations (0.1 and 0.5 g/l), as shown in **Table (1)**.

Table 1. Effect of antioxidant treatment (PVP) on survival, growth value and degree of browning of date palm juvenile leaf explants.

Concentrations of PVP (g/l)	Survival %	Growth value	Degree of browning
0.0	35.2 d	21.4 de	5.11 a
0.1	81.0 c	28.7 c	4.90 ab
0.5	90.0 ab	67.5 b	2.80 c
1.0	100.0 a	88.0 a	1.0 d

These findings corroborated those of **Zaid (1984)**, who discovered that adding PVP to the culture medium inhibited explant browning. According to **Zaid (1987)** the browning of date palm tissue and the surrounding media is caused by phenolic compound oxidation. Browning and consequent explant mortality is a major problem in woody plant tissue culture, which is often related to phenolic chemicals **(Hesami *et al.*, 2020)**. Phenolic substances may activate the polyphenol oxidase enzyme, change cellular metabolism and ultimately oxidase to form the brown precursor Quinine. Antioxidants limit phenolic compound oxidation by altering the oxidation potential of phenolic compound explants, hence alleviating browning symptoms **(Raj *et al.*, 2020)**. Similarly, in *Curculigo latifolia*, adding PVP to the culture media at a concentration of 0.5% decreased browning and infection in shoot-tip explants **(Babaei *et al.*, 2012)**. Antioxidant substances may influence explant growth by promoting the leaf expansion process and encouraging callus formation **(Huh *et al.*, 2017)**.

Thus, the beneficial impact of PVP on lowering the degree of browning in date palm leaf primordial explants was attributed to the explants' ability to absorb phenolic substances. As a result, adding PVP to culture media at a concentration of 1.0 g/l may be reported as the treatment with the lowest infection rate and the greatest callus induction subsequently.

- Leaf segments implanted on ¾ MS medium supplemented with varying doses of NAA, 1.0 mg/l BA and 2.0 mg/l 2ip, demonstrated a variety of growth responses. Explants treated with 10.0 mg/l NAA, 1.0 mg/l BA and 2.0 mg/l 2ip had a considerably greater frequency of callus development on leaves (87.50%) than those supplied with 5.0 mg/l NAA, 1.0 mg/l BA and 2.0 mg/l 2ip (44.65%), as shown in **Table (2)** and **Fig. (2)**. The medium supplemented with 2.0 mg/l NAA, 1.0 mg/l BA and 2.0 mg/l 2ip exhibited the minimal explant response (13.20%).

According to the findings in **Table (2)**, plant growth regulators (NAA, BA and 2ip) were beneficial at initiating callus formation. On the surface of the leaf explants, the maximum percentage of callus induction (81.90%) was found in ¾ MS medium supplemented with 10.0 mg/l NAA, 1.0 mg/l BA and 2.0 mg/l 2ip; callus induction was lower in other treatments. The control treatment did not result in the formation of the callus.

Table 2. Effects of different concentrations of NAA with added BA at 1.0 mg/l and 2ip at 2.0 mg/l on callus initiation of date palm leaf primordial explants of Zaghloul cv. cultured in vitro for 4 months.

Treatments	Growth value	Callus initiation %
Control	0.00 d	0.00 d
2.0 NAA	13.20 c	24.33 c
5.0 NAA	44.65 b	50.67 b
10.0 NAA	87.50 a	81.90 a

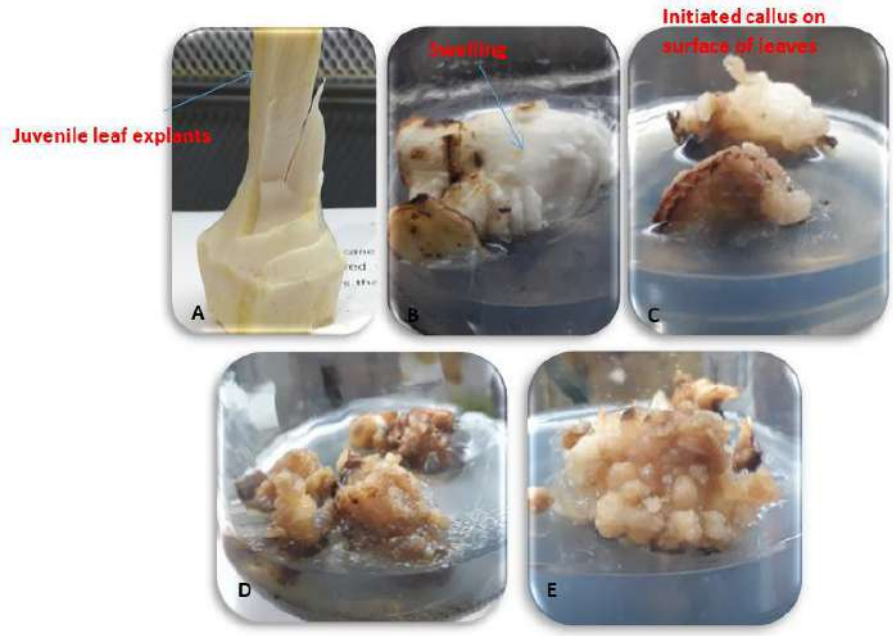

Fig.2: Induction of callus from juvenile leaf explants of date palm cv. Zaghlol; a) Juvenile leaf explants were around the shoot tip, B) the swelling of explants, C) initiated callus on the surface of leaf explants after six weeks on induction medium, D) The control treatment did not resulted in the formation of callus, E) the maximum percentage of callus induction was recorded in MS medium supplemented with 10.0 mg/l NAA, 1.0 mg/l BA and 2.0 mg/l 2ip.

The previous research has shown that plant growth regulators have an effect on the induction callus. The kind and dosage of growth regulators, as well as the type of explants, are the most critical factors in callus formation **(George *et al.*, 2008)**. Additionally, the amount of endogenous auxin and the position of explants **(Almeida *et al.*, 2012)**, particularly the kind and characteristics of tissue vascularization, are likely to affect *in vitro* responses. Numerous variables influence callus initiation, including plant species, culture conditions, explant age and explant placement **(Shin *et al.*, 2019)**. Auxin's beneficial impact on callus induction may be due to low endogenous auxin levels in the explants. Exogenous auxin may influence the amount of endogenous auxin by regulating a range of auxin-related enzymes **(Machakova *et al.*, 2008)**.

Gueye *et al.* (2009) established callus from seedling leaves of the cultivar Ahmar. A rather high auxin concentration, 15.0 mg/l NAA, either alone or in conjunction with cytokinin, significantly increased callus initiation and growth (BA or Kin). The leaf explants grown on Eeuwen's medium at various NAA concentrations (5.0–20.0 mg/l) developed callus at all NAA levels **(Asemota *et al.*, 2007)**. To date, the most frequently employed growth phyto-hormones for plant regeneration are 2,4-D, BA and NAA. In this investigation, we evaluated several combinations of NAA, 2ip and BA to see which combination produced the highest callus percentages **(Liu *et al.*, 2018)**. According to **Liu *et al.* (2021)**, an adequate concentration of auxin induced callus formation in *F. mandshurica*. The proportion of callus induction increased significantly with increasing NAA levels in the range of 0.1 to 0.15 mg/l.

- The callus began to form during the fourth week of culture on induction medium. After 4 sub-cultures, the callus generation rate remained consistent but callus weight maintained growing. The maximum development value (94.50%) of embryogenic callus was obtained on ¾ MS medium combined with 5.0 mg/l NAA and 2.0 mg/l BA. The leaf primordial explants, revealed callus development rates of 100.0%. Additionally, with this treatment resulted in the best fresh weight of callus (3.53 g), as shown in **Fig (3)**.

The Lowest concentration of BA at 0.5 mg/l with NAA at 5.0 mg/l, exhibited the least reaction as growth value of callus embryogenesis (25.33%), percentage of callus formation (43.56%) and fresh weight of callus was (1.02 g). By raise the concentration of BA to 1.0 mg/l with adding 5.0 mg/l NAA on MS medium recorded the satisfactory findings, as shown in **Table (3)**. The control treatment did not exhibited any callus development activity on the explants.

As a result, it is possible to consider that the mixture of NAA and BA acts as a critical hormone in the development of embryogenic callus.

Table 3. Effects of different concentrations of BA with added NAA at 5.0 mg/l on callus growth of date palm leaf primordial explants of Zaghloul cv. cultured in vitro for 4 months.

Treatment (mg/l)	Growth value	Callus formation%	Fresh weight (g)
Control	0.00 d	0.00 d	0.00 d
5.0 NAA + 0.5 BA	25.33 c	43.56 c	1.02 bc
5.0 NAA + 1.0 BA	38.00 b	56.77 b	1.98 b
5.0 NAA + 2.0 BA	94.50 a	100.0 a	3.53 a

Cytokinins are necessary for the formation of calluses and cell division **(Minocha, 1987)**. The addition of BA to the culture medium in combination with auxin increased callogenesis rates in *Acacia raddiana* **(Sane *et al.*, 2006)**. Callus development requires a balance of auxin and cytokinin administration **(Rout, 2004)**.

Balzon *et al.* (2013) demonstrated that lowering the auxin concentration was critical for establishing repetitive cycles of cell division and inhibiting differentiation processes, therefore enabling *E. guineensis* calli to proliferate. **Salim (2014)** cleared that among the combinations of NAA and BA evaluated for callus induction on various explants (seed, node and internode), results indicated that NAA concentrations of 0.3 and 0.4 mg/l were the most effective for callus induction. **Al-Mayahi (2015)** has observed embryos regeneration from cv. Quntar leaf explants. This innovative method has the potential to significantly improve date palm cultivar *in vitro* propagation. Nonetheless, further research is required to enhance the incidence of somatic embryogenesis from leaf explants.

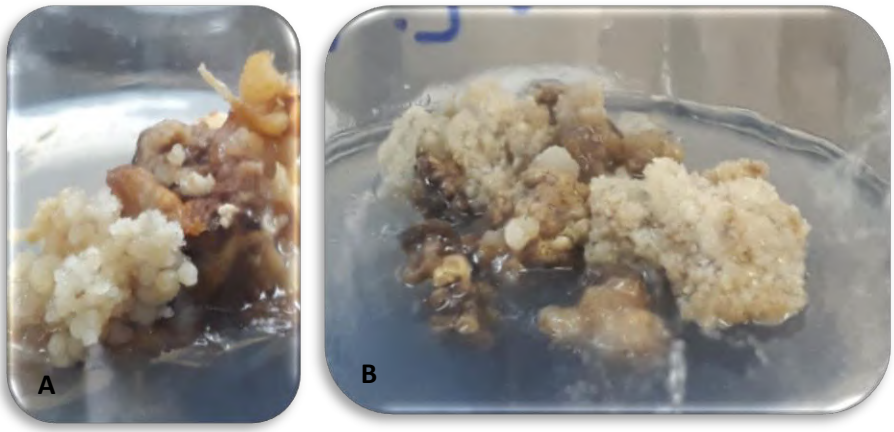

Fig.3: The embryogenic callus production from leaf explants, A) MS basal medium containing BA at 0.5 mg/l with NAA at 5.0 mg/l, exhibited the lowest result as growth value of callus embryogenesis (25.33%), B) the maximum embryogenic callus formation was obtained on ¾ MS medium combined with 5.0 mg/l NAA and 2.0 mg/l BA after 3 subcultures.

In the somatic embryogenesis of oil palm, callus formation occurred in the addition of auxin, either at full strength or ½ MS basal medium **(Rival and Parveez, 2005)**. Notably, the presence of BA to the medium seems to give additional options for the regeneration of plants. Additionally, the usage of various quantities of plant growth regulators had a substantial effect on the development of callus **(Liu *et al.*, 2018)**.

3.2- Somatic embryos proliferation

The somatic embryos were seen in this work by incubating embryogenic calluses on MS medium supplemented with various doses of TDZ, 1.5 mg/l BA and 0.5 mg/l NAA. The embryos were white in color, globular in form as they matured and appeared alone or in clusters. According to the findings in **Table (4)**, the TDZ concentration had an effect on the proliferation percentage, the number of somatic embryos and the length of somatic embryos.

Concerning the percentage of somatic embryos that proliferated, the best results 65.67% was obtained on MS medium supplemented with 2.0 TDZ, 1.5 BA and 0.5 NAA, as compared to the other TDZ doses. While raising the TDZ concentration to 3.0 mg/l lowered the proliferation rate of embryos to 40.51%, as shown in **Fig (4)**.

On the other hand, TDZ concentrations had an effect on the number of somatic embryos; MS medium contained 2.0 mg/l TDZ, 1.5 BA and 0.5 NAA was shown to be the optimal medium for increasing the number of embryos to 32.10 embryos/culture. By increasing the TDZ concentration to 3.0 mg/l, the number of embryos per culture was reduced to 23.16 embryos. Low doses of TDZ (0.5 and 1.0 mg/l) in the presence of 1.5 BA and 0.5 NAA produced the fewest somatic embryos (11.20 and 20.00 embryos/culture, respectively).

In terms of somatic embryo length, MS medium supplemented with 2.0 TDZ, 1.5 BA and 0.5 NAA (mg/l) resulted in embryos measuring 1.5 cm in length, compared to 0.5 cm in the control treatment. TDZ concentrations of 0.5 and 1.0 mg/l resulted in embryos ranging in length from 0.8 to 1.2 cm.

Table 4. Effect of different concentrations of TDZ with added 1.5 BA and 0.5 NAA (mg/l) on somatic embryos proliferation of Zaghloul date palm cultivar after 12 weeks of culturing.

Treatment (mg/l)	Somatic embryos proliferation %	Number of somatic embryos	Length of somatic embryos (cm)
Control	8.00 e	4.33 d	0.5 cd
0.5 TDZ, 1.5 BA, 0.5 NAA	22.15 d	11.20 c	0.8 c
1.0 TDZ, 1.5 BA, 0.5 NAA	30.10 c	20.00 b	1.2 b
2.0 TDZ, 1.5 BA, 0.5 NAA	65.67 a	32.10 a	1.5 a
3.0 TDZ, 1.5 BA, 0.5 NAA	40.51 b	23.16 b	1.0 bc

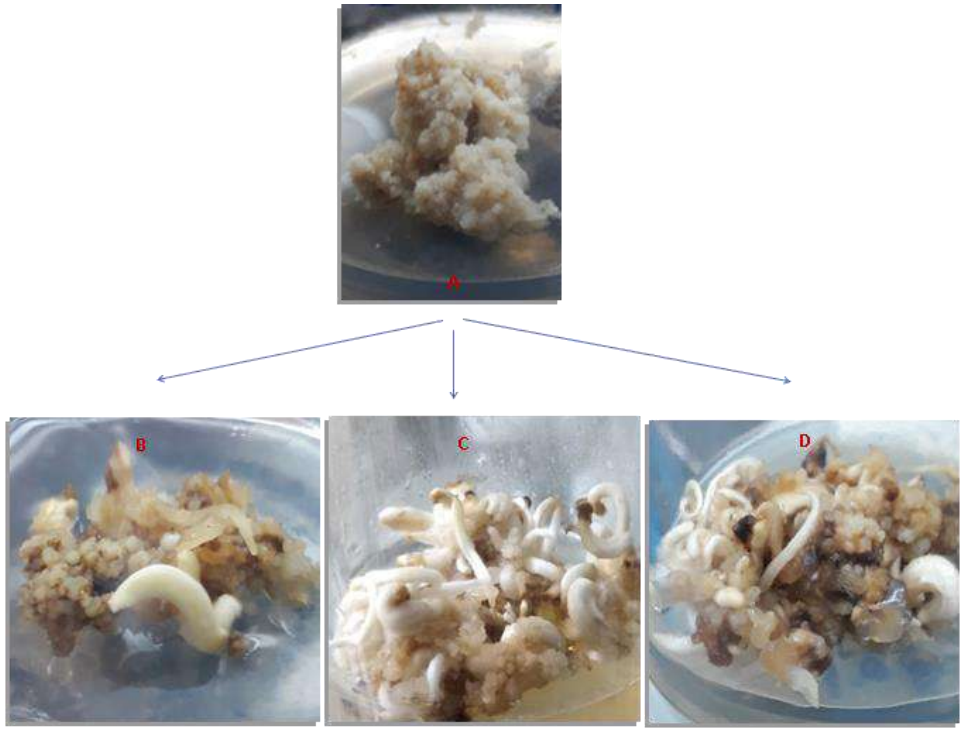

Fig. 4. The embryogenic callus germinated, A) Embryogenic callus was formed, B) The control treatment recorded the fewest number of somatic embryos, C) MS medium supplemented with 2.0 TDZ, 1.5 BA and 0.5 NAA shown to be the optimal medium for increasing the number of embryos, D) By increase the TDZ concentration to 3.0 mg/l lowered the proliferation rate of embryos.

In previous research, **Rival and Parveez (2005)** somatic embryogenesis in oil palm as a multistep process that includes induction, embryogenesis, somatic embryo development and maturation, shoot development and rooting. The explants are stimulated to develop primary callus in auxin-containing media under dark conditions during the induction stage. The callus is also transferred to auxin-containing media during the embryonic stage, however at lower concentrations and often under light conditions. This stage promotes the development of proliferating callus into embryogenic cell clusters.

Organogenesis requires a ratio of auxin to cytokinin of 10/0.4/0 or equal. In *Damask rose*, combining TDZ and BA resulted in considerably more shoots per explant than the most optimal BA treatments alone **(Mamaghani *et al.*, 2010)**. Additionally, treatment with TDZ promoted development and increased the quantity of somatic embryos. TDZ has been proposed to enhance nucleoside levels, purine cytokinin accumulation or synthesis, or to accelerate adenine to adenosine conversion **(Capelle *et al.*, 1983)**. TDZ enhanced the explants' embryogenic response throughout normal growth and development; same effect has been reported in a variety of different species. TDZ is very stable in culture medium and has a long half-life in plant tissues **(Mok and Mok, 1985)**. **Victor and colleagues (1999)** demonstrated that TDZ efficiently promoted somatic embryogenesis in peanut after a minimal exposure period. TDZ may have a double function in the induction of somatic embryogenesis: 1) cytokinin-like activity that stimulates cell division and differentiation and 2) a modest auxin-like activity that seems to be required for the development of embryogenic competence.

Wójcikowska *et al.* (2013) discovered that auxin treatment accelerated somatic embryogenesis by activating transcription factors, particularly leafy cotyledon 2, a transcription factor that regulates IAA production in explants. In oil palm, somatic embryogenesis has been described mostly as an indirect process **(Hilae and Te Chato, 2005)**. According to **Baharan *et al.* (2015)**, plant growth regulators (2,4-D, TDZ and BA) were effective in inducing callogenesis. The maximum regeneration ratio was seen in medium supplemented with 5.0 mg/l BA or 5.0 mg/l TDZ, whereas other treatments induced fewer calluses. However, when shoot tips were cultured with 2,4-D and TDZ at 10.0 mg/l, somatic embryogenesis occurred **(Sidky and Eldawyati, 2012)**.

3.3- Somatic embryo maturation and germination

MS basal medium supplemented with BA at 0.5 mg/l, kin at 0.1 mg/l and NAA at 0.05 mg/l was examined for further enhancement and maturation of somatic embryogenesis, and then these embryos formed shoots concurrently. After three weeks of culture, the mature

embryos developed a green color. Germination of somatic embryos was detected in control and MS medium treated with combinations of plant growth regulators, with germination percentages ranging from 32.4 to 87.5%. According to **Table (5)**, the most leaves number (20.11 leaf/jar) were found in MS basal medium with BA at 0.5 mg/l, kin at 0.1 mg/l and NAA at 0.05 mg/l, as shown in **Fig (5)**. In comparison, the medium devoid of plant growth regulators produced the fewest leaves (8.43 leaf/jar).

Table 5. Effect of BA at 0.5 mg/l, kin at 0.1 mg/l and NAA at 0.05 mg/l on germination of somatic embryos of Zaghloul ate palm cultivar after three subcultures.

Treatment (mg/l)	Somatic embryos germination (%)	Number of shoots	Number of leaves	Length of leaves (cm)
Control	32.4 b	2.50 b	8.43 b	1.60 b
0.5 BA, 0.1 kin, 0.05 NAA	87.5 a	8.00 a	20.11 a	3.33 a

Fig. 5: Improved the somatic embryos production and shoots regeneration of date palm, cv. Zaghloul after three sub-cultured on MS medium supplemented with BA at 0.5 mg/l, kin at 0.1 mg/l and NAA at 0.05 mg/l.

Numerous variables have been related with somatic embryo germination in prior studies. In terms of PGRs, **Othmani *et al.* (2009a)** recommended an NAA concentration of 1.0 mg/l for date palm cv. Boufeggous, whereas **Zouine and El Hadrami (2007)** enhanced somatic embryo germination in date palm cultivars Bousthami Noir and Jihel, using a mixture of NAA, IBA and BA. **Fki *et al.* (2003)** observed that when germinated date palm cv. Deglet nour embryos were transferred to a medium enriched with 1.0 mg/l NAA and BA, healthy plantlets with balanced shoot and root development were produced.

Sane *et al.* (2006) reported on the effect of NAA on the conversion of developed somatic embryos in date palm cv. Amsekchi and cv. Boufeggous cultures. When 1.0 mg/l NAA was administered, the average number of somatic embryos was 50.66 embryo/culture, the frequency of germination (83.50%) and the frequency of conversion (94.50%) were determined **(Othmani *et al.*, 2009b)**. Similar needs for cytokinins such as 2ip **(Badawy *et al.*, 2005)**, BA **(Zouine *et al.*, 2005)** and Kin **(Meziani *et al.*, 2015)** have been identified to stimulate growth, development, shoot morphogenesis and maturation into date palm.

BA is considered a possible cytokinin capable of accelerating the differentiation and development processes by causing fast cell division. **Aslam and Khan (2009)** discovered that BA was more effective than kinetin in increasing the frequency of numerous shoots in date palm, corroborating the current study's findings. The various doses of 2ip alone or in combined with Kin or IBA were extremely efficient in germination and development of somatic embryos and plantlets in both types tested **(D'Onofrio and Morini, 2005)**. We investigated somatic embryo germination and plantlet conversion using a variety of BA and Kin concentrations and discovered that BA was particularly efficient in promoting germination and plantlet conversion. Previously, it was found that the same cytokinins, alone or in association with auxin, were quite successful in somatic embryogenesis in a variety of plants **(Nasim *et al.*, 2009; Ghanti *et al.*, 2010)**.

Transfer of date palm cultivar embryos to MS medium supplemented with 1.0 mg/l NAA and 1.0 mg/l BA resulted in the formation of viable plantlets with uniform shoot and root development **(Zouine *et al.*, 2005)**. Recently, a somatic embryogenesis pathway was reported using leaf segments from *in vitro* shoots of date palm cv. Quntar **(Al-Mayahi, 2015)**; these explants would be ideal for large-scale propagation because their somatic origin, seasonal independence and availability. **Mazri *et al.* (2017)** also detected somatic embryogenesis in proximal leaf segments isolated from *in vitro*-grown shoots.

Letouze *et al.* (2000) demonstrated that when the hormonal combination during the induction stage is changed appropriately, the embryogenic callus pathway

may result in the development of somatic embryos and organogenesis. The dynamic equilibrium in the auxin and cytokinin concentrations in the media would have resulted in fast shoot and root growth, hence reducing the total time required for regeneration, which is rather unique in this research. Our findings indicated that 32.4 to 87.5 (%) of somatic embryos germinate. Other date palm cultivars have been documented to have varying rates of somatic embryos germination. **Al-Khayri and Al-Bahrany (2012)**, for example, observed germination rates of 17.5–72.5 (%) in cv. Naboul Saif. Germination frequencies of somatic embryos derived from cvs. Khusab, Berny and Barhee were between 60% and 75% **(Al-Khayri, 2011)**.

Mazri *et al.* (2018) found that MS medium supplemented with 2.5 µM NAA and 2.5 µM BA had the greatest rate of somatic embryo germination (52.0%). For the cv. Safawi, the optimal medium for promoting somatic embryos germination (%), shoots development and shoots length was 6.0 mg/l 2iP + 3.0 mg/l Kin + 0.5 mg/l IBA. While the ideal medium comprised 6.0 mg/l 2iP + 2.0 mg/l Kin + 1.0 mg/l IBA for cv. Magdoul **(Metwali *et al.*, 2020)**.

3.4- Regeneration and shoots multiplication

The results demonstrated the critical importance of using the optimal hormone combinations for successful shoots regeneration. After three sub-culturing, the cluster of shoots cultured on MS basal medium supplemented with BA at 2.0 mg/l and NAA at 0.5 mg/l possessed the best values for leaf number (34.25) and leaf length (4.75 cm), as shown in **Fig (6)**.

Fig.6: The cluster of shoots cultured on MS basal medium supplemented with BA at 2.0 mg/l and NAA at 0.5 mg/l resulted the best shoots multiplication.

Numerous parameters, most notably the combination of the culture medium and genotype, have been identified to impact shoot multiplication in date palm in earlier research. The combination of differing auxin and cytokinin concentrations may have generated a hormonal balance that enhanced shoot proliferation. Cytokinins are well recognized for their ability to decrease apical meristem dominance and encourage the development of both axillary and adventitious shoots from meristematic explants **(Madhulatha *et al.*, 2004)**. Due to its efficiency and availability, BA is now the most extensively utilized cytokinin in the micro-propagation industry **(Bairu *et al.*, 2007)**.

However, **Al-Khateeb (2006)** observed that low hormone concentrations stimulated the production of new buds, but high hormone concentrations resulted in aberrant growth devoid of budding or shoots development. Clearly, low auxin concentrations in the presence of cytokinin promoted adventitious buds proliferation **(Al-Najm *et al.*, 2018)**. For shoots multiplication, **Beauchesne *et al.* (1986)** recommended ½ MS medium supplemented with 2.0 mg/l NOA, 1.0 mg/l NAA, 1.0 mg/l IAA, 0.5 mg/l BA, 1.0 mg/l 2iP and 1.0 - 5.0 mg/l kin.

Similarly, **Taha *et al.* (2001)** developed an efficient quick technique for *in vitro* multiplication of date palm shoot buds cv. Zaghlool. They employed a medium that had a high concentration of 2ip. **Khierallah and Bader (2007)** reported that a hormone combination of 1.0 mg/l NAA, 1.0 mg/l NOA, 4.0 mg/l 2iP and 2.0 mg/l BAP increased shoots multiplication in MS medium of date palm. **Othmani *et al.* (2009a)** revealed that high rates of shoots proliferation were attained in cv. Deglet Nour on medium containing 1.0 mg/l NAA and 1.0 mg/l BA. For the cultivar Khalas, **Aslam and Khan (2009)** stated that, on medium supplemented with 7.84 µM BA, the greatest shoots regeneration response (in terms of shoot development (%), shoots number and shoots length.

According to **Zaid *et al.* (2011)**, NAA, NOA, IAA, BAP and kin may be utilized at concentrations ranging from 0.5 to 5.0 mg/l for shoots bud proliferation. **Mazri and Meziani (2013)** discovered that ½ MS medium supplemented with 0.5 mg/l NOA and 0.5 mg/l Kin resulted in the formation of 23.5 shoot buds per explant after three months of multiplication in cv. Najda. Additionally, **Bekheet (2013)** observed that adding three various kinds of cytokinins to the growth medium, BA, Kin and 2ip, at concentrations ranging from 0.5 to 5.0 mg/l, increased shoot bud development in date palm cv. Zaghlool.

The optimal medium for shoots regeneration was ½ MS medium combined with 0.5 mg/l NOA and 0.5 mg/l kin, which resulted in an average of 23.5 shoots per explant after three months of multiplication **(Mazri and Meziani, 2013)**. **Al-Mayahi (2014)** proposed growing cv. Hillawi on MS medium with 1.0 mg/l BA and 0.5 mg/l TDZ, which

resulted in an average of 18.2 buds per culture. **Mazri (2015)** suggested that MS medium with 2.5 µM IBA and 2.5 µM BA for cv. 16-bis (22.3 shoot buds/culture), whereas cv. Boufeggous (22.9 shoot buds/culture) required ½ MS media supplemented with 3 µM IBA and 3 µM BA.

After about two subcultures into medium containing 2,4-D at 0.1 mg/l, subculture of secondary shoots on MS medium supplemented with NAA and BA at (1.0 mg/l) ensures both multiplication and development of shoots capable of conversion into plantlets **(Othmani *et al.*, 2018)**. **Baghdady *et al.* (2018)** investigated for shoots multiplication, transfer of cultures to MS medium enriched with BA and Kin, either alone or in combination, after direct appearance of 2-3 shoots. The findings demonstrated that MS medium supplemented with BA at any concentration outperformed the control therapy. In this respect, introducing BA at a concentration of 1.0 mg/l to MS medium resulted in the greatest shoots number and shoots fresh weight values.

3.5- Genetic stability between mother plant and regenerated date palm shoots induced from leaves explants

In **Table (6)** ISSR analysis was done on the DNA of Zaghlol cultivar shoots produced from leaf explants in tissue culture. The ISSR profiles of tissue culture-derived shoots, particularly those induced from leaf explants, clearly showed a high similarity of 97% to the mother tree as shown in **Fig. (7)**.

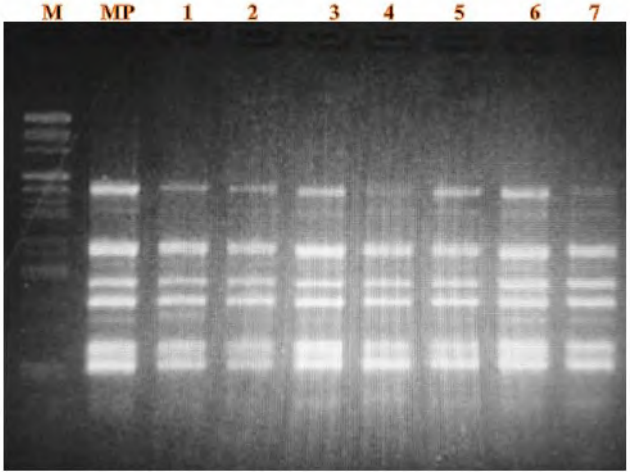

Fig. 7. ISSR profile of regenerated date palm shoots Zaghlol cv. from the leaves explants in comparison to their mother plant. M: 1kp DNA marker, MP: mother plant, Lanes 1 to 7: the regenerated date palm shoots induced from leaves explants. There were no differences in genetic variation (97%).

Table 6. Names and sequences of ISSR primers used in genetic stability among regenerated date palm shoots in comparison with their mother plant.

Primer name	Primer sequence	Annealing Temp. (°C)
DP1	(AGG)6	55
DP2	(AG)10G	60
DP3	(AG)10C	60
DP4	(AG)10T	57
DP5	(CT)10A	57
DP6	(CT)10G	60
DP7	(CT)10T	57

It is possible for regenerated somaclones to differ in chromosomal numbers and structures **(Hao and Deng, 2002; Mujib *et al.*, 2007)**. Fruit cultivars that have been micro-propagated have had their genetic variability studied using inter-simple sequence repeat (ISSR) markers.

Date palm cultivars can have polymorphisms found using the ISSR method **(Abd-Alla, 2010)**. ISSR is a practical and effective method used in many species to identify polymorphisms without knowledge of the DNA sequences. This technique is trustworthy for identifying date palm cultivars. Using ISSR, **Zehdi *et al.* (2012)** evaluated the genetic diversity of a collection of cultivars of Tunisian date palm.

Numerous authors have extensively studied the value of molecular analysis of *in vitro* regenerated plants **(Piatcza *et al.*, 2015; Bhalang *et al.*, 2018)**. Micro-propagated plants' genetic diversity has significant practical advantages and commercial implications.

A successful micro-propagation method should give true-to-type plantlets with no genetic or morphological alteration **(Prakash *et al.*, 2016; Safarpour *et al.*, 2017; Khatab and Youssef, 2018)**. The micro-propagated plants derived from shoot tips and axillary buds have been previously reported to maintain genetic stability **(Borsai *et al.*, 2020)**. Somaclonal variation is often induced by the culture media and subculture cycles **(Bidabadi *et al.*, 2010)**. Therefore, testing of genetic stability of *in vitro* raised plants is necessary to date palm plantlets production. However, in our study, no variability was detected among the plantlets by ISSR assay; Therefore, we can state that there were no somaclonal differences in the tissue culture-raised plantlets used in the current investigation.

IV. CONCLUSION

Explants of date palm Zaghloul cv. inner juvenile leaves were employed to develop an indirect somatic embryogenesis system through callus. When leaf segments were cultivated on MS medium, callus formation occurred on the surface of the leaves. MS medium supplemented with 10.0 mg/l NAA, 1.0 mg/l BA and 2.0 mg/l 2ip was used. Following that, the explants are transferred to MS medium containing 5.0 mg/l NAA and 2.0 mg/l BA to promote the development of embryogenic callus. To differentiate the embryogenic callus, it was grown on MS basal medium with 2.0 TDZ, 1.5 BA and 0.5 NAA. After that, the resulting somatic embryos were transferred to MS basal medium supplemented with BA at 0.5 mg/l, kin at 0.1 mg/l and NAA at 0.05 mg/l for maturation. The cluster of shoots cultivated on MS basal medium supplemented with BA at 2.0 mg/l and NAA at 0.5 mg/l exhibited the greatest regeneration. The date palm Zaghlol cultivar displayed the greatest genetic stability in the *in vitro* culture, according to the results of genetic variation detection using ISSR primers in date palm shoots induced from leaves explants. The detection of specific somaclonal variation in cloned plants using ISSR is another successful application.

KEY MESSAGE

In this paper, we used the juvenile leaves surrounding the shoot tips to study the capability of induction the somatic embryos or not on different media, then we did the ISSR analysis to study the genetic stability of shoots induced from leaf explants.

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Exploration of Road Traffic Emissions and Their Impact on Air Quality in and around of Annaba City (North East Algeria)

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Abstract— *Global climate is warming, and the effects of climate change are associated with many causes; among them are the Greenhouse gas emissions from motor vehicles and factories. The study of exposure to air pollution related to road traffic in Annaba's region is based on the results of air analyses, which shows that air temperatures are frail, accompanied by a very high humidity due mainly to the presence of the sea and many bodies of water (Fetzara Lake). This can allow the accumulation of certain pollutants such as carbon monoxide and dust (contents of CO and dust exceed the WHO standards), while some pollutants are present in small to negligible quantities such as NO, NO_2, and SO. The air quality of Annaba and its surroundings (El Bouni, Sidi Ammar) can be generally described as good. Nevertheless, the existence of strong pollution due to the dust is noted close to the industrial complexes specially El Hadjar.*

Keywords— ***Air quality, Annaba, Climate change, Fetzara Lake, Road traffic***

I. INTRODUCTION

Air quality is a matter of concern because of the health impacts of air pollution and its involvement in global warming through greenhouse gas emissions This effect has been widely studied [1], [2]

Climate change is defined as "any change in climate over time, whether due to natural variability or human activities (road traffic). Thus, climate change is reflected in several phenomena: modification of the Earth's surface temperatures, rise in sea level, melting of snow and ice, disruption of precipitation patterns, then multiplication and intensification of extreme events: floods, droughts [3]

Most urban pollution is due to road traffic, and its contribution is likely to increase further as more than 70% of the world's population will live in cities by 2050 [4]**.** The impact of traffic on the environment can be reflected in the chemical composition of water and air [5]. To better understand and control air pollution, it is necessary to understand, identify and quantify its sources. This way, actions can be taken to reduce emissions at source. Emissions inventories are also a necessary baseline for air quality assessments and for estimating the impacts of this pollution on health and ecosystems.

Road traffic emissions from tailpipes and exhaust sources have a significant impact on the concentration of particles in the urban atmosphere. This effect has been widely studied [6], [7]. In addition, very fine particles emitted by various processes related to road traffic (wear of brakes, for example) can penetrate human organs, many studies have been done on this impact: [8], [9], [10].

Knowing the physicochemical properties and sources of particles is essential to determine the effects of particles on the environment [11]. The exposure to diesel exhaust and other combustion products also increased the risk of lung

cancer. The results of Nyberg *et al*., 2000 indicate that urban air pollution increases the risk of lung cancer and that vehicle emissions may be particularly important.

At the global level, the nightmare lies ahead. With currently more than a billion cars in circulation in the world and an annual production of around 70 million new cars every year, the propagation of automobile is becoming the main culprit of climate change (Figure.01). Taking an average of CO_2 emissions of around 1.8 T per year per car, we can estimate CO_2 emissions from the automobile on a planetary scale at around 2 billion T of CO_2/year [12].

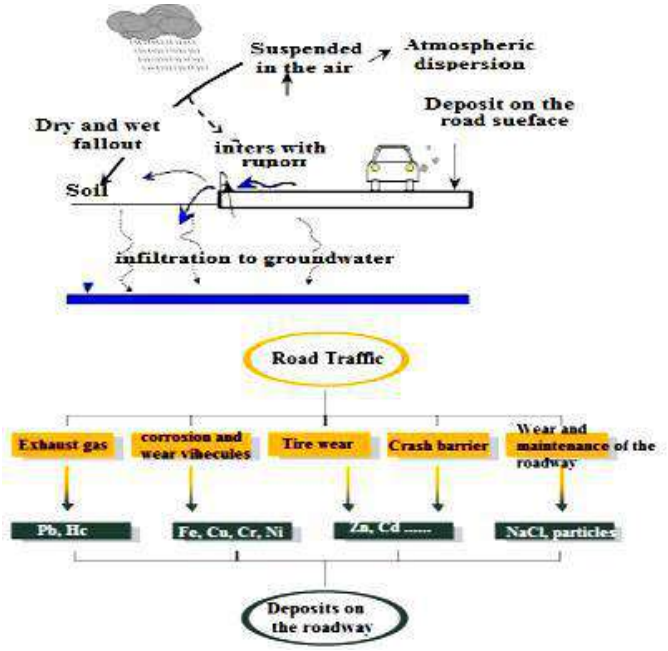

Fig.1 Method of pollutant transfer emissions to soils, air and groundwater [13].

Of course, the automobile is not the only cause of CO_2 emissions, but the continued growth of the automobile fleet and the global production of cars seems to have a definite impact on the growth of both CO_2 emissions and CO_2 concentration in the atmosphere (Figure.02). In 2006, the average carbon dioxide (CO_2) content of the Earth's atmosphere reached the highest levels ever recorded 381.2 ppm (WMO).

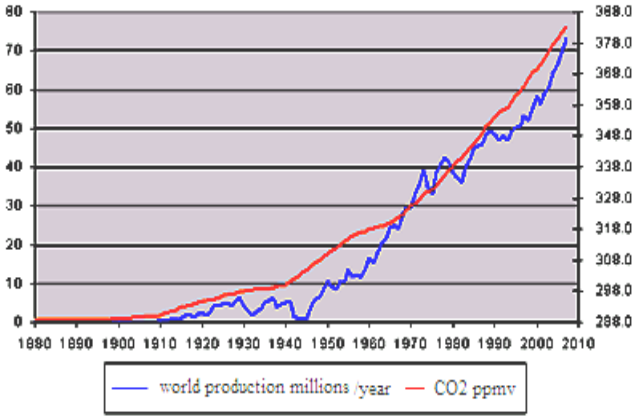

Fig.2 Comparative evolution of global car production and CO_2 concentration in the atmosphere

According to a recent study, the current concentration of carbon dioxide is 27% higher than the maximum reached during the last 650 thousand years. However, the correlation between the increase in the concentration of CO_2 in the atmosphere and the increase in the Earth's temperature has been proven since the four reports of the IPCC (Intergovernmental Panel on Climate Change). The last ten years (1998-2007) are the warmest years ever observed on a planetary scale [14].

II. MATERIALS AND METHODS

2.1 Context of the study site

Annaba city is located 600 km from the capital Algiers, in the extreme east of Algeria, open to the Mediterranean coast for 80 km. It covers 1 439 km_2 or 0.06% of the national territory between latitudes 36 °30' N and 37 °30' N, and longitudes 07 °20' E and 08 °40' E, with 12 municipalities. It is geographically limited by the Mediterranean Sea in the north, Guelma in the south, El-Taref in the east, and Skikda in the west (Figure.3).

Fig.3 Geographical location of Annaba city [15].

The assessment of air pollution in the region of Annaba is subject to permanent monitoring through the establishment of an air quality monitoring system called *SamaSafia*since June 2002.

The installation of the air quality-monitoring network was commissioned in March 2002. For a trial period of three months and began the actual operation in June 2002, but it was stopped in May 2007, this network consists of three stations: Annaba city, El Bouni, Sidi Ammar and Salines (Figure.4)

Fig.4 Location map of the air quality monitoring stations.

2.2 The climate of study area

The climate of the city of Annaba is temperate with an aridity index of DE.Martone located in the range 20<I<30, the study region is characterized by average annual rainfall is estimated at about 800 mm with irregular rainfall distribution. The average annual temperature is around 17.7°C. The Annaba plain is dominated by North to North-East, South-West to North winds [13].

The strongest winds occur in winter and the weakest in summer with a few episodes of SIROCCO, which increase the temperature (Figure.5) and (Figure.6).

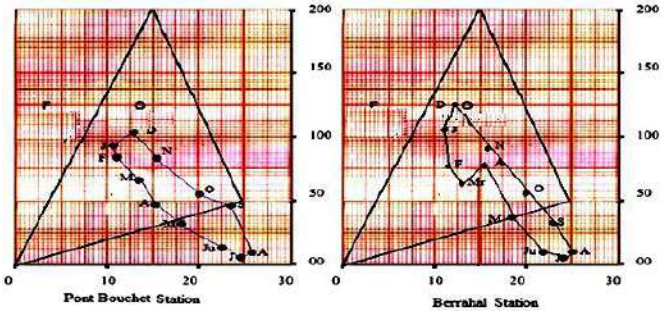

Figure.5 Climogram of PEGUY

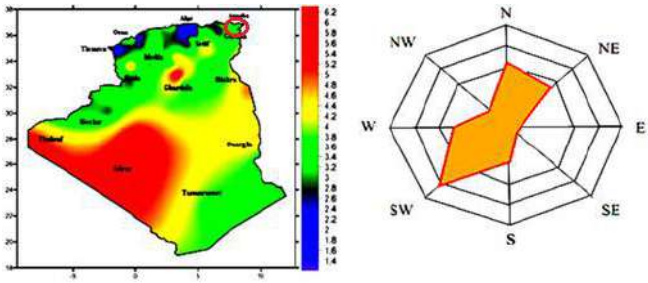

Fig.6 Speed and direction of the wind at Annaba

2.3 Evaluation of the impact of road traffic on air quality in the city of Annaba

2.3.1 Annual statistics of the vehicle fleet in the study area

The city of Annaba is considered one of the most polluted cities in Algeria. On the one hand, there is the existence of a steel complex and a very large fleet of vehicles compared to the distances traveled; on the other hand, certain topographical and climatic characteristics create a climate conducive to pollution [16].

According to the national service of statistics (2014), we could collect the different types of vehicles and their ages as well as the evolution of their number according to time during a period of 14 years (Figure.7a), (Figure.7b). At the level of the Wilaya of Annaba, the fleet has more than 170 thousand vehicles; tourist vehicles occupy 68% of the total number of vehicles in the wilaya. With its numerous traffic

jams, arterial congestion, and challenging parking, the road traffic in Annaba city and its surrounding areas has grown to be quite complex. Long periods of time passed with no medium- or long-term plans for solving this problen **(**Figure.8).

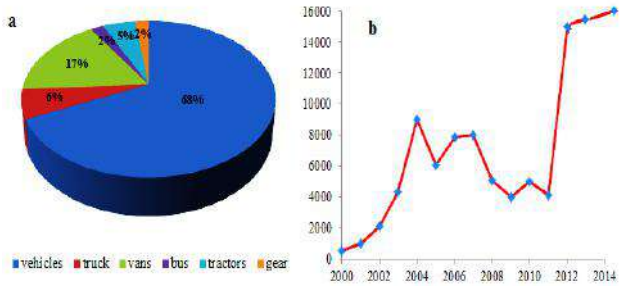

Fig.7 a. Number of vehicles by type. B. Annual evolution of the number of vehicles.

Fig.8 Traffic jams in Annaba city

2.3.2 Estimation of CO_2 release by vehicles according to their age and the type of fuel consumed

The amount of CO_2 generated by a vehicle is directly proportional to the amount of fuel consumed. The release of CO_2 is related to the distance (Figure.9), (Figure.10); the estimated release of CO_2 is increasing exponentially from a distance 10000 km to another 40000 km [17].

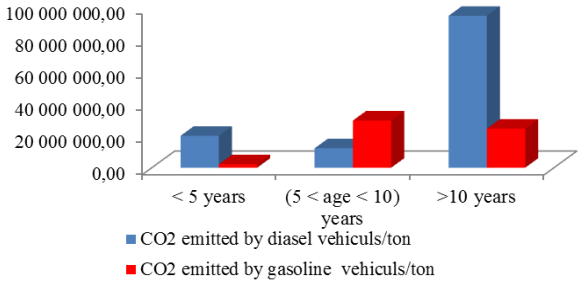

Fig.9 Quantity of CO_2 emitted by type and age of the vehicle

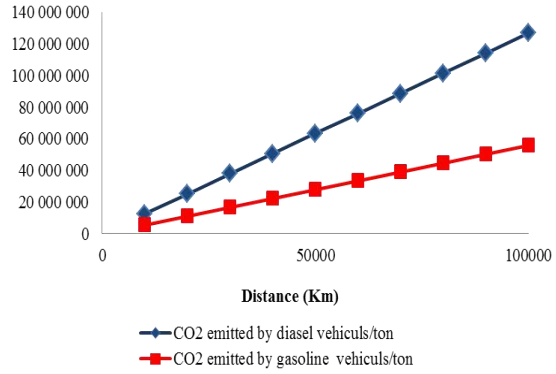

Fig.10 Quantity of CO_2 released by type of vehicle according to the distance traveled

2.3.3 Data analysis

The average values of the concentrations of gas emitted were estimated using MS-Excel, also calculated and represented in the form of graphs. The concentrations were compared to those of the WHO guideline values

III. RESULTS AND DISCUSSION

3.1 Nitrogen Monoxide Variation (NO)

The variation of NO is in function of time (Figure.11) shows that for the station of Sidi Ammar, the NO's concentrations recorded are higher compared to other stations. This pollution is generated by road traffic (motor vehicles), in some boilers and industrial engines, and during some processes of the chemical industry (Arcelor Mittal plant of the industrial complex El Hadjar).

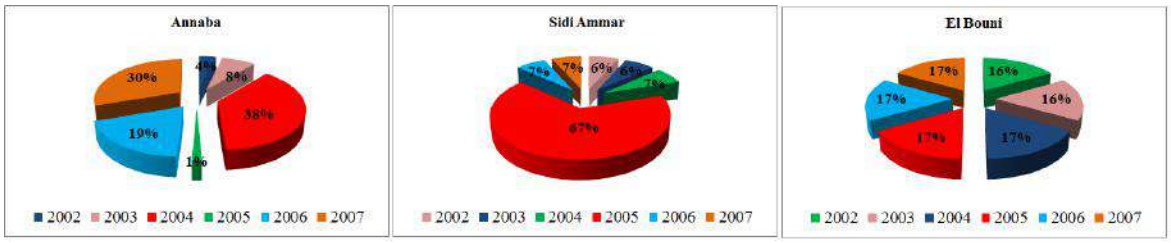

Figure.11 NO annual variation

3.2 Nitrogen Dioxide variation (NO_2)

Nitrogen dioxide (NO_2) associated with fuel combustion is partly emitted directly from the tailpipe and partly formed indirectly in the atmosphere from nitrogen monoxide. The NO and NO_2 concentrations generally increase in cities during peak hours (Figure.12).

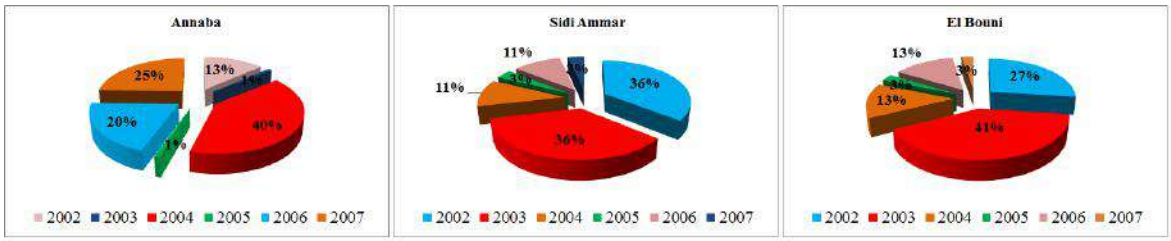

Fig.12 NO_2 annual variation

The station of Annaba city always records important values (Figure.12), but these values remain always weak compared to the standards of WHO (200 µg/m^3).

3.3 Dust Annual variation

The annual variation of dust at the three stations indicates a strong dust pollution (higher than the WHO standard: 50 µg/m^3) at the station of Annaba city, this value reaches 215 µg/m^3 in 2004 (Figure.13).

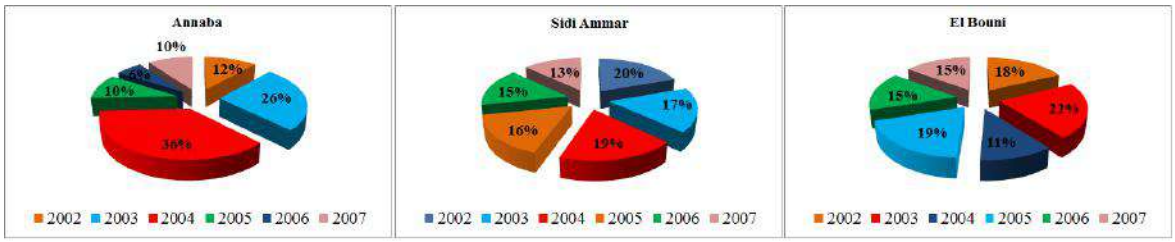

Fig13 Dust annual variation

3.4 SO_2 Annual variation

The annual variation of SO_2 (Figure.14) shows that for the three stations the concentrations remain below the WHO standards (125 µg/m^3).

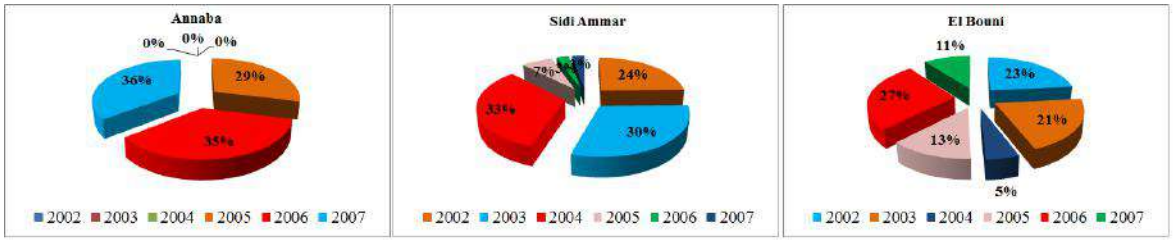

Fig.14 SO_2 annual variation

At the level of Annaba's station, the values vary from 0 µg/m3 (2002, 2003, and 2004), 21.75 µg/m3 (2005) to 22. 1 µg/m^3 (2006, 2007), on the other hand in El Bouni, they range from 22.5 µg/m^3 (2002) to 16 µg/m^3 (2003), with a

maximum recorded in 2004 (24.1 µg/m3), and dropped to 2 µg/m^3, while at the station of Sidi Ammar, they vary from a minimum of 4 µg/m^3 (2004) to a maximum of 28.4 µg/m^3 (2007).

The highest concentrations of SO_2 are recorded in the night phase. They are explained first by the production of primary pollutants at night since most of the factories in the industrial areas El Hadjar and Sidi Ammar operate 24/24h, at night, the thermal inversion, the low speed of the land breeze or mountain present favorable conditions for the accumulation of primary pollutants (SO_2). The latter, whether emitted at night or during the day, are redirected by the night breeze towards the sea after having travelled through the agglomeration [18].

3.5 CO Annual variation

According to the graphical representation of the annual variation of CO recorded at three stations: Annaba city, El Bouni and Sidi Ammar (Figure.15), we notice that the CO is present in the air with strong contents, exceeding the standard of the WHO (0.125 µg/m^3).

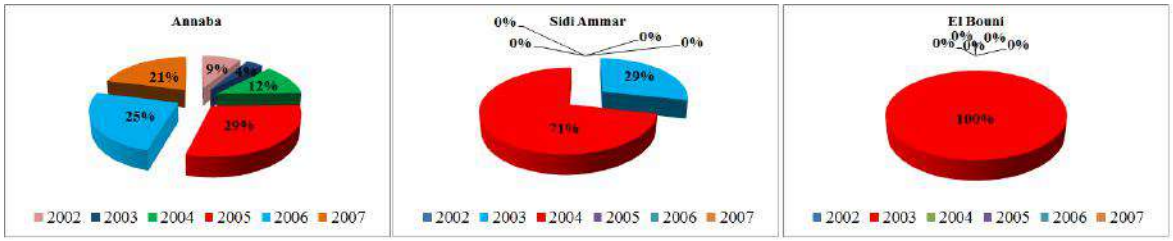

Fig.15 CO annual variation

The highest levels are recorded at Annaba's station with a value of 20 µg/m^3 and reach 2.0 µg/m^3 at the El Bouni station and 0.5 µg/m^3 at the Sidi Amar station. These high concentrations are probably generated by the action of vehicles, especially in Annaba city, the humidity that prevents the dilution of CO in the air as well as weak winds, which promote the accumulation of pollutants.

1.6 Air Quality Indices

How is the AQI Calculated? The index for a given pollutant is its concentration expressed as a percentage of its limit value.

AQI= (Pollutant $_{Concentration}$/Pollutant $_{Standard}$)*100

Pollutant emission limit values have been established by a specific monitoring station. To assess overall air quality, an index is calculated for each pollutant measured and the maximum is considered the air quality index for that monitoring station, as it represents the worst of the pollutants measured.

The frequencies of the air quality indices for Annaba's region and its agglomerations are presented in Figure 16. In total, the air quality in Annaba's region varies from excellent to fairly good with a rate of 81%. The poor quality is due in the majority of cases to dust levels and to a lesser extent to photochemical pollution during the summer season (Figure.16).

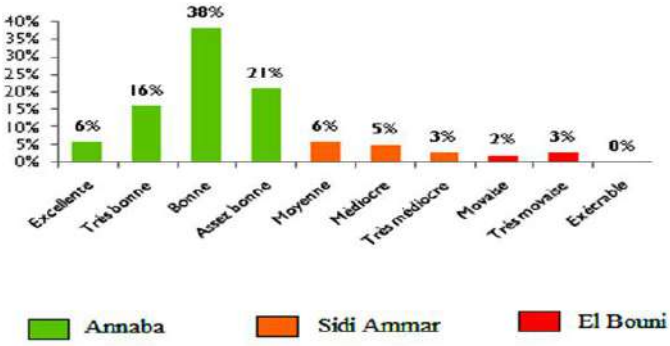

Fig.16 Air quality indices for the city of Annaba and its surroundings

IV. CONCLUSION

The study of air pollution exposure is based on the results of the air analysis conducted over the period (2002-2007). These results show that the air temperatures are very low accompanied by a very high humidity due mainly to the presence of the sea and many bodies of water (Lake Fetzara swamps ...).

This can allow the accumulation of some pollutants such as carbon monoxide and dust (CO and dust levels exceed WHO standards), while some pollutants are present in small to negligible quantities such as NO, NO_2, and SO_2.

Air quality in Annaba and surrounding areas (El Bouni, Sidi Ammar) can be described as good overall. However, in the vicinity of industrial complexes, such as El Hadjar, there is evidence of heavy dust pollution.

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Genetic Diversity 20 Bean Varieties using Microsatellite Technique (SSR)

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Abstract— *Agro-morphological characters and PCR based markers have provided valuable information about genetic diversity of bean collection in HATRI . Analysis on SSR molecular markers: out of a total of 44 primers conducted genetic diversity studies, only 28 primers amplified the product on 20 bean varieties. Through the SSR marker data with 28 primers used, 20 varieties are classified into 4 main groups. In the subgroup of the SSR on 28 molecular markers are noted with 4 distinct groups. Molecular markers to be able to indirectly assess the presence or absence of selected genes thanks to markers without environmental influences. The diversity index analyzes according to the high SSR method (H = 0.384) while the diversity index of stick beans. The results presented here are the first steps towards a better understanding of varieties introduced from countries and may help guide future research into the crop.*

Keywords— ***bean , gene source diversity, SSR.***

I. INTRODUCTION

The world is facing food insecurity due to climate change and nearly 800 million people from developing countries go to bed hungry (Khush et la.,2012). The world's population is growing rapidly and is estimated to reach 10 billion people by 2050. Therefore, it is necessary to increase world food production by 60%–110% to meet projected food demand by 2050 (Tilman et al.,2011). To meet global food demand, it is necessary to harness plant genetic diversity. Characterization of the gene pool is a strategy in this regard as it helps to discover genotypic and phenotypic variants that can be effectively selected by the breeding community for use (Nadeem et al., 2020, Baloch et al.,2017). Almost 41,500 gene sources from the genus *Phaseolus* are present at the International Center for Tropical Agriculture (CIAT) (Islam, et al., 2006); In addition, there are hundreds of local varieties present in the fields of farmers in bean-growing countries. Most of the genes available at gene resource centers breed quite race.Gene pool characterization has always been one of scientists' favorite methods of investigating new variants that can be used to develop improved plant varieties that exhibit higher yields with better quality, bio-stress resistance and abiotics (Nadeem et al., 2020, Nadeem et al.,2018,). A large number of studies have been conducted to explain morphological, phenomenological and agronomic variation among local bean populations in parts of the world (Boros et al.,2014; Madakba et al.,2011). Rana et al.,2015 explored agronomic and morphological variations in the bean variety and proposed several genotypes that work well for a breeding perspective and (Bozo et al.,2011) used the Turkish bean variety to explore phenotypic variations and report the existence of a variety of phenotypic diversity. Characterization and evaluation of diversity among traditional varieties will provide plant breeders information necessary in the identification of initial materials for hybridization to produce varieties with improved productivity and quality.The objectives of the study are: to evaluate genetic diversity of the bean varieties in the genebank of High Agricultural Technology Research Institute of Mekong Delta, CanTho (HATRI), Vietnam using morphological characters and microsatellite markers. To study correlation among the characters for

application in plant breeding, and and to relate results between morphological characters and molecular markers.

II. MATERIAL AND METHOD

The materials used include 20 bean seed samples collected at the gene bank of the HATRI. The experiment was conducted at the High Agricultural technology Research Institute of Mekong Delta (HATRI). Morphological and agronomic characterization of imported pea gene banks and selected plant varieties are carried out according to plant variety evaluation criteria developed by the International Institute of Plant Genetic Resources (IPGRI 1982) and European Community Plant Varieties (EU-CPVO), (2013). All characteristics are measured on 10 representative individual plants, for 1 variety. A total of 10 seeds were randomly selected, fully developed and undamaged. Ten plants of each genotype were sown, with a distance of 30 cm X 30 cm, completely randomized block design (CRBD) with three iterations. All characteristics are measured on 10 representative individual plants, for 1 variety. The qualitative characters differ as measured according to the description IPGRI (1986) for beans.A total of 10 randomly selected, fully developed and seeds were used to measure seed length, width, and height using a digital Vernier caliper. Root length (cm) was measured by following the methodology of Aghamir et al 2016, (8) Width of fruit, (9) number of fruits per string, (10) number of seeds per tree (11) Yield of fruit (kg) and (12) Yield of hectares (tons/ha).

DNA extraction The DNA molecule for PCR analysis is prepared according to the simplified miniscale process. A sample of fresh, young leaves (2 cm) was collected and placed in a test tube, centrifuged 1.5ml with markings, in ice. The leaves are ground in a mortar and pestle (Spot Test Plate -Thomas Scientific) after 400 (l buffer solution is added (50 mM Tris-HCl pH 8.0, 25mM EDTA, 300mM NaCl and 1% SDS). Grind the sample until the buffer solution is green. Add 400(l of buffer solution and mix well. Transfer 400 (l lysate to a test tube with the original leaf sample. Lysate triggers a protein splitting reaction by adding 400(l chloroform. The floating object (supernatant) is transferred into a new test tube (1.5 ml) and DNA is agglomerated using ethanol alcohol. The DNA sample was dried by wind and agglutinated in 50(l of buffer TE (10mM Tris-HCl pH 8.0, 1mM EDTA pH 8.0). Use aliquot 1(l for PCR analysis. DNA samples are stored in a refrigerator -20°C deep for use. 28 primer pairs were used for evaluation according to table 1.

PCR Tests PCR amplification was performed in 10mM Tris-HCL (pH 8), 50mM KCl, 1.5mM MgCl2.1 units of Taq HATRI, 4 nmol dNTP, 10pmol primer and 50ng genomic DNA. PCR cycles: double wire separation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 60 seconds, 36°C for 60 seconds and 72°C for 120 seconds. The final cord extension is 72°C for 5 minutes. Add 13(l buffer solution (98% formamide, 10mm EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol) after PCR. Polymorphism in the PCR product was detected by ethidium bromide dye after electrophoresis above 5% agarose gel. Data analysis: based on NTSYS-pc software version 2.1.

PCR reaction products with SSR marker

To detect polymorphisms of 20 varieties of stick beans, 44 primers were used in a PCR reaction on DNA genome obtained from leaf samples of 20 varieties of stick beans. The amplification product generated from these primers was observed on 1.5% agarose gel. Observations showed that 28 primers amplified over 100% of the samples, while 20 primers did not amplify any band. The number of DNA fragments produced in a reaction is noted on the table (table 1). Based on the differences between alleles shown in the bandages on the gel, it is possible to determine the differences between the breeds genetically.

After the PCR reaction is complete, 2.5 µl of 6X loading dye solution (MB1 Ferment Inc., Maryland, USA) is added to the PCR product and 1.5% agarose gel electrophoresis (m/v) with 1X TAE buffer solution, then the gel is stained with ethidium bromide and the results are recorded using the system (Syngene, Cambridge, UK). Repeat the electrophoresis process 3 times to observe the ice locations accurately as well as the concentration of each plant with each primer.

PCR reaction products with SSR marker To detect polymorphisms of 20 varieties of stick beans, 44 primers were used in a PCR reaction on DNA genome obtained from leaf samples of 20 varieties of stick beans. The amplification product generated from these primers was observed on 1.5% agarose gel. Observations showed that 28 primers amplified over 100% of the samples, while 20 primers did not amplify any band. The number of DNA fragments produced in a reaction is noted on the table (table 1). Based on the differences between alleles shown in the bandages on the gel, it is possible to determine the differences between the breeds genetically. After the PCR reaction is complete, 2.5 µl of 6X loading dye solution (MB1 Ferment Inc., Maryland, USA) is added to the PCR product and 1.5% agarose gel electrophoresis (m/v) with 1X TAE buffer solution, then the gel is stained with ethidium bromide and the results are recorded using the system (Syngene, Cambridge, UK).

Table 1: Molecular markers from SSR used in bean experiment and polymorphic index and PIC diversity index

No.	Primers	sequencing	replication	No of band	polymorphisum	Retio	PIC	reference
1	BM 200	F-TGTAAACGACGGCCAGTATGCGGTTGGGAAGCCTCATACAG R-ATCTTCGACCCACCTTGCT	(TCT) 10	3	4	68.59	0.32	Rauscher et al.,2013
2	BMd45	F-TGTAAACGACGGCCAGTATGCGGTTGGGAAGC R-CTCATACAG ATCTTCGACCCACCTTGCT	$(AG)_5$	3	9	67.687	0.36	Rauscher et al.,2013
3	PV-ag003	F-TGTAAACGACGGCCAGTATGCTCACGTACGAGT R-TGAATCTCAGGATGGTGTCGGAGAGGTTAAGGTTG	$(AG)_8$	3	8	70.5	0.39	Rauscher et al.,2013
4	BMd10	F-TGTAAACGACGGCCAGTATGCG R-CTCACGTACGAGTTGAATCTCAG	$(GA)_8$	3	9	75.93	0.41	Rauscher et al.,2013
5	BM156	F-TGTAAACGACGGCCAGTATGCCTTGTTCCACCTCCCATCATAGC R-ATCTGAGAGCAGCGACATGGTAG	$(CT)_{32}$	3	7	70.88	0.39	Rauscher et al.,2013
6	GATS91	F-TGTAAACGACGGCCAGTATGCGAGTGCGGAAGCGAGTAGAG R-TCCGTGTTCCTCTGTCTGTG	$(GA)_{17}$	2	12	76.40	0.42	Rauscher et al.,2013
7	BMd47	F-TGTAAACGACGGCCAGTATGCACCTGGTCCCTCAAACCAAT R- CAATGGAGCACCAAAGATCA	$(AT)_5$	10	11	80.99	0.51	Rauscher et al.,2013
8	BMd17	F-TGTAAACGACGGCCAGTATGCGTTAGATCCCGCCCAATAGTC R-AGATAGGAAGGGCGTGGTTT	$(CGCCAC)_6$	3	6	67.67	0.35	Rauscher et al.,2013
9	PVBR107	F-TGTAAACGACGGCCAGTATGCCCCCTTTCTCACCACTTCAG R-ACCAAAAACGGTGCTCAAAC	$(CT)_{16}$ $(GT)_4$	2	7	66.45	0.39	Rauscher et al.,2013
10	BM175	F-TGTAAACGACGGCCAGTATGCCAACAGTTAAAGGTC R-GTCAAATT	$(AT)_5$ $(GA)_{19}$	2	8	85.00	0.44	Rauscher et al.,2013

		CACTCTTAGCATCAACTGGA						
11	J01263	F-ATGCATGTTCCAACCACCTTCTC R-GGAGTGGAACCCTTGCTCTCATC	$(ATCC)_3$ $(AG)_2$	3	2	72.8	0.42	Yu et al.,2000
12	J04555	F-AGGGTGTTTCACTATTGTCACTGC R-TTCATGGATGGTGGAGGAACAG	$(CTT)_3(T)_3$	2	10	75.2	0.38	Yu et al.,2000
13	K03288	F- TGCCACCACAGCTTTCTCCTC R-TATGAGAGAAGCGGTTGGCACG	$(ATGC)_4$	2	2	43.39	0.35	Yu et al.,2000
14	K03289	F-AGCTTTCACACTATGACACCACTGG R-TGCGACATGAGAGAAAGACACGG	$(ATGC)_4$	2	5	65.46	0.39	Yu et al.,2000
15	M18093	F-CCAGCTACCATCTCCTCCATCG R-TAGTGGTGGAGGTGGAGATTT	$(CCA)_6$	2	3	59.00	0.38	Yu et al.,2000
16	M18094	F-TAATTTCTCTCTTCCCATCCCAAAC R-GTAGTAATAAGGAGGAGGCGGTGAG	$(ATCT)_3$	7	15	55.74	0.35	Yu et al.,2000
17	SS715647275	F-ATCTGAGAGCAGCGACATGGTAG R-TATACACACGAACTTTGCATTCCG	$(CT)_8$	12	17	79.25	0.48	
18	SS715649259	F-ACATGCAAGTTCACACGGTCCTC R-ACCTAGAGCCTAATCCTTCTGCGT	$(TCTTTC)_6$	12	17	82.4	0.55	
19	M75856	F-CAATCCTCTCTCTCTCATTTCCAATC R-GACCTTGAAGTCGGTGTCGTTT	$(GA)_{11}$	4	19	54.46	0.32	Yu et al.,2000
20	U10419	F-TGGAGCCATCTGTCTCTTACCCAC R-GAGCACGAGTCACGTTTGCAAC	$(AAAT)_3$	2	6	55.4	0.38	Yu et al.,2000
21	U18349	F- CTGAAGCCCGAATCTTGCGA R- CGCGAGAGGTGAACGAAAGC	$(GGC)_5$	2	9	66.24	0.35	Yu et al.,2000
22	U18791	F- GGGAGGGTAGGGAAGCAGTG R- GCGAACCACGTTCATGAATGA	$(TA)_{22}$	8	7	57.68	0.33	Yu et al.,2000
23	U28645	F-GCAAGAGAACACTGAAGAGGATCG R-GACATTACTCATTTCATCATCTAC	$(CCA)_5$	3	15	74.51	0.41	Yu et al.,2000

		TACACG						
24	U34754	F-GTTTCTTCCTTATGGTTAGGTTGTTTG R-TCACGTTATCACCAGCATCGTAGTA	$(AT)_8$	4	17	68.45	0.36	Yu et al.,2000
25	U54703	F- CGAGGAGGAAGGAGAAGACGG R-GAGGGTTATCACAAGGAAGACACG	$(TTA)_4$	4	12	61.23	0.35	Yu et al.,2000
26	U77935	F- CGTTAGATCCCGCCCAATAGT R- CCGTCCAGGAAGAGCGAGC	$(GCCACC)_5$	2	6	66.57	0.36	Yu et al.,2000
27	X04001	F-TCACGTACGAGTTGAATCTCAGGAT R-GGTGTCGGAGAGGTTAAGGTTG	$(AG)_8$	2	6	58.56	0.33	Yu et al.,2000
28	X04660	F- TTGATGACGTGGATGCATTGC R-AAAGGGCTAGGGAGAGTAAGTTGG	$(AG)_8$	6	8	74.25	0.49	Yu et al.,2000
Total				**111**	**257**	**65.65**	**0.384**	

Statistical analysis The SSR tape will be displayed based on molecular mass and in kilo base (kb) based on the scale as a marker. The data is processed using MS Excel software to calculate the polymorphic tapes of each individual primer, polymorphic average and polymorphic scale. Analysis of potential information of molecular markers and genetic diversity in assessed genotypes including the number of alleles on the desired locus–Aep according to (Weir BS et al 1996), Shannon diversity index- (Martynov SP et al. 2003) genetic diversity/diversity index- Hep, marker index (MI) and polymorphic content - PIC is calculated for each primer on a plant based on the allele frequency on the locus of each plant.

Cluster Analysis

SSR will be encrypted in binaries 0 and 1. On horizontal electrophoresis gels, the sample with the tape is recorded as 1, the sample without the tape is recorded as 0. Based on the tape results, the matrix represents the genetic correlation for all pairs from Euclidean Distance and is used to construct the family tree schema according to the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The data will be analyzed using IBM SPSS Statistics 20 software. The data from the SSR marker will be processed together

III. RESULTS AND DISCUSSION

The variance analysis (ANOVA) showed a statistically significant phenotype ($p < 0.05$) for all characteristics of the variety, date of first flowering, plant height, flowers per plant, fruit weight (Table 2). Based on morphological parameters, the average value of characteristics related to flowering date, height of plant, number of fruits on plant with 20 varieties of beans is analyzed on table 2. The bean-like genotype has the very early appearance of 50% of flowering flowers (20 days after seeding) for Alubia beans, while the bean variety imported from the United States recorded the longest flowering period (40 days after seeding) of 50% female flowering. The number of days of flowering ranged to 24 days for Alubia (23.93) and the United States (40.66), respectively, while 13 days were found to be the average number of days to appear. The number of flower varieties also varies HaLan (78.76 flowers) varieties with the number of l cotton t ...The variety with the lowest L number of flower is the Cong

Ty bean variety (36.39 flower per string).The color of seed flowers is also rich white, black and brown .

Table 2. Evaluation of physiological characteristics of 20 varieties of Bean sticks in the Winter-Spring crop 2021 planted in Can Tho

no	lines	Flowering (days)	Hight plants (cm)	Color flower	Color seed	Second branch	No flower
1	Alubia	23,92d	193,19b	white	white	1,57ab	44,54b
2	Osu544C	35,91b	221,04ab	white	white	3,80c	69,43ab
3	Hà Lan	30,87c	300,40a	white	Large white	4,33efg	78,76ab
4	Pháp	32,24ab	188,47b	white	Small white	3,58def	56,76ab
5	Brasil	36,89ab	194,76ab	Purple	black	5,90de	77,51ab
6	Áo	39,58a	184,75b	red	White and spot red	2,78ab	75,02ab
7	HoaKỳ	40,66a	198,68ab	Gray	gray	7,03fg	40,05ab
8	Anh	33,56ab	196,85ab	Purple	gray	5,90h	67,84ab
9	Ấn Độ	39,91a	198,81ab	Whie	gray	7,06fg	68,92ab
10	Bhatle	37,90a	203,41ab	red	black	4,20bc	55,32ab
11	Chiese Long	31,30c	190,71ab	red	Black	7,60def	66,12ab
12	WhiteOP	30,43c	172,15b	White	White	6,85g	68,31ab
13	DHundi	37,66a	194,35ab	Purple	There are black spots	8,10d	75,95ab
14	LB39	36,51ab	192,25ab	Whte	White	4,11a	66,38ab
15	Chaumese	41,11a	189,681b	red	black	7,16fg	68,99ab
16	Thái Lan	37,50a	203,47ab	white	white	5,50bc	66,32a
17	Bolu (HQ)	31,20c	190,71ab	white	white	7,65def	56,12ab
18	Philippine	30,53c	222,25a	white	white	6,45g	48,13ab
19	Đài Loan	37,55a	197,36ab	white	white	8,18d	55,95a
20	CôngTy	36,69ab	194,23ab	white	white	4,47a	36,39ab
	CV (%)	14,01	8,63			2,05	12,27

Note: Numbers that follow the same character have no statistical significance difference at 5%. - **Yield and yield composition recorded**: the number of seeds/fruits ranges from 5 to 11 seeds. The Bhatle bean-like genotype exhibits a maximum average weight (1.87 g), The highest fruit length is the Osu544C and Brasil bean variety long (17.9-17.44 cm) repectity . The width ranges from 1.47 – 2.23cm. Productivity ranges from 0.70 to 1.8kg/wire. The tallest breed is still the Bhatle. Through the analysis of agronomic traits, yield and yield composition of 20 varieties, we show that Bhatle variety followed by White OP is somewhat superior to other varieties, Dai Loan bean variety is the lowest yielding variety (Table 3).

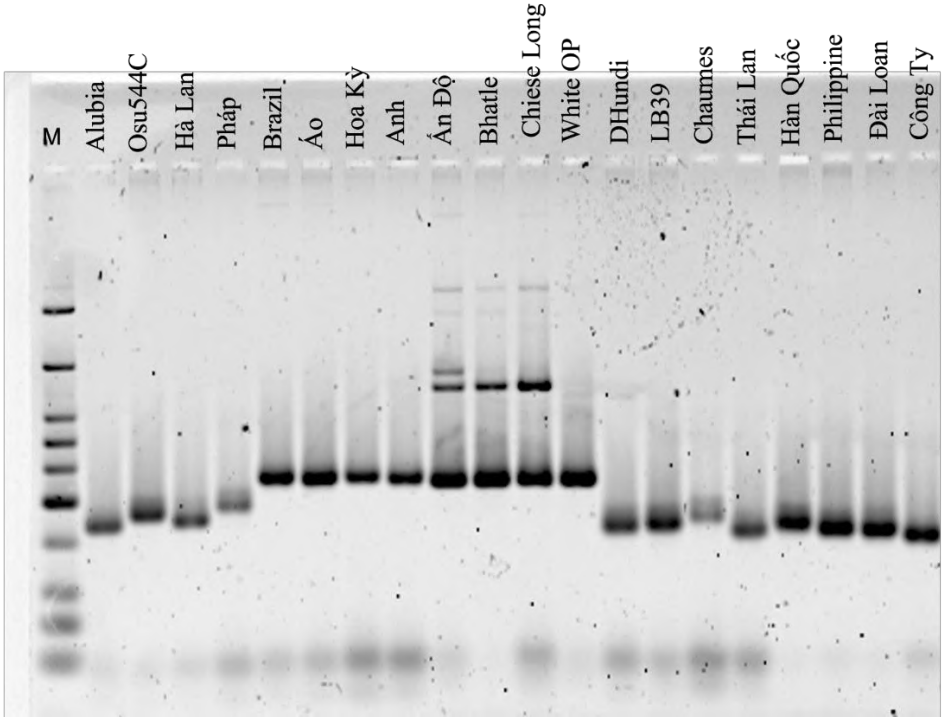
Table 3: Yield and yield composition of 20 varieties of bean melons in the Winter Spring crop 2021 in Can Tho

no	Giống	Numbers seeds/ pod	Length fruits (cm)	wide (cm)	Number pod / plant	Yield/plant (kg)	Yield/ ton/ ha
1	Alubia	8d	14.23d	2.23a	80	0.79h	8.47e
2	Osu544C	9c	17.90b	1.47b	58	1.51d	11.53a

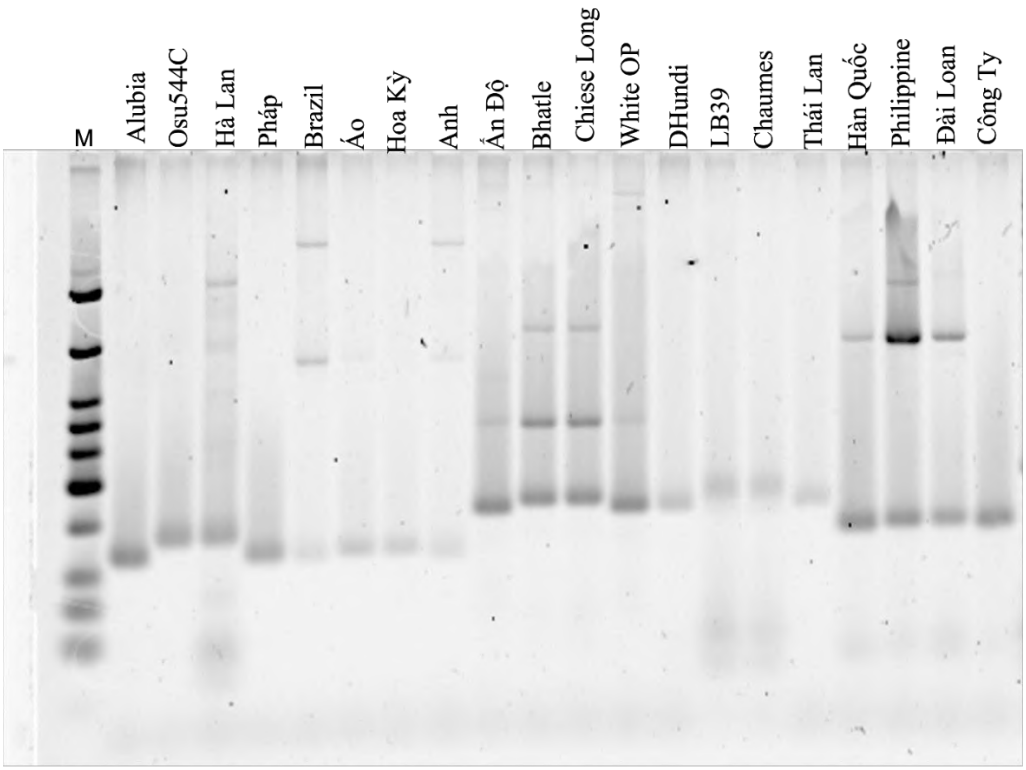
3	Hà Lan	8d	16.10bc	2.56a	37	1.35e	9,23d
4	Pháp	8d	15.82c	1.41b	42	0.70h	8.66e
5	Brasil	8d	17.44ab	1.09c	42	0.77h	7.97f
6	Áo	5g	14.17d	1.97ab	40	0 .83h	9.13d
7	Hoa Kỳ	7e	16.33bc	1.92ab	50	1.18g	9.90d
8	Anh	4h	14.46d	1,79ab	82	1.02g	9.39d
9	Ấn Độ	9c	16.96abc	1.88ab	32	1.56c	11.86b
10	Bhatle	11a	14.40d	1.07c	41	1.86a	12.94a
11	Chiese Long	8d	13.28de	1.49b	60	1,04g	7.04f
12	White OP	8d	12.24e	1.79b	59	1.78b	11.33b
13	DHundi	8d	13.50de	1.87ab	41	1.10g	10.52c
14	LB39	10b	13.07de	1.06c	40	1.64c	11.38b
15	Chaumese	6f	15.34c	1.12c	39	1.69c	8.55 e
16	Thai Lan	5g	11.6d	1.5b	41	0.85h	7.14f
17	HanQuoc	6f	15.20c	1.65b	37	1.17g	9.15d
18	Philippine	6f	13.67de	1.32b	72	0.8h	8.14e
19	DaiLoan	7e	16.12bc	1.41b	35	1.13g	5.85g
20	Công Ty	5g	16.11bc	1.6b	33	0.94h	7.36f
	C%	**4.33**	**5.45**	**4.11**	**2.16**	**1.12**	**11.28**

Note: Numbers that follow the same character have no statistical significance difference at 5%.

PCR reaction products with SSR marker method Use 24 SSR primers to amplify stick bean plants, in which all eSSR products are recorded for polymorphism over 20 bean stick varieties (P = 100%). For SS715649259 DNA amplification for the product reaches 100% The product amplifies 12 alleles with molecular sizes ranging from 180bp to 400bp. Alubia (210 bp), OSU 544C (220bp); Ha Lan(210bp), Phap(250bp), Brazil (300bp), Austria (300bp), Hoa Ky(300bp), Anh (300bp), India (300;320,350,400 bp); Bhatle (300;350,400), Chiese Long (300;350,400); White OP (300bp); Dhundi (210bp); LB39 (210bp), Chaurmes (220bp); Thailand (200bp), South HanQuoc(210bp), Philippines (200bp), Đai Loan(200bp), Cong Ty(180bp) (Figure 1A). For SS 715647275 DNA amplification for the product reaches 100% The product amplifies 12 alleles with molecular sizes ranging from 250bp to 320bp. Alubia (210 bp), OSU 544C (220bp); Netherlands (220;400bp), France (210bp), Brazil (210,350,400bp), Austria (2050bp) United States (205 bp) Anh (210,450 ...) Ấn Độ (250; 330,360bp); Bhatle(260;300,380bp), Chiese Long (260;300,380bp); White OP(250,300bp) Dhundi(250bp); LB39(270bp), Chaurmes(270bp); Thai Lan (206bp), Han Quoc (240bp,380), Philippine (240bp,380), Đài Loan (240,380bp), Công Ty (240bp) (figure 1B).



(A)



(B)

Fig.1: PCR products of SS715649259(A) and SS715647275(B) on 20 different bean varieties separated on polyacrylamide gel with silver nitrate dyeing

Genetic diversity assessment of 20 stick bean varieties using NTSYSpc.2.1 software The results of grouping 20 bean varieties based on phenotypic data and calculation results using NTSYSpc.2.1 software are

presented as subgroup trees combining phenotype and genotype. Figure 2. Subgroup trees show the relationship of genetic distance between varieties based on the correlation between varieties and their contribution to the diversity index of molecular markers. The results of genetic grouping based on genotype show that: at a genetic distance of about 0.74 breeds are divided into 4 very pronounced groups: Groups A, B, C, and D. Thus, with 28 primers, 20 varieties are classified into 4 main groups, in which the correlation between varieties ranges from 0.39 – 1.0, indicating that the breeds have high genetic diversity. The degree of similarity between the varieties was as low as 0.39% and some varieties in group A had the highest degree of similarity of nearly 100%. Genetic differences between breeds in group A are higher than in breeds in group C, the coefficient of similarity.Genetic differences between breeds in group A are higher than in breeds in group C, the similarity coefficient between group A and group B compared to group C is 0.41 and breeds in group B can be considered intermediate breeds between group A and group C. This means that if the breeds in groups A and C are crossbred with group C B can produce more individuals with many desirable traits because the longer the genetic distance, the higher the likelihood of hybrid superiority (Bui Chi Buu et al. 2003).

Genetic subtypes are based on their ability to combine with primers, at similarities 0 to 0.41 varieties are divided into two main groups A, B, C, D:

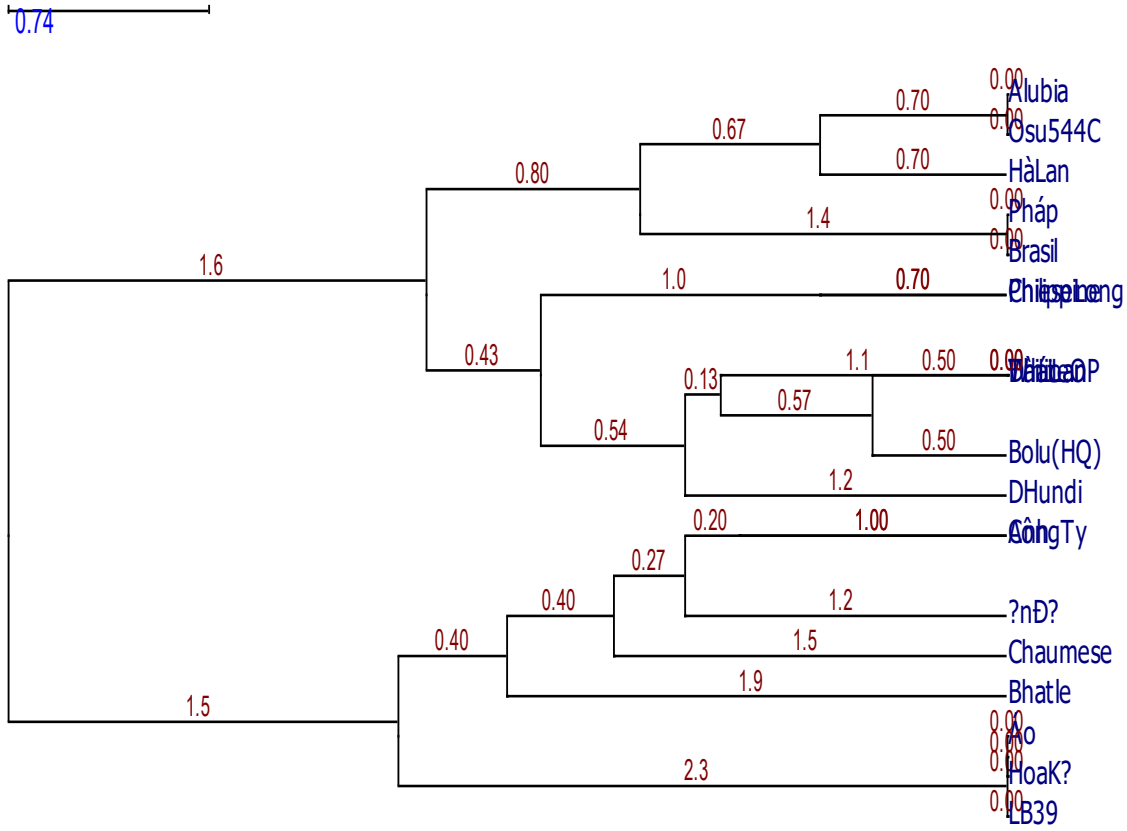


Fig.2. The genealogy schema shows the genetic correlation between 20 bean varieties based on the Jaccard Homology Index using the SSR marker (UPGMA). Four groups when considering a similarity coefficient less than 0.74

- Group A: Group A has a yearly similarity in the range of 0.00-0.40, including 3 varieties: LB39, stick beans imported from the Hoa Ky and Ao (with indicator SS715649259) with a molecular size of 300bp. Group B: there are 5 varieties: Bhatle, Chaumese, DaiLoan, An Do, cong ty . Group C: is divided into three groups C1, C2 and C3 at a similarity coefficient of 0.50. Group C1 has 1 variety of Dhundi. Group C2 includes the following

varieties: 2 varieties: Bolu (HQ) and Thai. Group C3: White OP, UK Group D: includes 2 varieties: Chiese Long and Philippne. Group E: includes 5 varieties: stick beans from Brazil, Phap, HaLan, OSU 544 C and Alubia. Group E1 includes 2 varieties: Brazilian, Phap. Group E2: includes three varieties: Dutch, OSU 544 C and Alubia.

IV. DISCUSSION

Phenotypic analysis noted bean varieties with different meanings of different fruit forms. Among the 28 primers (Fig. 1) show the lines of the trees in a uniform way. SSR primers with a total of 257 band lines have 111 alleles that exhibit polymorphism. Polymorphism is expressed through markers of varieties valued from 28.57% (EC45). The average number of DNA fragments amplified by the SSR marker in this study ranged from 2 to 12 alleles (size 180-400bp). With 28 markers, there is a high variability of DNA fragments produced from the SSR marker, which can be attributed to differences in attachment sites across the entire set of alleles of different bean varieties. The effectiveness of the primers used in SSR is to determine the quantity by estimating based on differences in genetic parameters (table 1). In the analysis of genetic diversity divided into important components: allele frequency and polymorphism in the group. To assess genetic diversity between different breeds/lineages of geographical origin in the many traits are abundant and find genetic gaps to establish plausibility for later hybrid material. For SSR for polymorphic locus ratio:

At a significance level of 1%, the percentage of polymorphic loci among seed groups ranged from 59% to 100%. Polymorphism manifests itself most importantly in different groups of breeds. Average number of alleles per locus: In general, in most loci there are two or three alleles, except for the alele on molecules SS715649259 and SS, which 715647275 have 12 alleles. The genetic diversity index is (H) H = 0.384. However, the Shannon Diversity Index, which evaluates only on primers in different melon varieties, indicates the similarity of narrow genetics of the plants studied. When comparing PIC values, recorded a wide range of PIC values in this topic with 20 varieties ranging from 0.32 (BM 200) to 0.55 (SS 715647275) with an average of 0.384. Genetic diversity (Hep) is polymorphic, demonstrating the effectiveness of the eSSR loci information in this study also noted polymorphisms with high on primers such as M75856.

In this study, Hep showed remarkable homogeneity across each SSR primer and ranged from 0.85% (BM175) Furthermore, Hep showed a positive and significant association (r = 0.914, P < 0.001) with allele/locus influence (11.32 to 11.95). The marker's parameter was calculated to detect the characteristics of each individual using a separate primer to identify polymorphic loci on different melon varieties. Based on the number and frequency of scoring of DNA fragments, polymorphism rates, and other efficacy parameters after combining, it appears that these SSR instructions suggest that polymorphisms are best effective markers and can be used to screen molecules in later high-yielding melon gene pools. The electrophoresis tape analyzed by UPGMA method (figure 1) shows the genotypic grouping process into 4 main groups. There is a strong association between the genotypes that have been documented. Such genetic correlation is very significant, it provides for breeds in the crossing program.

All traits (except dates of appearance) reflect significant genotyping, indicating the existence of genotypic variation useful for breeding purposes (Table 2). The genotypes observed in this experiment were found to be consistent with previous reports (Okii etal.,2018). In this study, all traits were found to be hereditary except for dates of occurrence, root length, and secondary branches on a scale of (Robinson et al.,1966) (Table 2). The degree of heredity is mainly governed by the degree of genetic variation, while higher heredity leads to a lower environment on a particular trait (Phuke et al.,2017). Variance analysis (ANOVA) for most of the traits studied reflected that genotypic variance was significant within as well as across the environment, indicating a higher degree of their heredity. The findings of this study are consistent with previous studies (Okii et al.,2018, Wondimu et al., 2017) stating that in the days leading up to maturity, fruit per tree, number of seeds per tree, and weight of 100 seeds are less affected by environmental forces and are highly heritable traits over many years/places.

The wide range of phenotypic values obtained for the 12 traits reflects the occurrence of important variation for different agromorphological traits in the study of bean sprouts of many countries, which was found to be consistent with previous studies (Rana et al., 2015, De La Fuente et al.,2013). Seed-related characteristics are considered important for stick beans and are considered a major determinant of the commercial acceptability of commercial varieties (Rana et al.,2015). Fruit / string weight is an important characteristic, which has a positive and significant impact on stick bean yield. The average weight of 100 grains (42.2 g) resulted in this study which was significantly higher than previous studies (Bozo ̆glu et al.,2011, Yeken et al.,2019, Yeken, et al.,2018). The higher average seed weight noted in this study may be due to the inclusion of large numbers of joinings that have larger seed sizes than in previous studies. Voysest et

al.,1983 claim that p...Given that the weight of 100 grains of regular beans can vary between < 15 to > 90 g per 100 grams

In addition to seed color, growth habits also reflect variations in the height of the plant. Climatic conditions and human preferences can play an important role in the distribution of bean in a particular area (Rana et al.,2015). In this study, climatic conditions noted French, Austrian varieties of determined growth with early maturity. The length and width of the left remain in the shell. Similar findings were reported by Balkaya and Ergün (Balkaya et al.2008) on the use of genotypes of variable-length creeping pea varieties (Table 2) Selecting the gene pool that performs best and is stable in multiple environments per year is also one of the objectives of this study.

V. CONCLUSTION

-Morphological parameters, average values of characteristics related to flowering date, string tall, number of leaves, number of cotton of genotypes on 20 varieties of beans are analyzed. The Alubia genotype has a very early appearance of 50% of flowering female flowers (23.92 days after sowing), while the Chaumese variety recorded the longest flowering period (41.11 days after sowing).

- Analysis on SSR markers: Through the SSR marker data with 28 primers used, 20 varieties are classified into 4 main groups. The SSR marker has proven to be a powerful tool for determining how to genetically diversify across 20 varieties of beans to better manage the bean gene bank also to promote the use of imported varieties in future breeding programs. In the subgroup of the SSR on 28 molecular markers are noted with 4 distinct groups. A grouping map that coordinates two molecular marker methods is also established with four different groups. Relying on molecular markers to be able to indirectly assess the presence or absence of selected genes thanks to markers without environmental influences.

- Diversity index analyzed by high SSR method (H = 0.384) while the diversity index of beans. The results presented here are the first steps towards a better understanding of bean varieties introduced from countries and may help breeding program in future.

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Response of lowland rice to phosphate amendments in three acidics agroecological zones of Côte d'Ivoire: Man-Gagnoa-Bouaké

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Abstract— *The need to achieve self-sufficiency in rice led producers to overexploit soils and to use excessively chemical fertilizers, which deplete soils and make phosphorus unavailable to crops. As an alternative, eight phosphate amendements made from phosphate rocks from Morocco (MPR), Triple Superphosphate (TSP) and NPK (T0a (0%MPR + 0%TSP without NPK); T0 (0%MPR + 0%TSP+ NPK); T1 (100%MPR + 0%TSP+ NPK); T2 (90%MPR + 10%TSP+ NPK); T3 (80%MPR + 20%TSP+ NPK); T4 (40%MPR + 60%TSP+ NPK); T5 (20%MPR + 80% TSP + NPK); T6 (0%MPR + 100%TSP+ NPK) were applied to the field and their agronomic efficiencies were evaluated in Man (very acidic soil). Gagnoa (moderately acidic soil) and Bouaké (weakly acidic soil). After three cultivation cycles, results designate Man and Gagnoa as more productive zones with respectively 5.04 t ha^{-1} and 4.36 t ha^{-1} grain yield (GY), comparatively to Bouaké (3.74 t ha^{-1}). Likewise, straw yields (SY) are 9.68 t ha^{-1} at Man and 6.06 t ha^{-1} at Gagnoa, comparatively to Bouaké (5.85 t ha^{-1}). Treatments T3 and T4 were more productive in all zones with respectively GY of 7.50 t ha^{-1} and 6.50 t ha^{-1} in Man, 5.54 t ha^{-1} and 5.91 t ha^{-1} in Gagnoa and 5.55 t ha^{-1} and 4.84 t ha^{-1} in Bouaké. This disparity is due to the chemical properties of the soils. In Man, Gagnoa and Bouaké, the combination 80% MPR + 20% TSP and 40% MPR + 60% TSP seem to improve better the yield of lowland rice.*

Keywords— ***Acid soil, Lowland rice, Man-Gagnoa-Bouaké, Phosphate amendments, phosphate rock of Morocco, Triple Superphosphate***

I. INTRODUCTION

In West Africa, about 80 % of rice production surface accounted for upland rainfed rice cultivation (Koné et al. 2013). With increasing land shortages, the length of fallow between periods of cultivation has declined from 12 years in the 1980s to less than two years at present (Koné et al. 2013). This intensification of land use in the low-input systems causes declining yield levels, which are associated with a reduced soil fertility and an enhancement of soil acidification (Koné et al. 2016; Vitousek et al. 2010). These high acidity of soils favors high available P fixation by iron (Fe) and aluminum (Al) oxides and hydroxides (Koné et al., 2013). Therefore, low P concentration and P deficiencies are commonly observed. This is a major soil constraint to sustainable rice production in the tropical humid agroecosystems (Sahrawat et al. 2001). Phosphate fertilization is, therefore, strongly recommended to increase crop yields. Chemical fertilizers used until then, such as triple superphosphate (TSP), although effective for to remedy this problem are prohibitively expensive for small African farmers. An alternative and cheaper source of P is Morocco phosphate rock (MPR), which abounds in West Africa (Asuming-Brempong and Anipa, 2014). Morocco

Phosphate rock (MPR) adoption by smallholder farmers is, however, limited particularly because of the large quantities required due to its low solubility, thus making its use impossible for farmers (Debrah 2000). Numerous studies have been conducted to increase the availability and solubility of P from native sources PR. Among these, organic amendments, including animal manure, plant residues and green manure (Adesanwo et al. 2 012), composts (Saleem et al. 2013), bacterial inoculation (Abbasi et al. 2015) and the combined application of water-soluble P fertilizers (Koné et al. 2011; Mashori et al. 2013) are considered beneficial for improving the P efficiency. However, the MPR dissolution and P solubilization and availability is influenced to a large extent by physico-chemical and biological properties of the soil (Richardson et al. 2009). The aim of this study was to evaluate the agronomic effectiveness of different types of phosphates amendments resulting to the combinaison of differents proportions of Morroco phosphate rock (MPR) and of triple super phosphate (TSP) on rice yield in acidic soils in three agroecological zones.

II. MATERIALS AND METHODS

2.1. Experimental site

The experiment was conducted in Ivory Coast from 2019 to 2021 in three agroecological localities where soils pH were acidic (pH <6): Man (strongly acidic), Gagnoa (moderately acidic) and Bouaké (weakly acidic) (TABLE 1). The experiment was an on-farm study carried out in the Center (Bouaké : 6°41'37"N. 5°01'49"O), Center-West (Gagnoa 6°07'54"N. 5°57'02"W) and West (Man : 7°24'45"N. 7°33'13"W) part of Ivory Coast. Man and Gagnoa are in tropical humid forest agro-ecosystem with a mono modal and a bimodal rainfall pattern respectively. While Bouake is in tropical humid savannah with a mono modal rainfall pattern. In each locality, five fields were selected per agroecological zones for expérimentation.

Table 1. Physical and chemical characteristics of soils at 0-20 cm depth before experimention.

Parameters	Man	Gagnoa	Bouaké
Clay (%)	23.70	28.50	12.50
Silt (%)	24.10	22.50	15.00
Sand (%)	52.20	49.00	72.50
pHwater	4.93	5.18	6.11
pH_{KCl}	4.21	4.70	5.42
Total P (mg kg^{-1})	300.53	286.43	313.60
Assimilable P (mg kg^{-1})	20.96	12.55	21.15
Total N (g kg^{-1})	2.11	2.17	2.31
Organic C (g kg^{-1})	24.40	23.15	24.30
Organic matter (g kg^{-1})	41.97	39.82	41.80
C/N (mg kg^{-1})	11.56	10.67	10.52
K^+ ($mmol^+$ kg^{-1})	5.37	3.33	8.34
Na^+ ($mmol^+$ kg^{-1})	0.19	0.90	0.13
Ca^{++} ($mmol^+$ kg^{-1})	47.51	30.22	29.20
Mg^{++} ($mmol^+$ kg^{-1})	1.76	1.05	3.20
Al^{+++} ($mmol^+$ kg^{-1})	5.28	2.83	0.49
CEC ($mmol^+$ kg^{-1})	36.68	28.99	34.91

2.2. Plant material

The plant material is the WITA 9 rice variety also called Nimba. It was developed in 1984 by the International Institute of Tropical Agriculture (IIAT) by mixing the variety HI 2042-178-1 and the variety CT19 is an improved variety which was chosen mainly for its short cycle (90 days). It's average yield of 6 t ha^{-1} and its potential yield of 10 t ha^{-1}. The seed of WITA 9 provided in National Center of Agricultural Research (CNRA) in Man.

2.3. Chemical and natural materials

We used two types of phosphate amendments provided by OCP-Africa (Office Chérifien des Phosphates): TSP (Triple Superphosphate) a chemical fertilizers containing 30% P_2O_5, and a natural fertilizer Morocco phosphate rock (MPR) containing 30% of P_2O_5 with a solubility of 3% in water. Another chemical fertlizers such as Urea 46% N and NPK 15/15/15 were used in this study. The chemical composition of the MPR is presented in TABLE 2.

Table 2. *Chemical composition of Morocco phosphate rock*

Chemical	P_2O_5	CO_2	SO_3	SiO_2	CaO	MgO	Fe_2O_3	Al_2O_3	F	H_2O
Contents (%)	30	6.44	1.29	6.64	49.54	1.16	0.2	0.41	2.21	2.13

2.4. Field Experimentation

Fallow land of 500 m^2 was cleaned and tilled manually every year (2019 to 2021). The following eight treatments resulting to the combination of different proportions of MPR and TSP were implemented on-field before planting rice cultivar sativa : Absolute control (T0a : 0 % MPR and 0% TSP without NPK) ; reference control (T0 : 0% MPR and 0% TSP + NPK) ; T1 (100% MPR and 0% TSP+ NPK) ; T2 (90% MPR and 10% TSP + NPK) ; T3 (80% MPR and 20% TSP + NPK) ; T4 (40% MPR and 60%TSP + NPK) ; T5 (20% MPR and 80%TSP + NPK) ; T6 (0% PR and 100%TSP + NPK). In field a randomized complete block design with five replications was considered, with each field as a replicate. The MPR-TSP combination was designed to provide 90 kg ha^{-1} of P_2O_5 or 300 kg ha^{-1} of MPR or TSP. Each treatment was applied to an elementary plot of 25m² (8m × 3.13m) which included 600 pockets spaced 20cm apart from each other. Three yield squares of 1m² each were demarcated by processing along a diagonal of each elementary plot. One-meter canals separated the elementary plots framed by bunds formed to avoid contamination due to overflowing water in the event of rain.

Two days before sowing the 15-day-old rice plants, NPK 15-15-15 (200 kg ha^{-1}) and the different treatments were applied, as background fertilizer for each plot except for the absolute control. The transplanting was carried out at a rate of 02 plants per pocket 100 kg ha^{-1} of 46%N urea were spread in two stages: 50 kg ha^{-1} at the tillering stage and 50 kg ha^{-1} at the bolting stage. TABLE 3 gives the composition of the different treatments applied. In order to avoid competition between rice and weeds, a layer of water 10 to 15 cm high was maintained until heading and manual weeding was carried out if necessary. No insecticide or fungicide was applied to the plots. The transplants were carried out according to the cultural calendar of each study area. At maturity the agronomic parameters were measured.

- Plant height (H)

Heights (cm) were measured using a measuring rod from the base to the limit of the highest leaf of each plant located in the yield squares. i.e. seventy-five pockets per elementary plot due to twenty-five plants per square (IRRI, 2014). An average of the values obtained for each treatment was taken.

- Number of tillers (Til) and Number of panicles (Pan)

Tilling density is the number of tillers formed by a rice plant. The total number of tillers and the number of panicles are determined simultaneously with the harvest by counting on each plant the twenty-five pockets located in each yield square. An average of the values obtained per m² is made by multiplying the average by the number of pockets.

- Straw yield (SY)

On each site and for each treatment the biomass consisting of panicle straw tillers and leaves from the three yield squares was collected and dried in the open air then weighed in order to determine the straw yield (SY) per m².

- Grain yield (GY)

Grain yield (GY) was determined after drying the grains in the open air then in an oven at 65°C for 72 hours. Grain yield (GY) was calculated after reducing grain weights to 14% moisture (Koné et al. 2010; Akassimadou et al. 2017).

$$GY\ (14\%) = \frac{P1\ (100-h1)}{100-14} \qquad (1)$$

- Harvest Index (HI)

HI is defined as the ratio between grain yield and the sum of GY and SY.

$$HI(\%) = \frac{GYx}{GYx + SYx} \times 100 \qquad (2)$$

GYx is the Grain Yield of a treatment at a given x dose; SY: Straw yield of the same treatment at x dose.

- Yield gain (Yg)

Yield gain (Yg) of the GY of each treatment compared to the control treatment T0 was determined by modifying the formula of Morel and Fardeau (1991) using yields instead of P exports (Koné et al. 2010) :

$$Yg = \frac{GYx-GY0}{GY0} \times 100 \qquad (3)$$

Yg were calculated in relation to the T0 treatment in order to be able to subtract the share of NPK + Urea in the yields.

2.6. Statistical analyzes

Analyzes of variance were carried out using SAS (Statistical Analysis System) software using mixed models on the data from each campaign. Following linear models, the average values of each measured variable were determined depending on the treatment and the experimental site. Student Newmann_Keuls (SNK) test was used to compare these means using the least significant difference (ppds) method at the 5% significance level.

Table 3. *Treatments composition and values of fertilizers and nutrients applied on plots*

	Quantities of fertilizer applied (kg ha^{-1})					Quantities of each nutrients (kg ha^{-1}) in each treatments		
Treatments	**MPR**	**TSP**	**NPK**	**Urea**	**Total quantities of fertilizer**	**N**	**P**	**K**
T0a (soil potential)	0	0	0	0	0	0	0	0
T0 (reference control)	0	0	200	100	200 NPK + 100 Urea	76	13.2	24.9
T1	300	0	200	100	300 MPR + 200 NPK + 100 Urea	76	52.8	24.9
T2	270	30	200	100	270 MPR + 30 TSP + 200 NPK + 100 Urea	76	52.8	24.9
T3	240	60	200	100	240 MPR + 60 TSP + 200 NPK + 100 Urea	76	52.8	24.9
T4	120	160	200	100	120 MPR + 180 TSP + 200 NPK + 100 Urea	76	52.8	24.9
T5	60	240	200	100	60 MPR + 240 TSP + 200 NPK + 100 Urea	76	52.8	24.9
T6	0	300	200	100	300 TSP + 200 NPK + 100 Urea	76	52.8	24.9

III. RESULTS

3.1. Effect of treatments on the agro-morphological parameters of lowland rice

3.1.1. Tillers and panicles

TABLE 4 shows that panicle production is proportional to tiller production. Ours results indicated that all treatments amended with phosphate amendments (PAs) produced very hight significantly ($p<0.0001$) more tillers and panicles than control treatments T0 and T0a Treatments. However, when PAs contains more than 80% of TSP (T5 and T6), le number of tillers and panicles is significantly lower than when phosphate amendments (PAs) were enriched with Morroco phosphate rock (MPR) e.g. T1, T2, T3 and T4. Generally, the number of tillers and panicles are highest under T3 (339.76 to 564.9 m^{-2} for tillers and 291.78 to 519.0 m^{-2} for panicles) and T4 (316.1 m^{-2}to 557.6 m^{-2} for tillers and 291.8 m^{-2} to 499.4 m^{-2}) whatever the study site. However, ours results showed that le number of tillers and panicles produced in very (Man : 404.6 tillers and 362.7 panicles per m^2) or moderately (Gagnoa : 264.1 tillers and 231.8 panicules per m^2) acidic soils are higher than in weakly acidic soil (Bouaké) with 263.6 tillers and 226.9 panicules per m^2 (TABLE 4).

3.2.2. Plant height

Whatever the agroecological zone, a significant ($p<0.05$) increase of height have been observed, when the soils are amended with MPR and/or TSP, comparatively to soils unamended (T0 and T0a) (TABLE 4). However, the rice plants heights are significantly lower (80 to 89 cm), when soils are amended with TSP only (T6) or with 80% TSP+20%MPR (T5) than the height of plant when soils are amended with more than 40% MPR (T1, T2, T3, T4) with an average 99.3 to 109.1 cm (TABLE 4). Likewise, regardless of treatment applied, rice plants height are higher in very acidic soil (Man : 95.34 cm) than in moderately acidic soil (Gagnoa : 89.8 cm) and than in weakly acidic soil (Bouaké : 88.5 cm) (TABLE 4).

Table 4. Effect of treatments on number of tillers and panicles, and plants height in each agroecological zone after three cultural cycles

	Tillers /m^2			Panicles s /m^2			Heigtht (cm)		
Treatments	Man	Gagnoa	Bouaké	Man	Gagnoa	Bouaké	Man	Gagnoa	Bouaké
T0a	165.9^{c}	155.1^{c}	148.9^{c}	124.2^{c}	113.8^{c}	110.0^{c}	80.4^{b}	64.0^{c}	62.6^{c}
T0	237.6^{c}	194.6^{cb}	241.1^{b}	203.6^{c}	180.8^{b}	160.5^{cb}	84.4^{b}	74.7^{c}	72.4^{c}
T1	483.2^{a}	321.2^{a}	308.9^{a}	444.4^{a}	275.86^{a}	285.9^{a}	101.2^{a}	99.3^{ba}	100.4^{ba}
T2	531.9^{a}	322.5^{a}	312.7^{a}	491.9^{a}	287.4^{a}	281.8^{a}	105.8^{a}	103.2^{a}	100.3^{ba}
T3	564.9^{a}	343.4^{a}	339.8^{a}	519.0^{a}	313.6^{a}	303.2^{a}	109.1^{a}	108.2^{a}	105.9^{a}
T4	557.6^{a}	329.4^{a}	316.1^{a}	499.4^{a}	291.8^{a}	292.8^{a}	108.2^{a}	107.6^{a}	104.4^{a}
T5	357.2^{b}	229.4^{b}	219.0^{b}	319.2^{b}	199.62^{b}	195.4^{b}	89.1^{b}	81.0^{b}	80.8^{b}
T6	340.1^{b}	216.9^{cb}	222.3^{b}	300.0^{b}	193.6^{b}	187.0^{b}	84.6^{b}	80.4^{b}	81.2^{b}
Means	404.8	264.1	263.6	362.7	231.8	226.9	95.3	89.8	88.5
CV (%)	24.3	21.4	18.5	26.6	22.9	24.8	19.6	14.1	18.8
Lsd$_{.05}$	92.57	53.28	29.78	90.72	52.93	32.6	16.0	16.7	8.0
Pr	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***

*Values in the tables are means values of agronomic parameters mesured during experiments. Means within the same row with different superscripts letters are different (p<0.05) as indicated by Student Newman-Keuls test. *** probability very highly significant at 0.05;*

3.3. Yields and Harvest Index

3.3.1. Paddy rice grain yields (GY), straw yield (SY) and Harvest Index (HI)

The ANOVA carried out on yield data indicates a very highly significant effect of phosphate amendments ($p<0.0001$) on grain yields (GY) and straw yield (SY) of rice under soils amended (4.95 to 7.5 t ha^{-1} for GY and 9.11 to 11.76 t ha^{-1} for SY), comparatively to soil unamended (controls treatments T0 and T0a) with 1.52 to 3,49 t ha^{-1} for GY and 2.49 to 8.76 t ha^{-1} for SY, whatever the agroecological zone (TABLE 5). On the other hand, when soils are amended with phosphate amendments enriched with TSP (T5 and T6), grain yield and straw yield are significantly lower than when it's amended with phosphate amendments enriched with MPR (T1, T2, T3 and T4). The high GY and SY appeared under T3 and T4 Treatments whatever the the agroecological zone (TABLE 5). HI values follow the same trends as GY and SY for each treatment. Thus, the lowest HI values are obtained in Bouaké compared with Man and Gagnoa (TABLE 5). Irrespective of the agro-ecological zone, the treatments have a highly significant effect on HI. Treatments T3 and T4 achieved the highest HIs in Man (T3: 0.43 and T4: 0.41) and Gagnoa (T3: 0.4 and T4: 0.39), while in Bouaké the HIs under treatments T3 and T4 were 0.38 and 0.36 respectively (TABLE 5).

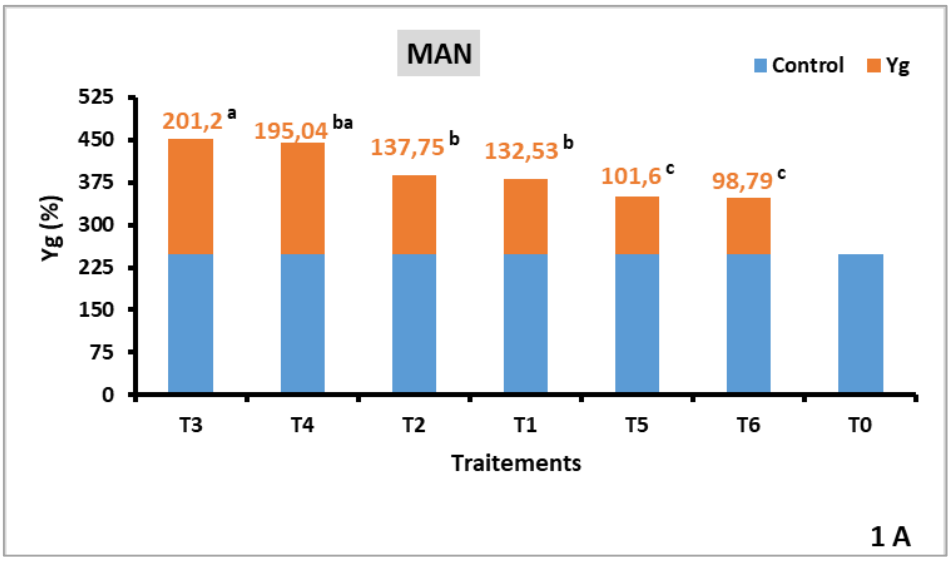
3.3.2. Yield gain

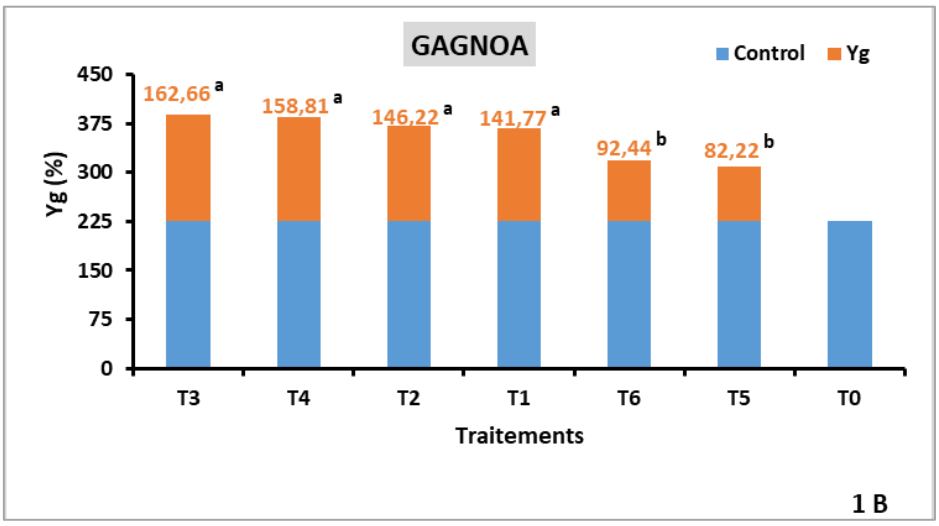
The analysis of Fig.1 shows that yield gain values vary depending on each treatment and the agroecological zones. But the greatest gains are obtained on the strongly acidic soils of Man (138.8%) followed by the moderately acidic soils of Gagnoa (130.66%) then come the weakly acidic soils of Bouaké (115.27%). We also note that Yg under T3 and T4 in Man (T3: 201.2%, T4: 195.04%) and Gagnoa (T3: 162.66%. T4: 158.81%) are greater than Yg under T3 and T4 in Bouaké (T3: 173.39%. T4: 169.44%). On the same agroécological zone, treatments rich in MPR (T1, T2, T3 and T4) significantly increased yields compared to control T0 and compared to treatments T5 and T6 enriched in TSP (Fig.1).

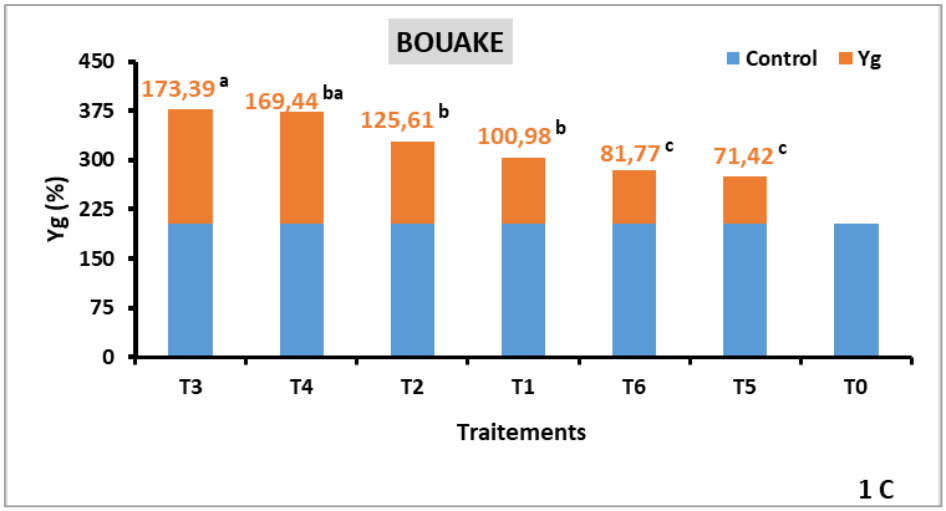
Table 5. Effect of treatments on GY. SY and HI of rice in each agroecological zone after three cultural cycles

	GY (t ha^{-1})			SY (t ha^{-1})			HI		
Treatments	Man	Gagnoa	Bouaké	Man	Gagnoa	Bouaké	Man	Gagnoa	Bouaké
T0a	2.17^{d}	1.52^{d}	1.69^{d}	4.12^{e}	2.49^{c}	3.36^{e}	0.28^{f}	0.3^{d}	0.22^{e}
T0	3.49^{d}	2.67^{d}	2.03^{d}	8.76^{d}	4.38^{c}	4.04^{ed}	0.3^{e}	0.33^{c}	0.27^{d}
T1	5.79^{bc}	5.54^{ba}	4.08^{bc}	10.99^{b}	7.24^{a}	6.15^{bc}	0.38^{c}	0.39^{a}	0.34^{b}
T2	5.92^{bc}	5.68^{ba}	4.58^{bac}	11.34^{a}	7.86^{a}	6.92^{bac}	0.4^{b}	0.39^{a}	0.34^{b}
T3	7.50^{a}	5.91^{a}	5.55^{a}	11.76^{a}	8.21^{a}	8.17^{a}	0.43^{a}	0.4^{a}	0.38^{a}
T4	6.50^{b}	5.82^{a}	4.84^{ba}	11.5^{a}	7.95^{a}	7.35^{ba}	0.41^{ba}	0.39^{a}	0.36^{ba}
T5	5.02^{c}	4.33^{bc}	3.69^{bc}	9.88^{c}	5.88^{b}	5.47^{dc}	0.37^{c}	0.36^{b}	0.33^{b}
T6	4.95^{c}	4.10^{c}	3.48^{c}	9.11^{c}	5.52^{b}	5.34^{dc}	0.35^{d}	0.32^{c}	0.31^{c}
Means	5.17	4.45	3.75	9.68	6.19	5.85	0.37	0.36	0.32
CV (%)	26.63	24.97	25.87	22.04	21.60	22.09	15.4	8.13	8.62
Lsd$_{.05}$	0.91	0.83	1.02	1.21	1.67	1.23	0.031	0.027	0.031
Pr	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	0.002**	<0.001***	<0.001***

*Values in the tables are means values of agronomic parameters mesured during experiments. Means within the same row with different superscripts letters are different (p<0.05) as indicated by Student Newman-Keuls test. *** probability very highly significant at 0.0 ;*







Values in the graphic are means values of agronomic parameters mesured during experiments. Means within the same row with different superscripts letters are different ($p<0.05$) as indicated by Student Newman-Keuls test.

Fig.1. Yield gain under treatments compared to control T0 after three cultural cycles

IV. DISCUSSION

4.1. Soil properties

The acidic pH of soils in the 0-10 cm horizon constitues a favorable factor for the solubilization of the MPR contained in applied treatments. The high levels of assimilable P (Passi > 20 mg kg^{-1}) and N (2‰ < N < 3‰) in the soil indicate that these two elements cannot be considered as a limiting factor in the assimilation of nutrients by rice plants. The fairly high percentages of clay in soils of Man (23.7%) and Gagnoa (28.5%) coupled with their so-called average ECE (10 meq.$100g^{-1}$ < EC < 25 meq.$100g^{-1}$) indicate that these clay levels could constitute nutrient reserves. However the low clay content (12.5%) of soil of Bouaké could be a limit to water retention as well as nutrient retention (Kaboré et al. 2020).

4.2. Efficiency of PAs on agro-morphological parameter of rice

Statistical tests carried out showed that treatments increased not only the production of tillers and panicles of the cultivated rice but also the height of rice plants. This increase which is greater on more acidic soils of Man and Gagnoa than that of Bouaké. demonstrates that MPR is more effective in acidic soils. These results show that the more pronounced acidity of the soils of Man and Gagnoa favored the solubilization of the MPR as well as the provision of the released P for the nutrition of rice plants (Wafaa et al. 2011). Obtaining larger plants as well as high number of tillers and panicles produced under treatments (T1, T2, T3 and T4) richer in MPR indicates that the higher the percentage of MPR in the fertilizer the more the plants are larger and the higher their biomass production. In fact the phosphorus released after dissolution of the PRM is involved in vegetative growth (Wafaa et al. 2011; Kotchi et al. 2018). These results agree with those of several authors such as Useni et al. (2012) and Sanogo et al. (2020) who showed that high doses of phosphorus induce elongation of rice plants. Sanogo et al. (2020) also demonstrated that the number of tillers in lowland rice is increased by high doses of phosphate and nitrogen fertilizers. A study conducted by Saito et al. (2029) from 2014 to 2015 showed that the application of phosphate fertilizer increases production of tillers, panicles and the height of rice plants of the WITA 9 variety used in our study. The highest production of panicles per m^2 and larger rice plants under T3 and T4 in Man (very acidic soil) and in Gagnoa (moderately acidic soil) shows that their production would be influenced by pH and doses of MPR applied. This highest production would also be under the influence of other chemical parameters of the soil such as the initial values of N, P, K, Ca and Mg as demonstrated by Yang and Zhang (2010), Koné et al. (2013).

Treatments rich in TSP (T5, T6) produced shorter plants fewer tillers and panicles per m^2 compared to treatments rich in MPR. It is because the greater aqueous solubility of TSP favors the quick release of P for rice nutrition compared to PRM but its effect fades quite quickly over time because the majority of this released P forms complexes with Fe^{2+}, Al^{3+} (Wafaa et al. 2011; Aallam et al. 2023).

4.3. Yield parameters

Yield parameters subjected to analysis of variance indicate that the contributions of treatments on the different plots favor the productivity of lowland rice compared to controls (T0 and T0a). This productivity in terms of grain (GY) and straw (SY) which is even higher under T1, T2, T3 and T4 which contain between 50 and 100% MPR is the result of the provision of P contained in MPR after dissolution. In fact P intervenes during rice growing phase to promote flowering and consequently grain production as indicated by Mollier (2016) who states that 65% of the P supplied to crops in the form of fertilizer is found grains. Thus P is assimilated in large quantities by rice which like wheat is a very voracious crop in nutrients to satisfy its needs (Bihari et al. 2021). Therefore to maintain good rice productivity it would be necessary to add fertilizer annually or at each rice cultivation cycle because meeting its nutritional needs tends to considerably reduce soil nutrients (Nayak et al. 2022). This explain the low productivity of rice in treatments T5 and T6 richer in TSP because P released by this chemical fertilizer applied all at once is not enough to satisfy the nutritional needs of rice over a long period. This low grain and straw productivity can also be linked to the complexation of a large part of the P released by the TSP as highlighted by Wafaa et al. (2011). The large production of straw and grain observed on strongly acidic soils of Man and moderately acidic soils of Gagnoa compared to the weakly acidic soil of Bouaké demonstrates that MPR is more effective in an acidic environment (Kotchi et al. 2010; Maharana et al. 2020) because the application of PR on acidic soils allows the release of hydrogen ions which promote the conversion of P into forms assimilable by plants during more than one year (FAO, 2004). Treatments T3 and T4 which stand out from other treatments by their higher GY and SY indicate that it would be advantageous to constitute treatments composed of 60 to 80% MPR to increase the yields of lowland rice. But beyond the pH and fertilizer doses taking into account the interaction between the chemical elements of the soil proves necessary in order to make the application of phosphate fertilizers agronomically profitable.

Due to their highest Yg an HI under treatments rich in MPR and on strongly acidic soils of Man and those moderately acidic of Gagnoa compared to the weakly acidic soils of Bouaké show that application of MPR on plots favors rice productivity as demonstrated by Sanogo et al. (2020).

Treatments T3 (80%RP+ 20%TSP) and T4 (60%RP+ 40%TSP) having obtained the highest Yg rate shows that a fertilizer in the proportions of 240 kg ha^{-1} RPM + 60 kg ha^{-1} TSP in proportions of 180 kg ha^{-1} RPM + 120 kg ha^{-1} TSP would be best suited to considerably increase rice grain production in Man Gagnoa and Bouaké (Alam et al. 2009; Elkheir et al. 2018). However thus proportions are low compared to 300 kg ha^{-1} of phosphate from Tilemsi used by Koné et al. (2010) to produce 2.5 t ha^{-1} of rice. Our results corroborate those of Taktek (2015) who asserted that it is neccesary to reduce about 50% of phosphate rock to improve the productivity of rice. All this disparities between production in each agroecological zone bear out that the efficiency of phosphate rock varie depending to soils characteristic.

Treatments T3 and T4 which have produced more GY, SY and Yg appear to be appropriate to improve lowland rice cultivation in Man Gagnoa and Bouaké.

V. CONCLUSION

Amendment of lowland soils with MPR combined with TSP is more effective than applying MPR or TSP alone to lowland soils for rice cultivation. The treatments resulting from the MPR-TSP combination considerably increased the production of tillers panicles as well as the GY and SY of rice especially under those rich in MPR (T1, T2, T3 and T4). After three cultivation cycles treatments T3 and T4 are those which increase the GY more compared to the control T0 whatever the agroecological zone with respective GYs of 7.50 t ha^{-1} and 6.50 t ha^{-1} to Man; 5.54 t ha^{-1} and 5.91 t ha^{-1} in Gagnoa then 5.55 t ha^{-1} and 4.84 t ha^{-1} in Bouake. But the consideration of chemical (initial N, P, K, Ca and Mg contents) and biological (BSP) factors is necessary to recommend the MPR-TSP combination adapted to each type of rice soil.

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Health effects and toxicological outcomes of exposure to inhalable particulate matter from urban air pollution: A mini review

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Abstract**—** *Inhalable air pollution consists of a complex mixture of solid PM (Particulate Matter) or liquid and gaseous components originating from a myriad of natural and anthropogenic sources that cause harmful to humans, animals or plants. From the environmental aspect, air pollutants include PM, carbon monoxide, ozone, nitrogen dioxide and sulfur dioxide. Ambient levels and composition often vary greatly depending on the emission sources and meteorological conditions. There are many hazardous air pollutants such as benzene, dioxin, asbestos and metals; however, the mass of PM is one of the most widely accepted indicators of air quality monitoring and regulation. Epidemiological and toxicological studies have identified an association between elevated levels of PM in the "respirable size fraction" and adverse health outcomes in the general population. For this reason, airborne PM has recently been listed as a potentially carcinogenic agent by the International Agency for Research on Cancer (IARC) because of its heterogeneity and variation of chemical composition over space and time.*

Keywords**—** ***urban air pollution, particulate matter, health effects***

I. INTRODUCTION

Ambient PM sources, composition and classification of particulate matter (PM_{10}, $PM_{2.5}$, $PM_{0.1}$)

Ambient PM is a complex mixture consisting of solid particles and liquid droplets of varying size and composition, which is dispersed in the air. PM mainly originate from three sources; (i) **natural sources** such as forest fires, windblown dust, soil erosion, volcanoes and seas, (ii) **anthropogenic** sources such as power plants, refuse incinerators, motor vehicles, construction activity and residential heating, (iii) **formation of secondary aerosols** in the atmosphere by transformation of gases into liquids or solids e.g. SO_2, NO_X, and NH_3 (Feng et al., 2016; Thangavel et al., 2022). Being emitted from various sources, PM therefore differs in its chemical and physical properties such as size, morphology, crystal structure, surface charge and chemical composition. These characteristics strongly impact on sedimentation time, i.e. persistence of PM in the air. More importantly, because of the great diversity of particle sources, the composition of ambient PM is complex (Li et al., 2022; Sarti et al., 2015; Valavanidis et al., 2008). The size of the PM varies and the term *ultrafine* particles, i.e. those < 0.1 μm in aerodynamic diameter, *fine* particles, i.e. those < 2.5 μm, and those with larger diameters as coarse particles and is generally referred to as $PM_{0.1}$, $PM_{2.5}$ and PM_{10}, respectively (Cho et al., 2022; ; Johnson et al., 2022; US EPA, 2016). $PM_{2.5}$ and ultrafine particles are essentially produced by combustion processes directly or indirectly from precursor substances, while coarse particles (PM_{10}) originate from biological and mechanical processes (e.g. pollen or dusts from opencast mining, dumping of debris and tailings, demolition processes, as well as sand storms or volcanic eruptions). The size of the particles determines the site in the respiratory tract they will deposit; PM_{10} particles mainly deposit in the upper respiratory tract, while $PM_{2.5}$ and $PM_{0.1}$ particle is able to reach lung alveoli. The adverse effects of

inhaled PM are highly dependent on the deposition and retention of particles in the lung. Identifying and quantifying the influences of specific chemical components or source-related mixtures on measures of health-related impacts represents one of the most challenging areas of environmental health research (Byeon et al., 2015; Kelly and Fussell, 2012).

Legal thresholds for PM concentrations in ambient air

According to the preamble to Directive 2008/50/EC on ambient air quality and cleaner air for Europe of the European Union, the main goal of air quality control is "to reduce pollution to levels which minimize harmful effects on human health, paying particular attention to sensitive populations, and the environment as a whole". Therefore, air quality research focuses on particles which can cause damage to human health. From this point of view, it is of particular interest how the levels of ambient air particles are legally regulated based solely on their mass per cubic meter in the air for both daily and annual average concentration. Thresholds for $PM_{2.5}$ and PM_{10} have been indicated by the World Health Organization (WHO, 2021) to protect the health of the population. For PM_{10}, the permissible short-term guideline (24 h average) is 50 $\mu g/m^3$, while the value for $PM_{2.5}$ is 25 $\mu g/m^3$. The annual average concentration chosen as the long-term guideline value for $PM_{2.5}$ is 10 $\mu g/m^3$ and the annual average concentration chosen for PM_{10} is 20 $\mu g/m^3$. However, it might be more beneficial for public health stakeholders to focus on reducing emissions from specific sources.

II. DISCUSSION

Role of PM physical and chemical characteristics causing health effects

Epidemiological and experimental studies *in vivo* and *in vitro* provide increasing evidence for the importance of physical and chemical characteristics in particle-induced biological effects (Hadrup et al., 2020; Schwarze et al., 2007). The physical and chemical characteristics of inhaled particles both at urban and rural sites have shown that there is a strong link between exposure to coarse, fine, and ultrafine particulate matter and mortality (Arif et al., 2017; Hufnagel et al., 2021; Jiang et al., 2022; Petersen et al., 2019; Stafoggia et al., 2017; Valavanidis et al., 2008; Yorifuji et al., 2016). Moreover, small particles exhibiting a large surface area per mass have been found to induce a more pronounced pro-inflammatory response than larger particles of the same material because the deeper the deposition site in the respiratory tract, the slower the clearance rate and the higher the probability of particle-cell interactions (Schmid and Stoeger, 2016).

Ultrafine particles as compared to engineered nanoparticles

Ultrafine particles are defined as particles with diameters below 100 nm. They are chemically heterogeneous and polydisperse materials that can be engineered or naturally occurring, e.g. atmospheric ultrafine particles are formed during combustion. The physical and chemical properties of these particles play an important role in determining their health implications (Mossman et al., 2007). Engineered nanoparticles are described as inorganic ingredients of high uniformity with at least one critical dimension below 100 nm that are specifically engineered for commercial applications (Montes-Burgos et al., 2009). They are intentionally designed and created with physical properties to meet the requirements of a specific application. The physical and chemical properties of these particles are extremely important for determining their performance in the product where they are incorporated.

Chemical composition and source comparison of urban air particles

Particle composition is also responsible for particle-associated adverse health effects. Ambient particles are composed of thousands of chemicals and constituents, including inorganic ingredients, transition metals, crystal compounds, elemental carbon and various organic compounds, such as polycyclic aromatic hydrocarbons (PAHs), nitro-and oxy-PAHs and endotoxins have been reported to influence particle-induced inflammation (Arif et al., 2018; Becker et al., 2002; Borm et al., 2007; Donaldson et al., 2005; Maschowski et al., 2020, 2019; van Berlo et al., 2009). Therefore, different physical and chemical PM properties, including size, surface area, number, composition, crystal structure, and particle agglomeration/aggregation can play a significant role in initiating PM toxicity and can induce adverse health effects (Chen et al., 2016).

The main anthropogenic sources of airborne PM include combustion processes, especially those of high-sulfur fuels used extensively in gasoline-powered generators, traffic, construction and solid waste incineration (Zeb et al., 2018). Particles such as quartz and calcite may be originating from earth's crust and construction activities. Somewhat regular spherical shapes indicate anthropogenic origin e.g. traffic-related soot from vehicle exhaust. Therefore, understanding the properties, chemical compositions and origins of different - source-related urban air particles is significantly important both for an environmental and health risk assessment.

III. CONCLUSIONS

This review considers PM in ambient air and particles that can actually be inhaled in the environment. The WHO states that the presence of PM in ambient air is a problem in both big and small cities around the world. In addition, only with this information can strategies aimed at effectively addressing the threat of particulate matter in the environment can be developed, thereby ensuring environmental sustainability, can be developed. It will also provide evidence that will inform policy in the Establishing standard guidelines for the biological and chemical constituents of particulate matter components of particulate matter rather than for the total mass of particulate matter in the environment. further studies are needed to better understand to better understand the contribution of the combination of biological and chemical components of particulate matter to the documented health endpoints that have not yet been fully elucidated.

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Levels of Heavy Metals in Selected Canned Fish on Cape Coast Market, Central Region, Ghana

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Abstract— *Background and objectives: The sea is polluted with heavy metals that accumulate in fishes. Consumption of these fishes pose health risk. The aim of this study is to respectiely assess the concentrations and health risks of lead (Pb), zinc (Zn), iron (Fe), tin (Sn), manganese (Mn), and mercury (Hg) in canned fish samples. Methods: The study focused on Mackerel, Sardine and Tuna. Mackerel brands are African Queen, Geisha, Ena Pa, and Milano; Sardine includes Titus, Festiva, Ohemaa, and Princess, while Tuna were Lele and Star Kist. Flame Atomic Absorption Spectrophotometry was used. Results: The mean concentrations of lead and mercury in the Mackerel, Sardine and Tuna were respectively 0.142± 0.017, 0.122±0.034, 0.141±0.006 µg/g and 0.126± 0.017, 0.132± 0.012, 0.263± 0.006 µg/g below the recommended limits of 0.3 and 0.5 µg/g by EU Reg. No 1881/2006. The concentrations of zinc, iron, tin, and manganese were within the acceptable respective limits. A health risk assessment based on the criteria established by the US EPA revealed no significant health risks associated with the concentrations of the metals. Conclusion: The canned fish samples exhibited low levels of heavy metal contamination, indicating that the fish samples pose no significant health risks to consumers.*

Keywords— ***lead, canned fish, contamination, health risk, safety standards, heavy metals.***

I. INTRODUCTION

Canned fish, is a convenient food choice that offers essential nutrients, protein, vitamins, and minerals (Newman et al., 2020; Johnson, J. L., & Brown, D (2018)[1,2] . However, the presence of heavy metals, such as lead (Pb), zinc (Zn), iron (Fe), tin (Sn), manganese (Mn) and mercury (Hg) has raised concerns about potential health risks for consumers (Roberts, T. 2017); Boadi et al., 2021)[3,4] . Fish may be contaminated by toxic elements during fish growth, transportation, storage and canning processes, for instance Lead poisoning is generally ranked as the most common health hazard (Boadi et al., 2021; Food Safety Authority 2019; Anderson, E. J. 2016)[5,6,7]. Ghana serves as a significant distribution center for canned fish, catering for a diverse consumer population including Cape Coast in the Central region. Ensuring the safety and quality of canned fish in this market is crucial to protect public health (Smith, A. B. 2021; Martin, S. A. 2021)[8,9] . The objective of this study is to examine the concentrations of these metals in canned fish samples obtained from the Cape Coast market in the Central region of Ghana. The analysis involved mackerel, sardines, and tuna fish, which are commonly consumed by local residents[8].Thus, it is necessary to evaluate and monitor the levels of heavy metals, in these products to establish compliance with national and international safety standards. (Harris, E., & Thompson, K. 2022; Food Safety Authority 2019; ISO. 2020; Jones et al., 2023)[10,11,12,13]. The findings of this study will contribute to the existing knowledge regarding heavy metal contamination in canned fish products, and for stakeholders to make informed decisions regarding the safety and quality of canned fish. The result shall also help protect public health, instill consumer confidence, and contribute to a safer and healthier food market.

II. MATERIALS AND METHODS

2.1 Reagents

All reagents utilized were of analytical reagent grade. Standard stock solutions of lead (1000 mg/l), mercury, zinc, manganese, tin and iron were prepared from commercially available sources and diluted to the desired concentrations for the analysis. Working solutions were freshly prepared by diluting appropriate aliquots of the stock solutions using 10% nitric acid.

2.2 Sample preparation and digestion

A total of ten (10) canned fish samples were used in the study, including four (4) mackerel canned fishes [African Queen, Geisha, Ena Pa, and Milano], four (4) sardines [Titus, Festiva, Ohemaa, and Princess], and two (2) tuna fish [Lele and Star Kist]. Each can's contents were thoroughly homogenized in a stainless steel food blender. A representative sample (2 ± 0.001 g) was weighed and promptly digested.

2.3 Chemical analysis

Mercury, was determined by Cold Vapor Atomic Absorption Spectrophotometer using the Direct Mercury Analyzer. Lead, tin, zinc, manganese, and iron were determined using a UNICAM 969 Flame Atomic Absorption Spectrometer. The detection limits in µg/mL for the metals were 0.010 for lead, 0.002 for zinc, 0.002 for manganese, 0.005 for iron, and 0.001 for mercury.

2.3.1 Quality assurance

To ensure the accuracy of the analytical procedure, sample duplication was performed, and analytical validation was conducted using certified reference samples for lead, tin, zinc, manganese, mercury and iron. Method blanks were carried out for lead, tin, zinc, manganese, and iron determinations in fish samples. Prior to the metal determinations, blank solutions were analyzed under the same experimental conditions as the samples.

2.4 Statistical Methods

Descriptive statistics (mean) and one-way analysis of variance (ANOVA) were conducted using SPSS and Excel software. A one-way ANOVA statistical procedure was employed to assess the variation in metal concentrations among canned fish of the same brand and across canned fish of different brands.

III. RESULTS

Table 1: Descriptive Statistics of Heavy Metal Concentrations in the Samples

Fish Type	*Pb (µg/g)*	*Zn (µg/g)*	*Fe (µg/g)*	*Sn (µg/g)*	*Mn (µg/g)*	*Hg (µg/g)*
Mackerel	M = 0.142 SD = 0.017	M = 0.137 SD = 0.048	M = 7.279 SD = 6.832	M = 16.550 SD = 3.700	M = 0.017 SD = 0.014	M = 0.126 SD = 0.017
Sardine	M = 0.122 SD = 0.034	M = 0.140 SD = 0.039	M = 13.261 SD = 9.945	M = 14.482 SD = 10.202	M = 0.024 SD = 0.014	M = 0.132 SD = 0.012
Tuna	M = 0.141 SD = 0.006	M = 0.178 SD = 0.022	M = 12.115 SD = 1.985	M = 16.024 SD = 0.195	M = 0.022 SD = 0.004	M = 0.263 SD = 0.006
Recommended limits [EU Reg. No 1881/2006]	0.3	100	Not specified	200	Not specified	0.5

Note: M = mean, SD = standard deviation

Source: Bartels/ Apaah/ Gadzekpo, 2023 statistical analysis

Table 2: ANOVA Results for Heavy Metal Concentrations among Fish Types

Variable	Sum of Squares	df	Mean Square	F	p-value
Pb	0.015	2	0.0075	1.23	0.305
Zn	0.158	2	0.0790	4.55	0.024*
Fe	5.291	2	2.6455	9.82	<0.001**
Sn	18.920	2	9.4600	7.76	0.004*
Mn	0.002	2	0.0010	0.42	0.664
Hg	0.000	2	0.0000	0.01	0.990

Note: * $p < 0.05$, ** $p < 0.001$.

IV. DISCUSSION

The results obtained from the analysis of heavy metals in canned fish from the Cape Coast market provide valuable insights into the levels of contamination and potential health risks associated with their consumption. Table 1 presents the descriptive statistics of heavy metal concentrations in the analyzed canned fish samples. The results indicate that mackerel samples had a relatively higher mean concentration of lead [0.142± 0.017]. Zinc concentration was highest [0.178± 0.022] in tuna samples, while sardine samples exhibited the highest mean concentration [13.261±9.975] of iron. Mackerel samples showed the highest mean concentration [16.550±3.700] of tin, and tuna samples had the highest mean concentration [0.263±0.006] of mercury (Smith, P. 2021; Johnson, J. L., & Brown, D. 2018)[14,2] .These variations in heavy metal concentrations among different fish types highlight potential differences in contamination levels across the analyzed canned fish samples.

ANOVA results presented in Table 2 reveal significant variations in the concentrations of zinc and iron among the different fish types. This suggests that the type of fish and their respective environments may influence the accumulation of these heavy metals. However, no significant differences were observed for lead, tin, manganese, and mercury concentrations among the fish types. This implies that the fish species examined in this study did not exhibit significant variations in the accumulation of these particular heavy metals.

As shown in Table 1, the results highlights the compliance of lead concentrations in the canned fish samples with the regulatory limit set by the European Union (0.3 µg/g). This indicates that the analyzed canned fish products meet international safety standards, ensuring consumer safety in terms of lead exposure. The concentrations of zinc, iron, manganese, tin, and mercury fall within acceptable ranges reported in previous studies or can be compared with relevant standards, indicating no significant health risks associated with their consumption. (Garcia et al., 2018; Roberts, T. 2017)[15,4].

The findings underscore the importance of further investigation into the sources of zinc and iron contamination in canned fish products, as significant variations were observed in their concentrations across fish types. This investigation will help identify potential risks and implement measures to mitigate these heavy metals' presence.

The concentrations of tin in the analyzed canned fish samples ranged from 14.482 to 16.550 µg/g. Although no specific international standard exists for tin in canned fish products, guidelines for other food commodities can be referenced. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established a provisional tolerable weekly intake (PTWI) of 14 µg/kg body weight for tin (JECFA, 2011)[16]. This PTWI represents the amount of tin that can be consumed on a weekly basis without significant health risks. In the absence of a specific regulatory limit for tin in canned fish products, it is important to compare the observed tin concentrations with other relevant standards. The European Union (EU) has established regulatory limits for tin in various food commodities, including canned fruits and vegetables. For example, EU Regulation No. 1881/2006 sets a maximum limit of 200 µg/kg for tin in canned fruits and vegetables. Although this limit is not directly applicable to canned fish, it provides a reference point for assessing the acceptability of tin concentrations in food products. Comparing the observed tin concentrations in the canned fish samples with the EU regulatory limit for canned fruits and vegetables (200 µg/kg), it is evident that the tin levels in the analyzed samples are significantly below the established limit. This indicates that the tin concentrations in the canned fish samples are well within acceptable ranges and pose no significant health risks to consumers. Furthermore, considering the PTWI established by JECFA for tin (14 µg/kg body weight), the observed tin concentrations in the canned fish samples are also well below this safety limit. This suggests that the consumption of the analyzed canned fish products would not result in excessive tin intake or pose any adverse health effects.

V. CONCLUSION

The present study aimed to determine the levels of heavy metals, specifically lead (Pb), in canned fish. Ten samples were analyzed, including three mackerel canned fish, four sardines, and three tuna fish.

1. The results showed that the concentrations of lead, as well as other metals, varied among the different canned fish brands.
2. These levels were found to be either below or within the recommended limits
3. Significant differences in metal concentrations were observed across the different canned fish brands analyzed in this study. This suggests variations in the manufacturing processes, sourcing of raw materials, and potential environmental factors that can contribute to the metal content in canned fish.
4. The levels of heavy metals in the analyzed canned fish varieties do not pose a serious health risk to consumers.

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RECOMMENDATION

- The study recommends regular monitoring of heavy metal levels in canned fish from the Cape Coast market to ensure compliance with safety standards.
- Stringent quality control measures, such as sourcing fish from low-metal suppliers and conducting regular testing, should be implemented during production.
- Public awareness campaigns are needed to educate consumers about the risks of heavy metal contamination.
- Collaboration between regulatory bodies, research institutions, and the seafood industry is crucial for identifying contamination sources and developing mitigation strategies. Safety standards should be periodically reviewed and updated, and international cooperation and training programs can enhance analysis and quality control measures.

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Natural Concentrations of Iodine in Common Salts Produced from Popular Lagoons in Ghana and their Potential to Eliminate Iodine Deficiency Disorders

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Abstract— *Background and objectives: Iodine deficiency is a global public health concern. This deficiency if not cured results in illness like goiter, cretinism and reduced intelligence quotient that disrupts normal physical and mental functions. The USI programme was introduced to improve household's iodine intake, in Ghana the mandatory salt iodisation law that specifies that all salts meant for consumption must be iodised has been long passed to give credence to the USI. Yet, some Ghanaian families are still deliberately or ignorantly consuming salt containing less or no iodine at all with the perception that common salt naturally contains enough iodine. Hence, this study assesses the levels of iodine in salts from popular lagoons, which are major salt producing centres in Ghana. Methods: Two hundred (200) samples of salts were collected in six (6) months from popular major salt producing lagoons, namely, Ada-Songhor, Nyanyano, Keta, Amisa and Benya lagoons. In addition, five (5) brands of packaged refined salts also sampled for their iodine for comparative analyses. Questionnaire was administered to household's women in charge of meal preparation to solicit information about preference for salt and knowledge about IDD. Statistical Package for Social Sciences (SPSS) version 16 and Microsoft Excel were used in data analysis. Results: The lagoon salt samples did not record any measurable iodine (0 ppm). Ninety-three percent of respondents were ignorant about benefits of iodised salt, whilst 50.4% prefer non-iodised salt. Conclusion: The ignorance about the absence of iodine in crude salt could influence the extent of the ignorance about benefits of iodine and preference for non - iodised salt.*

Keywords— Lagoon, Common salt, Iodine Deficiency Disorders, Survey, iodine

I. INTRODUCTION

Common salt is important for human, animal and industry. One of the sources of common salt in Ghana predominantly is the seawater, primarily consists of sodium chloride (Osborne et al., 2023)[1]. Ghana started salt production in the nineteenth century. It has been a substantial economic activity, in the major salt producing lagoon centres, namely, Ada-Songhor, Keta, Densu Delta areas, Nyanyano, Amisa and Benyah lagoons (Amadu, A.K. 2019, Atta-Quayson & Baidoo, 2020)[2,3] . National production levels are estimated around 250,000 MT annually with potential of 2-3 million Tonnes (GEPC 2009)[4].

Iodine is a necessary trace mineral received through diet or supplements because the body cannot synthesize it. It is required for the creation of thyroid hormones (De Escobar et al., 2004)[5] which control several body processes, including metabolism. Iodization, the process of iodizing salt, used to alleviate iodine deficiency. In locations where iodine shortage is a problem, iodized salt is frequently used (Chirawurah et al.,201 5)[6]. A third of the world's population lives in regions with low natural iodine levels,

necessitating interventions to guarantee a sufficient iodine supply (Doku & Bortey (2018)[7].

Globally, efforts are made to promote the consumption of iodized salt to improve iodine intake (Zimmermann, M. B. (2009)[8], through USI programme. The USI has a target of 90% household use of sufficiently iodized salt. To achieve this, a legislation was passed in Ghana requiring iodine fortification of salt meant for humans and poultry (Ghana Public Health Act. (2012)[9]. To guarantee safe usage, the Ghana Standards Authority has established standards for the iodine level in salt (Ghana News Agency (2010)[10].

This notwithstanding, there is the perception that common salt contains iodine naturally which is enough for the body's physiological processes, contrary to established fact that unprocessed salt does not contain iodine (IGN, 2019)[11]. The aim of the study is to analyse the iodine in common salt from the major salt producing lagoons in Ghana, and then assess the impact of this perception in eliminating IDD in Ghana. The results will enrich the data on IDD about Ghana, and also enable policy makers and stake holders in their decisions.

II. MATERIAL AND METHODS

2.1 Study Design and Participants

Quantitative determination of the iodine levels in the salt was by titration (WHO).

Questionnaire designed to solicit information from respondents about their knowledge on IDD and choice for salt type. One hundred and thirteen (113) respondents who were women responsible for household meal preparations were interviewed.

2.2 Study Area and Sampling

One hundred (100) packaged salt samples were obtained from shops. They were six (6) different brands and coded for analysis.

In addition, two hundred (200) samples of common salt was collected in 6 months from major salt producing lagoons across Ghana.

The locations and the coordinates are as follows:

The Ada- Songhor lagoon is located at Ada East District in the Greater Accra Region (GSS, 2010)[12] with coordinates 5.82^{O}N 0.47^{O}E (en.m.wikipedia.org)[13].

The Keta Lagoon is located at Keta Municipal District in the Volta region, the coordinates are 5º53'16"N 0º49'36"E. (en.m.wikipedia.org)[13]

Nyanyano lagoon at Gomoa East district in the Central region having coordinates 5º30'89" N 0º25'2.9"W(en.m.wikipedia.org)[13]

Amisa lagoon in the Mfantsipim district of Central region with coordinates latitude 5º12'16.49"N longitude -1º0'38.45"W. (en.m.wikipedia.org)[13]

Benya lagoon in Elmina of Komenda /Edina/ Eguafo/ Arem distrct in the Central region, latitude is 5.05º4.92"N longitude -1º 21'3.35"W. (en.m.wikipedia.org)[13]

2.3 Chemical Analysis

Samples A, B and C were subjected to chemical analysis for the presence of iodine them in the research Lab of the department of Laboratory Technology, University of Cape Coast.

Twenty grams (20g) of sample C was weighed into 250mL conical flask and stoppered. Thirty

milliliters (30 mL) of distilled water was added and swirled to dissolve. Thirty milliliters (30 ml) distilled water was added to make up 60 mL. One milliliter (1mL) of 1M H_2SO_4 and 5mL of 10% KI solution was added. The flask was stoppered and placed in the dark for 10 minutes. It was titrated against 0.01M $Na_2S_2O_3$ until the solution turned pale yellow. Two (2) mL of starch indicator solution was then added, and titration continued until the solution became colorless. This was repeated for samples A and B. (Ahiadeke et al., 2012)[14].

The qualitative analysis depended on the reaction:

$$IO_3^- + 5I^- + 6H + \rightarrow 3I_2 + 3H_2O.$$

The yellow or brown colouration indicates the presence of free iodine liberated on addition of acid, and kept in a dissolved state by potassium iodide (Doku & Bortey, 2018)[7],(University of Canterbury, College of Science. 2021)[15]. The liberated free iodine reacts with the amylose component of starch (indicator) to give the characteristic dark purple (blue - black) coloration. Addition of thiosulphate solution ($Na_2S_2O_{3\,(aq)}$) reduces excess iodine, the dark blue – black colour disappears leaving a colourless endpoint. (University of Canterbury, College of Science. 2021)[15].

The unrefined salt showed no formation of a yellow colouration from the reaction, this indicates a negative qualitative analysis, for the industrially iodated salt, a yellow coloration was observed after the initial reaction indicating the presence of iodine, hence a positive qualitative analysis.

2.4 Calculations

The equations below explain the reaction:

$$IO3^-(aq) + 5I^-(aq) + 6H + (aq) \rightarrow 3I_2(s) + 3H_2O(L). \quad (1)$$

$$I_2(s) + 2S_2O_3^{2-}(aq) \rightarrow 2I^-(aq) + S_4O_6^{2-}(aq) \ldots\ldots\ldots\ldots (2)$$

The number of moles of thiosulfate required for reaction with each mole of iodate in the initial salt solution was

calculated using the redox equations above, as was the average volume of thiosulfate solution used from concordant titres. (University of Canterbury, College of Science. 2021)[15]. The amount of thiosulfate, in moles, reacting was calculated first, followed by the amount of iodate in moles in the salt solution. The iodate concentration in the salt solution was obtained in mol L^{-1} , then the concentration of iodine in the salt solution was calculated, using the equation:

Iodine (I) content = iodate (IO_3^-) content × 126.9/174.9

2.5 Data Analysis

The Statistical Package for Social Sciences (SPSS) version 16.0 was used to analyze the

Data. Statistically, $p < 0.05$ was reflected as significant.

III. RESULTS

Table 1: Average Iodine Content (ppm) of Brands of Salt

Sample	Iodine content
Salt from lagoon	
Ada-Songhor	**0.0**
Nyanyano	**0.0**
Keta	**0.0**
Amisa	**0.0**
Benya	**0.0**
Crude salt from market	
A	**0.0**
B	**0.1±0.00**
C	**0.1±0.00**
D	**0.0**
E	**0.0**
F	**0.0**
G	**0.1±0.00**
Refined iodised salt	
Annapurna	**39 ± 0.01**
U2	**36.3 ± 0.02**
Crude iodised salt	
Iodised sea salt	**33.2 ± 0.01**
Ghana Standard Authority	**25-50**

Source: Bartels/Vanderpuye/Gadzekpo, Laboratory results, 2023

Table 2: Knowledge about Iodised Salt and Preference for Choice of Brand

Parameter	Respondents	Percentage, %
Benefits of iodised salt		
No idea	**105**	**93**
Improve IQ	**5**	**4**
Prevents goitre	**3**	**3**
total	**113**	**100**
Knowing salt is iodised		
Labelled as iodised	**60**	**53.1**
Assumed iodised	**53**	**46.9**
total	**113**	**100**
Preferred salt brand		
iodised	**46**	**49.6**
Non-iodised	**67**	**50.4**
total	**113**	**100**

Source: Bartels/ Vanderpuye/Gadzekpo Statistics, 2023

IV. DISCUSSION

4.1 Iodine content in lagoon salt

As shown in Table 1, all the samples from the lagoons, namely, Ada-Songhor, Nyanyano, Keta, Amisa and Benya did not record any measurable or detectable concentration of iodine, that is, zero (0) ppm of iodine content. The absence of iodine, however, suggests that the salts do not naturally contain any appreciable level of iodine, this corroborates earlier study (IGN, 2019) [11], and even though the sea contains about 2 ppm iodine content (Medeiros-Neto et al., 2016)[16]. Therefore, non-iodised salt cannot serve as a standalone remedy to fight IDD. This finding presupposes that consuming non-iodised salt do not provide iodine to combat IDD and that the perception that non-iodised salt contains iodine is false.

Moreover, results from salt samples A, B, C, D, E, F and G from various markets in Ghana also revealed the absence of iodine as shown in Table 2. Such are salts directly sold on the market from the lagoons without being iodised.

As in Table 2, for example, the 50.4% of the respondents who preferred non-iodised salt for cooking, would be deprived of the iodine required by law to fight IDD and defeat government efforts to effectively implement USI in Ghana. This could affect Ghana's grading of iodine deficiency status.

4.2 Iodine content in iodised salt

As shown in Table 2, two of the most patronized iodised salt brands in Ghana, namely, Annapourna and U2 had iodine content of 39 ± 0.01 and 36.3 ± 0.02 respectively. These concentrations are within the GSA limit of 25-50 ppm required for the maximum benefit of the consumption of iodised salt. It could be inferred that the 7% respondents could benefit from the consumption of iodised salt.

It must be emphasized that refined salt often undergoes a purification process where impurities are removed and essential micronutrients are added for fortification (Ghana Statistical Service: Ghana Demographic and Health Survey Report. (2014)[17].

Ghana, to combat iodine deficiency disorders, many countries including Ghana have implemented iodization programs, which involve adding iodine to refined salt to ensure an adequate intake of this essential nutrient.

4.3 Iodised and non-iodised salt

The difference in iodine content between the iodised and non-iodised salt sample demonstrates the effectiveness of the iodization process. Iodised salt is deliberately (Ghana Health Service/ USAID/FANTA II, 2023)[18] fortified with iodine to provide a reliable source of this micronutrient to the general population. (Ghana Statistical Service (2014)[19]. This approach helps prevent iodine deficiency, which can lead to various health problems, particularly affecting the thyroid gland. (De Escobar et al., 2004)[5] .

The WHO estimates a 20% loss from production level to household and a further 20% loss during cooking. WHO also predicts that, in order to meet an average daily requirement of 150µg iodine from an average salt intake of 10g of salt a day, iodine concentration in salt at the point of production should be within the range of 20 - 40ppm. (Doku & Bortey (2018)[7].

Households that cook meals with non-iodised salt are exposed to risks of Iodine Deficiency Disorders.

4.4 The survey

4.4.1 Benefits of iodised salt consumption

Out of the 113 surveyed, 105 constituting 93% stated that they had no knowledge about the benefits of iodine in the salt being consumed. Such ignorance involving majority of the respondents suggests lack of iodine nutrition awareness programmes in the communities of the respondents, and therefore embolden the perception that non-iodised salt contains natural iodine.

This ignorance and perception may be attributed to factors, such as, inadequate public health campaigns, insufficient dissemination of information about iodine nutrition.(Chirawurah et al., 2015)[6] , and lack of cooperation or flow of knowledge between industry and academia.

Universal salt iodization has been one of the most effective public health interventions adopted

by WHO/UNICEF/ ICCIDD for the global eradication of iodine deficiency disorders (IDDs). (Osborne et al., 2023), GEPC (2009)[4,1]. The progress of this intervention was assessed by interviewing women in control of household diet preparations, the study revealed that 53.1% have knowledge about iodized salt, as compared to earlier studies in Hohoe in the Volta and Gushegu in the Northern regions of Ghana that recorded 59.3 and 53.5% respectively. (University of Canterbury, College of Science. 2021), Medeiros-Neto et al., 2016)[16,15]

In spite of this observation, the 53.1% does not translate into use as 50.4% prefer non- iodised salt for cooking as shown in Table 2. Such behavior may also be influenced by the perception that non-iodised salt contain natural iodine, and may eventually impact negatively on the USI programme in Ghana.

V. CONCLUSION

1. Salt samples (common salt) from all the lagoons did not contain iodine. This finding does not support the assertion that raw salt contains iodine, required to eliminate iodine deficiency disorders (IDD).

2. Raw salt (samples A- G) from these lagoons that are sold on the markets also did not record significant level of iodine. Consumption of such salts therefore does not have the potential to eliminate Iodine Deficiency Disorders (IDD).

3. Concerning the survey, majority of respondents had no knowledge about the benefits of iodised salt, while many prefer non-iodised salt.

4. Annapurna most patronized refined iodised salt in Ghana had iodine content within the Ghana Standard Authority Limit. Consumption of such could eliminate IDD.

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RECOMMENDATION

- The need for effective collaboration between industry and academia for dissemination of information, and research findings as applied to salt.
- The need for relevant agencies to intensify education on iodine nutrition, emphasizing on the negative impact of the perception that raw salt contains enough iodine to fight iodine deficiency disorders

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The Effect of Organic fertilizers on Growth Quality of Sweat corn (*Zea mays saccharata* L)

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Abstract— *The experiment was conducted to study the effect of organic waste from rice husk, corn straw and sugarcane leaves as compost for sweat corn growth. The experimental design used in the study was a factorial randomized block design with two factors (compost types and doses), nine treatments, and three replications, P1: rice straw compost; P2: sugarcane leaves compost; P3: corn straw compost then doses were D1: 7,5 t ha^{-1}; D2: 15 t ha^{-1}; D3: 23 t ha^{-1}. The result showed that the compost types and dose treatments had a significant effect on the maize growth, including the height, leaf area Index, stem diameter, and dry weight on specific observations without any interaction. Besides, the treatments also affected the yield of corn production. The highest yield was found at 23 t ha^{-1} (D3) dose in all types of compost treatments. Meanwhile the highest to the lowest yields were, respectively, P3 (9,29 t ha^{-1}), P1 (8,72 t ha^{-1}), and P2 (8,00 t ha^{-1}).*

Keywords— ***Organic fertilizer, Sweat Corn, Organic Compost.***

I. INTRODUCTION

Corn, commonly known as maize, plays a crucial role as a primary cereal crop, serving as a staple food source for a significant portion of the global population. It ranks third in worldwide production, trailing only wheat and rice. Maize kernels consist of approximately 80% starch, 10% proteins, 4.5% oil, 3.5% fiber, and 2% minerals (Wangmo et al., 2020). In most of developed nations, around 90% of maize is allocated for animal feed and various industrial by-products. In contrast, a substantial proportion, ranging from 80% to 90%, is utilized for direct human consumption worldwide, unlike in developed countries (Grote, 2021). Several key factors contributing to diminished maize yields include declining soil fertility and insufficient use of fertilizers, leading to severe nutrient deficiencies.

The production of corn, rice and sugar cane is massive in most of asian and eroupean countries then resulting waste along with the number of its productivity. At least 50% of the plant's production are waste consisting of stems, leaves and roots (Amie & Nugraha, 2014; Aziz et al., 2014). Corn waste contains at least N (0.81%), P (0.16%) and K (91.33%) (Sianipar et al., 2020), while rice; N (0.5-0.8 %), P (0.070.12 %), K (1.2-1.7 %) (Abdel-rahman et al., 2016), as well as sugar cane ; N (0.3%), P (0.15 %), (K 0.53 %), (Mentari et al., 2021). Several research state that the use of corn, rice and sugar cane stover compost may increase the yield, growth and production of sweet corn (Ernita et al., 2017; Helmi et al., 2022; Suryani et al., 2022).

This research was carried out not only to determine the effect of compost on the growth and yield of sweet corn, but also to see the potential of fertilizer along with the correct and efficient dosage.

II. MATERIAL AND METHODS

This study was conducted in a field experiment at the University of Brawijaya, Malang City, East Java, from June 2021 to March 2022. The materials used were starter liquid decomposer then organic wastes including rice straws, corn husks, and sugarcane leaves.

Research design

The experiment was conducted using two factors with a factorial randomized block design (FRBD). The first one

was the compost types (P), P1: rice straw compost, P2: corn husk compost, P3: sugarcane leaf compost, and the second one was the compost dose (D), D1: 7.5 t ha-1, D2: 15 t ha-1, D3: 23 t ha-1. There were nine treatments, each repeated thrice therfore it has 27 treatment units.

Compost Preparation

Composting was completed for more than two months in a greenhouse on a box-shaped tarpaulin with a 1 x 3 m size using the anaerobic method. To ensure the compost ready to use, physical observation was conducted by observing colour, temperature , smell and texture (Angraeni et al., 2020).

Land Preparation

Sterilization procedures were applied which included tasks such as weed removal, trench digging, and waste removal. The entire experimental area covered 600 square meters, comprising 27 plots measuring 5.5 meters in length and 3 meters in width."

Planting Method

Organic fertilizers (compost) were applied 14 days before planting. The plant spacing implemented was 70x25 cm. Moreover, plant protection and treatments such as weeding, spraying pesticides, and watering were also conducted. NPK pearl fertilizer at 16:16:16 was given at 14 DAP (day count after planting) and 30 DAP. The plants were harvested at age 65 DAP, at the final stage of the generative phase marked by the ripening seeds in the cob (Motasim et al., 2022)

Soil Analysis

The sample of soil were collected before fertilizer was applied on the field. At the first was collected randomly on the surface area within 0-15 cm depth. While the last stage of harvesting, soil samples were collected randomly but nearby the canopy of corn leaves (James & Wells, 1990).

The chemical characteristics of soil were analyzed considering pH (H2O) and (KCl) using a digital pH meter (1:2.5; soil: solution), organic C (Walkley and Black method), total N (Kjeldahl method), and available P (Olsen method) (Cahyani et al., 2022)

BRIX index

Brix or sweetness content of corn is measured immediately after harvest using a manual hand refractometer with a scale range of 0.0-32.0% and a minimum scale: brix 0.2%.

Data Observation

Furthermore, the growth parameters observed included the plant height, number of leaves, leaf area, stem diameter, Crolophyl index, and dry weight of plants. Plant growth was observed four times 15, 30, 45, and 60 DAP. The yield parameters observed included the corn cob length, diameter, and crop yield per treatment. Plant height was observed using a ruler (up to 100 cm). The number of leaves was observed by using the Leaf collar method (counting visible leaves of Corn)(Schepers et al., 1992). The leaf area was analyzed using Leaf Area Meter (LAM). The diameter of corn fruit and stem was observed by using a vernier caliper. Meanwhile, the dry weight of corn was obtained by drying the plants in the oven until their maximum drying limit, then weighed using digital scales. Yield of corn was observed on 65 DAP including corn cob length, diameter, and crop yield per treatment.

Data analysis

The observation results that had been collected were analyzed using the Analysis of Variance (F test) at the 5% level. If the test results obtained a significant difference, it would proceed with a comparison test using the Least Significant Difference (LSD) test at the 5% level

III. RESULTS AND DISCUSSION

Plant heights

The results of the Analysis of Variance exposed that the types of compost did not significantly affect the height of the corn plants at 15 and 30 DAP (day count after planting). Nevertheless, the observation showed that the types of compost affected plant height at 45 and 60 DAP. The sugarcane and corn compost were not significantly different at 45 DAP but significantly different from the rice compost. At 60 DAP, rice, and sugarcane composts were not significantly different. However, both were significantly different from corn compost. Despite that, the compost dose affected plant height. At 15 DAP, the dose of D3 and D2 were not significantly different until the age of 30 DAP. The treatment dose was significantly different at 45 and 60 DAP.

In summary, the types and dose of compost affected plant height at a specific dose and age. The average height of sweet-corn plants is presented in Table 3. Applying organic matter as a compost would increase the nutrients and growth of corn plants (Singer et al., 2004). Giving corn husk compost can increase N, P, and K in the soil used by plants in the growth process (Chen et al., 2014).

Table 1. Plant heights as a result of compost types and dosage

Treatment	Plant height (cm) (DAP)			
	15	30	45	60
P1	14,82	38,27 a	92,49 a	135,66 a
P2	15,15	36,02 a	101,77 b	136,11 a
P3	15,68	35,42 a	105,76 b	144,29 b
LSD 5%	ns	ns	9,19	6,83
D1	13,81 a	37,70 a	86,48 a	126,31 a
D2	15,78 b	36,08 a	101,17 b	139,82 b
D3	16,06 b	35,93 a	112,36 c	149,93 c
LSD 5%	1,52	3,73	9,19	6,83
CV (%)	9,96	10,21	9,19	5,01

Note: Values followed by the same letter in the same column are not significantly different based on the 5% of LSD test, ns = not significant, DAP = day after planting. P1: rice straws, P2: sugarcane leaves, P3: corn husks, D1: 7,5 t ha^{-1} D2: 15 t ha^{-1} D3: 22,5 t ha^{-1}

Leaves area

The results of the Analysis of Variance revealed that the corn leaf area did not significantly impact the types of compost at the age of 15 to 30 DAP. However, there was a significant response at the age of 45 and 60 DAP. Due to the corn compost (P3) given, the leaf area was 5% higher than P2 and P1 treatments at the age of 45 DAP at an average value of 468 cm2 and 12% at the age of 60 DAP compared to P2 and P1 with an average value of 404 cm2. The treatment dose of D1 and D2 were not significantly different at the age of 15 DAP, with an average of 14.24, 21% lower than the D3 treatment. At the age of 30,45, and 60 DAP, the treatment dose significantly affected the leaf area of the corn. It disclosed that the treatment of D3 was 6% higher than D2, while D2 was 13% higher than D1.

Table 2. leaves area as a result of compost types and dosage

Treatment	Leaf area (cm2) (DAP)	
	30	60
P1	182	388,03 a
P2	192,29	420,25 b
P3	209,49	456,44 c
LSD 5%	ns	27,97
D1	176,29 a	381,70 a
D2	188,59 ab	417,96 b
D3	218,9 b	465,07 c
LSD 5%	28,11	27,97
CV %	15,99	6,63

Note: Values followed by the same letter in the same column are not significantly different based on the 5% of LSD test, ns = not significant, DAP = day after planting. P1: rice straws, P2: sugarcane leaves, P3: corn husks, D1: 7,5 t ha^{-1} D2: 15 t ha^{-1} D3: 22,5 t ha^{-1}

In conclusion, the compost treatment types only significantly affected the leaf area at 45 and 60 DAP. On the other side, the treatment dose significantly affected all ages during the observation. The highest value in the types of compost treatment was found in the corn compost, while the compost dose was found at 22 t ha^{-1}. It was obtained that the K elements in rice, corn, and sugarcane compost were as follows: 3349, 5562, and 3857 ppm. Due to that case, it

emerged an assumption that the K element capitalized on the growth of leaf area with that the reason that the element contributed to helping the photosynthesis process of plants was by increasing the leaf area index; thus, the process of CO2 assimilation and translocation of photosynthetic products increased (Clover & Mallarino, 2013).

Stem Diameters

The results of the Analysis of variance revealed no significant effect on the types and dose of compost treatment at the age of 15-30 DAP (see Table 7). The actual effect was only seen at 60 DAP on the types of compost treatment. The type of compost P3 was significantly different from P2 and P1. P2 treatment was 3% higher than P1. Meanwhile, the dose of D3 compost had a significant effect on stem diameter compared to D2 and D1. Likewise, at the age of 60 DAP, the D1 and D2 treatments were not significantly different.

The stem diameter was related to the growth of sweet-corn plants. One of the factors that affected the diameter of the stem was the nitrogen content of the plant. The application of compost with good N could affect the diameter of corn because the element of N played an important role in compiling amide acids, nucleotides, and nucleoproteins and was essential for cell division and enlargement.

Table 3. Stem diameter as a result of compost types and dosage

Treatments	Dry weight (DAP)			
	15	30	45	60
P1	3,03	19,51 a	37,61 a	85,80 a
P2	3,09	21,07 ab	45,45 b	91,74 ab
P3	3,17	23,77 b	51,91 b	93,01 b
LSD 5%	ns	3,36	7,48	7,08
D1	2,73 a	18,53 a	34,90 a	83,22 a
D2	3,18 b	21,3 a	43,40 b	89,53 b
D3	3,37 b	24,53 b	56,67 c	97,8 b
LSD 5%	0,37	3,36	7,48	7,08
CV %	11,87	15,64	16,62	6,25

Note: Values followed by the same letter in the same column are not significantly different based on the 5% of LSD test, ns = not significant, DAP = day after planting. P1: rice straws, P2: sugarcane leaves, P3: corn husks, D1: 7,5 t ha^{-1} D2: 15 t ha^{-1} D3: 22,5 t ha^{-1}

Dry weight of plants

The results of the Analysis of Variance disclosed that the types of compost did not affect the plant's dry weight at the age of 15 DAP. The type of P3 compost was significantly different, 25% higher than the P1 compost at the age of 30-60 DAP with an average of 59 and 47 g. Meanwhile, 16% compared to D2 with an average of 59 and 51 g. The dry-weight corn significantly responded to the compost dose treatment at all ages in the observation. It was known that D2 treatment was not significantly different from D3 at the age of 15 and 60 DAP but significantly different from D1, which was 19% at the age of 15 DAP and 12% at the age of 60 DAP with an average value of 93.66 g.

Table 4. Plant dry weight due to compost types and dosage

Treatments	Dry weight (DAP)			
	15	30	45	60
P1	3,03	19,51 a	37,61 a	85,80 a
P2	3,09	21,07 ab	45,45 b	91,74 ab
P3	3,17	23,77 b	51,91 b	93,01 b
LSD 5%	ns	3,36	7,48	7,08
D1	2,73 a	18,53 a	34,90 a	83,22 a
D2	3,18 b	21,3 a	43,40 b	89,53 b

D3	3,37 b	24,53 b	56,67 c	97,8 b
LSD 5%	0,37	3,36	7,48	7,08
CV %	11,87	15,64	16,62	6,25

Note: Values followed by the same letter in the same column are not significantly different based on the 5% of LSD test, ns = not significant, DAP = day after planting. P1: rice straws, P2: sugarcane leaves, P3: corn husks, D1: 7,5 t ha^{-1} D2: 15 t ha^{-1} D3: 22,5 t ha^{-1}

At the age of 30 and 45 DAP, the D3 treatment was 3% higher than D2 and D1 treatments, with an average value of 40.6 g. This summarized that the types and dose of compost treatments significantly affected the dry weight of the plants. The type of P3 compost was not much different from the P2 compost, but it was significantly different from the P1 compost. The treatment dose had a significant effect on the dry weight of the corn. The highest values were found in the D3 and D2 treatments at 15 and 60 DAP. In detail, D2 treatment was significantly different from D1 in all ages in the observation, except at 45 DAP. On the other hand, the D3 treatment was significantly different from D1 at all ages in the observation and was significantly different from D2 at 45 DAP.

For further information, the dry weight measured the plant growth and development as the dry weight reflected the accumulation of organic compounds synthesized by plants. Plant dry weight reflects the plants' nutritional status and acts as an indicator that determines whether the plants' growth and development were better .

BRIX index

The results shows that there is no significant interaction between the type and dose of fertilizer on Brix index. In addition, there is also no significant effect of both the dose and type of fertilizer.

Table 6. Brix index value of sweet corn due to type and dosage

Treatments	Brix Index
Fertilizer	Value
P1	13,33
P2	13,51
P3	13,80
LSD 5%	ns
Doses	
D1	13,44
D2	13,21
D3	13,99
LSD 5%	ns
CV (%)	4,77

Note: Values followed by the same letter in the same column are not significantly different based on the 5% of LSD test, ns = not significant, DAP = day after planting. P1: rice straws, P2: sugarcane leaves, P3: corn husks, D1: 7,5 t ha^{-1} D2: 15 t ha^{-1} D3: 22,5 t ha^{-1}

Growth rates

Based on the Analysis of Variance results, the growth rate at 15-30 DAP did not have a significant response to the types or dose of compost treatment. The growth rate had a significant response at 30-45 and 45-60 DAP, where P3 significantly differed from P2 and P1. In the treatment of compost dose, it was indicated that the dose of D1 and D2 were not significantly different. A significant response was found in the D3 treatment, where it was 21% higher than D2 and D1.

Table 7. Growth rates of sweet corn plants as a result of compost type and dosage

Treatments	Crop Growth Rate (CGR)		
	15-30	30-45	45-60
P1	18,26	15,44 a	37,82 a
P2	19,23	17,79 ab	38,15 a
P3	22,49	19,77 b	48,51 b
LSD 5%	Ns	3,15	5,09
D1	19,29	16,96 a	36,29 a
D2	18,57	15,84 a	38,36 a
D3	22,11	20,20 b	49,83 b
LSD 5%	ns	3,14	5,09
CV (%)	18,80	17,81	12,26

Note: Values followed by the same letter in the same column are not significantly different based on the 5% of LSD test, ns = not significant, DAP = day after planting. P1: rice straws, P2: sugarcane leaves, P3: corn husks, D1: 7,5 t ha^{-1} D2: 15 t ha^{-1} D3: 22,5 t ha^{-1}

Plant Yields

Based on the results from analysis of variance, there is a significant interaction between dosage and fertilizer type. At dosage D1, fertilizer types P2 and P3 showed no significant difference, but both were significantly different from fertilizer P1. Then, at dosage D2, fertilizer type P1 and D2 did not differ significantly. Fertilizer type P3 performed better than both of them at dosage D2. Meanwhile, at dosage D3, all three fertilizer types differed from each other.

Table 8. Plant production due to types and dosage

Plant Production (gr)			
Treatments	Corn Productivity (t ha^{-1})		
	D1	D2	D3
P1	100,44 a	142,01 b	183,86 ed
P2	127,19 b	142,11 b	168,42 cd
P3	129,39 b	159,65 c	195,52 e
LSD 5%		9,22	
CV %		6,16	

Note: Values followed by the same letter in the same column are not significantly different based on the 5% of LSD test, ns = not significant, DAP = day after planting. P1: rice straws, P2: sugarcane leaves, P3: corn husks, D1: 7,5 t ha^{-1} D2: 15 t ha^{-1} D3: 22,5 t ha^{-1}

Soil Contents

The sample of soil at the end of the research was collected when the corn reached 60 DAP at the end of research. Each fertilizers had a significant effect on the CEC, organic C, C/N ratio and N, P, K values of the soil. The highest average values were in treatments P3, P2 and P1 respectively. Meanwhile, D1, D2, and D3 also had significant differences in the CEC, organic C, C/N ratio and soil N, P, K values. Therefore, the dosage stages of fertilization also increase the nutrient value and CEC of the soil.

Table 9. Chemical soil content due to fertilizer aplication

Treatments	Soil contents after fertilizer aplication					
	CEC	C.Organic (%)	C/N	N (%)	P (ppm)	K (ppm)
Compost Types						
P1	27,19 a	3,36 a	5,79 a	0,27 a	19,12 a	33,99 a
P2	28,51 ab	3,64 b	6,28 b	0,31 b	21,20 b	36,56 b
P3	29,95 b	4,04 c	6,97 c	0,34 c	22,51 c	37,12 b
LSD 5%	1,82	0,12	0,20	0,02	0,66	1,00
Dosage						
D1	26,09 a	3,41 a	5,88 a	0,28 a	20,75 a	33,60 a
D2	28,8 b	3,73 b	6,43 b	0,30 b	20,91 b	35,79 b
D3	30,77 c	3,90 c	6,73 c	0,32 c	21,17 c	38,27 c
LSD 5%	1,82	0,12	0,20	0,02	0,66	1,00
CV (%)	6,73	3,13	3,13	5,12	3,16	2,78

Note: Values followed by the same letter in the same column are not significantly different based on the 5% of LSD test, ns = not significant, DAP = day after planting. P1: rice straws, P2: sugarcane leaves, P3: corn husks, D1: 7,5 t ha^{-1} D2: 15 t ha^{-1} D3: 22,5 t ha^{-1}

IV. CONCLUSION

Base on the result of experiment, it conclude that fertilizer made from corn straw was better than rice and sugracane compost at most of growth parameters in similar dosage. However there is no significant changes at Brix and stem diameter of corn. The dosage 22,5 t ha^{-1} was highly recommended in all types of compost for better productivity. In addition, the use of different compost and dosage resulting significant nutrient values and made improvement of soil content at certain types and dosages of compost.

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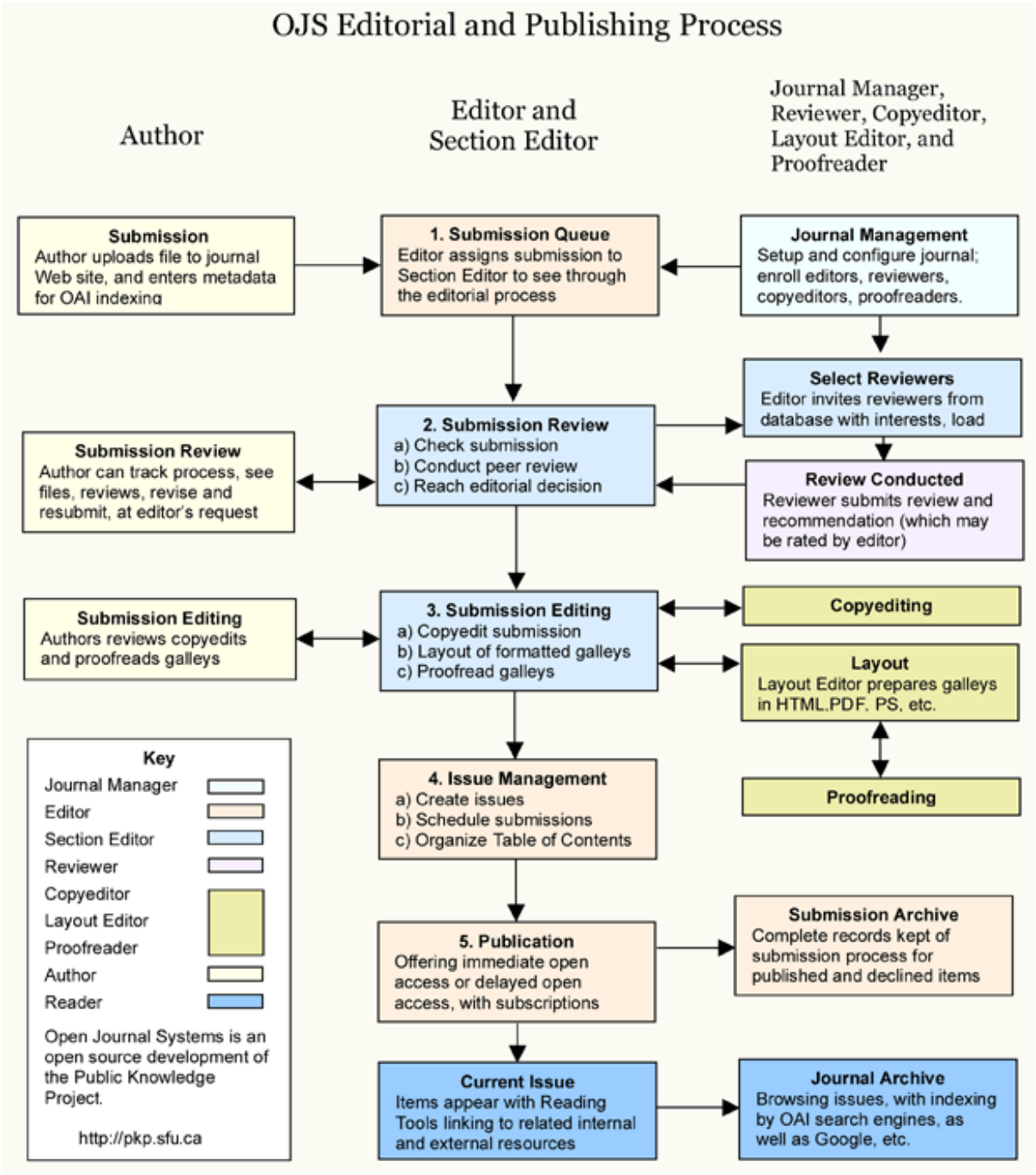

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