Preface

We would like to present, with great pleasure, the inaugural volume-6, Issue-12, December 2020, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas.

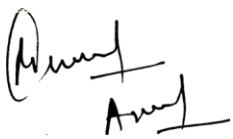
Environmental Research:

Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

Agriculture Research:

Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.



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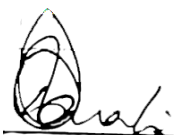

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Agricultural Sciences	
Soil Science	Plant Science
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Production Manager Zeal Food & Beverages Industry Aladand Road, Amandara Batkhela Malakand Agency KP. He developed the skills for Preparation of different fruit pulp preserve Orange, Peach, Guava, Strawberry, and orange squash, orange marmalade.

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Profile of the Tribal Families from Palghar District

S.K Deshmukh[1], J.R Kadam[2], S.G Bhave[3]

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Abstract— *This paper examines the profile of the tribal families from Palghar district. The study was conducted at the Palghar district. A sample of 120 tribal families were considered as respondents for present study. The respondents were interviewed with the help of specially designed schedule. Collected data was classified, tabulated and analysed by using various statistical method. The result of the study showed that most of the respondents have 'medium' family education status, 'medium' family size, 'medium' annual family income, 'cultivation' as their major occupation, 'marginal' land holding, 'fair' cropping pattern, 'medium' farming experience and 'low' social participation. The extension workers should consider these facts while planning and executing programmes for development of the tribal families living in Palghar district.*

Keywords— Profile, Tribal families.

I. INTRODUCTION

Food consumption pattern and food habits is an essential part of any culture. An important part of healthy lifestyle is proper intake of food. The benefits of proper intake of food are observed in wide range of studies. There is a large difference in food consumption pattern of our country India. A balanced diet is required because organs and tissues need proper nutrition to work effectively. The tribal communities have vast knowledge about the importance of consumption of wild plants. These groups are homogenous, culturally firm and wish to survive and live their own lifestyle. The choice of food is deeply related to the lifestyle of an individual. Food habits and consumption pattern is greatly influenced by thoughts, beliefs, notions, traditions and taboos of the society. Apart from these socio-cultural barriers, the religion, education, and economic factors do alter the food behaviour. Government has taken number of measures to overcome hunger and malnutrition. The National Food Security Act, 2013, is the Act of Parliament of India which aims to provide subsidized food grains to approximately two third of India's population. It includes Midday Meal Scheme, Integrated Child Development Services scheme and the Public Distribution System. Even though there are many schemes and nutritional programmes to serve the people, there is a great bulk of illness in our country. Hence, eating good food in one of the pleasures of life.

II. OBJECTIVE

To access the profile of the tribal families from Palghar district.

III. METHODOLOGY

The research work was purposively conducted in Palghar district of Konkanregion of Maharashtra State. Two tahasils Mokhada and Vikramgad having maximum tribal population were selected to carry out the research. Six villages from each tahasil were selected randomly to carry out the present study. A total of 120 tribal families were considered as respondents for the present study. The data was collected with the help of a specially designed interview schedule by keeping in view the objective of the study. Collected data was classified, tabulated and analysed by using various statistical method. 'Ex-post facto' research design was used to conduct the present study.

IV. RESULT AND DISCUSSION

The data in Table 1 indicated that 'majority' (74.16 per cent) of the respondents belonged to 'medium' family education status, while '16.66 per cent' respondents belonged to 'low' family education status and '9.18 percent' of the respondents belonged to 'high' family education status. The average family education status of the respondents was found to be up to 5th standard.

TABLE 1
PROFILE OF THE TRIBAL FAMILIES

Sr. No.	Category	Respondents (N=120)	
		Number	Percentage
Family Education Status			
1.	Low (up to 2nd std.)	20	16.66
2.	Medium (3rd – 7th)	89	74.16
3.	High (8th and above)	11	9.18
Mean= 5th Standard Total		120	100
Family Size			
1.	Low (up to 3)	14	11.66
2.	Medium (4-6)	78	78
3.	High (7 and above)	28	23.34
Mean= 5.4 Total		120	100
Annual income of the family			
1.	Low (up to Rs. 24,556/-)	1	0.83
2.	Medium (Rs. 24,557/- to 2,09,083/-)	100	83.34
3.	High (2,09,084/- and above)	19	15.83
Mean= 1,16,820 Total		120	100
Major occupation			
1.	Labour	40	33.34
2.	Caste occupation	0	0.00
3.	Business	2	1.66
4.	Independent profession	9	7.50
5.	Cultivation	57	47.50
6.	Service	12	10.00
Total		120	100
Land holding			
1.	Landless (No land holding)	25	20.83
2.	Marginal (up to 1 ha)	82	68.33
3.	Small (1.01 to 2.00 ha)	11	9.16
4.	Semi-medium (2.01 to 4.00 ha)	2	1.68
5.	Medium (4.01 to 10.00 ha)	0	0.00
6.	Big (Above 10.00)	0	0.00
Mean= 0.65 ha Total		120	100
Cropping pattern			
1.	Poor (Up to 2)	27	22.5
2.	Fair (2.1 to 7.9)	70	58.33
3.	Good (8 and above)	23	19.17
Mean= 5.01 Total		120	100
Farming experience			
1.	Low (Up to 5 years)	42	35
2.	Medium (6 to 14 years)	48	40
3.	High (15 years and above)	30	25
Mean = 10 years Total		120	100
Social Participation			
1.	Low (Up to 1)	70	58.34
2.	Medium (2 to 3)	32	26.66
3.	High (4 and above)	18	15
Mean = 2.28 Total		120	100
Resource availability			
1.	Low (Up to 9)	34	28.33
2.	Medium (10 to 11)	48	40.00
3.	High (12 and above)	38	31.67
Mean=10.74 Total		120	100

Regarding family size 'majority' (78.00 per cent) of the tribal family respondents have 'medium' family size, while '23.34 per cent' respondents had 'high' family size and '11.66 per cent' respondents had 'low' family size.

It could be observed that, 'majority' of the tribal family respondents (83.34 per cent) had 'medium' annual family income, while '15.83 per cent' respondents had 'high' family income and only '0.83 per cent' of the respondents had 'low' annual family income. The average annual family income of the tribal families was 'Rs.1,16,820/-'.

The data presented in Table 4 that revealed that, 'nearly half' of the respondents (47.50 per cent) had 'cultivation' as their major occupation, while '33.34 per cent' were 'labours', '10.00 per cent' of them were engaged in 'service', '7.50 per cent' families were engaged in independent profession, only '1.66 per cent' tribal families had 'business' as their profession, and 'no respondents' were engaged in 'caste occupation'.

The data presented reveals that, 'majority' (68.33 per cent) of the respondents had 'marginal' land holding. While '20.83 per cent' of the respondents were 'landless' and '9.16 per cent' and '1.68 per cent' belonged to 'small' and 'semi-medium' category of land holding. No respondents had more than 4 ha of land holding. The average land holding of the respondents was 0.65 ha.

It could be seen that, 'majority'(58.33 per cent) of the respondents had 'fair' cropping pattern, while '22.50 per cent' of respondents had 'poor' and '19.17 per cent' of the respondents have 'good' cropping pattern respectively. The average cropping pattern score of the respondents is 5.01.

It is indicated that, 'majority' of the tribal family respondents (40.00 per cent) had 'medium' farming experience. While '35.00 per cent' had 'low' farming experience and '25.00 per cent' had 'high' farming experience. The average farming experience was 10 years.

It is indicated that, majority of the tribal family respondents (58.34 per cent) had 'low' social participation. While '26.66 per cent' had 'medium' social participation and '15.00 per cent' had 'high' social participation.

V. CONCLUSION

The result of the study showed that most of the respondents have 'medium' family education status, 'medium' family size, 'medium' annual family income, 'cultivation' as their major occupation, 'marginal' land holding, 'fair' cropping pattern, 'medium' farming experience and 'low' social participation. The extension workers should consider these facts while planning and executing programmes for development of the tribal families living in Palghar district.

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In-Vitro Evaluation of selected Fungicides on the Growth and Sporulation of *Alternaria alternata* causing Blight Disease of Broad Bean (*Vicia faba* L.)

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Abstract— *Broad bean (Vicia faba L.) is an important leguminous cold season crop cultivated widely in different parts of the world and in India. This crop is grown especially in U.P., Bihar, Punjab, Haryana and in the foot hill ranges of Himalayan region including north eastern states. In Manipur, it is an important winter vegetable cum pulse crop. However, this crop suffers attack of various diseases of fungi, viruses and nematodes resulting in substantial reduction in yield. Hence, an in-vitro evaluation of selected fungicides on the Growth and Sporulation of Alternaria alternata causing blight disease of broad bean (Vicia faba L.) was under taken in the present investigation. A judicious application of Tricyclazole and Copper oxychloride at 1000ppm can effectively manages the blight disease of broad bean and prevent economic loss due to disease condition.*

Keywords— Alternaria alternata, broth media, solid media, sporulation, mycelium mat, radial growth, inhibition, fungicides, per cent disease incidence index.

I. INTRODUCTION

Broad bean (*Vicia faba* L.) is an important leguminous cold season crop cultivated widely in different parts of mild sub-tropical and temperate regions of the world. In India, this crop is grown especially in U.P., Bihar, Punjab, Haryana and in the foot hill ranges of Himalayan region including north eastern states. In Manipur, it is an important winter vegetable cum pulse crop. The protein rich tender green pods were consumed as vegetable and seeds as dal and snacks. However, this important pulse or vegetable crop suffers attack of various diseases of fungi, viruses and nematodes resulting in substantial reduction in yield. Important diseases of broad bean include leaf blight caused by *Alternaria alternata*, Rust caused by *Uromyces fabae*, Leaf spot caused by *Phoma exigua*, powdery mildew caused by *Erysiphe polygoni*, root knot diseases caused by *Meloidogyne javanica*, mosaic virus and little leaf virus diseases (Gupta, 1985). Among these diseases leaf blight caused by *Alternaria alternata* was found most serious in India and reported to cause losses upto 80% in sunflower (Agrawal, et al., 1979).

II. MATERIAL METHODS

The research work was carried out in the field and laboratory of Department of Plant Pathology, KVK-Senapati district, Manipur, ICAR, ATARI, Zone-VII, from 2017-2019. The details of experimental procedures adopted during the course of investigations are described as follows.

2.1 Collection of diseased specimen

During the month of October to December of *rabi* season 2017, Broad bean leaves showing typical blight symptoms was collected from the experimental plots of KVK-Senapati, Manipur and brought in the laboratory for isolation of fungus.

Isolation and purification of fungus associated with blight disease of Broad bean:

The blight disease infected leaves were cut into small bits of 2-3 mm and surface sterilized in 1% sodium hypochlorite for 1-2 minutes and wash with sterile water three times and then inoculated in sterilized petriplates containing fresh potato dextrose agar (PDA) and then incubated at 25 ± 10C for 72 hours. The fungus was purified by single hyphal tip culture method. The fungal culture thus obtained was stored at room temperature and periodically subcultured on fresh potato dextrose agar (PDA) slants from time to time.

2.2 Disease symptoms:

The disease first appeared as small circular usually at the leaf margin or tips or as scattered dark brown spot on surface of the broad bean leaves and progressed towards the mid rib. The spots coalesce together to form an elongated, irregular necrotic dark brown lesions. The affected leaf sometime showed chlorosis and later fell off or dries up along with the leaf stalk still

clinging on the plants. In severe condition young plant shows pale greenish leaves, start drooping, wilted and died. {Photo-I, II, III, IV].

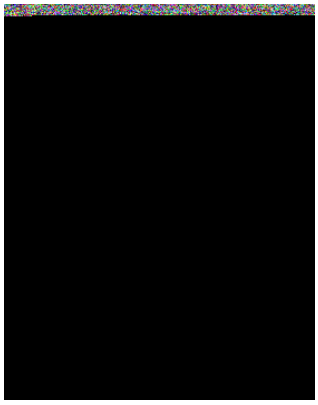

PHOTO (I). Initial blight symptom

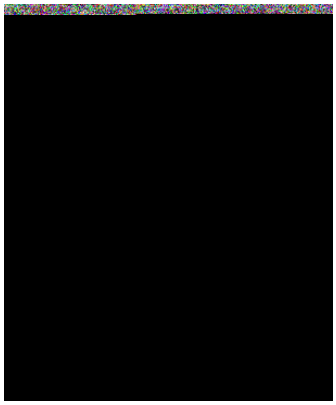

PHOTO (II). Leaf blight advance stage

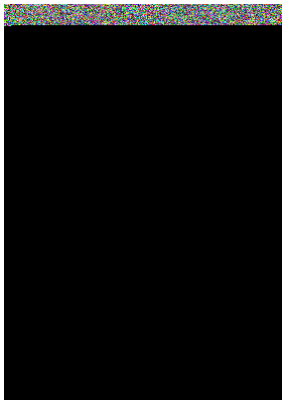

PHOTO (III). Wilting of blight infected plant

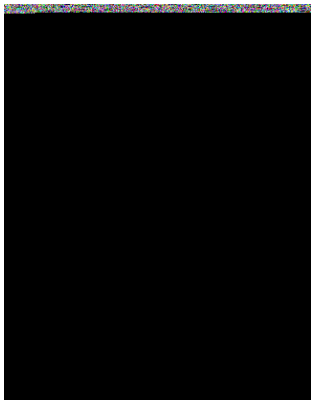

PHOTO (IV). Death of blight infected plant

2.3 *In vitro* evaluation of selected fungicides on the Growth and Sporulation of *Alternaria alternata*

2.3.1 Broth media test:

The efficacy of selected 5 fungicides on the growth and sporulation of fungus was studied by using poisoned food technique (Sharvelle, 1960). The selected fungicides viz. Dithane M-45 (Mancozeb), Blitox 50 (Copper Oxychloride), Topsin M (Thiophenate methyle), Bavistin (Carbendazim), Beam (Tricyclazole) was prepared at uniform concentration of 1000ppm. 50ml of potato dextrose broth was dispelled in conical flasks and plugged with non absorbent cotton and autoclaved at 15 lb pressure per square inch for 20 minutes. And after cooling down at 45ºC each of these selected fungicides were incorporated and then with the help of sterilized inoculating needle three days old mycelial disc of 5mm diameter cut out by using sterilized cork borer was transferred aseptically. The medium without fungicides served as control. For each treatment four replication was maintained. The experimental flasks were incubated at 25±10C for 10 days shaken for 2 minutes at 24 hrs intervals. On completion of the incubation period mycelium mats was harvested by filtering through pre-weighed filter paper Qualigens No.651 A (11 cm diameter) and dried in hot air oven at 600C for 72 hours and then kept in a desiccator for 24 hours and weight was taken again. The differences of weight between pre-weight filter paper and filter paper weight containing dry fungal mycelium determine the test fungicides and its efficacy on growth inhibition of the test fungi. The per cent growth inhibition over control was calculated by following method described by Vincent (1927).

$$I = \frac{100(C - T)}{C}$$

Where I=per cent growth inhibition of test fungi, C=growth of test fungi in control, T=growth of test fungi in treatment fungicides.

2.3.2 Solid media test:

50ml of molten potato dextrose agar (PDA) was dispelled in 100ml capacity conical flasks and autoclaved at 15 lb per square inch for 20 minutes. After cooling down at 45ºC required concentration (1000ppm) of test fungicides was added to each of the experimented flasks and mixed thoroughly by gently shaking in circular motion. The poisoned molted about 15ml were then poured in 9cm diameter sterilized petriplates. The medium without fungicide served as control. Each poisoned media plates was inoculated with 5mm diameter mycelial discs of 3 days old test fungal culture cut with the help of sterilized cork borer. The mycelial discs were placed at the center of the plate facing the mycelial mat with the media. For each treatment four replication was maintained. The inoculated plates were incubated at 25 ± 10C and observations were made at 24 hours interval till the fungus in the control plate covered the whole plate. The per cent inhibitions of fungal radial growth were calculated by following method described by Vincent (1927). For estimation of fungal spores, method described by Devi (1991) was followed where 1sq.cm mycelial block was cut out from each of the experimented plates and the mycelial mats was detached and then transferred into a test tube containing 5ml distilled water and vigorously shaken for 5 minutes. The

spore density/ml of this solution was estimated by using Haemocytometer. Four replications were made while counting of the spores.

III. RESULTS AND DISCUSSION

TABLE 1
EFFICACY OF DIFFERENT FUNGICIDES ON THE GROWTH AND SPORULATION OF FUNGUS (BROTH MEDIA)

Treatment	Dose (%)	Growth (mycelium dry weight) mg*	% inhibition over control
Bavistin (Carbendazim)	1000ppm	257.5 (16.0)	55.2
Beam (Tricyclazole)	1000ppm	2.0 (1.5)	99.6
Blitox 50 (Copper oxychloride)	1000ppm	13.5 (3.7)	97.6
Dithane M-45 (Mancozeb)	1000ppm	250.0 (15.8)	56.5
Topsin M (Thiophenate methyl)	1000ppm	496.0 (22.2)	25.9
Control	-	575.0 (23.9)	-
$C.D._{5\%}$	3.3		

Mean of four replication

Figure in the parenthesis are the square root transform value

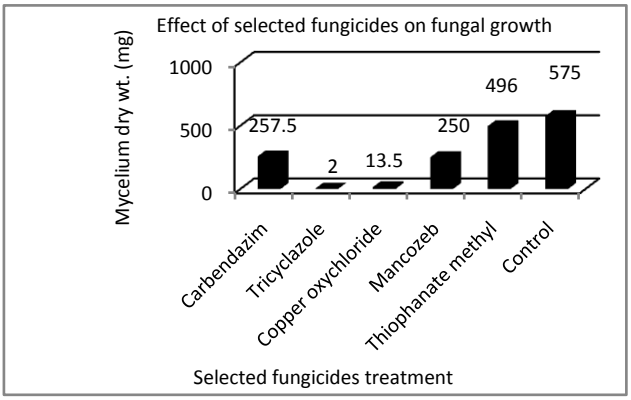

FIGURE 1: Selected fungicides and mycelium dry weight (mg)

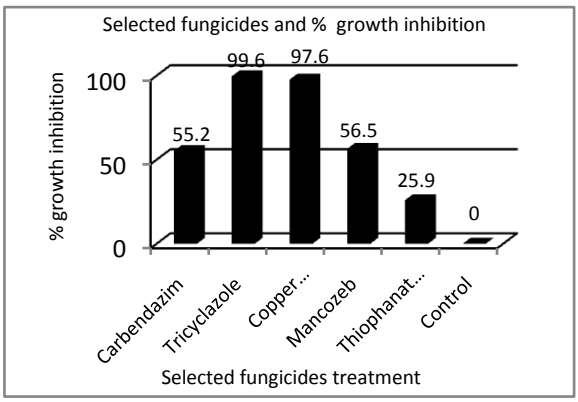

FIGURE 2: Per cent growth inhibition over control

The data presented in the above Table (1) and Fig.(1&2) is the result of efficacy of different fungicides on mycelium growth of *Alternaria alternata* in broth media. Among the selected fungicides, statistically highest significant inhibition on mycelial growth (minimum mycelium dry weight) was found in Tricyclazole (1.5mg) which was closely followed by copper oxychloride (3.7mg) with per cent growth inhibition of 99.6 and 97.6 respectively over untreated control whereas Mancozeb (15.8mg) and Carbendazim (16mg) with respective per cent growth inhibition of 56.5 and 55.2 and minimum significant mycelium growth inhibition was recorded in Thiophenate methyl (22.2mg) with per cent growth inhibition of 25.90 over untreated control. The present finding agreed with that of Misra and Singh (1965) who asserted fungicides like Blitox 50, Captan, Mancozeb and Zineb have got effective inhibitory properties on different isolates of *Alternaia tenui* during the *in vitro* experiment. Similar report was also reported by Paul and Mishra (1993).

TABLE 2

EFFICACY OF SELECTED FUNGICIDES ON GROWTH AND SPORULATION OF FUNGUS (SOLID MEDIA)

Treatment	Dose (%)	Colony diameter (mm)*	Spores/ml*	% inhibition over control	
				Colony diameter	Number of spores
Bavistin (Carbendazim)	1000ppm	55.2 (7.4)	1.1 x 10^3	38.2	82.5
Beam (Trycyclazole)	do	0.0 (0.7)	0.0	100.0	100.0
Blitox 50 (Copper oxychloride)	do	14.7 (3.8)	0.0	83.6	100.0
Dithane M-45 (Mancozeb)	do	41.7 (6.4)	0.6 x 10^3	53.6	90.0
Topsin M (Thiophanate methyl)	do	72.2 (8.5)	3.4 x 10^3	19.7	20.0
Control	-	90.0 (9.5)	6.3 x 10^3	-	-
C.D. 5%		0.4	-	-	-

Mean of four replication

Figure in the parenthesis is the square root transform value

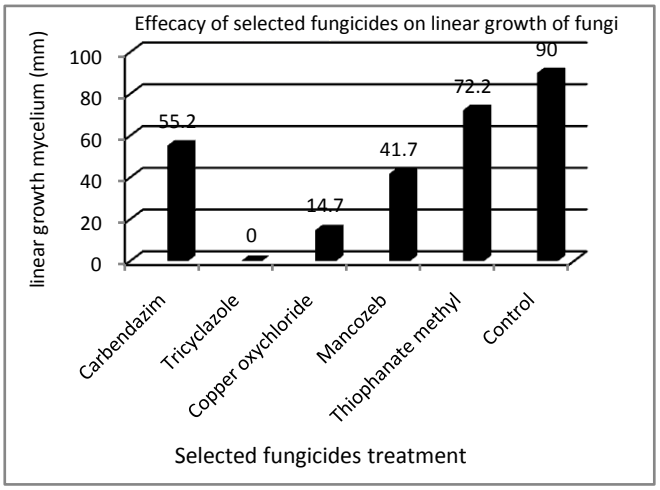

FIG.3.Selected fungicides on linear growth of fungi (*Alternaria ataernata*)

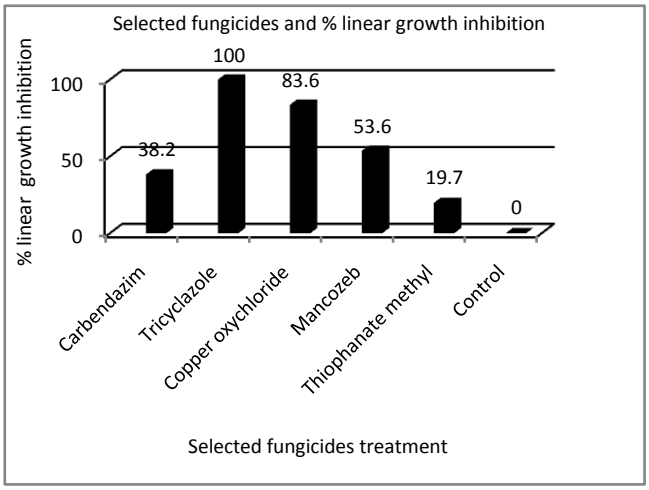

FIG.4. Selected fungicides and per cent linear growth inhibition

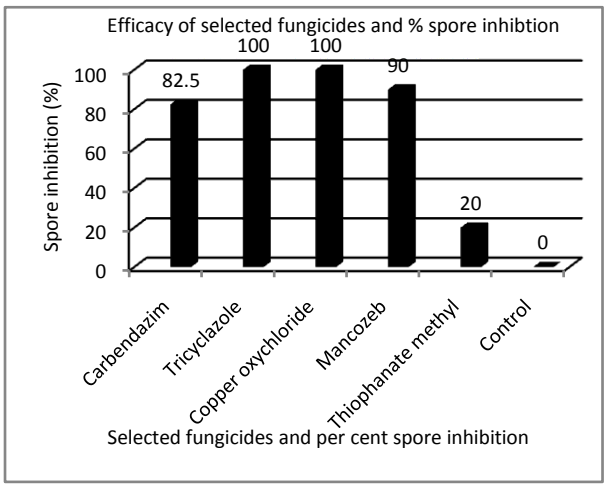

FIG.5. Selected fungicides and percent Spore inhibition of fungi (*A.alternata*)

Plate1.Mancozeb
Plate2.Tricyclazole
Plate3.Carbendazim
Plate4.Copper oxychloride
Plate5.Thiophanate methyl
Plate6.Control

PHOTO (V). Selected fungicides treatment and linear growth inhibition of *Alternaria alternate*

The data presented in the above Table (2), Fig. (3,4 &5) and Photo (V) is the result of the effect of different fungicides on growth and sporulation of *Alternaria alternata* in solid media test. Among the selected fungicides statistically highest significant inhibition was found in Tricyclazole (0mm) where radial growth of mycelium and sporulation was completely suppressed followed by Copper oxychloride (14.7mm) with 83.6% radial growth inhibition with no formation of spore whereas Mancozeb (41.7mm) and Carbendazim (55.2mm) radial growth with respective radial growth inhibition of 53.6% and 38.2% and per cent spore inhibition of 90% and 82.5% over untreated control. A minimum significant effect on radial growth and sporulation of test fungus was recorded in Thiophanate methyl (72.2mm) with radial growth inhibition 19.7% and per cent spore inhibition of 20 over untreated control.

IV. CONCLUSION

Our present finding was in agreement with Misra and Singh (1965) who reported that fungicides like Blitox, Captan, Mancozeb and Zineb have got effective inhibitory properties on different isolates of *Alternaia alternata* during the *in vitro* experiment. Similar report also found by Paul and Mishra (1993). Rao and Rajagopalan (1982) reported Thiram was found most effective against the growth of *Alternaria helianthicola* with 95.9% inhibition of mycelium growth and 89.7% of spore germination during the *in vitro* test whereas Giri and Peshney (1993) reported Carbendazim, Mancozeb, Fosetyl-A and Iprodione could inhibit spore germination and mycelial growth of *Alternaria alternata* causing leaf spot of mungbean. Similary, Singh (1994) who reported Dithane M-45 @ 0.2% could completely inhibit mycelial growth and sporulation of *Alternaria Alternata* causing stalk rot of sunflower.

The present investigation therefore, revealed a judicious application of Tricyclazole and Copper oxychloride at 1000ppm can effectively manages the blight disease of broad bean and prevent economic loss due to disease condition and also will prevent indiscriminate used of non effective option of available fungicides against the target diseases.

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Effect of *Pseudomonas Fluorescens* in the Germination and Growth of *Prosopis Laevigata* under Greenhouse Conditions

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***Abstract*—** *Mesquite (Prosopis laevigata) is a tree of arid and semi-arid areas of northern and central Mexico. This species allows erosion control, atmospheric nitrogen fixation, and improves soil quality. Pseudomonas fluorescens is a rhizobacterium that favors plant growth-promoting rhizobacteria (PGPR). Also, promotes seed germination and development of Mesquite plants under adverse environmental conditions.*

The aim is to evaluate the role of bacterial strains A7 and Sv of P. fluorescens, using two types of soil (vertisol and phaeozem), and adding vermicompost (0, 25, 50, 75 and 100 tons/ha) in the germination and growth stages of mesquite (Prosopis laevigata).We tested the characteristics developed by the plants over 180 days. A randomized experimental design with four repetitions was used to test the seed germination rate and 16 more variables in the greenhouse, such as morphology, dry biomass accumulated, and morphological indices through the randomized factorial experimental design with three factors, 2x3x5x3.

Regarding the control treatment, the use of the bacterial strain A7 of P. fluorescens inhibited the germination of mesquite seeds, while the strain Sv favored seedlings development. We observed opposite effects; inhibition and growth in the germination stage, and development of the seedlings observed at 180 days when using the A7 and Sv strains of P. fluorescens.

***Keywords*— Arid and semi-arid areas, Bacterial strain, Biofertilizer, Mesquite, Plant growth-promoting Rhizobacteria (PGPR), Pseudomonas fluorescens, Vermicompost.**

I. INTRODUCTION

The mesquite [*Prosopis laevigata* (Humb. Et Bonpl ex Wild.) M.C. Johnst], a multi-purpose tree (Rodríguez-Sauceda *et al*., 2014), is used a**s** a source of energy, a natural barrier, feed for livestock, getting gums and for medicinal (Prieto-Ruíz *et al*., 2013). In addition, it has important ecological functions because it allows erosion control, atmospheric nitrogen fixation and improves soil quality (Stanton *et al*., 2001; Buendía-González *et al*., 2012; Palacios-Romero *et al*., 2017). All these characteristics make it a species of interest to exploit and cultivate in arid and semi-arid areas, since according to Villanueva-Díaz *et al*., (2004), cited by Palacios-Romero *et al*., (2017), this woody species has a wide distribution in arid and semi-arid areas of northern and central Mexico.

However, cultivating species in arid and semi-arid environments requires generating new technologies (Prieto-Ruíz *et al*., 2013). Mia *et al*., (2012) stated that the development of any plant species depends on various factors such as the vigor of the seed for effective germination, and the rapid establishment of the plant (Bécquer *et al*., 2013). In this sense, with the purpose of new production options, the use of plant growth-promoting microorganisms (PGPM) as biofertilizers (Afzal and Bano, 2008) they considered a solution and an alternative to promote plant growth and nutrition (Vessey, 2003; Jaiswal *et al*., 2016). In fact, the use of such fertilization sources has attracted the attention of researchers because of their success in crop development and their low ecological footprint (Egamberdieva, 2008; Karakurt *et al*., 2011; Radhapriya *et al*., 2015) in relation to chemical fertilizers (Vessey, 2003; Dadrasan *et al*., 2015).

Among the plant growth-promoting microorganisms, it gives great emphasis to the study of the group of fluorescent Pseudomonas bacteria, either to increase agricultural production or for its various related benefits by the association of bacteria on plant roots (Carrillo-Castañeda *et al*., 2000; 2011). For leguminous species, many authors report that *P. fluorescens,* selected as plant growth-promoting Rhizobacteria, are a source of nutrients, where plant root colonization (Siddiqui *et al*., 2001; Egamberdieva *et al*., 2013) stimulates growth (Vessey, 2003) and direct or indirect production (Shaukat *et al*., 2006, Afzal and Bano, 2008; Iqbal *et al*., 2012).

They have shown the plant growth-promoting capacity of bacteria of the genus *Pseudomonas* through the "biofertilizing effect" and also for its ability to antagonize multiple pathogenic microorganisms (Pal *et al*., 2001). In addition, they have considered that natural reforestation in eroded areas of the desert is very difficult because, in these degraded soils, the surface layer of the soil has lost the microorganisms that promote the development of plants. That is why, by artificially reforesting these areas, we recommend inoculating the seeds of the plants with microorganisms to return to the soil part of their fertility and potential for the development of plants. Drezner, (2006) mentioned that only good irrigation with water will not restore fertility and soil microbial communities.

That is why we considered in this work pertinent to determine the specificity and positive or negative effects of bacterial cells associated with mesquite seeds and roots as an ecological strategy to promote the growth of plants within an utilization perspective of natural and biological fertilizers. Thus, in this study, we tested the significant importance of an inoculation with cells of the bacterial strains A7 and Sv of *P. fluorescens* in two physiological stages, germination, and the development of mesquite. In addition, we suggested two hypotheses. The first is that the use of bacterial strains favors the germination process of mesquite. Meanwhile, the second one is that the use of these plant growth-promoting microorganisms favors the initial development stage of this tree species.

II. MATERIALS AND METHODS

2.1 Soil collection site

The soils used originate from two agricultural production sites, within the low micro-basin of the Rio Grande, Tulancingo, state of Hidalgo, northeast of Mexico City. The geographical coordinates and the surface of the collection sites (soil and soil samples) were 20° 11'39.96' 'N, 98° 26' 38.62" W and 0.86 ha for the vertisol soil, while for the Phaeozem soil was 20° 12'27.18' 'N, 98° 27' 21.50" W and 1.18 ha. We carried out the soil classification according to the edaphological vector data set, scale 1: 250 000 series II of the INEGI (Valdez-Pérez *et al*., 2016).

We collected the soils in November 2016. Each sampling site comprised one hundred and twenty soil samples, with two hundred and forty samples, with forty sub-samples for section 0-5 cm deep, forty for section 5-10 cm and forty for that corresponding to 10-40 cm (Gardezi *et al*., 2009). We describe the physicochemical characteristics in (Table 1), which shows that phosphorus and nitrogen levels were three times higher in the vertisol soil in relation to the phaeozem soil, than and twice as important as potassium.

2.2 Vermicompost used as organic matter

We prepared the vermicompost, used as organic matter, with 60 kg of bovine manure (Bos taurus), 25 kg of melon residues (*Cucumis melo*) and 15 kg of wheat residues (*Triticum* sp.), which interacted with Californian earthworm (*Eisenia fetida*) during the months of September to December 2016. Table 1 includes the physicochemical characteristics of vermicompost.

TABLE 1
PHYSICAL-CHEMICAL PROPERTIES OF VERMICOMPOST AND TWO AGRICULTURAL SOILS OF THE LOW MICRO-BASIN OF THE RIO GRANDE, TULANCINGO, MEXICO

Properties	Vermicompost	Soil samples					
		Vertisol			Phaeozem		
		0-5 cm	5-10 cm	10-40 cm	0-5 cm	5-10 cm	10-40 cm
Organic matter (%)	7.93	5.51	3.03	3.63	3.36	3.16	3.23
pH	7.08	6.99	7.16	7.27	6.39	6.28	6.35
Electrical conductivity dSm^{-1}	2.52	0.14	0.12	0.12	0.05	0.06	0.05
Total Nitrogen (N)%	0.34	0.27	0.06	0.13	0.11	0.06	0.07
Assimilable Phosphorus (P) mg kg^{-1}	145.68	39.77	28.85	26.36	11.95	8.79	8.55
Exchangeable potassium (K) mg kg^{-1}	8543	5659	5394	5203	2714	2741	3323
Lead (Pb) mg kg^{-1}	2.55	3.89	0.77	2.25	2.89	3.48	4.9
Chrome (Cr) mg kg^{-1}	0	0	0	0	0	0	0
Cadmium (Cd) mg kg^{-1}	0	0.26	0	0	0	0	0
Nickel (Ni) mg kg^{-1}	1.03	0.49	0.56	0.43	0.9	1.26	0.8
Cobalt (Co) mg kg^{-1}	0.12	0.14	0.11	0.12	0.14	0.08	0.09

2.3 Origin of mesquite germplasm and bacterial strains of *P. fluorescens*

The biological material used had two different origins. We collected the seeds of *P. laevigata* from the trees in September 2016, and the vertisol and phaeozem soils came from the micro-basin of the Río Grande, Tulancingo. We got the bacterial strains A7 and Sv of *P. fluorescens* from the collection of the Molecular Biology Laboratory, Institute of Genetics, Postgraduate College, Montecillo Campus.

2.4 Experimental design

In January 2017, we inoculated the *P. laevigata* seeds with cells of the Sv and A7 strains of *P. fluorescens*, subsequently transferred to the greenhouse for germination and growth. We used a randomized experimental design with four repetitions, used in the inoculation and germination of seeds, comprised three treatments, the first corresponded to the inoculation of *P. laevigata* roots with the bacterial strain A7 of *P. fluorescens*, and the second consisted of the inoculation of the roots of *P. laevigata* with the bacterial strain Sv of *P. fluorescens*; and the control treatment. The ***P.fluorescens*** culture was carried out in the King B base culture medium prepared in a liquid state (Carrillo-Castañeda *et al*., 2011).We incubated this cultures in a shaker at 150 opm at an average temperature of 30°C for 72 hours. The turbidity of the bacterial cultures was adjusted to turbidity (660 nm) of 1.8 for strain Sv and 1.2 for strain A7.

Prior to the inoculation, we treated the *P. laevigata* seeds with 120 mL of 70% alcohol for one minute, followed by washing with distilled water at an average temperature of 60°C (Quiñones-Gutiérrez *et al*., 2013) for five minutes and drying for four hours. In addition, twelve 15 ml bottles were prepared (four bottles per treatment) to perform the inoculation of the seeds and added 10 ml of bacterial cell suspension (A7 or Sv) for the two treatments (eight bottles), distilled water and 70 seeds. The twelve bottles (four with strain A7, four with strain Sv, and four without bacterial strain) were put to rest for two hours. Then we transferred the seeds of each bottle to pre-identified Petri dishes and provided them with a triple layer of absorbent paper.

2.5 Greenhouse experiment

We placed the twelve Petri dishes prepared in the laboratory in a greenhouse germination chamber of the Postgraduate College at an average temperature of 18.9°C and a humidity of 57.3%. We watered the seeds every 24 hours with 3 mL of distilled water. The daily germination percentage of *P. laevigata* seeds was determined for 20 days. We placed the seeds in germination beds, and after thirty days, the homogenous seedlings were selected. Then transplanted into polyethylene bags filled with nine kilograms of soil.

We implemented the greenhouse phase from January to July 2017, with the help of a randomized experimental design with a 2x3x5 factorial arrangement with three repetitions. The study factors were bacterial strains A7 and Sv of *P. fluorescens*, soils vertisol, phaeozem, and 0, 25, 50, 75, and 100 tons/Ha of vermicompost. We measured sixteen dependent variables, divided into three groups, in the experimental units or calculated after 180 days, either in group A «morphological variables (height (cm), diameter (mm), the number of leaves and root length (cm)) », group B« dry biomass (dry biomass weight of leaves (g),

leaf litter (mg), stems (g), roots (g), nodules (mg), aerial (g), underground (g) and total (g)) »and group C« indicators and indices (leaf area (cm^2), air weight ratio between radical weight, slenderness index, and Dickson index) ».

2.6 Statistical analysis

We performed the data analysis for the two experimental designs using R software (R Core Team, 2013). We subjected these to the homogeneity tests of variances by Bartlett's test and graphic methods (residual against predicted). In addition, we corroborated their normality by the Shapiro-Wilk test. Various transformations combined with the "power transform" function of Box-Cox (Table 2) were used to satisfy the assumed assumptions (Gurka *et al*., 2006) of the models used. As suggested by Rodríguez-Sauceda *et al*., (2019), the Tukey multiple comparisons ($p \leq 0.05$) were used to match the effects of the control treatment, strain A7, and strain Sv of *P. fluorescens* of the experimental design used in the germination stage. For the greenhouse experiment, we determined significant differences ($p \leq 0.05$) of the bacterial, soil, and vermicompost factors. We compared the means of the three levels of the bacterial strains factor, the factor of interest in this study.

TABLE 2
VARIABLES OF TRANSFORMATION: COMPLIANCE WITH THE ASSUMPTIONS OF THE MODELS OF ANALYSIS OF VARIANCE (ANOVA)

Determinations at 456 h	Variables of transformation
Random experimental design	
Variables	$y = (456h)^{9.8098}$
Random factorial experimental design	
A. Morphological variables	
Diameter (mm)	$y = (((Diameter\ (\text{mm})) - 7)^2)^{0.241}$
Number of leaves	$y = ((\log(Number\ of\ Leaves) - 5.9)^2)^{1.1539}$
B. Dry biomass	
Dry weight of leaves (g)	$y = (((\text{Log}(Dry\ weight\ of\ leaves\ (\text{g}) + 0.7)) - 1)^2)^{0.3156}$
Dry weight of leaves litter (Mg)	$y = (Dry\ weight\ of\ leaf\ litter\ (\text{g}))^{0.2551}$
Dry weight of stems (g)	$y = ((\text{Log}(Dry\ weight\ of\ stems\ (\text{g})) - 1)^2)^{0.3552}$
Dry weight of nodules (Mg)	$y = 1/(Dry\ weight\ of\ nodules\ (\text{Mg}) + 1)^{8.4901}$
Aerial dry weight (g)	$y = ((\text{Log}(Aerial\ dry\ weight\ (\text{g})) - 2)^2)^{0.1909}$
Total dry weight (g)	$y = ((\text{Log}(Total\ dry\ weight\ (\text{g})) - 2)^2)^{0.3686}$
C. Indicators and morphological indices	
Dickson Index	$y = (Dickson\ index)^{0.0292}$
Slenderness index	$y = ((\text{Log}(\text{Slenderness index}) - 3)^2)^{0.1337}$
Aerial/radical weight ratio	$y = 1/(\text{Aerial}/radical\ weight\ ratio)^{0.3766}$
Foliar area (cm^2)	$y = ((\text{Log}(Foliar\ area\ (\text{cm}^2)) - 6)^2)^{0.074}$

III. RESULTS

3.1 Germination of *P. laevigata* seeds

When analyzing the trend during the germination stage from initial to end, the results allowed us to discard our first hypothesis proposed at the beginning of this study for two reasons. First, the germination of *P. laevigata* seeds inoculated with cells of the bacterial strains A7 and Sv, was inhibited, 22.81 and 18.16% respectively, showing the results of the analysis of variance showed significant effects ($p \leq 0.05$) between treatments (Table 3). The second, the absence of significant favoring or inhibition of germination at 120 hours, and for the period 168-360 hours (Table 3). Corresponding to our results of the inhibition of the germination process, the tendency of the observations agreed with those made by Carrillo-Castañeda *et al*., (2000; 2011), who concluded that there is a capacity for an inhibition of specific strains of *P. fluorescens* for certain plant species. However, among the limitations found in this study were the exclusive use of the A7 and Sv strains of *P. fluorescens*, which suggest future experimentation with other bacterial strains to corroborate the tendency of these plant growth-promoting microorganisms (PGPM) in the germination stage of mesquite (*P. laevigata*).

TABLE 3
GERMINATION OVER A PERIOD OF 456 HOURS OF *P. LAEVIGATA* SEEDS INOCULATED WITH CELLS SUSPENSIONS OF THE INDICATED BACTERIAL STRAINS

Determination of germination after the number of hours indicated	ANOVA Pr(>F)	Comparison of means: germination percentage		
		WI	A7	Sv
24	0.0270 *	5.71 a	3.22 ab	1.43 b
48	0.00755 **	31.07 a	11.79 b	15.72 b
72	0.0237 *	43.22 a	18.93 b	26.79 ab
96	0.0933.	50.00 a	29.29 b	40.72 ab
144	0.0700.	67.15 a	42.14 b	53.57 ab
384	0.0671.	92.50 a	83.22 b	88.57 ab
408	0.0822.	92.50 a	83.57 b	88.57 ab
432	0.0672.	92.86 a	83.57 b	88.57 ab
456	0.0394 *	93.22 a	83.57 b	88.57 ab
120, 168-360	>0.3186	NS	NS	NS

The means with an identical letter in the same row are statistically equal (Tukey). NS = Not significant. WI = without inoculation. Significance code: '': 0.001, '**': 0.01, '*': 0.05, '.': 0.1, ' ': 1.***

3.2 Seedling growth of *P. laevigata*

When dimensioning and quantifying separately the effect of the three factors used in the corresponding experimental design in the greenhouse growth stage, the soil and vermicompost factors showed significant differences (see p value in table 4) for 81.25% and 68.75% of the 16 variables tested (Table 4), respectively. When considered the bacterial factor, we only observed significant differences ($p \leq 0.05$) in 56.25% of the analyzed variables (Table 4). Although in third position, when compared with the soil and vermicompost factors, the use of strains A7 and Sv together open the way to explore the development of environmentally friendly production technologies focused on the use of plant growth-promoting bacteria(PGPB), which have been widely used to improve plant growth (Egamberdieva, 2008; Karakurt *et al*., 2011; Radhapriya *et al*., 2015).

TABLE 4
ANALYSIS OF VARIANCE OF MORPHOLOGICAL VARIABLES, DRY BIOMASS AND MORPHOLOGICAL INDICATORS AND INDICES AT 180 DAYS OF GROWTH IN THE GREENHOUSE OF *P. LAEVIGATA*

Variable	Soil	Bacteria	Vermicompost
A. Morphological variables			
Height (cm)	1.81e-05 ***	0.2395	0.0305 *
Diameter (mm)	0.000963 ***	0.024451 *	0.000487 ***
Number of leaves	0.32762	0.00473 **	0.02485 *
Root length (cm)	0.0932.	0.5814	0.0565.
B. Dry biomass			
Dry weight of leaves (g)	1.08e-08 ***	0.2903	0.0580.
Dry weight of leaves litter (Mg)	0.4109	0.0509.	0.1004
Dry weight of stems (g)	< 2e-16 ***	0.00679 **	0.00182 **
Dry weight of roots (g)	3.48e-10 ***	0.0148 *	1.18e-05 ***
Dry weight of nodules (Mg)	1.14e-07 ***	0.0214 *	0.4127
Aerial dry weight (g)	9.58e-05 ***	0.04279 *	2.71e-06 ***
Underground dry weight (g)	1.55e-09 ***	0.0162 *	1.40e-05 ***
Total dry weight (g)	<2e-16 ***	0.1848	0.5536
C. Indicators and morphological indices			
Dickson Index	5.57e-05 ***		4.57e-06 ***
Slenderness index	0.141	0.344	0.793
Aerial/radical weight ratio	0.00131 **	0.47089	0.19293
Foliar area (cm^2)	3.18e-09 ***	0.2957	1.48e-05 ***

Significance code: '': 0.001, '**': 0.01, '*': 0.05, '.': 0.1, ' ': 1.***

Among the 56.25% of variables classified as significant, we observed two patterns of prime effect that allowed us to respond to our second hypothesis. The first, favoring, since in relation to the control treatment, the inoculation of the mesquite seeds with cells of the **Sv** strain of *P. fluorescens* in mesquite roots (*P. laevigata*) improved significantly(see p value in table 5) 43.75% of the variables analyzed in this study. Specifically, for group A) morphological variables: an increase of 17.89% and 19.48% in diameter (mm) and the number of leaves; Group B) dry biomass: an increase between 23.81% and 44.27% of the dry weight of leaves litter (mg), stems (g), roots (g), and underground (g). Finally, group C) indicators and morphological indices: the same trend, an increase of 28.02% in the Dickson index (Table 5). While, for the second pattern, inhibition, in relation to strain Sv, we found that strain A7 significantly decreased (see p-value in table 5) 12.5% of the analyzed variables, 48.10% and a 16.73% for the dry weight of nodules (mg) and aerial (g), respectively (Table 5). Only the first pattern of this research work related to the trend of inoculation work with plant growth-promoting bacteria (PGPB) in legumes reported in the literature, for example, for the growth of the air section of Bashan *et al*., (2012), cited by Radhapriya *et al*.,(2015), height (Iqbal *et al*., 2012), and anhydrous weight (Dileep Kumar *et al*., 2001).

The results obtained allowed us to delimit the degree of action corresponding to the use of bacterial strains as a natural source of nutrients (Afzal and Bano, 2008; Iqbal *et al*., 2012) to increase the growth of mesquite trees. However, although the Sv strain was the pioneer treatment when the effects of the bacterial factor were significant in relation to the control treatment (43.75% of the variables), we must take into account that this factor is in the last position if we consider the effects of the soil and vermicompost factors.

TABLE 5
COMPARISON OF BACTERIAL FACTOR MEANS OF MORPHOLOGICAL VARIABLES, DRY BIOMASS AND MORPHOLOGICAL INDICATORS AND INDICES AT 180 DAYS OF GROWTH IN THE GREENHOUSE OF *P. LAEVIGATA*

Variable	Bacteria		
	Witness	A7	Sv
A. Morphological variables			
Height (cm)	83.44 a	82.46 a	93.29 a
Diameter (mm)	5.22 b	5.74 ab	6.35 a
Number of leaves	36 b	50 a	45 a
Root length (cm)	58.20 a	60.14 a	55.83 a
B. Dry biomass			
Dry weight of leaves (g)	2.25 a	2.47 a	2.85 a
Dry weight of leaves litter (Mg)	101.62 b	131.74 ab	182.34 a
Dry weight of stems (g)	4.89 b	5.27 b	6.41 a
Dry weight of roots (g)	3.61 b	4.10 ab	4.86 a
Dry weight of nodules (Mg)	85.74 ab	45.46 b	87.59 a
Aerial dry weight (g)	7.24 ab	7.87 b	9.45 a
Underground dry weight (g)	3.70 b	4.14 ab	4.95 a
Total dry weight (g)	10.94 a	12.01 a	14.40 a
C. Indicators and morphological indices			
Dickson Index	0.67 b	0.79 ab	0.93 a
Slenderness index	16.13 a	14.44 a	15.87 a
Aerial/radical weight ratio	2.14 a	1.94 a	2.00 a
Foliar area (cm^2)	273.13 a	293.60 a	334.41 a

The means with an identical letter in the same row are statistically equal (Tukey).
Significance code (p value): '': 0.001, '**': 0.01, '*': 0.05, '.': 0.1, '': 1.***

IV. DISCUSSION

4.1 Germination of *P. laevigata* seeds

In this study, we observed a significant decrease ($p \leq 0.05$) of 9.65% of the germination percentage of mesquite seeds inoculated with cells of the bacterial strain A7 in relation to the seeds without inoculation after 456 hours (Table 3). However, our results opposed previous research by authors such as Bashan *et al*.,(2012), Radhapriya *et al*., (2015), Elekhtyar (2015), and Kumar (2016), who pointed out that plant growth-promoting Rhizobacteria (PGPR), including *P. fluorescens*,

increase seed germination. However, Villegas-Espinosa *et al*., (2014) mentioned that in different investigations carried out with plants and beneficial microorganisms, they observed inhibitory or positive effects on germination.

4.2 Seedling growth of *P. laevigata*

Another aspect to consider is that the results obtained only apply to plant production under greenhouse conditions described in the method of this document. Given the need to find answers for different environmental conditions, as stated by Bécquer *et al*., (2013) and Villegas-Espinosa *et al*., (2014)**.** It requires future research at the field level to assess the effects of the interaction of plant growth-promoting microorganisms (PGPM) and mesquite trees, both exposed, for example, to the environmental conditions of the site of origin.

V. CONCLUSION

This study allowed us to reach two main conclusions regarding the control treatment. The first one: the use of bacterial strains called plant growth-promoting rhizobacteria showed opposite effects, inhibition-favoring, in the germination and growth stage tested at 180 days; however, the benefit of the cells associated with the roots of the plants could be presented in the phenological stages of the crop. The second conclusion: the use of bacterial strains proved to be an alternative as a biofertilizer to stimulate the growth of 43.75% of the tested variables, which shows that the plant-bacterial interaction of *P. laevigata* and *P.fluorescens* can be used as a biological method to contribute to the balance of soil fertility.

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Effect of Industrialization and Urbanization on Agriculture

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Abstract— *Industrialization and urbanization becomes a bane for the agriculture now days. With increase in industrialization and urbanization, the growth of agriculture sector decline continuously. Large area of land is covered under industry and infrastructure, which results in shortage of agriculture productive lands. The waste products of industry and urban areas are flowed in water bodies such as river, lakes and ponds which pollute them and make them unsuitable for any kind of use. The harmful effluents released from industry contaminate the air with harmful gases and suspended material. These gases and suspended particles affect the growth and development of plants and animals. The suspended particles are inhale during breathing and cause blockage in veins and arteries of animals and humans. To feed the ever increasing population of our country, the farmer put high pressure on shrinking land to get higher output. For this, farmer use modern technology and chemicals which reduce the productivity and fertility of soil. The polluted water of lakes and ponds become poisonous for the water living entities and results in death of plants and animal species. The waste effluents of urban areas and industry have high concentration of heavy metals which are very poisonous for animals and plants which survive under water and on land. The growth of plants and microbes in soil is reduced due to increase in concentration of heavy metal in soil. The effects of industries and urbanization need to be decreased to get a healthy environment for plants and animals. Special management practice needs to be developed to suppress this increasing problem to survive on earth*.

Keywords— industrialization, urbanization, suspended particles, productivity, fertility, heavy metals, etc.

I. INTRODUCTION

World population has been rising continuously since the end of the Black Death, around the year 1350 (Dunham, 2008). Population began growing rapidly in the Western world during the industrial revolution. The most significant increase in the world's population has been since the 1950s, mainly due to medical advancements (Greenwood, 2014) and increases in agricultural productivity (Armelagos *et al.,* 1991). Population growth is the increase in the number of individuals in a population. Global human population growth amounts to around 83 million annually or 1.1% per year. The global population has grown from 1 billion in 1800 to 7.774 billion in 2020. It is expected to keep growing, and estimates have put the total population at 8.6 billion by mid-2030, 9.8 billion by mid-2050 and 11.2 billion by 2100 (Nations, 2017). The 29 % (149 million km^2) surface of the earth is covered with land. Out of 29 %, 71 % is habitable land and 10 % under glaciers and 19 % under barren condition. Out of this 71 %, 50 % is under agriculture and rest under shrubs and forests. Only 23 % of this 50 % is under crop and rest 77 % is under livestock and dairy (Ritchie and Roser, 2013). In this way, only 11 million km^2 is under crop production. This area is used to feed the ever increasing population of our world. To feed that amount of population, farmer use heavy machinery and chemicals to increase the productivity of land. This rapid increasing population and food shortage leads to industrialization. It is believed that industrialization is the root of urbanization (Mitra and Sato, 2007, Li *et al.,* 2014).

Industrialization has historically led to urbanization by creating economic growth and job opportunities that draw people to cities. Urbanization typically begins when a factory or multiple factories are established within a region, thus creating a high demand for factory labour. Other businesses such as building manufacturers, retailers, and service providers then follow the factories to meet the product demands of the workers. This creates even more jobs and demands for housing, thus establishing an urban area (Invetopedia, 2019).In 1900, worldwide, there were 6.7 rural dwellers to each urban dweller; now there is less than one and projections suggest close to three urban dwellers to two rural dwellers by 2025 (Satterthwaite *et al.,* 2010). This fast growing urban areas and industries result in shortage of agriculture land. The continuous expansion in urbanization and the increasing demand for agriculture production are in continuous race with each other in competing for scare natural resources such as land and water. The shrinkage resource base for agriculture production on the one hand and the increasing demand for food production and population place severe pressure on both the quantitative and qualitative aspects of land and water resource (Balasubramanian and Choi, 2010). Urbanization and industrialization not only contaminates the land and water bodies but also change the crop and land use pattern. Kurucu and Christina (2008) reported change in the land use patter in metropolitan cities around the world.

II. INDUSTRIALIZATION

Industrialization is the process by which an economy is transformed from primarily agricultural to one based on the manufacturing of goods. Individual manual labour is often replaced by mechanized mass production, and craftsmen are replaced by assembly lines (Investopedia, 2019). Characteristics of industrialization include economic growth, more efficient division of labour, and the use of technological innovation to solve problems as opposed to dependency on conditions outside human control. Industrialization is most commonly associated with the Industrial Revolution of the late 18th and early 19th centuries. Industrialization has rapidly taken the stage of public attention and debate in the past few years (Drabenstott, 1995).Industrialization enhances productivity, raised per capital income and accelerates the pace of saving and capital formation. It is the key for the development of a community (Holkar *et al.,* 2018).

III. EFFECT OF INDUSTRIALIZATION ON AGRICULTURE

Industrialization has both positive as well as negative effect on agriculture. It plays an important role in the development of a nation by generating employment and by utilizing the resource available which help in expansion of business (Holkar *et al.,* 2018). It helps in earning foreign exchange and enhances the system of farming by developing modern machinery and inputs. However, continues utilization of these machinery and input degrade the fertility and productivity of soil. Because of uncontrolled and unorganised industrial advancement in developing countries, destructive effect on the environment, wildlife and biodiversity was reported by Hatami and Shafieardekani (2014). Zhang *et al.* (2015) reported that with rapid industrialization in the recent years, China is now facing great challenge in heavy metal contamination in farmland soil.

Particularly, in the recent years the use of chemical fertilizer, sewage irrigation and pesticide is increased. The use of these products disturbs the natural ecosystem and pollutes the environment and soil (Liu *et al.,* 2014). The waste materials of industries have high concentration of heavy metal which degrades the physical, chemical and biological properties of plants (Sethy and Ghosh, 2013). High concentration of Cd and Pb in various industrial area of Pondicherry was reported by Devy (2002). Concentration of heavy metal beyond the prescribed standard in soil destroys the soil structure, fluctuate the pH and Ec of the soil and reduced the plant water and nutrients uptake (Sahid *et al.,* 2015). After uptake inside the plants, they form complexes which affect the photosynthesis and chlorophyll content in leaves. Toxicity of heavy metals results in poor seed emergence and seedling growth which cause reduction in yield. Pokharel *et al.* (2000) observed that the root growth and the number or root per bulb in onion is in all effluents as compared to growth. Seed germination and seedling growth in some agricultural crop is reduced due to the addition of brewery industry effluent (Acharya, 2001).Ghani (2010) reported decreased dry matter and seed yield, reduced nitrogen content in plant tissues, and lowered protein content in seeds of maize under treatment of heavy metal. Significant reduction in the yield of crop was reported by Fathi *et al.,* 2011 and Okoye *et al.,* 2019 due to heavy metals.

Rapid advancement in industrialization shrinks the forest area and change the land use pattern. Significant changes in the arable land and land cover due to industrialization was reported by Lu *et al.,* 2011. High quality cultivated land was changed to developed land and low quality land generated from unused land has resulted a serious threat to food supplies (Wand *et al.,* 2011). The shrinkage of forest area and polluted environment results in extinction of several wildlife and water species. The indiscriminate and untreated discharge of industries and municipal solid waste is the principal source of surface water contamination (Abbasi *et al.,* 2002).Paints industries use numerous chemical for the production of paints which are responsible for high concentration of organic compounds, suspended particles, coloured materials and other hazardous material which when discharge into environment may penetrate and leach in to the subsurface environment and leach down in to water bodies (Olaoye *et al.,* 2015).

Non-treated discharge of industrial waste into lagoons, river and streams had become a treat for the aquatic environment. These water bodies have high chemical oxygen demand (COD), high biological oxygen demands (BOD), less dissolve oxygen and nitrate and phosphate concentration. Aniyikaiye *et al.*(2019) evaluated that the effluents discharge of five industries have BOD of 840.6, 502.9, 162.8, 974.7 and 595.8 mg/L which was very high above the BOD standard of WHO (60 mg/L). High COD and BOD values was reported by Jordao *et al.,* (2005) in Uba stream (Brazil) because of a great load of organic untreated pollutant was flowed from kaolin processing plant. Microorganism activities in the water bodies decrease the amount of oxygen which cause suffocation and death of the aquatic organisms. Excess of nitrate and phosphate results in heavy growth of algae on the water surface also deplete the dissolve oxygen and create suffocating condition (Bhateria and Jain, 2016). Industrial waste products also have oil and greases which remain suspended on the surface of water bodies and restrict the penetration of sunlight which is necessary foe photosynthesis of water (Aniyikaiye *et al.,* 2019). The effluents from the industry contains large amount of bacteria and fungi. Some of them are beneficial help in

decomposition of organic matter but some are pathogenic to plants. They cause different diseases to plants.

Similar to the water, the quality of air is also affected by the industrialization. At the end of nineteenth century, a large number of industries such as calcium carbide, pesticide and fertilizer industries was established which release a huge load of harmful gases in the environment. The emission from these industries increases as the demand of the product is increased. The concentration of CO_2increased from 365.26 ppm to 416.18 ppm from 1998 to 2020 (CO_2 earth, 2020) due to continues emission of gases (oxidized and reduced forms of carbon (CO_2, CO, CH_4), SO_2, O_3, C_6H_6 vapours, Hg, etc.), particulate matter (PM_{10} and $PM_{2.5}$) and radioactive isotopes (Agbaire and Esiefarienrhe, 2009). These poisonous gases emitted by industries in the atmosphere cause acid rain which is rich in sulphur and other chemicals. Microorganism which add nutrients to soil eventually die because of acid rain (Manisalidis *et al.,* 2020). The acid rain acidifies the water and soil environment, damage the tree and plantations. Suspended particle and gases are the main reason of depletion of ozone layer and cause global warming result in increases the temperature of earth. Depletion of ozone layer exposed the crop to harmful UV radiation which causes significant reduction in the yield of crop (Teramura, 1983). Harmful gases and PMs are also responsible for vegetable injury and yield loss (Joshi and Swami, 2007) and also affect the germination of seed and flowers in inflorescence (Nithamathi and Indira, 2005). SO_2 is the major component of air pollutant which affects the morphological character of plants such as number of leaves, length of shoot and root, leaf area and number of flower and fruits (Wali and Iqbal, 2004).

Air pollutants also affect the photosynthetic rate by decreasing the chlorophyll content of the leaf (Joshi and Swami, 2007). Air pollutant also leads to stomatal closure which reduces the entry of CO_2 in the plant leaves and inhibits the carbon fixation (Seyyednejad *et al.,* 2011). Reduction in the area of leaf also reduces the photosynthetic activity due to decreasing in the net radiation absorption (Tiwari*et al.,* 2006). Chapla and Kamalakar (2004) reported reduction in the total chlorophyll content, RuBisCo enzyme activity and net photosynthetic rate under fumigation of 40, 80 and 150 ppbv concentration of O_3. Decrease in the chlorophyll content increase the respiration rate and CO_2 fixation which result in reduction of total soluble sugar. Joshi and Swami (2009) also found reduction in the carotenoid content in the leaves of six tress species which are exposed to air pollutant. Sravanakumar (2014) also found decrease in the photosynthetic pigment in plant leaves around urban and industrial sites. High exposer of plant leaves to air pollutant also produces ROS (Woo *et al.,* 2007) which destroy the cell wall and cell membrane (Tiwari *et al.,* 2006).

The yield of crops is also affected all over the world by the industrialization. There are numbers of study in which reduction in the yield of crop was due to industrial air and water pollutant. Khai and Yabe (2013) reported 12% reduction in the yield and 26% profit loss in rice crop due to water pollution in Vietnam. Significant reduction in the grain yield of 8.46 % in rice and 9.52 % in wheat and straw yield of 11.08% in rice and 12.24% in wheat were reported by Malay et al.. (2017). Chakrabarti *et al.* (2014) indicated that straw yield was reduced up to 17.6% and 24.5% rice and wheat crop respectively due to air pollution. Raja *et al.* (2014) also showed that similar types of results, he reported that straw yield of rice was significantly reduced over control. Mills *et al.* (2018) also estimated the reduction of 12.4%, 7.1%, 4.4% and 6.1% in the yield of soybean, wheat, rice and maize, respectively due to high concentration of ozone.

IV. URBANIZATION

Urbanization refers to the population shift from rural to urban areas, the decrease in the proportion of people living in rural areas, and the ways in which societies adapt to this change. It is predominantly the process by which towns and cities are formed and become larger as more people begin living and working in central areas (Wikipedia, 2020).The United Nations projected that half of the world's population would live in urban areas at the end of 2008.By 2050 it is predicted that 64.1% and 85.9% of the developing and developed world respectively will be urbanized (Science daily, 2008). Urbanization is closely linked to modernization, industrialization, and the sociological process of rationalization. Urbanization can describe a specific condition at a set time, i.e. the proportion of total population or area in cities or towns, or the term can describe the increase of this proportion over time. Urbanization is not merely a modern phenomenon, but a rapid and historic transformation of human social roots on a global scale, whereby predominantly rural culture is being rapidly replaced by predominantly urban culture.

V. EFFECT OF URBANIZATION ON AGRICULTURE

Agriculture is the backbone of Indian economy, which provides livelihood to 65 to 70% of the total population and employ about 52% population of the country (Pramanik and Sarkar, 2011). With the onset of British government and industrial revolution in the 18th century, the relationship between the population and agriculture was broken and an unprecedented

growth in the urban population takes place over the course of 19th century both through continued migration from the countryside and due to the tremendous demographic expansion that occurred at that time. The urban population of the world will grow by more than billion between 2010 and 2025, whereas the rural population will hardly grow at all (Satterthwaite *et al.,* 2010). Rapid urban population growth because of continue migration results in increase in the demand of land, particularly for housing, water and energy (Iheke and Ihuoma, 2016). Iheke and Nto (2010) reported that urbanization is an important driving force in migration and community.

Urbanization has led to conversion of agriculture land into non agriculture purpose such as factories, buildings, residential or other commercial use (Malik and Ali, 2015). Ho and Lin (2004) found that the urban population growth was the cause of farmland conversion into coastal cities. Han and He (1999) found a significant positive relationship between urban population growth and farmland conversion in to cities. They also found that real estate speculation also result in agriculture land conversion. Land conversion (conversion of agriculture land into urban land) has negative impact on the agriculture land. Uncontrolled land conversion has greater impact on environment and agriculture yield. Loss in the prime agriculture land reduces the agriculture crop production, agriculture job employment (Malik and Ali, 2015). Land conversion increases the pressure on shrinking land to feed the increasing population which decline the health of land. These lands were put into uses which benefit the urban people neglecting agriculture use. Land conversion result in fragmentation of land, change in land supply and increment in land values (Iheke and Ihuoma, 2016).

Rapid population growth and migration also cause a serious threat to the environment which directly and indirectly affects the properties of soil and plants which cause reduction in yield. Expansion of urban area results in increase in the temperature of atmosphere with usage of modern equipment. Han *et al.* (2019) found that the station surrounded by large urban land experience rapid warming and high temperature. Much of the solar heat reached on the surface of earth is used in evaporation of water from vegetation and soil. In cities, where vegetation is less and exposed soil, most of the solar energy is absorbed by buildings and asphalts, result in increases the temperature of surface (Kolokotroni and Giridharan, 2008, Peng *et al.,* 2013).Vehicles, factories and industries also release more heat. This rising temperature results in depletion of soil moisture and reduction in reabsorption of CO_2emission (Sanders, 2004). Urbanization also results in eutrophication in water bodies. When rain occurs in urban areas, the rain filters the pollutant in the air onto the ground below. Then this running water enters into the rivers, ponds and lakes causing in decline in the quality of water and marine ecosystem (Jiang *et al.,* 2008).The ocean also absorbs small quantity of CO_2 released by human which help in maintaining the balance of CO_2in nature (Abas *et al.,* 2014). Eutrophication results in increase in the pH of the ocean water (Feely *et al.,* 2010) which inhibits the formation of calcium carbonate which is a crucial component for many marine organisms for the formation of shells and skeleton (Anderson *et al.,* 2015).

Urbanisation also results in the alteration in the physical, chemical and biological properties of soil (Liu *et al.,* 2016; Luo *et al.,* 2020). Cutting of trees, construction, mining activities changed the soil structure, soil porosity and contaminate the soil with harmful substances. Urbanization led to change in the hydrological cycle, reduced the infiltration rate of the soil and changes the stream flow (Marcotullio *et al.,* 2008). Heavy rainfall in the cities creates water logging condition because of high compaction and less infiltration rate of soil. Wang *et al.* (2018) also found high compaction and less infiltration rate of soil in urban area. Water logging condition also creates anaerobic condition which inhibits the growth of plant roots and soil microorganism. The soil of urban area easily prone to soil erosion poses serious threat to life and property (Yao *et al.,* 2015). Poor vegetation cover increase the impact of raindrop on soil result in disintegrates the soil aggregate. Because of less vegetation cover and heavy traffic burden on soil increase the surface crust formation which reduces the infiltration rate (Marcotullio *et al.,* 2008). Surface crust formation restricts the exchange of O_2 and CO_2between the soil and atmosphere. Rapid urbanization decreases the organic carbon status of soil. Less vegetation and microbial activities reduced the easily oxidising organic carbon status of the soil in urban areas. Luo *et al.* (2020) found that the 20 % loss in the easily oxidising organic carbon in the soil whish id surrounding to the cities. However, the total carbon content was found higher in the soil of urban area (Lorenz *et al.,* 2006; Asabere *et al.,* 2018; Liu *et al.,* 208). The decomposition process of urban litter was very slow because of repressed soil organism.

Urbanization affects both the crop and livestock sector by affecting the demand and supply of agriculture and livestock products. Because of extension in the urban area, the grazing area is shrinking. Land availability per livestock is decreased as farmer minimized the herd size to grow crops (Swain and Teufel, 2017). With expending urbanization, the demands of the concentrated feed also increased. Farmer feed higher amount of concentrated feed to the milch animal to get more milk which decreased the quality of milk. Tian *et al.,* 2017 found that long term feeding of high concentrated diet decreased the milk fats concentration. Decreased in the yield and milk fat percentage in the milk of dairy cows due to concentrated feed of

Alfalfa was observed by Khafipour *et al.,* (2009).Urbanization has significantly affected the cereal grain consumption in many countries. Urbanization leads to a significantly reduction in the demand of cereal food while the demand of non-cereal food was increased (Swain and Teufel, 2017). Huang and David (1993) also found that urbanization had a negative impact on the consumption of rice and coarse grain. Mottaleb and Mishra (2016) also reported that urbanization leads to a dramatic shift in the rice consumption pattern in the major rice producing countries of Asia and the world. Mottaleb *et al.,* (2018) also found that education, income and urbanization were the main driving forces behind the changing food and cereal consumption in Bangladesh.

VI. CONCLUSION

With the increase in the population, the demands for food and land were increased. To feed the increasing population, chemical fertilizers are used which decrease the fertility of the soil and crop productivity. This increasing demand of chemicals and processed food result in establishment of industries. These industries have both positive as well as negative impact. The positive impact is the generation of employment and processed products for the people. The negative impact is that they release poisonous gases and suspended particle in the environment and climate change. These gases result in global warming which increases the temperature of earth. Harmful gases and suspended particles deteriorate the quality of environment which affects the functioning of plant and animals. Poisonous gases and suspended particle also cause acid rain which decreases the pH of soil and water bodies. The heavy metals destroy the soil structure, fluctuates the pH of soil and affect the metabolic activities of plants and animal by forming complexes inside them. The discharge effluents of industries contain organic material, oil, grease and high concentration of heavy metals. These materials pollute the natural environment of water bodies which become lethal for aquatic species.

Increasing population and industrialization result in migration of people from rural to urban area. It is considered that industrialization is the root of urbanization. However, urbanization enhances the living and education standard of people, but it also pollutes the natural environment. With rapid urbanization, agriculture land is converted into non-agriculture land as land was covered under houses, industries, roads and buildings. Urbanization affects the properties of soil by decreasing its pore space, infiltration rate, easily oxidizing organic carbon, increasing surface compaction and bulk density. Vehicles, household emission, air conditioners, etc. release the green-house gases in environment which cause global warming. The polluted environment of urban area affects the crop and livestock production. Decreasing in the cereal consumption and the quality of milk was the harmful effect of urbanization and industrialization.

Industrialization and urbanization are necessary for the development of a nation. Both of them play an important role in increasing the GDP of the nation. Excess of both degraded the environment. Special measure and techniques needs to be developed to overcome this problem. The waste of industries and urban area should be dumped outside the city and away from the water bodies after the proper treatment. The area of land should be reserved for the agricultural and forests which maintain a healthy living environment on earth.

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Extraction and Formulation of Perfume from Lemongrass

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Abstract— *Perfume extraction is the extraction of aromatic compounds from raw materials, using methods such as distillation, solvent extraction etc. The extracts are essential oils, absolutes, butters, depending on the amount of waxes in the extracted product. Here, in this work solvent extraction, Enfleurage method, hydrodistillation and steam distillation methods were used to extract essential oil from lemongrass leaves. Distillation based recovery processes such as steam and vacuum distillation are preferred for the extraction of essential oils from plant materials. Other methods include solvent extraction, expression or enfleurage. In the present work, four methods are used for oil extraction namely solvent extraction, hydrodistillation and enfleurage. By using solvent extraction, 2.07% yield of essential oil was obtained. In enfleurage method, we obtained 1.957% oil yield. 0.946% yield of oil was obtained by hydro distillation process. The steam distillation process gave 0.70% yield of oil. From the analysis solvent extraction gave the highest yield because of the less exposure air and heat and this confirm the literature value. The extracted essential oil was formulated into perfume using a fixative and carrier solvent.*

Keywords— Perfumes, Lemongrass, Enfleurage method, hydrodistillation.

I. INTRODUCTION

The problem of perfume extraction process is the distortion of the odor of the aromatic compounds obtained from the raw materials. This is due to heat, harsh solvents and also through the exposure to oxygen which will denature the aromatic compounds. These will either change their odor, character or render them odorless. The problem of formulation of perfume involves knowing the proportion in which essential oil, and other materials to be mixed to avoid skin irritation and increase the intensity and longevity of the perfume. Most imported perfumes are synthetic odorant which are not pure chemical substance but are mixture of organic compounds that are harmful when applied. There are limited perfume plants, from which perfume can be made; this can lead to importation of perfume thereby causing the decline of foreign reserves and unemployment.

This project focuses on the production of perfumes from natural/plant sources as against synthetic chemicals thereby will reduce any side effect resulting from synthetic chemicals.

This project work is on how perfumes are extracted and formulated from lemongrass. It further entails; the synthetic and aromatic sources of perfumes. The composition of perfumes and its concentration. The extraction methods and formulation process involved .The economic importance of lemongrass and the uses of lemongrass oil in perfume production process.

II. METHODS OF EXTRACTION

Fragrance extraction refers to the extraction of aromatic compounds from raw materials, using methods such as distillation, solvent extraction, expression or enfleurage. To a certain extent, all of these techniques tend to distort the odour of the aromatic compounds obtained from the raw materials. Heat, chemical solvents, or exposure to oxygen in the extraction process denature the aromatic compounds, either changing their odour character or rendering them odourless

Before perfumes can be composed, the odorants used in various perfume compositions must first be obtained. Synthetic odorants are produced through organic synthesis and purified. Odorants from natural sources require the use of various methods to extract the aromatics from the raw materials. The results of the extraction are essential oils, absolutes, concretes, or butters, depending on the amount of waxes in the extracted produced.

2.1 Solvent Extraction

This is most used and economically important technique for extracting aromatics in the modern perfume industry. Raw materials are submerged in a solvent that can dissolve the desired aromatic compounds. Fragrant compounds form woody and fibrous plant materials are often obtained in this manner as are all aromatics from animal sources. The technique can also be used to extract odorants that are too volatile for distillation or easily denatured by heat. Commonly used solvents for maceration/solvent extraction include hexane, and dimethyl ether. The product of this process is called a "concrete".

2.2 Distillation

The process in which a liquid or vapour mixture of two or more substance is separated into its component fractions of desired purity, by the application and removal of heat. Distillation is a common technique for obtaining aromatic compounds from plants, such as orange blossoms and roses. The raw material is heated and the fragrant compounds are re-collected through condensation of the distilled vapour. There are two types of Distillation for extracting. Steam Distillation and Hydro-Distillation.

2.3 Steam Distillation

Steam from boiling water is passed through the raw material for 60-105 minutes, which drives out most of their volatile fragrant compounds. The condensate from distillation, which contains both water and the aromatics, is 42 settled in a Florentine flask. This allows for the easy separation of the fragrant oils from the water as the oil will float to the top of the distillate where it is removed, leaving behind the watery distillate. The water collected from the condensate, which retains some of the fragrant compounds and oils from the raw material, is called hydrosol and is sometimes sold for consumer and commercial use. This method is most commonly used for fresh plant materials such as flowers, leaves, and stems.

2.4 Hydro-Distillation

Mostly used by small scale producers of essential oils in water / hydro distillation the plant material is almost entirely covered with water as suspension in the still which is placed on a furnace. Water is made to boil and essential oil is carried over to the condenser along with the steam. It is useful for distillation of powders of spices and comminuted herbs etc. The Deg Bhabka method of India using copper stills is an example of this technique. Some process becomes obsolete to carry out extraction process like Hydro Distillation which often used in primitive countries. The risk is that the still can run dry, or be overheated, burning the aromatics and resulting in an Essential Oil with a burnt smell. Hydro distillation seems to work best for powders.

2.5 Enfluerage

This is the absorption of aroma materials into solid fat or wax and extracting the odorous oil with ethyl alcohol. Extraction by enfleurage was commonly used when distillation was not possible because some fragrant compounds denature through high heat. This technique is not commonly used in the present day industry due to its prohibitive cost and the existence of more efficient and effective extraction methods. Enfleurage is a two-step process during which the odour of aromatic materials is absorbed into wax or fat, and then extracted with alcohol.

III. FORMULATION OF OIL TO PERFUME

Formulation is a mixture of ingredients prepared in a certain way and used for a specific purpose**.** 10ml of lemongrass essential oil extract were measured and placed in a 120ml beaker containing 5ml of Methanol. 5ml of the Fixatives were added to the mixture (to improve the longevity of the perfume). The solution were shaken and poured into a 50ml bottle.

IV. PROCEDURES

4.1 Solvent Extraction

1. Weigh 150g of the dry sample of lemongrass from the sliced lemongrass sample and placed in 1 litre flat bottom flask.
2. Take 500ml of N-Hexane solvent & pour into the flask.
3. The flask and content are allowed to stand for 36 hrs; this is done to extract all the oil content in the lemongrass and for complete extraction.
4. After this decant the extract into another 1 litre beaker add 200ml of Ethanol to extract the essential oil since essential oil is soluble in Ethanol
5. The mixture is then transferred to 500ml separating funnel and separate by a process called liquid/liquid separation process. The content of the separating funnel are allowed to come to equilibrium, which separates into two layers (depending on their different density)
6. The lower Ethanol extract and the upper Hexane layer are collected into two separate 250ml beaker and are placed in a water bath at 78^{o}C. This is done to remove the Ethanol leaving only the natural essential oil. The yield of oil is

determined by weighing the extract on an electronic weighing balance. The difference between the final weight of the beaker with extract and the initial weight of the empty beaker gave the weight of essential oil.

FIGURE 1: Solvent Extraction

4.2 Steam Distillation

1. Place 150 grams of fresh lemongrass sample into a 1 litre round bottom flask containing 250ml of distilled water.
2. The flask was fitted with a rubber stopper connected to condenser and heated. Water at 0oC flowed counter currently through the condenser to condense the ensuring steam.
3. When the water reached 100oC it started boiling ripping off the essential oil from the lemongrass.
4. When the lemongrass got heated up, the essential oil that was extracted from the leaf mixed with the water vapor. Both passed through the condenser and the vapor was condensed into liquid. With the use of ice block, cooling was made possible and volatilization of the essential oil was avoided.
5. The condensate was directly collected using a 500ml beaker and then poured into a separating funnel. This formed two layers of oil and water.
6. The tap of the separating funnel was opened to let out the water while the oil was immediately collected into a 100ml stoppered bottle. The bottle was closed tightly to prevent vaporization of the essential oil. The oil was collected and the volume of oil obtained was weighed.

FIGURE 2: Steam Distillation

4.3 Enfleurage

1. 140 gram of the dry sample of lemongrass was pounded with mortar and pestle to reveal the tighter inner stem and to increase the absorption area.
2. 70ml of light-flavored olive oil were warmed and mixed with the mashed lemongrass (to allow for efficient absorption of the essential oil).
3. The aluminum foils were used to cover beaker. Then it was shaken for distribution of the lemongrass.

4. It was then allowed to stand for 24 hours at room temperature.
5. 140 ml ethanol was added to absorb the essential oil leaving behind the light-flavoured olive oil and the lemongrass residue.
6. The yield of oil was determined.

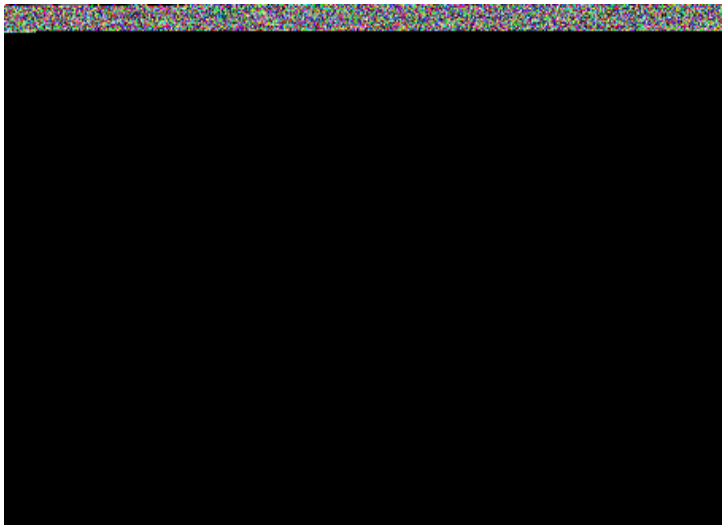

FIGURE 3: Enfleurage

4.4 Hydro-Distillation

1. 500 ml of distilled water and 140 gram of fresh lemongrass sample were placed into a round bottom flask.
2. The flask was fitted with a rubber stopper and connected to a condenser and heated. Water was allowed to flow counter currently through the condenser.
3. After reaching appropriate temperature, the essential oil, mixed with the water vapor was extracted from the leaves.
4. The oil-water overhead product was passed through the condenser. The vapours were condensed.
5. Volatilization was avoided by cooling with ice cubes. The condensate was collected using a beaker.
6. The condensate was then separated by a separating funnel. The oil was immediately collected into a stoppered bottle and closed tightly.

FIGURE 3: Hydro-Distillation

V. RESULT

The result obtained from the study is shown in the table 1 below

TABLE 1
EXTRACTION AND FORMULATION OF PERFUME

Methods	Yield %
Solvent Extraction	2.07
Steam Distillation	0.70
Enfluerage	1.957
Hydro-Distillation	0.957

VI. FUTURE SCOPE

This project work is on how perfumes are extracted and formulated from lemongrass rather than using synthetic chemicals thereby reduce the side effects.

The success of this work will stimulate the development of the perfume industry locally because of available, cheap raw materials.

More people will get engaged in cultivation of Lemongrass and more jobs will be created in Perfume Industry and help in development of local small scale industries in India.

VII. CONCLUSION

The experiment was carried out for the extraction of essential oil from lemongrass. Analyses carried out were to determine the various oil yields using different extraction methods and the formulation of perfume with the essential oil produced. The result from the experiment yielded more with solvent extraction, followed by the effleurage method and the hydro distillation method. The extraction of essential oils by distillation is governed by the sensitivity of the essential oil to the action of heat, water and alcohol. All these methods of extraction are special type of separation process used for heat sensitive materials like essential oils, resins, hydrocarbons, etc. which are insoluble in water and may decompose at their boiling point. The temperature of the steam must be high enough to vaporize the essential oil present, yet not destroy or burns the essential oils. In summary these methods used chemical engineering unit operations of leaching, liquid-liquid extraction and evaporation techniques.

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One Case of Internal Fixation Treatment for Tibia and Fibula Fracture of Dogs

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***Abstract*—** *Tibia and fibula fracture is a common fracture of hind limb in dogs. It is caused by external force on the hind limb. After fracture happens, external fixation is often used for treatment, and the effect is ideal. However, if the fracture site is close to the joint, we suggest to use internal fixation as far as possible, so as not to cause sequelae of joint stiffness. The author diagnosed the fracture of tibia and fibula in the left hind limb of the poodle through combining the incidence, clinical manifestations and DR examination, and then used the internal fixation plate for internal fixation. Under postoperative care, the dog recovered well.*

Keywords*— *fracture, tibia and fibula, internal fixation, internal fixation plate.

I. INTRODUCTION

Fracture refers to the bone state in which the integrity or continuity of bone tissue is destroyed by external force or pathological factors. Fracture is accompanied with different degrees of injury of soft tissue around the bone [1]. Most fractures occur in limb bones, which are caused by external injuries, that is, direct external violence, such as vehicle collision, trampling, rolling, falling, hammering and squashing. Fractures may also be caused by strong contraction of muscles such as running, twisting and sudden stop. For tibia and fibula fracture, we usually use external fixation in clinic, but if the fracture site is close to the joint, it is easy to cause dead joint, and in this case, we mostly use internal fixation. The author diagnosed and treated a case of tibia and fibula fracture caused by traffic accident. After using the internal fixation plate to carry out internal fixation, the effect was good.

II. INCIDENCE

Poodle, 9 months old, male, 3.4 kg. Hit by the battery car, after which its left hind limb could not touch the ground. There was obvious hematoma in some part of the distal end of tibia and fibula, and dislocated movement appeared. When the swelling part moves up and down, bone fricative sound can be heard. When the dog walking, it showed supporting limb lameness. Once the dog the left hind limb was touched, the dog barked in pain.

III. CLINICAL DIAGNOSIS

After the preliminary clinical diagnosis, the dog was diagnosed with fracture. In order to further clarify the location and condition of the fracture, we examined it by X-ray. Through the blood routine index examination and the blood biochemical index examination, we judged whether we could perform an operation for the dog normally.

3.1 Blood routine index examination

The number of white blood cells increased, indicating that there is inflammation in the body of the dog, so we need to carry out infusion for the dog for anti-inflammatory. Mean corpuscular hemoglobin concentration decreased, indicating that there was a mild anemia. The test results of other items were in the normal range. There was no effect on the operation.

3.2 Blood biochemical index examination

In the biochemical examination items, except for glucose (GLU) index, which decreased, the results of all the other items were normal. GLU decrease is often due to malnutrition, dyspepsia and chronic anemia, which has no effect on the operation.

3.3 Digital Radiography (DR) examination

DR examination: it can be seen from Figure 1 and Figure 2 that the tibia and fibula of the left hind limb is fractured. Because the fracture site is close to the joint, the operation is difficult and it was preliminarily assessed that the operation risk was high. After discussing with the owner of the dog, we finally decided the operation plan. We decided to let the dog be hospitalized for observation for 3 days, and we would first carry out routine infusion to relieve inflammation and pain. Three

days later, we performed internal fixation with internal fixation plate to treat the tibia of the dog. The fibula does not need to be treated.

FIGURE 1: Preoperative lateral position

FIGURE 2: Preoperative anteroposterior

IV. OPERATIVE TREATMENT

4.1 Preoperative preparation

After the examination was completed, an indwelling needle was given into the dog first to facilitate the fluid infusion for the dog during the 3-day observation before the operation. It was used for anti-inflammatory, pain relief (hypodermic injection of 0.4ml tilidine), hemostasis (0.6ml etamsylate) and infusion.

Antiphlogistic: 30ml 0.9% NaCl injection, 2ml ceftiofur, 30ml Metronidazole and sodium chloride injection.

Nutrient solution: 30ml 5% glucose injection, respectively 0.6ml (0.2ml/kg) of ATP-COA (adenosine triphosphate, coenzyme A for injection) and Vc, 0.6ml (0.2ml/kg) of ethylphenesulfonate injection.

Routine surgical instruments disinfection: Before the operation, the dogs were forbidden to eat for 12 hours and drink water for more than 3 hours.

4.2 Anesthesia

Ten minutes before the operation, the dogs were subcutaneously injected with 0.45 ml sedative drugs (0.1 ml/kg atropine sulfate and 0.05 ml/kg scepromazine) and 0.6 ml ethamsylate (0.2 ml/kg), 10 minutes later, inject 15 mg propofol slowly, which was used for induction anesthesia then we conducted endotracheal intubation and connected it to the ventilator and anesthesia machine, and then isoflurane was used to maintain the anesthesia during the operation; and then we injected 2 ml 2% lidocaine hydrochloride into different points around the operation site for local anesthesia. After connecting the ventilator and anesthesia machine, let the dog lie on the right side and fix it. Control the amount of oxygen and anesthesia, if the dog has signs of recovery during the operation, we should increase the amount of anesthesia as appropriate.

4.3 Treatment of the operative site

After anesthesia, we shaved the operative site and the whole left hind limb and cleaned it up and then used clean gauze to wipe dry the affected limb. Then we used 75% alcohol cotton to disinfect the operative site in a spiral way (from inside to outside) and then used iodophor cotton to disinfect the left hind limb for 2-5 minutes. Before operation, we used 75% alcohol for deiodination and disinfection.

4.4 Operation process

4.4.1 Before operation

The operator should first wear sterile gloves, and then wear sterile surgical gown, masks and hats. Then open the instrument set. After disinfecting the affected limb wholly, use iodophor cotton to pinch its toe, and then the operator takes out the sterilized self-adhesive bandage from the instrument set, and uses the self-adhesive bandage to wrap the distal end of the hind limb, and then lay the operation towel well.

4.4.2 During operation

First, make a skin incision parallel to the tibia on the anteromedial side of the tibia, separate the subcutaneous connective tissue and separate the fascia on the surface of the bone (Fig. 3). In the process of operation, the operator should pay attention to avoid the saphenous nerve and blood vessels in the middle and lower parts. If there is bleeding, use hemostatic forceps to clamp the blood vessels in the bleeding site to stop bleeding. The operator passively separates the muscle to expose the broken ends of fractured bone (Fig. 4).

Secondly, on the oblique side of the operation towel, by pinching and pressing the salt water bottle, use sterile normal saline (a needle was inserted into the saline bottle) to repeatedly wash the surgical incision site. At the same time, check whether there are small broken bone pieces and blood clots in the affected part. If there are, clean them, and then integrate and fix the broken ends of fractured bone (Fig. 5).

Then, determine the internal fixation plate to be used. Because the fracture site is relatively special and close to the joint, we chose the 7-holes T-shaped bone plate without holes in the middle for internal fixation. Use the electric bone drill to drill the tibia at the corresponding position of the internal fixation plate holes with the help of a drill guide (Fig. 6). On the proximal side of the tibia, the drill bit was led to the proximal tibia at an angle of 15 degrees to the horizontal direction; on the distal tibia side, the drill bit was about 30 degrees to the plane of the steel nail on the proximal side, and the drill bit was led to the distal tibia at an angle of 15 degrees to the horizontal direction. Screws were led from both ends of the internal fixation plate to the middle. After the screws were led, we examined the fit degree between the plate and the tibia and the broken ends.

Finally, use absorbable surgical sutures to intermittently suture the periosteum and the muscle tissue that was previously bluntly dissected. Suture the skin with sterilized silk thread, smooth the incision (Fig. 7) and drop anti-inflammatory drugs, and then spray a layer of aluminum on the suture and around the suture .The operation was complete.

4.4.3 After operation

After the operation, we can take two more DR images to check the operation results (Fig. 8 and Fig. 9), and analyze and evaluate the prognosis of operation.

FIGURE 3: After being bluntly dissected

FIGURE 4: The broken ends of fractured bone

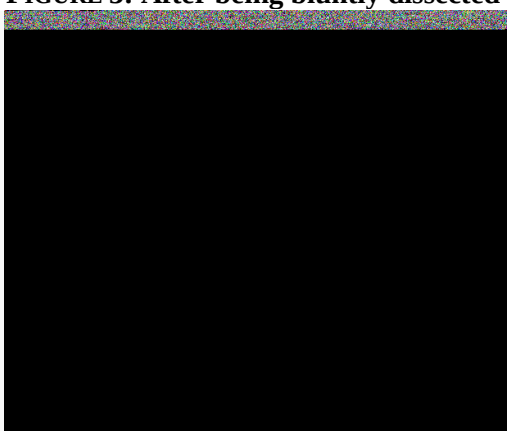

FIGURE 5: Integrate and fix the broken ends

FIGURE 6: Tibia perforation

FIGURE 7: Suture the incision smoothed

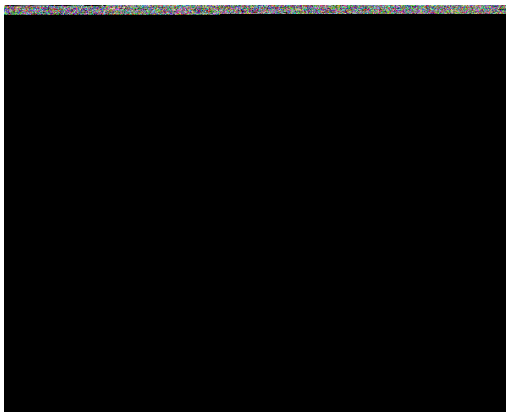

FIGURE 8: Anteroposterior side after operation

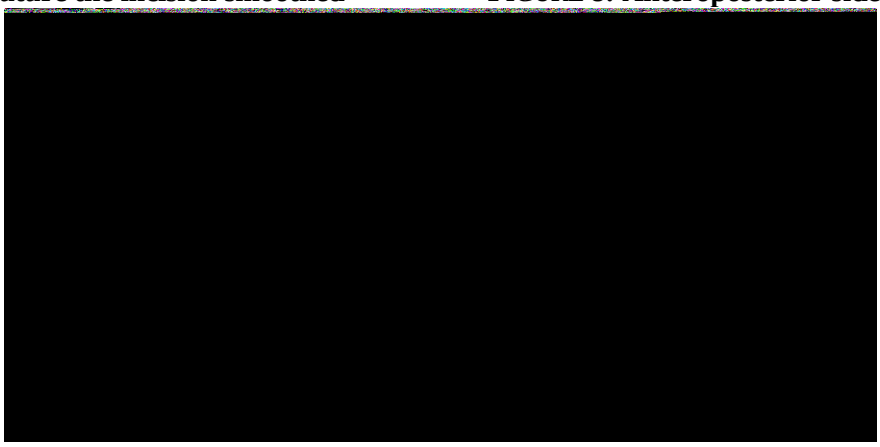

FIGURE 9: Lateral side after operation

V. POSTOPERATIVE CARE

Let the dog wear Elizabethan ring, so as to prevent the dog from licking the surgical suture wound, resulting in infection of the surgical wound. Keep the dog in cages to limit its movement. It was hospitalized for a total of seven days. On the seventh day, we took out the suture and let it leave hospital. In two weeks after that, limit its exercise. For one month, the dog was prohibited from strenuous exercise. Feed high calcium food and nutritious food to help physical recovery and bone healing.

5.1 Daily care

Every day, use iodophor to disinfect the wound; clean the hair and scab on the wound to keep the wound dry and clean; observe whether there is infection and pus on dog's wound; drip anti-inflammatory drugs on the wound to observe the dog's eating, drinking, defecation, urination and limb movement. On the second day after the operation, the dog ate a small amount of food, but did not drink water. After a few days, the appetite gradually recovered, the water drinking became normal, and the defecation and urination returned to normal. On the third day after the operation, the left hind limb of the dog could walk gently; on the fifth day after the operation, the left hind limb of the dog, as a force supporting point, could bear the load; on the seventh day after the operation, the wound had no infection and healed well, so we took out the suture. After the suture was removed, apply erythromycin ointment to the sutureing holes. On 9th day, remove the Elizabethan ring.

5.2 Daily anti-inflammatory

Every day, give the dog infusion for anti-inflammatory, to promote bone growth and healing and pain relief. The infusion drugs were as follows: 50ml 0.9% NaCl injection, 2ml ceftiofur; 30ml metronidazole sodium chloride injection; 30ml 0.9% NaCl injection and 2ml ossotide injection.

VI. SUMMARY AND DISCUSSION

6.1 Reasons for choosing internal fixation

For this case, the stability of the fixation provided by plaster and splint were insufficient. As for external fixation, it is usually used in dogs without dislocation on the fractured ends. Fixation of plaster and splint is only suitable for some tibia and fibula fractures, and strict nursing care is needed, which may lead to poor alignment or that the broken bones are not nonunion.

6.2 The choice of operation time

Because of the body condition, bleeding and edema of the dog with bone fracture, it is very important to choose the right time of operation for internal fixation. Relevant medical data show that it is more appropriate to carry out operation one week after fracture. In this way, the weak dog is easy to supplement nutrition and the swelling of fracture end may disappear. However, in clinical, it is more often to carry out operation immediately after the fracture, which is conducive to the recovery of the fracture end. If the operation is carried out after 3 days, the main purpose is to eliminate the swelling of the affected part and reduce the bleeding and the interference of bleeding on the operation. However, according to the author's experience, if the operation is carried out after more than 4 days, there will be a small amount of callus formation or tissue hyperplasia at the fracture end in most cases, affecting the operation.

6.3 Anesthesia and aseptic operation

Good anesthesia is a necessary condition for the operation to be carried out smoothly. The sensitivity of dogs to anesthetics is different. If the anesthesia is not enough, it will directly affect the operation process. If the anesthesia is excessive, it will easily lead to the death of dogs. So anesthesia must be appropriate, so that the operation can be carried out smoothly. In addition, aseptic operation is very important, because aseptic operation can prevent the occurrence of fracture healing difficulty and healing delay. If the aseptic operation is improper, it may lead to osteomyelitis and other serious sequelae.

6.4 Reduce the surgical injury

In this operation, the medial incision method is used, and there are less tissues to be cut and it can easily expose the fracture ends. When opening the affected area, we should try our best to use blunt dissection to reduce muscle injury; protect the lateral saphenous vein, and according to the need of exposure the operative field, we should as far as possible avoid it or transecte ir after ligation, so as to protect the peripheral nerve from injury.

6.5 Postoperative care

After the internal fixation is completed, we can take appropriate external fixation to enhance its stability. After the operation, apply antibiotics to the whole body of the dog to prevent and control the infection; strengthen the feeding management and nutrition, and supplement vitamin A, vitamin D and calcium preparations; limit its activity for 2 weeks. After operation, X-ray film should be taken regularly to check the recovery. The dog was in its infancy, so the bone healing and growth were faster. Removed the internal fixation steel plate two months after the operation.

ACKNOWLEDGMENT

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Rubber Tree Cultivation and Improvement: Rootstock-Scion Compatibility between *Hevea* Species and Cultivated Planting Materials

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Abstract— *Rootstocks have a clear effect on rubber tree growth and development during the seedling and immature stages. However, the exploration of Hevea species as rootstocks is relatively uncommon in the general practices in the cultivation and improvement programmes in Malaysia. Hevea species were tested in this research including Hevea brasiliensis, Hevea benthamiana, Hevea camargoana, Hevea guianensis, Hevea nitida, Hevea pauciflora, Hevea rigidifolia and Hevea spruceana. This research examined the successful bud-grafted percentage between scion and rootstock of different Hevea species and cultivated planting materials. The results demonstrated that rootstock-scion of H. benthamiana-PB 260 achieved the highest successful bud-grafted percentage at 94.5%, followed by H. nitida-RRIM 2001 (93.8%), H. nitida-PB 350 (92.3%) and H. pauciflora-PB 260 (90.8%). The lowest successful bud-grafted percentage came from H. benthamiana-RRIM 2025 at 51.1 %. Therefore, the exploration of Hevea species as potential rootstocks based on the successful bud-grafted percentage between rootstock-scion and their compatibility could be applied as a speed indicator for rubber nurseries to produce high quality planting materials.*

Keywords— bud-grafted, ***Hevea species, rootstock-scion.***

I. INTRODUCTION

Among the famous events in the history of natural rubber is the massive amount of natural rubber produced by rubber plantations to satisfy the high demand during The Industrial Revolution. Natural rubber is a chain of polymers that exhibits high resilience, impact resistance, elasticity, stretchy strength, as well as low heat swelling during manufacturing processes. This is attributable to the unique molecular structure of the rubber, which is difficult to be matched by synthetic rubber derived from the petroleum sources. The use of natural rubber can be seen in various domestic and industrial products nowadays. This started in 1876 when Henry Wickham collected about 70,000 rubber seeds (*H. brasiliensis*) near the Tapajos River in Brazil, and attempted to sow them in the United Kingdom. This small population of sowed and germinated seedlings were later transported to Ceylon (Sri Lanka) and Singapore in 1876. Eventually, 22 seedlings survived during the transportation journey and arrived in Kuala Kangsar, Malaya (Malaysia) in 1877, since then these seedlings have formed the genetic base of rubber trees in the country (Baulkwill 1989; Barlow 1978; Loadman 2005, MRB 2005; Priyadashan, 2011). Prior to the 1950s, seedlings that germinated from rubber seeds were widely accepted as cultivated planting material, either by rubber plantations or by smallholdings. For example, the seeds obtained from established experimental gardens at Rubber Research Institute of Malaysia (RRIM) have been accepted as being good in quality and recommended for extensive planting (Heuser 1932; RRIM 1957: Ng, 1983; Ong and Shamsul, 2013). In general, seedling trees would generate variable yield and unpredicted characteristics such as growth rate, canopy density, branching habit, bark thickness, wind damage tolerance, disease tolerance *etc*., whereas cultivated planting materials recurrently showed uniformity on the mentioned characteristics.

II. BUD-GRAFTING TECHNIQUE IN RUBBER TREES

Bud-grafting technique was gradually tested and recognized as a useful way to multiply planting materials for large quantities during 1950s in Malaysia, which involved cultivated planting materials or clones that produced high latex yields for cultivated planting such as Tjirandji 1, RRIM 501, RRIM 526, AVROS 157 and PR 107 (RRIM, 1952; Webster and Baulkwill, 1989). Subsequently, planting materials derived and multiplied from the bud-grafting technique rapidly replaced the unselected seedlings from the experimental gardens and rubber nurseries, where the majority of the rubber tree improvement programmes at that period were initiated and established at RRIM (Burkill, 1959; RRIM, 1952; RRIM, 1963,

RRIM, 1965; Ng, 1983; RRIM 1995; MRB 2009; Ong and Shamsul, 2013). Later, RRIM 500 Clone Series, RRIM 600 Clone Series, RRIM 700 and other cultivated planting materials were introduced and multiplied extensively through bud-grafting technique. Eventually, the cultivated planting materials and planted expansively in the country were mostly originated from the improvement programmes and experiments including RRIM 600, RRIM623, RRIM 703, RRIM 901, RRIM 2001, RRIM 2002, RRIM 2025, PB5/51, PB 260, PB 235, PB 314, PB 350, PB 355 (Ramli *et al*., 1996; MRB, 2009; Shamsul and Ong, 2014). Recently, MRB Planting Recommendations emphasized the selection of Latex Timber Clones (LTC) as good planting materials that would generate high latex yield and rubberwood through their breeding programmes (Table 1). Latex yield remains as the most important characteristic, and rubberwood yield is also taken as one of the good characteristics in order to produce Latex Timber Clones for commercial planting (RRIM, 1995; MRB, 2009; Ratnasingam *et.al*., 2011). However, the successful bud-grafted percentages of different rootstock-scion were varied and unpredictable even among the rubber planting materials within the same clone series. This is regularly due to the compatibility issues between rootstocks and scions, through the observations made at rubber nurseries over the years. A rootstock and a scion in a polybag that were prepared for bud-grating procedure in the rubber nursery as showed in Figure 1.

TABLE 1
CULTIVATED PLANTING MATERIALS DEVELOPED FROM BUD-GRAFTING TECHNIQUE AND PLANTED EXTENSIVELY OVER THE YEARS IN MALAYSIA.

Planting material	Parents	Country of Origin
RRIM 600	Tjir 1 x PB 86	Malaysia
RRIM 623	PB 49 x Pil B 84	Malaysia
RRIM 703	RRIM 600 x RRIM 500	Malaysia
RRIM 901	PB 5/51 x RRIM 600	Malaysia
RRIM 2001	RRIM 600 x PB 260	Malaysia
RRIM 2025	IAN 873 x RRIM 803	Malaysia
PB 5/51	PB 56 x PB 24	Malaysia
PB 260	PB 5/51 x PB 49	Malaysia
PB 235	PB 5/51 x PB S/78	Malaysia
PB 314	RRIM 600 x PB 235	Malaysia
PB 350	RRIM 600 x PB 235	Malaysia
PB 355	PB 235 x PR 107	Malaysia

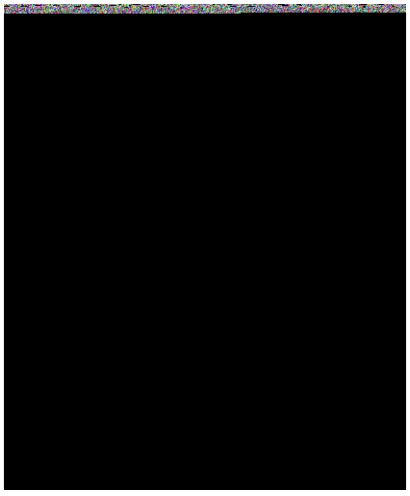

FIGURE 1: A rootstock and a scion in polybag that were prepared for bud-grafting.

III. MATERIALS AND METHODS

3.1 Rootstock-scion Compatibility between *Hevea* Species and Cultivated Planting Materials

The bud-grafting technique in rubber trees is a method used to asexually multiply rubber planting materials by grafting vegetative buds onto a rootstock in a polybag at a young age (Leong and Yoon, 1979; Jeffree and Yeoman, 1983; Ng, 1983; Webster and Baulkwill, 1989; Mercykutty and Gireesh, 2015). The bud-grafting technique was carried out in this research, where the standard methods were followed and adopted, as applied in a local rubber nursery. Seeds of rootstocks from rubber species were gathered and sowed in the seedbeds at a small-scale rubber nursery. When these rootstocks reached an age of five to six months, they were prepared to receive scions at their basal portion. Scions were prepared in the nursery from budwood that was still green in colour and not older than eight weeks. In this procedure, the basal portion of each rootstock was cleaned, and vertical budding panels of about 5 cm long and 1 cm wide were created with a knife. In a separate operation, a bud-patch carrying a vegetative bud of the scion was stripped off from the budwood collected earlier, and trimmed to a size matching that of the budding panel on the rootstock. The bud-patch was inserted into the budding panels and the region grafting was securely bound with transparent polythene tape. The budding panel was examined after 21 days through the transparent bandages, which can be removed for clearer observation if required. The retention of the green colour of the scion on the budding panel is a sign that bud-grafting procedure was successful, and the shoot portion of the original rootstock can then be cut off. This would allow a new shoot to emerge from the budding panel. The shoot was allowed to develop until the emergence of two whorls of leaves. Each *Hevea* species was tested as a rootstock and paired with different scions of selected cultivated rubber planting materials such as PB260, PB350, RRIM 2001 and RRIM 2025. These scions were collected from only fresh and healthy budwoods, to ensure their viability after the bud-grafting practice. Furthermore, they were derived from the recommended cultivated planting materials with proven records of high latex yields from RRIM (RRIM, 1995; MRB, 2005). The compatibility between rootstock-scion could be observed and calculated through the percentage of successful bud-grafted. Nevertheless, the number of bud-grafted seedlings in different rootstock-scion combinations tested was not in equal numbers because of the varied seed germination rates between the cultivated planting materials, and also because of losses from pest attacks during the sowing stages when this research was carried out.

IV. RESULTS

The observation on the successful bud-grafted percentage of different rootstock-scion combinations is presented in Table 2. Cells in the bud-grafted panel would form a cambial bridge of new vascular tissues that connected to the old cambium and vascular tissues on the rootstock and scion. Typically, cell division of parenchyma cells occurred within days after bud-grafted, and new callus tissue would continue to develop between rootstocks and scions for up to 21 days. If there was no sign of yellowish colour on the scions, an indication of rootstock-scion in high state of compatibility, that they survived through the bud-grafting procedure. The results demonstrated that rootstock-scion of *H. benthamiana*-PB 260 achieved the highest successful bud-grafted percentage at 94.5%, followed by *H. nitida*-PB 2001 (93.8%), *H. nitida*-PB 350 (92.3%) and *H. pauciflora*-PB 260 (90.8%). Meanwhile, *H. rigidifolia* showed low compatibility (<80%) relatively three of the cultivated planting materials *i.e.*, PB 350, RRIM 2001 and RRIM 2025. Nevertheless, the lowest successful bud-grafted percentage was found between *H. benthamiana*-RRIM 2025 at 51.1%. Therefore, this research revealed that *H. benthamiana*, *H. nitida* and *H. pauciflora* worth to be explored as the potential rootstocks, since they were highly compatible with different scions of cultivated planting materials. Interestingly, RRIM 2025 was one of the high latex-yielding cultivated planting materials in the country, but it was seen as mediocre with low successful bud-grafted percentage (<80%) when its scions were bud-grafted with different rootstocks of *Hevea* species.

TABLE 2
SUCCESSFUL BUD-GRAFTED PERCENTAGE BETWEEN DIFFERENT ROOTSTOCKS (*HEVEA* SPECIES) AND SCIONS (CULTIVATED PLANTING MATERIALS).

Rootstock	Scion	Total number of bud-grafted (n)	Total number of successful bud-grafted (a)	Successful bud-grafted after day-21 (%)
H. benthamiana	PB 260	91	86	94.5
	PB 350	46	37	80.4
	RRIM 2001	74	58	78.4
	RRIM 2025	45	23	51.1
H. brasiliensis	PB 260	121	107	88.4
	PB 350	163	143	87.7
	RRIM 2001	86	76	88.4
	RRIM 2025	96	53	55.2
H. camargoana	PB 260	173	155	89.6
	PB 350	126	113	89.7
	RRIM 2001	144	128	88.9
	RRIM 2025	140	102	72.9
H. guianensis	PB 260	35	25	71.4
	PB 350	47	42	89.4
	RRIM 2001	45	39	86.7
	RRIM 2025	39	24	61.5
H. nitida	PB 260	67	58	86.6
	PB 350	52	48	92.3
	RRIM 2001	48	45	93.8
	RRIM 2025	75	56	74.7
H. pauciflora	PB 260	65	59	90.8
	PB 350	46	34	73.9
	RRIM 2001	63	55	87.3
	RRIM 2025	72	57	79.2
H. rigidifolia	PB 260	58	48	82.8
	PB 350	66	52	78.8
	RRIM 2001	54	40	74.1
	RRIM 2025	53	37	69.8
H. spruceana	PB 260	185	146	78.9
	PB 350	193	168	87.1
	RRIM 2001	149	127	85.2
	RRIM 2025	162	139	85.8

V. DISCUSSION

The successful bud-grafted rootstocks and scions in this research were detailed by: (1) sturdy cohesion between the rootstock and scion at the grafting panel, (2) continuous multiplication of callus cells at the grafting panel, (3) the development of new vascular cambium tissues at the grafting panel. Besides, the practice of bud-grafting technique can be carried out year-round, and rubber planting materials can be produced in large quantities under a controlled environment, and uniformity in growth when raised in the polybags. One major problem of rubber nurseries in Malaysia is the quality and authenticity of the rootstocks. The quality of rootstocks normally receives little attention, as long as there are seeds available for germination and used as rootstocks at most of the rubber nurseries in Malaysia. Even though *H. benthamiana*, *H. nitida*, and *H. pauciflora* worth to be explored as the potential rootstocks, other *Hevea* species that were not highlighted in this research, might have other uses that yet unexplored. There is limited literature describing latex yield, wood production, ornamental use, photosynthesis efficiency, water-use or even ability of drought tolerance of these species. On the other hand, cultivated

planting materials that have proven records of high latex yields might not necessary compatible with rootstocks of *Hevea* species, and extra efforts are needed to find specific rootstocks, in order to produce high quality seedlings and increase the production at rubber nurseries.

VI. CONCLUSION

The combinations rootstock-scion of *H. benthamiana*-PB 260, *H. nitida*-PB 2001, *H. nitida*-PB 350 and *H. pauciflora*-PB 260 showed high successful bud-grafted percentage that should be focused by rubber plant breeders because these neglected *Hevea* species have the potential to contribute to a higher level than they currently do. From a crop improvement perspective, the genetic potential of *Hevea* specie is massively determined by the combination of genes that they have contained. Quantitative Trait Loci (QTLs) analysis and Marker-Assisted Selection (MAS) were introduced in rubber improvement programmes in many decades ago, in attempts to verify the purity of rubber planting materials, selection of parentage, population diversity analysis, and increase desired traits for commercial purposes. However, there exist no successful MAS to detect genes make-up for rootstock-scion compatibility in rubber trees. This is because of the low power of QTL detection, whereas complex traits such as callus cells multiplication and vascular cambium tissue development that alleged to associate with a series of QTLs. In short, the successful bud-grafted percentage between rootstock-scion, regardless of *Hevea* species or commercial planting materials, could be applied as a speed indicator for rubber nurseries to produce high quality planting materials.

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Criteria for the Selection of Vegetable Growth-Promoting Bacteria to be applied on Roselle Crop (*Hibiscus Sabdariffa* L.) and Bioremediation

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Abstract**—***In order to define which are the most important criteria for the selection of plant Growth-Promoting bacterial strains of the Hibiscus sabdariffa L. crop (Roselle), bacterial strains isolated from the roots of Roselle plants of two varieties (Creole and Spider) were used, collected in the community of Río de los Peces, municipality of Candelaria Loxicha, Oaxaca and seeds of the same varieties. To characterize the varieties, the following were determined: total germination percentage (TGP), germination speed (GS), the root length(RL), the stem length (SL), the dry root biomass (DRB), the dry stem biomass (DSB) and the chlorophyll content (CC). Three types of LED lamps were used to illuminate the seedlings. The seeds inoculated with cells of six selected bacterial strains were grown in a greenhouse to determine: the stem length (SL) at 3, 45 and 65 days after sowing (das). The treatments were distributed under a completely random design and comparison of means (Tukey, p = 0.05). The TGP, DSB and DRB parameters were not useful in the selection process of the strains that promoted plant growth to a greater degree. The GS and SL to be considered safe criteria or not, what is important is the relationship of what happens at the time of germination and development of the seedlings in the laboratory and greenhouse. The SL of the plants in the greenhouse showed differences between strains, but not regarding the control and also only observed in the first days of development (3 das). The CC did not prove to be a good selection criterion either. The lamp composed of 15% white light, 27% blue light and 58% red light was the one that most promoted root growth.*

Keywords—Plant Growth-Promoting bacteria strains, Hibiscus sabdariffa L, Bacterial strains selection criteria, LED lamps.

I. INTRODUCTION

The study of microorganisms that promote plant growth has gained importance worldwide because of the multiple advantages they represent (Ortiz-Texon et al., 2016). Currently, research is focused on the evaluation of various rhizosphere microorganisms, selecting those most efficient in inoculation experiments under controlled environmental conditions in the laboratory, greenhouse and in the field. This seeks to increase yield and reduce the amount of agrochemicals used (PazosRojas et al., 2016).

Plant Growth-Promoting Rhizobacteria (PGPR) are bacteria influenced by exudates from plant roots that can improve plant growth in the short term (Molina-Romero et al., 2017), through the production of plant Growth-Promoting substances, which are synthesized in different structures of the plant. These molecules exhibit effects on plant physiology, such as increasing root volume and root respiration rate, resulting in the absorption of soluble mineral elements (Molina-Romero et al., 2015). The beneficial bacteria applied to agricultural crops allow the phytostimulation and bioremediation of toxic compounds associated with plants; having a positive impact on human health and the environment (Sing and Trivedi, 2016; Pazos-Rojas et al., 2016). These can interact effectively with plants in contaminated agricultural soils, carrying out the degradation of pollutants and increasing the yield of crops (Báez-Rogelio et al., 2016).

Among the satisfactory results for the control of phytopathogenic microorganisms is the genus *Pseudomonas spp*. (Anguloa, et al., 2014). They are a group of bacteria that can exert a direct beneficial effect, through the synthesis of phytohormones and vitamins, stimulation of seed germination and emergence of seedlings, inhibition of ethylene synthesis, solubilization of inorganic phosphorus (P). Indirectly, they exercise the function of controlling pathogenic microorganisms through the synthesis of antibiotics and fungicides, competition for nutrients, production of siderophore or by inducing systemic resistance to pathogens (Alcarraz-Curi et al., 2019). For example, in *P. fluorescens* G20-18 the ability to efficiently control infection by *P. syringae* has been identified, which allows the maintenance of tissue integrity, reflecting on the biomass yield (Großkinsky et al., 2011). Some criteria commonly used to test a crop are:

Total germination: it is the maximum germination percentage got under previously defined conditions (Durán and PérezGarcía, 1984).

Germination speed: Maguire (1962) defines it as the ratio of the number of germinated seeds to the germination time.

$$M = \sum \left(\frac{n_i}{t}\right)$$

where M = germination speed, n = number of seeds germinated on day i, t = germination time from sowing to germination of the last seed.

1.1 Accumulation and distribution of dry matter

The accumulation of dry matter is commonly used as a parameter to characterize growth, because it usually has great economic significance (Ñústez et al., 2009). The distribution of dry matter plays an important role in the final yield of a crop, since it is given by the ability to accumulate biomass in the organs that are destined for harvest (Barrientos-Llanos et al., 2015).

1.2 Chlorophyll content

Allows us to relate it to the nutritional level of the plants, it also has a close relationship with the photosynthesis index and these two factors, considerably influence the performance of a plant, both in its development and in the final yield of harvest (López-Tolentino et al., 2016).

Roselle (*Hibiscus sabdariffa L.*), is a species belonging to the Malvaceae family (Ríos et al., 2013). It is native to India and Malaysia. It has been widely distributed in the tropics and subtropics of both hemispheres, in addition, it has become naturalized in many areas of the Antilles and Central America (Morton, 1987). During the colonial era, the Spanish were the ones who introduced Roselle to Mexico (Romano-Cadena et al., 2017). It is a crop that is currently gaining more importance in Mexico and is part of the sector of spices and medicinal plants (Sánchez-Prado et al., 2019). In recent years it has had a potential use for lowering cholesterol and hypertension, in addition, it is attributed diuretic and antipyretic properties (Caamal et al., 2020). These benefits are supported by various scientific investigations that relate them to compounds such as vitamins E and C, polyphenolic acids and antioxidants such as flavonoids and anthocyanins (Cid and Guerrero, 2012). However, the information devoted to the study of Plant Growth-Promoting Bacteria in this crop is almost nil. Considering the above, the present work was developed, with the aim of defining which are the most important criteria for the selection of bacterial strains that promote plant growth in seedlings of two varieties of Roselle.

II. MATERIALS AND METHODS

2.1 Biological material and experiment location

Roselle seeds (*Hibiscus sabdariffa* L.), Creole and Spider varieties, were obtained from the community of Río de los Peces, municipality of Candelaria Loxicha, Oaxaca. And the roots used in this research were also collected there.

2.2 Selection of plants to get root samples for the isolation of bacteria

The selection of the plants was carried out in a plot of approximately 1 hectare, choosing the largest plants, with more foliage and a healthy appearance, 5 of each variety (Spider and Creole) to collect the tips of 3 roots of each variety, about 10 cm long.

2.3 Getting root samples from Roselle plants

To get the bacteria present, the roots of each plant were placed in sterile test tubes and washed with 5 mL of sterile distilled water, shaking the tubes in a vortex for 2 minutes. An aliquot sample of 500 µL was taken from each of the 10 tubes, to make

dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. The roots that remained in the test tubes were rinsed 4 times with sterile water, vortexing for 30 seconds and, after removing the water, were macerated with sterile glass rods. 2 mL of sterile water were added to each tube and 500 µL aliquots were taken from this liquid to make the 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions.

2.4 Getting bacterial isolates

Aliquots of 100 µL were taken from each of the dilutions (from the series of the washing liquid and from the macerates) to spread them on the surface of King's B culture medium in Petri dishes. The Petri dishes were incubated at a temperature of 28-30°C to allow the development of the cultures for 48h and to perform the colony count using the plate count method of Allaert and Escolá (2002).

2.5 Identification and isolation of bacterial colonies

Petri dishes were observed under UV light to identify fluorescent colonies (Fig 3). With sterile wooden sticks, 5 samples of completely isolated colonies were taken from each of the Petri dishes, getting 200 isolates from the samples of the washings and the root maceration.

A bacterial bank prepared in sterile 2 mL Eppendorf tubes containing the bacterial suspensions in 1 mL of sterile distilled water was used to keep the 200 bacterial isolates got in the refrigerator, before making the bank in tubes with slanted agar of the selected isolates.

2.6 Identification of bacterial isolates that promote germination and vigor of Roselle seeds

From the temporary bacteria bank, the bacterial cultures were prepared to inoculate the seed samples of the 2 varieties of Roselle. The seed inoculation was carried out in 4 blocks. In the first block, 20 strains were tested, 4 from the root washing and 16 from the maceration; In the second block, 30 strains were tested, all from the root maceration; In the third and fourth block, 24 and 23 strains were tested respectively, all from the maceration. From the total of the 97 strains, 5 strains were selected for the Creole variety and 3 for the Spider variety. Subsequently, of the 5 previously selected strains, only 3 were chosen for the Creole variety, considering only the percentage of total germination as a criterion.

To carry out the seed inoculation, the bacterial cultures were prepared in Petri dishes containing King's B medium. From the cultures, after 24h of incubation at 28-30^{o}C, bacterial suspensions were prepared in sterile distilled water adjusted between 0.8 and 1 turbidity (660 nm).

The seeds were mixed with the bacterial suspension (25 seeds mixed with 400 µL of bacterial suspension), preparation that was left for 60 minutes at room temperature. Subsequently, the inoculated seeds were placed on two sheets of filter paper moistened with 4 mL of distilled water in 9 cm diameter plastic Petri dishes. The seeds were placed in a germination chamber set at 28-30^{o}C and the seeds that had germinated were counted daily (seeds in which the root tip was already visible) (Fig 4).

2.7 Lighting type

Three types of LED lamps were used to illuminate the Roselle seedlings previously inoculated with the 6 selected strains (3 for each variety Creole and Spider), to observe if the type of lighting also influenced the dry stem biomass (DSB), dry root biomass (DRB), stem length (SL), root length (RL) and chlorophyll content (CC). The lamps used were of LED light: one composed of 100% white light; the second composed of 15% white light, 27% blue light and 58% red light and the third composed of 29% blue light and 71% red light. The seedlings were placed at a distance of approximately 30 cm from the lamps for 4 days.

2.8 Establishment of greenhouse cultivation

Seeds inoculated with cells suspensions of the 6 strains selected for the two varieties were used and germinated in plastic Petri dishes in a germination chamber set at 28-30^{o}C for 3 days. The seedlings obtained were transplanted into polyethylene bags and their growth was observed during the first 65 days after sowing (das). Irrigation was carried out every third day, using a half-liter container so it was homogeneous in all the pots.

2.9 Variables tested

The variables were: total germination percentage (TGP), which was calculated by adding the daily germination values up to the third day; germination speed (GS) was calculated with the formula of Maguire, (1962); root and stem length were

measured using a sheet of millimeter paper (Fig 1); To get the dry root biomass and the dry stem biomass, these were separated and preserved in an oven set at a temperature of 45°C for 5 days and then in another oven set at a temperature of 70°C, for 4 days; the chlorophyll content was obtained using the Konica Minolta SPAD 502 PLUS meter, considering the average of three readings per seedling; stem length of the plant (SL) in the greenhouse was measured with a ruler at 3, 45 and 65 das.

2.10 Statistical analysis

The analysis was divided into three parts. In the first, 10 treatments with 3 repetitions were considered, the variables TGP, GS, RL, SL, DRB and DSB were analyzed and the variation factors were varieties and strains; In the second, 8 treatments with 3 repetitions were considered (the repetitions represented the type of lighting used), the variables TGP, GS, RL, SL, DRB, DSB and CC were analyzed and the variation factors were varieties, strains and type of lighting; in the third, 8 treatments with 3 repetitions were considered, only the variable SL was analyzed at 3, 45 and 65 das and the variation factors were strains and varieties. The treatments were distributed under a completely random design. For the analysis of variance and comparison of means (Tukey, p = 0.05), the ANOVA procedures of the statistical software package SAS (Statistical Analysis System) version 8.0 (SAS, 1999) were used.

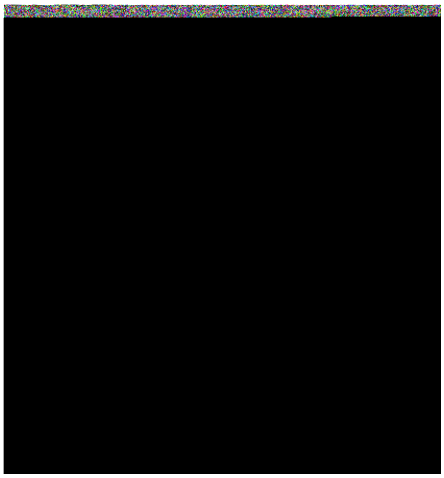

FIGURE 1: Appearance of the Roselle seedlings developed in Petri dishes from which the root and stem lengths were obtained

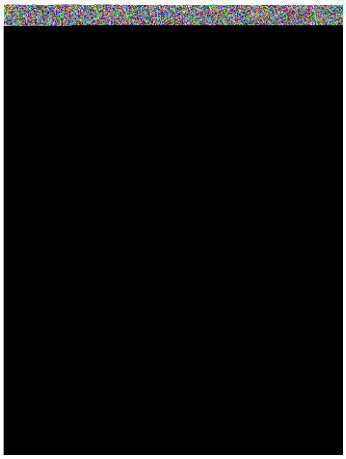

FIGURE 2: Appearance of the Roselle seedlings developed in a greenhouse from which the stem lengths of Roselle plants were obtained

III. RESULTS AND DISCUSSION

A total of 271 isolates derived from washing and macerated root preparation of the two Roselle varieties were obtained, of which 7.7% corresponded to isolates of fluorescent strains from the root washing and 14.6% from the root maceration preparations (Table 1).

When the variables TGP, GS, DSB, DRB, SL and RL were tested, it was found that the type of variety had a significant effect ($p \leq 0.05$) on the germination speed, the dry biomass of the stem and root and the stem and root length (Table 2), getting higher values for the Spider variety in all the tested criteria. However, when the strains were tested, a significant difference was observed between strains on all the tested criteria (Table 3). However, no strain showed a significant effect when compared with its respective control (M67, L168, M82_C, M3 and M61 were tested with the Creole variety and M83, M88 and M82_A with the Spider variety) on total germination, germination speed, stem dry weight, root dry weight and root length.

The only significant positive effect of strain M88 was obtained on stem length when compared to the Spider Control. There was a significant effect of the strains on most of the criteria tested, but without considering the effect of the variety (Table 3). Smith and Goodman (1999) pointed out that the genotype of the organisms involved play an important role in the association between microorganisms and plants, determining the biological result of said association. Total germination percentage was not influenced by the type of variety or by inoculation with strains used in the same variety, which is under what was reported by Méndez and Campos (2007), who got unsatisfactory results when testing germination percentage, stem length, radicle length and fresh stem biomass in the laboratory.

TABLE 1
BACTERIAL ISOLATES GOT FROM THE ROOT TIPS OF TWO VARIETIES OF ROSELLE

Process	Number of bacterial isolates	Number of fluorescent bacterial isolates	Percentage of fluorescent bacterial isolates
Root washing	169	13	7.7
Root macerate	103	15	14.6

A significant effect ($p \leq 0.05$) of the type of variety was obtained in the germination speed, dry biomass of the stem and the root, root length and chlorophyll content (Table 4). Getting higher values for the Spider variety in all the tested criteria. No differences were observed between varieties when testing the total germination percentage and the stem length. Light is a vital environmental factor that affects the growth and development of plants by acting not only as the only source of energy for photosynthesis but also as a type of external signal (Ding et al., 2010; Liu, 2012). The type of lighting did not have a significant effect on the Roselle seedlings (Table 4). Only differences in root length were observed (Table 5), achieving greater root growth with the lamp composed of 15% white light, 27% blue light and 58% red light. Xiaoying et al., (2012) mention that the combination of red-blue and red-blue-green LEDs were shown to be beneficial factors in the growth and photosynthesis of cherry tomato (*Solanum esculentum*var. *cerasiforme*) seedlings.

FIGURE 3: Fluorescent bacterial isolates

FIGURE 4: Aspect of the germinated seeds of the two varieties of Roselle
(a: Creole variety; b: Spider variety)

Table 6 shows that there was no positive effect of the strains on the total germination percentage and the stem length, however, on the germination speed positive effects were observed when inoculating the Creole variety with the M67 strain, which speed up germination with regarding the Creole Control. In contrast, strains M83, M88 and M82_C strains compared to the Spider Control did not show significant effects. The dry stem biomass, dry root biomass and chlorophyll content showed significant differences between the type of cells of the strains used but not between those used for the same variety. So, the variety influences the cells of the strains and for this reason, this difference is obtained. The cells of the M83 strain had a positive effect on the root of the seedlings, increasing their length considerably regarding the Control Spider. In contrast, there was no strain that promoted root growth in the Creole variety.

To confirm the effect of the strains on the stem length of the Roselle seedlings, the stem lengths of the plants were measured in the two varieties established in the greenhouse during their first days of development (Fig 2). No significant differences were obtained on the stem lengths of both varieties (Table 7). However, when testing the effect of inoculation with cells of bacterial strains, if there were contrasts between the strains M67, M83 and M82_A at 3 das (Table 8), but when comparing them with the controls, no significant differences were obtained. At 45 das and 65 das, in the same way, no differences were perceived. It reflects the above shows that the effect of inoculation with cells of bacterial strains only in the first days of plant development. Méndez and Campos (2007) got similar results when testing the growth of Roselle plants at the field level, where the greatest difference in plant height was obtained at 8 das.

TABLE 2
DETERMINATION OF THE PARAMETERS SHOWED ON THE VARIETIES OF ROSELLE CREOLE AND SPIDER

Variety	Total Germination (%) **		Germination speed		Drystem biomass (mg)		Dryroot biomass (mg)		Stem length (mm)		Root length (mm)	
Creole	4.02	a*	9.76	b	39.50	b	6.72	b	36.59	b	24.48	b
Spider	4.47	a	16.11	a	72.12	a	12.29	a	44.55	a	44.63	a
MSD	1.06		2.94		2.55		3.87		1.78		4.18	

**** Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula ROOT (100- (TGP / 25 * 100)). * Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula SQRT (100- (TGP / 25 * 100)).***

TABLE 3
TESTED CHARACTERISTICS OF THE SEED INOCULATED WITH CELLS OF THE SHOWED STRAINS

Strain	Total germination (%) **		Germination speed		Dry stem biomass (mg)		Dry root biomass (mg)		Stem length (mm)		Root length (mm)	
M67	5.40	a*	8.70	c	37.50	b	9.17	bcd	36.33	c	19.80	d
L168	4.31	ab	9.33	c	38.67	b	3.33	d	36.33	c	32.80	bcd
M82_C	4.28	ab	9.87	c	38.17	b	3.50	d	34.27	c	23.67	cd
M3	4.12	ab	9.93	c	42.33	b	7.83	cd	37.67	c	24.33	cd
M61	1.82	b	11.93	bc	40.33	b	7.50	cd	39.53	bc	22.80	cd
Creole Witness	4.20	ab	8.80	c	40.00	b	9.00	bcd	34.50	c	23.47	cd
M83	3.64	ab	17.93	a	76.00	a	15.17	abc	41.00	bc	52.33	a
M88	5.29	a	14.87	ab	70.83	a	22.16	a	51.33	a	42.33	ba
M82_A	4.28	ab	16.33	ab	68.00	a	15.16	abc	45.00	ba	46.20	ba
Spider Witness	4.76	a	15.30	ab	73.67	a	16.67	ab	40.87	bc	37.67	abc
MSD	2.57		4.63		9.20		7.92		6.83		16.02	

**** Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula ROOT (100- (TGP / 25 * 100)). * Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula SQRT (100- (TGP / 25 * 100)).***

TABLE 4
CRITERIA TESTED IN THE SEEDS OF TWO VARIETIES OF ROSELLE

Variety	Total germination (%) **		Germination speed		Dry stem biomass (mg)		Dryroot biomass (mg)		Stem length (mm)		Root length (mm)		Chlorophyll Content (SPAD Units)	
Creole	3.93	a*	13.27	b	78.83	b	14.33	b	53.62	a	32.71	b	36.33	b
Spider	4.49	a	17.22	a	139.04	a	30.21	a	55.46	a	69.22	a	40.76	a
MSD	0.80		1.57		29.32		8.64		2.33		5.66		2.18	

**** Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula ROOT (100- (TGP / 25 * 100)). * Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula SQRT (100- (TGP / 25 * 100)).***

TABLE 5
TESTED CHARACTERISTICS OF THE DEVELOPMENT OF THE SEEDLINGS OF TWO VARIETIES OF ROSELLE ILLUMINATED WITH THE TYPE OF LIGHT SHOWED

Lighting type	Dry stem biomass (mg)		Dryroot biomass (mg)		Stem length (mm)		Root length (mm)		Chlorophyll Content (SPAD Units)	
100% White	110.31	a*	19.69	a	52.94	a	40.24	b	37.22	a
15% White, 27% Blue, 58% Red	108.12	a	23.12	a	58.37	a	54.92	a	39.18	a
29% Blue, 71% Red	108.37	a	24.00	a	57.30	a	43.97	a	39.24	a
MSD	43.63		12.85		3.43		8.33		3.20	

**** Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula ROOT (100- (TGP / 25 * 100)). * Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula SQRT (100- (TGP / 25 * 100)).***

TABLE 6
CRITERIA TESTED IN THE SEEDS THAT HAD BEEN PREVIOUSLY INOCULATED WITH CELLS OF THE SHOWED STRAINS

Strain	Total germination (%) **		Germination speed		Dry stem biomass (mg)		Dry root biomass (mg)		Stem length (mm)		Root length (mm)		Chlorophyll Content (SPAD Units)	
M67	3.04	a*	16.83	ab	77.83	b	14.83	b	54.87	a	28.90	e	37.84	ab
L168	3.43	a	14.10	abc	78.50	b	15.00	b	53.03	a	33.63	de	32.74	b
M82_C	4.85	a	12.93	bc	80.67	b	12.83	b	54.63	a	33.07	de	36.70	ab
Creole Witness	4.38	a	9.20	c	78.33	b	14.67	b	51.93	a	35.23	cde	38.06	ab
M83	5.01	a	15.83	ab	144.67	a	30.83	a	56.60	a	89.10	a	39.60	a
M88	4.43	a	17.83	ab	136.33	a	29.00	a	55.57	a	63.50	abc	42.13	a
M82_A	4.21	a	16.8	ab	133.33	a	30.33	a	53.80	a	66.26	ab	40.89	a
Spider Witness	4.31	a	18.43	a	141.83	a	30.67	a	55.87	a	58.00	bcd	40.41	a
DMS	2.82		5.19		81.27		20.96		8.12		28.64		6.09	

**** Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula ROOT (100- (TGP / 25 * 100)). * Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula SQRT (100- (TGP / 25 * 100)).***

TABLE 7
STEM LENGTHS OF PLANTS OF TWO VARIETIES OF ROSELLE ESTABLISHED IN THE GREENHOUSE AT 3, 45 AND 65 DAS

	Stem length (cm)					
Variety	**3 das**		**45 das**		**65 das**	
Creole	2.2083	a*	5.275	a	8.358	a
Spider	2.167	a	4.95	a	8.558	a
MSD	0.624		1.147		1.781	

****Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula ROOT (100- (TGP / 25 * 100)). * Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula SQRT (100- (TGP / 25 * 100)).***

TABLE 8
STEM LENGTHS OF PLANTS OF TWO ROSELLE VARIETIES ESTABLISHED IN THE GREENHOUSE AT 3,45 AND 65 DAS INOCULATED WITH CELLS OF THE SHOWED STRAINS

	Stem length (cm)					
Strain	**3 das**		**45 das**		**65 das**	
M67	1.33	b*	5.167	a	8.667	a
L168	2.33	ab	5.5	a	7.667	a
M82_C	3	ab	5.77	a	9.167	a
Creole witness	2	ab	4.67	a	7.93	a
M83	1.33	b	4.5	a	8.667	a
M88	2.167	ab	4.9	a	7.73	a
M82_A	3.5	a	5.067	a	8	a
Spider Witness	1.83	ab	5.33	a	9.83	a
MSD	2.04		3.746		5.818	

**** Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula ROOT (100- (TGP / 25 * 100)). * Means with the same letter in a column are not significantly different (Tukey, p = 0.05). MSD = Minimum significant difference. ** TGP was transformed with the formula SQRT (100- (TGP / 25 * 100)).***

IV. CONCLUSION

The criteria of total germination percentage, dry stem biomass and dry root biomass are not safe criteria for the selection of Plant Growth-Promoting Strains, since the seedlings from Roselle seeds inoculated with cells of the bacterial strains did not show significant differences with respect to the controls corresponding to each variety. It must be taken into account that the benefit that the plant obtains from bacteria must occur after they colonize its roots more than just during the germination process. The germination speed and the stem length of the seedlings showed significant differences when inoculating the seeds, therefore, selection criteria could be considered, however to consider them safe or not, the important thing is the relationship of what happens at the moment of the germination and development of the seedlings in the laboratory and what happens in the development of the seedlings in the greenhouse, because, when measuring the stem length of the plants in the greenhouse, it was concluded that differences between strains can be noticed, but not regarding the control and that it is also only observed in the first days of development (3 days after sowing). Subsequently, no significant differences are shown. The same happened with the root length, they only showed favorable results in the experiments carried out in the laboratory, but in the same way it would have to be verified if the same effect had in the greenhouse. Chlorophyll content also did not show to be a safe selection criterion, since there were no significant differences between strains applied to the same variety. The type of lighting did not have a significant effect on the Roselle seedlings. Only, the lamp composed of 15% white light, 27% blue light and 58% red light was the one that most promoted root growth. The mean values of most of the criteria tested were higher for the Spider variety. Therefore, it is important to consider the type of variety used when making the selection of strains.

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Symptoms and their Assessment of Sugarcane Pokkah Boeng

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Abstract— *Sugarcane (Saccharum officinarum L.) is one of the main important commercial crops, mainly grown in tropical and subtropical countries in the world, because these areas provide suitable conditions for obtaining the best yield and productivity. Sugarcane is affected by many pathogens such as fungi, bacterial and viral diseases and fungal diseases are increasingly being affected internationally, affecting the quantity and/or quality of harvested crops. Among the fungal diseases, pokkah boeng have become the main problems faced by sugarcane growing countries, causing serious yield losses. However, there are many reports of an outbreak of the disease, which looks spectacular, but it caused trade and industrial losses. In this review we highlight the importance of sugarcane and the symptoms of the Pokkah Boeng disease tend to develop during period of rapid crop growth.*

Keywords— Sugarcane, Pokkah Boeng, Symptoms, Pathogen, Fusarium.

I. INTRODUCTION

Sugarcane (*Saccharum officinarum L.*) is one of the most important commercial crops and the primary producer of sugar in the world, accounting for around 70% of the world's overall sugar supply. Sugarcane is cultivated primarily for sugar, which is an important cooking material in the modern world. Sugarcane is a member of the *Poaceae* family which consists of six perennial grass species of the genus *Saccharum L*. It is a long-term crop of 10-12 months, so it is susceptible to a number of diseases, and the importance of diseases as a restrictive factor in the development of sugar cane has been widely established. A substantial number of diseases have a major effect on the production of sugar cane **[1]**. This is partially due to their nature, reproduction, cultivation and management methods.

The crop has an unparalleled record of dealing with new diseases, some of which have caused major losses or more widespread. The increase in sugarcane land used as a commercial crop is expected to cause more disease problems in many countries. The losses caused by these diseases may vary from place to place and depend on the type of crop. Therefore, these diseases cannot be ignored or neglected due to their impact on the quality and quantity of sugarcane.

Disease is one of the major problems affecting sugarcane productivity. Sugarcane disease not only caused a decline in production, but also had a major impact on variety development plans. The fungal pathogens of sugarcane are known to spread to different continents and different sugarcane varieties. Many primitive noble canes are susceptible to some serious diseases, but their hybridization with wild canes improves their toughness. Among fungal diseases, pokkah boeng has become a serious problem for sugarcane-growing countries, which can lead to severe yield losses.

When the plant grows rapidly, Pokkah boeng may become obvious during wet periods. However, the plants can be restored and have little effect on yield. In particular, resistant varieties may affect the disease resistance or resistance of plants to pathogens, and moderate safety in controlling plant diseases should be considered. Planting healthy seed materials/using drug-resistant materials and following comprehensive disease management measures is the best way to prevent the occurrence of diseases **[2]**. This chapter initially briefly introduced the economic importance of sugarcane. Subsequently, the pokkah boeng of sugarcane is described in detail, including its disease symptoms and pathogens. Biological control has become the ultimate long-term solution to the problems of sugarcane pokkah boeng because it is safer than chemical pesticides and is considered to be less polluting to the environment. However, due to severe constraints, the above-mentioned disease control measures cannot be implemented, so some alternative sustainable strategies are necessary.

II. SYMPTOMS

Fungal infections may typically cause noticeable signs of abnormal development, patterns and colors, but non-biological issues can cause normal, stable symptoms. Pathogens can also be present on symptoms such as fungal growth, bacterial exudate, etc. The magnitude of symptoms varies with the species' propensity and the environmental factors that regulate the growth of the organism. The symptom is easy to recognize because it affects the top of the plant and the young leaves begin to chlorosis. The death of host cells and tissue is triggered by constant activation of environmental factors. This

contributes to the development of symptoms that may result in damage or death to the whole plant or portions of it. The initial sign is easy to recognise because it involves the top portion and the chlorosis region at the base of the young leaf. If the fungus is confined to the leaves, the plant will normally recover, else internal ladder-like lesions can occur in the stem [3]. Heavily infected plants showed a malformed or damaged top and stalk may occur in highly susceptible varieties.

2.1 Chlorotic Phase

Severely infected plants exhibit top deformities or damage, and stalks can occur in highly susceptible varieties. The earliest symptom of Pokkah boeng is the appearance of green leaf disease on the roots of young leaves, occasionally occurring in other parts of the leaves. It is often seen that the bottom of the affected leaf is narrower than the bottom of a normal leaf. The first sign of Pokkah boeng is the green state toward the base of the young leaves, which rarely occurs in other parts of the leaves. The roots of affected leaves are usually narrower than the roots of normal leaves. Ladder like shaped lesion on the spindle-shaped leaves is obviously yellow, the spindle is wrinkled, twisted or tangled, red streaks appear, the leaves become shorter and the young leaves are deformed. As the leaves mature, irregular reddish stripes and spots will appear in the chlorosis part.

2.2 Top-Rot Phase

The most advanced and severe stage of Pokkah Boeng is the top rot phase. Leaf infection sometimes continues downward and penetrates into the stem through the growth point. In the late stage of infection, the entire root of the spindle and even the growth point showed leaf deformities, and the spindle blades were obviously wrinkled, twisted and rotted. Red spots and streaks also appeared. In the late stages of the disease, decay will appear. The growth points are killed and the plants die.

The young spindle was killed, and the entire upper mold died. Leaf sheaths may also chlorosis and develop reddish asymmetric necrotic areas. The reddish tissue forms a stepped lesion, usually with darker edges. These lesions sometimes penetrate the surface of the peel. Sometimes, pathogens also attack the spindle and move down from there to the end of the stem, causing the top to rot. The decayed apical rot is through the rot of the spindle leaf, obvious red streaks and white spore clumps, necrosis and bud sprouting. The spots and flow channels combine to form large red-brown wilted tissues. Later, irregular red streaks and spots are formed in the chlorosis part, forming a lens or diamond-shaped hole **[4]**. The top part of the stem is severely damaged.

2.3 Knife-Cut Phase

The symptoms of knife-cut stage are observed in association with the acute phase of the disease characterized by one or two or even more transverse cuts in the rind of the stalk /stem in such a uniform manner as if, the tissues are removed with a sharp knife. Usually, the infection occurs evenly on top of the sugar cane, which is obviously the cause of the spread of the disease. This is the exaggerated stage of the typical stepped lesions of pokkah boeng disease. The infection in the spindle sometimes continues down to the stem, and dark red stripes may be found to extend across multiple internodes. When the leaves are peeled off, a large horizontal cut is formed on the stem. As the name suggests, the most obvious function of pokkah boeng is to deform the top of the sugar cane. The earliest symptoms appear on young leaves, which are chlorosis to the base, twisted, wrinkled, narrower and shorter than normal leaves. Irregular reddish streaks may appear in the fading area. If the infection is limited to the leaves, the plant usually recovers little and is damaged [5]. However, the infection may progress to the stem, where internal and external ladder-like (knife cut) lesions may appear. The most serious damage occurs when the fungus penetrates the growth point, which may die and cause the top to rot. Infection is achieved by washing air-borne spores between partially unfolded leaves to the roots of the spindle during rapid growth in hot conditions, and then rain or irrigation. Then, the spores germinate and infect the young tissues of the spindle [6]. Pokkah boeng disease shows several post-inflection points in morphology, anatomy, biochemistry and physiology. The occurrence of Pokkah boeng disease is more pronounced in the dry season followed by the humid season. Under these conditions, leaf infections develop rapidly, and even resistant varieties sometimes show typical leaf symptoms. It is generally observed that when conditions favorable for plant growth occur, the affected plants recover from the disease. The infection of this disease is caused by spores or ascospores.

Pathogens enter the host tissue through any damage caused by insects or borers or natural growth cracks. The severity of symptoms varies with changes in drug susceptibility and environmental conditions, and determines the development of pathogenic organisms. After entering, the infection line will develop normal hyphae, which will grow in the host tissue for a period of time, and then come out of the cells and reach the outer surface, forming the cervix. Upon close inspection, the

deformed leaf blade showed extensive damage on the leaf base, resulting in white plaques, blackening of the affected lamellar area and extensive black vein necrosis.

Rain and heavy dew will usually wash the nodules and microspores that develop between the nodes, and the spores will stay around the nodes behind the leaf sheath. Due to severe PB infection, the extension of intermodal transportation in the affected straw area has been drastically reduced. Depending on the severity of the PB, at most five or six nodes show shortened nodes. Spores germinate, mycelium forms bud scales, roots are primitive or leaf scars, and then enters the plant tissue. Associated with the increase in *fusarium* are several cultural practices that may contribute to the increase in the disease: maximum farming, high nitrogen fertilizer, high plant populations, and continuous cropping. In the process of fungal penetration and growth inside plants, *fusarium* protease and mycotoxins play a complementary role in host defense inhibition and intracellular colonization of spikelets, and play a strategic cooperative role in the process of ear and core colonization.

III. MEANS OF DISPERSAL

The pathogen of the disease is spread by moving spores from one place to another by air currents **[7]**, and will settle on the leaves, flowers and stems of plants **[8]**. For spores to take off, it depends on the environmental conditions, which requires different propagation strategies **[9]**. The fungus splashed by rain is based on the "puff" and "tap" mechanisms, which cause dry spores to spread into the air, and the spores are usually bent like *Fusarium* **[9]**. Pokkah boeng disease of sugarcane may also be transmitted from seeds contaminated with fungi **[10]**. The spread of spores depends on various environmental conditions and may require different spreading strategies. However, many have active or passive means of diffusion in the atmosphere and are common among settlers of aerial plant parts, where they can cause diseases of great economic significance. Infection usually occurs through the spindle along the edge of a partially expanded leaf. The spores that enter the spindle germinate and grow into the internal tissue of the spindle leaf. **[11]** reported that adults of the and sugarcane stem bore can also transmit fungi. The top bore worm is called Chilo spp. It usually results in leaf deformation and shortening, which is similar to leaf deterioration and shortening caused by pokkah boeng disease.

IV. CAUSAL ORGANISMS

Although the disease is caused by *Fusarium*, there is some controversy about the species **[12]**. In Malaysia, the pathogenic organism of Pokkah boeng is called *Fusarium moniliforme var. Subglutinans* **[13]**.**[2]** studied the morphological and pathogenicity of different isolates of *F. moniliforme* associated with pokkah boeng disease collected from various places in Maharashtra. According to reports, the causative organism of Pokkah Boeng disease belonging to the Section Liseola is *Fusarium sacchari* found on sugarcane in Asia **[13]**. **[12]** proved the association between *F. sacchari* and pokkah boeng disease in their work. Pathogens can be spread by air currents **[4,5]** and airborne spores will colonize the leaves, flowers and stems of plants **[8].** The curved structure of the large conidia of *Fusarium* species is easily scattered by rain.

V. CONTROL MANAGEMENT

Spraying different fungicides, such as Bavistin (1 g l-1 water) or Blitox (2 g l-1 water) or copper oxychloride or Dithane M-45 (3 g l-1 water), can effectively reduce pokkah boeng disease **[15]**. Spraying 2 to 3 times every 15 days can reduce the propagation of pathogens, thereby reducing sugarcane yield and quality loss. Therefore, paired rows or larger planting intervals are necessary to promote plant protection operations. When they are observed, the sticks showing the highest decay should be driven out of the field immediately. Planting healthy seed material/using drug resistance and following comprehensive disease management practices is the best way to prevent disease from occurring **[2]**.

Only disease-resistant varieties can be controlled. Generally, the resistance of the seedlings to pokkah boeng is tested by injecting a suspension of the conidia of G. fujikuroi conidia into the leaf spindle 10 cm below the highest visible leaf joint. In most breeding institutions, susceptible new varieties are discarded in the selection process, which provides sufficient control **[1]**. In addition, the fungus *F. moniliforme* can be spread horizontally through airborne spores or crop residues, and vertically through seed blocks. For these seeds, it is important to use resistant varieties and apply fungicides. Both control methods are limited. Therefore, it is important to develop novel and environmentally sound strategies to control this and other sugarcane diseases. Burkholderia isolates from sugarcane plants are essential for further isolation of these isolates for biological control of pokkahboeng and other sugarcane diseases. Because of the high frequency of Burkholderia in endophytic bacteria from sugarcane plants and its strong growth inhibitory activity against *F. moniliforme*, these isolates are potential candidates for disease control.**[16]** The endophytic bacterial community associated with sugarcane has multiple genera and has the potential to promote plant growth and control disease.

VI. POTENTIAL UTILIZATION OF SUGARCANE

Today, sugarcane agriculture has become an important economic activity in more than 100 countries especially in developing economies. Sugarcane is responsible for raw sugar production worldwide **[17]**. Dryness, winter, and chemical maturity all slow down growth and increase sugar concentration. In addition, chopped sugarcane stalks are widely used as cattle feed, especially during dry seasons when pastures are unavailable for grazing. Sugarcane is considered the first generation of biofuel crops. The world's demand for sugar is the main driving force for sugarcane agriculture. In addition to sugar, products extracted from sugar cane include witch falernum, molasses, rum, cachaça, bagasse and ethanol.

It is one of the most efficient photosynthesis in the plant kingdom. This is a C4 plant that can convert solar energy into chemical energy. The sugarcane pathway-C4 or dicarboxylic acid pathway-also functions in other species and appears to have some unique anatomical features. It has been recognized as an important energy crop and has recently been enhanced by large-scale production of sugarcane-based ethanol from molasses and directly from cellulose. Bagasse is the fibrous material remaining after crushing sugarcane. Generally, for every 10 tons of sugarcane crushed, 3-4 tons of wet bagasse are left. It has a high moisture content, usually 40% to 50%, and is usually stored before further processing. Molasses is another important by-product of the sugar industry. The mother liquor remaining after the crystallization of sucrose cannot economically recover more sucrose from it. Due to the total sugar content of molasses, it is a valuable raw material for the production of many value-added products. The main products that can be produced from molasses are breweries, acetic acid, fuel alcohol, biogas from sewage treatment, cattle feed, ethanol, Baker's yeast, lactic acid, citric acid, etc. Ash and filter mud are also used as fertilizer. Boiler ash is "scrubbed" from the mill stacks, and the filter residue/filter cake is the residue left after sugar clarification. In the early stage, the processing of press mud posed a problem in the sugar mill, not only related to the processing volume, but also related to the sugar processing volume. Now, due to technological advancement, pressed mud is widely used as fertilizer as well as wax and compost industry. In many countries, many sugar manufacturing units have transformed themselves into sugar agricultural industrial parks, producing various chemicals and practical products from sugar cane. In southern China, a typical subtropical climate region, the epidemic of pokkah boeng disease is more serious, which seriously threatens the production of sugarcane plantations and the huge losses of the sugar industry. The growth of susceptible varieties led to significant losses of pokkah boeng in the humid climate followed by the dry season. Carry out appropriate planning and environmental risk assessments to expand sugarcane into new areas, improve land use practices to reduce soil erosion and nitrogen pollution, properly protect streams and riparian ecosystems, prohibit sugarcane burning practices, and fair working conditions for sugarcane cutters. Due to the production of sugar, the chemical composition of the ethanol is usually available. It can be used as a biofuel substitute for gasoline and is widely used in Brazilian cars. It is a substitute for gasoline and may become the main product of sugarcane processing instead of sugar. Molasses-based industries mainly produce edible alcohol, acetic acid, fuel ethanol, cattle feed and many pharmaceutical products for use in distillers. The pressed mud-based industries mainly produce fertilizer, wax and compost industries as animal feed.

VII. CONCLUSION

From the sugarcane samples exhibiting Pokkah boeng symptoms, a phytopathogenic fungal strain belonging to the complex of Fusarium and *Gibberella fujikuroi complex* was isolated through morphological and molecular techniques, which may be *Fusarium verticillioides*. The existence of this pathogen is unknown. Therefore, this report opens the door to different viewpoints, such as researching the biology of the pathogen and the diseases it produces, and formulating mitigation and control strategies to avoid economic losses and food contamination problems produced by the fungus and its impact on the health of consumers, all of them of highly importance for the industry and public health.

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Study on the Extraction Technology of Ginkgo Biloba Leaf Extract by Enzymolysis Combined with Fermentation

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Abstract**—** *In this paper, we select Ginkgo biloba leaves in Taizhou as raw materials and use cellulase and pectinase to hydrolyze Ginkgo biloba leaves, and then the Ginkgo biloba leaves extract was prepared by microbial fermentation. Firstly, cellulase and pectinase were selected for single factor experiment and orthogonal experiment to determine the effect of enzyme dosage, enzymolysis time, temperature and pH value on the extraction rate of Ginkgo biloba leaves; then, microbial fermentation was used to study the effect of optimal temperature, time and pH value on the extraction rate of Ginkgo biloba leaves. The results showed that: the optimal enzyme content was 0.2%, the time of enzymolysis is 2 h, the temperature of enzymolysis was 4 ^{o}C, the pH of enzymolysis was 4.5; the optimal microorganism content of fermentation was 4%, the temperature of fermentation was 30^{o}C, the time of fermentation was 8 D, the pH of fermentation was 5， and extraction rate was 18.56%.*

Keywords— Ginkgo biloba; enzymolysis; fermentation; Ginkgo biloba extract.

I. INTRODUCTION

Ginkgo biloba is the dry leaf of Ginkgo biloba, a plant of the Ginkgo family, which likes to grow in sunny soil. Ginkgo biloba can promote blood circulation to remove blood stasis, relieve collaterals and relieve pain, and is mostly used for hyperlipidemia. Ginkgo biloba mainly has the effects of improving cardiovascular and cerebrovascular functions, anti-aging, and anti-tumor [1]. At present, the preparation of Ginkgo biloba extract mainly includes chemical methods, microwave, and enzymatic methods [2]. In order to make full use of the effective substances in Ginkgo biloba leaves, enzymatic hydrolysis is used to hydrolyze cellulose and pectin to turn large molecules into small molecules. Fermentation is used to remove toxic substances and the conditions are mild.

II. MATERIALS AND METHODS

2.1 Material

TABLE 1
MATERIAL

Materials and Reagents	Specifications	Manufacturer
Ginkgo biloba Leaf	Food Grade	Picked from Jiangsu Institute of Agriculture and Animal Husbandry Technology
Cellulase	5×10^{4}U/g	Nanning Pangbo Biological Co., Ltd.
Pectinase	3×10^{4}U/g	Nanning Pangbo Biological Co., Ltd.
S. cristatum	food grade	Nanning Pangbo Biological Co., Ltd.

2.2 Equipment and instruments

TABLE 2
EQUIPMENT AND INSTRUMENTS

Equipment and Instruments	Specifications	Manufacturer
Electronic balance	AL204 type	METTLER TOLEDO Instruments Co., Ltd.
Portable high-speed Chinese medicine grinder	FW135 type	Wenling Linda Machinery Co., Ltd.
Electric heating blast drying oven	101A-3E type	Wenling Linda Machinery Co., Ltd.
High performance liquid chromatograph	Lab Tech type	Lab Tech type Shanghai Tongwei Analytical Technology Co., Ltd.

2.3 Method

2.3.1 Technological process

Drying of Ginkgo Leaves → Smash → Enzymolysis → Fermentation → Extraction

↓

Cellulase and Pectinase

2.3.2 Operating points

Ginkgo biloba leaf pretreatment Screen the picked ginkgo leaves, rinse them with water, put them in a drying box for low-temperature drying, and then crush them with a Chinese medicine grinder, and pass through a 60-mesh sieve.

Ginkgo biloba enzymatic hydrolysis Take a certain amount of ginkgo biloba, add different amounts of enzyme (cellulase: pectinase=1:1), and add the same amount of water for enzymatic hydrolysis.

Using high performance liquid method, C18 column (5μm, 150mm×4.6mm), column temperature 25^{o}C.

2.3.3 Single factor test of ginkgo biloba enzymatic hydrolysis

The effect of the added amount of enzyme on the extraction rate of Ginkgo biloba enzymatic hydrolysis. The fixed enzymatic hydrolysis time is 2h, the enzymatic hydrolysis temperature is 45^{o}C, pH5, and the amount of added enzyme is 0.1%, 0.15%, 0.2%, 0.25%, 0.3% , 0.35%, 0.4%.

The effect of enzymolysis time on the extraction rate of Ginkgo biloba enzymatic hydrolysis. The amount of immobilized enzyme is 0.25%, the enzymatic hydrolysis temperature is 45^{o}C, and the pH is 5. The enzymatic hydrolysis time is respectively 0.5h, 1h, 1.5h, 2h, 2.5h, 3h, and 3.5h.

The effect of enzymatic hydrolysis temperature on the extraction rate of Ginkgo biloba enzymatic hydrolysis. The amount of immobilized enzyme is 0.25%, the enzymatic hydrolysis time is 2h, and the pH is 5. The enzymatic hydrolysis temperature is respectively 30^{o}C, 35^{o}C, 40^{o}C, 45^{o}C, 50^{o}C, 55^{o}C, 60^{o}C.

The effect of pH of enzymatic hydrolysis on the extraction rate of Ginkgo biloba leaves. The dosage of immobilized enzyme is 0.25%, the enzymatic hydrolysis time is 2h, the enzymatic hydrolysis temperature is 45^{o}C, and the enzymatic hydrolysis pH is 3, 3.5, 4, 4.5, 5, 5.5 , 6, 6.5 respectively.

2.3.4 Ginkgo biloba enzymatic hydrolysis response surface optimization scheme

On the basis of single factor test, select enzyme addition, enzymolysis time, enzymolysis temperature, enzymolysis pH as factors A, B, C, and D respectively to carry out a four-factor three-level response surface analysis test. The extraction rate of Ginkgo biloba flavonoids Y value, further optimize the best plan obtained by single factor, and analyze the interaction between each factor.

TABLE 3
ENZYMATIC HYDROLYSIS RESPONSE SURFACE ANALYSIS FACTORS AND LEVEL DESIGN

Level	Factor			
	A Enzyme dosage (%)	B Enzymatic hydrolysis time (h)	C Enzymolysis temperature (^{o}C)	D Enzymatic hydrolysis pH
-1	0.2	1.5	40	4
0	0.25	2	45	4.5
1	0.3	2.5	50	5

2.3.5 Enzymatic hydrolysis extraction rate algorithm

Extraction rate= Flavonoid content in filtrate/ Weight of Ginkgo biloba leaves×100%

2.3.6 Single factor test of ginkgo leaf fermentation

The influence of the amount of microorganisms on the extraction rate of Ginkgo biloba fermentation. Fixed fermentation temperature 25^{o}C, fermentation time 9d, fermentation pH5, select ginkgo biloba leaves after enzymatic hydrolysis (according to the optimal formula enzymatic hydrolysis), the amount of added microorganisms is 1 %, 2%, 3%, 4%, 5%, 6%, 7%.

The influence of fermentation temperature on the extraction rate of Ginkgo biloba fermentation. The amount of immobilized microorganisms is 4%, fermentation time is 9 days, and fermentation pH is 5. Select the ginkgo biloba after enzymatic hydrolysis (according to the optimal formula enzymatic hydrolysis), and the fermentation temperature is 10^{o}C respectively, 15^{o}C, 20^{o}C, 25^{o}C, 30^{o}C, 35^{o}C, 40^{o}C.

The influence of fermentation time on the extraction rate of Ginkgo biloba fermentation. The amount of immobilized microorganisms is 4%, the fermentation temperature is 25^{o}C, and the fermentation pH is 5. Select the Ginkgo biloba after enzymatic hydrolysis (according to the optimal formula), and the fermentation time is 2d respectively , 4d, 6d, 8d, 10d, 12d, 14d.

The influence of fermentation pH on the extraction rate of Ginkgo biloba fermentation. The amount of immobilized microorganisms is 4%, the fermentation temperature is 25^{o}C, and the fermentation time is 8d. Select the ginkgo biloba after enzymatic hydrolysis (according to the optimal formula enzymatic hydrolysis), and the fermentation pH is 3.5, 4, 4.5, 5, 5.5, 6, 6.5.

2.3.7 Ginkgo leaf fermentation response surface optimization plan for extraction rate

On the basis of the single factor test, select the microbial addition amount, fermentation temperature, fermentation time, and fermentation pH as factors A, B, C, and D to conduct a four-factor three-level response surface analysis test. The extraction rate of ginkgo leaf flavonoids is Y value, further optimize the best plan obtained by the single factor, and analyze the interaction between the factors. The response surface analysis factors and level design are shown in Table 4.

TABLE 4
FERMENTATION RESPONSE SURFACE ANALYSIS FACTORS AND LEVEL DESIGN

Level	Factor			
	A Microbial addition (%)	B Fermentation temperature (^{o}C)	C Fermentation time (d)	D Fermentation pH
-1	3	20	7	4.5
0	4	25	8	5
1	5	30	9	5.5

2.3.8 Fermentation extraction rate algorithm

Extraction rate= Flavonoid content in filtrate/ Weight of Ginkgo biloba leaves × 100%

III. RESULTS AND ANALYSIS

3.1 Enzymatic single factor test

3.1.1 The effect of different enzymes on the extraction rate of ginkgo flavonoids

FIGURE 1: The effect of enzyme addition on the extraction rate

As the amount of enzyme increases, the extraction rate first rises and then becomes gentle. When the added amount of enzyme reaches 0.25%, the extraction rate reaches the highest (Figure 1). It may be that cellulose can degrade the cellulose skeleton of the cell wall into glucose. As the amount of enzyme increases, it will destroy the cell wall more effectively and increase the dissolution of active substances in the cell [3]. Therefore, the added amount of selected enzyme is 0.25%.

3.1.2 The effect of different enzymatic hydrolysis time on the extraction rate of ginkgo flavonoids

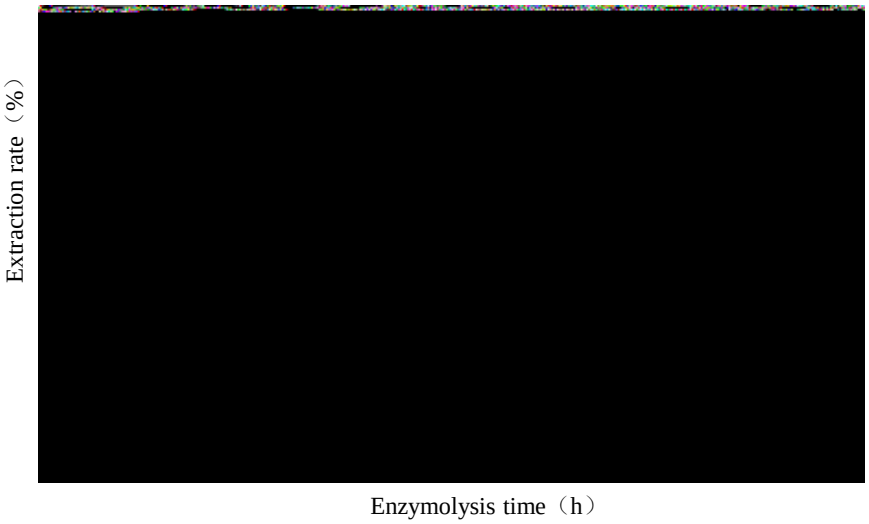

FIGURE 2: The effect of enzymolysis time on extraction rate

With the increase of enzymatic hydrolysis time, the extraction rate of ginkgo flavonoids increased significantly, and the overall trend increased first and then leveled off (Figure 2). When the enzymatic hydrolysis time is 2h, the extraction rate increases the most, and with the extension of time, the extraction rate tends to be flat. It may be that the high enzyme activity in the early stage of enzymatic hydrolysis makes the flavonoids continue to dissolve [4], and the extraction rate continues to increase, and then the time continues to increase, most of the substrates are enzymatically hydrolyzed, and the extraction rate decreases. Therefore, the optimal enzymatic hydrolysis time is 2h.

3.1.3 The effect of different enzymatic hydrolysis temperature on the extraction rate of ginkgo flavonoids

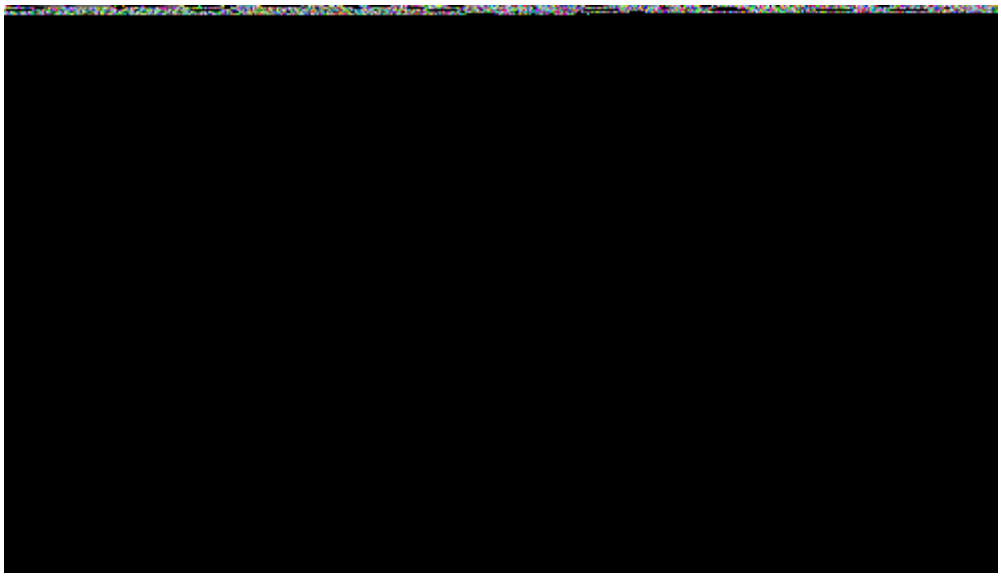

FIGURE 3: The effect of enzymatic hydrolysis temperature on the extraction rate

The temperature increases, the extraction rate first increases and then decreases (Figure 3). When the temperature reaches 45°C, the extraction rate is highest. When the temperature is between 30°C and 45°C, as the temperature increases, the enzyme activity continues to increase, causing the cell wall to continue to degrade and increase the extraction rate of ginkgo flavonoids [5]. When the temperature is between 45°C and 60°C, the temperature increases continuously and the enzyme activity decreases, thereby reducing the extraction rate of ginkgo flavonoids. Therefore, the optimal enzymolysis temperature is 45^{o}C.

3.1.4 The effect of different enzyme hydrolysis pH on the extraction rate of ginkgo flavonoids

FIGURE 4: The effect of enzymatic hydrolysis pH on the extraction rate

The pH of enzymatic hydrolysis increases, the extraction rate first increases and then decreases (Figure 4). When the pH of the enzymatic hydrolysis is 4.5, the extraction rate of ginkgo flavonoids reaches the maximum. At this time, the enzymatic hydrolysis is complete, the efficiency of destroying the cell wall increases sharply, and the mass transfer resistance is reduced [6]. When the pH of enzymatic hydrolysis is in the range of 4.5-6.5, the pH of enzymatic hydrolysis continues to increase, the enzyme activity decreases, and the extraction rate of ginkgo flavonoids decreases. Therefore, choose the best enzymatic hydrolysis pH 4.5.

3.2 Enzymatic hydrolysis response surface test results

3.2.1 Response surface analysis of the extraction rate of enzymatic hydrolysis

TABLE 5
DESIGN RESULTS OF ENZYMATIC RESPONSE SURFACE TEST

Test number	Factor				Extraction rate（%）
	Enzyme addition (%)	Enzymatic hydrolysis time (h)	Enzymolysis temperature (^{o}C)	Enzymatic hydrolysis pH	
1	0.2	1.5	45	4.5	12.82
2	0.3	1.5	45	4.5	13.23
3	0.2	2.5	45	4.5	13.92
4	0.3	2.5	45	4.5	11.84
5	0.25	2	40	4	12.65
6	0.25	2	50	4	12.84
7	0.25	2	40	5	13.42
8	0.25	2	50	5	12.34
9	0.2	2	45	4	12.22
10	0.3	2	45	4	11.55
11	0.2	2	45	5	13.36
12	0.3	2	45	5	12.88
13	0.25	1.5	40	4.5	13.11
14	0.25	2.5	40	4.5	12.44
15	0.25	1.5	50	4.5	11.64
16	0.25	2.5	50	4.5	11.89
17	0.2	2	40	4.5	12.22
18	0.3	2	40	4.5	12.34
19	0.2	2	50	4.5	11.67
20	0.3	2	50	4.5	12.44
21	0.25	1.5	45	4	14.22
22	0.25	2.5	45	4	14.06
23	0.25	1.5	45	5	10.98
24	0.25	2.5	45	5	11.21
25	0.25	2	45	4.5	16.42
26	0.25	2	45	4.5	15.67
27	0.25	2	45	4.5	17.36
28	0.25	2	45	4.5	14.83
29	0.25	2	45	4.5	16.66

3.2.2 Establishment and analysis of a fitting model for the extraction rate of flavonoids from ginkgo leaves by enzymatic hydrolysis

Perform regression analysis on Table 5 to obtain the second order multiple numbers of the extraction rate (Y) and the independent variable enzyme addition amount (A), enzymolysis time (B), enzymolysis temperature (C), enzymolysis pH (D) The regression equation is:

$$Y=16.19-0.16A-0.053B-0.28C-0.28D-0.62AB+0.16AC-0.048AD+0.23BC+0.097BD-0.32CD-1.84A^2-1.73B^2-2.02C^2-1.68D^2$$

TABLE 6
ANALYSIS OF VARIANCE TABLE

Source of Variance	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-value	P-value	Significance
Model	59.74	14	4.27	3.68	0.0102	*
A Enzymatic hydrolysis addition amount	0.31	1	0.31	0.27	0.6128	
B Enzymatic hydrolysis time	0.034	1	0.034	0.029	0.8662	
C Enzymolysis temperature	0.94	1	0.94	0.81	0.3827	
D Enzymolysis pH	0.95	1	0.95	0.82	0.3841	
AB	1.55	1	1.55	1.34	0.2667	
AC	0.11	1	0.11	0.091	0.7671	
AD	9.03E-03	1	9.03E-03	7.79E-03	0.9309	
BC	0.21	1	0.21	0.18	0.6756	
BD	0.038	1	0.038	0.033	0.8588	
CD	0.4	1	0.4	0.35	0.5646	
A^2	21.88	1	21.88	18.89	0.0007	**
B^2	19.36	1	19.36	16.72	0.0011	**
C^2	26.54	1	26.54	22.91	0.0003	**
D^2	18.34	1	18.34	15.83	0.0014	**
Residual	16.22	14	1.16			
Lack of fit error	12.45	10	1.25	1.32	0.423	
Pure error	3.76	4	0.94			
sum	75.96	28				
R^2	0.7625					
R^2adj	0.5782					

*****Indicates extremely significant difference (P<0.01), *Indicates a significant difference (P<0.05)***

The p-value is used to test the importance of influencing factors, and its value can also reflect the interaction between influencing factors. The smaller the p-value, the more important this influencing factor is. It can be seen from Table 6 that the addition amount of enzymatic hydrolysis (A), enzymatic hydrolysis time (B), enzymatic hydrolysis temperature (C), enzymatic hydrolysis pH (D), enzymatic hydrolysis addition amount and enzymatic hydrolysis time (AB) contribute to the extraction of flavonoids from Ginkgo biloba leaves Rates have an impact, while other factors have less impact. The contribution rate is tested by the F value, and the order of the significance of each response factor to the response value is B>A>C>D, and the F value of the model is 3.68.

3.2.3 Response surface interaction of Ginkgo flavonoid extraction rate

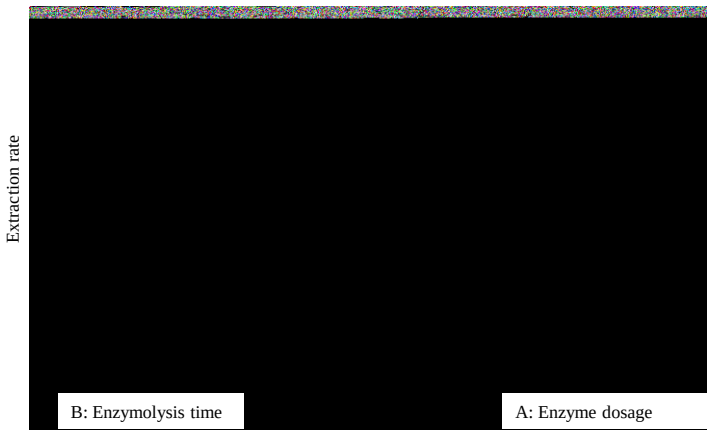

FIGURE 5: The effect of addition amount of enzymatic hydrolysis and enzymatic hydrolysis time on extraction rate

The oval shape of the graph is not obvious, indicating that the interaction of the two factors is not obvious (Figure 5). The extraction rate of ginkgo flavonoids first increased and then decreased with the amount of enzymatic hydrolysis, and first increased and then decreased with the enzymatic hydrolysis time; the two factors increased in similar magnitude

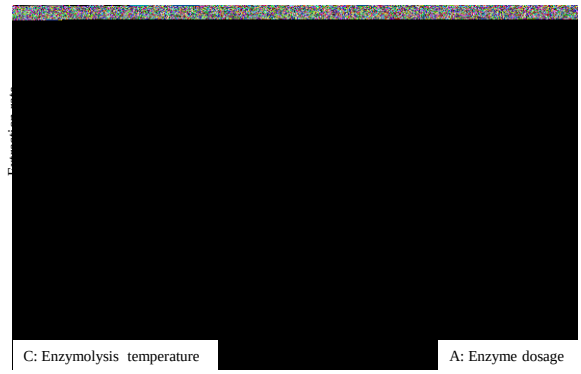

FIGURE 6: The effect of the amount of enzymatic hydrolysis and the temperature of enzymatic hydrolysis on the extraction rate

The ellipse of the graph is not obvious, indicating that the interaction of the two factors is not obvious (Figure 6). The extraction rate of ginkgo flavonoids first increased and then decreased with the amount of enzymatic hydrolysis, and first increased and then decreased with the enzymatic hydrolysis temperature; the two factors increased in similar degrees.

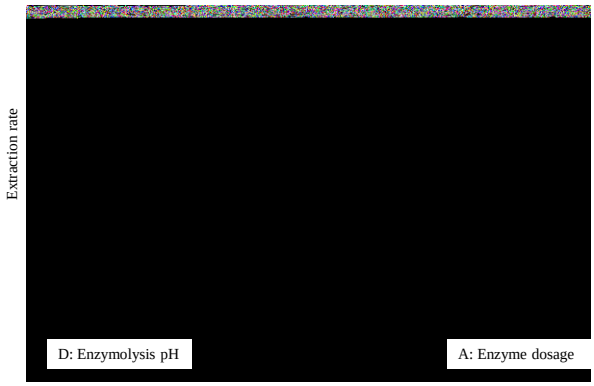

FIGURE 7: The effect of enzyme hydrolysis addition amount and enzyme hydrolysis pH on the extraction rate

The oval shape of the graph is not obvious, indicating that the interaction of the two factors is not obvious (Figure 7). The extraction rate of ginkgo flavonoids increased first and then decreased with the amount of enzymatic hydrolysis, and the pH increased first and then decreased with the enzymatic hydrolysis; the two factors increased in similar ranges.

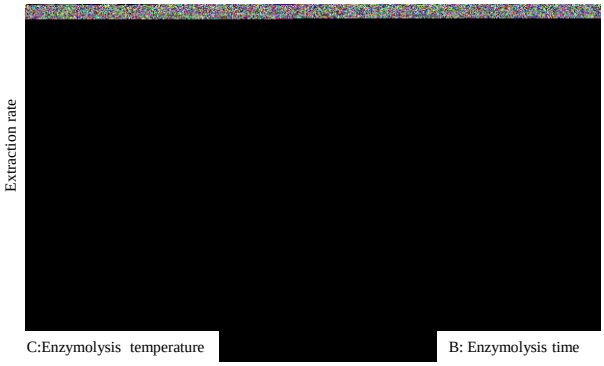

FIGURE 8: The effect of hydrolysis time and temperature on the extraction rate

The oval shape of the graph is not obvious, indicating that the interaction of the two factors is not obvious (Figure 8). The extraction rate of ginkgo flavonoids first increased and then decreased with the enzymatic hydrolysis time, and first increased and then decreased with the enzymatic hydrolysis temperature; the two factors rose similarly.

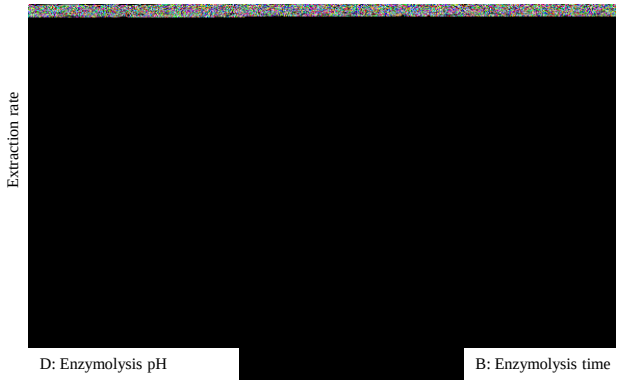

FIGURE 9: The effect of enzymolysis time and pH on the extraction rate

It can be seen from Figure 9 that the ellipse of the figure is not obvious, indicating that the interaction of the two factors is not obvious. The extraction rate of ginkgo flavonoids first increased and then decreased with the enzymatic hydrolysis time, and the pH increased first and then decreased with the enzymatic hydrolysis; the two factors had similar rises.

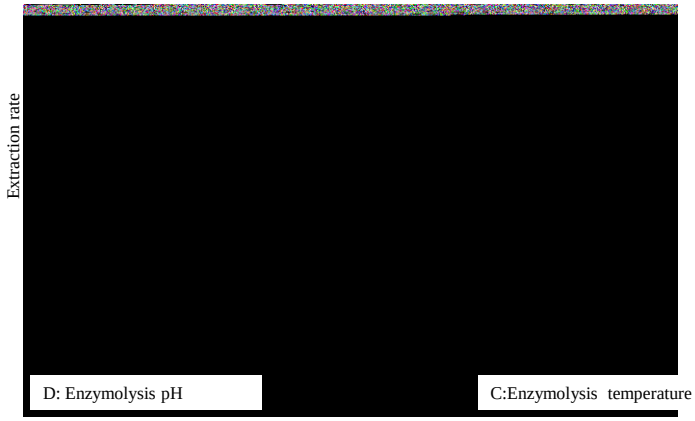

FIGURE 10: The effect of enzymolysis temperature and pH on the extraction rate

The ellipse of the figure is not obvious, indicating that the interaction of the two factors is not obvious (Figure 10). The extraction rate of ginkgo flavonoids first increased and then decreased with the enzymatic hydrolysis temperature, and the pH increased first and then decreased with the enzymatic hydrolysis; the two factors had similar rises.

According to response surface analysis, the optimal amount of enzyme addition is 0.2%, the enzymolysis time is 2h, the enzymolysis temperature is 45^{o}C, and the enzymolysis pH is 4.5.

3.3 Fermentation single factor test

3.3.1 The effect of different microorganisms on the extraction rate of ginkgo flavonoids

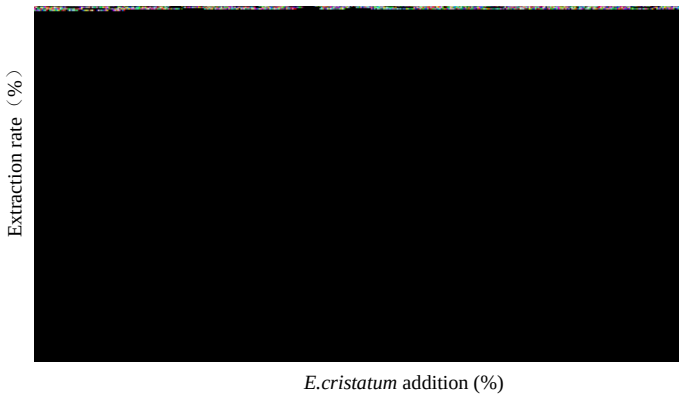

FIGURE 11: The effect of microbial addition on the extraction rate

With the increase of the amount of microorganisms added, the extraction rate shows a trend of increasing first and then gentle (Figure 11). When the amount of microorganisms added is in the range of 1% to 4%, the amount of microorganisms continues to increase and the extraction rate continues to rise. When the amount of microorganisms added was 4%, the amount of microorganisms continued to increase, and the extraction rate rose slowly and was not significant. Therefore, a 4% microbial addition was selected [7].

3.3.2 The effect of different fermentation temperature on the extraction rate of ginkgo flavonoids

FIGURE 12: The influence of fermentation temperature on extraction rate

As the fermentation, temperature increases the extraction rate of ginkgo flavonoids increases first and then decreases. When the fermentation temperature reaches 25°C, the extraction rate is significantly higher than that at other fermentation temperatures (Figure 12). It shows that at this time, the microorganisms reach the optimum growth temperature, and increasing the temperature will hinder the growth of the microorganisms [8], and the extraction rate will decrease. Therefore, the optimal fermentation temperature was selected as 25°C.

3.3.3 The effect of different fermentation time on the extraction rate of ginkgo flavonoids

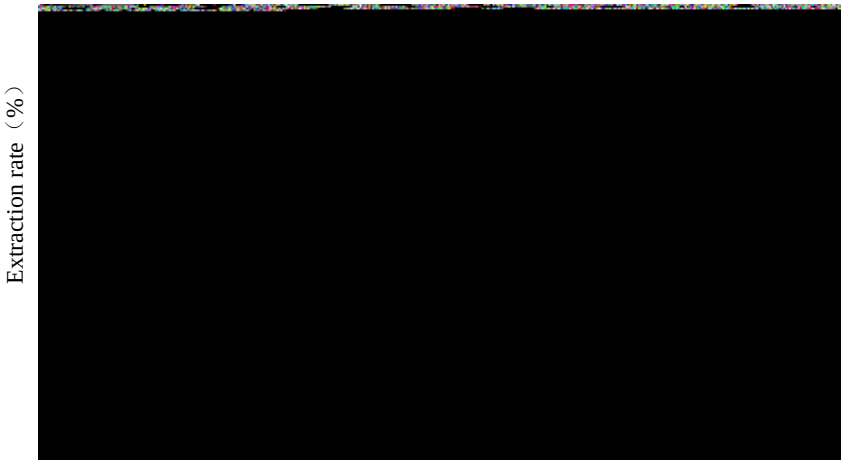

FIGURE 13: The effect of fermentation time on extraction rate

As the fermentation time continues to increase, the extraction rate of ginkgo flavonoids increases first and then becomes gentle (Figure 13). When the fermentation time is within 2-8 days, the extraction rate increases with the increase of the fermentation time, and when the fermentation time continues to increase, the extraction rate does not increase significantly. Probably because the fermentation product grew well in the early stage of fermentation [9], the extraction rate increased. Therefore, the fermentation time is 8 d.

3.3.4 The effect of different fermentation pH on the extraction rate of ginkgo flavonoids

FIGURE 14: The influence of fermentation pH on extraction rate

As the fermentation pH increases, the extraction rate of ginkgo flavonoids increases first and then decreases (Figure 14). When the fermentation pH is in the range of 3.5 to 5, the extraction rate will continue to increase as the fermentation pH increases, and when the fermentation pH continues to increase, the extraction rate will continue to decrease. Probably because of the good growth environment at the initial stage of fermentation, the extraction rate continued to increase [10]. Therefore, a fermentation pH of 5 was selected.

3.4 Analysis of fermentation response surface test results

3.4.1 Response surface results of fermentation extraction rate

TABLE 7
DESIGN SCHEME AND RESULTS OF FERMENTATION RESPONSE SURFACE

Test number	Factor				Extraction rate (%)
	Enzymatic hydrolysis addition amount (%)	Fermentation Temperature (^{o}C)	Fermentation Time (d)	Fermentation pH	
1	3	20	8	5	12.78
2	5	20	8	5	13.23
3	3	30	8	5	13.92
4	5	30	8	5	11.84
5	4	25	7	4.5	12.56
6	4	25	9	4.5	12.78
7	4	25	7	5.5	13.42
8	4	25	9	5.5	12.34
9	4	25	8	4.5	12.22
10	5	25	8	4.5	11.78
11	3	25	8	5.5	13.36
12	5	25	8	5.5	12.86
13	4	20	7	5	13.21
14	4	30	7	5	12.44
15	4	20	9	5	11.64
16	4	30	9	5	11.87
17	3	25	7	5	12.22
18	5	25	7	5	12.34
19	3	25	9	5	11.86
20	5	25	9	5	12.44
21	4	20	8	4.5	14.22
22	4	30	8	4.5	14.06
23	4	20	8	5.5	11.38
24	4	30	8	5.5	11.51
25	4	25	8	5	16.32
26	4	25	8	5	15.67
27	4	25	8	5	16.63
28	4	25	8	5	14.97
29	4	25	8	5	16.16

3.4.2 Establishment and analysis of a fitting model for the extraction rate of flavonoids from fermented ginkgo leaves

Regression analysis is performed on Table 7, and the second order polynomial regression equation of extraction rate (Y) for the number of microorganisms (A), fermentation temperature (B), fermentation time (C), and fermentation pH (D) coding values is:

$$Y=15.95-0.16A-0.068B-0.27C-0.23D-0.63AB+0.12AC-0.015AD+0.25BC+0.072BD-0.32CD-1.71A^2-1.56B^2-1.93C^2-1.51D^2$$

TABLE 8
ANALYSIS OF VARIANCE TABLE

Source of Variance	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-value	P-value	Significance
Model	51.65	14	3.69	4.43	0.0043	**
A Enzymatic hydrolysis addition amount	0.29	1	0.29	0.35	0.5636	
B Fermentation temperature	0.056	1	0.056	0.067	0.7991	
C Fermentation time	0.89	1	0.89	1.06	0.32	
D Fermentation pH	0.63	1	0.63	0.76	0.3991	
AB	1.6	1	1.6	1.92	0.1874	
AC	0.053	1	0.053	0.064	0.8047	
AD	9.00E-04	1	9.00E-04	1.08E-03	0.9742	
BC	0.25	1	0.25	0.3	0.5924	
BD	0.021	1	0.021	0.025	0.876	
CD	0.42	1	0.42	0.51	0.4881	
A^2	19.05	1	19.05	22.87	0.0003	**
B^2	15.73	1	15.73	18.89	0.0007	**
C^2	24.16	1	24.16	29	< 0.0001	**
D^2	14.77	1	14.77	17.72	0.0009	**
Residual	11.66	14	0.83			
Lack of fit error	9.98	10	1	2.37	0.2101	
Pure error	1.68	4	0.42			
sum	63.31	28				
R^2	0.7539					
R^2adj	0.5263					

*****Indicates extremely significant difference (P<0.01), *Indicates a significant difference (P<0.05)***

Table 8 shows that the amount of microorganisms added (A), fermentation temperature (B), fermentation time (C), fermentat ion pH (D), amount of microorganisms added and fermentation time (AC), amount of microorganisms added and fermentatio n pH (AD), Fermentation temperature and fermentation time (BC) have a significant effect on the extraction rate of ginkgo fl avonoids. From the F value test of the contribution rate, the order of the significance of each response factor to the response v alue is C>D>A>B.

3.4.3 Response surface interaction of extraction rate of fermented ginkgo leaves

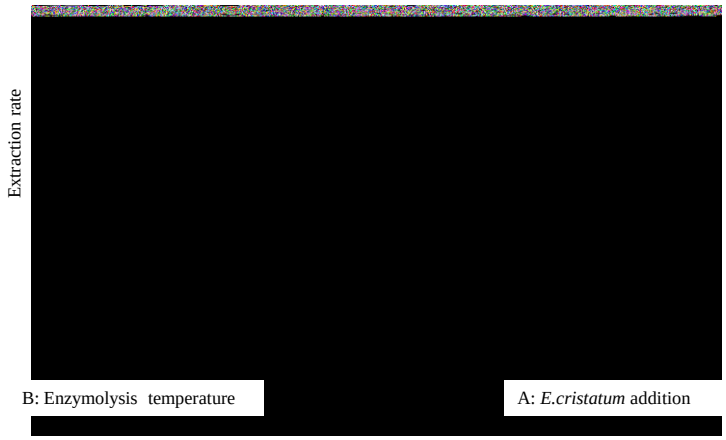

FIGURE 15: The effect of microbial addition and fermentation temperature on extraction rate

The oval shape of the graph is not obvious, indicating that the interaction of the two factors is not obvious. The extraction rate of ginkgo flavonoids first increased and then decreased with the amount of microorganisms added, and first increased and then decreased with the fermentation temperature (Figure 15).

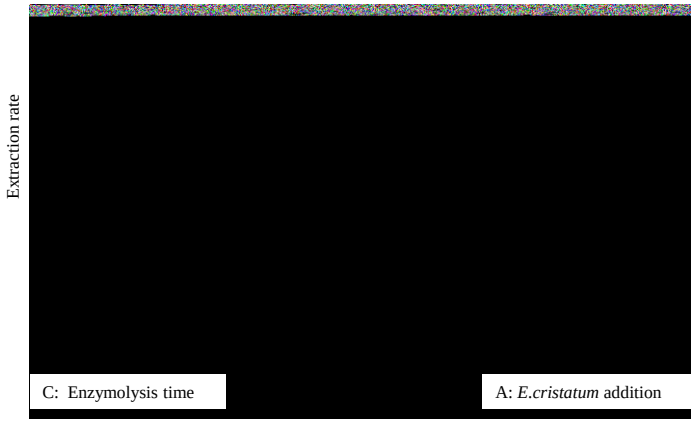

FIGURE 16: The effect of microbial addition and fermentation time on extraction rate

It can be seen from Figure 16 that the ellipse of the figure is not obvious, indicating that the interaction of the two factors is not obvious. The extraction rate of ginkgo flavonoids first increased and then decreased with the amount of microorganisms added, and first increased and then decreased with the fermentation time.

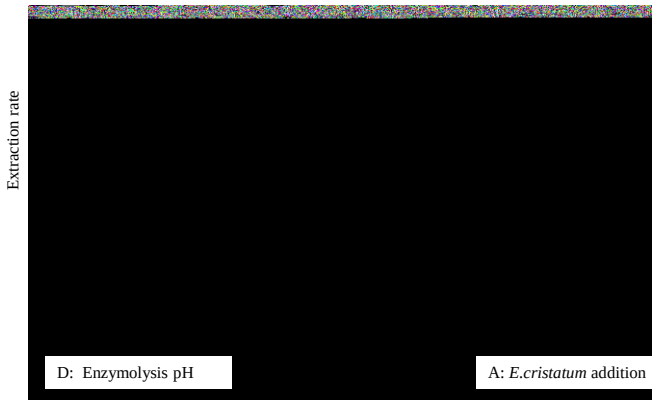

FIGURE 17: The effect of microbial addition and fermentation pH on extraction rate

It can be seen from Figure 17 that the oval shape of the graph is not obvious, indicating that the interaction of the two factors is not obvious. The extraction rate of ginkgo flavonoids first increased and then decreased with the amount of microorganisms added, and the pH of the fermentation first increased and then decreased.

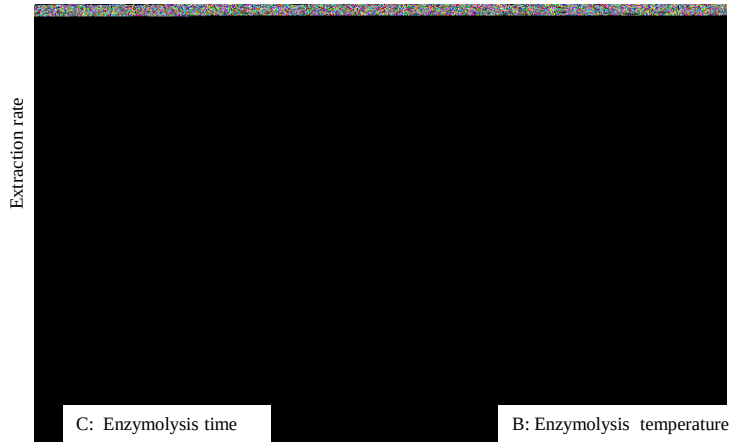

FIGURE 18: The influence of fermentation temperature and fermentation time on extraction rate

The ellipse shape of the graph is obvious, indicating that the two factors interact significantly. The extraction rate of ginkgo flavonoids first increased and then decreased with the fermentation temperature, and first increased and then decreased with the fermentation time (Figure 18).

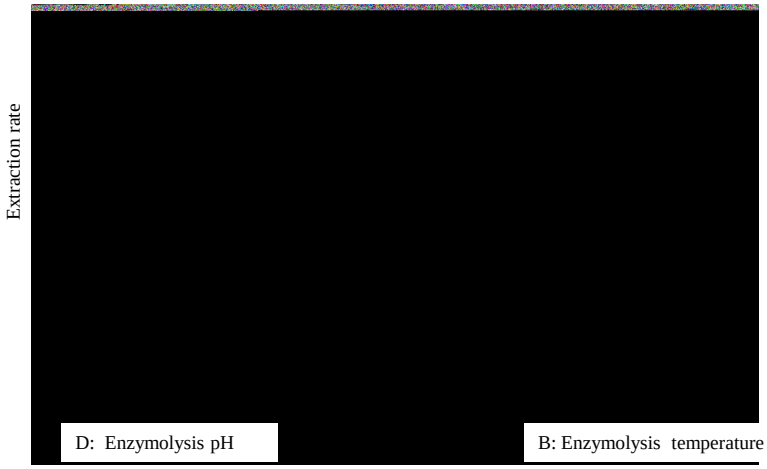

FIGURE 19: The influence of fermentation temperature and fermentation pH on extraction rate

The ellipse of the graph is not obvious, indicating that the interaction of the two factors is not obvious. The extraction rate of ginkgo flavonoids first increased and then decreased with the fermentation pH, and first increased and then decreased with the fermentation temperature (Figure 19).

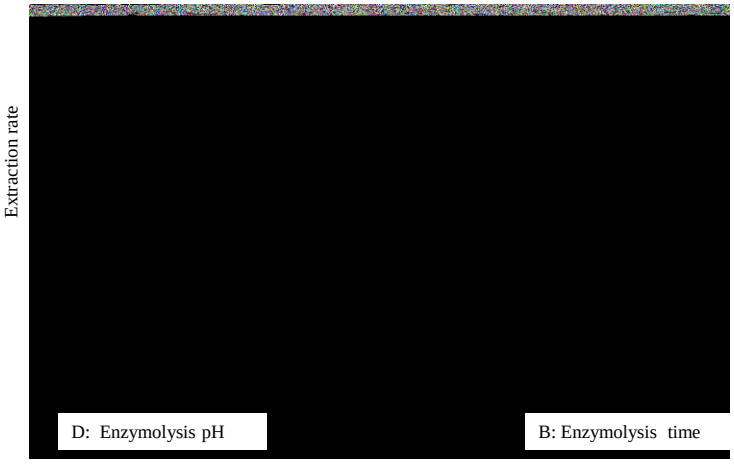

FIGURE 20: The influence of fermentation time and fermentation pH on extraction rate

It can be seen from Figure 20 that the oval shape of the graph is not obvious, indicating that the interaction of the two factors is not obvious. The extraction rate of ginkgo flavonoids first increased and then decreased with the fermentation time, and the pH increased first and then decreased with the fermentation.

Based on response surface analysis, the optimal fermentation conditions are 4% microbial addition, fermentation temperature 30^{o}C, fermentation time 8d, and fermentation pH5.

IV. CONCLUSION

In this paper, ginkgo was used as raw material, and the results of enzymatic hydrolysis and fermentation were determined according to the extraction rate through single factor experiment and orthogonal experiment of enzymatic hydrolysis and fermentation. The optimal enzyme addition amount for enzymolysis is 0.2%, the enzymolysis time is 2h, the enzymolysis temperature is 45^{o}C, and the enzymolysis pH is 4.5; the optimal microorganism addition amount for fermentation is 4%, the fermentation temperature is 30^{o}C, the fermentation time is 8d, and the fermentation pH is 5, The extraction rate was 18.56%.

ACKNOWLEDGMENT

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Social and Environmental Concerns of Flower Farms in Central Ethiopia

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Abstract— *The extensive use of fertilizers and pesticides in the flower farming industries has been linked to negative environmental and social impacts. The cross-sectional study was conducted to assess social and environmental concerns of flower farms in Central Ethiopia using questionnaires, focus group discussion and field visits. This study revealed that 317 (52.75%) of respondents reported that flower farms have been disposing of their flower residue of in the open field. The findings of this study showed that 216(36%) of inhabitants buy or receive empty chemical bags and containers that had been disposed by the flower farms. Focus Group Discussion participants perceived the decrease in volume and quality of groundwater, a decrease in productivity, land degradation, and increased emerging diseases due to the existence of flower farms in the area.. In addition, they reported abuse of employee rights, displacement of farmers from fertile land, death of cattle and fish, loss of acceptance for their agricultural and fish products. In conclusion, this study revealed that there are a poor waste management and unsustainable activities by the flower farms. The government should closely monitor these farms and undergo a holistic study to quantify environmental and local inhabitant's opportunity costs of flower farming activity.*

Keywords— Flower farm, Waste management, Environmental pollution, Pesticides, Fertilizer, Human health.

I. INTRODUCTION

Ethiopia started to enter the flower export market in the mid- 1990s at the time when the European Union (EU) market was much more demand-driven, and as a result, increasingly stringent standards and regulations had been instituted. In less than a decade, the country became the fifth largest non-EU flower exporter to the EU market and the second-largest exporter from Africa, surpassing all early exporter countries except Kenya (Gebreeyesus & Sonobe, 2012; Gezmu, 2013). Ethiopia generated over 178 million USD from flower exports. Although the contribution of the sector to GDP growth is undeniable, many scholars are doubtful about the long-term impacts of this sector on the environment and welfare of the rural families, in areas where flower farms are developed (Gezmu, 2013).

Due to the rapid growth of the floriculture industry, flower farms in Ethiopia have imported 96 types of insecticides and nematicides, and 105 types of fungicides from 2007 to 2014 (Tilahun 2013; MoA, 2014). Most growers rank pesticides second on their list of expenditures, next to international (air) transport costs (Mengistie *et al*., 2017). As a result, many have become concerned about the potential adverse environmental impacts of flower farms. Fertilizers and pesticides used extensively in the industry have been linked to negative environmental and health impacts (Getu, 2009; Gadaa, 2010; Hatch & Wells, 2012).Pesticides (including herbicides, insecticides, fungicides, etc.) can contaminate organisms, soil, water, turf, and other vegetation (Hatch & Wells, 2012). The adverse effect of pesticide use includes degrading water and soil quality, the effect on non-targeted lives like soil organisms, aquatic life, human beings, insects, cattle, etc, air pollution, and increase of pesticide resistance by targeted pests (Getu, 2009; Tilahun, 2013).

On other hand, the fact that they are often harmful to the environment, fertilizers are used in many different forms of agriculture to increase the level of crop production by adding nutrients to the soil that benefit the growth of plants (Getu, 2009). The residue of these fertilizers can cause water pollution, eutrophication of freshwaters, and increased nitrate concentrations in ground and surface waters (Hatch & Wells, 2012). The long-term use of inorganic fertilizers can also be detrimental to the soil because it can kill nitrogen-fixing bacteria and other beneficial organisms (Pimentel *et al*., 1995). As a result, more fertilizers are applied each year to make up for the loss of natural microorganisms and micro-nutrients (Getu, 2009; Hatch & Wells, 2012).

Many studies were performed focusing on occupational health, employee's rights, water pollution, soil pollution, waste management, and so on. However, there are none or a few who collected data from the surrounding residents who can give better testimony regarding the health impacts, the local inhabitants' benefit, the solid waste management practice, and social

complaints of flower farming industries. It is important to collect data from a different source to generate reliable information. The local inhabitants are the mosaic of the industry employee, the farmers, and other residents; they can be taken as watchdog that is following what is happening inside the compound as well as the surrounding environment. Therefore, in this study, the social and environmental concerns witnessed by nearby inhabitants of flower farms were tried to be assessed.

II. MATERIAL AND METHODS

2.1 Study Areas

This study assessed the environmental and social consequences inhabitants living around five flower farms (Farm 1, Farm 2, Farm 3, Farm 4, and Farm 5) in Central Ethiopia. The flower farms' location was depicted in Figure 1. Farm 1 and Farm 2 are found in the Southwest Shewa zone (Woliso Woreda and Bacho Woreda, respectively). Farm 3 and Farm 4 are found in the West Shewa zone (Walmera Woreda). And, Farm 5 is located in the East Shewa zone, Adami Tulu Jido Kombolcha Woreda. These flower farms were purposely selected for this study based on the intensity of the social complain as per the local Environment, Forest, and climate change authority recommendation.

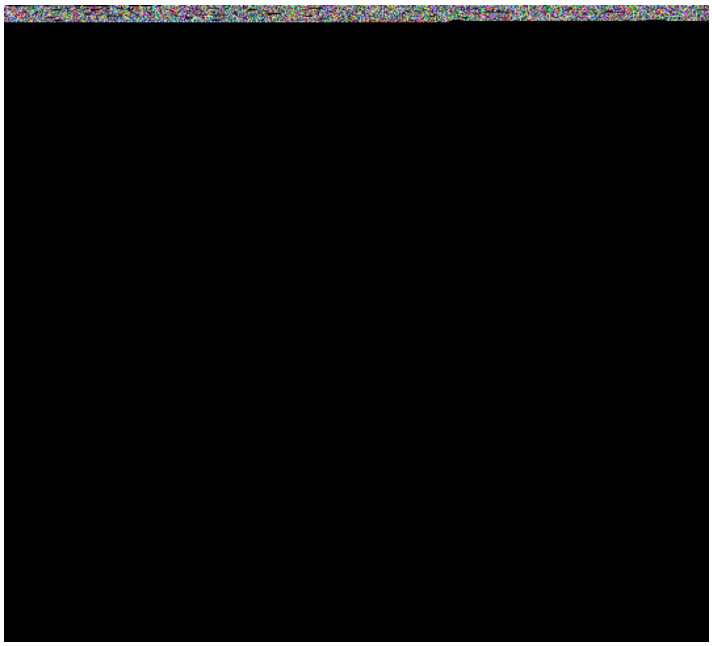

FIGURE 1: Study areas

2.2 Study Design and Period

The cross-sectional study was conducted to assess social and environmental consequences observed by nearby inhabitants living within a 2 km radius of flower farms in 2019 using questionnaires, focus group discussion (FGD), and field visit.

2.3 Sample Size Determination

The sample size was determined using a Cochrans formula (G*len, 2020)* at a 95% confidence interval and 4 margin of error. Systematic random sampling techniques were employed to determine the number of samples per study site. A total of 601 sample size was determined which is allocated to the flower farms based on the land surface area the flower farms have occupied.

2.4 Sampling Technique

It is not appropriate to use the whole residents of a kebele to determine study subjects for this specific study. Therefore, we have set a benchmark indicating a 2 km distance from the flower farm in four directions using GPS and collected data from those living in the specified distance. The sample size per flower farm was determined based on the assumption of "The land area the flower farms are using is proportional to the number of residents affecting." Accordingly, the data were collected from 53, 53, 53, 53, and 389 inhabitants in a 2km radius of Farm 1, Farm 2, Farm 3, Farm 4, and Farm 5, respectively.

2.5 Data Collection Process

Data was collected by urban health extension professionals and experts from concerned woreda Environment, Forest, and climate change Authority who has taken detail orientation on data collection tool. Data was gathered from randomly selected individuals of inhabitants around flower farms by questionnaire and four Focus group discussion (FGD) discussion having 15 members in which farmers, residents, and concerned experts have participated. In addition, field observations were also held using checklist and camera.

III. RESULTS AND DISCUSSION

3.1 Socio-demographic characteristics of the respondents

A total of 601 households (HHs) were included in this study. Among households' study participants, 317(52.75%) were male and 284 (47.25%) were females. The majority of the study participants were found in the age range of 20-45 (77.7%). Regarding the occupational and educational status of respondents, 14(2.33%), 125(20.80%), 224(37.27%), 97(16.14%), of the respondents were government workers, private business, farmer, and unemployed, respectively. Among study participants, 147(24.46%), 217(36.11%), 136(22.63%), 52(8.65%) of them reported as they had grade 9-12, grade 1-8, illiterate, can read and write, respectively. Four hundred ninety-eight (82.86%) respondents were lived in the area for more than 11 years. The results are presented in Table 1.

TABLE 1
SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE RESPONDENTS

Characteristics		IA Farm 1		IA Farm 2		IA Farm 3		IA Farm 4		IA Farm 5		Total	
		Frequency	%	Frequency	%	Frequency	%	Frequency	%	Frequency	%	Frequency	%
Gender	Male	40	75.47	33	62.26	31	58.49	32	60.38	181	46.53	317	52.75
	Female	13	24.53	20	37.74	22	41.51	21	39.62	208	53.47	284	47.25
	Total	53	100	53	100	53	100	53	100	389	100	601	100
Age	20-35	9	16.98	21	39.62	15	28.30	20	37.74	189	48.59	254	42.26
	36-45	19	35.85	18	33.96	17	32.08	19	35.85	140	35.99	213	35.44
	>45	25	47.17	14	26.42	21	39.62	14	26.42	60	15.42	134	22.30
	Total	53	100	53	100	53	100	53	100	389	100	601	100
Educational status	First Degree	0	0.00	2	3.77	0	0.00	0	0.00	8	2.06	10	1.66
	10 + 3	0	0.00	0	0.00	0	0.00	0	0.00	40	10.28	40	6.66
	Grade 9 to 12	17	32.08	11	20.75	14	26.42	9	16.98	96	24.68	147	24.46
	Grade 1 to 8	18	33.96	11	20.75	20	37.74	15	28.30	153	39.33	217	36.11
	Illiterate	2	3.77	25	47.17	10	18.87	25	47.17	74	19.02	136	22.63
	Read and write	16	30.19	4	7.55	9	16.98	4	7.55	19	4.88	52	8.65
	Total	53	100	53	100	53	100	53	100	389	100	601	100
Type of job	Government	0	0.00	0	0.00	1	1.89	0	0.00	13	3.34	14	2.33
	Private business	2	3.77	7	13.21	0	0.00	3	5.66	113	29.05	125	20.80
	Farmer	46	86.79	43	81.13	31	58.49	28	52.83	76	19.54	224	37.27
	Unemployed	0	0.00	1	1.89	0	0.00	20	37.74	76	19.54	97	16.14
	Other	5	9.43	2	3.77	21	39.62	2	3.77	111	28.53	141	23.46
	Total	53	100	53	100	53	100	53	100	389	100	601	100
Residence duration in years	<5	0	0.00	0	0.00	0	0.00	3	5.66	8	2.06	11	1.83
	5 to 10	0	0.00	2	3.77	0	0.00	13	24.53	77	19.79	92	15.31
	11 to 20	7	13.21	10	18.87	2	3.77	7	13.21	104	26.74	130	21.63
	>20	46	86.79	41	77.36	51	96.23	30	56.60	200	51.41	368	61.23
	Total	53	100	53	100	53	100	53	100	389	100	601	100

3.2 Waste management gap of flower farms

Waste can be produced during each process of flower farming. It is estimated that up to 500 tons of residues per hectare per year are generated from flower farms (Tilahun, 2013). The Anano village residents, one of Adami Tullu Jido Kombolcha district villages, use flower farm residue to feed their cattle as an alternative feedstuff especially during the scarcity of fodder (Figures 2a and 2b). It is becoming common to see when cattle are eating flower residue from the flower farms in the village. During field observation, the residents reported that they had encountered scarcity of fodder to feed their cattle especially in dry seasons; when most fields are bared for grazing. Consequently, the inhabitants are forced to use cut flower residue that they get it by purchase. Even this harmful flower waste is not affordable for most farmers to buy. The residents added that the flower farm they are buying smells like a dead body. This could be an indication of chemically contaminated wastes. The residents of the village were asked if the flower residue feedstuff has solved their problem. Accordingly, they have replied that the feedstuff has helped them to sustain the life of their cattle. On another way, the users of the cut flower residue reported that they haven't enough awareness if their practice can harm cattle and human health in the long term. However, plants grown in farms where pesticides are applied may thus become contaminated and consequently, pesticide residues are transferred to milk when these plants are fed to cows (Ismail, 2009). Several studies in tropical areas showed positive milk samples (Asselt *et al*., 2016).

FIGURE 2: Pictures showing cattle feeding on flower residue

In addition, we observed wastewater is discharged from the compound of some flower farms. As it was tried to illustrate in Figures 3a and 3b, this discharged wastewater is added to the nearby water body, drink up by cattle, or fetched by residents for uses.

FIGURE 3: Pictures showing the waste management gap of flower farms

3.3 Respondents flower farm empty chemical container use status

Another environmental concern in the flower farming is the unsafe management of pesticide containers (Tilahun, 2013; Mengistie *et al*., 2017). To assess whether they receive/buy chemical bag/containers from flower farms, residents living

around flower farms were asked. The result showed that 216 (35.94%) of inhabitants receive/buy the chemical bags/containers, respectively. Residents reported that they get the materials from guards and employees of the flower farms (Figure 4).

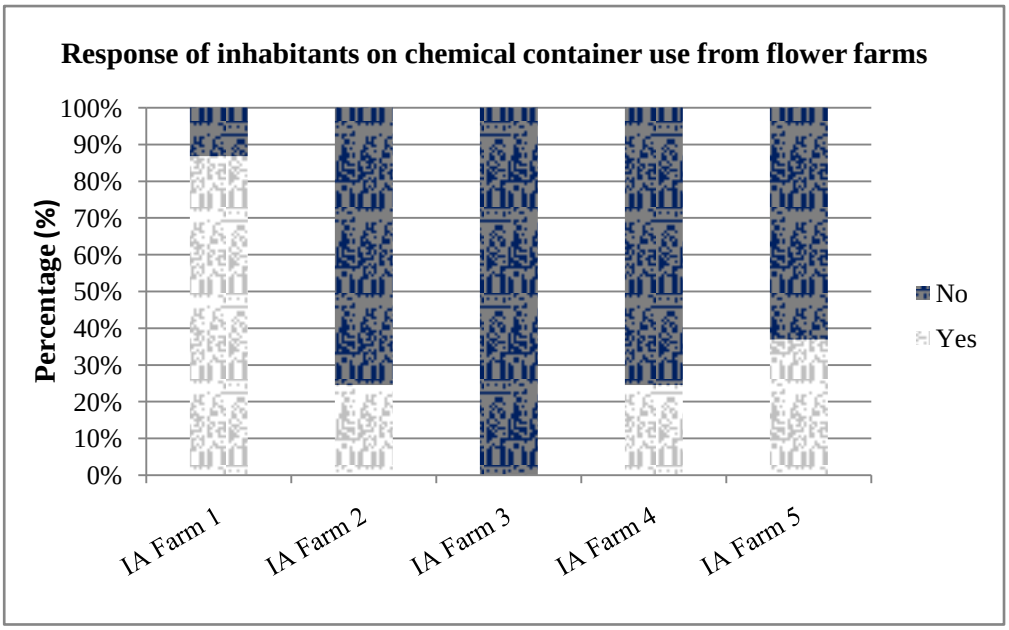

FIGURE 4: Response of inhabitants on chemical container use discharged from flower farms.

The local inhabitants were also asked for what purpose they use the chemical container they received/bought. Accordingly, 28(60.87%), 11(23.91%), and 7(15.22%) of respondents around Farm 1 use it to fetch and store water, to make and store Tella and Areki (Cultural Alcoholic drink in Ethiopia), and for sale, respectively. Whereas all inhabitants around Farm 2, reported they use it to fetch and store water, only. Regarding inhabitants around Farm 4, 8(61.54%) and 5(38.46%) use it to fetch and store water, and to make and store Tella and Areki, respectively. While, 102(70.83%), 17(11.81%), and 15(10.42%) of inhabitants around Farm 5 use it to fetch and store water, for house shade, and to make and store Tella and Areki, respectively (Figure 5).

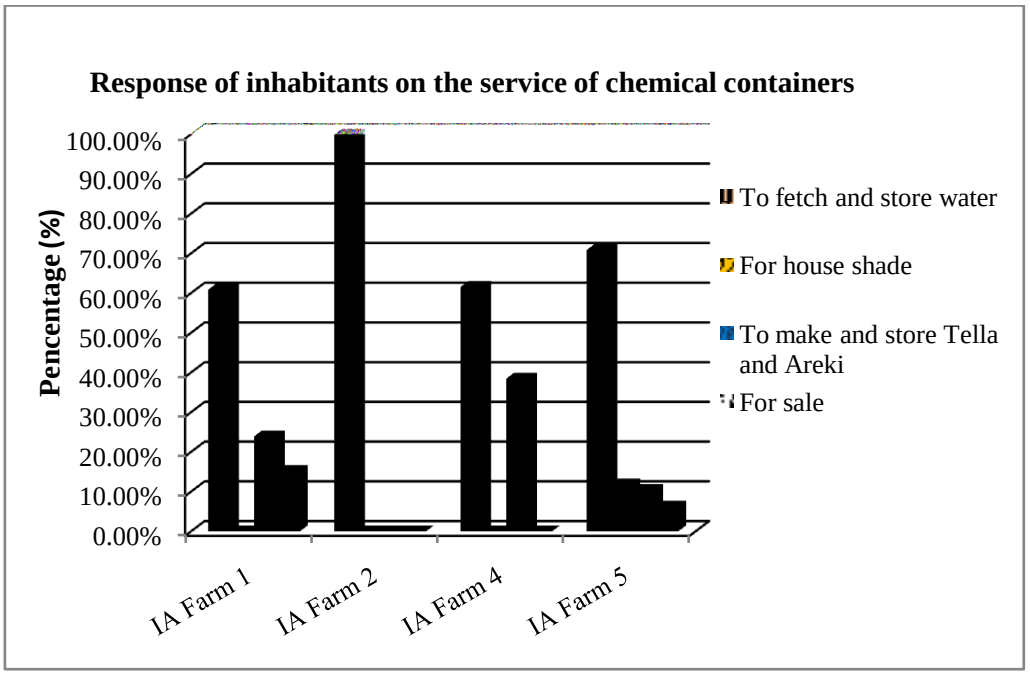

FIGURE 5: Response of inhabitants on the service of chemical containers

The researchers have tried to make field visits in the vicinity around flower farms to check if empty chemical containers are haphazardly disposed of in the immediate environment. As depicted in Figure 6(a) empty chemical bags were disposed of

haphazardly which further enter into the water body or eaten by cattle's grazing around the compound. Among empty chemical containers, we had a chance to take pictures of residents fetching water with Jerry Cans from which chemical was emptied (Figure 6b).

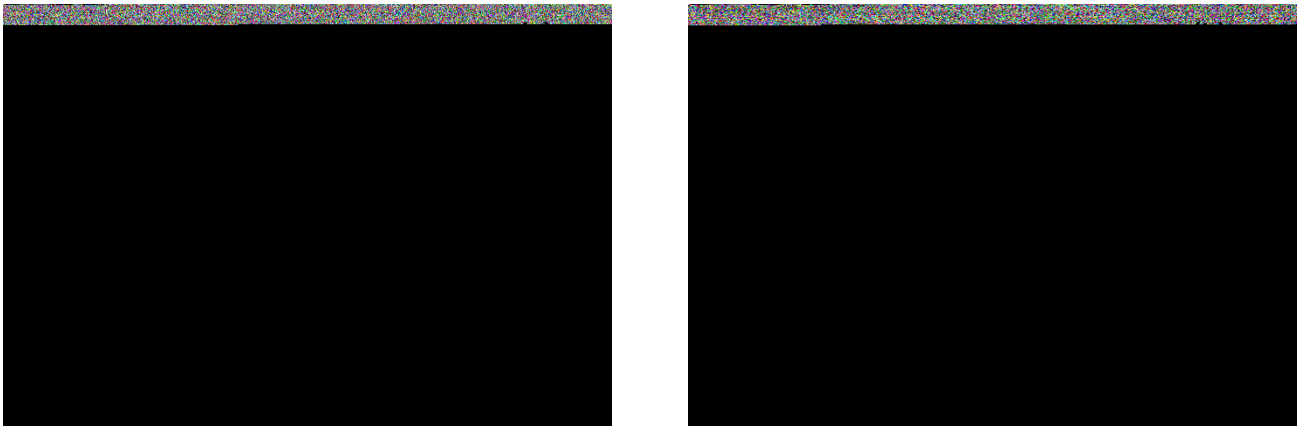

FIGURE 6: (a) Picture showing an empty chemical bag is haphazardly disposed of in the immediate environment. (b) Picture showing a person fetching water with Jerry cans from which chemical was emptied

3.4 Social complaint on the flower farms

In this survey, we have tried to identify how many of the inhabitants around the flower farms raised complain regarding the flower farm problems in their vicinity. Accordingly, 463 (77.04%) of inhabitants have reported they had raised a complaint. Whereas, 57(9.48%) of inhabitants have reported they have no complaint. The results are shown in Figure 7.

FIGURE 7: Report of inhabitants if they had risen to complain about flower farms

The respondents were asked for the cause of complaint in their vicinity. Accordingly inhabitants around flower farms reported high flood from the greenhouse (Farm 1; Farm 2), high water abstraction (Farm 1; Farm 3), chemical contamination of nearby lands (Farm 1; Farm 2; Farm 4; Farm 5), loss of local vegetables and fish acceptability on the market (Farm 1; Farm 2), decrease of crop yield (Farm 3; Farm 4). Also, unfair compensation (Farm 1; Farm 2), unwillingness to implement its promises (Farm 1; Farm 2), unfair wage (Farm 1), loss farmland (Farm 4) were raised as a complain. Furthermore, occupational injury, abuse of employee rights, health problems, death of cattle and fish, funeral area demolishment, chemical odor problem, reduced drinking water resource due to contamination were listed by inhabitants around Farm 5. This result is in agreement with the findings of Gezmu (2013); Hatch & Wells (2012), Mengistie *et al*. (2017), Gudeta (2012), and NAPE (2012).

3.5 Inhabitants flower farms benefits perception

It is undeniable that cut flower production is now a major part of the Ethiopian economy and has shown considerable potential for Ethiopia in terms of creating employment opportunities and foreign exchange earnings (Zegeye 2013).This study revealed that 27(50.94%), 10(18.87%), and 10(18.87%) inhabitants around Farm 1 have confirmed that they get a job opportunity, drinking water, and school, respectively. Among inhabitants around Farm 2, 23(43.40%) and 25(47.17%) has confirmed that they got job opportunity and water supply, respectively. Similarly, 23(43.40%) of inhabitants around Farm 4 have confirmed that they get the job opportunity. While, 30(56.60%) responded that they get nothing from the flower farm, respectively. On another hand, 209(53.73%) and 117(30.08%) inhabitants around Farm 5 have reported that they have got job opportunities, and school, respectively. Also, inhabitants around Farm 3 were asked to list the benefit they get as a resident of the vicinity. Accordingly, 19(35.85%) have confirmed that they got job opportunities. The result is presented in Figure 8.

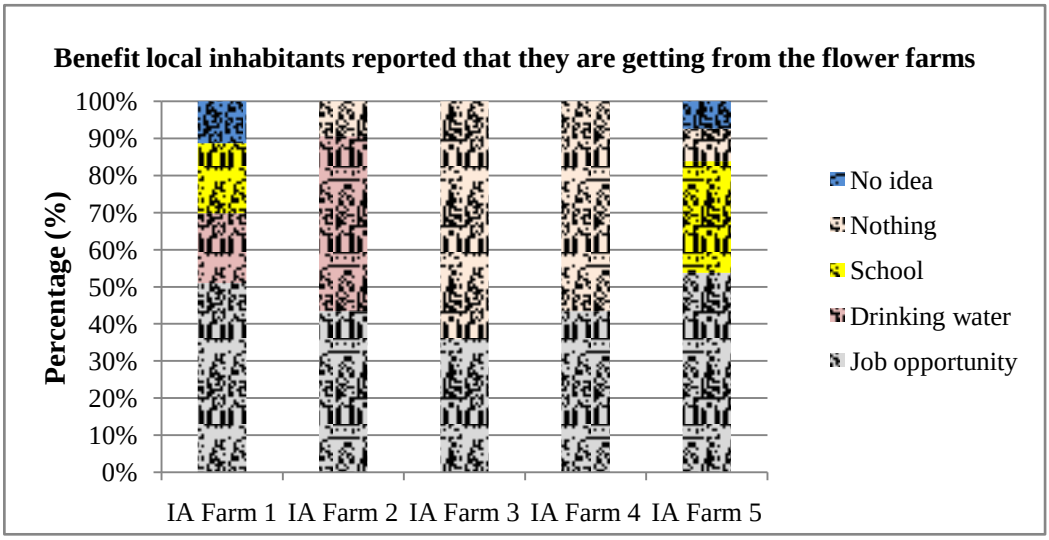

FIGURE 8: Benefit local inhabitants reported that they are getting from the flower farms

3.6 Local Inhabitant's degree of satisfaction about the flower farm activity

A developmental project should ensure the benefit of the local community besides its national role. Accordingly, residents around flower farms were asked about their satisfaction level 213 (35.44%), and 342(56.91%) of HHs were responded that they were "Slightly satisfied" and "Not at all satisfied", respectively. The results are presented in Figure 9.

FIGURE 9: Satisfaction level of inhabitants with the existence of the flower farm in their vicinity

The respondents were asked to report diseases and injuries they have perceived it was occurring newly or increasing due to flower farms in their vicinity. Consequently, the inhabitants residing around the flower farms reported Eye irritation (Farm 1; Farm 5), Asthma (Farm 1; Farm 2; Farm 5), Bad smell (Farm 1; Farm 4), cough (Farm 1; Farm 2; Farm 5), skin lesion (Farm 1; Farm 5), lung disease (Farm 2), and Malaria (Farm 1; Farm 2). Exclusively, inhabitants living around Farm 5 flower farms reported weight loss, headache, miscarriage, disability and death, shortness of breath, diarrhea, convulsion, and wounding of hands and other body. Studies also indicate that the neighboring communities complain of a smell from pesticides spray in the greenhouses (Tilahun, 2013; Tizazu & Workie, 2018). Literatures do support the findings of this study regarding the possible health impacts of pesticide. Pesticide can cause acute effects such as nerve, skin, and eye irritation and damage, headaches, dizziness, nausea, fatigue, vomiting, abdominal pain, and systemic poisoning. Major acute effects can cause respiratory problems, nervous system disorders, and aggravation of pre-existing conditions such as asthma (Singh *et al*., 2017).

A focus group discussions held on April 2019 revealed the existence of significant complaints in the community regarding the flower farm industry. These complaints traverse a wide range of issues and aspects related to the flower farms. Participants in the focus group discussions referred to environmental, wellbeing, financial, and personal issues.

A common dominator of the views expressed by all focus group participants regarding perceived flower farms negative impact is the decreased volume and quality of groundwater, a decrease of productivity, land degradation, increased emerging diseases, abuse of employee right, displacement of farmers from fertile land, death of cattle and fish, loss of acceptance for their agricultural and fish product. Participants reported perceived changes in their environment attributed to flower farms such as a change in color and odor of water body, spring has terminated, decreased water table, bad smell, decreased groundwater yield, and decrease in fish production. The key words most commonly brought up across all four focus groups included water, health problems, occupational injury, abuse of employee rights, farmland, cattle death, productivity, and fertility.

IV. CONCLUSION AND RECOMMENDATIONS

In this study it was tried to show the social and environmental issues such as waste management, empty chemical bag/container misuse, social grievance of the farm, residents benefit from the farm, and inhabitant's degree of satisfaction around flower farms. Accordingly, the main issues reported were high flood from the greenhouse, unfair compensation, uncontrolled water abstraction, unfair wage for the employee, chemical contamination of nearby land and water, loss of local vegetables and fish acceptability on the market, loss farmland, decrease of crop yield, occupational injury, abuse of employee rights, health problems, death of cattle and fish, and chemical odor problem.

In general, it was reported that there is a poor waste management and unacceptable activities by the flower farms. As a result, inhabitants around flower farms broadly manifest high social grievance and dissatisfaction. Every developmental activity has its own negative impact, which ranges from low to high, reversible to irreversible and short-term to long-term. The cost-benefit analysis of such a sector should be well examined and recognized. The fact that this study has tried to hear from the community, the government should strongly and closely monitor these farms if the firms are acting according to their environmental management plan. Also, detail and holistic study is still highly required to quantify environmental and social opportunity costs of flower farming activity.

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Effects of Feeding Ice Fish and Feed on the Flavor of Chinese Crab

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Abstract— *Eriocheir sinensis is an important aquaculture animal in China. In order to compare the effects of feeding chilled fish and feed on the flavor of Chinese mitten crab, this experiment compared the volatile flavor substances, sensory evaluation and the differences of amino acids (AA), fatty acids (FA) and nucleotides. As a result, the sweet taste, fresh taste and grass flavor of Chinese chelate crab in the feed group were significantly higher than those in the ice fish group (P<0.05). The fishy smell of the feed group was significantly lower than that of the ice fish group (P<0.05). Amino acids in feed group and chilled fish group were not significantly different. Only 5'-adenosyl monophosphate (AMP) was found to be significantly different between the two groups (P<0.05), and the AMP content in feed group was significantly higher than that in ice fish group. The fatty acid composition of feed group and chilled fish group varied greatly. compared with the chilled fish group, saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) in feed group decreased significantly (P<0.05), while high unsaturated fatty acids (PUFAs) increased significantly (P<0.05). Gas chromatography-mass spectrometry (GC-MS) was used to study volatile small molecules in muscle difference, compared with the ice fish group, the content of aldehydes in the feed group increased significantly (P<0.05), and the content of ketones and nitrogen compounds decreased significantly (P<0.05). The enzyme (lipoxygenase) that catalyzes the formation of aldehydes from polyunsaturated fatty acids was further analyzed. Compared with the ice fish group, the expression of LOX 5 genes and proteins and LOX enzyme activity in the feed group were significantly increased (P<0.05).*

Keywords— Chinese mitten crab, Eriocheir sinensis, amino acid, fatty acid, flavor.

I. INTRODUCTION

Chinese mitten crab *(Eriocheir sinensis)* is favored by consumers because of its rich nutritional value and unique flavor, and is one of the important economically farmed crabs in China [1,2]. In 2015, the total output of river crabs in China reached about 823,000 tons, most of which came from pond culture [3]. For a long time, traditional feeds such as chilled miscellaneous fish, corn, wheat, soybeans and cakes have been mainly used for breeding river crabs in ponds. However, these traditional feeds have some shortcomings such as unstable sources, unbalanced nutrient composition, low feed utilization rate and easy to cause water quality deterioration, which easily lead to the disease of river crabs and unstable quality of adult crabs [4-6]. Traditional breeding mode has become one of the important factors restricting the sustainable development of Chinese crab breeding industry [7,8]. Although the artificial compound feed has been gradually applied to the production of river crabs with the continuous improvement of the culture concept and technical level, in the late stage of culture, especially after the reproductive molting of river crabs, farmers still generally use a large number of Iced fish for fattening [9]. At present, there are two kinds of fattening feed on the market: puffed feed has good stability in water, less anti-nutritional factors in raw materials, which is beneficial to digestion and absorption of aquatic animals, and has the advantages of high utilization rate of raw materials, less pollution, safety and hygiene, etc. The processing of hard pellet feed has low requirements on equipment and relatively low manufacturing cost, and can also avoid oxidation loss of vitamins and fatty acids caused by puffing processing technology [10]. At present, Chinese feed manufacturers mostly adopt the processing technology of hard pellet feed. There are few reports on the effect of feed on the flavor of river crabs. In this experiment, hard pellet feed and Iced fish were used to feed river crabs, and their effects on the flavor of river crabs were compared to provide scientific basis and practical reference for the cultivation and quality control of river crabs.

II. MATERIALS AND METHODS

2.1 Experimental design and sample collection

The *Eriocheir sinensis* purchased from a local farm in Pukou District, Nanjing. During the experiment, the crabs were fed with ice fish (purchased locally) or artificial compound feed (Jiangsu Haipurui Feed Co., Ltd.) every day. The main nutritional components are shown in Table 1, and the experimental crabs were given two weeks to adapt to the experimental conditions. 80 crabs (25.33±0.79g) were evenly distributed to 8 cement ponds (1.5×1.5×0.5m, length: width: height). These crabs were divided into two groups, with four repetitions in each group: one group was fed with ice fish, and the other group was fed with compound feed for 12 weeks. During the experiment, the water temperature was controlled at 24±2^{o}C, pH 8.5-8.6, and dissolved oxygen was more than 5 mg/L.

TABLE 1
COMPARISON OF NUTRITIONAL COMPONENTS BETWEEN COMPOUND FEED AND ICED FISH

Projects	Compound Feed	Iced Fish
Protein	42.89±0.12^{b}	64.55±0.72^{a}
Total fat	8.09±0.56^{b}	13.98±1.52^{a}
Crude ash	12.96±0.04^{b}	14.98±0.45^{a}
Moisture	9.35±0.18^{b}	77.51±0.82^{a}

After the experimental breeding stage, the crab was put on ice for 10 minutes to reduce its vitality. Each crab was weighed separately, the length and width of the shell were measured, and then killed. The muscles of single crab were dissected on ice, washed thoroughly with 0.89g/L NaCl, frozen in liquid nitrogen immediately after treatment and stored at -80^{o}C for subsequent analysis.

2.2 Sensory analysis

We invited 30 ordinary diners to do sensory analysis. Diners should not eat, smoke or drink within 1 hour before the evaluation. The *Eriocheir sinensis* used for sensory analysis was washed and steamed for 20 minutes without adding any spices or flavoring agents. In order to prevent crabs of different genders from interfering with the taste, the female crab and the male crab are separated during cooking. The cooked crab is divided into the following parts: breastplate, leg, body muscle and hepatopancreas, which are placed in numbered dishes for diners to evaluate. After tasting a sample, the participating diners need to gargle with purified water before evaluating the next sample.

2.3 Analysis of free amino acids

Use 3 to 5mL of 80% ethanol per 0.5g of dry tissue. Then the tissue was homogenized in an ice bath with a ceramic homogenizer for 5 minutes. The homogenate was centrifuged at 3000r/min for 15 minutes, and then the clear supernatant was centrifuged again, which was repeated 3 times. After taking supernatant and vacuum drying to remove ethanol, the residue was dissolved in 8 ml of 6 mol/L HCl and placed in a 40mL hydrolysis tube. Then, the hydrolysis tube was vacuumized and filled with nitrogen at 110^{o}C for 24 hours. After hydrolysis, 1mL of hydrolysate was taken out and dried by vacuum evaporation at 50^{o}C to remove HCl. The hydrolysate was dissolved in 5ml of 0.02m HCl, and 50μL of supernatant was used for amino acid analysis by Biochrom 30 automatic amino acid analyzer (Cambridge, United Kingdom). Set the detection wavelength to UV 570 and 440nm (for Pro). All analyses were performed in triplicate. The characteristics and quantity of amino acids were determined by comparing with the retention time and peak area of each amino acid standard.

2.4 Fatty acid composition analysis

The fatty acid was analyzed by Morrison and Smith(1964) [11], which was methyl esterified with 14% boron trifluoride (BF3) methanol solution to produce fatty acid methyl esters, FAME). The fatty acid composition of fat source, liver and muscle was determined by gas chromatography [12]. The gas chromatograph model used is agilent 6890 (agilent technologies, Santa Clara, ca, USA), and the column model is Omegawax 320(30m×0.32mm; Supelco, Billefonte, PA, USA).

2.5 Statistical analysis

The experimental data are expressed by (mean standard error). After considering the normality of distribution and homogeneity of variance, SPSS 19.0 software is used to analyze all the data by independent sample T-test. Let the significance level be 0.05.

III. RESULTS AND ANALYSIS

3.1 Sensory evaluation

Sensory evaluation is a traditional experimental method often used to describe the smell and taste of crab muscles [13]. Each sample was evaluated in this test, and the results are shown in Figure 1. It can be found that the scores of bitter taste and salty taste of ice fish group and feed group are similar, while the scores of sweet taste and savory taste of feed group are higher than those of ice fish group. Ice fish group and feed group showed similar scores in meat flavor and fat odor, while compared with ice fish group, feed group could experience higher grass flavor and lower fishy smell.

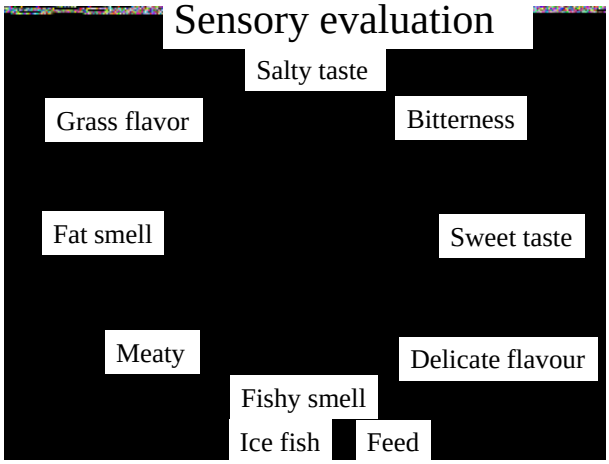

FIGURE 1: Radar image of sensory evaluation of *Eriocheir sinensis*

3.2 Comparison of fatty acid composition

The FAs composition in muscle of *Eriocheir sinensis* fed with compound feed and Iced fish for 8 months is shown in Table 2. In order to further understand the changes of FAs composition in crab muscle, we also detected FAs composition in Iced fish and feed, and the results are shown in Table 3.

According to the analysis of fatty acid saturation, compared with the ice fish group, the muscle of crab fed with feed group contains lower proportion of SFA and MUFA (Table 3), while the proportion of PUFA in feed group increases significantly ($P<0.05$). In this study, there was no significant difference in EPA content between ice fish group and feed group [14]. Compared with the ice fish group, the content of ALA and AA diets increased significantly ($P<0.05$), while the content of DHA decreased significantly ($P<0.05$). As shown in Table 1, the content of PUFA in compound feed (51%) is 14 percentage points higher than that of Iced fish (37%).

In terms of FAs composition, the feed group showed higher PUFA and lower SFA and MUFA than the ice fish group.

TABLE 2
FATTY ACID COMPOSITION OF ICED FISH AND COMPOUND FEED AND FATTY ACID COMPOSITION% OF *ERIOCHEIR SINENSIS* AFTER 8 MONTHS

Fatty acid	Iced fish	Compound Feed	Eight months(Iced fish)	Eight months(compound feed)
C12:0	0.110	0.044	0.0647±0.005	0.053±0.007
C14:0	5.602	1.449	1.171±0.117	0.542±0.068*
C15:0	0.728	0.159	0.375±0.017	0.257±0.010*
C16:0	24.804	16.238	16.741±0.271	15.320±0.183*
C17:0	0.695	0.238	0.759±0.044	0.619±0.023*
C18:0	4.791	4.326	8.320±0.371	8.619±0.224
C20:0	0.533	0.371	0.190±0.016	0.114±0.005*
C22:0	0.143	0.394	0.072±0.006	0.088±0.013
C16:1	5.816	1.936	3.435±0.499	2.790±0.452
C18:1	11.080	22.506	21.980±0.499	22.502±0.363
C20:1	3.385	0.724	1.925±0.17	0.817±0.049*
C22:1	4.785	0.448	0.702±0.088	0.169±0.008*
C18:2	1.028	40.095	6.804±1.074	13.631±0.491*
C20:2	0.163	0.092	1.417±0.033	2.018±0.095*
C18:3n6	0.130	0.061	0.083±0.003	0.090±0.010
C18:3n3	0.463	4.912	0.580±0.178	0.991±0.099
C20:3	0.040	0.041	0.032±0.005	0.042±0.005
C22:3	0.141	0.051	0.885±0.083	0.700±0.073
C20:4	1.255	0.381	3.249±0.364	3.916±0.103
C22:4	0.477	0.084	0.210±0.029	0.243±0.011
C20:5	10.658	2.410	14.329±0.565	12.988±0.673
C22:5	1.012	0.299	0.435±0.037	0.530±0.011*
C22:6	22.167	2.746	16.24±0.696	12.963±0.703*
SFA	43.220	25.153	27.691±0.330	25.613±0.073*
MUFA	19.249	23.677	28.042±0.181	26.282±0.731*
PUFA	37.533	51.170	44.277±0.273	48.118±0.733*

Note: * It shows significant difference between ice fish group and feed group (P<0.05).
SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: Polyunsaturated Fatty Acids

TABLE 3
FLAVOR NUCLEOTIDES AND EQUIVALENT FLAVOR CONCENTRATION EUC (n=3) IN CHINESE MITTEN CRAB MEAT AFTER FEEDING ICED FISH AND FEED FOR 8 MONTHS

Tasty nucleotide	Iced fish	Compound Feed
GMP (mg/100g)	41.62±2.05	36.98±1.65
IMP (mg/100g)	394.95±46.69	530.19±62.55
AMP (mg/100g)	253.56±61.28	617.84±81.41*
EUC (g MSG/100g)	163.8±0.95	215.9±1.20*

Note: "*" shows significant difference between ice fish group and feed group (P<0.05).
Imp: 5'-inosine monophosphate; Gmp: 5'-guanosine monophosphate; AMP: 5'-adenosine monophosphate; Ump: 5'-uridine monophosphate.

3.3 Comparison of free amino acids and nucleotides

We detected 17 kinds of amino acids in crabs, and the results are shown in Table 2. There is no significant difference in the content of 17 AAs between ice fish group and feed group (Table 4). Besides AA, we also detected three nucleotides (AMP, IMP, GMP), and the results are shown in Table 3 [15]. AMP concentration in feed group was significantly higher than that in ice fish group (P<0.05), but there was no significant difference in IMP and GMP concentration between the two groups.

TABLE 4
AMINO ACID COMPOSITION OF *ERIOCHEIR SINENSIS* AFTER FEEDING ICE FISH AND FEED FOR 8 MONTHS

Amino acid	Taste	Iced fish	Compound Feed
Asp	Fresh	1.654±0.123	1.501±0.077
Glu	Fresh	2.498±0.121	2.304±0.115
Ser	Sweet	0.704±0.036	0.653±0.032
Thr	Sweet	0.774±0.057	0.677±0.032
Gly	Sweet	0.935±0.097	0.907±0.054
Ala	Sweet	1.089±0.021	1.066±0.044
Cys	Sweet	0.178±0.009	0.169±0.006
Val	Bitter	0.776±0.058	0.707±0.034
Met	Bitter	0.597±0.075	0.532±0.059
Ile	Bitter	0.722±0.067	0.683±0.055
Leu	N.A.	1.248±0.081	1.161±0.068
Tyr	N.A.	0.731±0.069	0.67±0.028
Phe	N.A.	0.828±0.065	0.736±0.054
Lys	N.A.	1.198±0.063	1.046±0.08
His	N.A.	0.471±0.057	0.368±0.029
Arg	N.A.	1.761±0.1	1.589±0.107
Pro	N.A.	0.623±0.066	0.606±0.024
Total		16.788±0.9	15.376±0.836

Note: N.A.: No.

3.4 Comparison of volatile compounds

The volatile compounds in meat were detected by SPME-GC-MS, and the results are shown in table 5. A total of 55 volatile compounds (2549.32±12ug/kg in ice fish group; 2392.62±4.85ug/kg in feed group, including 16 aldehydes (878.98±5.75ug/kg in ice fish group; The feed group was 962.26±8.52μg/kg/kg) and 6 kinds of ketones (the ice fish group was 104.11 4.72 ug/kg; 72.44±3.77ug/kg in the feed group) and 6 kinds of alcohols (149.31±6.12ug/kg in the ice fish group; 163.47 3.2 ug/kg in the feed group) and 6 aromatic hydrocarbons (119.45 1.05 ug/kg in the ice fish group; 120.61±1.97ug/kg in the feed group), 5 compounds containing n (599.65±18.03ug/kg in the ice fish group; The feed group is 371.26±16.17ug/kg), and 10 kinds of hydrocarbons (the ice fish group is 587.4±11.17ug/kg; The feed group is 592.13±0.3ug/kg) and the other 6 species (ice fish group is 110.41±3.85ug/kg; The feed group was 110.45±4.95ug/kg). Compared with the ice fish group, the contents of total volatile compounds and aldehydes in the feed group increased significantly ($P<0.05$), while the contents of ketones and nitrogen compounds decreased significantly ($P<0.05$). Odor activity value (OAV) is an index to evaluate whether each volatile compound reaches the taste concentration, which can be calculated by dividing the concentration of the compound by the odor threshold of the compound, and if the value is greater than 1, the compound presents taste [16]. Nineteen AAC were selected from all 55 volatile compounds in each group, including 14 aldehydes, 1 alcohol, 1 N-containing compound, 1 hydrocarbon and 2 other compounds.

TABLE 5
CONCENTRATION OF VOLATILE COMPOUNDS IN *ERIOCHEIR SINENSIS* AFTER FEEDING ICE FISH AND FEED FOR 8 MONTHS

Compound	LRI	Threshold (ng/g)	Authenticate	Iced fish	Compound feed	ACCs
Aldehydes						
2-Methylbutanal	665	1	MS, RI	7.41±1.45	7.28±1.17	Y
Pentanal	699	9	MS, RI	31.07±2	27.27±1.24	Y
2-Methyl-2-butenal	740	458.9	MS	52.7±2.04	63.48±1.62*	N
Hexanal	800	2.8	MS, RI	14.52±0.58	67.02±4.95**	Y
4-Heptenal	901	4.2	MS, RI	4.23±0.51	7.02±0.37*	Y
Heptanal	903	2.8	MS, RI	25.02±0.56	18.62±1.13*	Y
Benzaldehyde	971	41.7	MS, RI	222.08±1.47	229.53±2.83*	Y
Octanal	1002	0.587	MS, RI	154.74±2.69	179.69±4.36*	Y
2,4-Heptadienal	1019	15.4	MS, RI	19.44±1.05	17.07±1.72	Y

Benzeneacetaldehyde	1030	4	MS	22.25±1.09	21.5±0.5	Y
Nonanal	1102	1.1	MS, RI	137.05±5.49	153.38±6.09	Y
2, 6-Nonadienal	1116	0.15	MS, RI	14.84±1.55	21.59±0.9*	Y
Decanal	1220	0.1	MS	114.4±7.1	94.41±2.73	Y
Undecanal	1315	5	MS, RI	15.65±1.42	10.76±0.93*	Y
2,4-Decadienal	1329	0.07	MS, RI	24.95±0.74	23.46±1.3	Y
Hexadecanal	1820	N.A.	MS, RI	18.62±0.67	20.17±0.45	N.J.
Subtotal (16)				878.98±5.75	962.26±8.52**	
Ketones						
Acetone	<500	14500	MS	34.11±1.24	23.74±1.5*	N
2-Butanone	589	35400	MS, RI	8.49±0.74	5.86±1.28	N
2-Octanone	994	50.2	MS, RI	36.39±2.91	21.14±1.12*	N
2-Nonanone	1091	38.9	MS, RI	17.3±0.69	13.88±0.57*	N
3,5-Octadien-2-one	1102	150	MS	4.87±0.2	4.85±0.67	N
2-Decanone	1190	7.94	MS, RI	2.95±0.15	2.97±0.97	N
Subtotal (6)				104.11±4.72	72.44±3.77*	
Alcohols						
1-Penten-3-ol	682	358.1	MS, RI	22.76±1.7	22.46±0.7	N
1-Pentanol	675	150.2	MS, RI	1.71±0.64	1.75±0.19	N
1-Octen-3-ol	978	1.5	MS, RI	86.86±2.93	101.65±1.74*	Y
1-Heptanol	981	N.A.	MS	4.52±0.8	4.93±0.5	N.J.
2,4-Undecadienol	1071	N.A.	MS, RI	4.69±0.47	5.86±0.66	N.J
Cedrol	1792	N.A.	MS	28.78±3.77	26.82±1.54	N.J
Subtotal (6)				149.31±6.12	163.47±3.2	
N-containing compounds						
Trimethylamine	<500	2.4	MS, RI	540.33±18.34	320.4±15.69*	Y
Pyridine	775	2100	MS, RI	13.08±0.81	8.83±0.28*	N
2-Ethylpyridine	909	57	MS, RI	23.31±0.89	21.42±0.49	N
2,5-Dimethylpyrazine	916	1700	MS, RI	2.91±0.65	2.41±0.52	N
2,3,5-Trimethylpyrazine	1005	350.12	MS, RI	20.02±1.09	18.2±0.62	N
Subtotal (5)				599.65±18.03	371.26±16.17*	
Aromatics						
Benzene	668	1500	MS, RI	72.85±1.17	74.49±2.56	N
Toluene	770	1550	MS, RI	19.78±0.99	15.8±1.27	N
Ethylbenzene	865	2205.25	MS, RI	8.56±0.69	8.9±0.98	N
P-Xylene	873	450.23	MS, RI	10.66±0.85	11.28±1.13	N
Xylene	879	N.A.	MS, RI	5.25±0.58	7.62±0.8	N.J
Naphthalene	1215	60	MS, RI	2.34±0.23	2.51±0.07	N
Subtotal (6)				119.45±1.05	120.61±1.97	
Hydrocarbons						
2,4-Dimethyl-heptane	840	N.A.	MS, RI	21.72±0.87	21.41±1.13	N.J
Limonene	1038	10	MS, RI	117.4±3.77	117.25±4.28	Y
Undecane	1102	1170	MS, RI	12.43±0.3	14.44±0.46*	N
Dodecane	1201	2040	MS, RI	22.42±0.48	20.7±0.1*	N
Tridecane	1297	2140	MS, RI	5.96±0.64	5.1±0.63	N
Tetradecane	1398	N.A.	MS, RI	1.72±0.1	1.9±0.07	N.J.
Pentadecane	1500	N.A.	MS, RI	25.35±2.03	23.35±2.03	N.J
Hexadecane	1602	N.A.	MS, RI	35.95±2.6	34.34±3.39	N.J

2,6,10,14-Tetramethylpentadecane	1702	N.A.	MS, RI	4.03±0.71	6.73±0.3	N.J
Eicosane	1999	N.A.	MS, RI	340.41±13.01	346.91±3.56	N.J
Subtotal (10)				587.4±11.17	592.13±0.3	
Other				0±0	0±0	
2-Acetylthiazole	1205	10	MS, RI	3.2±0.3	3.87±0.24	N
2-Ethylfuran	700	2.3	MS, RI	5.85±0.27	5.05±0.41	Y
2-Pentylfuran	992	5.8	MS, RI	57.55±3.26	56.68±2.82	Y
Iodomethane	586	N.A.	MS, RI	24.81±0.44	23.99±1.14	N
Phthalic acid, butyl tetradecyl ester	1570	N.A.	MS	13.85±0.77	15.41±1.1	N.J
Hexadecanoic acid, methyl ester	1917	N.A.	MS	5.14±0.42	5.44±0.71	N.J
Subtotal (6)				110.41±3.85	110.45±4.95	
Total				2549.32±12	2392.62±4.85*	

Note: *P<0.05, **P<0.01.

IV. DISCUSSION

4.1 Sensory changes

After sensory evaluation, it can be clearly found that the bitter taste and salty taste of crabs in ice fish group and feed group are almost unchanged, while the delicious taste and sweet taste of Chinese mitten crab fed with feed are more prominent. Shi Jing et al. reported similar results, that is, Chinese mitten crab fed with compound feed had a higher taste and sweetness score than that fed with ice fish. The scores of meat smell and fat smell of the two groups were similar, while the experimental members tasted lower fishy smell and higher grass smell in the crabs of the feed group.

4.2 Fatty acid changes

The fatty acids of Chinese mitten crab under two feeding modes have great changes. Compared with the ice fish group, the muscle of Chinese mitten crab fed with feed contains a lower proportion of SFA ($P<0.05$). It may be that SFA in compound feed is lower than that in Iced fish. Many studies in recent years have confirmed an undisputed view that foods rich in SFA will have negative effects on human health [17]. Therefore, from the perspective of healthy diet, artificial compound feed can improve the nutritional value of *Eriocheir sinensis*. Confusingly, the proportion of MUFA in compound feed is higher than that of Iced fish, while the proportion of MUFA in feed group is lower than that of Iced fish group. Further analysis shows that the higher proportion of MUFAs in compound feed is mainly due to the fact that the proportion of oleic acid in compound feed (22%) is twice that of Iced fish (11%). Oleic acid (OA, C18: 1) can be converted into linoleic acid (LA, C18: 2) catalyzed by desaturase. The LA content in the feed group was twice as high as that in the ice fish group (6.80%), which indicated that Chinese mitten crab was more inclined to deposit OA-transformed LA in muscle in the form of PUFA instead of MUFA, which was the main reason why Chinese mitten crab was rich in polyunsaturated fatty acids. The impact of MUFA on human health is still controversial, and there is no unified view at present. However, in clinical investigation, it is found that the proportion of MUFAs is better than 30, otherwise it will induce cardiovascular diseases. As we all know, PUFA has many benefits to human health, including preventing chronic diseases and promoting brain development. Compared with ice fish group, the proportion of PUFA in feed group increased significantly ($P<0.05$). This may be related to the difference of PUFA content between oleic acid synthesis and diet. As shown in Table 3, the content of PUFA in compound feed (51%) is 14 percentage points higher than that of Iced fish (37%). Among various polyunsaturated fatty acids, there are four main types of fatty acids, which are very important for maintaining cell morphology and preventing chronic diseases [18]. (ν)-linolenic acid (ALA, 18: 3n-3) is the precursor of n-3- family, eicosapentaenoic acid (EPA, 20: 5n-3) or docosahexaenoic acid (DHA, 22: 6n-3), while linoleic acid (LA, 18: 2n-6) is arachidonic acid AA and DHA are the main components of membrane phospholipids, and the phospholipids of central nervous system membrane are mainly long-chain PUFA. In this study, there was no significant difference in EPA content between ice fish group and feed group. Compared with the ice fish group, the content of ALA and AA diets increased significantly ($P<0.05$), while DHA content decreased significantly ($P<0.05$).

4.3 Changes of free amino acids and nucleotides

It is reported that the content of amino acids (AAs) has a strong correlation with the taste of crabs. In particular, some flavor amino acids (glutamic acid, aspartic acid, glycine, serine, alanine and proline) contribute greatly to the sweetness and umami taste of crabs. There was no significant difference in the content of 17 AAs between ice fish group and feed group. The AA composition of aquatic animals is closely related to the protein sources in the diet [19]. At present, fishmeal is the main and necessary protein source in the compound feed of *Eriocheir sinensis*. Fish meal is mainly processed from Iced fish, so it has similar AA composition to Iced fish. In this study, the consistent AA composition between the ice fish group and the feed group may be attributed to fish meal being selected as the main protein source for preparing feed. As far as amino acids are concerned, the compound feed developed by our laboratory has no significant effect on muscle taste.

Besides AA, disodium salt of 5'- nucleotide has great influence on the taste of Chinese mitten crab. Among the three nucleotides, AMP is considered to have the greatest influence on the flavor of crab muscle, because AMP is detected as the highest content in all tissues of *Eriocheir sinensis*. From the results of three disodium salts of 5'- nucleotides (AMP, IMP and GMP), it can be seen that AMP concentration in feed group is significantly higher than that in ice fish group ($P<0.05$), but there is no significant difference in IMP and GMP concentration between the two groups. The significant increase of AMP content may be related to the higher PUFA content in compound feed. Many studies have confirmed that PUFA can activate AMPK, which indicates that AMP content increases [20]. Nucleotides have a unique synergistic effect between AA to synergistically increase the flavor. Monosodium glutamate equivalent (EDC) is the flavor intensity given by the mixture of amino acids and 5'- nucleotides, which is considered as a very useful tool to evaluate the flavor of food. As shown in Table 3. Compared with the ice fish group, the EUC of the feed group increased significantly ($P<0.05$), which was consistent with the analysis of sensory evaluation of higher umami score in the feed group. From the point of view of free amino acids and nucleotides, the compound feed used in our laboratory improved the delicious taste of Chinese mitten crab, which was mainly due to the increase of AMP content [21].

4.4 Changes of Volatile Compounds

Comparing the volatile small molecules of the two groups, it can be found that the contents of total volatile compounds and aldehydes increased significantly ($P<0.05$), while the contents of ketones and nitrogen-containing compounds decreased significantly ($P<0.05$). Compared with other volatile compounds, aldehydes are considered to contribute the most to crab flavor because of their higher content and lower threshold [22]. In this study, the total content of aldehydes in feed group was significantly higher than that in ice fish group ($P<0.05$). The contents of 7 aldehydes (2- methyl -2- butenal, hexanal, 4- hexenal, benzaldehyde, nonanal, octanal aldehyde and 2,6- nonadialdehyde) in the feed group were significantly higher than those in the ice fish group ($P<0.05$). The higher content of these aldehydes may be caused by the feed group of *Eriocheir sinensis* eating compound feed rich in PUFA. PUFA in compound feed (51.170%) was higher than that in wild fish (37.533%). Meanwhile, the contents of two aldehydes (Heptanal and Undecanal) in the feed group were significantly lower than those in the ice fish group ($P<0.05$).

Besides aldehydes, ACCs also includes 2- ethyl furan, 2-pentylfuran, limonene, 1- octene -3- alcohol and trimethylamine. 2- ethyl furan and 2-pentylfuran, which belong to furan, also contribute greatly to the taste of crabs, and have similar performances in several crabs. Limonene, the only hydrocarbon in AAC, is common in *Eriocheir sinensis* [23]. In this study, compared with feed group, the content of trimethylamine in ice fish group increased significantly ($P<0.05$), which was consistent with the sensory evaluation of ice fish group with higher fishy smell score. The higher content of trimethylamine in ice fish group may be due to the consumption of ice fish by Chinese mitten crab, so crabs contain more fish compounds than feed group. It is reported that only one alcohol, 1- octene -3- ol, exists in *Eriocheir sinensis*. In this study, the content of 1- octene -3- ol in feed group was higher than that in ice fish group. Aldehydes can be converted into corresponding alcohols by alcohol dehydrogenase. The higher content of 1- octene -3- ol may be related to the increase of aldehyde [24-25].

Based on the experimental data, we speculated that the difference of aldehyde was the main reason for the flavor change between the ice fish group and the feed group. Previous studies have confirmed that aldehydes produced by PUFA catalyzed by lipoxygenase play an important role in forming unique aroma in fruits and vegetables.

V. CONCLUSION

It was found that the Chinese mitten crab in the feed group had higher sweetness, umami taste and grass fragrance, while the fishy smell was lower than that in the ice fish group. There was no significant difference in AA components between the two groups, but the PUFA ratio of Chinese mitten crab fed with feed increased significantly, while the ratio of SFA and MUFA

decreased significantly. In addition, AMP and EDU increased significantly in the feed group. Aldehydes are one of the important factors that can produce unique flavor of Chinese mitten crab. Aldehydes increase significantly after feeding Chinese mitten crab with compound feed, which may be related to the activity of lipoxygenase. Feeding feed can completely replace the traditional Iced fish. Feeding feed is easy to operate and store, and Chinese mitten crab has more flavor.

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Relationship between Profile and Food Consumption Pattern of Tribal Families of Palghar District

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***Abstract*—** *This paper examines the relationship between profile and food consumption pattern of the tribal families from Palghar district. The study was conducted at the Palghar district. Samples of 120 tribal families were considered as respondents for present study. The respondents were interviewed with the help of specially designed schedule. Collected data was classified, tabulated and analysed by using various statistical methods. The result of the study showed that the relationship between family education status and food consumption pattern, family size and food consumption pattern, annual income of the family and food consumption pattern, cropping pattern and food consumption pattern, resource availability and food consumption pattern was found to be 'positive' and 'significant'. The relationship between major occupation and food consumption pattern, land holding and food consumption pattern, farming experience and food consumption pattern, social participation and food consumption pattern was found to be 'non-significant'. The extension workers should consider these facts while planning and executing programmes for development of the tribal families living in Palghar district.*

***Keywords*— Profile, Food consumption pattern, tribal families, Relationship.**

I. INTRODUCTION

India has the second largest tribal population in the world, next to Africa. Our country represents over 700 tribal groups (including sub-groups) with a population of 84.3 million (8.2%) population in almost all states except in Haryana and Punjab. Bhil, Gond-Madia, Katkari, Koli, Oraon, Warli are the major tribes of Maharashtra. These communities are mostly dependent on wild plants for many purposes. The tribal communities have vast knowledge about the importance of consumption of wild plants. Nutritional status of the population largely depends on the consumption of food in relation to their needs, which is influenced by availability of food and purchasing power. With respect to food consumption, people living in urban areas have better access to variety of food items while tribal people living in remote and isolated areas have different dietary habits. The choice of food is deeply related to the lifestyle of an individual. Food habits and consumption pattern is greatly influenced by thoughts, beliefs, notions, traditions and taboos of the society. Apart from these socio-cultural barriers, the religion, education, and economic factors do alter the food behaviour. Maharashtra is becoming one of the game-changers in agriculture-nutrition in India. For one component of the Grand Challenge Initiative funded by Biotechnology Industry Research Assistance Council (BIRAC) and the Gates Foundation, MSSRF has been working to address malnutrition through the underlying cause of agriculture, gender and biofortification. Food consumption pattern of the tribes needs to be assessed because it helps us understand the major effect on the nutritional status of the population. Also, there is an urgent need to document the data regarding consumption pattern of food that will give an understanding of the existing health. Also, there is a need to spread awareness about the health care and food consumption pattern through educational programmes in the tribal areas. The intervention of these programmes can help them identify their problems and find an appropriate solution for these problems.

II. OBJECTIVE

To ascertain the relationship between profile and food consumption pattern of the tribal families

III. METHODOLOGY

The research work was conducted in Palghar district of Konkan region of Maharashtra state because it has maximum tribal population in the Konkan region. Two tahasils Mokhada and Vikramgad having maximum tribal population were selected to carry out the research. Six villages from each tahasil were selected randomly to carry out the present study. A total of 120 tribal families were considered as respondents for the present study. The data was collected with the help of a specially designed interview schedule by keeping in view the objective of the study. To determine the relationship among the independent and dependent variables correlation analysis was worked out. For the study nine independent variables namely Family education status (X1), Family size (X2), Annual income of family (X3), Major occupation (X4), Land holding (X5),

Cropping pattern (X5), Farming experience (X6), Social participation (X6) and Resource availability (X7) were selected and their relationship with dependent variable food consumption pattern was worked out. 'Ex-post facto' research design was used to conduct the present study.

IV. RESULT AND DISCUSSION

The predictive power of each variable was estimated by working out the value of co-efficient of determination (R^2). Independent variables together contributed 26.00 per cent variation in the food consumption pattern of the tribal families. This implies that the selected independent variables explained the variation in the food consumption pattern of tribal families to the extent of 26.00 per cent.

TABLE 1
CORRELATION COEFFICIENT OF INDEPENDENT VARIABLES WITH FOOD CONSUMPTION PATTERN

S. No.	Independent Variables	Variable Code	Correlation coefficient (r)
1.	Family education status	X1	0.21456 *
2.	Family size	X2	0.21549 *
3.	Annual income of family	X3	0.31835 *
4.	Major occupation	X4	-0.05055 NS
5.	Land holding	X5	0.17250 NS
6.	Cropping pattern	X6	0.27477 *
7.	Farming experience	X7	-0.04462 NS
8.	Social participation	X8	-0.01762 NS
9.	Resource availability	X9	0.29027 *

R^2 = 0.2600 F = 4.296
****= Significant at 0.05 level***
NS = Non significant

- The correlation coefficient computed between Family education status (X1) and food consumption pattern (Y1) at 0.05 level of significance is 0.21456 which is greater than the table value 0.174.
- A significant and positive relationship was found between Family size (X2) and food consumption pattern (Y1) at 0.05 level of significance. The correlation coefficient computed is 0.21549 which is greater than the table value 0.174.
- The correlation coefficient computed between Annual family income (X3) and food consumption pattern (Y1) at 0.05 level of significance is 0.31835 which is greater than the table value 0.174.
- The correlation coefficient computed between Major occupation (X4) and food consumption pattern (Y1) at 0.05 level of significance is -0.05055 which is less than the table value 0.174.
- The correlation coefficient computed between Land holding (X5) and food consumption pattern (Y1) at 0.05 level of significance is 0.17250 which is less than the table value 0.174.
- The correlation coefficient computed between Cropping pattern (X6) and food consumption pattern (Y1) at 0.05 level of significance is 0.27477 which is greater than the table value 0.174.
- The correlation coefficient computed between Farming experience (X7) and food consumption pattern (Y1) at 0.05 level of significance is -0.04462 which is less than the table value 0.174.
- The correlation coefficient computed between Social participation (X8) and food consumption pattern (Y1) at 0.05 level of significance is -0.01762 which is less than the table value 0.174.
- The correlation coefficient computed between Resource availability (X9) and food consumption pattern (Y1) at 0.05 level of significance is 0.29027 which is greater than the table value 0.174.

V. CONCLUSION

The result of the study showed that the relationship between family education status and food consumption pattern, family size and food consumption pattern, annual income of the family and food consumption pattern, cropping pattern and food consumption pattern, resource availability and food consumption pattern was found to be 'positive' and 'significant'. The relationship between major occupation and food consumption pattern, land holding and food consumption pattern, farming experience and food consumption pattern, social participation and food consumption pattern was found to be 'non-

significant'. The extension workers should consider these facts while planning and executing programmes for development of the tribal families living in Palghar district.

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Aspects for Agricultural Water Management in Water Stress Conditions: Case Study of Konya Plain, Turkey

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Abstract— *The major aim of the study was to propose sustainable agro-water management strategies, particularly for water poor-ecologies. In current work, information was obtained from worldwide previous findings of studies relevant to the water management. In order to maximize water productivity in those environments exposing climate changes following applicable suggestions were presented: changing crop pattern in accordance of available current water resources, increasing utilization areas in favor of modern irrigation systems, if possible converting of water delivery networks to pipe systems, improving share of low water consuming crops in current crop patterns, practicing deficit irrigation program, collecting water charges based on volumetric basis, more uses of rainwater harvesting systems, training of farmers about irrigated agriculture, and if possible transferring some water from neighbor basins to irrigation farms.*

Keywords— Climate Change, Crop Pattern, Irrigation, Water Management, Water Shortage.

I. INTRODUCTION

Climate change is one of major global crisis and its effects have increased gradually due to global warming. Agriculture is one of the activities mostly affected by climate change. The spatial and temporal fluctuations of precipitation together with rising in temperature may have a negative impact on crop yield and quality [1]. As known, the amount of water resources on earth surface is constant but rainfall distribution is not homogenous in time and space. The amount of water on global about $1.35x10^9$ m^3/year but the most of it, about 97.4%, is available at seas with saline form. The most of fresh water about 69% exists in glacier, 30% of at groundwater reservoir, and the rest (1%) at surface water supplies. The only 5% of groundwater resources are available at present [2]. Climate change has impacts on many sectors such agriculture, fishery, forestry, and so on. Agriculture, relies directly on environmental factors, has been influenced by climate change and in turn, has also had an impact on climate change [3]. There are three climates in Turkey namely, Mediterranean, Black Sea and Continental climates. Continental climate has observed mainly at inlands with large-sized farmlands having hot in summer, and cold in winter [4] so farming activities based on water savings are vital important for sustainable utilization of water resources particularly at those kinds of water-starved ecologies.

In recent years, water reductions of wells are getting increase gradually in Turkey. Annual depletion of groundwater level is about 1.5 m depending on regions. Konya Closed Basin is forefront in groundwater reduction within whole river basins. The reasons behind such water depletion could be increase of crop patterns in favor of high-water consuming crops, and unnecessary or poor uniformity of rainfall. This situation has forced farmers to extract over water pumping from groundwater resources which resulted in land subsidence in some part of the region. In basin, total available water potential is about $4.5x10^9$ m^3/year, but actual use is about $6.5x10^9$ m^3/year. In that regard, there is $2x10^9$ m^3/year water deficiency in basin resources each year.

The farmers have great experiences about all farming activities in such basin. They produce plenty different field crops such as sugar beet, corn, alfalfa, squash, sunflower, cereals, legumes etc., and various vegetables such as tomato, lettuce, pepper, carrot, egg plants, and plenty fruit plants such as apple, cherry, plum, apricot, grapes, pear and so on. In addition, having the highest animal population in Turkey that basin is well-known as an animal production center. Due to the recent climate changes, all crops including cereals have produced under irrigation for obtaining economical benefits [5]. It is noted that irrigation is main factor increasing crop yield particularly in arid and semi-arid climates such as Konya plain of Turkey. Like average of worldwide, more than 70% of fresh water resources have been used in irrigation practices in those environments [6, 7, 8].

In Konya basin, there are about 10-25 mm rainfall reductions in last 30 year sifting such basin to arid climate. Rainfall of basin is not uniform and insufficient and only 30% of rainfall has recorded at crop vegetation period [9]. Over water extraction from groundwater reservoir for irrigation purpose has resulted serious environmental problems [10]. Sprinkler irrigation systems are common irrigation method in region. Drip irrigation system is gaining the popularity for irrigation of some field crops such as corn and sunflower, and vegetables since water application efficiency is about greater than 90% in that system under well management.

In water shortage environments, growing drought resistance crops, changes in calendar of sowing date in accordance of crop patterns, mixing crop system, improvement of water productivity and planting of trees are practical solutions for minimizing climate change inverse impacts particularly in water scant ecologies [11].

The main target of this paper is to present practical recommendations for enhancing water productivity in water scant ecologies.

II. MATERIALS AND METHODS

In this study, water saving strategies was examined particularly at arid and semi-arid regions such as Konya plain of Turkey. The sample region, Konya basin, is semi-arid climate characteristics in accordance of long-term average annual rainfall of about 320 mm. It is within the second drought areas of Turkey and irrigation is very important role to play for obtaining satisfactory production. Even, it is almost impossible for farmers to get desired economical returns without irrigation particularly in semi-arid Middle Anatolian Region of Turkey. In current study, findings of previous studies relevant to agricultural water management were used and practical recommendations about productive water utilization in agriculture were analyzed with detail for water-starved environments.

III. RESULTS AND DISCUSSIONS

The five main effective aspects for better agricultural water management particularly for arid or semi-arid environments were as follows;

3.1 Changing crop pattern in favor of less water use crops

The land sizes of farmlands with high water consuming crops such as sugar beet, alfalfa and corn are big in Konya basin due to the resulting well economical returns. Konya province is also in first rank in accordance of animal breeding in Turkey. However, the highest cost for animal sector is feeding materials in Turkey so farmers have to produce most feeding materials under irrigation. The irrigation cost is maximum share within whole production costs in agriculture particularly by using pressured irrigation systems. Alfalfa having rich of nutrient contents and silage corn are first preference forage plants in region. Sugar beet, alfalfa and corn crops have used high water around 800-1400 mm in season. Therefore, productive utilization of irrigation water has resulted water and energy savings as well as reducing irrigation energy costs. Increase of farming areas of sugar beet, alfalfa and corn plants has resulted also serious environment problems such as groundwater depletion or formation of sinkholes in Konya plain. In result, reason behind over water pumping from ground water reservoir is current crop pattern.

In brief, increasing of production areas with low water consuming crops like pumpkin [12], sunflower, and cereals can be practical way for sustainable use of water resources in semi-arid environments such as Konya plain, Turkey.

3.2 Practicing water saving irrigation technologies

It is recommended to use pressurized irrigation systems in arid and semi-arid regions. There are plenty advantages including water saving, more uniform water application for crops, very little labor requirement, productive utilization of plant nutrients due to the almost no fertilizer percolation towards to lower parts of root systems, resulting high and quality yields and so on by use of those irrigation techniques under well management.

Those irrigation systems are strongly recommended for better water productivity, even at least 50% water saving could be accomplished by correct selection of irrigation system such as drip irrigation [13] in areas having limited irrigation water resources.

Irrigation system performance can be maximized with proper selection of irrigation system, adequate design, right choice and dimensioning of system components, proper installation and management. As a result, uniform water application by irrigation systems for crops has resulted desired crop yield and quality [14].

In Konya plain, permanent sprinkler irrigation systems with sprinklers having low flow rates are getting popularity due to the less labor requirement. Sprinkler irrigation systems have used in large farmlands of Konya plain especially for field crops. The application efficiency in such irrigation technology is satisfactory, even higher than world average, at semi-arid Konya region. Labor cost in agriculture is also very high in Turkey so irrigation systems requiring less manpower result more economical benefits. Beside that drip irrigation is getting great interests and has been used for irrigation of some field crops such as maize and sunflower, and some vegetables plants likes tomato, pepper, egg plant in Konya province.

3.3 Practicing of deficit irrigation

There is no doubt that maximum and qualified yield can be obtained from full irrigation treatment. Therefore, full irrigation can be applied particularly in regions having no water stress conditions [15]. The performance of deficit irrigation, DI, is highly relevant to cost of applied water, and price of agro-products as well as yield response to deficit irrigation.

Field trials relevant to different irrigation regimes effect on water productivity, WP, have indicated that increments of WP were 28-29% for wheat crop, and 24% for corn plant under 33% and 20% DI, respectively. The average yield reductions for wheat and corn plants were around 15%, and 2.7%, respectively in those treatments [16]. DI by drip-irrigated sugar beet, grain maize, potato and pumpkin plants at semi-arid Konya Plain of Turkey showed that 25% deficit irrigation had no result remarkable yield reduction comparison to full irrigation treatment so it was highly recommended in case the main goal of the farmers is to put more areas into the production consequently obtaining higher economical incomes at water shortage regions [17 - 20]. DI has caused also reducing irrigation energy cost, maximum rate within the all agro-production inputs in Turkey, due to the less amount of applied water during the irrigation season. In that regard, DI irrigation is friendship irrigation strategy for sustainable water utilizations of current water supplies particularly at water poor regions.

3.4 Water charge in accordance of volumetric basis

Water fees in accordance of amount of water use may be a realistic and practical solution that would push farmers to make more efficient use of irrigation water. By this way, farmers will apply water to crops with great care for minimizing irrigation cost.

Types of water resources influence water charges e.g taking water from groundwater had resulted 2.5 times greater water charging per ha over gravity water in Turkey [21]. Water price in accordance of applied water will improve water productivity thereby increase irrigation areas as well as enhancing works relevant to the evaluation and water collection dues so it can be recommended [22].

3.5 Training farmers about water management at farm levels

In farmlands, end user of water is a farmer so deep experiences of farmers have affected water productivity positively. Particularly pressurized irrigation systems need technical information. In that regards, farmers should be trained about agricultural water management at farm level. Proper training program for farmers can be done by organizing field days on sample farms. In addition, farmers should be supported by videos showing correct design, installation, and maintenance-repair works of those irrigation systems. In result, training of farmers with visual documents will be very efficient way. The more training farmers about irrigation water management leads to more water savings.

IV. CONCLUSION

Water productivity in agriculture is necessarily prerequisites particularly in water shortage ecologies. Farming activities have used about 70% fresh water in worldwide so water saving should be done in irrigation at first. In arid and semi-arid climates, following suggestions could be underlined for sustainable uses of water resources: 1- Land sizes of cultivated crops with irrigation should be planned in accordance current water resources of region, 2- New crop varieties having tolerant to the dry environments should be developed, 3- Areas practicing innovative irrigation technologies resulting better water savings should be enlarged, 4- Training activities for farmers about irrigation water management in field conditions should be

increased, 5- Deficit irrigation should be applied for some crops e.g. 25% deficit irrigation can be recommended for some field crops such as sugar beet, corn, sunflower, potato and so on, 6- Fresh water from neighbor basins can be brought to water shortage farmlands, and 7- Rainwater harvesting techniques could be viable solution for improvement of rainwater effectiveness for crop production.

V. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Preparation of Crop Calendar on Mangalbari Town under Matiali Block, Jalpaiguri District

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Abstract— *The crop calendar in a single word is time-table providing periodical information of sowing, growing and harvesting of different crops in relation to the climatic conditions of a particular area in advance. It also enhances the crop productivity and determines the appropriate distribution of labor, application of manures in the field as well as the wholesome development of the agronomy of a specific area. The present work is an effort to highlight the present pattern of agricultural practice as well as to identify different types of crops are produced in the Mangalbari town of Jalpaiguri, West Bengal. The investigation also focuses on the assessment of crop combination, crop specialization & crop diversification in the study area to end with the preparation of crop calendar. The entire work concludes with précised suggestive measure for the development of agronomy in the area.*

Keywords— Crop Calendar, Crop Combination, Crop Specialization, Crop Diversification, Agronomy.

I. INTRODUCTION

'Crop Calendar' is a tool as well as a completion of work that provides timely information about seeds to promote local crop production. This tool contains information on planting, sowing, and harvesting menses of locally adapted crops in particular agro-ecological zones. It also provides information on the sowing rate of seeds and planting equipment, and the staple agricultural practices. This work supports many cultivators and agricultural extensionists across the world in taking correct decisions on crops and their sowing periods, respecting the agro-ecological dimensions. It also provides a solid base for emergency planning of the exoneration of farming systems after disasters.

The present paper is an attempt to study the pattern of the agricultural practices as well as the differential production varieties in the area under review. In addition to that, it highlights on the duration of crops along with their sowing and harvesting point of time organizing in a particular schedule i.e. Crop Calendar.

II. LITERATURE REVIEW

Generally, through a Crop Calendar we get to know the Crop Combination, Crop Specialization, Crop Diversification, Crop Concentration etc. At the same time, we can get an idea of the climatic characteristics there through the crop produced. The importance of a Crop Calendar in geography is huge, because most developing countries depend on the crop production, the economy of that country, like India. So, it can be said that a big part of the economy comes from the Agricultural Field. So, creating a Crop Calendar of any particular region is very important. Some Geographers and Economists worked at the macro level in the Jalpaiguri District. **Prof. Bipul Chandra Sarkar** identified the agricultural regions of Jalpaiguri at Block Level and gave a brief description. He Said, "Agriculture is the backbone of economy of the district of Jalpaiguri and the land use pattern quite differs from that of West Bengal due to its diversity in relief and climate."

Moreover, Professors of Economics **Debasis Mithiya and Kumarjit Mandal** worked on Agricultural activities on West Bengal. The concept of crop concentration or the geographic/regional concentration is based on a specific crop. Regional concentration of a specific crop reflects the distribution of its regional shares. A highly concentrated crop will be a very large part located in a small number of regions. In other words, crop concentration means the variations in the density of any crop in a region at a given point of time (**R.K Banerji, et al.).** "Therefore, crop concentration and crop diversification are the two fundamental elements of agricultural economy that determine the cropping pattern of Jalpaiguri. Consequently, knowledge about crop concentration and diversification in Jalpaguri may be considered very useful in proper agricultural land use planning. So, the knowledge of crop concentration and diversification not only provides an idea of crops that dominate a

particular region but also guides the land use planning and thereby strengthen the agricultural economy" (**Basu Roy P, 2014**). Researchers have worked on many other fields at micro level study in the study area previously, except Preparation of Crop Calendar. But, a large part of demand for the Jalpaiguri's as well as West Bengal's markets comes from the Mangalbari Town in the Dooars.

So, the present work tries to study & prepare Crop Calendar at a micro level in the Mangalbari Town. Through this crop calendar, it has been highlighted not only the Major Crops in the study area, but also the Amount of cultivated area of different crops, Production amount of crops, Type of Cultivation, Sowing-Growing-Harvesting season of crops, Economy structure etc. Besides, Literacy rate of Mangalbari Town is 62 per cent (Average) & most of the people here are clearly involved with the agricultural field.

III. OBJECTIVES

- To study the general pattern of agriculture and to recognize different types of crops produce in the area;
- To assess the crop combination, identify the crop specialization & crop diversification;
- To prepare crop calendar of the particular study area;
- Finally, to suggest some measures to develop the agro-economy on the basis of result and findings.

IV. RESEARCH METHODOLOGY

Field study requires certain processes and methods within this and should be systematically followed in order to have an organized field report. The three stages include-

4.1 Pre-field Work

This involves an idea about the place before visiting it, with location, topography, soil, climate, economy etc. from secondary data source. It also involves the collection of base map *(Mouza Map)* and Topographical Sheets collected from the SOI *(Survey of India)* and other secondary information from district gazetteers & district census handbook. In addition a structured questionnaire schedule was also prepared.

4.2 Field Work

It involves the collection of both primary and secondary data from field, showing of maps and conducting primary survey with structured questionnaire schedule. Primary data were done by random sampling of house-holds nearby the agricultural fields. The agricultural pattern was observed by visiting the farmers and interviewing them.

4.3 Post-field Work

It is the most vital part of the study. The primary data collected from the field is processed and analyzed using statistical and cartographic techniques and the crop calendar is prepared with proper illustrations.

V. DATABASE

The database of the study incorporates the primary as well as the secondary data sources:

a) The secondary data sources are different types of maps collected from Survey of India, information about the place from district gazetteers, old maps and route charts from websites, the economic backdrops and the agricultural aspects from different published and unpublished literatures.

b) Apart from the secondary data, the entire work is based on the data and information collected from the field visit or primary data source. Structured questionnaires were generated to study the area in a comprehensive way.

VI. BRIEF LAYOUT OF THE STUDY AREA

The present study in an attempt to explain the crop concentration and diversification in agriculture of Mangalbari under Matiali Block, Jalpaiguri District of West Bengal, it's one among the 3 towns of Matiali Block of Jalpaiguri District. As per government register, the town number of Mangalbari is 307021. The town has 1390 households.

The study area stretches within three blocks of Jalpaiguri District (i.e. Mal, Maynaguri & Matiali) extending from 26^0 25' N to 27^0 N and 88^0 30' E to 89^0 E. Geographically the area is well known as 'Dooars'. The total area of Mangalbari Town is 8.5^2 KMs. Out of these 4.5^2 KMs are Agricultural and Farm Lands and 4 KMs are Reserve Forests.

FIGURE 1: Location Map

Source: Compiled by Author

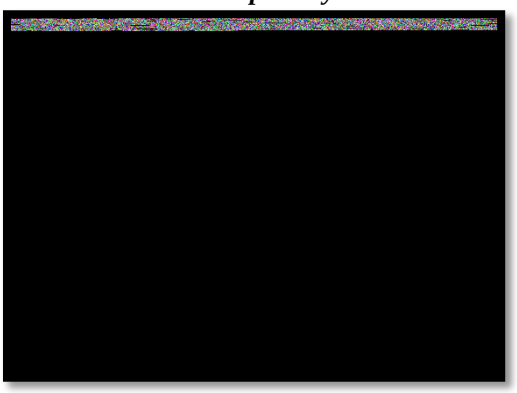

FIGURE 2: Survey Points of the Study Area

Source: Compiled by Author

6.1 Landscape

This area is almost flat nature and suitable for cultivating a variety of vegetables, although it is not suitable for the cultivation of other crops, but the crop of rice flour is cultivated throughout the year.

6.2 Climate

This region is suitable for cooler climate and for Tea cultivation. Apart from tea other crops are suitable for cultivation. The highest warmth is 29^0C and the minimum temperature is 18^0C. *Aman* rice and many types of vegetables are good quality in this climate; there is a lot of rainfall here which is suitable for tea cultivation throughout the year.

6.3 Soil

The soil here is iron clay so it's suitable for few specific types of cultivation. Huge organic carbon concentration in the top layer facilitates cultivation of multiple types of vegetation. But leaching and sheet erosion are very prominent and as a result of high amount of rainfall amount of organic matter in soil slows down. These soils are reclaimed and are prepared again by stubble mulching process for the tea plantation & other crop production. This process involves the remnants of crops (crop residues) over the agricultural fields during fallow period.

6.4 Population

According to census 2011, Mangalbari's population is 5934, out of which, 2972 are males and the 2962 females. This town has 661 children in the age group of 0-6 Years. Among them 321 are boys & 340 are girls.

VII. RATIONALE BEHIND SELECTION OF THE STUDY AREA

The area under review has large amount of agricultural prospects as the soil is rich in organic matter and irrigational water is provided by nearby river and well water.

a) It is evident that according to census 2011 the count of employed people of Mangalbari is 2276 while 3658 are unemployed. Again out of 2276 working individual 994 individuals are completely reliant on agriculture.

b) Huge rainfall in the wet months in this region is the source of large amount of water for irrigation, but the method of water conservation is absent. About 99 per cent of the residents depend on the monsoon for cultivation and tank irrigation is very popular source of irrigation here. *Kharif* crops are moderate in production, but there is lot of cultivation of Rabi grains by irrigation from rivers and tube-wells.

c) The versatile nature of crops produced here is another important reason behind the selection. Although, tea plantation is dominant in nature, but during field survey it's observed that multiple types of crops are produced here. Jute cultivation is very common along with plantation of Rubber and Betel Nuts orchards in almost every household. The cultivation of various kinds of vegetables and fruits throughout the year in the area would be suitable in the generation of the crop calendar.

VIII. ECONOMY OF THE STUDY AREA

Manufacturing of Tea is the major economy of Mangalbari. The raw material for these tea industries is tea leaf, which is abundantly produced and supplied to the factories. Moreover, the cultivation of the horticulture is also promoted thereby serving the district's economy to a large extent. Besides these, Jute and Pepper is an additional product here much of which is exported. The orchards fruits (mainly Betel nuts, Maize & Banana) draw a large quantity of export income. Hence the economy of Mangalbari is agriculture based.

Besides, there are plenty of Tea factories in the Mangalbari strengthen the economic system of local inhabitants. Now-a-days, Dooars is very famous and popular tour destination. The economy of the study area is also advanced in the tourism industry. Throughout the year, many tourists come here, but October to January to peak season. The cottage made of tin roof and cement is truly astonishing tourist here and it's said to be developed from the economic aspects of Dooars, and people here, especially the indigenous people, make cut woods from the forests & sometimes they are thumbnails from the surface for the animal species do not come in the cottage. In one word the economy of this place is strong.

IX. AGRICULTURE AS A PART OF ECONOMY IN THE STUDY AREA

Agriculture is the most important sector of the study area. Here many people are dependent upon agriculture as mentioned previously. Major crops are Tea, Rice, Wheat, Maize, Vegetables etc. Tea is the main important crop as the industrial sector is developed here and many large private Tea Estates are flourished here by the companies like Goodricke, Lipton etc. Apart from this, the Rice, Jute, Black Pepper, Betel Nut etc. holds the people's economy. Cultivation or the horticulture is also promoted thereby serving the district's economy to a large extent.

Farmers are habituated with indigenous methods for sowing, growing & harvesting of the crops. The Tea Estates are covered by small shading trees everywhere in between the gardens and they control the pests by wrapping the lower part of the tree trunk using yellow colored paper with special type of adhesives. Rubber plantation and Betel nut orchards amidst the tree estates is very common scenario over here. These kinds of eco-friendly procedure are very familiar here. They practice traditional techniques like stubble mulching, strip cropping, conservation tillage etc., which not only helps in fertilizing the soil but also prevents soil erosion due to heavy run-off in the area.

TABLE 1
PRODUCTION AMOUNT OF DIFFERENT MAJOR CROPS AND THEIR TYPE OF CULTIVATION AT MANGALBARI TOWN, DOOARS

Major Crops	Productive Amount (Tonnes)	Type Of Agriculture
Cereals	54.733	Sustainable Agriculture
Pulses	0.5	Sustainable Agriculture
Jute	4.00	Commercial Agriculture
Tea	53.72	Commercial Plantation Agriculture
Fruit	0.9	Sustainable Agriculture
Vegetables	1.02	Sustainable Agriculture
Others	27	Commercial Agriculture

Data Source: District Official Website of Jalpaiguri, 2020

It is evident from the above table that sale their products to the local markets however, return is insufficient.

X. CROP COMBINATION AT MANGALBARI TOWN, JALPAIGURI

10.1 Crop Combination method of Weaver

In the field of Agricultural geography **Weaver (1954)** was the first to use statistical technique to establish the crop combination of the Middle West of USA. Weaver computed the per cent of total harvested cropland occupied by each crop that held as much as 1 per cent of the total cultivated land. In his work Weaver calculated deviation of the real per cents of crops (occupying over 1 per cent of the cropped area) for all the possible combinations in the component areal units against a theoretical standard are as follows:

Monoculture = 100 per cent of the total harvested crop land in one crop.

2-crop combination = 50 per cent in each of two crops.

3-crop combination = 33.33 per cent in each of three crops.

4-crop combination = 25 per cent in each of four crops.

…

10-crop combination = 10 per cent in each of ten crops and so on.

For the determination of the minimum deviation the standard deviation method was used. The actual formula he used was excludes the square root as variance.

$$D = \text{Summation } d^2/n$$

Where d is the difference between the actual crop per cent and the appropriate per cent in the theoretical standard in a given aerial unit and n is the number of crops in a given combination.

Monoculture = (100 - 1st crop's land use value)^2/1

2-crop combination = (50 - 1st crop's land use value)^2 + (50 - 2nd crop's land use value)^2/2 and so on.

10.2 Crop Combination method of Rafiullah

Looking the weakness of Weaver's method which tends to include all or most of the crops and over generalization, **Rafiullah (1956)** developed a new deviation method. The technique devised by him may be expressed as follows:

$$d = (\text{summation } D^2p - D^2n) / N^2$$

Where d is the deviation, Dp is the positive difference and Dn is the negative difference from the median value of the theoretical curve value of the combination, N is the number of crops in the combination. Rafiullah's combination method is known as maximum positive deviation method. From the calculation, maximum value is taken into consideration.

Monoculture= (1st crop's land use value-50)^2/ N ^{2}

2 crop combination= {(1st crop's land use value-25)^2 - (2nd crop's land use value-25) ^{2}}/N^2

…

The Statistical technique advocated by Rafiullah is more accurate, objective, and scientific and therefore quite popular for the delineation of crop combination regions.

TABLE 2
TOTAL AREA OF DIFFERENT MAJOR CROPS (IN HECTARES AND PER CENT)

Major Crops	Total Area of Different Crops (In Hectares)	Total Area of Different Crops (In Per cent)
Cereals	8.2005	6.34
Pulses	1.665	1.29
Vegetables	2.6315	2.03
Jute	4.792	3.70
Tea	107.1035	82.80
Fruits	0.643	0.50
Others	4.322	3.34

Data Source: Computed by Author from the District Official Website of Jalpaiguri, 2020

In Weaver's combination analysis 5 main crops of the Mangalbari town is taken into consideration which occupy more than 1 per cent area of the Gross Cropped Area. These are Cereals, Pulses, Vegetables, Jute, & Tea. By the calculation procedure following table is generalized.

TABLE 3
CROP COMBINATION FOR MANGALBARI TOWN (2020) AFTER WEAVER

S. No	Town	Mono	2 Crop	3 Crop	4 Crop	5 Crop	Remarks	Combination Crops
1	Mangalbari	295.84	1491.02	1351.23	1167.59	1013.82	**Mono-crop**	T

Data Source: Computed by Author
***T = Tea**

Rafiullah method of crop combination is more accurate and practical in the town of Mangalbari. His Maximum positive deviation method is tabulated below:

TABLE 4
CROP COMBINATION FOR MANGALBARI TOWN (2020) AFTER RAFIULLAH

S. No	Town	Mono	2 Crop	3 Crop	4 Crop	5 Crop	Remarks	Combination Crops
1	Mangalbari	1075.84	748.16	454.76	294.82	204.29	**Mono-Crop**	T

Data Source: Computed by Author
***T = Tea**

XI. CROP SPECIALIZATION AT MANGALBARI TOWN, JALPAIGURI

Crop specialization is done to determine the special crops in that regional unit. It is perhaps done from combination table. Up to last combination level it is counted as 100 per cent area. Again calculation is done for cropping area. Three hierarchical conditions are assumed. High specialization (more than 66.67 per cent), moderate (33.33 - 66.67 per cent) and low (less than 33.33 per cent). In the Mangalbari Town one type of crop is specialized rather than crop diversification. Following table will highlight the condition of crop specialization in the town.

TABLE 5
CROP SPECIALIZATION FOR MANGALBARI TOWN (2020) AFTER WEAVER

S. No	Town	Mono	2 Crop	3 Crop	4 Crop	5 Crop	Degree of Specialization		
							High (>66.67)	Moderate (33.33-66.67)	Low (<33.33)
1	Mangalbari	86.11	6.59	3.85	2.11	1.34	Tea	-	-

Data Source: Computed by Author

XII. CROP DIVERSIFICATION AT MANGALBARI TOWN, JALPAIGURI

Crop Diversification is a concept which is opposite to Crop Specialization. The farmers all over the world, especially in the developing countries, try to grow several crops in their holdings in an agricultural year. The level of crop diversification largely depends on the geo-climatic/socioeconomic conditions and technological development in a region. In general higher the level of agricultural technology, lesser the degree of diversification. Moreover, the rich farmers prefer to specialize in agricultural enterprise while the poor and subsistence farmers are generally more interested in the diversification of crops.

For the measurement of Crop Diversification, **Bhatia (1965**) developed a formula based on the gross cropped area. The formula has been expressed as:

Index of Crop Diversification (ICD) = Per cent of sown area under crops / Number of 'x' crops

In general it's assumed by Bhatia that 'x' crops are those crops that individually occupy 10 per cent or more of the gross cropped area in the area under study. The degree of Crop Diversification is closely influenced by the soil characteristics, soil moisture, amount of rainfall received, the availability of irrigation facilities, the accessibility of the arable land and the technology deployed by the cultivators. The areas of extreme wet or extreme dry climate are least conductive for crop diversification.

According to the Bhatia's concept (1965) there is very low diversification in Mangalbari Town. Only Tea crop occupies more than 10 per cent of the gross cropped area. So, there is clearly seen that the one specialization crop is Tea *(82.80 per cent).*

But, **Jasbir Singh (1976)** has modified the technique of Bhatia. In his modified technique, the crops, which occupy individually less than 5 per cent, are not considered for calculating the index of diversification.

According to the Jasbir Singh's concept (1976) there is also very low diversification in Mangalbari Town but, Tea and Cereals crops are occupy more than 5 Per cent of the gross cropped area. So, there is clearly seen that the two specialization crops are Tea and Cereals at the Mangalbari Town, Jalpaiguri.

**ICD = (82.80 per cent + 6.34 per cent) / 2 = 44.57 per cent.*

XIII. CROP CALENDAR AT MANGALBARI TOWN, JALPAIGURI

Crop calendar is the fundamental element of Agricultural Geography because it helps to know cropping model of a region in a very comprehensive manner as it provides knowledge about concentration, rotation and diversification of agriculture in a simple and single graphic representation of a region.

Crop concentration refers to the spatial density of individual crop or it may be stated as the variation in the density of any crop in a region at a fixed time span. On the other hand, crop diversification means cultivation of various crops in a particular soil regime. Thus, it refers to growing of varieties of crops either in a region or in the same agricultural field.

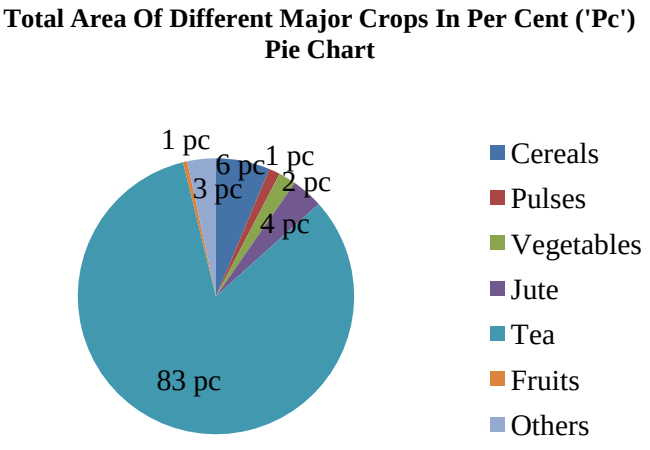

FIGURE 3: Total Area of Different Major Crops in Per cent.
(*Source: Compiled by Author)*

Basically, during the period of Green Revolution in the late sixties, there was a surge for diversified agricultural system to rejuvenate agricultural economy and for that purpose; it becomes necessary to diversify the cropping pattern to country's growing demand and to increase income by earning foreign exchange. Consequently, crop concentration and diversification do not only provide that idea of a region dominated by particular crop but also play a role of guide to strengthen agricultural economy and land-use planning.

Crop Calendar assists the economic planners in proper agricultural land-use planning. The crop calendar is also efficient in assessing the exact timings of sowing, growing, and harvesting of a particular crop along with the climatic elements such as temperature, rainfall and relative humidity for the beneficiaries, mainly the cultivators of the area.

TABLE 6
AVERAGE TEMPERATURE (in ^{0}C), RAINFALL (in mm), AND RELATIVE HUMIDITY (in Per cent) IN THE STUDY AREA (MANGALBARI TOWN, DOOARS)

Months	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Temperature (in ^{0}C)	17.25	20.29	24.46	26.47	21.64	28.72	29.05	28.44	27.97	26.45	23.10	19.70
Rainfall (in mm)	8.17	4.15	17.30	119.75	201.15	371.68	512.08	405.37	372.90	125.07	15.75	00.00
Relative Humidity (in Per cent)	88	81	74	75	75	84	87	90	81	83	79	79

Data Source: Regional Meteorological Centre, Kolkata, 2019-20

TABLE 7
SOWING, HARVESTING AND TOTAL CULTIVATION SEASON OF MAJOR CROPS IN THE STUDY AREA (MANGALBARI TOWN, DOOARS)

Items	Season (Total)	Sowing	Harvesting
Cereals	July to December	July 1st Week	December Last Week
Vegetables	January to December	Any Time	Any Time
Jute	December to May and July to November	July 1st Week & December 1st Week	November Last Week & May Last Week
Tea	July to December	July 1st Week	December Last Week
Fruits	January to November	January 1st Week	November Last Week
Others	January to December	Any Time	Any Time

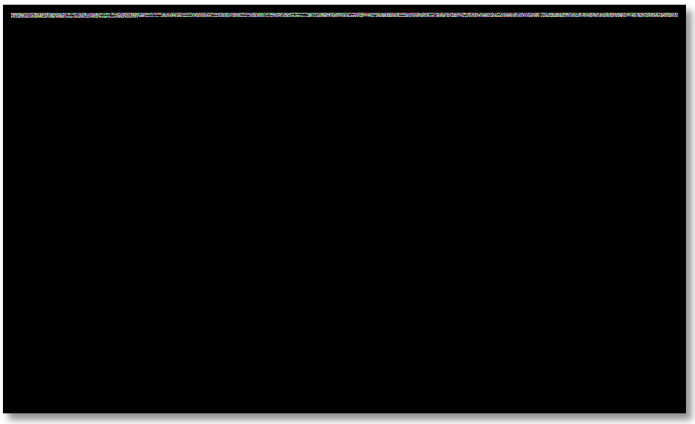

FIGURE- 4: *'ERGOGRAPH'*, Showing The Relationships Between Rainfall, Temperature, Relative Humidity and Cultivation of Major Crops in the Study Area (Mangalbari Town, Dooars)

XIV. RESULTS AND CONCLUSION

The crop calendar that has been prepared on the basis of secondary as well as primary data related to the agriculture in Mangalbari Town. The secondary data and information about weather elements, we have collected from the **NRSC's** website and crop related data of Mangalbari under Jalpaiguri district has been collected by field survey.

14.1 Climatic Characteristics

The diagram depicts that the highest temperature in the study area found to be in the month of July is 29.08^{0}C and the lowest temperature is found to be in the month of January is 17.25^{0}C. Rainfall scenario of the area is very unpredictable as highest rainfall (512.08 mm) is found in the month of July but in the month of December the amount of Rainfall is almost deficient. In Jalpaiguri, the highest Relative Humidity is found to the month of January (87 per cent) on the other hand, lowest Relative Humidity is (74 per cent) found in the month of March.

14.2 Pattern of Crops

The crop calendar of Mangalbari has various types of crops production throughout the year. Tea is the most dominant crop of

the area which (82.80 per cent) of the total produced crops.

The growing season is July to December. The second dominant is Cereals (6.34 per cent) of the total gross shown area. Then the amount of Jute occupies (3.70 per cent) of total gross shown area. Other crops (Viz. Black Pepper, Betel Nuts etc.) are produced in the part of study area (3.34 per cent). Few quantities of Vegetables (2.03 per cent) and Fruits (0.50 per cent) are also produced in this area. Although, the amount of Vegetables are insignificant to the total amount of crop production, but the area under review comprises production of multiple types of fruits and vegetables. In spite, some quantities of Pulses (1.29 per cent) are also produced here.

XV. SUGGESTIONS

Suggestions that are absolutely needed in the future for farming and agro-economic development in this area:

- Increase the quality of education.
- Agricultural fields will have to be used for advanced technology.
- The quantity of improved seeds and organic fertilizers should be increased.
- Many crops should be cultivated in greater quantities.
- For farmers, necessary allowance should be made from the government.
- In the field of agriculture, farmers should be encouraged.
- All the benefits will be given from the government.
- The demand of the market has to be increased and the farmers have to pay a fair price.
- Communication needs to be improved so that primary materials can be easily accessed in the nearby industries and markets.
- The fallow land must be filled through agricultural work.

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