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## Preface

We would like to present, with great pleasure, the inaugural volume-7, Issue-8, August 2021, 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, Terrestrial 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.*

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*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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(Managing Editor)



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Sustainable Natural Resource Utilisation	Management of the Environment
Agricultural Management Practices	Agricultural Technology
Natural Resources	Basic Horticulture
Food System	Irrigation and water management
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# Mass Production of *Paecilomyces Lilacinus* by using Different Cultivation Media as an Alternative of Incubator

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**Abstract**— *Paecilomyces lilacinus* is a common saprophytic, filamentous fungus. Morphological characters of *Paecilomyces lilacinus* were separate mycelium, hyaline, conidia white to pink colored and formation of phialides. The growth of *Paecilomyces lilacinus* carried out on SDA media at room temperature was better than incubator. Various solid substrates like Rice, Wheat bran, and Sorghum were evaluated for the mass multiplication of fungus *Paecilomyces lilacinus*. Added dextrose and antibiotics in solid media for mass multiplication at room temperature. Among all the substrate Wheat bran recorded the maximum spore count of  $7.1 \times 10^8$  spore/ml followed by Sorghum  $5.4 \times 10^8$  spore/ml and Rice  $5.1 \times 10^8$  spore/ml after 20 days. Also dry mycelia weight or biomass of fungus *Paecilomyces lilacinus* without an incubator was more than using an incubator.

**Keywords**— *Paecilomyces lilacinus*, filamentous fungus, phialides, biomass of fungus, incubator.

## I. INTRODUCTION

In recent years, few environmental issues have aroused the concern of the public as much as pesticides, especially in relation to the health of children. In spite of the many published studies on the subject of pesticides and human health, there remains deep controversy surrounding these crops. They are in a dilemma to either sacrifice a significant share of their crops to pests or use highly toxic pesticides that can harm human health and the environment. Bio pesticides are key elements of incorporated insect management programs, and are receiving much practical attention as a means to reduce the fill of artificial chemicals being used. After twenty years it was found that the level of synthetic pesticides were building and were not biodegradable and their harmful effects started coming out. There is a need to create bio pesticides which are effective, eco-friendly and do not leave any harmful effect on the environment. 'Bio pesticides' are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. Bio pesticides also play an important role in providing pest management tools in areas where pesticide resistance, niche markets and environmental concerns limit the use of chemical pesticide products. The most widely Known microbial pesticides are varieties of the bacterium *Bacillus thuringiensis*, or BT, which can Control certain insects in cabbage, potato, and other crops.

Bio pesticides can be considered as dividing into three major classes:

1. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest. For example, there are fungi that can control certain weeds, and other fungi that can kill specific insects.
2. Biochemical pesticides are naturally occurring substances that control pests by non-toxic mechanisms. Conventional pesticides, by contrast, are generally synthetic materials that directly kill or inactivate the pest. Biochemical pesticides include substances, such as insect sex pheromones, which interfere with mating, as well as various scented plant extracts that attract insect pests to traps.
3. Plant-Incorporated-Protectants are pesticidal substances that plants produce from genetic material that has been added to the plant. For example, scientists can take the gene for the B.T.pesticidal protein, and introduce the gene into the plant's own genetic material. Then the plant, instead of the B.T.bacterium, manufactures the substance that destroys the pest.

They impede the take up of water and nutrients and weaken the stand ability of affected plants. The nematode species involved are worldwide in their distribution and collectively cause billions of dollars of crop damage every year. The plant parasitic nematodes, the hidden enemies of farmers, cause an average annual loss of about 8. Plant nematodes are one of the most important and difficult pests to control in agriculture. Vegetative hyphae are branched and septate. *P.lilacinus* occurs naturally in soil, in egg clusters contained in the gelatinous egg mass of root-knot nematodes, and in cysts of *Globodera* spp. and *Heterodera* spp. In this respect *Paecilomyces lilacinus*, a facultative fungal parasite on eggs and females of root-knot nematodes, is a promising tool. Carrier used in the mass production system and application technology determines the successful use of bio agents against nematodes. It has been found in nematode eggs and occasionally from females of root knot and cyst nematodes. In addition, it has been frequently detected in the rhizosphere of many crops. It has a wide pH tolerance and can grow on a variety of substrates. *P.lilacinus* has shown promising results as a biocontrol agent to control the growth of destructive root knot nematodes.

## II. REVIEW OF LITERATURE

Lysek first reported the association of the fungus *Paecilomyces* with the eggs of *Meloidogyne* spp. was affected by naturally occurring *P.lilacinus* and *Verticillium* sp. in soils of peanut collected from fields of Alabama, USA. Stirling and West reported that the considerable numbers of *Meloidogyne* eggs were parasitized by *P.lilacinus* in tropical and subtropical soils in Australia. The fungus *P.lilacinus* was reported to parasitize eggs of root knot nematode *M.incognita* (Jatala et al., 1979, Jatala, 1982, Gintis et al., 1983; Goodey et al., 1983). They also studied the effect of temperature on their growth and bio efficacy and reported that maximum growth as determined by dry weight of mycelium has occurred from 26-30o C where as it was least at 12- 36 C. Jatala reported that application of *P.lilacinus* to rhizosphere of oranges and lemons in Peru and reduced the damage caused by *M.incognita* and *Tylenchus semipenetrans*. Stephan and Al Din, standardised the optimum temperature requirement for the growth of *P.lilacinus* and reported it to be between 20-250 C and sporulation between 10-300 C. Mani et al. indicated that wheat, bajra, rice and jowar were suitable substrates for multiplication of *P.lilacinus* and also reported reduction of *Tylenchus semipenetrans* population with increased levels of *P.lilacinus*. Daisy worked on mass production of nematophagous fungi, *Paecilomyces farinosus* and *P.lilacinus*. Among different media tested for the mass production of *P.lilacinus*, 6% molasses medium supported significantly less mycelial growth compared to PDB whereas the biomass production and spore production were significantly high on 6% molasses. Among the oil cakes, *P.lilacinus* produced significantly more spores on cotton seed cake followed by groundnut and coconut cake. Sugarcane press mud, an Agricultural waste, supported the growth as well as significantly greater spore production of *P.farinosus* and *P.lilacinus* (10. Prabhu et al. mass produced *P.lilacinus* in different liquid media and evaluated them for growth and spore production. Viability of the spores was tested at frequent intervals and shelf life was up to 120 days in talc and flyash. Amala evaluated various solid substrates like rice bran, wheat bran, gingelly oil cake, coir pith and neem cake for the mass multiplication of *Paecilomyces lilacinus*. Mojumder et al. reported the application of neem and biocontrol agents *P.lilacinus* and *Verticillium chlamydosporium* singly or in combination reducing the reniform Nematode population on egg plant. Sixty days after sowing, the growth of okra plants was greater and the root knot nematode population was reduced in all the treatments compared to untreated control. Raja and Ranganathan evaluated the biocontrol potential of *Paecilomyces lilacinus* in field conditions in two seasons during 2005-2008 at Annamalainagar district of Tamilnadu. In the field evaluation of different doses and application methods of *P.lilacinus* viz., seed treatment, seedling treatments, soil application treatments and the integration treatments produced mixed results on the growth of okra. Rao et al. studied the bio-efficacy of a bio-nematicide *P.lilacinus* (*Purpureocillium lavenderum* Luangsa-Ard) for the management of *Meloidogyne incognita* on tomato at IIHR Bangalore and Kanpur.

## III. MATERIAL AND METHOD

### 3.1 Material

- Glassware: - petri plate, test tube, beaker, flask, spreader, inoculating loop, funnel.
- Other: what man paper, aluminum foil, micro pipette, tips
- Equipment: - Autoclave, Laminar air flow, Incubator, weighing balance, burner.

### 3.2 Media

#### 3.2.1 Solid media

- SDA: Composition for 1 liter: Peptone- 10 g, Dextrose- 40 g, Agar- 30 gm.
- Wheat bran
- Sorghum powder & sorghum seed
- Rice powder

#### 3.2.2 Antibiotic

Chloramphenicol, Tetracycline, Amoxicillin, Gentamicin, Mancozeb+Metalaxyl, Fluconazole.

### 3.3 Methods

- Isolation Method
- Subculture Method
- Mass Multiplication Method
  1. Multiplication of *P.lilacinus* on sorghum powder with incubator
  2. Multiplication of *P.lilacinus* on sorghum powder without incubator
  3. Multiplication of *P.lilacinus* on wheat bran with incubator
  4. Multiplication of *Paecilomyces lilacinus* on wheat bran without incubator
  5. Multiplication of *P.lilacinus* on rice (poa) with incubator
  6. Multiplication of *P.lilacinus* on rice (poa) without incubator
  7. Mass multiplication on A media given by vise innovative enterprise Pvt. Ltd to observe *P.lilacinus* growth on different content of moisture:
    1. Take 20 gm of A media powder in each 4 flask.
    2. Add different content of *P.lilacinus* suspension in each four A media flask.
    3. Add 4ml, 6ml, 8ml, 9ml suspension of *P.lilacinus*. In each 20gm of A media flask under laminar air flow and cap with cotton plug and apply aluminum foil paper.
    4. After 25°C, 7 days of incubation, the fungal biomass of *P.lilacinus* along with A media.

### 3.4 Parameters

#### 3.4.1 CFU count by Hemocytometer

- Loading the Hemocytometer
- Counting cells in a hemocytometer:

$$Total\ cells/ml = Total\ cells\ counted \times \frac{dilution\ factor}{number\ of\ squares} \times 10,000\ cells/ml$$

So, for example, if you diluted your sample 1:1 with Trypan blue, and you counted 325 cells in 4 corner square plus the central big square.

$$Total\ cells/ml = 325\ cells \times \frac{2(dilution\ factor)}{5\ squares} \times 10,000\ cells/ml = 130 \times 10^4\ cells/ml$$

If you want to know how many cells you have in your original sample, just multiply the cell concentration by total sample volume. For example, if your original sample volume is 5 ml, than your sample has a total of:

$$130 \times 10^4\ cells/ml \times 5ml = 650 \times 10^4\ cells$$

### 3.4.2 Microscopic Examination

### 3.4.3 Biomass calculation of *Paecilomyces lilacinus*:

#### 3.4.3.1 *Paecilomyces lilacinus* growth with incubator:

The dry weight of the fungus was calculated by using the following formula:

$$\text{Dry weight} = (\text{weight of petri plate with mycelium}) - (\text{weight of petri plate})$$

#### 3.4.3.2 *Paecilomyces lilacinus* growth without incubator:

The dry weight of the fungus was calculated by using the following formula:

$$\text{Dry weight} = (\text{weight of petri plate with mycelium}) - (\text{weight of petri plate})$$

## IV. RESULT

### 4.1 Zone of inhibition on different antibiotics dose on *Paecilomyces lilacinus*:

Gentamicin, Mancozeb, Mancozeb + Metalaxyl, and fluconazole which are antibacterial antibiotics which reduce the contamination of bacteria in culture plates during incubation periods.

**TABLE 1**  
**ZONE OF INHIBITION ON DIFFERENT ANTIBIOTICS DOSE ON *PAECILOMYCES LILACINUS***

Antibiotics	Zone of inhibition for 10 ml (mm)	Zone of inhibition for 100 ml (mm)
Mancozeb + metalaxyl	0.05	0.1
Gentamicin	0.02	0.3
Fluconazole	0.01	0.1
Mancozeb	0.5	0.8

[Zone of inhibition of different antibiotic dose on *P.lilacinus* on culture plate using Disc Diffusion method day 3 results]

- 100 ml and 10 ml concentration of different antibiotic dose combination :

Zone of inhibition on *P.lilacinus* culture plate by using 100ml and 10ml concentration of different antibiotic dose combinations did not appear but inhibited contamination of bacteria.

### 4.2 Biomass calculation of *Paecilomyces lilacinus* with incubator and without incubator:

$$\text{Dry weight} = (\text{weight of petri plate with mycelium}) - (\text{weight of petri plate})$$

### 4.3 Effect of different grain substrate medium on sporulation of *Paecilomyces lilacinus* with incubator:

*P.lilacinus* maximum spore production on wheat bran  $3.4 \times 10^{-8}$ . Rice and sorghum also produce good spore production.

**TABLE 2**  
**DIFFERENT GRAIN SUBSTRATE MEDIUM ON SPORULATION OF *PAECILOMYCES LILACINUS* WITH INCUBATOR**

Grain (20g)	Spore count ( $\times 10^{-4}$ ) (Series 1)	Spore count ( $\times 10^{-6}$ ) (Series 2)	Spore count ( $\times 10^{-8}$ ) (Series 3)
Rice	4.1	3.6	2.8
Wheat bran	5.9	4.02	3.4
Sorghum	4.8	4.01	3.2

### 4.4 Effect of different grain substrate medium on sporulation of *Paecilomyces lilacinus* without incubator:

*P.lilacinus* maximum spore production on wheat bran  $7.1 \times 10^{-8}$  which are more than with incubator method. On rice and sorghum spore production is also more than with the incubator method.

**TABLE 3**  
**DIFFERENT GRAIN SUBSTRATE MEDIUM ON SPORULATION OF *PAECILOMYCES LILACINUS* WITHOUT INCUBATOR**

Grain (20g)	Spore count ( $10^{-4}$ ) (Series 1)	Spore count ( $10^{-6}$ ) (Series 2)	Spore count ( $10^{-8}$ ) (Series 3)
Rice	6.5	5.8	5.1
Wheat	9	8.4	7.1
Sorghum	7.3	6.6	5.4

**4.5 Mass multiplication on A media given by vise innovative enterprise Pvt. Ltd to observe *P.lilacinus* growth on different content of moisture:**

Various concentration volume of suspension in A media and measured growth of *P.lilacinus*.

**TABLE 4**  
***P.LILACINUS* GROWTH ON DIFFERENT CONTENT OF MOISTURE**

Volume of Suspension	Growth of <i>P.lilacinus</i>
4 ml	60%
6 ml	69%
8 ml	86%
9 ml	90%

## V. DISCUSSION

Recently, *Paecilomyces lilacinus* based different formulations are used for the control of nematode diseases, which is economical, eco friendly and sustainable in the long run. Among the microorganisms, bioagents are having great promise with the dual advantage of plant growth promotion and plant disease suppression. First, observed Zone of inhibition of different antibiotics 100 ml and 10 ml concentration for reducing contamination of bacteria. Different antibiotics did not reduce the growth of fungus *P.lilacinus*, only inhibited bacterial contamination. Evaluated Biomass of *P.lilacinus* without incubator results were better than with incubator. Also find out that the growth of *P.lilacinus* appeared earlier on without incubator culture plates. For reducing contamination of bacteria using different combinations of antibiotics and measuring biomass of *P.lilacinus* fungus on SDA media plates. Various solid substrates like Rice, Wheat bran and Sorghum were evaluated for the mass production of fungus *P.lilacinus*. Various solid substrates like Rice, Wheat bran and Sorghum were evaluated for the mass production of fungus *P.lilacinus*. Added dextrose and combination of antibiotics, without incubator production, among all substrate Wheat bran recorded maximum spore count of 7.

## VI. SUMMARY AND CONCLUSION

The present investigation "Mass production of *Paecilomyces lilacinus* by different methods given by vise organization" was carried out at the Department of Microbiology, Aspee Shakilam Biotechnology Institute, Navsari Agricultural University, Surat, and Gujarat, India during 2018. The results obtained from the present study can be summarized and concluded here. Nematophagous fungus *Paecilomyces lilacinus* infect nematodes with their spores which either adhere to the surface of nematodes or are swallowed by them. Ultimate result of nematode infection in any way is always the death of the host. Different antibiotics did not inhibit the growth of fungus *P.lilacinus*, only reduced contamination of bacteria, which helped in the mass multiplication of *P.lilacinus* without an incubator. Various solid substrates like Rice, Wheat bran, and Sorghum were evaluated for mass multiplication and observed without incubator growth of *P.lilacinus* was better than incubator. Also observed without incubator growth of fungus *P.lilacinus* starts earlier than with the help of incubator. From this data concluded that without incubator also carried out mass multiplication of *P.lilacinus* fungus, which very helpful to industry and also farmers can easily produce biopesticides. Which helps in reduced cost of biopesticide

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# Effect of time and proportion of leaf harvest on pest, forage and root yields of sweetpotato (*Ipomoea batatas* L.) in the inland valley swamp and upland ecologies of Njala

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**Abstract**— Dearth of knowledge exists regarding the leaf harvest intensity and frequency thresholds that support optimum forage and fresh storage root yields in Sierra Leone. A study was carried out to assess the effects of leaf harvesting time and proportion on *Cylas puncticollis* infestation, growth and yield of sweet potato in the inland valley swamp and upland ecologies of Njala. Treatment combinations comprised of two varieties (“Kabia” and “Gbanie”), four leaf harvest regimes: 0, 30 60 and 90 days after planting (DAP); and four-leaf harvest intensities (0, 25, 50 and 100%). The experiment was laid out in a randomized complete block design (RCBD) with three replications. Data collected included *Cylas puncticollis* severity on vines and storage roots, root dimensions and numbers, fresh foliage and storage root yields. The results revealed that leaf harvesting twice at 25 and 50% contributed more to optimum forage and storage root yields and related attributes of sweet potatoes compared to other treatments. The present study suggests that good agronomic management of sweet potato that supports optimum forage and storage root yields should be selected to meet the dual purpose for which it is grown. These findings serve as good guide for incorporation of leaf harvesting time, proportion of leaf harvest in germplasm assessment and new population development objectives.

**Keywords**— leaf harvest, regimes, intensities, root yield, pest, sweetpotato.

## I. INTRODUCTION

Sweetpotato (*Ipomoea batatas* L) is a dicotyledonous root crop that belongs to the family Convolvulaceae that comprises about 50 genera and more than 1000 species. *Ipomoea batatas* is the only crop of major importance in the Convolvulaceae family [1]. The crop is native to Central and South America [2] and cultivated in over 100 countries of the world. Sweetpotato is extensively grown in tropical and subtropical regions particularly in Asia, Africa and the Pacific; with Asia and Africa continents accounting for 95% of total global production [3]. Sweetpotato is the fifteenth most important food security crop, and the third most important root and tuber crops, grown on 8.6 million hectares worldwide, with production and average yield of about 106 million tons and 12.2 t ha<sup>-1</sup>, respectively [4]. China is the major producer of sweetpotato in the world, followed in order by Nigeria, Tanzania, Indonesia, Uganda, Ethiopia, Angola, India, the United States of America, Vietnam and Madagascar [4].

In Sierra Leone, the crop ranks as the second most important root and tuber crops after cassava, and is cultivated in all the provinces [5]. Both the leaves and roots of sweetpotato are consumed in Sierra Leone. Leaf harvesting from sweetpotato during its vegetative growth is common in most parts of the country. The harvested leaves are either used for vegetable or for fodder. Leaves harvested for livestock feed involves plucking the fully expanded mature leaves [6], whereas the immature leaves are used for human consumption. According to Kiozya *et al.* [7] leaf harvesting in sweetpotato reduces root yield by 43%. Moreover, Masumba [8] also noted that harvesting of a certain number of leaves from root crop reduces photosynthates thereby contributing to reduction in root yield.

Despite the dual-purpose potentiality of the leaves and roots of sweetpotato in contributing to food and nutrition security, no comprehensive study has been done on the effects of leaf harvest frequency and intensity on the growth and yield of sweetpotato. Even though earlier works by Kiozya *et al.* [7] suggests that leaf harvesting in sweet potato reduces root yield by 43%; and by Masumba [8] that increased leaf harvest intensity decreases photosynthate translocation to the roots thereby contributing to reduction in root yields, these are yet to be tested using different genotypes, ecologies and leaf harvest intensities and frequencies in Sierra Leone.

The identification of dual-purpose leaf harvest frequency and intensity with optimum economic forage and fresh root yields contributes to increasing food and feed production and productivity that meet the demands of various actors in the sweetpotato value chain. The identification of the leaf harvest frequency and intensity with optimum economic forage and fresh storage root yields also facilitate ready availability of planting materials, food and feeds, thereby contributing to increased food production and productivity. Thus, the main aim of the study was to investigate the effects of leaf harvesting time and proportion on *Cylas puncticollis* infestation, forage yield, fresh storage root yield and related attributes of sweetpotato grown in the inland valley swamp and upland ecologies of Njala. The specific objectives were to: (i) assess the effect of leaf harvest intensity and frequency on *Cylas puncticollis* infestation in sweetpotato; (ii) assess varietal response to leaf harvest intensity and frequency in sweetpotato; (iii) determine the effect of leaf harvest frequency and intensity on the forage yield of sweetpotato; and (iv) determine the effect of leaf harvest frequency and intensity on fresh storage root yield of sweetpotato.

## II. MATERIALS AND METHODS

### 2.1 Trial site description

A field trial was conducted at the inland valley swamp (IVS) and the upland of Njala during the third cropping season (dry season) of 2019 under irrigated conditions for the inland valley swamp and rain fed for the upland. Njala is situated at an elevation of 54 m above sea level on 8°06'N latitude and 12°06'W longitude. The most dominant vegetation at Njala is grassland, comprising mainly of *Andropogon gayanus* (gamba grass). Njala has two distinct seasons, like other parts of the country, including the rainy season, which starts from April to November, while the dry season lasts between December and March. The dry season trial was irrigated every other day to field capacity till establishment at 2 months after planting using watering cans. The mean monthly air temperature of Njala ranged from 23.4°C to 34.9°C, and the mean monthly relative humidity was 69.9% for the dry season trial; whereas the rainy season, the mean monthly air temperature ranged from 20.4°C to 33.8°C, and the mean monthly relative humidity was 83.9% (SLARI Weather Station, 2019). The average annual rainfall was about 2500 mm.

The soils of the experimental sites are slightly acidic and moderate in available plant nutrients. The soils from trial sites are well to moderately well-drained and low in plant nutrients [9].

### 2.2 Experimental design, treatments and management

The experimental site was manually brushed and cleared. The ridges were prepared using digging hoe, large clods were pulverized using garden hoe prior to planting. The experiment was laid out in a randomized complete block design with three replications. A total of 20 treatment combinations used in the experiment is shown in Table 1. The varieties used were “Gbanie” (V 1) and “Kabia” (V 2).

Healthy vine tip cuttings 30 cm long were used as planting materials, and planted in a slantwise orientation. Planting was done lowland ecology on the 7<sup>th</sup> of February 2019 on ridges at a plant spacing of 0.30 m × 1 m giving a plant density of 33,333 plants ha<sup>-1</sup>; and the second planting was done at the upland ecology on the 29<sup>th</sup> May 2019 with the same plant spacing and density. Weeding and earthing up to cover the exposed roots were done monthly, for both planting seasons. No fertilizer and pesticides were applied in both seasons.

### 2.3 Data collection

A total of six parameters were collected including two above ground (vine diameter, and fresh leaf yield) and above ground (tuber length, tuber diameter, number of tubers per plant and fresh storage root yield) traits. The root diameter was measured from the middle portion of the fresh storage roots. Both the root diameter and root length were measured using the vernier caliper.

At harvest, storage roots were counted and weighed using the weighing balance. Big, medium and small storage roots were randomly selected for diameter and length measurements. Harvesting at the lowland and upland ecologies was done at 120 DAP.

**TABLE 1**  
**TREATMENT COMBINATIONS FOR SWEETPOTATO TRIAL**

Variety	Leaf harvest regime	Leaf harvest proportion	Treatment combination
<b>Gbanie</b>	R0	P0	V1 P0R0
	R1,3	P25	V1 P25R1,3
	R2,3	P25	V1 P25R2,3
	R1,3	P50	V1 P50R1,3
	R2,3	P50	V1 P50R2,3
	R1,3	P100	V1 P100R1,3
	R2,3	P100	V1 P100R2,3
	R1,2,3	P25	V1 P25R1,2,3
	R1,2,3	P50	V1 P50R1,2,3
	R1,2,3	P100	V1 P100R1,2,3
<b>Kabia</b>	R0	P0	V2 P0R0
	R1,3	P25	V2 P25R1,3
	R2,3	P25	V2 P25R2,3
	R1,3	P50	V2 P50R1,3
	R2,3	P50	V2 P50R2,3
	R1,3	P100	V2 P100R1,3
	R2,3	P100	V2 P100R2,3
	R1,2,3	P25	V2 P25R1,2,3
	R1,2,3	P50	V2 P50R1,2,3
	R1,2,3	P100	V2 P100R1,2,3

*The R1,2 combinations were not included due to limited planting material and space; R0,1,2,3= leaf harvest regime at 0, 30, 60 and 90 days after planting*

## 2.4 Statistical analysis

The data collected were analyzed using analysis of variance (ANOVA). The data obtained were analyzed using the Genstat statistical package [10] and differences between treatment means were compared using the Least Significant Difference (LSD).

## III. RESULTS

### 3.1 Effects of ecology, leaf harvest frequency and proportion on severity of *Cylas puncticollis* infestation on vines and roots of sweetpotato

The infestations of *Cylas puncticollis* on vines and roots varied significantly ( $p < 0.001$ ) for the lowland and upland ecologies used in the study (Table 2). However, the infestations of *C. puncticollis* were non-significant for treatments ( $p = 0.847$ ) and ecology  $\times$  treatment interaction ( $p = 0.341$ ) for *C. puncticollis* on vines. Similarly, the infestations of *C. puncticollis* on fresh storage roots were non-significant among treatments ( $p = 0.981$ ) and ecology  $\times$  treatment interactions ( $p = 0.178$ ). The pest pressure on the vines and fresh storage roots was mild for all treatments during the rainy season cultivation in the upland ecology compared to the dry season treatments in the lowland ecology which had low attack of the pest for most treatment combinations.

### 3.2 Effects of ecology, leaf harvest frequency and proportion on leaf yield of sweetpotato

The leaf yields varied significantly ( $p = 0.013$ ) at the lowland and upland ecologies. There were also significant differences ( $p < 0.001$ ) among treatments and ecology  $\times$  treatment interaction for sweetpotato leaf yield per hectare. Leaf foliage harvest at 100% harvest intensity at 30, 60 and 90 DAP intervals (treatment V1P100R1,2,3) exhibited the highest leaf yield (26.30 t

ha<sup>-1</sup>) in the upland, whereas leaf harvest at 100% harvest intensity at 30 days after planting (treatment VIP25R1) was among treatments that produced the lowest leaf yield (1.11 t ha<sup>-1</sup>) at both ecologies (Table 3).

**TABLE 2**  
**EFFECTS OF *CYLAS PUNCTICOLLIS* SEVERITY ON VINES AND ROOTS OF SWEETPOTATO**

Treatment	<i>Cylas puncticollis</i> severity on vines		<i>Cylas puncticollis</i> severity on roots	
	Lowland	Upland	Lowland	Upland
V1P0R0	2.4	1.7	1.7	1.7
V1P100R1,2,3	2.7	1.3	2.2	1.3
V1P100R1,3	2.8	1.3	1.7	1.3
V1P100R2,3	2.8	1.5	1.8	1.6
V1P25R1,2,3	2.8	1.3	2.2	1.3
V1P25R1,3	2.9	1.1	2.3	1.1
V1P25R2,3	2.4	1.1	2.0	1.1
V1P50R1,2,3	2.7	1.4	1.5	1.3
V1P50R1,3	2.7	1.7	1.4	1.7
V1P50R2,3	2.6	1.4	1.5	1.4
V2P0R0	2.5	1.5	1.7	1.4
V2P100R1,2,3	2.8	1.0	2.4	1.0
V2P100R1,3	2.6	1.1	2.0	1.1
V2P100R2,3	2.8	1.2	2.1	1.2
V2P25R1,2,3	2.9	1.2	2.3	1.2
V2P25R1,3	2.5	1.3	1.7	1.3
V2P25R2,3	2.9	1.3	1.7	1.3
V2P50R1,2,3	2.9	1.2	1.7	1.2
V2P50R1,3	2.6	1.1	1.9	1.1
V2P50R2,3	2.7	1.1	1.8	1.1
<b>Mean</b>	<b>2.7</b>	<b>1.3</b>	<b>1.9</b>	<b>1.3</b>
Grand mean	2.0		1.6	
LSD0.05=E	0.13 <sup>***</sup>		0.16 <sup>***</sup>	
LSD0.05=T	.40 <sup>ns</sup>		0.52 <sup>ns</sup>	
LSD0.05=E×T	0.56 <sup>ns</sup>		0.73 <sup>ns</sup>	
CV(%)	17.3		28.3	

*R*=leaf harvest regime, 1, 2 and 3=30, 60 and 90 days after planting, respectively; P0=0% leaf harvest, P25=25% leaf harvest, P50=50% leaf harvest, P100=100% leaf harvest, E=ecology, T=treatment, ns=non-significant at  $p<0.05$ , \*\*\*=significant at  $p<0.001$

At 30 DAP, treatments V1P100R1,2,3 and V2P100R1,2,3 in both lowland and upland ecologies had significantly ( $p<0.001$ ) higher leaf yield than the remaining treatments except V1P100R1,3 and V2P100R1,3 (Table 3). The ecology and ecology × treatment were non-significant for leaf yield measured at 30 DAP sampling regime.

At 60 DAP, treatment V2P100R2,3 (11.1 t ha<sup>-1</sup>) significantly ( $p<0.001$ ) had the highest leaf yield followed by treatments V1P100R1,2,3 (5.57 t ha<sup>-1</sup>) and V2P100R1,2,3 (6.27 t ha<sup>-1</sup>) in the lowland (Table 4). However, in the upland, the highest leaf yielders were treatments V1P100R1,2,3 (11.85 t ha<sup>-1</sup>) and V2P100R2,3 (10.74 t ha<sup>-1</sup>). The ecology and ecology × treatment was non-significant for leaf yield measured at 30 DAP sampling regime. At 90 DAP, treatments V1P100R1,2,3 (11.48 t ha<sup>-1</sup>) and V1P100R2,3 (11.48 t ha<sup>-1</sup>) significantly ( $p<0.001$ ) exhibited the highest leaf yield in the lowland (Table 5). For the upland ecology, treatment V2P100R2,3 (10.73 t ha<sup>-1</sup>) significantly ( $p<0.001$ ) produced the highest leaf yield. The ecology and ecology × treatment interactions were significant ( $p<0.001$ ) for leaf yield measured at 30 DAP sampling regime.

**TABLE 3**  
**EFFECT OF ECOLOGY AND TREATMENT COMBINATIONS OF VARIETY, LEAF HARVEST FREQUENCY AND PROPORTION ON FRESH LEAF YIELDS (t ha<sup>-1</sup>) OF SWEETPOTATO AT 30 DAYS AFTER PLANTING**

Treatment	Leaf yield (t ha <sup>-1</sup> ) at 30DAP	
	Lowland	Upland
V1P0R0	-	-
V1P100R1,2,3	2.93	2.96
V1P100R1,3	2.57	2.59
V1P25R1,2,3	1.10	1.11
V1P25R1,3	1.10	1.11
V1P50R1,2,3	1.83	1.85
V1P50R1,3	1.83	1.85
V2P0R0	-	-
V2P100R1,2,3	2.93	2.96
V2P100R1,3	2.57	2.59
V2P25R1,2,3	1.10	1.11
V2P25R1,3	1.10	1.11
V2P50R1,2,3	1.83	1.85
V2P50R1,3	1.83	1.85
Mean	1.62	1.64
Grand mean	1.63	
LSD0.05=E	0.21 <sup>ns</sup>	
LSD0.05=T	0.55 <sup>***</sup>	
LSD0.05=E×T	0.78 <sup>ns</sup>	
CV(%)	29.2	

*R=leaf harvest regime, 1, 2 and 3=30, 60 and 90 days after planting, respectively; P0=0% leaf harvest, P25=25% leaf harvest, P50=50% leaf harvest, P100=100% leaf harvest, E=ecology, T=treatment, ns=non-significant, - =no leaf harvest, \*=significant at p<0.05, \*\*\*=significant at p<0.001*

**TABLE 4**  
**EFFECT OF ECOLOGY AND TREATMENT COMBINATIONS OF VARIETY, LEAF HARVEST FREQUENCY AND PROPORTION ON FRESH LEAF YIELDS (t ha<sup>-1</sup>) OF SWEETPOTATO AT 60 DAYS AFTER PLANTING**

Treatment	Leaf yield (t ha <sup>-1</sup> ) at 60 DAP	
	Lowland	Upland
V1P0R0	-	-
V1P100R1,2,3	5.57	11.85
V1P100R1,3	4.83	6.67
V1P25R1,2,3	1.47	1.11
V1P25R1,3	1.83	1.11
V1P50R1,2,3	2.20	1.85
V1P50R1,3	5.53	2.96
V2P0R0	-	-
V2P100R1,2,3	6.27	6.67
V2P100R1,3	11.1	10.74
V2P25R1,2,3	1.47	1.11
V2P25R1,3	1.83	1.48
V2P50R1,2,3	2.57	1.85
V2P50R1,3	4.43	2.22
Mean	<b>3.51</b>	<b>3.54</b>
Grand mean	3.53	
LSD0.05=E	0.90 <sup>ns</sup>	
LSD0.05=T	2.39 <sup>***</sup>	
LSD0.05=E×T	3.38 <sup>ns</sup>	
CV(%)	58.5	

*R=leaf harvest regime, 1, 2 and 3=30, 60 and 90 days after planting, respectively; P0=0% leaf harvest, P25=25% leaf harvest, P50=50% leaf harvest, P100=100% leaf harvest; E=ecology, T=treatment, - =no leaf harvest, ns=non-significant, \*=significant at p<0.05, \*\*\*=significant at p<0.001, V1= "Gbanie", V2="Kabia"*

**TABLE 5**  
**EFFECT OF ECOLOGY AND TREATMENT COMBINATIONS OF VARIETY, LEAF HARVEST FREQUENCY AND PROPORTION ON FRESH LEAF YIELDS (t ha<sup>-1</sup>) OF SWEETPOTATO AT 90 DAYS AFTER PLANTING**

Treatment	Leaf yield (t ha <sup>-1</sup> ) at 90 DAP	
	Lowland	Upland
V1P0R0	-	-
V1P100R1,2,3	11.48	4.07
V1P100R1,3	7.41	4.80
V1P25R1,2,3	11.48	3.33
V1P25R1,3	3.33	2.57
V1P50R1,2,3	2.96	3.33
V1P50R1,3	1.85	5.17
V2P0R0	6.30	1.47
V2P100R1,2,3	5.19	4.83
V2P100R1,3	3.70	4.43
V2P25R1,2,3	-	-
V2P25R1,3	10	4.8
V2P50R1,2,3	7.04	9.27
V2P50R1,3	9.63	10.73
Mean	2.22	2.2
Grand mean	2.22	
LSD0.05=E	2.59	
LSD0.05=T	6.30	
LSD0.05=E×T	2.96	
CV(%)	4.07	

*R=leaf harvest regime, 1, 2 and 3=30, 60 and 90 days after planting, respectively; P0=0% leaf harvest, P25=25% leaf harvest, P50=50% leaf harvest, P100=100% leaf harvest; E=ecology, T=treatment, - =no leaf harvest, \*\*\*=significant at  $p<0.001$*

### 3.3 Effects of ecology, leaf harvest frequency and proportion on root yield of sweetpotato

Fresh storage root yields differed significantly ( $p<0.001$ ) among treatments, but non-significantly varied for ecology ( $p=0.092$ ) and ecology  $\times$  treatment interaction ( $p=0.118$ ) (Table 6). “Gbanie” (14.89 t ha<sup>-1</sup>), “Kabia” (10.44 t ha<sup>-1</sup>) defoliated twice at 25% leaf harvest intensity at 30 and 90 DAP (i.e. treatments V1P25R1,3 and V2P25R1,3, respectively); and “Kabia” (12.45 t ha<sup>-1</sup>) defoliated twice at 50% leaf harvest intensity at 60 and 90 DAP (i.e. treatment V2P50R2,3) had similar fresh root yields as the non-leaf harvested plots in the lowland ecology. For the upland ecology, “Gbanie” (11.33 t ha<sup>-1</sup>) and “Kabia” (15.33 t ha<sup>-1</sup>) defoliated twice at 25% leaf harvest intensity at 30 and 90 DAP (i.e. treatments V1P25R1,3 and V2P25R1,3, respectively); and “Gbanie” (11.33 t ha<sup>-1</sup>) defoliated twice at 50% leaf harvest intensity at 60 and 90 DAP (i.e. treatment V1P50R2,3) had similar fresh root yields as the non-leaf harvested plots.

### 3.4 Effects of ecology, leaf harvest frequency and proportion on root yield attributes of sweetpotato

The fresh storage root length and width significantly ( $p<0.001$ ) varied among treatments studied, but did not vary for ecology  $\times$  treatment interactions of both traits. Three treatments (V2P50R2,3; V1P100R1,3 and V1P25R1,3) with storage root length ranging from 10.56 – 13.46 cm were similar to those of the non-leaf harvested plots in the lowland ecology (Table 6). For the upland ecology, “Gbanie” with no leaf defoliation (i.e. treatment V1P0R0) significantly produced the longest roots.

For storage root diameter, five treatments (V2P25R2,3; V2P50R2,3; V2P25R1,3; V1P50R1,3; and V1P25R1,3) with storage root diameter ranging from 6.68 – 8.84 cm had similar root diameters compared to the non-leaf harvested plots in the lowland ecology (Table 6). Treatments V1P25R2,3 (7.00 cm) and V2P25R1,3 (7.53 cm) had similar root diameters as the non-leaf harvested plots in the upland ecology. The fresh storage root number per plant significantly varied among ecology ( $p<0.001$ ), treatments ( $p<0.001$ ), and ecology  $\times$  treatment interaction ( $p=0.024$ ). Treatment V1P100R1,2,3 (2.55) had similar numbers of storage roots as the control plots in the lowland ecology. For the upland ecology, seven treatments (V2P50R2,3; V2P50R1,3; V2P25R1,3; V2P100R1,3; V1P50R2,3; V1P25R1,3; and V1P25R1,2,3) with number of storage roots ranging from 2.20 – 3.10 produced similar numbers of storage roots compared to the non-leaf harvested control plots (Table 6).

**TABLE 6**  
**EFFECT OF ECOLOGY AND TREAT COMBINATIONS OF VARIETY, LEAF HARVEST FREQUENCY AND PROPORTION ON FRESH ROOT YIELD AND ATTRIBUTES (t ha<sup>-1</sup>) OF SWEETPOTATO**

Treatment	Root length (cm)		Root width (cm)		Root no per plant		Root yield (t ha <sup>-1</sup> )	
	Lowland	Upland	Lowland	Upland	Lowland	Upland	Lowland	Upland
V1P0R0	12.51	13.63	7.63	<b>7.73</b>	2.33	2.33	<b>13.78</b>	<b>14.43</b>
V1P100R1,2,3	10.11	6.63	5.43	4.23	2.55	1.80	8.89	4.43
V1P100R1,3	11.77	6.60	6.43	3.80	2.00	1.33	9.11	3.57
V1P100R2,3	7.45	8.27	4.52	6.00	1.00	1.90	4.45	7.77
V1P25R1,2,3	8.36	9.43	4.86	5.83	1.22	2.80	3.33	10.67
V1P25R1,3	13.46	10.37	8.84	6.80	1.67	2.23	14.89	11.33
V1P25R2,3	8.10	9.57	5.56	7.00	1.33	1.90	6.22	10.00
V1P50R1,2,3	9.59	9.27	5.76	5.80	1.55	1.90	4.45	7.33
V1P50R1,3	9.47	7.57	7.37	6.03	1.45	1.87	8.67	7.57
V1P50R2,3	10.07	7.40	6.28	5.10	1.89	3.00	6.45	12.23
V2P0R0	10.30	11.20	8.13	<b>8.57</b>	2.89	<b>2.77</b>	<b>14.22</b>	<b>15.33</b>
V2P100R1,2,3	8.28	7.80	4.52	4.47	1.11	2.10	4.44	5.33
V2P100R1,3	8.30	7.70	6.46	4.87	1.22	2.47	4.22	5.13
V2P100R2,3	7.71	8.60	5.45	5.93	1.33	1.87	4.22	8.63
V2P25R1,2,3	8.05	8.37	4.77	5.70	1.00	1.67	3.55	6.47
V2P25R1,3	9.73	9.77	7.85	7.53	1.66	3.10	10.44	15.33
V2P25R2,3	8.28	7.73	6.68	5.60	1.00	2.23	2.44	8.23
V2P50R1,2,3	8.73	8.63	5.79	5.40	1.22	2.10	7.33	6.67
V2P50R1,3	7.43	7.13	4.95	4.63	1.55	2.23	4.22	5.80
V2P50R2,3	10.56	8.40	7.10	4.37	1.55	2.20	12.45	6.00
<b>Mean</b>	<b>9.41</b>	<b>8.70</b>	<b>6.22</b>	<b>5.77</b>	<b>1.58</b>	<b>2.19</b>	<b>7.39</b>	<b>8.61</b>
Grand mean	9.06		5.99		1.88		8.00	
LSD <sub>0.05</sub> =E	0.71*		0.54 <sup>ns</sup>		0.20***		1.43 <sup>ns</sup>	
LSD <sub>0.05</sub> =T	2.23***		1.70***		0.64***		4.52***	
LSD <sub>0.05</sub> =E×T	3.15 <sup>ns</sup>		2.41 <sup>ns</sup>		0.90*		6.39 <sup>ns</sup>	
CV(%)	21.4		13.3		29.4		15.3	

*R=leaf harvest, regime 1, 2 and 3=30, 60 and 90 days after planting, respectively; P0=0% leaf harvest, P25=25% leaf harvest, P50=50% leaf harvest, P100=100% leaf harvest; E=ecology, T=treatment, ns=non-significant, \*=significant at p<0.05, \*\*\*=significant at p<0.001*

#### IV. DISCUSSION

*Cylas puncticollis* infestation on the vines and fresh storage roots was mild for all treatments during the rainy season cultivation in the upland ecology relative to the dry season cultivation in the lowland ecology which exhibited low attack of the pest for most treatment combinations. Findings indicated that ecology and time of cultivation of sweetpotatoes contribute more to *Cylas puncticollis* infestation on vines and roots of sweetpotatoes rather than the combined effects of putative varieties, leaf harvest frequency and intensity.

Foliage yield of sweetpotato increased with leaf harvest frequency and intensity to varying degrees. Complete defoliation at 60- and 90-days increased foliage yield of both varieties at the two agro-ecologies indicating that two critical leaf harvest regimes of a putative variety good economic returns to producers targeting both foliage and root yields. Moreover, harvesting thrice mostly produced higher yields at different ecologies suggesting the dependence of foliage yield on the variety, frequency of leaf harvesting and proportion of leaf harvest. Findings concur with those obtained by Kiozya [7] who opined that leaf harvesting frequencies exhibited higher foliage yields than the non-leaf harvest plots. The results are in concurrence with Olorunnisomo [11] who reported that foliage yield increased with delayed leaf harvesting. The results also partly agree with Lebot [12] who noted that leaves can be harvested thrice or four times per growing season at 50% defoliation and 20 days interval. In this study, some treatments with 50% leaf harvest done twice or thrice also produced reasonable yields. The slight variances might be due to different genotypes and environments where the studies were conducted.

In this study, higher storage root yields were obtained in the plots with foliage harvested twice at 30 and 90 days after planting and leaf harvest intensities of 25% in the lowland and upland ecologies. Delayed harvesting of foliage twice of "Kabia" at 60 and 90 days after planting and leaf harvest intensities of 50% resulted in higher storage root yield in the upland ecology. The results generally indicated that comparably high fresh storage root yields are obtainable in lower leaf harvest

intensities of 25% and 50% dependent upon the leaf harvest sampling interval, genotype and ecology. These findings agree with Lebot [12] who opined that sweetpotato defoliation of 50% promotes optimal leaf and root yields of the crop, since greater defoliation could reduce fresh storage root yields. Findings are also supported by Kiozya *et al.* [7] who noted that foliage harvest at 45, 75 and 105 days after planting decreased fresh storage root yields by 33, 25 and 15%, respectively. The reduction is probably attributable to translocation of most of the photosynthates to foliage production at the expense of storage root yields. These results suggest that cultivation of sweetpotatoes for forage and storage root yields require selection of desired variety, leaf harvest proportion and intensity.

Leaf harvesting affected fresh storage roots dimensions of sweetpotatoes to varying degree. High root length and diameter were obtained in treatments with foliage harvested twice at 30 and 90 days after planting and leaf harvest intensities of 25% in the lowland and upland ecologies. Delayed harvesting of foliage twice of “Kabia” at 60 and 90 days after planting and leaf harvest intensities of 50% resulted in high storage root dimension compared to treatments harvested at 30, 60 and 90 days after plant in the upland ecology indicating that frequent harvesting of leaves reduces storage root dimensions depending on variety and ecology [13] (Brazilian Archives of Biology and Technology, 2006). Findings in this study concur with those of Villordon *et al.* [14] who noted that radial diameter of storage roots is among main components of production and fresh storage root yields of sweetpotatoes. These results are also consistent with the suggestion by Kathabwalika *et al.* [15] that cultivars with similar storage root length and wide variations in diameters account for differences in root yields. Moreover, findings of the present study are in concurrence with the suggestion that root yield per plant is a function of number of roots per plant, root length, and root diameter [16, 17].

## V. CONCLUSION

The current study demonstrated that good agronomic management including choice of appropriate ecology, variety and leaf harvest frequencies and intensities depending on the desired produce, can contribute to optimizing forage and fresh root yields of sweetpotato. Where both forage and fresh root yields are desired, leaf harvesting should be kept at 25 and 50%. Sweetpotato foliage tends to be rejuvenated thereby producing more leaves for subsequent harvests. Dual purpose sweetpotato varieties can be conserved and genetically improved to support both forage production for ruminant animals throughout the year and food for humans. The ecology and time of cultivation of sweetpotatoes contributed more to *Cylas puncticollis* infestation on vines and roots of sweetpotatoes than putative varieties, leaf harvest frequency and intensity treatment combinations assessed. Findings of the present study demonstrated the incorporation of leaf harvesting time, proportion of leaf harvest in germplasm assessment and new population development objectives for dual purpose sweetpotato varieties utilized by various end users.

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# Review Article: Effect of Biochar on Growth and Yield of Agricultural Produce

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**Abstract**— Biochar is a boon for agricultural crops. Biochar is baked biomass that you can add to soil. It is a biomass that is thermally altered in the absence of oxygen, it is baked and not burned and flammable gasses are released (hydrogen, carbon dioxide). Heat transforms plant carbon (found in the cellulose and lignin) into fused aromatic carbon rings that are very stable. Biochar are made from different feedstocks at different physical and chemical properties. In carbon cycle almost all of the carbon returns to the air. Green plants remove carbon dioxide from the atmosphere via photosynthesis and convert it into biomass. Virtually all of that carbon is returned to the atmosphere when the plants die and decay, or immediately if the biomass is burned as a renewable substitute for the fossil fuels. While in the biochar cycle up to half of the carbon is sequestered, green plants removed and sequestered as biochar, while the other half is converted to renewable energy co-products before being returned to the atmosphere. Biochar retains soil moisture of the agricultural field. Worms loves biochar, it works best when composted with other organic matter before adding to garden soil. This allows life to colonize the biochar. Biochar composted with animal manure, it is inoculated with compost tea. Biochar composted with food waste and bokashi (anaerobic lactobacillus fermentation). Other activities include minerals, NPK, fungi, worm castings, fish emulsion, urea, etc. biochar can be added to soils to improve fertility. Reduces emissions from the biomass. Improves the water quality and quantity. Helps to improve the agricultural productivity. Valuable resource reduces the forest fires. Value added product for urban and rural agriculture and forest communities.

**Keywords**— Biochar, Biomass, Green plants, Agricultural, Carbon, Sequestered.

## I. INTRODUCTION

Biochar is considered by many scientists to the “black gold” for agriculture. It is the future of sustainable Agriculture. Green parts of solid waste are deposited inappropriately near the rural areas or cities, contributing to environmental impact. Biochar is a form of carbon, somewhat like charcoal, which can be made by heating wood with limited air. It differs from coke in that is very porous, having a very high surface can serve as a template for the growth of microorganisms such as bacteria and fungi, and can actively adsorb fertilizers. For this certain reason, it is valuable as an aid to farming and lumbering too. Initially, heat must be applied to start vaporization of volatile components. As the temperature increase, chemical reactions begin which liberate heat and form several products as volatile vapors, a portion of which can be condensed to form. Bio-oil is a liquid which can be burned or further refined, and biochar is a charcoal – like solid, of agricultural value. The relative amounts of these can be controlled by selecting heating rates and temperatures. The heat supplied by burning the vapors is more than sufficient to continue the process, so no net external energy is needed. Aggregation of soil is one of the crucial processes that facilitate carbon sequestration and maintain the soil fertility. The effect of biochar amendment on soil aggregation will remain inconclusive. Biochar application to soil is a carbon negative technology used to tackle climate change while sustainability improving soil fertility (Lehmann et al., 2005). There is a general agreement that the low degradability of biochar, like other types of black carbon, derives mainly from its specific chemistry, which is dominated by fused aromatic ring structures (Haumaier and Zech, 1995); Glaser et al., 2000; Brodowski et al., 2005). Despite its intrinsic low biodegradability, the introduction of biochar to soil does often result in an increase in carbon dioxide emissions in the

short – term (Sagrilo *et al.*, 2014). Among explanatory factors, the positive priming of biochar on the positive priming of biochar on the decomposition of native soil organic matter (Maestrini *et al.*, 2014) and the abiotic release of carbon dioxide from the reaction of carbonates in the biochar after amendment to acidic soil (Brunn *et al.*, 2014) were identified, nevertheless, the main source of the increase in carbon dioxide emissions from a biochar amended soil seems to be the microbially mediated decomposition of labile biochar constituents (e.g., Cross and Sohi, 2011; Hilscher and Knicker, 2011). Overall, the net increase in carbon dioxide release following the application of biochar to soil appears to be a short – lived effect, while for incubations over a longer time period (>200 days), the average emission of carbon dioxide is usually not or even negatively affected for large application rates (Sagrilo *et al.*, 2014). In a meta- analysis of forty-six studies, Sagrilo *et al.* (2014) showed that large additions of biochar relative to native soil organic carbon (SOC) content did not significantly affect for large application rates (Sagrilo *et al.*, 2014). Fabbri *et al.* (2012) related the mineralization rates of twenty biochar to their chemical composition and found biochars with higher concentrations of proteins and sugars (from incomplete transformation by pyrolysis) to be associated with the largest mineralization rates. In contrast, biochar produced at a higher temperature resulted in lower carbon dioxide emissions (Fabbri *et al.*, 2012), probably related to an increasing degree of aromatic condensation (Keiluweit *et al.*, 2010; Wiedemeier *et al.*, 2015) and the relative decrease of the labile fraction of biochar. To explain the result, Sagrilo *et al.* (2014) proposed that a major part of the labile fraction of biochar might have been consumed over two hundred days. Another possible explanation is that N deficiency eventually occurs after prolonged incubation of biochar amended soil (Ameloot *et al.*, 2015), as most biochar have high C:N ratios. In their survey, Sagrilo *et al.* (2014) showed that soils with a C:N ratio < 10 were much more subject to an increase in carbon dioxide emissions after addition of biochar, which corroborates this assumption. Despite an already overwhelming number of studies on the effect of biochar on the soil biology and greenhouse gas emissions, most data originate from short – term experiments in the laboratory conditions (Sagrilo *et al.*, 2014), although biochar persists in soil for centuries (Singh *et al.*, 2012) and therein lies exactly its premise to abate net carbon dioxide emission. Since properties of biochar change over time (Joseph *et al.*, 2010), long- term implications of biochar soil amendment are very likely to differ from short- term effects. For instance, positive priming has only been observed shortly after addition of fresh biochar to soil and does not seem to last over long periods of time (Hamer *et al.*, 2004; Wardle *et al.*, 2008; Zimmerman *et al.*, 2011). More importantly, on long timescales after the addition of biochar to soil, a decrease of metabolic quotient defined as microbial activity reported to soil biomass or even a lower absolute amount of respired carbon was observed in biochar rich terra preta soils (Jin, 2010; Liang *et al.*, 2010). Nevertheless, data from the Amazonia cannot be extrapolated to other soil and climate conditions with very different land – use histories. Additionally, several types of organic and inorganic household waste other than biochar were involved in the genesis of terra preta soils (Glaser, 2007), which makes it nearly impossible to isolate the effect of biochar from the effect of these other inputs. Very few studies have studied carbon turnover and soil biology at historic charcoal kiln sites in comparison with adjacent charcoal- free soils. Kerre *et al.* (2017) measured a smaller total of carbon dioxide emissions from soil than in reference soils and a smaller mineralization of fresh maize soil organic matter traced by <sup>13</sup>C isotope signature when added to a pre – industrial soil. As biochar application is mainly intended to cropland soils, these sites represent a critical source of information to unravel the long – term fate of biochar in soil and its effect on soil properties field experiment in agricultural soil (Hardy *et al.*, 2017; Kerre *et al.*, 2017). They related it to an increased sorption of dissolved organic carbon, with a preferential adsorption of the dissolved organic carbon rich in aromatics. Hence, proved by several scientists that biochar is produced by thermal decomposition of biomass under oxygen – limited conditions pyrolysis, and it has received attention in soil remediation and waste disposal in recent years. Biochar plays an vital role on growth and yield on agricultural crops.

**How is biochar generated:** Gasification is one of the dominant thermal decomposition processes producing gas along with biochar. Gasification is the process of converting solid fuels to gaseous fuel. The process involves drying, pyrolysis, combustion with air, reduction into combustible gases, (Carbon monoxide, hydrogen, methane, some higher hydrocarbons) and inert, (carbon dioxide and nitrogen). Biochar is produced from a range of organic materials under different conditions, showing variable properties (Guerrero *et al.*, 2005). The currently used feedstock at a commercial scale and for different research facilities may include chips, pellets, bark of tree, and also the agricultural wastes including crop residues such as nutshells, straw, switch grass etc. The organic wastes including sugarcane bagasse, waste use of chicken litter proposed by (Das *et al.*; 2017) and other biomassare dairy manure, as well as sewage and sludge. The agricultural waste biochar does not cause any notable greenhouse gas emissions. There are a considerably higher yield and porosity of biochar derived from the biomass having more lignin and lesser cellulose. (Nartey and Zhao; 2014). The biochar is produced by the thermal

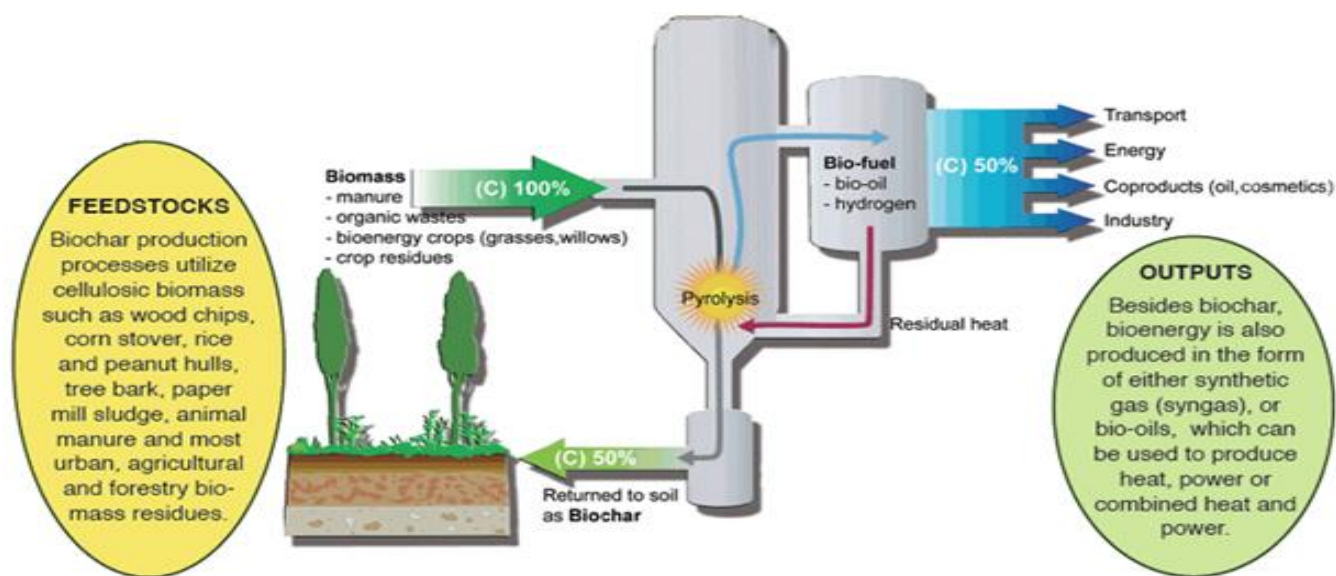
decomposition of waste biomass and the temperatures between 200-900° C in the presence of very little oxygen gas which is required for the biochar generation. The conversion of biomass into biochar takes place by the use of pyrolysis methods. The pyrolysis of waste biomass avoids the production of gasses like carbon dioxide and nitrogen oxide of greenhouse and also retains half of the carbon fixed by plants during photosynthesis. The biomass is helpful in the formation of biochar by slow or fast pyrolysis process and it also produces bioenergy as a byproduct. Bioenergy serves as an alternative form of fossil energy with low carbon dioxide emissions after combustion. The production of 35% biochar by pyrolysis, a maximum energy output of 8.7MJ kg<sup>-1</sup> has been recorded in the form of bioenergy like liquid fuels (Woolf; 2008), (Zhang et al; 2012). Carbonized organic matter can essentially have different physical and chemical properties based on the technology eg. torrefaction, a pyrolytic process primarily at low temperature, slow pyrolysis, fast pyrolysis, gasification, hydrothermal carbonization, or flash carbonization used for its production. In contrast to considerable research, this has already been carried out to assess the value of biochar as soil amendment (Luo et al;2013).

*Fast and slow pyrolysis*

BIOMASS -----> BIOCHAR+ BIOENERGY

**1.1 Characterization of Biochar**

**What good is biochar:** For those who are interested in preparing bio-oil, the biochar often about 35% of the yield, it is a undesirable by- product. It is frequently burned to recover its energy content. We believe this is a waste of a valuable resource, having unique properties that are beneficial for agriculture. It makes more sense to obtain the energy otherwise. As well as documented in the order practices of natives in the Amazon and by modern studies in Asia, Europe, and the United States., there are great benefits arising from adding biochar to the soil. Some are enhancement of growth rates of plants and trees, greater quality and nutrient density of food crops. Decrease in needs for fertilizers, decrease in run-off of fertilizers to streams. The biochar in the soil remains as a stable solid for indefinite periods of time. The benefits greatly outweigh those derived from the energy that might be obtained by burning it. A good sustainable biochar is a powerfully simple tool that can fight global warming, produce a soil enhancer that holds carbon and makes soil more fertile, reduce agricultural waste, produce clean, renewable energy. In some biochar systems all four objectives can be met, while in others a combination of two or more objectives will be obtained. The efficiency and effectiveness of the process of its creation and use can vary and the specific biomass sources used can affect the characterization and usability of the biochar (McLaughlin et al., 2009). It has been predicted that the stable portion of biochar has a mean residence time of greater than hundred years (Spokas et al., 2013). All biochar are not created equal they differ on their pH, surface are, Ash content, water holding capacity, cation exchange capacity (CEC), H/C ratio and C/N ratio. All a function of pyrolysis temperature highest treatment temperature (HTT), pyrolysis method, residence time and feedstock (McLaughlin et al., 2009). Following above characteristics are required for a good biochar.



**FIGURE 1: Manufacturing of Biochar.**

Source: International Biochar initiative <http://www.biochar-international.org/biochar/soils>.

## 1.2 Quantification of biochar

Quantification major main is to distinguish biochar from soil organic matter and from other forms of black carbons produced from varieties of biomasses. Many of the potential techniques depend on spectroscopic from soil organic matter and from varieties of biomasses. Many of the potential techniques depend on spectroscopic characteristics rather than physical separation or isolation. Some of the techniques that most effectively distinguish types of biochar can also be used to characterize individual biochar wastes or collection of fragments recovered from both soil and solution systems. An assessment of pure samples removes the matrix effects, but where function of a recalcitrant component depends on its surface characteristics or those of accessible pores, separation of active and inactive components presents a significant challenge (Lou and Yang *et al.*, 2012). Classifying biochar is principally problematic on the basis of its chemical complexity and diversity, yet characteristically uncreative nature. Due to its recalcitrance nature, biochar cannot eloquently be extracted from soil using chemical methods, though potential biomarkers may be. The result from studies using the physical location of biochar within a soil matrix (Smernik *et al.*, 2002, Kroger *et al.*, 2013) suggest that usefulness of physical separations using density or means other than hand sampling approach which is restricted to very small samples is susceptible to site factors. There is no difference of biochar age on soil physical and chemical properties. Biochar able to maintain structure as a stable lattice network. Strong ability to retain hydrocarbons and other organic compounds. High physical adsorption capacity within the macro pores to micro pores.

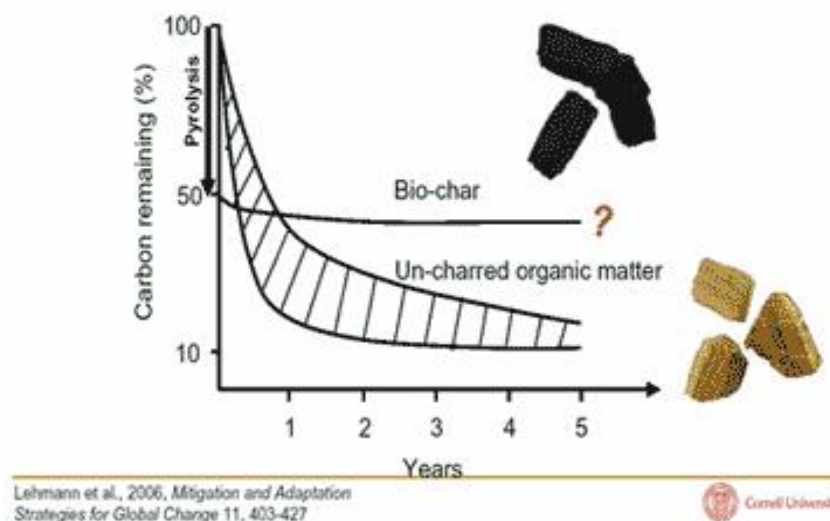
**TABLE 1**  
**ORGANIC CONTAMINATES ADSORBED BY BIOCHAR PRODUCED FROM DIFFERENT BIOMASS.**

Source of Bio-char	Organic pollutant sorbed	References
Pine needle	Naphthalene, nitrobenzene, and m-dinitrobenzene from waste water.	B.L. Chen <i>et al.</i> , (2008)
Bamboo	Pentachlorophenol	Lou and Yang <i>et al.</i> , (2012)
Bamboo, Brazilian pepper wood and sugarcane bagasse.	Sulfamethoxazole from waste water.	Yao <i>et al.</i> , (2012)
Wheat straw	Hexachlorobenzene	Wild <i>et al.</i> , (2012)
Hardwood, Softwood and grass	Ctechol and humic acid	Zimmerman <i>et al.</i> , (2010)

## 1.3 Properties of Biochar

Biochar is commonly alkaline. The pH values of biochar at different pyrolysis temperature ranged from slightly alkaline (=8.2) to highly alkaline (=11.5) across a wide variety of feedstocks (Yaun *et al.* 2011). Biochar shows positive effect in the case of acidic soils compared to alkaline soils (Biederman and Harpole 2013). Biochar addition can reduce the bioavailability of toxic forms of Al, Cu, and Mn and increase the availability of essential nutrients such as Na, K, Ca, Mg, and Mo, thereby rendering a favorable environment for plant growth (Altkinson *et al.*2010). The physical structure of biochar can be described by scanning electron microscopy (SEM). The physiochemical properties of biochar vary with the temperature at which it forms and the type of feedstock involve in its production. Most of the biochar is produced at the temperature between 300°C to 500°C possess alkaline pH (Brown *et al.*, 20211)and also depending on the type of feedstocks, the biochar prevails PH range of 6.1 to 11.6 which is considered to be alkaline. This alkaline character of biochar is due to the presence of carbonates and alkaline elements such sodium, potash, calcium and magnesium present, which forms during thermo-chemical conversion of the biomass. The other properties of biochar due to its alkaline nature are the high total carbon content which is reflected in C:N ratio of 200:1 and lower total nitrogen 1.3 g kg<sup>-1</sup>(Chen *et al.*, 2008). The type of feedstock used for the biochar also affects the energy content of biochar which may range from 30 to 35 MJkg<sup>-1</sup>(Ryu *et al.*, 2015). (Sohi *et al.*, 2010) observed that the ash content of this black material also increases with increasing temperature.

## The essential stability of bio-char



**FIGURE 2: The essential stability of Bio-char.**

Source: international Biochar initiative <http://www.biochar-international.org/biochar/soils>

### II. BIOCHAR AND THE ENVIRONMENT

Stability of biochar relates to carbon structure, as it constitutes the carbon structure. It is the main factor that must be focused on prior application is stability. Biochar is utilized for many purposes, its influence on the environment must be analysed properly to avoid its negative impacts (Cross and Sohi., 2013). The dissolved organic matter released from biochar maintains a high degree of aromaticity, resistance and stability. When biochar is utilized for treating wastewater, the carbon content of water increases due to the release of carbon by biochar. The biochar produced from sludge containing heavy metals may percolate during the treatment process thus causing heavy metal contamination. Aromaticity and aromatic condensation are the main measures of biochar carbon structures (Vikrant et al., 2018). When the biochar acts as a catalyst, the stability gradually decreases on reusing biochar several times. The instability of biochar may be due to structural damage also. Hence, stability of biochar plays an important role in the environmental concern. In addition, the toxicity of biochar to soil microbes must be investigated before application (Premarathna et al., 2019). (Gong et al., 2019) observed that the physicochemical properties of biochar vary with different biomass, it is important to study in detail the toxic effects of biochar on environment. A different toxicity test can be performed using fungi, algae, bacteria, fish etc. (Venegars et al., 2016) suggested that supporting carbon sequestration and reducing the effect of green house gases. Biochar is a powerful simple tool to fight global warming. Transfers agricultural waste into a soil enhancer that can hold carbon, boost food security, and discourage deforestation (Biochar International 2012). Biochar have potentially to enhance seed germination and plants growth which very beneficial and significant for making our environment green and clean, while some biochar could have negative influence too.

### III. EFFECT OF BIOCHAR ON AGRICULTURE FIELD

Biochar enhances the health of soil and agricultural crops as well. Increases the pH of acidic soil since, biochar is typically alkaline. It also increases the water and nutrient retention of soil. Biochar carbon is chemically altered during gasification and thus are resistant to attack by micro-organisms. Biochar carbon can remain stabilized for long years of time hundred to thousand years. Is therefore an important way of storing carbon that has been scavenged from the atmosphere during photosynthesis. Biochar may have the potential to reduce leaching of nutrients from agricultural soils (Lehmann et al., 2007). This possibility is suggested by the strong adsorption affinity of biochar for soluble nutrients such as ammonium, nitrate, phosphate and other ionic solutes (Radovic et al., 2001). Lehman et al (2003) found that "cumulative leaching of mineral N, K and Mg in the Amazonian Dark Earth was only 24, 45 and 7%, respectively, of that found in a Ferralsol. All biochar showed significant increase in soil organic matter rather than control. Biochar did not show any significant effect on moisture content of soil. Kadam biochar showed relatively less changes in soil nitrogen and phosphorus compared to other. Activated

biochar is added into soils of agricultural field to improve soil structure. Biochar itself is a porous material, it can adsorb and retain huge amount of water. Research suggests that even low rates of biochar application can significantly increase crop productivity (*Winsley., 2007*). The biochar application in soil not only helped in isolating carbon in soil, it also enhanced the quality of soil by neutralizing the pH, increasing soil cation exchange capacity and strengthening the microbial growth in soil. The functional groups such as carboxylic, hydroxyl and phenolic groups present in biochar interact with hydrogen ions in soil and reduces concentration of hydrogen ions in soil and reduces concentration of hydrogen ion thus increasing the pH. Carbonates, bio-carbonates and silicates in biochar react with hydrogen ions and neutralizes soil pH (*Ahmad et al., 2012*). Hence, biochar application in remediating soil in agricultural fields has increased interest owing to its surface properties, elemental composition, enhancing the soil fertility and improving crop productivity.

#### IV. USE OF BIOCHAR TO MANAGE WATER QUALITY

Biochar may be beneficially take the edge off spread contamination arising from farming all the time distribution in solum from which adulterating essential come to light. It could possibly to make use of its natural action magnitude to flush out the pollutants in the water reception procedure. Disquisition a particular illustrate the volume for bio-char to separate the nitrates (*Mizuta, 2004*) and phosphate (*Beaton, 1960*) in this circumstances has been adverted, and in over-passing the convolution of the soil system, squeeze ability is registered.

#### V. BIOCHAR IN THE TIME OF CORONAVIRUS

Density of people have lost asked that if how can biochar will be helpful during this pandemic. Accommodating a contagion that has departed intercontinental is an entirely dissimilar calamity. Nonetheless, there are other ways that biochar can be beneficial to help. Masks made with biochar activated carbon have long been used in personal protective gear (PPE) and there is some indication that certain types of carbon may be effective in activated viruses (*Matsushita et al 2013*). A truly innovative, reusable mask that contains biochar which could be made in mobile manufacturing faculties was pioneered. Carbonizing contaminated medical waste, the high heat temperatures should eliminate the virus and the resulting biochar could be used for a variety purposes including potentially masks. Carbonizing human feces, there is some indication that human feces may contain traces of virus through more needed. To be on the safe side, putting sludge through a thermo-chemical conversion system would eliminate the risk and also provide substantial other benefits as well.

#### VI. CARBONIZATION VERSUS CREMATION

Funeral homes are overloaded in hot spot zones for the pandemic. Through the concept of carbonizing human remains is still largely theoretical, it is feasible. Keeping part of a loved-one's carbon from returning to the atmosphere might seem like purgatory to some, but it might be one small way to help rebalance carbon levels. Farmers in the Northern Hemisphere are just about ready to start planting and going without fertilizer is causing many to panic. However, those that know how to convert biomass to biochar and blend it with locally available nutrients such as manure, compost, urine, compost, etc. can wean themselves off their dependency on imported fertilizer. This not only increases their resiliency but also reduces costs and emissions related to fertilizer use. It can also alleviate leaching of excess nutrients into local water bodies.

#### VII. CONCLUSION

This concluded that the efficient use of biochar by converting it as a useful source of soil amendment is one way to manage the soil health, fertility and crop productivity. One of the approaches for efficient utilization of biochar involves carbonization of biomass to highly stable carbon compound biochar. Use of biochar in the agricultural systems is one viable option that enhances the nutrient availability, increases soil carbon levels, moisture retention, cation exchange capacity, improve soil quality and natural rate of carbon sequestration in the soil. Biochar reduces the emission gasses, decreases toxicity of aluminium and other metals, decrease tensile strength and bulk density of soil and provides numerous benefits to the soil. Measurable and verifiable improves agricultural productivity. Research gaps are still an evident and hold strong ethics to the people. Further, inter -disciplinary research has to be taken up for studying the long term impact of biochar application on soil physical properties, nutrient availability, soil microbial activities, carbon sequestration potential, crop productivity and green house mitigation.

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# Degradation of Nevirapine and Trimethoprim from Aqueous Solutions using Selected Microorganisms

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**Abstract**— Together with pharmaceutical residues, personal care products encompassing prescription drugs, fragrances, and cosmetics have been detected in groundwater and other aquatic environments, hence compromising the quality of water. Their classification as micropollutants is due to their antibacterial resistance potential, persistence, and ecotoxicity. Biodegradation has been identified as a potential mechanism in their removal. The focus of this study focus was bioaugmentation; (*Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*) to enhance the degradation of Nevirapine and Trimethoprim in model aqueous solutions. A liquid chromatography-tandem mass spectrometer (LC-MS/MS) was used to determine the pharmaceuticals. The efficacy of the bacterial strains to degrade selected drugs was evaluated by making the two drugs the sole source of energy and carbon. From the experimental data, the highest percentage biodegradation was recorded; *Pseudomonas aeruginosa* (86 %) and *Staphylococcus aureus* (79 %) for TMP and NVP respectively.

**Keywords**— Biodegradation, efficacy, LC-MS/MS, model solutions, pharmaceutical.

## I. INTRODUCTION

The occurrence of pharmaceutical residues in surface waters is an emerging environmental concern (Zhou, Lutovsky, Andaker, Gough, & Ferguson, 2013). The main sources of these residues include wastewaters from hospitals, drug production facilities as well as agriculture. Owing to growth in population, coupled with the emergence of new ailments, many pharmaceutical products are being manufactured today for the protection of humans and animals. Low concentrations of pharmaceutical residues have deleterious effects on aquatic biota. It also has adverse effects on human health (Wang, Hu, & Wang, 2018).

Some pharmaceutical residues are partially broken down by animals. However, most are eliminated in their original forms. The residual pharmaceutical compounds in animal manure can easily penetrate the terrestrial environment and are readily transported into aquatic environs through direct runoff and leaching. Recent studies have also revealed that many pharmaceuticals are degraded partially since most municipal wastewater treatment plants are not designed for the removal of pharmaceuticals (Chefetz, Mualem, & Ben-Ari, 2008).

Trimethoprim and Nevirapine are widely used antibiotics and anti-retroviral respectively. Different techniques have been used for their removal including biological, physical, and chemical processes. Amongst the physicochemical methods adopted include, sorption by special materials, advanced oxidation processes (AOP), and photodegradation (Basha *et al.*, 2010; Chefetz *et al.*, 2008; Klavarioti, Mantzavinos, & Kassinos, 2009). While biodegradation of trimethoprim and nevirapine has been reported in several publications and reviews, only a few microalgae, bacterial and fungal species have been found to degrade them (Göbel, McArdeell, Joss, Siegrist, & Giger, 2007). Of great consideration in the removal of organic micropollutants from wastewater are their water solubility, tendency to volatilize, hydrophobicity, and biodegradability.

The dynamics of bacterial populations exposed to different concentrations of antibiotics have been examined and modeled in relation to the minimum inhibitory concentration (MIC). Without drugs, the growth rate of cells is higher than the death rate, and thus a bacterial population always grows. When drug concentration increases, as long as the concentration remains below the MIC, the growth rate is higher than the death rate and thus a population still grows, albeit at a slower rate. When the drug

concentration increases further and reaches the MIC, the growth rate becomes equal to the death rate, and the population size is maintained at a constant level. Only at drug concentrations above the MIC does a bacterial population decline (Magiorakos *et al.*, 2011). In this study pure cultures of *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* commonly found in the water were used to evaluate the biodegradability of Nevirapine and Trimethoprim.

## II. MATERIALS AND METHODS

### 2.1 Microbial Assay Preparation

Microorganisms all American Type Culture Collection (ATCC); *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (27853) were obtained from the botany laboratory at Jomo Kenyatta University of Agriculture and Technology. The microorganisms were cultured in an optimal nutrient medium to generate a healthy breed of microorganisms that can withstand the toxicity of the pharmaceuticals. Culturing was performed in a laminar flow hood to ensure a germ-free environment. The healthy isolates were then transferred into other plates and stored in a freezer. The medium used was Mueller–Hinton agar jells which was prepared in de-ionized water. Minimum mineral salt medium (MMSM) prepared in de-ionized water contained the following compounds;  $\text{KH}_2\text{PO}_4$ ,  $\text{NH}_4\text{Cl}$ ,  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ ,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and  $\text{K}_2\text{HPO}_4$ . The medium's pH was thereafter adjusted to 7.0 and then autoclaved for 20 min at 121°C to kill the existing microorganisms (Sharma *et al.*, 2020).

### 2.2 Acclimatization of Microorganisms to the Pharmaceuticals

Frozen microorganisms meant for future studies were removed and transferred into conical flasks containing minimum mineral salt medium together with some amount of glucose to alleviate the microorganisms from starvation. The pharmaceuticals were added to each flask in concentrations to be used for biodegradation experiments (0.5, 1.0, and 1.5 mg/ml). The microorganisms were allowed to grow in presence of the pharmaceuticals for several days and then removed and stored at a temperature of 4°C.

### 2.3 Evaluation of the Pharmaceuticals Tolerance on Microorganisms

Effects of pharmaceuticals concentration on the growth of the microorganisms were evaluated by inoculating acclimatized microorganisms into flasks containing a small amount of glucose and MMSM. This was preceded by the separate addition of Nevirapine and Trimethoprim drugs into these flasks in a range of 0.5-10 mg/ml upon which the flask's optical densities were measured at 600 nm.

### 2.4 Biodegradation Experiments

Into 500 ml conical flasks, (98.0) ml of MMSM spiked with different concentrations of the selected pharmaceuticals was added followed by corking to prevent contamination. Acclimatized bacterial isolate (2.0 ml) was then placed in each flask to make up a volume of 100 ml. To prevent possible photodegradation, the flasks were covered with aluminium foil. The temperature was set at 25°C with a rotating speed of 150 rpm.

### 2.5 Control Experiments

Three control experiments were set up: without the microorganisms to account for the drug's abiotic degradation, with dead biomass to account for sorption to the biomass and autoclaving at temperatures of 121°C to kill other microorganisms present (Al-Gheethi *et al.*, 2019; Gauthier, Yargeau, & Cooper, 2010). For the autoclaving and non- autoclaving experiments, two model solutions were prepared in conical flasks containing 50% de-ionized water and 50% methanol and a concentration of 0.5 mg/L of each pharmaceutical. Flask A was autoclaved while flask B was not autoclaved. These flasks were taken under the same conditions as those involved in biodegradation experiments. 1.0 ml of each solution was filtered with a PVDF filter and put in an HPLC vial and its concentration was determined using LC-MS/MS.

### 2.6 Monitoring Bacterial Growth

Assessment of bacterial growth was done by diluting 1.0 ml of biodegradation contents in the flasks with 2.0 ml of deionized water and measuring the absorbance using a UV-vis spectrophotometer at a wavelength of 600 nm. This was done until the optical density began to decline.

### 2.7 LC-MS/MS Determination

Trimethoprim and Nevirapine were determined using a Waters Micromass Quattro Ultima mass spectrometer coupled with an 1100 Agilent series HPLC (USA). The experimental conditions were mobile phase 30 % methanol (B) and 70 %

deionized water (A) with a column Evo C18 (100 mm x 3.0 mm, 5 mm particle size 100 Å) at a flow rate of 0.45 ml/min. The injection volume was 10 mL. External temperature was maintained at 40 °C.

### III. RESULTS AND DISCUSSION

#### 3.1 Evaluation of Tolerance to Pharmaceuticals by Microorganisms

The use of indigenous microorganisms and optimization of their biodegradation parameters such as concentration of microorganisms, temperature, pH, and time has a great influence on biodegradation efficiency. Furthermore, they enhance the quality of the degradation process without necessarily polluting the environment (Al-Gheethi *et al.*, 2019). To determine the levels of pharmaceuticals that could be tolerated by the microorganisms, each microorganism was subjected to various concentrations of the pharmaceuticals and their growth was monitored. Presence of glucose in the media results in the generation of enough biomass that adsorbs on target compounds. In effect, low concentrations of the pharmaceuticals were utilized coupled with a reduction in toxicity. However, under the conditions used (1 mg/ml of each drug) in the biodegradation experiments, the microorganisms were only minimally affected leading to a conclusion that lower concentrations should be used in the biodegradation experiments. The MIC used ranged from 0.5 mg/ml-1.5 mg/ml. Figures (1-8) illustrate the effect of 1 mg/ml, 4 mg/ml, and 10 mg/ml TMP/NVP on the growth of microorganisms.

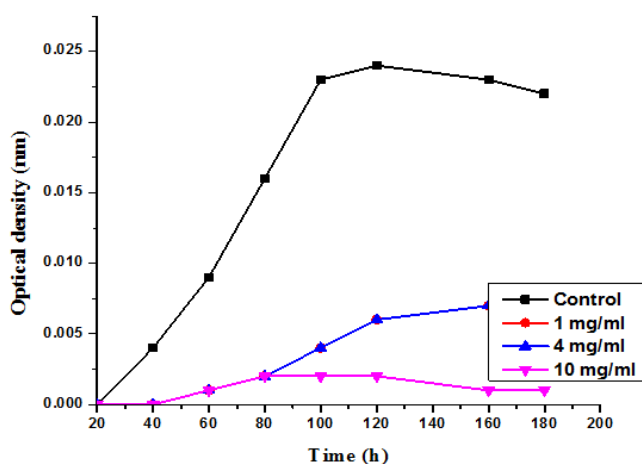


FIGURE 1: Effect of 1 mg/ml, 4 mg/ml, and 10 mg/ml TMP on the Growth of Microorganism *P. aeruginosa*

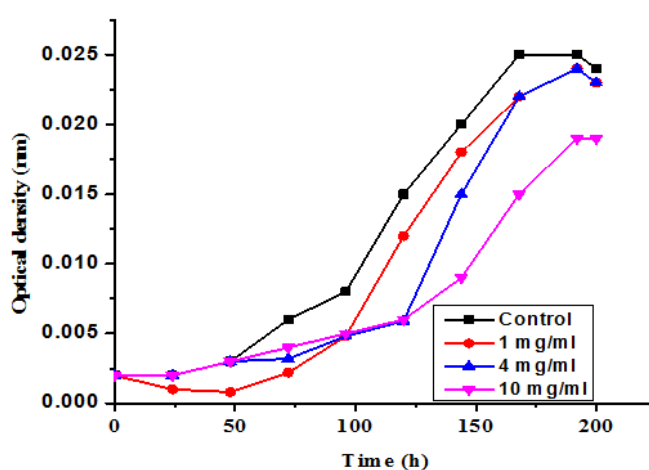


FIGURE 2: Effect of 1 mg/ml, 4 mg/ml, and 10 mg/ml NVP on the Growth of Microorganism *P. aeruginosa*

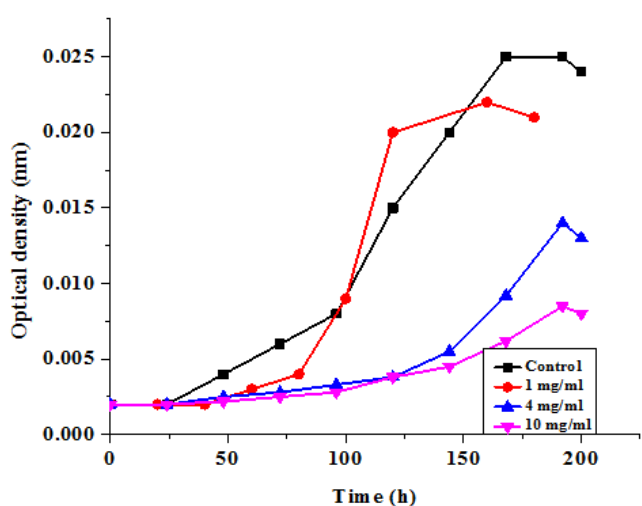


FIGURE 3: Effect of 1 mg/ml, 4 mg/ml, and 10 mg/ml TMP on the Growth of Microorganism *Bacillus subtilis*

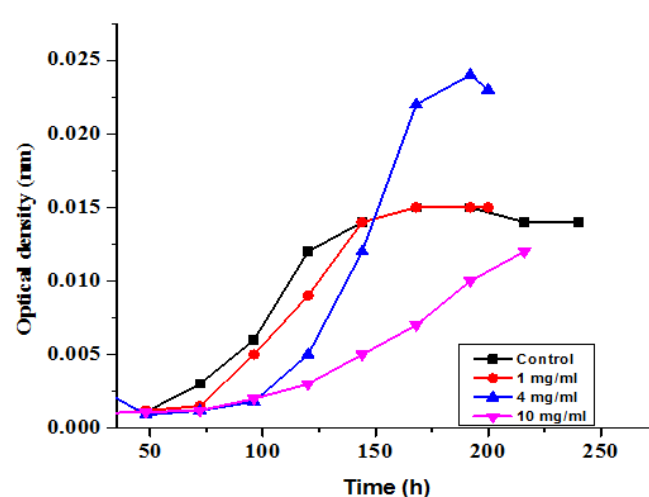


FIGURE 4: Effect of 1 mg/ml, 4 mg/ml, and 10 mg/ml NVP on the Growth of Microorganism *Bacillus subtilis*

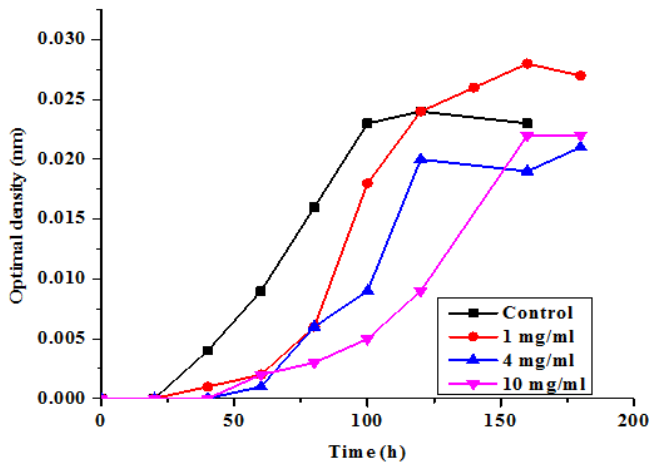


FIGURE 5: Effect of 1 mg/ml, 4 mg/ml, and 10 mg/ml NVP on the Growth of Microorganism *E. coli*

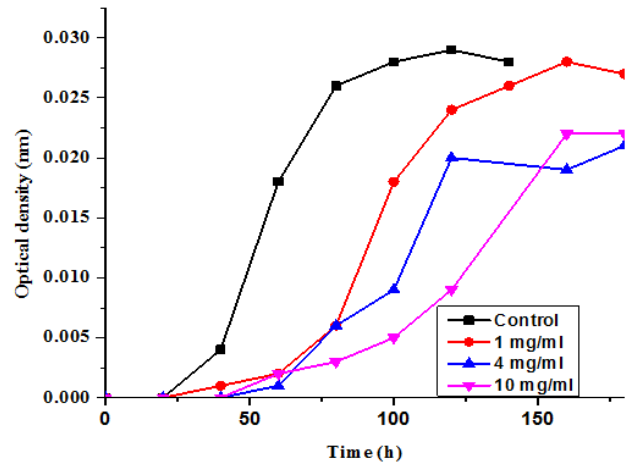


FIGURE 6: Effect of 1 mg/ml, 4 mg/ml, and 10 mg/ml TMP on the Growth of Microorganism *E. coli*

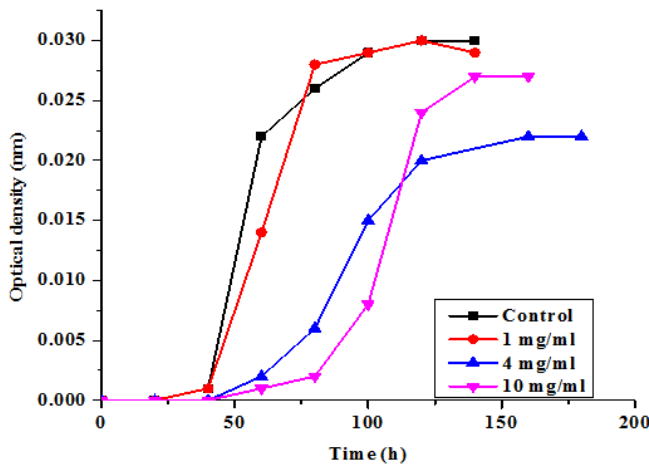


FIGURE 7: Effect of 1 mg/ml, 4 mg/ml, and 10 mg/ml TMP on the Growth of Microorganism *Staphylococcus aureus*

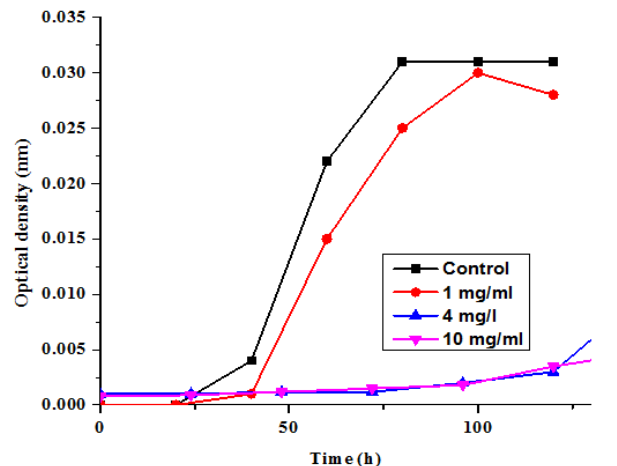


FIGURE 8: Effect of 1 mg/ml, 4 mg/ml, and 10 mg/ml NVP on the Growth of Microorganism *Staphylococcus aureus*

3.2 Biodegradation Studies

The biodegradation of pharmaceuticals was studied in 500 ml Erlenmeyer flasks for a period of one week and the percentage removal calculated (equation 1) after measuring the residual pharmaceuticals by LC-MS/MS. Representative chromatogram depicting the biodegradation of Trimethoprim and Nevirapine is shown on figure's 9 and 10 respectively.

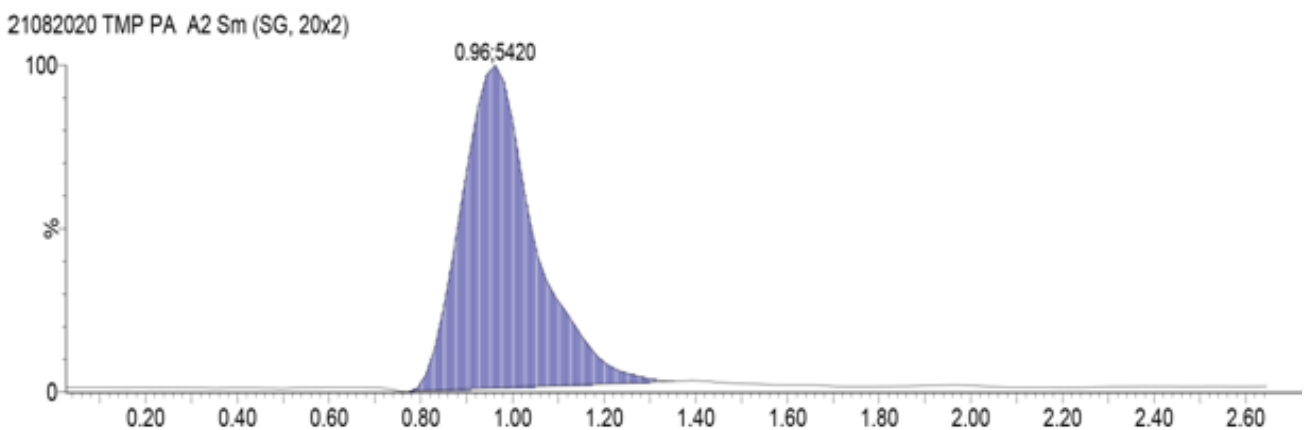
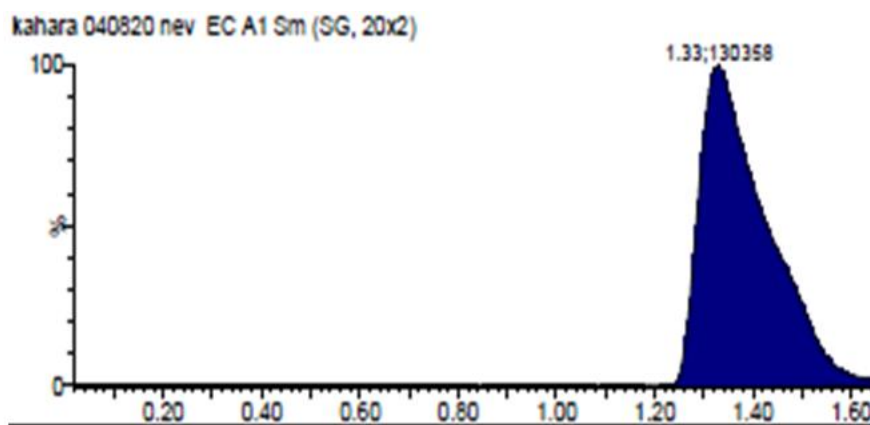


FIGURE 9: Biodegradation of Trimethoprim by *Pseudomonas aeruginosa*

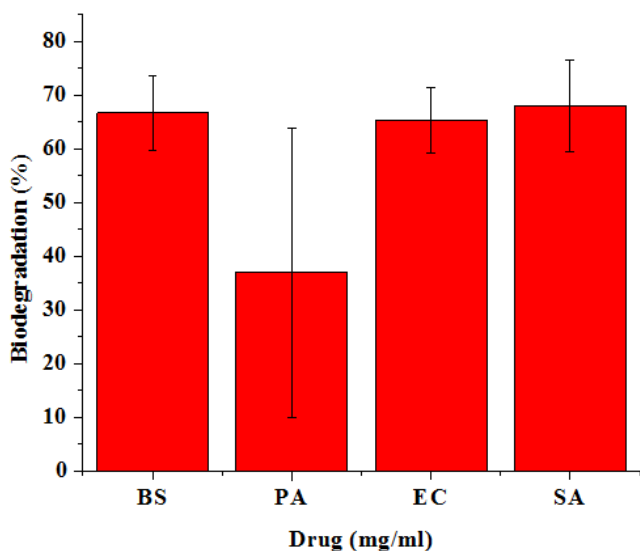


**FIGURE 10: Biodegradation of Nevirapine by *E. coli***

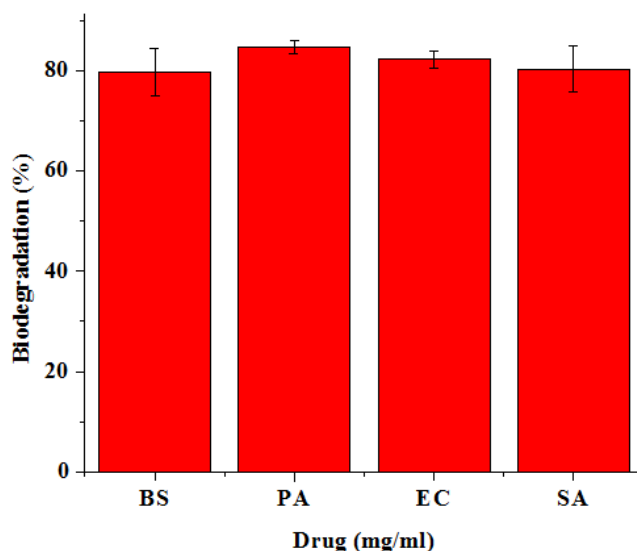
Three important control experiments were set up: without the microorganisms to account for the drug’s abiotic degradation, with dead biomass to account for sorption to the biomass, and autoclaving at temperatures of 121°C (Autoclaved TMP- 0.44±0.02, Non-autoclaved TMP-0.42±0.01; Autoclaved NVP- 0.45±0.01, Non-autoclaved NVP- 0.43±0.01) to kill other microorganisms present (*Gauthier et al., 2010*). From the control experiments, autoclaving had minimal effect on the biodegradation of the pharmaceuticals. Percentage biodegradation rate was much faster with *Staphylococcus aureus* (79 %) and least in *Pseudomonas aeruginosa* (35 %) for nevirapine over one week. Over the same time frame, with Trimethoprim, *Pseudomonas aeruginosa* (86 %) was the highest and least in *Bacillus subtilis* (73 %).

Equation 1 below was used to tabulate percentage biodegradation of the selected pharmaceuticals.

$$\text{Biodegradation \%} = \left( \frac{\text{Initial Concentration} - \text{Final Concentration}}{\text{Initial Concentration}} \right) \times 100 \tag{1}$$



**FIGURE 11: Percentage Biodegradation of Nevirapine with selected Microorganisms**



**FIGURE 12: Percentage Biodegradation of Trimethoprim with selected Microorganisms**

**IV. CONCLUSION**

Results show that *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* can degrade biodegrade. Highest percentage biodegradation were recorded for *Pseudomonas aeruginosa* 0.5 mg/ml (86 %) and *Staphylococcus aureus* 1.5 mg/ml (79 %), TMP and NVP respectively. Of late, the necessity for fresh biotechnological tools to get rid of pharmaceuticals from the environment, with less harm and negative impacts, has risen. Utilizing indigenous microorganisms can be a viable solution to tackle this menace. Based on the experimental data, it is evident that higher pharmaceutical concentrations have extreme effects on the microorganisms.

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# Analysis of the relationship between the Socio-Economic Characteristics of Rice Farmers and Soil Management Practices in Abuja, Nigeria

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**Abstract**— *The study examined the analysis of the relationship between the Socio-Economic Characteristics of Rice Farmers and Soil Management Practices in Abuja. The study was conducted in rural communities in Abuja, Nigeria. Two objectives guided the study. The study adopted descriptive and logistic regression research design. Multistage sampling technique was employed to select the farming communities for the study. Twelve (12) agricultural wards (Chuwkuku, Gaube, Bamushin, kotunku, Pai, Dafa, Bako, Dobi, Paso, Chibiri, Gadabiu and Paikon) were randomly selected giving a total of thirty-six (36) agricultural wards. Five blocks were randomly picked from each of the agricultural wards making the total of 180. Lastly two (2) circles were randomly selected from each of the blocks resulting to three hundred and sixty (360) respondents who were randomly selected from the chosen circles. The results show that 58.06% of the respondents were male while 36.13% of the respondents were between the ages of 30 and 39 years. Also, 47.74% of them were married with 40% of the respondents having an average of 5 people in their households. 61.94 had at most a national certificate of education (NCE). Majority (44.84) of the respondent had a farm size of between half a hectare and two hectares. The study recommended that manual tillage should be mostly carried out by rice farmer to improve the level of production and also soil rotation should be practiced where soil is much available to reduce the level of degradation.*

**Keywords**— *Smallholders, soil management, rice farmers, food security, adoption.*

## I. INTRODUCTION

Rice is a staple food in Nigeria, and the average consumption is 200kg/capita/year. However, rice productivity has declined dramatically in recent years due to incessant killing of smallholder farmers on the farmland. Food security can be reached by improving the technical efficiency of rice farming, especially in rice farming centers Nigeria. Prokopy *et al.* (2008) and Baumgart-Getz *et al.* (2012) argue that the key capacity variables considered to be important in influencing farmers' adoption decisions include age, education (formal education and farmer [extension] training), income, farming experience, tenure, social networks, labor, capital and information. While both Prokopy *et al.* (2008) and Baumgart-Getz *et al.* (2012) use this concept (capacity) to combine both farmer and farm characteristics, most adoption literature separates them (Reimer *et al.* 2012; Meijer *et al.* 2015). In this study, we chose to adopt the latter categorization since one of the categories (farmer characteristics) relates to the management ability of the farmer, while the other category (farm characteristics) relates to farm resources (Chomba, 2004). Adoption literature of agricultural technologies posits that the decision to adopt technologies including Integrated Soil Management Fertility Management (ISFM) practices, is affected by both farmer and farm attributes (Meijer, *et al.*, 2015). For instance, based on household size, households with more adults are more likely to adopt ISFM since many of the ISFM practices are labour intensive (Kassie, *et al.*, 2013). As household size increases, the likelihood of adoption of ISFM practices is expected to be high.

Household heads are the final decision makers regarding choice of soil fertility practices and technologies. While most adoption studies have found a negative effect of age to adoption of soil conservation (Kassie *et al.* 2013) and others have found age to be insignificant. This implies that the influence of age on adoption of technologies is inconclusive and warrants a more nuanced study. In almost every adoption study, education of the farmer is considered to positively influence the



farmer's likelihood of adopting a new technology or practice because farmers with better education have more exposure to new ideas and information, and thus have better knowledge to effectively analyse and use available information (Kassie *et al.* 2011). While most studies consider education in terms of number of years of formal education, the categorization of education by Baumgart-Getz *et al.* (2012) seems more appropriate. In contrast to formal education, it reflects knowledge farmers attain through other means such as extension programs, workshops, and field days.

Important to adoption of soil fertility practices and technologies is farmers' experience. As a farmer grows older, (s) he has generally been exposed to more ideas, information (Prokopy *et al.* 2008) and production practices thereby being more efficient and accurate in judgment of expected benefits (Kassie *et al.*, 2015). This, in turn, facilitates the potential to adopt new technologies. A meta-analysis by Knowler and Bradshaw (2007) found that farmers' experiences positively influence adoption soil conservation practices. However, other meta-analyses on the same parameter have found quite inconclusive results. For instance, Prokopy *et al.* (2008) reviewed adoption literature of best management practices within the US, and found farmers' experience to have mixed results. Baumgart-Getz *et al.* (2012) found farming experiences were not significantly related to adoption, thus calling for further studies (Prokopy *et al.* 2008).

### 1.1 Household of Adoption of Soil Management for Rice Production

We consider household wealth to include livestock ownership, farm size (acres) farming come and equipment. With respect to wealth, it is regularly theorized that adoption of any new technology requires sufficient financial well-being, particularly if new equipment is needed (Knowler, 2015; Knowler & Bradshaw, 2007). Several analyses of the role of income and farm profitability on adoption have revealed a positive influence (Baumgart-Getz *et al.* 2012; Knowler & Bradshaw 2007; Prokopy *et al.* 2008). In relation to ISFM in many developing countries, the presence of livestock plays a key role in adoption of animal manure since the animals not only contribute synergistic crop-animal production interaction, but, cattle and oxen can also be a source of draft power (Kassie, *et al.*, 2013). Size of the farm (acreage) as a measure of physical capital has been found to be a best (financial) predictor of adoption (Baumgart-Getz *et al.* 2012) since it can be used as collateral to access credit for investments in soils.

Labour is a major production cost in agriculture. The lack of sufficient labour on the farm is theorized to impede the use of various soil fertility management practices (Kamau and Ayuo, 2013). In many developing countries, families continue to provide the bulk of farm labour for most farm operations because many households cannot afford to hire wage labourers. This implies that the lack of family labour coupled with family liquidity constraints to hiring labour greatly affect the adoption of ISFM practices. However, when addressing farm labour concerns, it is important to identify other community adaptive mechanisms through which labour is mobilized on farms. Mugwe *et al.* (2009) observed that farmers sometimes trade their labour for food or make reciprocal arrangements in which they pool their labour efforts together through their farmer-to-farmer local network systems and work on each other's fields during peak labour requirement periods. This could help in ascertaining whether such labour arrangements favour adoption of specific soil fertility management practices at the expense of others within the package of ISFM. Therefore, the purpose of this study is to find out the relationship between the Socio-Economic Characteristics of Rice Farmers and Soil Management Practices in Abuja, Nigeria. The specific objectives of this study are to:

- Describes the socio-economic characteristics of rice farmers in the study area.
- Analysis the influence of farm size on effectiveness of soil management practices study area.

## II. MATERIALS AND METHODS

### 2.1 The Study Area

The Federal Capital Territory, Abuja is located in the geographical centre of Nigeria with a land area of 8,000 square kilometres and lies between latitude 9° 10' north of the equator and longitude 7° 11' east (FCT, 2007). It is bounded in the North by Kaduna State, in the West by Niger State, in the East by Nasarawa State and in the South by Kogi State; and is made up of six area councils namely Gwagwalada, Kuje, Kwali, Bwari, Abaji and Abuja Municipal Area Councils. The major communities with high intensity of farming activities are Nyanya, Karu, Gwagwalada, Kuje, Abaji, Karshi, Bwari, Kwali and Garki (AGIS, 2004). It had a population of 1,408,239 persons according to 2006 population census but has grown to 2,245,000 in 2010.

Gwagwalada Area Council has an area of 1,043 km<sup>2</sup> and a population of 157,770 at the 2006 census. Gwagwalada area council lies between latitude 070.57°N and longitude 070.7°E. Kuje area council comprises of 162 communities widely

spread within a land mass of about 1,800 km<sup>2</sup> and a population of over 420,000 at the 2006 census. Kwali area council has an area of 1,206 km<sup>2</sup> and a population of 85,837 at the 2006 census. The vegetation of these area councils combines the best features of the Southern tropical rain forest and Guinea savannah of the North (Aiyedun, 2003). This reflects the full transitional nature of the Area as between the Southern forest and Northern grassland which have the woods and shrubs respectively. The soil is reddish with isolated hills filled by plains and well drained sandy clay loams which support farming of rice and other crops. Gwagwalada, Kuje and Kwali area councils have been known for agricultural activities over the years, informed of the areas agro-climatic conditions and rural characteristics with about 85% of the population who are mostly indigenes engaged in farming activities which includes the cultivation of cereals, tuber/root crops and legumes it make the council areas suitable for this study.

## 2.2 Population of the study and research design

All the rice farmers in FCT, Abuja constitute the population of the study. The research design for this study was descriptive survey method which involves the use of questionnaire and interview schedule to collect information on the soil management practices from rice farmers in FCT, Abuja. Kayode *et al.* (2017) used survey research design to investigate characteristics of population in a study to evaluate a study. It involves Participants answering questions administered through questionnaires, while researchers describe the responses given in order for the survey to be both reliable and valid. These questions are constructed properly and data collected was used to answer specific objectives of the study.

## 2.3 Sample Size and Sampling Techniques

Multi-stage sampling technique was used to select the sample. Firstly, out of the six area councils of Abuja (Gwagwalada, Kwali, Kuje, Abaji, Bwari and Abuja Municipal). three area councils (Gwagwalada, Kuje and Kwali) were randomly selected. Then from each of the selected Area Councils, twelve (12) agricultural wards (Chuwkuku, Gaube, Bamushin, kotunku, Pai, Dafa, Bako, Dobi, Paso, Chibiri, Gadabiu and Paiko) were randomly selected giving a total of thirty-six (36) agricultural wards. Five blocks were randomly picked from each of the agricultural wards making the total of 180. Lastly two (2) circles were randomly selected from each of the blocks resulting to three hundred and sixty (360) respondents who were randomly selected from the chosen circles.

## 2.4 Procedure for data collection

Primary information was obtained with a questionnaire by researchers during the survey. The activities covered include; direct personal observation, oral interview and discussion with village heads about farming activities in the area, soil management practices from land acquisition to crops storage, agricultural development of the area among others.

The questionnaire was divided into different sections: 1. Data on the Social-economics characteristics of farmers (gender, age, marital status, name of town, education background, marital status, house hold size, among others); 2. Socio-economic factors that influence the effectiveness of soil management practices in the study area.

## 2.5 Method of Data Analysis

Both descriptive and inferential statistics were used to actualize the objectives of the study. The data obtained from the field survey was analysed using descriptive statistics like; simple frequency distribution and percentage was used to analyse social economic variables and management practices. Logistic Model analysis observed the relationship between the socio-economic characteristics of rice farmers and their soil management practices as was explained by (Adekayode and Akomolafe, 2011).

Binary responses are commonly studied in many fields. Examples include the presence or absence of a particular event. Often one wishes to study how a set of predictor variables  $X$  is related to a dichotomous response variable  $Y$  for convenience we define the response to be  $Y = 0$  or  $1$ , with  $Y = 1$  denoting the occurrence of the event of interest. Often a dichotomous outcome can be studied by calculating certain proportions, for example, the proportion of soil management practice among rice farmers. However, in many situations, there are multiple descriptors, or one or more of the descriptors are continuous. Without a statistical model, studying patterns such as the relationship between age and occurrence of a good soil management practice, for example, would require the creation of arbitrary age groups to allow estimation of prevalence as a function of age.

Letting  $X$  denote the vector of predictors  $\{X_1, X_2, \dots, X_k\}$ , a first attempt at modelling the response might use the ordinary linear regression model

$E\{Y|X\} = X_{\beta}$ , (10.1) since the expectation of a binary variable  $Y$  is  $\text{Prob}\{Y = 1\}$ . However, such a model by definition cannot fit the data over the whole range of the predictors since a purely linear model  $E\{Y|X\} = \text{Prob}\{Y = 1|X\} = X_{\beta}$  can allow  $\text{Prob}\{Y = 1\}$  to exceed 1 or fall below 0. The statistical model that is generally preferred for the analysis of binary responses is instead the binary logistic regression model, stated in terms of the probability that  $Y = 1$  given  $X$ , the values of the predictors. With aid of Statistical Package for Social Science (SPSS) version 24 the data were analyzed and the descriptive statistics were used to present the results.

### III. RESULTS AND DISCUSSION

#### 3.1 Socio-Economic Characteristics of Respondents

##### 3.1.1 Gender Distribution

Table 1 shows that 360 questionnaires were administered from which 180 respondents representing 58.06 % are males while 130 representing 41.94% are females. This trend in gender involvement shows that men (males) are more involved in Rice farming in the Area Councils than women (females). This might not be unconnected with the prevailing Islamic religion practice which forbids women in “pudah” from engaging in any form of work including light Agriculture works s discussed in (Fashola *et al.*, 2007). The difference is not in any way related to preference of men to women in the studies. Although (Gomiero *et al.*, 2011) pointed out those women contribute significantly to observing the soil management practice of rice farming even though their number may not be much compared to that of men.

**TABLE 1**  
**SOCIO-ECONOMIC CHARACTERISTICS OF RESPONDENTS**

Items	Total	%	Mean
<b>Gender:</b>			
Male	180	58.06	
Female	130	41.94	
<b>Age:</b>			
0-19	9	2.90	36.00
20-29	81	26.13	
30-39	112	36.13	
40-49	64	20.65	
50-59	44	14.19	
60 and above	-	-	
<b>Education:</b>			
Primary Cert/SSCE	88	28.39	
NCE	104	33.55	
OND	30	9.68	
HND/B.Sc	28	9.03	
Others	08	0.89	
Adult Education	15	2.58	
M.Sc/Ph.D	17	5.48	
None	20	6.45	
<b>Farm Size:</b>			
Less than half hectare	118	38.06	1.20
Half hectare and two hectare	139	44.84	
Above two hectare	53	17.10	

Source: Field Survey, 2020

##### 3.1.2 Age Distribution

The result in Table 1 shows that the mean age of respondents in the study area was 36years. And from the table we see that majority (36.13%) of rice farmers fall within the age bracket of 30-39 of the total respondents. This followed by age bracket 20-29 representing 26.13%. Thus, it became obvious that the age bracket 20 and 49 formed the largest part of the participants accounting for 82.91% of the total participants. It can be therefore safely inferred that the majority of rice farmer in the study area are youths who are strong enough to carry out the practice. Rice farming is labour intensive and requires much labour which employ the services of many youths. These compromise the views of (Ladha, 2014) which states that age factor determine the rate of farming. But it correlates with the views of assertions of (John *et al.*, 2019) which opine that farming is not gender related.

### 3.1.3 Education Qualification

The table 1 shows that 5.48% of the respondents were highly educated people and don't engage in farming as observed in the study area by (Adewole and Anyahara, 2010). In Abuja, majority of those that have High qualification engage in administrative work and as such, Secondary School certificate holders recorded the highest respondents in farming accounting for 33.55%, followed by respondents with Primary School Leaving Certificate with 28.39% due to the low level of education of 33.55% of the farmers, the mostly employ traditional means of soil management system which may not be effective as the scientific method as observed by (Alfred, 2018). The NCE and OND had thirty and twenty-eight participants' representing 33.55% and 9.68% respectively are more engaged in teaching work in primary and secondary schools, though few of them go to farm after their teaching work and other businesses they may be engaged in. Respondents with HND/BSc have 9.03% and it make up full administrative workers in most off the government agencies and private offices in Abuja. 6.45% of them are into businesses, this agrees with the views of (Kayode *et al.*, 2017). Other qualifications making up 0.89% are those with Islamic certificate and other skill acquisition certificates who have also impact majorly in the farming of rice in the study areas. Participants having adult education and other education represented 5.48% and 2.58% respectively as this have little impact on the farming in the area and those don't have education (20) accounted for 6.45% of the total respondents and Given this educational qualification distribution, 33.55% of respondents in the sampled areas are literates low academic qualifications.

### 3.1.4 Farming size of the Respondents

Small farm size is mostly observed in the study area 44.84 of the farmers are subsistence farmers. As shown in the table, of farmland between half hectare and one hectare. 38.06% had less than half hectare while the remaining 17.10% cultivates above one hectare of land. The result further reveals that the mean farm size of respondents is 1.2 hectares. From the analysis, one can easily infer that these farmers will not only feed themselves total respondents.

### 3.2 Socio Economic Factors that Influence the Effectiveness of Soil Management Practices in the Study Area

Table 2 shows the result of the logistic regression analysis on the influence of socio economic variables on effectiveness of soil management practices in the study area. The result shows that Cox and Snell  $R^2$  have a value of 0.154 which means that 1.54% of the variations in the dependent variable can be predicted by the independent variable. The remaining 44.9% was due to error or variables not captured in the model. The result shows that Gender, age and education were all significant at 1% while farm size was significant at 5%.

**TABLE 2**  
**LOGISTIC REGRESSION SHOWING INFLUENCE OF SOCIO-ECONOMIC VARIABLES ON EFFECTIVENESS OF SOIL MANAGEMENT PRACTICES**

Variable	B	S.E.	Wald	P value (Sig.)
Gender	.912	.287	10.136	.000**
Age	-.686	.159	18.733	.000**
Education	-.367	.210	12.732	.000**
Farm Size	-.152	.131	.342	.011*
Marital status	.180	.208	.746	.388
Household size	-.124	.089	1.928	.165
Constant	-.616	.622	.981	.322

\*significant at 5%

\*\*significant at 1%

$R^2 = 0.154$

Source: Field Survey, 2020

#### 3.2.1 Influence of Gender on the Effectiveness of Soil Management Practices

The regression result reveals that Gender was positive (.000) and significant at 1% probability. Hence, for a unit increase in Gender, if every other variable is constant, there will be a 0.91 unit increase in the effectiveness of soil management practices in the study area. This implies that the effectiveness of soil management practices in the study area is directly influenced by the Gender of the farmers. The result shows that soil management practices are more effective among male farmers in the study area.

### 3.2.2 Influence of Age on Effectiveness of Soil Management Practices

The result in Table 2 showed that age was negative (.000) and significant at 1% probability. The result shows that for a unit increase in age, assuming all other variables remain constant, there will be a 0.68 unit decrease in the effectiveness of soil management practices in the study area. This implies that younger farmers tend to effectively soil management practices more than the older farmers. This is probably due to the fact that younger farmers are more open to adopting new farm practices than the older farmers. The older farmers tend to stick with practices they are already familiar with, rather than try out new ideas (Fashola *et al.*, 2007).

### 3.2.3 Influence of Education on Effectiveness of Soil Management Practices

The regression result reveals that education was negative (.000) and significant at 1% probability. This means that education has an inverse relationship with the effectiveness of soil management practices in the study area. Hence, for a unit increase in education, if every other variable is constant, there will be a 0.36 unit decrease in the effectiveness of soil management practices in the study area. This could be a result of the fact that most of the respondents in the study area had at most a national certificate of education (NCE), with only a few of the respondents possessing higher educational qualifications. This is probably why the effectiveness of soil management practices reduces with increase in education, since most of the respondents possess lower education qualifications.

### 3.2.4 Influence of Farm Size on Effectiveness of Soil Management Practices

Farm size was significant at 5% but had a negative relationship with the effectiveness of soil management practices in the study area. This shows an inverse relationship between farm size and the effectiveness of soil management practices and this implies that for a unit increase in farm size there will be a 0.15 decrease in the effectiveness of soil management practices in the study area. This implies that the more the farm size of the respondents, the less they are effective with soil management practices. This is probably because larger farm sizes require more energy. While farmers may be able to effectively adopt soil management practices for small farms, it becomes more tedious and drudgery sets in with larger farms.

### 3.3 Test of Hypothesis

From Table 2, the level of significance is considered to be (P-value =0.05) and from the Logistic regression view, if the P-value is less than or equal to 0.05 (P-value  $\leq$  0.05), the test is significant we accept the alternate hypothesis. And if the P-value is  $>$ 0.05, the test is not significant we accept the null hypothesis. Since the soil management is regressed by the repressors (gender, age, education, farm-size, marital status and household-size). The p-values of Gender, age, education and farm size are less than 0.05, therefore the test is significant, and therefore we reject the null and accept the alternate hypothesis for the significant variables in the study area. The p-values for marital status and the household size are higher than 0.05; this means that they do not significantly influence the effectiveness of soil management practices among respondents in the study area, therefore we accept the null hypothesis for these variables.

## IV. CONCLUSION AND RECOMMENDATION

Considering the soil management practice of rice farmers in FCT, Abuja, there is the need to adopt good soil management measures like manual tillage and mechanical tillage that will improve the rice production. Minimum and zero tillage which preserve the land are not very advisable for rice production. The research has been able to identify gap and area of agreement, the finding of the study shows that the physical and chemical fragility is observed in some areas, minimum tillage was practice in some places where surface hoeing involving manual stirring of soil surface with hoe to the depth of about 4cm is normally done to conserve soil water, improve macro porosity, reduce bulk density and give better yield. In other places, Row (strip) tillage is done to improve on-zero tillage and control some problems associated with it, this is also done to conserve soil water. Manual heaping or ridging to conserve soil fertility and maintain high yield. In some areas the return of plant residues and mulching are used in place of fertilizer to increase the soil nutrients by farmers in Kuje, Gwagwalada and Kwali Area Council.

## V. RECOMMENDATION

Base on the peculiar soil management problems of the tropical soil, experience have shown that most soil have lost their fertility and better soil management of rice farming, the following recommendations are made:

1. Manual Tillage should be mostly carried out by rice farmer to improve the level of production.
2. Soil rotation should be practiced where soil is much available to reduce the level of degradation.

3. Organic farming should be practiced to reduce the application of chemical that will constitute hazard to the soil.
4. Cover cropping should be practiced to reduce the water loss from the soil surface and to prevent the direct effect of solar radiation the soil organic component.

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# Testing the ability against *Bacillus cereus* of actinobacteria strains isolated from sponges in Kien Giang Sea, Vietnam

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**Abstract**— This study aimed to test the antibacterial activity of *Bacillus cereus* of actinobacterial isolates isolated from marine sponges in the Kien Giang Sea, Vietnam. That can select the strains with high resistance to identify them. There were 198 actinobacterial isolates tested. Based on the ability of antimicrobial activity to *B. cereus*, 82/198 had the against *B. cereus*, in which there were six isolates with high (7.3%), 52 medium (25.6%), and 21 weak resistance (67.1%). Selection of six isolates with the best resistance to *B. cereus* (ND1.7a, ND2.7c, HD1-3e, HD1-6a, HD2.3b, and H6b) identified by PCR and 16S rRNA gene sequencing. The results identified five strains of *Streptomyces* (*Streptomyces tateyamensis* ND1.7a, *Streptomyces althioticus* HD1.3e, *Streptomyces flaveolus* HD1.6a, *Streptomyces olivaceus* HD2.3d, and *Streptomyces albidoflavus* H6b) and one strain of genus *Microbacterium* (*Microbacterium tumbae* ND2.7c).

**Keywords**— Antimicrobial activity, *Bacillus cereus*, Kien Giang Sea, sponge, *Streptomyces*.

## I. INTRODUCTION

According to the World Health Organization [1], more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Microorganisms have the potential to cause diseases. The human body is very prone to viral, bacterial, and fungal infections. The discovery of antibiotics in the early twentieth century provided an increasingly important tool to combat bacterial diseases. However, due to the indiscriminate use of commercial antibacterial drugs treated for infectious diseases, resistance is becoming more common and severe [2]. Microbial natural products have been the source of most of the antibiotics in current use for the treatment of various infectious diseases. *Bacillus cereus*, family Bacillaceae, order Bacillales, class Bacilli, phylum Firmicutes, is a Gram-positive, rod-shaped, facultative, an anaerobic, motile, beta-hemolytic, spore-forming bacterium commonly found in soil and food. Some strains are harmful to humans and cause foodborne illness, while others can be beneficial as probiotics for animals [3].

Until recently, the majority of antimicrobial compounds were isolated from terrestrial microorganisms. The aquatic environment is now becoming increasingly appreciated as a rich and untapped reservoir of useful novel natural products. The marine environment alone is known to contain taxonomically diverse bacterial groups which exhibit unique physiological and structural characteristics that enable them to survive in extreme environmental conditions, with the potential production of novel secondary metabolites not observed in terrestrial microorganisms [4].

Marine bacteria are considered to play a central role as symbionts of most marine invertebrates and also represent one of the most novel biomedical resources remaining to be explored [5]. Marine microorganisms have been an important study in recent years because of the production of novel metabolites which represent various biological properties such as antiviral, antitumor, or antimicrobial activities. These secondary metabolites serve as model systems in the discovery of new drugs [6]. The studies of the secondary substances produced by marine microorganisms have obtained many significant achievements in the world [7]. Among the secondary metabolites from marine microorganisms, many compounds are having interesting biological activities that should be useful for development for their pharmaceutical uses.

Therefore, in this study, the presence of potent antimicrobial metabolite-producing microorganisms with *Bacillus cereus* was reposted, a human pathogenic, especially microbes symbiosis in sponges at Kien Giang Sea, that is a resource not studied yet.

## II. MATERIALS AND METHODS

### 2.1 Materials

The actinobacterial strains were isolated from sponge [8]. The *Bacillus cereus* (ATCC 11778) used for testing the agent of antibacterial isolates.

### 2.2 Screening assays for antibacterial activity

The liquid cultures were grown with shaking at 150 rpm for one day at 30°C. The broth was centrifuged at 5,000 rpm, 15 minutes. The supernatant was stored at 4°C. The bacterial test organism (*Bacillus cereus*) was plated in the LB medium. The antimicrobial extract was added to the wells, the plates were incubated at 4°C for 2h for the diffusion of antimicrobial extract and observed for the zones of inhibition at 28°C for 48h.

### 2.3 The agar well diffusion method

The active isolates were cultured by the method given in the previous step. The supernatants were used for testing extracellular antimicrobial activity by the agar well diffusion method. By using a sterile cork borer, wells were punctured in the appropriate agar medium previously seeded with one of the test organisms. One hundred microliters of the culture supernatants were added to each well. The plates were then incubated at 4°C for at least 2 h to allow the diffusion of crude extracts followed by incubation for 24 h at 37°C for bacteria and 48 h at 28°C for yeast. The diameters of inhibition zones were monitored and measured [9]. Positive control was penicillin.

Screening of isolated microorganisms for inhibitory activity the isolates were screened for antibacterial metabolite production using the agar well diffusion method. The inoculate was prepared by growing the various test organisms on separate agar plates and colonies from the plate were transferred with inoculating loop into 3 mL of normal saline in a test tube. The density of these suspensions was adjusted to 0.5 McFarland standards.

Using a sterile cork borer wells (8 mm in diameter) were made in the agar and filled with 0.2 ml of 72 h culture of the isolated microorganism. Two replicates of the experiment were done, and the plates were incubated at 37°C for 18 h. The diameters of the zone of growth-inhibition produced were measured and the mean values calculated (Table 1).

### 2.4 Genomic DNA extraction

Bacterial cells from these cultures were collected by centrifugation, and genomic DNA was extracted [10].

### 2.5 16S rDNA gene amplification and sequencing

The PCR was performed in a final volume of 25 µl which was composed of about 50ng template DNA, 1.5 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 200 pM of Actinomycetes specific primers S-C-Act-0235-a-S-20 (5'-CGCGGCCTATCAGCTTGTTG-3') and S-C-Act-0878-a-A-19 (5'-CCGTACTCCCCAGGCGGGG-3') [11] and 1U of Taq polymerase with the appropriate reaction buffer under the following conditions: initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 50s, annealing at 52°C for 50s, and 72°C for 90s. The amplified products were separated by gel electrophoresis in 1.2% agarose gels which were stained with Safeview dye.

### 2.6 Sequence analysis

The 16S rRNA gene sequences compared with those from the type strains available in NCBI (<http://www.ncbi.nlm.nih.gov/>) using the Basic Local Alignment Search Tool (BLAST) [12].

For phylogenetic analysis, multiple sequence alignment performed using CLUSTALX, version 1.81. The Phylogenetic tree constructed using Mega 7.0. The consistency of the trees was verified by bootstrapping (1000 replicates) for the UPGMA method.

### 2.7 Statistical analysis

The experimental results analyzed the ANOVA with the isolates and levels of diameters of inhibition zones. All analyses conducted using the statgraphics program. The data were considered significantly different at  $P < 0.01$ . Duncan's test at  $P = 0.01$  using to differentiate.

## III. RESULTS AND DISCUSSION

### 3.1 Screening assays for antibacterial activity

There were 82/198 actinobacterial isolates with antimicrobial activity against *Bacillus cereus* (41.4%) (Table 1). Among 82 isolates, there were 6/82 (strong resistance), 55/82 isolates (medium resistance), and 21/82 (resistance); 116 isolates were



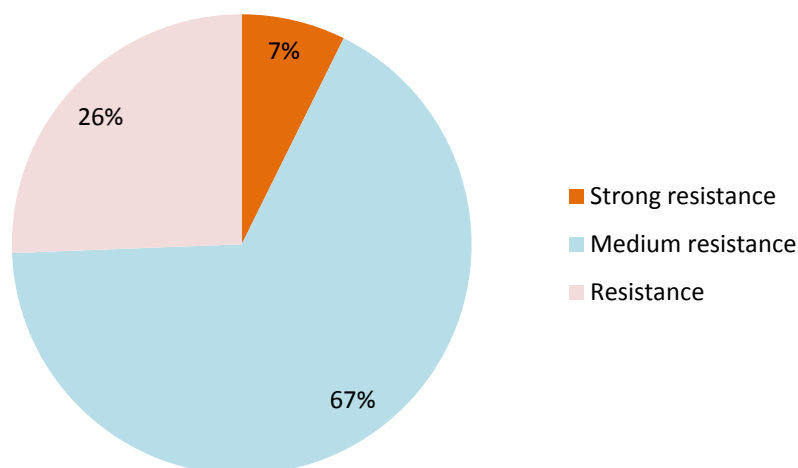
without resistance. Six isolates had the ability resistance to *Bacillus cereus* through a diameter of halo [sterile ring] (Table 1, Figure 1).

**TABLE 1**  
**ANTIMICROBIAL ACTIVITY OF 82 ACTINOBACTERIAL ISOLATES TO *BACILLUS CEREUS***

No	Bacterial isolates	Inhibition zone	Antibacterial Level*	No	Bacterial isolates	Inhibition zone	Antibacterial Level*	
01	ND1.1a	15.0 f	++	42	HD1.6a	21.0 a	+++	
02	ND1.1b	16.0 e	++	43	HD2.1a	14.0 h	++	
03	ND1.3b	14.0 h	++	44	HD2.2b	7.0 q	++	
04	ND1.4a	5.3 rs	+	45	HD2.3a	13.0 i	++	
05	ND1.4c	5.7 rs	+	46	HD2.3b	20.0 b	+++	
06	ND1.5a	17.0 d	++	47	HD2.3c	18.0 c	++	
07	ND1.5b	9.0 m	++	48	HD2.3e	6.0 r	++	
08	ND1.5c	15.0 f	++	49	HD2.4a	17.0 d	++	
09	ND1.5d	13.0 i	++	50	HD2.5a	15.0 f	++	
10	ND1.6b	10.0 l	++	51	HD2.5b	5.0 st	+	
11	ND1.7a	21.0 a	+++	52	HD2.5c	8.0 op	++	
12	ND1.7b	13.0 i	++	53	HD2.5d	15.0 f	++	
13	ND1.7c	4.0 u	+	54	HD2.6b	4.0 u	+	
14	ND2.6a	15.0 f	++	55	HD2.7c	7.0 q	++	
15	ND2.6c	17.0 d	++	56	HD2.7d	16.0 e	++	
16	ND2.7b	4.0 u	+	57	HD2.8a	6.0 r	++	
17	ND2.7c	21.0 a	+++	58	HD2.8p	14.0 h	++	
18	ND2.8a	14.0 h	++	59	HD2.9a	7.0 q	++	
19	ND2.8c	3.0 v	+	60	HD2.9c	8.0 op	++	
20	RL1c	4.0 u	+	61	H6a	18.0 c	++	
21	RL2b	14.0 h	++	62	H6b	20.0 b	+++	
22	RL3a	3.0 v	+	63	H10a	10.0 l	++	
23	RL3d	5.3 rs	+	64	N1a	10.0 l	++	
24	RN1a	5.7 rs	+	65	N2a	7.0 q	++	
25	RN1c	10.0 l	++	66	N6a	14.3 gh	++	
26	RN1d	4.0 u	+	67	N7a	5.3 rs	+	
27	RN1f	5.0 st	+	68	N7b	9.3 lm	++	
28	RN3a	5.7 rs	+	69	N8b	11.0 l	++	
29	RN3c	4.0 u	+	70	N8c	9.0 m	++	
30	RN4c	6.0 r	++	71	N8d	14.0 h	++	
31	RN5a	14.0 h	++	72	N8e	8.0 op	++	
32	RN5c	3.0 v	+	73	N9a	8.7 mo	++	
33	RN6a	7.0 q	++	74	N9c	10.0 l	++	
34	RN6b	4.3 tu	+	75	N9d	11.0 k	++	
35	HD1.2a	4.0 u	+	76	N9e	3.0 v	+	
36	HD1.2c	9.0 m	++	77	N9f	6.0 r	++	
37	HD1.3d	15.0 f	++	78	N9g	7.3 pq	++	
38	HD1.3e	20.0 b	+++	79	N9h	14.0 h	++	
39	HD1.4b	6.0 r	++	80	N10b	7.0 q	++	
40	HD1.4d	4.0 u	+	81	N10d	12.0 j	++	
41	HD1.5c	17.0 d	++	82	N11b	6.0 r	++	
CV (%) = 4.22%				Positive control				8.0 op

*In Means within a column followed by the same letter/s are not significantly different at p < 0.01*

*Inhibition zone: diameter [D = d<sub>1</sub> - d<sub>2</sub>] (mm)*



**FIGURE 1: Ratio of number of actinobacterial isolates against *Bacillus cereus***

Six best isolates as ND1.7a, ND2.7c, HD1-3e, HD1-6a, HD2.3b and H6b with diameter of sterile ring (20-21 mm) were chosen to identify by PCR technique and sequencing.

### 3.2 Identify actinobacterial isolates

The result from Table 2 showed that 5/6 strains belonged to *Streptomyces*, and one strain was *Microbacterium*.

**TABLE 2**

**PHYLOGENETIC AFFILIATION OF 6 ACTINOBACTERIAL ISOLATES ON THE BASIS OF 16S rDNA GENE SEQUENCES BY USING BLAST PROGRAMME IN THE GENBANK DATABASE BASED ON SEQUENCE SIMILARITY.**

No	Actinobacterial isolates	Closest species relative	Similarity (%)
<b>Actinomycetaceae</b>			
1	ND1.7a	<i>Streptomyces tateyamensis</i> strain 18I (MG009024.1)	100
		<i>Streptomyces chumphonensis</i> strain HQA999 (MH041238.1)	100
2	HD1.3e	<i>Streptomyces althioticus</i> P54-7 (LC551871.1)	100
		<i>Streptomyces griseoincarnatus</i> P49-18 (LC551868.1)	100
3	HD1.6a	<i>Streptomyces flaveolus</i> strain ADIP1 (KF732809.2)	100
		<i>Streptomyces ambofaciens</i> strain M (MK929483.1)	100
4	HD2.3b	<i>Streptomyces olivaceus</i> strain LEP7 (MW767828.1)	100
		<i>Streptomyces coelicoflavus</i> strain ROA061 (MW757213.1)	100
5	H6b	<i>Streptomyces albidoflavus</i> strain HQA017 (KT758349.1)	100
		<i>Streptomyces saprophyticus</i> strain DE2 (MW797316.1)	100
<b>Microbacteriaceae</b>			
6	ND2.7c	<i>Microbacterium tumbae</i> strain C3 (MG958700)	100
		<i>Microbacterium kyungheense</i> strain MK (MF373498)	100

The results from Table 2 showed that 5/6 belonged to *Streptomyces*, and 1/6 was *Microbacterium*. The phylogenetic tree of 6 strains showed that 2 clusters: cluster A: 5 strains were genus *Streptomyces* and cluster B: *Microbacterium tumbae* ND2.7c.



#### IV. CONCLUSION

In conclusion, the culturable diversity of sponge-associated actinobacteria from the Kien Giang Sea was established. Streptomyces isolates were found as the predominant strains showing antibacterial activity. Besides, Microbacterium tumbae performed as a rare actinomycete which displayed antifungal activity. It is indicated that marine sponges are a potent source of endophytic actinomycetes with wide biological activity against pathogenic fungi as well as Gram-positive bacteria, Bacillus cereus. This makes it a promising application of such newly functional sponge-associated actinobacteria as a novel source of bioactives.

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# Improving Fruit Quality and Nutritional Value of Deglet Nour dates subjected to Salt Stress by using Phospho-Potassium Fertilization (Biskra south-east of Algeria)

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**Abstract**— A field study was carried out during the two consecutive years (2015-2016) in the region of Biskra, southern east of Algeria on date palms of Deglet-Nour variety, grown in a salty environment. To study the combined effect of salinity and phospho-potassium fertilization on the quality and nutritional value of dates, two sites of different salinity, occupied by 54 date palms variety Deglet-Nour has been selected. The palms were fertilized by receiving three doses of potassium (0, 2 and 3 kg / palm) as potassium sulphate  $K_2SO_4$  (50%) combined with three levels of phosphorus (0, 1 and 2 kg / palm) as superphosphate (TSP 46%). The results revealed that applying 2 kg of potassium/palm in an excessively salty environment and 3 kg/palm in a low or unsalted environment associated to 1 kg of phosphorus in the two different cases of salinity of the two sites S1 and S2 improving the fruit traits.

**Keywords**— Salinity, dates, date palm, quality of dates, phosphorus, potassium.

## I. INTRODUCTION

The problem of salinity is multiple, because in addition to the toxicity of  $Na^+$  and  $Cl^-$  ions (dissolved in the irrigation water or present in the soil solution) and the perturbations of the mineral nutrition (following the interactions between ions), plants have difficulty absorbing soil water because of its high osmotic pressure, and this is justified by water stress in addition to salt stress, thus complicating and altering their physiological state exponentially and causing multiple perturbations on the metabolism, growth and development of plants at the molecular, biochemical and physiological levels (**Winicov 1998 Munns 2002, Tester and Davenport 2003**).

In arid regions in addition to drought and heat that inhibits growth and productivity of the date palm saline stress mainly affects its vitality. **Furr (1975)** reported that it is evident that the date palm is more salt tolerant than barley and that it can be the most salt-tolerant of all cultivated plants but increasing soil salinity is beginning to have a negative impact on the agro-ecosystem of date palm in the arid region, particularly in the Middle East (**Dakheel, 2005**). In Algeria in the Ziban oases (Biskra region) mismanagement of irrigation and drainage water has detrimental consequences on the phoenicultural environment (**Munier, 1973**), it has led to soil salinization, falling yields and poor-quality dates (**Dutil, 1971; Dubost, 1991**). Relative yields become null if the farmer uses salted soils by the usual method and it allows to obtain some harvests, but extremely low and of poor quality because of the salt content.

In the context of improving fruit quality and the nutritional value of Deglet Nour dates, phospho-potassium fertilization is considered an important factor that affects fruit quality and date palm productivity.

The objective of this study is to spatialize salinity, characterize soil in an irrigated palm grove and then adapt a phospho-potassium fertilization program aimed at improving the production and quality of Deglet-Nour dates grown in saline soil.

## II. MATERIALS AND METHODS

The present study was conducted during the successive seasons of 2015 and 2016 in private orchard with an area of 21, 90 ha located in Biskra in the southern east of Algeria (Fig.1).

- For this purpose, the salinity map was established to study the spatial distribution of salinity in the orchard to select the suitable site for the study. The thematic map of the "CE" is interpolated with spatial analyst of Surfer 14 (Golden Software, LLC) (Fig.2).
- To meet the objectives of our study, it was necessary to locate two sites S1 and S2 of different salinity class in the same plot (from the established salinity map):

**Site S1:** Soil salinity > 16 dS/m, occupied by 27 palms.

**Site S2:** Soil salinity between (4-8 dS/m), occupied by 27 palms (uniform as possible, healthy of any infection, subjected to the same cultural practices, palm tree were planted at spacing 9x9 meters apart and irrigated by drip system).

The palm was fertilized with superphosphate (46%) as a source of phosphorus and potassium sulphate (K<sub>2</sub>SO<sub>4</sub> 50%) as a source of potassium. The soil analysis of the two studied sites is presented in Table 1.

Nine soil application treatments were arranged in completely randomized design with three replicates (1replicate = 1 palm) per treatment (i.e :1x3x9 =27), the treatments were as follow:

<b>T1:</b> unfertilized tree (control),	<b>T5:</b> 2kg K + 1kg P
<b>T2:</b> 0kg K + 1kg P	<b>T6:</b> 2kg K + 2kg P
<b>T3 :</b> 0kg K + 2kg	<b>T7:</b> 3kg K + 0kg P
<b>T4 :</b> 2kg K + 0kg P	<b>T8:</b> 3kg K + 1kg P
	<b>T9:</b> 3kg K + 2kg P.

The treatments were added in either one dose to a depth of 40 cm from the soil surface and 50 cm apart from the palm trunk.



FIGURE 1: Location of the study plot (Extract from Google Earth)

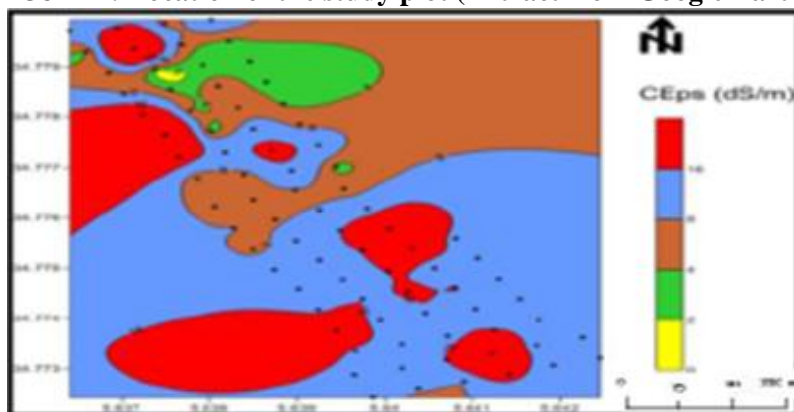


FIGURE 2: Salinity map of the study area (Interpolation of the EC)

**TABLE 1**  
**SOIL AND IRRIGATION WATER ANALYSIS OF THE TWO EXPERIMENTAL SITES**

Properties	Site S1 (EC <sub>Soil</sub> > 16dS/m)	Site S2 (EC <sub>Soil</sub> 4-8dS/m)
pH	7.98	7.96
Na( meq/l)	37.3	6.87
Ca( meq/l)	6.8	11.06
Mg (meq/l)	30.53	24.2
K (meq/l)	2.06	0.6
Cl (meq/l)	67.33	7.66
SO <sub>4</sub> (meq/l)	17.78	12.56
HCO <sub>3</sub> ( meq/l)	2.5	1.5
Gypsum%	60.10	57.33
Total calcareous %	13.01	9.45
OM%	0.53	1.19
EC <sub>irrigation water</sub> (ds/m)	5.5	

### 2.1 Chemical characteristics of fruits

A sample of 20 mature dates for each replicate was used to determine the chemical characteristics of the fruits. The fruits were cut into pieces with a clean knife; five grams were taken from fresh fruits to extract reducing sugars with water at 85°C and 3,5- dinitrosalicylic acid to extract total sugar (**Barbin, 2006**). The percentage of reducing sugar and the amount of total carbohydrates were determined according to **AOAC (1995)**. Acidity (as malic acid) was determined according to **AOAC (1995)**.

### 2.2 Moisture and mineral elements of fruits

A sample of 20 fruits from each replicate was taken and washed with tap water, rinsed twice in distilled water cut into small pieces with a clean knife. Then, an amount of the fresh sample was weighed (fresh weight) and dried at a constant weight (g) in an air-drying oven at 70°C and weighed (dry weight). The moisture of the fruit was calculated as follows:

$$M \% = \frac{M1-M2}{P} \times 100 \quad (1)$$

Dried fruits were digested with H<sub>2</sub>O<sub>2</sub> and H<sub>2</sub>SO<sub>4</sub> according to **Evanhuis and Waard (1980)**. Phosphorus was determined by ascorbic acid using the method of **Murphy and Riley (1962)**. Potassium was determined with a flame photometer.

### 2.3 Statistical analyses

The data were processed by the analysis of variance technique (ANOVA) by Xlstat 2016 (Addinsoft, 2016, data analysis and statistical solution for Microsoft Excel). Treatment averages were separated and compared using significant differences at 0.05 level of significance according to **Snedecor and Cochran (1989)**.

## III. RESULTS AND DISCUSSION

The results obtained show the positive impact of phospho-potassium fertilization on chemical parameters by increasing levels of total and reducing sugar in both sites compared to control date palms (Figures 5.6, 7, 8, 9, and 10)

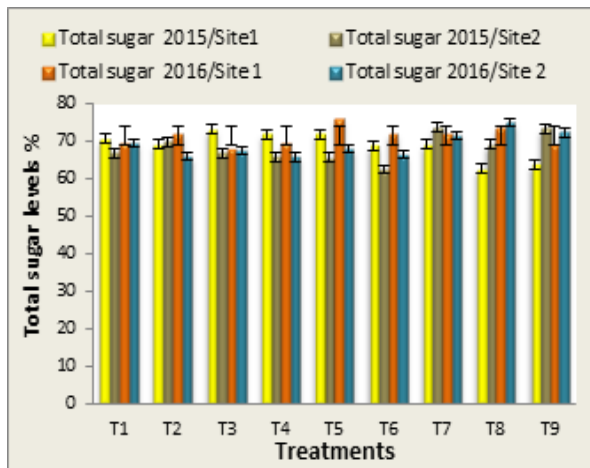
However, the differences are not significant between the treated date palms and the control, and this explains why the response of palms to nutrients may not be clear in the first years of addition, and this is even more so as trees that have not been fertilized for a fairly long time are beginning to normalize and compensate for nutritional deficiencies and then show the good effect of fertilization (**Ibrahim, 2008**).

**André (1994)** noted that in very poor soil, do not try to correct these soils quickly, poor soil quickly captures nutrients like a sponge but its redistribution to plants is not as fast and is partial. The example of phosphorus is the most demonstrative in this regard; it is better to make corrections over several years than over one or two years.

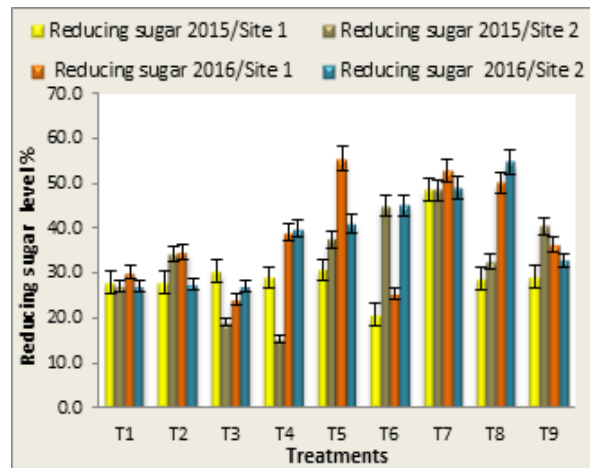
Similar results were found by **Hussein et al (1977)** on the Khunaizi and Sukkari varieties, by **Bacha et al (1982)** on the Khudari variety and by **Furr et al (1955)** on the Deglet Noor date variety. These same authors confirmed that the quality of

the fruit of palm trees fertilized by mineral fertilizers was not significantly different from that of the control date palm. On the other hand **Harhash (2000)**, **El-Shazly (1999)**, **Bliss and Mathez (1983)**, **Sinclair et al (1981)** obtained the same results and reported the desirable effect of the different levels of phosphorus and potassium in the formation of sugars.

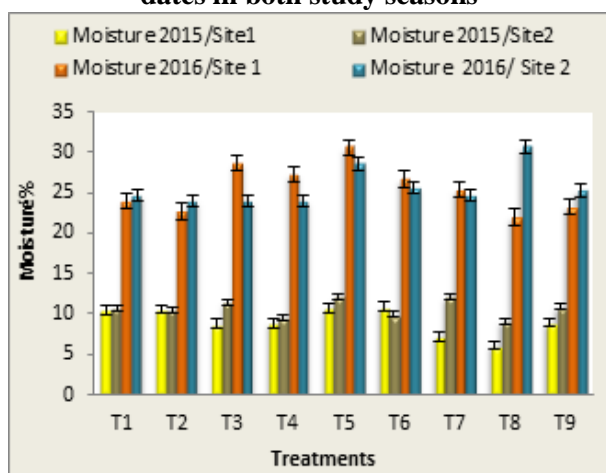
However, these results do not agree with those of **Al kharusi et al (2009)**, **Saleh (2009)**, **Dialami and Mohebi (2010)** who reported that the acidity of dates is positively affected by the application of fertilizing elements.



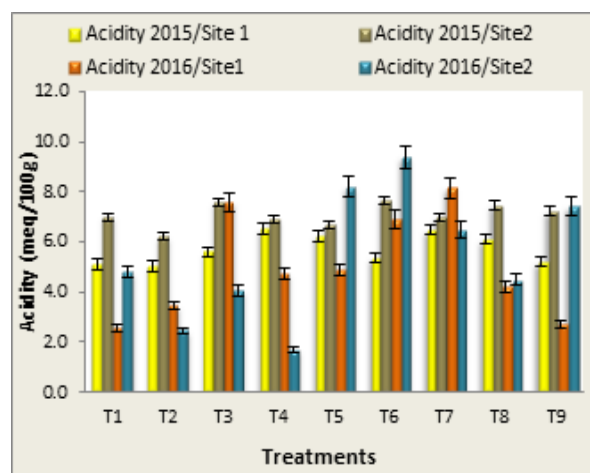
**FIGURE 3: Total sugar levels of dates in both study dates in both study seasons**



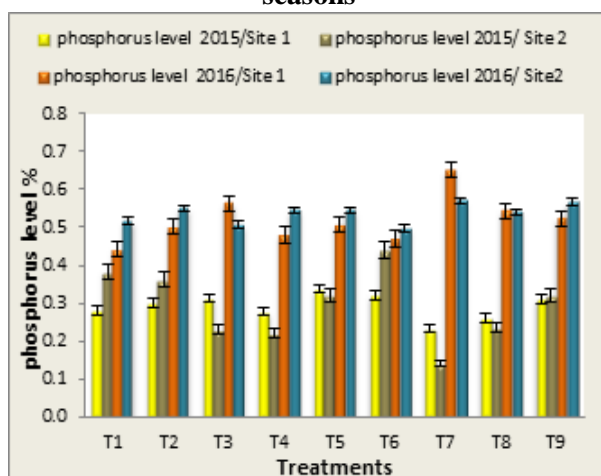
**FIGURE 4: Reducing Sugar levels of seasons**



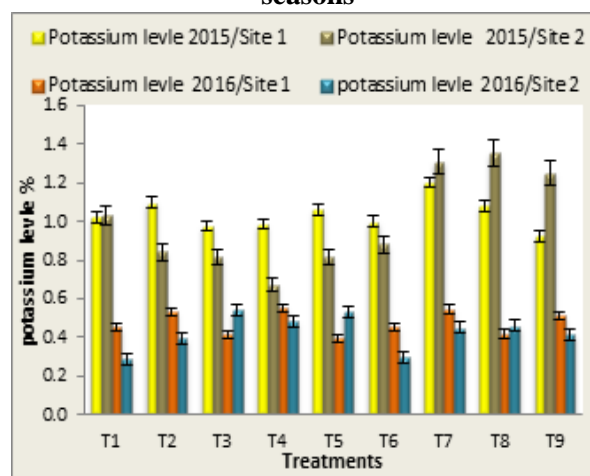
**FIGURE 5: Water content of dates in both study seasons**



**FIGURE 6: Acidity level of dates in both study seasons**



**FIGURE 7: Phosphorus levels of dates in both study seasons**



**FIGURE 8: Potassium level in both study seasons**



### 3.1 Moisture and mineral elements

The results obtained show a significant increase in the fruit's water content during the second year, which shows the effect of mineral fertilization on date palm from the second year.

The contribution of 2 kg of potassium sulphate and 1 kg of phosphorus (TSP) in the site S1 coincides with extremely high moisture level of 30%, while the contribution of 3 kg of potassium sulphate and 1 kg of phosphorus (TSP) to site S2 has moisture level of 28%. These results are similar or higher than those reported by other researchers in other countries (**Al-Shahib et Marshall, 2003; Aidoo et al, 1996; Ahmed et al, 1995; Youssif et al, 1989**).

The results obtained indicate a significant increase in the concentration of phosphorus contained in dates during the second season in the two sites studied; but no significant difference in phosphorus concentration is found between the two salinity classes; on the other hand, statistical analyses show a significant difference between the treatments on phosphorus concentration, the highest value is marked by the T7 treatment in site S1 with 0.65% followed by the T7 and T9 treatments in site S2 with 0.57%. The results also show that phosphorus concentration in fruits increased significantly after potassium sulphate contribution, these results are in agreement with those obtained by **Kassem (2012), Kassem et al (1997) and Epstein, (1972)** who reported that the content of N, P,K, Fe, Zn in leaves and fruits increases through the application of potassium which strongly influences nutrient absorption and the translocation and distribution of other cations. The T9 treatment shows a concentration equal to that obtained by the T7 treatment in the S2 site, which may explain why the plant uses the minimum of the phosphorus brought by the fertilizers compared to the reserve phosphorus in the soil (**Anonymous, 1985**); it also appears that the addition of potassium stimulates the absorption of reserve phosphorus compared to the phosphorus of the fertilizer compared to the phosphorus of the fertilizer which is exposed to precipitation reaction stresses in the alkaline and calcic medium hence the need to use fertilizer phosphorus in the low-salt environment.

The potassium analysis results showed a significant decrease in the potassium level in the fruits during the second season and this can be explained by the incomplete maturation of the dates and by the role of this element in the complete maturation of the fruits.

## IV. CONCLUSION

It is therefore necessary to emphasize that date palms, like other trees, need to be fertilized, especially since the palm tree needs nutrients continuously without any specific period, because its growth continues throughout year. At the end of this study, we were able to highlight the effect of phospho-potassium fertilization in improving date quality as an integrated action to minimize the consequences of different constraints.

The quality of the dates obtained in this study complies with the criteria for the qualitative evaluation of dates of Algerian, Moroccan, Tunisian, Egyptian and Iraqi cultivars reported by **Rygg, (1953); Meligi and Sourial, (1982) and Mohamed et al, (1983); Rayens et al (1994); Othman (1995)**.

To improve the quality and nutritional value of the fruits, it is recommended to apply 2kg of potassium sulphate/palm in an excessively salty environment and 3kg/palm in a non-salty environment with 1 kg of phosphorus in both situations.

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# Study of irrigation sources and cultivation area for Cereals & Pulses in the district of Meerut, Uttar Pradesh (India)

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**Abstract**— *Cereals and pulses play a significant role in the diet of population. As per WHO, the recommended ratio is 2:1 for cereals and & pulses. However, there are different reasons which have gone against the production of pulses in general. Cereals on the other hand, have picked up larger portion in overall cultivation and consequently, the gross & net sown area are more under the cultivation of cereals. Currently, the ratio between cereal to pulses production ranges from 8:1 to 6:1. In this paper, it is found that the ratio between cereals and pulses which was 7.3:1 in the year 2012-13 increased to 7.7 1 in the year 2018-19. The study found that there was not much change in the gross & net area sown in the district of Meerut from the year 2012-13 to 2018-19. Irrigated area was also constant in both the years. Furthermore, production of different cereals and pulses are studied to know whether there is any change in their production due to change in the availability of water for irrigation during studied years in the district of Meerut, Uttar Pradesh.*

**Keywords**— *Meerut, Crops, cereals, Pulses, Irrigated area, gross sown area, net sown area, irrigation sources, canals, tube wells.*

## I. INTRODUCTION

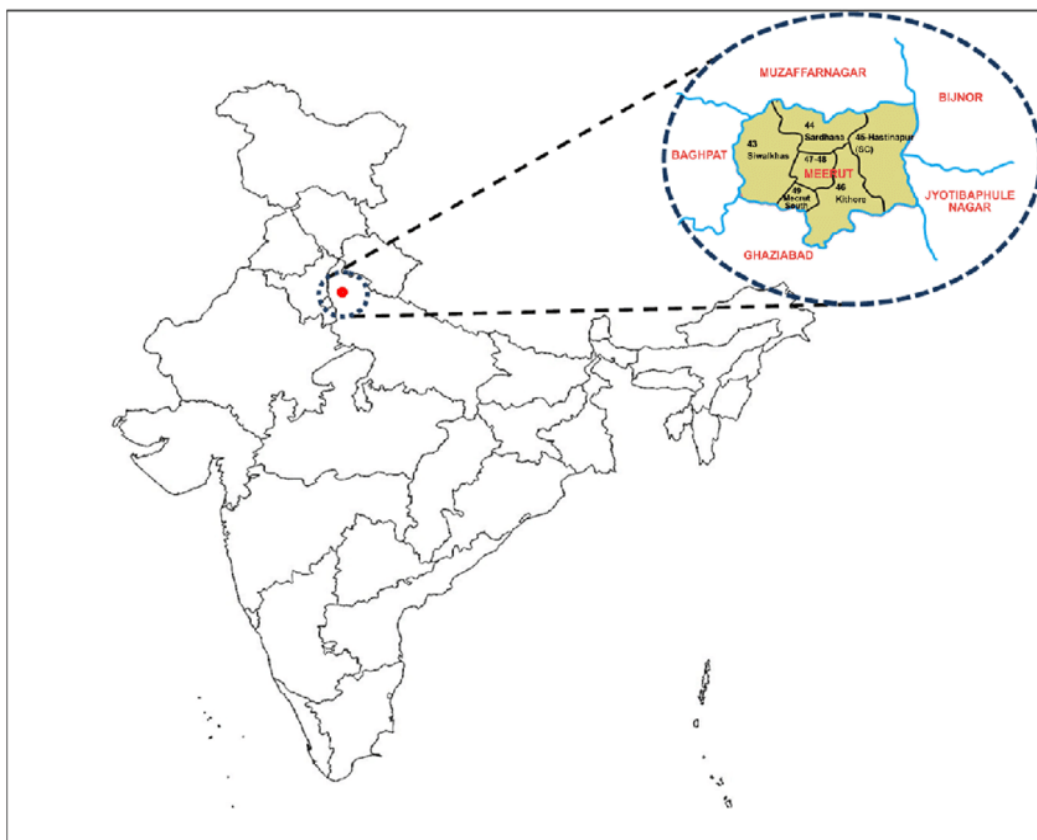
Humankind is primarily fed with agricultural products since ages. Cultivable land and resources required to cultivate crops has been limited to humankind. With ever increasing population, pressure is mounting up to produce more and more from every acre to feed population. For crop production, besides many other resources to ensure higher productivity, water is the most important critical resource. Besides a rain, which still is main source of water for agriculture; people at large have tried to innovate and build mechanical tools to pump out water from ground or taking water from one place to other through canals etc, since ages. History is filled with stances wherein rulers in the past have spent good amount of their revenues on ensuring water to farmers. This is continuing with modern day governments of every country.

All man-made means used to water agriculture fields are termed as irrigation source. Irrigation has become most critical input of agriculture production process. Canal is most critical among others. Canals have been in the past and now remained major resource. However, it is not easy to build canals on one hand and on the other hand, it is not possible to ensure canal reaching every field in nook & corner of the country. It has led to develop ways and means to ensure water reaching every possible field by other sources. In the past, means like Rahat, man-driven pulleys were used to lift water from underground sources. Mechanisation has made it little easy. Diesel generators have become common ways to pump put water now and perhaps easily accessible to farmers.

In general, all above mentioned types of irrigation are termed as Surface Irrigation Method. Surface irrigation has become the commonly used source to ensure water for cultivation. To summarise, surface irrigation includes canals, tube wells of all types, & traditional means of irrigation like Rahat etc. In the year 2018-19, irrigation from canals was 14.9%, government tube well 1.2% & private tube wells was 83.9%.

With decreasing level of availability in water at large and water for irrigation has been an area of concern for governments. Per capita availability of water which was around 5247 cubic meter in 1951 has gone down to 1453 cubic meter in 2015. Still with the above change, irrigation has positively influenced agriculture in Uttar Pradesh in general and district of Meerut in particular in last couple of years. Today, including Meerut district, Uttar Pradesh is naturally blessed to have a strong irrigation system, which is having the third highest gross irrigated area of 82.5% in 2014-15. In total, Uttar Pradesh has

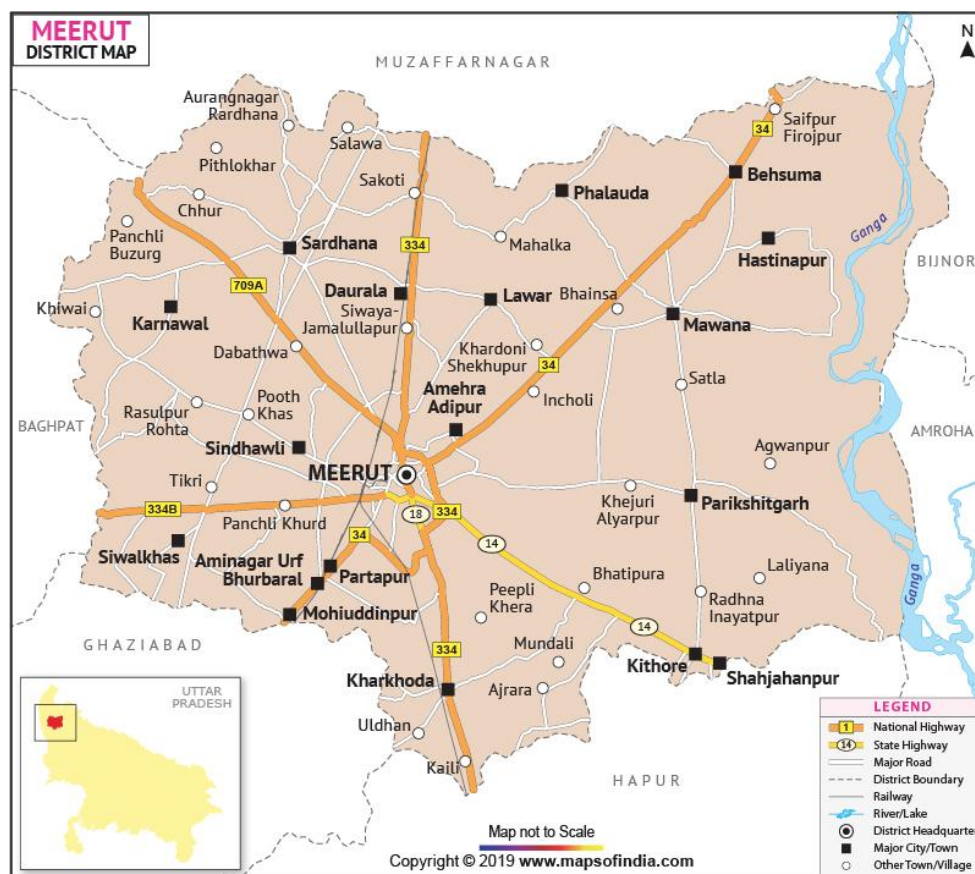
around 74659 kms of canals, 28 major and medium-lift canals, 249 minor lift canals, 69 reservoirs/budhis and about 32,000 running tube wells operated by the government. Tube wells are the major sources of irrigation. Tube wells have 80.2% share in the total irrigation sources, followed by canals which is 17.9%. Availability of water in the state and the district has made farmers growing crops like rice and sugar cane which require large quantities of water.



**MAP-1: Meerut district on India Map**



**MAP-2: Meerut district within the state of Uttar Pradesh, India**



**MAP-3: Meerut district map giving details of irrigation sources etc.**

## II. OBJECTIVES

- 1) To study change in areas of coverage under different irrigation sources
- 2) To study change in cultivation of crop pattern
- 3) To discuss impact of change in irrigation on cultivation pattern

## III. MATERIALS & METHODS

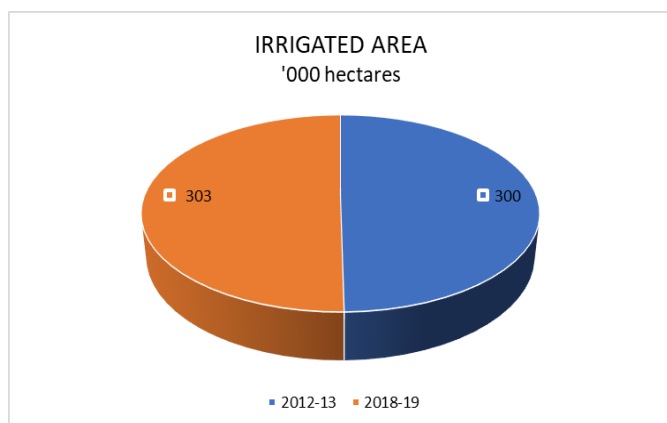
In this paper, relevant data about different irrigation sources for the years 2012-13 and 2018-19 are studied to find out impact of any change in irrigated area on crops under cereals & pulses. The data has been tabulated and presented in such a way that they provide basis of interpretation. Calculation is done based on percentage change in studied variables. This study is done based on secondary data, collected from different sources.

## IV. RESULTS & DISCUSSION

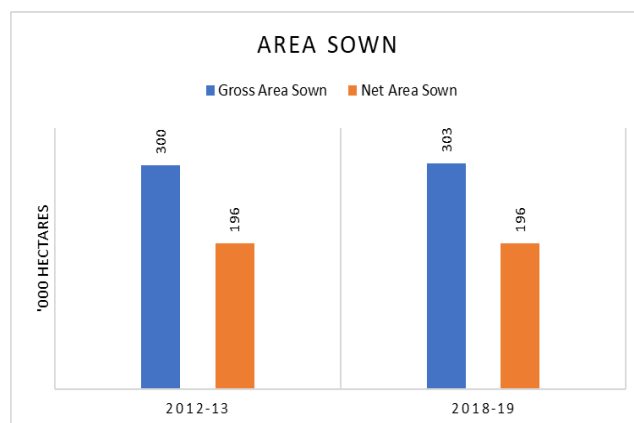
### 4.1 Gross & Net irrigated area

While studying net Irrigated area, the basic definition considered is that net irrigated area is the area irrigated through any source at least once in a year for a particular crop. From the Figure -1 below, the net irrigated area in the district of Meerut has remained mostly unchanged. It was 303 thousand hectares in 2012-13 which was marginally reduced to 300 thousand hectares in the year 2018-19. In percentage terms, there was a marginal decrease of 0.03% in net irrigated area from the year 2012-13 to 2018-19.

For, the net irrigated area had not changed much from the year 2012-13 to 2018-19; correspondingly change was there in the gross and net sown areas for the year 2012-13 to 2018-19 in the district. The gross sown area for the district was 300 thousand hectares in the year 2012-13 which marginally increased to 303 thousand hectares in the year 2018-19. However, no change was there in the net sown area in the years 2012-13 to 2018-19 in the district of Meerut. In absolute terms, net sown area was 196 thousand hectares in the year 2012-13 which remain unchanged in the year 2018-19, as presented in the Figure-2.



**FIGURE 1: Gross Irrigated Area for years 2012-13 & 2018-19 in Meerut District**



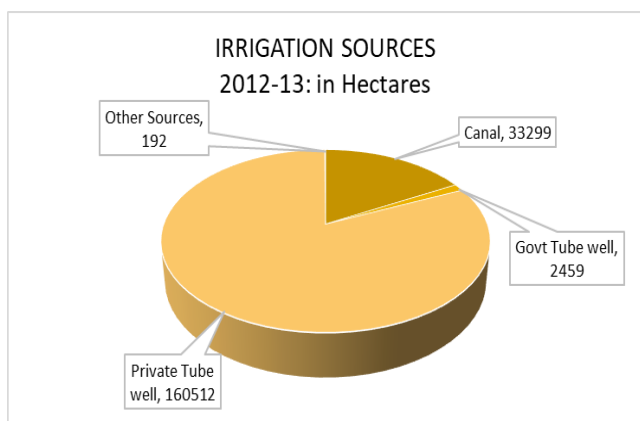
**FIGURE 2: Gross & Net Sown Areas for years 2012-13 & 2018-19 in Meerut District**

**4.2 Irrigation by different sources**

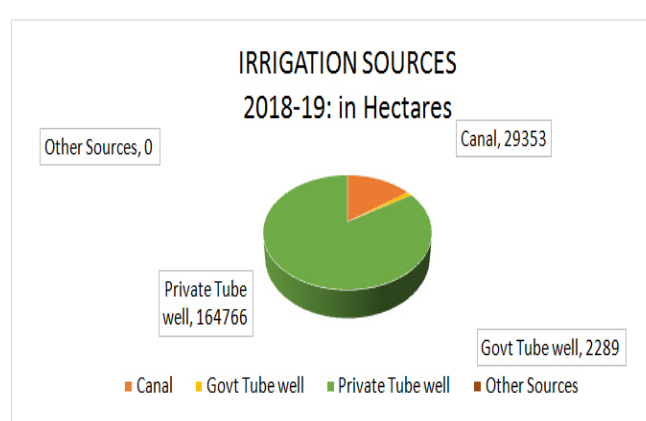
Water availability for cultivation in the district of Meerut, Uttar Pradesh is primarily from canals, government tube wells and private tube wells. There are few other traditional sources of irrigation which were used in the district.

In the year 2012-13, share of different sources of irrigation was like, from canal 33299 hectares of agricultural land was irrigated in the district of Meerut, Uttar Pradesh while government tube wells covered 2459 hectares of agriculture land. However, the maximum irrigation came from private tube wells which covered 160512 hectares of land which was approx. 82% of all irrigation sources in the district. Figure -3 below provides the relevant details.

In comparison, in the year 2018-19, irrigation from canals was reduced to cover only 29353 thousand hectares of land. Likewise, government tube wells share was also reduced to 2289 hectares. On the other hand, the share of private tube wells in overall irrigation increased by around 2% to approx. 84% of total irrigation water from different sources in the district of Meerut as per Figure-4 below.

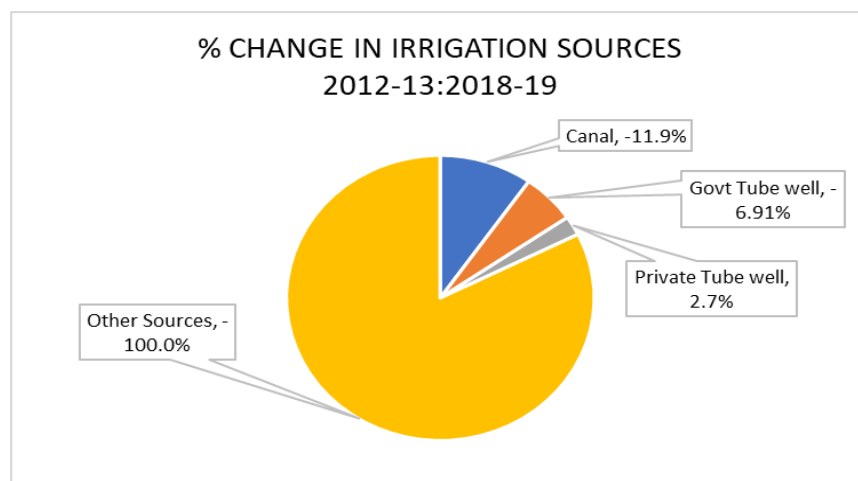


**FIGURE 3: Share of different Irrigation Sources in net irrigated area in the year 2012-13 in Meerut district**



**FIGURE 4: Share of different Irrigation Sources in net irrigated area in the year 2018-19 in Meerut district**

As mentioned, there was very minimal change in the net irrigated area in the district but the share of private tube wells among all irrigation sources rose to approx. 84% in the year 2017-18. Sources wise, canals saw reduction by 11.9%, government tube wells by 6.91% and other sources were having almost no share among irrigation sources in the district from the year 2012-13 to year 2018-18. In percent terms, the share of private tube wells in the district increased by 2.7% over the year 2012-13 details in Figure-5.

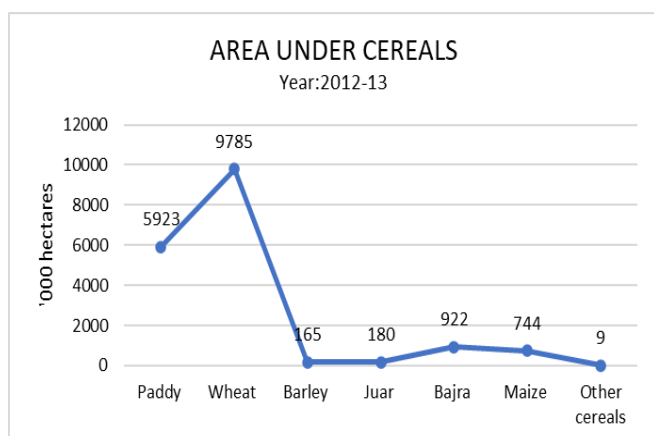


**FIGURE 5: Percentage change in Irrigation Sources from 2012-13 to 2018-19 in Meerut district**

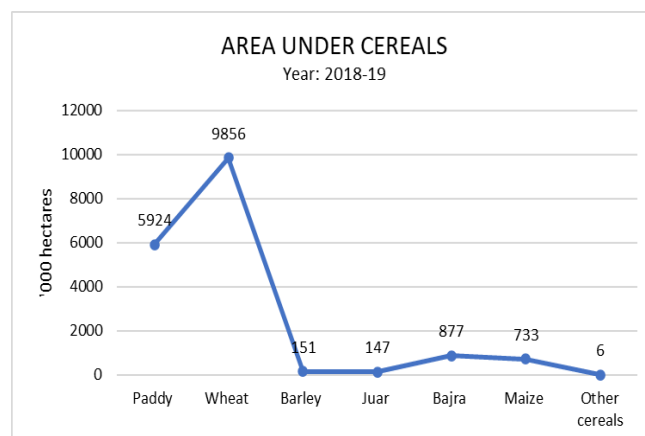
**4.3 Area under cultivation of Cereals**

Cereals are one of the major crops which are grown in the district of Meerut. This is the reason that cereals cover major agricultural land in the district. Among all cereals, wheat covers approx. 55% area under cultivation. In absolute terms, wheat had 9785 thousand hectares land under it, followed by paddy at 5923 thousand hectares. Other cereals, namely bajra, maize, juar and barley were having 922, 744, 180 and 165 thousand hectares of area under cultivation in the year 2012-13 as presented in the Figure-6.

In the year 2018-19, wheat as before continued to dominate crops in the district of Meerut to cover area under cultivation. Area under wheat was 9856 thousand hectares which was mostly the same with 55% share among all cereals, as was in the year 2012-13. All cereals however, saw decrease in the areas under their cultivation with respect to the year 2012-13. Paddy at 5924 thousand hectares was the second cereal to have maximum area under cultivation. Bajra at 877, maize at 733, barley at 151 and juar at 147 thousand hectares were other cereals, covering land for cultivation in the district. Details are presented in the Figure-7.

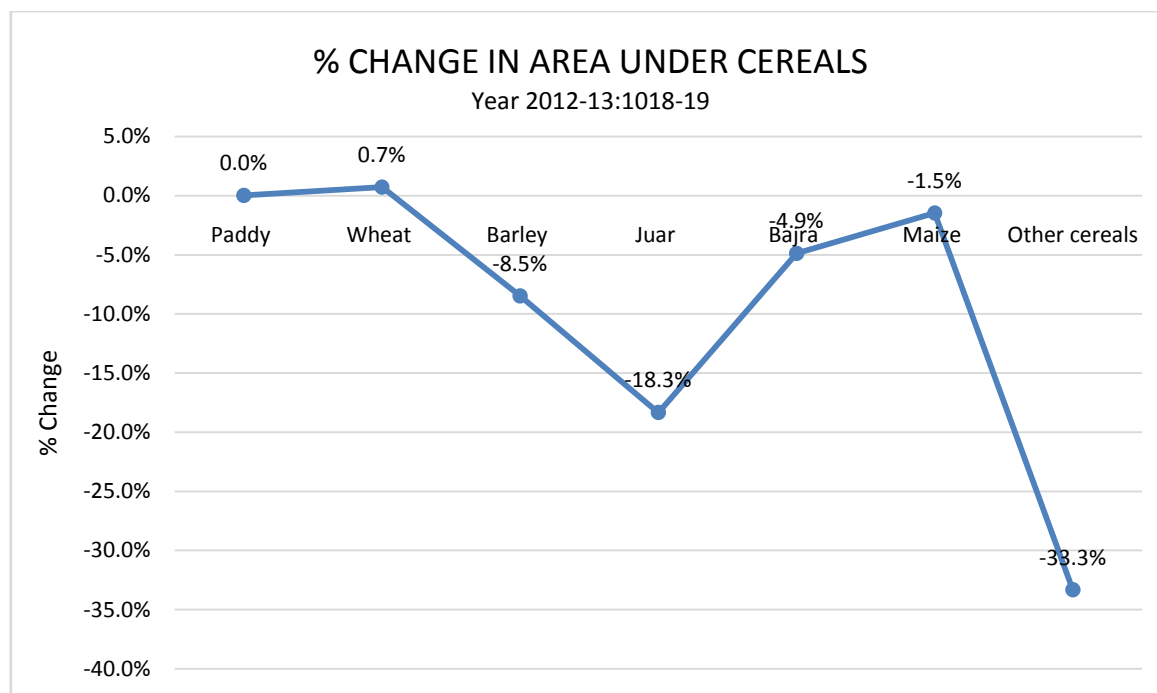


**FIGURE 6: Area under Cereals in the year 2012-13 in Meerut district**



**FIGURE 7: Area under Cereals in the year 2018-19 in Meerut district**

Although, area under cereals remained constant from the year 2012-13 to year 2018-19; but, area under wheat saw marginal increase of 0.7% in the year 2018-19 whereas, area under paddy was unaltered. Other cereals faced decrease in the area of their cultivation. Juar saw decrease of 18.3% in the year 2018-19 from the year 2012-13 which was maximum decrease among all major cereals in the district. Area under other cereals namely bajra witnessed decrease by 4.9%, barley by 8.5%, maize by 1.5%. Details are given in the Figure-8 below.

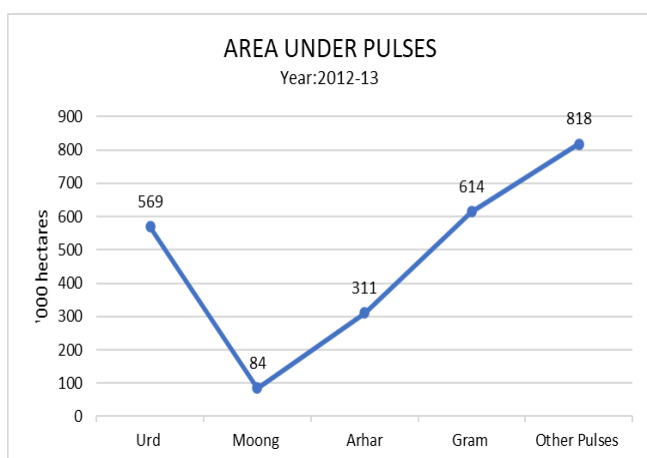


**FIGURE 8: Percentage change in Area under Cultivation of Cereals from 2012-13 to 2018-19 in Meerut district**

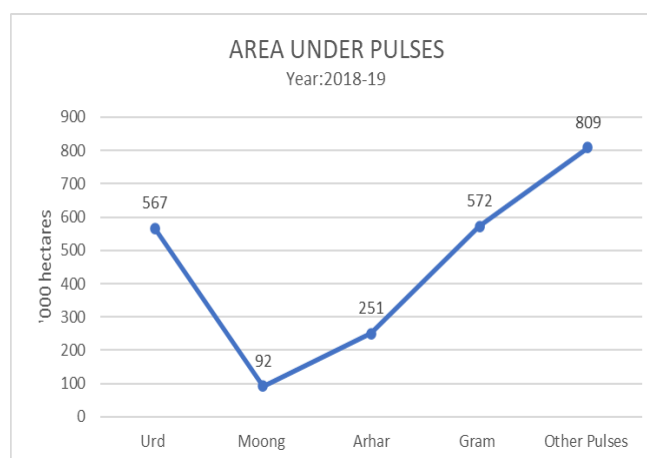
**4.4 Area under cultivation of Pulses**

Pulses are grown in a major way in the district of Meerut, besides cereals. Arhar, moong, urd and gram are major pulses grown primarily in the district. In the year 2012-13, gram was having 614 thousand hectares area under it. Urd with 569 thousand hectares followed by Arhar with 311 thousand hectares were second and third biggest in terms of covering area under cultivation in pulses category. Remaining crops under pulses were covering total 818 thousand hectares land under cultivation, as shown in the Figure-9.

Area under pulses witnessed decrease in the year 2018-19. Total area under cultivation of pulses decreased to 2291 thousand hectares. Areas under gram and urd were almost equal. It was 572 thousand hectares for gram and 567 thousand hectares for urd. Arhar was having area under cultivation of 251 thousand hectares in the year 2018-19. Other pulses in total covered area of 809 thousand hectares. Details are presented in the Figure-10 below



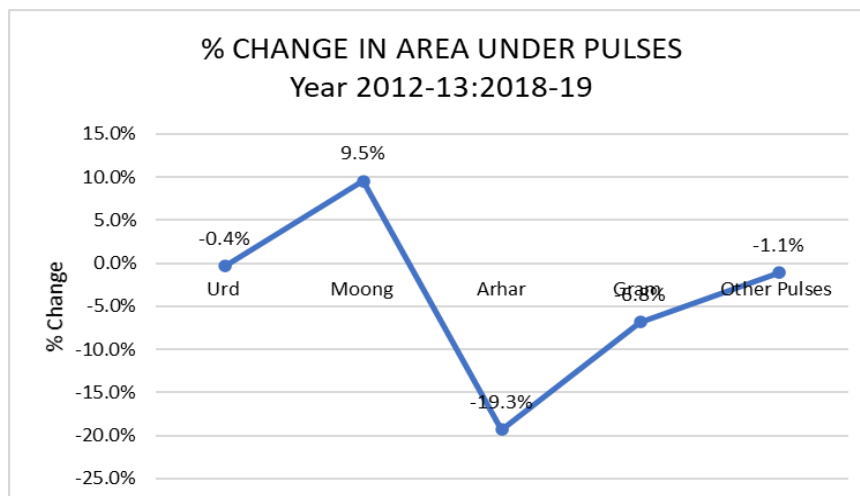
**FIGURE 9: Area under Pulses in the year 2012-13 in Meerut district**



**FIGURE 10: Area under Pulses in the year 2018-19 in Meerut district**



Study of below Figure reveals that area under pulses was reduced by around 5.2% in the year 2018-19. Maximum decrease in the area was seen for arhar. Decrease was by approx 19% in the year 2018-19 from the year 2012-13. Moong saw dip by 9.5% followed by gram which saw decrease of 6.8% in the area of cultivation, primarily. Details are given in Figure-11.



**FIGURE-11: Percentage change in Area under Cultivation of Pulses from 2012-13 to 2018-19 in Meerut district**

## V. CONCLUSION

The paper has studied availability of water through different irrigation sources in the district of Meerut in Uttar Pradesh (India) in the years 2012-13 & 2018-19 and found that there was no change in terms of net irrigated area. In the year 2012-13, the net irrigated area was 196462 hectares which remained almost same in the year 2018-19, i.e., 196408 hectares with marginal decrease of 0.03%. However, among different sources of irrigation, ratio of private tube wells increased in the year 2018-19 while share of canals & government tube wells decreased corresponding to the year 2012-13. The ratio of private tube wells saw a jump of 2.7% from the year 2012-13 to 2018-19. On the other hand, share of canals and government tube wells reduced by 11.9% & 6.9%. It means although there was apparent shift to ensure water for irrigation from private sources, the net irrigated area remained same in both the years. Consequently, it was it was the reason for net area remaining the same in both the years in the district.

Further, it can be concluded as there was no change in the gross & net area sown along with no major change in the availability of water for irrigation different sources in studied years in the district of Meerut, Uttar Pradesh, India, area under cultivation of cereals and pulses largely remained intact in both the years of 2012-13 & 2018-19. Area under cereals saw overall change by 0.2%, but pulses saw a decrease by 5.5% from the year 2012-13 to 2018-19. In absolute terms, where area under cereals was 17728 thousand hectares in the year 2012-13, it was 17696 thousand hectares in the year 2018-19. Among cereals, wheat & paddy were two major crops which were having majority of coverage in both the years. For other cereals, namely Juar, bajra, maize and barley, area under cultivation was reduced corresponding to the year 2012-13 in the year 2018-19. Reduction in the area under cultivation for juar was the highest among all with 18.3%.

Regarding pulses, area under cultivation witnessed a decrease by 5.2% from the year 2012-13 to the year 2018-19. Gram, urd & Arhar were grown primarily in the district of Meerut in both the years of study. In absolute terms, area under gram, urd and Arhar was 614, 569 & 311 thousand hectares in the year 2012-13. In the year 2018-19, it changed to 572, 567 & 251 thousand hectares for gram, urd & Arhar respectively. The study reveals that arhar saw major reduction in the area of cultivation from year 2012-13 to 2018-19. Reduction was by 19.3%. Gram was another pulses, which also observed reduction by 6.8% from year 2012-13 to 2018-19. Moong on the other hand, was one of the pulses in the district whose area under cultivation increased by 9.5% from the year 2012-13 to 2018-19. Urd observed marginal decrease by 0.4% in studied years.

It can overall be construed as there was no considerable change in the gross sown area, net sown area, irrigated area, irrigation sources in the district of Meerut, Uttar Pradesh, India; consequently, the cropping pattern remained almost same in the years of study, leading to area under cereals and pulses also seen no change from the year 2012-13 to 2018-19. However, within crop type, shifting from one crop to another is seen which is also marginal except for juar in cereals and arhar in pulses.

## VI. SUGGESTIONS

1. Cereals and pulses play a very significant role in the diet of people in the district and overall, in the country, therefore more attention to be given to pulses as their share is decreasing.
2. It seems that as cereals are seen as means to earn more, farmers are sticking to production of cereals and not pulses. Therefore, governments should motivate farmers to move to cultivation of pulses as well, so that diet can be balanced.
3. As reduction is observed in the contribution by canals in providing irrigation water, state & central governments should pay attention to this fact also. Else, the cost of production will keep increasing.
4. Likewise, government tube wells have seen decrease in their share to net irrigated area in the district of Meerut. It gives the impression that people are not having confidence on government system to ensure water for their crops.
5. Private tube wells are increasing contribution to net irrigated area in the district. It signifies two facts. One, people in the district are becoming self-sufficient and two, they do not believe in government sources of irrigation in fulfilling their needs of irrigation water. Increase in share of tube wells, certainly will increase cost of production. It will have circular repercussions on public at large.
6. It can also be construed from the study that gross sown area has reached its peak in the district of Meerut, Uttar Pradesh India as no change is observed during five years, i.e. from the year 2012-13 to 2018-19. Therefore, local governments should focus on providing training to farmers in the district on how to increase production of different crops by using modern day agricultural means and techniques.
7. Farmers should acquire knowledge about use of fertilisers, agrichemicals etc in such a way that should help in decreasing cost of production, as well as do not impact climate negatively. Local administration to help farmers in this area as well.

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# Effect of selected fungicides on Brown spot disease of rice caused by *Helminthosporium oryzae*

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Non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Abstract**— The in-vitro test of selected fungicides against brown spot disease incidence of rice and development of a disease prediction model base on weather variable was conducted during two Kharif seasons from 2014-2015 to 2015-2016. Results revealed that among the selected fungicides treatment lowest per cent disease incidence was found in Propiconazole in both the cropping season (2014-15) and (2015-16) with minimum mean per cent disease index (PDI) value bcd (7.76) and (7.03) with per cent disease control of 72.39 and 73.09 respectively over the control, followed by Propineb (PDI) value bcd (8.6) and (7.23) with per cent disease control of (69.40) and 73.09 respectively of the two cropping seasons. Among the fungicides treatment highest disease incidence was found in Thiophanate with maximum mean per cent disease index (PDI) value bcd (17.03) and (14.98) with per cent disease that control of 39.41 and 42.67 respectively in both the cropping seasons. It was also found disease intensity was higher during the first cropping season (2014-15) as indicated by higher mean per cent disease index (PDI) value abcd (12.5\*\*) whereas in the following cropping season (2015-16) with lower value of (PDI) value abcd (11.18\*\*).

**Keywords**— Brown spot, *Helminthosporium oryzae*, disease index, Fungicides, Rice, Fungal Diseases.

## I. INTRODUCTION

Proper evaluation of fungicides available in the market is required to identify the efficacy of a particular chemical against the target pest which will avoid economic losses as most chemicals are costly. Its indiscriminate use has serious effect on natural environment and is a global issue that needs to address through judicious application system. Brown spot disease of rice caused by *Heminthosporium oryzae* (Breda de Haan) is a major fungal disease which has been reported to occur in all rice growing countries including Japan, China, Burma, Sri Lanka, Bangladesh, Iran, Africa, South America, Russia, North America, Philippines, Saudi Arabia, Australia, Malaysia and Thailand (Ou, 1985; Khalili, *et al.* 2012). In India the disease was known to occur in all rice growing states but more severe in dry and direct seeded rice in the state of Bihar, Chhatisgarh, Madhya Pradesh, Orissa, Assam, Jharkhand and West Bengal (Gangopadhyay, 1983; Ou, 1985; Sunder, *et al.*, 2014). This particular disease has been reported to cause enormous losses in grain yield upto 90% particularly when leaf spotting phase assumes epiphytotic proportions as observed in great Bengal famine in 1942 (Ghose *et al.* 1960) and in general can cause yield loss upto 45% when no protection was given.

## II. METHODOLOGY

### 2.1 In-vivo test

Field trial was carried out in the experimental plot of Department of Plant Pathology, Allahabad School of Agriculture, SHUATS, Allahabad, U.P. for two consecutive cropping seasons of kharif (2014-15) and (2015-16) by using a susceptible Manipur Paddy cultivar *viz.*, Daramphou. Field layout was made in Randomized Block Design (RBD) with plot size (2x3) sq. m. 25 days old seedlings was transplanted with spacing 20 cm (row x row) and 15 cm (plant x plant) with 2-3 seedlings/hill. Five fungicides *viz.*, Thiophanate, Carbendazim, Myclobutanil, Propineb, Propiconazole at 1000ppm was sprayed at 10 days intervals from 48, 58 and 68 days after transplantation of the paddy and when prominent disease symptoms start appearing. Periodical monitoring on fixed plot was performed for obtaining real time data for rice brown spot disease incidence and severity in experimental plots. Observation was made one day ahead of each time of the treatment application and final observation was taken at 10 days after the final or third spray. For measuring disease progress 5 plants

per plot was tagged inside the field borders and one in the centre and top three leaves were taken into consideration in each time of disease rating during observation and data was systematically recorded and maintained as per the standard procedure.

The rating of the disease severity was done by using disease scoring scale of 0-4, based on percentage number of leaves showing symptoms according to Kalloo and Banerjee (2000) [where, 0=No symptoms observed, 1=1-25 % leaf area affected, 2=26-50 % leaf area affected, 3=51-75% leaf area affected and 4=75% and above leaf area affected]. Disease rating was recorded and the percent disease severity was worked out subsequently at every 10 days interval of the growth stage of the crop by following formula (Mc Kinny, 1923).

$$PDI (\%) = \frac{\text{Summation of numerical ratings}}{\text{Total number of leaves observed} \times \text{Maximum rating grade}} \times 100 \quad (1)$$

### III. RESULTS AND DISCUSSION

The results obtained during the course of investigation are presented in the following tables (1 and 2) and figures (1 and 2).

**TABLE 1**  
**SELECTED FUNGICIDES AND PER CENT DISEASE INCIDENCE OF BROWN SPOT OF RICE DURING FIRST CROPPING SEASON (2014-15)**

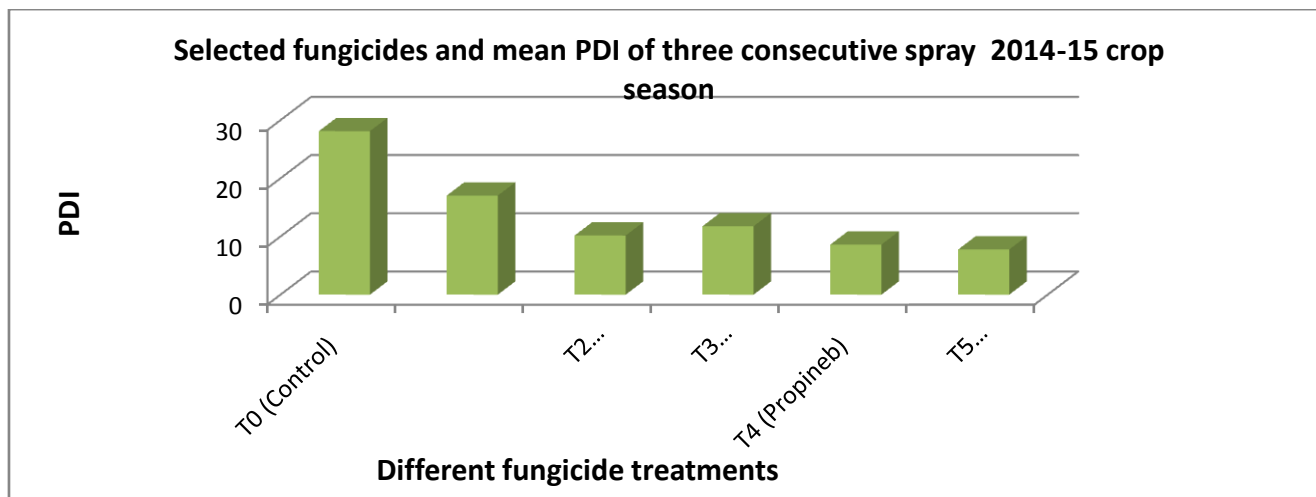
Sl. No.	Treatment	PDI					% Control
		BS* a	AFS* b	ASS* c	ATS* d	Mean (bcd)	
1.	T <sub>0</sub> (Control)	8.2	22.43	29.15	32.76	28.11	-
2.	T <sub>1</sub> (Thiophanate)	7.8	12.01	18.17	20.91	17.03	39.41
3.	T <sub>2</sub> (Myclobutanil)	9.16	6.96	11.83	11.69	10.16	63.52
4.	T <sub>3</sub> (Carbendazim)	8.6	6.91	15.01	13.33	11.75	58.19
5.	T <sub>4</sub> (Propineb)	8.2	4.69	10.72	10.39	8.6	69.40
6.	T <sub>5</sub> (Propiconazole)	7.8	4.44	9.92	8.94	7.76	72.39
	<b>Mean (abcd)</b>	8.29	9.57	15.80	16.33	12.50**	-
	<b>S.Ed (±)</b>	1.7	0.43	0.21	0.22	0.71	1.9
	<b>CD (0.05%)</b>	2.03 (NS)	0.61	0.29	0.32	0.40	5.60

*\*Mean value of four replication*

*BS-before spray, AFS-after first spray, ASS-after second spray, ATS-after third spray,*

*bcd-mean PDI value three observation after the spray*

*abcd-mean PDI value of four observation\*\* NS-non significant*



**FIGURE 1: Selected Fungicides and mean PDI of three consecutive spray (2014-15)**

The data presented on Table 1 is the per cent disease incidence of brown spot disease of rice and the selected fungicides at three consecutive schedule of spray at 48, 58 and 68 days and subsequent observation taken at 10 days interval *i.e.* 47, 57, 67 and 77 days after transplanting of the first cropping season (2014-15).

Results revealed that before the treatment was applied there was no significance difference among the treatment and between non treatment control plots concerning disease incidences. However, observation taken at 9 days after the first treatment found per cent disease incidence was lowest in Propiconazole (4.44) followed by Propineb (4.69), Myclobutanil (6.96) and highest incidence was observed in Thiophanate and Carbendzim treatment with per cent disease incidence of (12.01) and (6.91) respectively over the untreated control (22.43). However, all fungicides treatment was found significantly different among themselves as compared to untreated control. Similarly in the following second and third treatment on each time of observation taken at 9 days after the treatment application. It was observed that per cent disease incidence (PDI) was always found lowest in treatment with Propiconazole followed by Propineb and Myclobutanil and maximum disease incidence was observed in Thiophanate and Carbendazim. It is also evident from the mean PDI value of treatment (bcd) that lowest per cent disease index was found in Propiconazole (7.76) with per cent disease control (72.39) followed by Propineb (8.6) and Myclobutanil (10.16) with per cent control (69.40) and (63.52) respectively over control whereas maximum per cent disease index was found in Thiophanate (17.03) and Carbendazim (11.75) with per cent disease control of (39.41) and (58.19) respectively over the untreated control. However, in all cases all fungicides treatment was found significantly different among themselves as compared to untreated control (Fig.1).

**TABLE 2**  
**SELECTED FUNGICIDES AND PER CENT DISEASE INCIDENCE OF BROWN SPOT OF RICE DURING SECOND CROPPING SEASON (2015-16)**

Sl. No.	Treatment	PDI					
		BS* a	AFS* b	ASS* c	ATS* d	Mean (bcd)	% control
1.	T <sub>0</sub> Control	9.16	20.68	26.42	31.29	26.13	-
2.	T <sub>1</sub> Thiophanate	8.32	9.21	16.36	19.37	14.98	42.67
3.	T <sub>2</sub> Myclobutanil	8.36	5.94	7.59	9.04	7.52	71.22
4.	T <sub>3</sub> Carbendazim	7.64	6.91	12.55	11.1	10.18	61.04
5.	T <sub>4</sub> Propineb	7.65	4.70	8.31	8.70	7.23	73.09
6.	T <sub>5</sub> Propiconazole	8.12	4.23	8.76	8.12	7.03	73.09
	<b>Mean (abcd)</b>	8.20	8.61	13.33	14.60	11.18**	-
	<b>S.Ed (±)</b>	1.32	0.17	0.22	0.19	0.19	0.75
	<b>CD(0.05%)</b>	3.04 (NS)	0.51	0.68	0.57	0.58	2.32

\*Mean value of four replication

*BS-before spray, AFS-after first spray, ASS-after second spray, ATS- after third spray,*

*bcd- Mean PDI value three observation after spray*

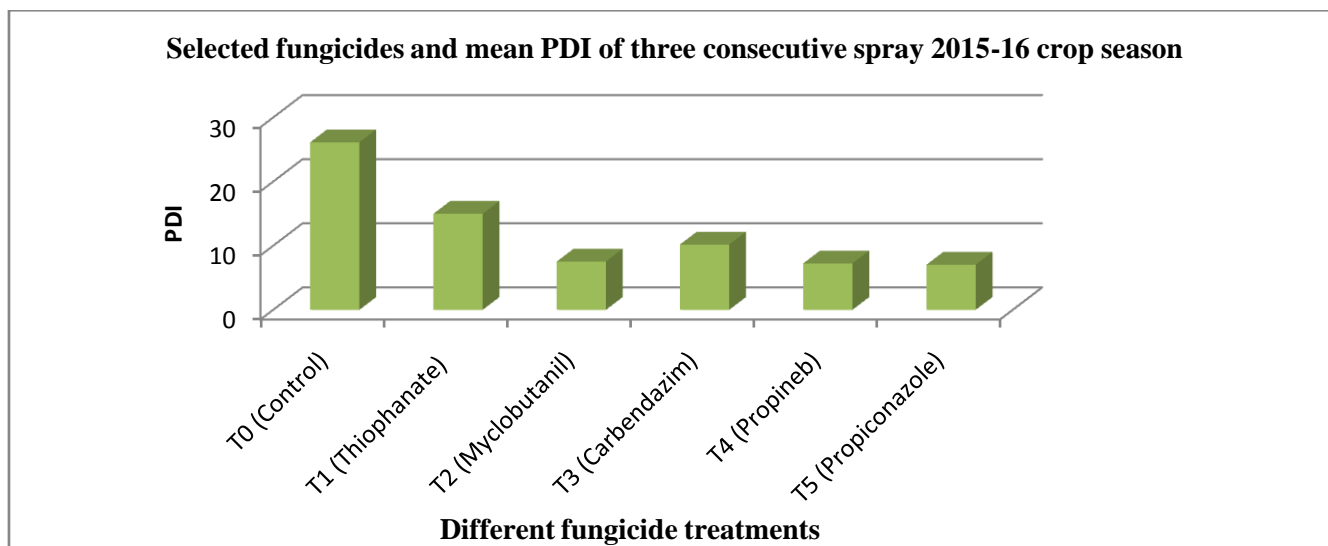
*abcd- mean PDI value of four observation\*\**

*NS - Non significance*

The data presented on Table 2 is the percent disease incidence of brown spot disease of rice and the selected fungicides at three consecutive schedule of spray at 48, 58 and 68 days and subsequent observation taken at 10 days interval *i.e.* 47, 57, 67 and 77 days after transplanting of the first cropping season (2015-16).

Results revealed that before the treatment was applied there was no significance among the treatment and non treatment control plots concerning disease incidences. However, observation taken at 9 days after first spray recorded that per cent disease incidence was lowest in Propiconazole (4.23) followed by Propineb (4.70), Myclobutanil (5.94) and highest incidence was observed in Thiophanate and Carbendzim treatment with per cent disease incidence of (9.21) and (6.91) respectively over the untreated control (20.68). However, all fungicides treatment was found significantly different among themselves as compared to untreated control. Similarly in the following second and third treatment taken at 9 days after the

treatment application it was observed that per cent disease incidence (PDI) was always found lowest in treatment with Propiconazole followed by Propineb and Myclobutanil and maximum disease incidence was observed in Thiophanate and Carbendazim. It is also evident from the mean PDI value of treatment (bcd) that lowest per cent disease index was found in Propiconazole (7.03) with per cent disease control (73.09) followed by Propineb (7.23) and Myclobutanil (7.52) with per cent control (73.09) and (71.22) respectively over control whereas maximum mean per cent disease index value bcd was found in Thiophanate (14.98) and Carbendazim (10.18) with per cent disease control of (42.67) and (61.04) respectively over the untreated control. However, in all cases all fungicides treatment was found significantly different among themselves as compared to untreated control (Fig.2).



**FIGURE 2: Selected Fungicides and mean PDI of three consecutive spray (2015-16)**

The analysis of the above results of the two crop seasons (2014-15) and (2015-16) of *in-vivo* test revealed that all selected fungicides significantly inhibit the disease incidence in all the three schedule of spray. However, among the treatments highest significant per cent reduction of brown spot disease incidence was recorded in Propiconazole followed by Propineb, Myclobutanil, Carbendazim and Thiophanate respectively. Our present finding are in corroborate with that of Percich (1989) who reported that foliar application with Propiconazole was found to have better results in management of brown spot disease of rice. Pannu *et al.* (2003) also reported that application of Propiconazole was found most effective against brown spot disease. Moletti *et al.* (1996) reported that application of Iprodione and Propiconazole was most effective against brown spot disease whereas Celmer *et al.* (2007) reported that Trifloxystrobin + Propiconazole can effectively control the brown spot diseases of rice. Kumar and Rai (2008) also reported that application of Antracol or Propineb and RIL-FA 200SC can effectively reduce brown spot incidence of rice. Sunder *et al.* (2005, 2010) reported that spraying of Hexaconazole and Propiconazole at early booting stage considerably reduced both leaf spot and stalk rot phase of brown spot disease of rice. The data shows that disease severity was more during first cropping season 2014-15 as revealed by higher mean PDI value of four observation abcd (12.50\*\*) whereas in the second cropping seasons 2015-15 lower mean PDI value of four observation abcd (11.18\*\*) recorded.

#### IV. CONCLUSION

Chemical indiscriminate use need to be addressed through proper screening and evaluation and its judicious application practices need to be advocated at the highest level by the end users or the farming community. In our present investigation among the selected fungicides, Propiconazole and Propineb at 1000ppm applied at 48 days after the paddy transplantation and consecutive two sprays at 10 intervals was found most effective against brown spot disease of rice and its severity.

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# Irrigation Water Quality Assessment for Water Resources used in Irrigation of Agricultural Fields in Mezitli Town of Mersin Province

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**Abstract**— This study was conducted for irrigation water quality assessment of water resources used in irrigation of agricultural fields in Mezitli town of Mersin province. Water samples were taken from 20 sampling points of surface water resources used for irrigations in irrigated farming lands of Mezitli town in 4 sampling periods (July – October). Samples were analyzed for pH, EC, water-soluble cations (Ca, Mg, Na, K) and anions ( $CO_3$ ,  $HCO_3$ , Cl and  $SO_4$ ), boron, %Na, SAR and RSC. Sample pH values varied between 7,05 - 8,26 and EC values varied between 292 - 1103  $\mu$ mhos/cm. According to US Salinity Lab Classification System, irrigation waters were classified as  $C_2S_1$  and  $C_3S_1$  (moderately and highly saline waters). Boron concentrations of all samples were below the threshold value of 0,67 ppm. Significant differences were not observed in water quality parameters throughout the irrigation season.

**Keywords**— Irrigation, irrigation water quality, saline irrigation water, boron.

## I. INTRODUCTION

Electrical conductivity (EC) is the most significant indicator of irrigation water quality. It is a measure of salinity with great impacts on crop productivity. Waters with high EC values reduce crop yields through reducing plant competition for water with the ions of soil solution. The greater the EC is, the less the water available for plants despite moist appearance of the soil [1].

Besides irrigation water quantity, irrigation timing and irrigation methods, irrigation water quality is also a significant parameter in modern irrigation systems [2]. When the sufficient quantity and quality irrigation water is not available, improper water resources are used in irrigations. Such waters then alleviate salinity problem. In a previous study, water samples were taken from 10 irrigation ponds in June, July, August and September to assess water quality of the ponds used for irrigation water supply in Hakkari province of Turkey. Samples were analyzed for EC, pH, anion and cations ( $Ca^{+2}$ ,  $Mg^{+2}$ ,  $K^+$ ,  $Na^+$ ,  $SO_4^{-2}$ ,  $NO_3^{-2}$ ,  $CO_3^{-2}$ ,  $HCO_3^-$  and Cl). Resultant values were used to calculate sodium adsorption ratio (SAR), residual sodium carbonate (RSC) and percent sodium (% Na) values. Research findings revealed that pH, EC, SAR, RSC and % Na values of irrigation ponds did not exceed threshold values, but  $Mg^{+2}$  and  $K^+$  values of the pond waters in Akçalı Village – Kanatlı locality and  $K^+$  values in Kırıkdağ Village - Şişer locality exceeded threshold values [2].

Total salt quantity of irrigation waters is expressed as electrical conductivity ( $EC \times 10^6$ ) in  $\mu$ mhos/cm ( $1000\mu$ mhos/cm=1mmhos/cm=1dS/m). Majority of waters used successfully in irrigated farming has a total salt concentration of less than 2250  $\mu$ mhos/cm. In terms only of total salt concentration, electrical conductivity of irrigation waters should be less than 750  $\mu$ mhos/cm. However, irrigation waters with electrical conductivity of between 750 - 2250  $\mu$ mhos/cm are largely used. Such waters may offer sufficient yield levels under proper drainage and operational conditions, but salinity problem may emerge if the sufficient leaching was not provided under improper drainage conditions [3].



Yeter and Yurtseven [4] investigated the effects of different quality irrigation waters on alfalfa plants and reported recessed growth, reduced yield and quality in alfalfa plant irrigated with saline waters. On the other hand, it was indicated that when sufficient leaching was provided and excess salt was removed from the rootzone, plant growth and development returned to normal levels. Researchers finally concluded that for high yield levels in alfalfa farming, irrigation water salinity should be less than  $1.5 \text{ dSm}^{-1}$ .

Salinity and alkalinity problems are largely encountered in irrigated farming lands of arid and semi-arid regions of the world. Low precipitation levels, poor-quality irrigation waters and high evaporation rates aggravate salinity and alkalinity problems of these regions. Such problems also destruct structural characteristics of the soils [5-6].

Gürcan [7] conducted a study to assess the water quality in irrigation district of Ankara Haymana Soğulca Village irrigation cooperative and classified irrigation water samples as  $C_3$  (highly saline) and indicated that these waters could not be used in fields with limited drainage facilities. It was also indicated that despite salinity problems in water resources of the irrigation district, salinity was not encountered in agricultural fields irrigated with these waters. However, it was recommended that closed or open drainage facilities should be constructed to prevent potential salinity problems in the future.

Topçu and Taş [8] conducted a study on Çanakkale Biga Plain and investigated electrical conductivity (EC), pH, potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), carbonate ( $\text{CO}_3$ ), bicarbonate ( $\text{HCO}_3$ ), chlorine (Cl), sulphate ( $\text{SO}_4$ ), nitrate ( $\text{NO}_3$ ) and boron (B) of water samples taken from 20 groundwater wells. Considering the classification system of Water Pollution Control Regulation (SKKY) and salinity parameters, 11 of 20 samples were classified as the second-class and the rest was classified as the first-class. Apart from nitrate pollution in groundwaters, a distinctive problem was not encountered in the research site overall.

Demir and Hepdeniz [9] investigated groundwater quality with the use of water samples taken from 21 groundwater wells in Isparta Plain and reported water quality class of some wells as  $C_3S_1$  (highly saline – slightly alkaline) and the rest as  $C_2S_1$  (moderately saline – slightly alkaline).

Dorak and Çelik [10] conducted a study to determine the effects of domestic and industrial wastewater effluents on Nilüfer Creek and took water samples from treated wastewater effluents of 5 treatment facilities and from the creeks to which treated wastewater effluents were discharged at 4 different sampling periods between August 2013 – May 2014. It was reported that water quality of Nilufer Creek and treatment facilities varied with the sampling periods, quality classes of water samples based on EC and SAR were identified as between  $C_2S_1$  -  $C_4S_4$  and discharged effluents negatively influenced especially pH, EC, ammonium, sulphate, boron and chlorine values of Nilfer Creek.

Akaroğlu and Seferoğlu [11] conducted a study in Sultanhisar town of Aydın province to assess irrigation water quality and reported that water quality classes varied between  $C_2S_1$  -  $C_3S_1$ , canal water quality influenced fruit quality and boron content of plants irrigated with these waters was greater than the boron content of control plants.

Aregahegn and Zerihun [12] took water samples from 17 sampling sites along the Awash River in four different sampling periods to assess the water quality of Awash River and tributaries. General water quality and suitability for irrigation were assessed with the use of several water quality parameters including pH, EC, SAR, RSC,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{++} + \text{Mg}^{++}$ ,  $\text{CO}_3^{2-}$ ,  $\text{HCO}_3^-$  and  $\text{Cl}^-$ . It was reported that entire quality parameters in Beseka Lake exceeded maximum allowable limits for irrigation, physicochemical characteristics of Awash River varied with the sampling sites and water quality parameters, pH and SAR values only of Beseka Lake and Meteka hot spring waters exceeded the allowable limits, Mojo, Wonji, Beseka, Melkasedi, Werer, Ambash, Meteka and Meteka hot spring waters had moderate-high salinity (EC) levels and very high RSC levels. It was recommended that industrial wastewater treatment facilities should be constructed to improve water quality of Awash River and tributaries.

## II. MATERIALS AND METHODS

Mezitli with wonderful natural beauties is located on the cost of Mediterranean Sea. Total surface area of the town is  $515.79 \text{ km}^2$  and average altitude is 3-5 m. About  $\frac{3}{4}$  of Mezitli soils are composed of mountains, plateaus and undulated topography.

The coastline between the mountain and the sea contracts toward the west. Taurus Mountains set a barrier in front of northerly winds and result in dominant Mediterranean climate in the region.

Mezitli is among the towns with the greatest sunshine durations. About 300 days of a year is shiny. Daily average sunshine duration is 7.4 hours and such duration is between 8 – 10 hours in summer months. Average relative humidity is 72% and monthly relative humidity is quite close to each other. Average monthly relative humidity varies between 65–75%. Annual average temperature is 18.4 °C. Average temperatures of summer months vary between 25 – 33 °C and average temperatures of winter months vary between 9–15 °C. Average sea water temperature is 20 °C. Sea water temperature reaches to 28 °C in summer months and such a temperature is maintained for a long time, thus prolong tourism season.

Annual average precipitation is 618.6 mm with the greatest precipitation in December and the least in August. There are no plains in the town and Gemrik, Garkın, Kalegediği, Gelin Kayası, Eyüp Kayası, Hazmur, Karagedik, Gıcık Kayası, Hürükızları Kepez, Manıt, Saladağ, Kuşkayası, Durnaz, Peynir, Koca Ellez Mountains are located on the north of the town. There are Kandak, Tece and Mezitli creeks in the town center.

Maquis, encountered at altitudes of 500 – 600 m, is the dominant ever-green typical plant cover of the Mediterranean region. Laurel, wild olive, carob, myrtle, rose laurel, banyan, blackberry and rosehip naturally grown in this zone. Forests start after maquis. Oak trees grow at altitudes of between 100 - 1000 m, Calabrian pine between 100 - 1200 m, black pine at 1500 m, cedar and juniper at 2000 m. Shrubs and pastures are encountered after 2500 m. Nomads (Yuruks) generally live on these high altitudes [13]. Location of the research site is presented in Figure 1.



**FIGURE 1: Location of the research site**

Water samples were taken from 20 sampling points of surface water resources used for irrigations in irrigated farming lands of Mezitli town in 4 sampling periods (July – October). Sample pH and EC readings were performed in each month. Water samples taken in August were also subjected to water-soluble cations (Ca, Mg, Na, K) and anions ( $\text{CO}_3$ ,  $\text{HCO}_3$ , Cl and  $\text{SO}_4$ ) and boron analyses. With the use of these analysis results, %Na, SAR and RSC values were calculated and irrigation water quality classes were determined.

### III. RESULTS AND DISCUSSIONS

Water samples were taken for 4 months throughout the irrigation season (July, August, September, October) from the water resources (groundwater wells) used in irrigation of agricultural fields.

The pH and EC values of irrigation water samples taken in July, September and October are provided in Table 1. Irrigation water pH values varied between 7,07 - 8,11 in July, between 7,14 - 8,15 in September and between 7,05 - 8,02 in October. Irrigation water EC values varied between 343 - 1045  $\mu\text{mhos/cm}$  in July, between 308-1103  $\mu\text{mhos/cm}$  in September and between 344-1056  $\mu\text{mhos/cm}$  in October.

In July, the greatest salinity values were observed in samples 16 and 17, EC values of samples 15 and 20 were greater than allowable limit value (750  $\mu\text{mhos/cm}$ ) and the rest was below the allowable limit (750  $\mu\text{mhos/cm}$ ), thus considered to be used in irrigation of agricultural fields without generating a salinity problem. In September, the greatest irrigation water salinity values were obtained from the samples 14, 15, 16 and 17, which were greater than the threshold salinity level (750  $\mu\text{mhos/cm}$ ) and the rest was below the threshold salinity level of 750  $\mu\text{mhos/cm}$ , which was considered to be used in irrigations. In October, the greatest salinity values were seen in the samples 14, 15, 16 and 17, which were greater than the threshold salinity level (750  $\mu\text{mhos/cm}$ ), but the rest was below the threshold value of 750  $\mu\text{mhos/cm}$ , which was considered to be used in irrigation of agricultural fields.

**TABLE 1**  
**EC AND pH VALUES OF IRRIGATION WATER SAMPLES TAKEN IN JULY, SEPTEMBER AND OCTOBER**

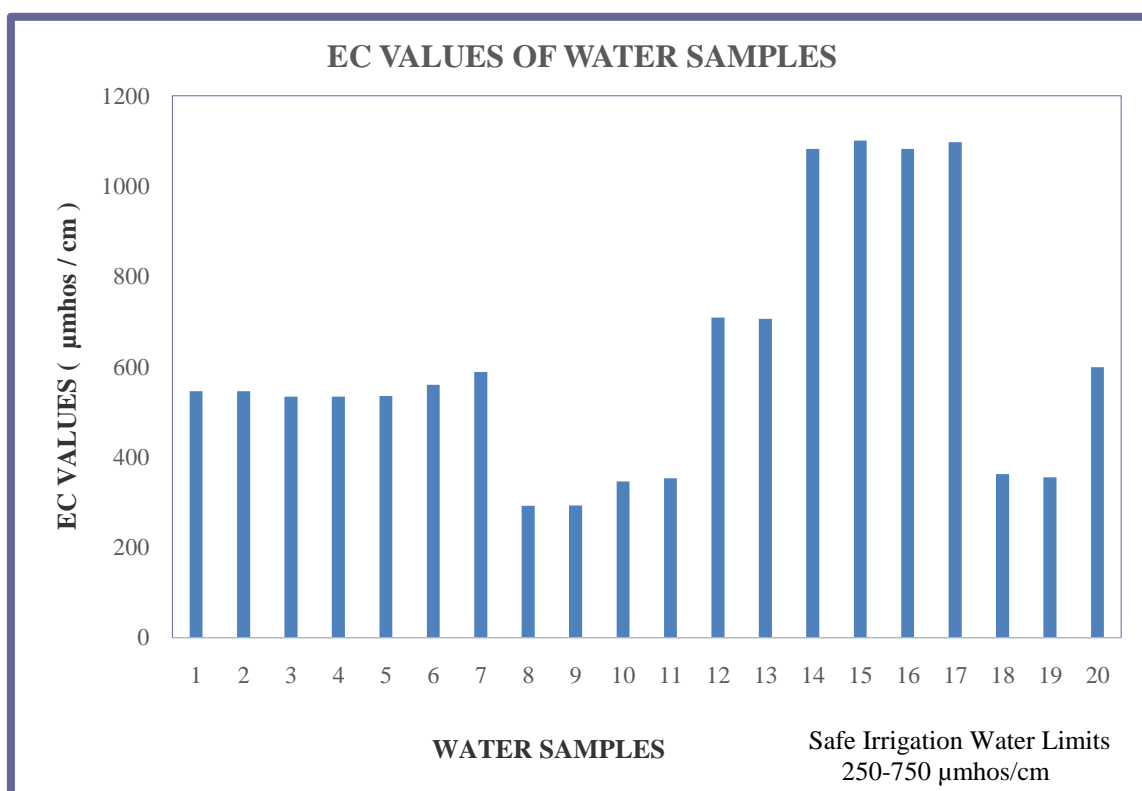
Sample No	July		September		October	
	pH	EC x 10 <sup>6</sup> $\mu\text{mhos/cm}$ 25 °C	pH	EC x 10 <sup>6</sup> $\mu\text{mhos/cm}$ 25 °C	pH	EC x 10 <sup>6</sup> $\mu\text{mhos/cm}$ 25 °C
1	8,11	549	7,93	551	7,86	548
2	7,72	553	7,88	547	7,98	556
3	7,60	452	8,11	525	8,02	498
4	7,65	435	8,15	516	8,01	502
5	7,40	452	8,08	519	7,88	503
6	7,09	585	7,20	554	7,15	576
7	7,08	582	7,23	570	7,05	565
8	7,60	351	7,82	311	7,88	346
9	7,56	351	7,90	308	7,75	344
10	7,30	343	7,44	353	7,50	349
11	7,31	349	7,50	351	7,66	359
12	7,90	715	7,48	724	7,60	710
13	7,80	717	7,39	730	7,48	720
14	7,11	724	7,15	1015	7,05	935
15	7,09	752	7,14	1040	7,11	1005
16	7,39	1045	7,56	1103	7,45	1018
17	7,31	1045	7,60	1094	7,55	1056
18	7,31	562	7,30	424	7,85	496
19	7,68	569	7,46	416	7,68	511
20	7,07	752	7,22	624	7,26	703

**TABLE 2**  
**CHEMICAL ANALYSIS RESULTS OF IRRIGATION WATER SAMPLES TAKEN IN AUGUST**

Samples No	pH	ECx10 <sup>6</sup> µmos/cm 25 °C	WATER SOLUBLE										RSC	SAR	%Na	Irrigation Water Class	Boron (mg/L)
			Cations (me/l)					Anions (me/l)									
			Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>+2</sup>	Mg <sup>+2</sup>	Total	CO <sub>3</sub> <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>-2</sup>	Total					
1	7,97	546	0,56	0,04	0,98	3,11	4,69	0,00	1,40	1,74	1,55	4,69	-	0,39	11,94	C <sub>2</sub> S <sub>1</sub>	<0,67
2	8,04	546	0,56	0,04	0,99	3,21	4,80	0,00	1,40	1,98	1,43	4,81	-	0,39	11,67	C <sub>2</sub> S <sub>1</sub>	<0,67
3	8,19	534	0,65	0,11	3,36	2,12	6,24	0,00	2,73	1,62	1,88	6,23	-	0,39	10,42	C <sub>2</sub> S <sub>1</sub>	<0,67
4	8,21	534	0,65	0,11	3,40	2,10	6,26	0,00	3,05	1,74	1,48	6,27	-	0,39	10,38	C <sub>2</sub> S <sub>1</sub>	<0,67
5	8,26	535	0,65	0,11	3,47	2,12	6,35	0,00	2,90	1,63	1,82	6,35	-	0,39	10,22	C <sub>2</sub> S <sub>1</sub>	<0,67
6	7,20	560	0,26	0,01	5,53	0,98	6,78	0,00	3,26	1,62	1,90	6,78	-	0,14	3,84	C <sub>2</sub> S <sub>1</sub>	<0,67
7	7,27	588	0,26	0,01	5,61	1,00	6,88	0,00	3,44	1,53	1,91	6,88	-	0,14	3,78	C <sub>2</sub> S <sub>1</sub>	<0,67
8	7,85	292	0,06	0,00	3,22	0,29	3,57	0,00	0,45	1,52	1,60	3,57	-	0,05	1,68	C <sub>2</sub> S <sub>1</sub>	<0,67
9	7,93	293	0,07	0,00	3,20	0,32	3,59	0,00	0,27	1,48	1,85	3,60	-	0,05	1,95	C <sub>2</sub> S <sub>1</sub>	<0,67
10	7,55	346	0,08	0,01	3,66	0,51	4,26	0,00	0,79	1,58	1,88	4,25	-	0,06	1,88	C <sub>2</sub> S <sub>1</sub>	<0,67
11	7,49	353	0,07	0,01	3,76	0,49	4,33	0,00	1,08	1,52	1,72	4,32	-	0,05	1,62	C <sub>2</sub> S <sub>1</sub>	<0,67
12	7,38	709	0,69	0,07	3,47	4,04	8,27	0,00	4,80	1,67	1,80	8,27	-	0,36	8,34	C <sub>2</sub> S <sub>1</sub>	<0,67
13	7,37	706	0,69	0,07	3,50	4,16	8,42	0,00	4,87	1,62	1,93	8,42	-	0,35	8,20	C <sub>2</sub> S <sub>1</sub>	<0,67
14	7,21	1082	1,64	0,05	5,24	5,43	12,36	0,00	8,80	1,84	1,73	12,37	-	0,71	13,27	C <sub>3</sub> S <sub>1</sub>	<0,67
15	7,16	1101	1,65	0,05	5,13	5,27	12,10	0,00	8,77	1,87	1,46	12,10	-	0,72	13,64	C <sub>3</sub> S <sub>1</sub>	<0,67
16	7,51	1082	5,12	0,08	1,96	4,12	11,28	0,00	7,80	1,81	1,67	11,28	1,72	2,94	45,39	C <sub>3</sub> S <sub>1</sub>	<0,67
17	7,55	1097	5,26	0,08	1,47	4,14	10,95	0,00	7,51	1,69	1,75	10,95	1,90	3,14	48,04	C <sub>3</sub> S <sub>1</sub>	<0,67
18	7,94	362	0,12	0,01	3,30	1,06	4,49	0,00	1,30	1,98	1,21	4,49	-	0,08	2,67	C <sub>2</sub> S <sub>1</sub>	<0,67
19	7,73	355	0,13	0,01	3,40	1,07	4,61	0,00	1,25	1,67	1,68	4,60	-	0,09	2,82	C <sub>2</sub> S <sub>1</sub>	<0,67
20	7,30	599	0,47	0,01	4,49	2,22	7,19	0,00	3,73	1,91	1,56	7,20	-	0,26	6,54	C <sub>2</sub> S <sub>1</sub>	<0,67

Chemical analysis results of irrigation water samples taken in August are provided in Table 2. Irrigation water pH values varied between 7,16 – 8,26 and EC values varied between 292 - 1101  $\mu\text{mhos/cm}$ . Boron concentration of all samples was below the threshold boron level of 0,67 ppm. In terms of water-soluble anions and cations, Ca was identified as the dominant cation and  $\text{HCO}_3^-$  as the dominant anion. Sodium adsorption ratios (SAR) of the samples varied between 0,05 – 3,14; % Na values varied between 1,62 - 48,04, residual sodium carbonate (RSC) values varied between 1,72 - 1,90 (in samples 16 and 17). According to US Salinity Lab Classification System, water samples taken in August were classified as  $\text{C}_2\text{S}_1$  and  $\text{C}_3\text{S}_1$ .

EC values of irrigation water samples taken in August are presented in Figure 2. The greatest salinity values were obtained from the samples 14, 15, 16 and 17, which were greater than the threshold salinity level (750  $\mu\text{mhos/cm}$ ) and the rest was below the threshold level of 750  $\mu\text{mhos/cm}$ , which was considered to be used in irrigations without a concern of salinity problem.



**FIGURE 2: EC values of irrigation water samples taken in August**

#### IV. CONCLUSION

Following conclusions were drawn from the present study conducted to assess irrigation water quality of water resources used in irrigation of agricultural fields of Mezitli town of Mersin province:

- Water samples with salinity levels of lower than the threshold salinity level (750  $\mu\text{mhos/cm}$ ) were classified as **moderately saline** ( $\text{C}_2$ ), thus they could be used in irrigations without posing a risk of salinity in surrounding fields. Rest of the samples with salinity values greater than the threshold salinity value of 750  $\mu\text{mhos/cm}$  was classified as **highly saline** ( $\text{C}_3$ ), thus salt-resistant plants should be selected or special measures should be taken for salinity control. EC values of present samples varied between 292 - 1101  $\mu\text{mhos/cm}$  and pH values varied between 7,05 - 8,26. Based on these values, irrigation water quality classes were identified as  $\text{C}_2\text{S}_1$  (moderately saline – low alkaline) and  $\text{C}_3\text{S}_1$  (highly saline – low alkaline).
- In terms of water-soluble anions and cations,  $\text{Ca}^{++}$  was identified as dominant cation and  $\text{HCO}_3^-$  as dominant anion. Sodium adsorption ratios (SAR) of the samples varied between 0,05 – 3,14; % Na values varied between 1,62 – 48,04 and boron concentrations of all samples was below the threshold boron level of 0,7 ppm.

## V. RECOMMENDATIONS

- a) Drainage facilities should be developed to prevent emergence of a salinity problem and periodical maintenance of available drainage facilities should be practiced.
- b) Soils should be enriched in organic matter and soil tillage methods should precisely be selected.
- c) Relevant measures should already be taken to prevent future salinity and alkalinity problems. Reclamation practices and leaching should be emphasized.
- d) To prevent yield losses, irrigation water should be applied through proper irrigation methods. Considering leaching practices, sprinkler irrigation should be selected in places with insufficient water resources and ponding irrigation (especially intermittent ponding) should be selected in places with sufficient water resources.
- e) Trainings on soil – plant – water relations and irrigation water quality should be provided by agricultural institutions and organizations to raise farmer’s awareness on these issues.

**Note: This paper was derived from the MSc Thesis of Onur AVCI.**

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# Barley Net Blotch Disease Management: A Review

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**Abstract**— Barley (*Hordeum vulgare* L.) is one of the ancient grain crops cultivated and used worldwide. In Ethiopia, barley is among important staple crops next to tef, maize, wheat and sorghum mainly grown on about 1 million ha of land with average yield of 2.1t ha. It is the predominant cereal in the high altitudes and it accounts nearly 25% of the total production in Africa.

The fungi *Pyrenophora teres* f. *teres* (Ptt) and *P. teres* f. *maculata* (Ptm) cause net form net blotch (NFNB) and spot form net blotch (SFNB) of barley, respectively. Net blotch is one of the most important barley diseases which reduce both quality and quantity of barley grain. Yield loss due to this disease reaches up to 100% in susceptible cultivars under severe epidemics. In Ethiopia, barley net blotch is among widely distributed and destructive diseases in cool highland areas and yield losses reaching about 67% have been recorded. Currently, the disease can be controlled using different approaches such as cultural, chemical and biological controls as well as using resistant cultivars of which development and deployment of resistant cultivars is the best management method. However, it is argued that using integrated disease management is one of the most important strategies that should be followed to reduce the effect of barley net blotch diseases. This review discusses recent information on economic importance, epidemiology, life cycle, host range, geographical distribution and disease management of barley net blotch disease. It also presents the barley net blotch disease management methods such as cultural, chemical, biological and use of host resistance methods. Under host resistance method, information on types of resistance, sources of resistance have been presented.

**Keywords**— Barley net blotch, Disease management, Methods, Cultural, Chemical, Host resistance.

## I. INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most important crops grown worldwide at an altitude ranging from 1400 to 4000 meters above sea level (Zemedu, 2002). At global level, barley ranks fourth among cereal crops in both yield and hectare coverage after wheat, rice and maize (Munck, 1981). In Ethiopia, barley grown from 1500 to 3500 meters above sea levels predominantly as food crops (Berhane, 1996) and ranks fifth after teff, maize, sorghum and wheat (Abdi, 2011). Barley is staple food for many people globally, especially for poor households, in addition to its uses in malting and as an animal feed (Newton et al., 2011). Barley grain is used for the preparation of different foodstuffs in Ethiopia, such as malt production, injera, porridge, roasted grains; and different local drinks and the straw and stem stub are good source of feed for animals and roof thatching, respectively, in Ethiopia (Fenta, 2018).

Barley productivity is low (1.97 t/ha) in Ethiopia as compared to world average of 3.1 t/ha and the reduction in productivity of the crop is mainly directed to multidimensional abiotic and biotic stresses (EIAR, 2019). In Ethiopia, net blotch is one of the most important barley diseases causing significant yield and quality loss (Yitbarek et al., 1996). Diseases such as scald, net blotch, spot blotch and rusts, can reduce yields by up to 67% (Chilot, 1998). Net blotch is one of the most widespread and a common foliar disease of barley, occurring in all barley-growing regions of the world (Weibull et al. 2003). This disease is of economic importance worldwide and can cause yield losses ranging from a trace to 100%, but typically cause losses from 10% to 40% (Mathre, 1997). The disease occurs in two forms, *Pyrenophora teres* f. sp. *teres* causes the net-form of net blotch (NTNB) and *P. teres* f. sp. *maculata* causes the spot-form of net blotch (STNB).

## II. ECONOMIC IMPORTANCE OF BARLEY NET BLOTCH DISEASE.

The ascomycete *P. teres* is the causal agent of net blotch on barley. During the last decades, *P. teres* has spread throughout the world and ravaged crops in many countries: Australia (McLean et al., 2010), Canada (Akhavan et al., 2016), Europe (Plessl et al., 2005), South Africa (Campbell et al., 1999), the United States (Lartey et al., 2012), Ethiopia etc.

The most serious effect of net blotch disease is a reduction in the quality of barley grain ((Jayasena et al., 2007). Net blotch reduces grain carbohydrate content thereby reducing brewing quality (Kamul and Naguib, 1957) and yield (Smedegaard-Petersen, 1974). For instance, in Australia, the economic losses are estimated to be \$ 117 × 106 per year (Murray and Brennan, 2010). In addition, yield losses might reach 40% in years with extensive rainfall in Germany (Plessl et al., 2005). Shipton (1966) reported a yield loss of 17% and a reduction in bushel weight, thousand-grain weight and grain size in net blotch-infected plots when compared to crops that were controlled by regular fungicide sprays. Jordan (1981) found that the greatest yield loss occurred when infection occurred at GS 30 (Zadoks *et al.*, 1974), before the end of tillering, causing a reduction in grain number, thousand-grain weight and an overall yield decrease of 19%. When infection occurred later (GS 45), yield was only reduced by 2.8%. These results agree with those of Rintelen (1969) who reported that with spring barley, yield losses of 20% resulted when plants were infected before tillering and losses of 10% resulted from when infection occurred between tillering and flowering. A reduction in leaf area, plant height and total weight result from seed infection (Wallwork *et al.*, 1995) and sowing infected seed enhances the losses associated with subsequent *P. teres* infections (Smedegaard-Petersen, 1974).

## III. EPIDEMIOLOGY OF BARLEY NET BLOTCH DISEASE.

*Pyrenophora teres* can persist as mycelia in seed, rendering it seed-borne, but it may also survive in crop debris between growing seasons (Ma et al. 2004; Steffenson 1997). Seed-borne inoculum serves to introduce net blotch to new fields, whereas conidia and ascospores formed in fields with a history of net blotch epidemics are considered to be the most important sources of primary inoculum (Steffenson 1997). Disease development in barley seedlings by seed-borne mycelium occurs best at temperatures of around 10-15 °C. Inoculum in crop debris generally survives as pseudothecia on the surface of infected barley stubble from one season to another. When conditions become favorable, cool and moist for a sustained period of time, ascospores are produced from the pseudothecia. As many as 400 ascospores may be produced per square centimeter of surface area of stubble (McLean et al. 2009). Infection of barley by ascospores requires free surface moisture or high (95-100%) relative humidity. Each ascospore will germinate to form an appressorium and an infection peg, which subsequently penetrates the epidermis of the host. Differences in growth patterns of *Ptt* and *Ptm* have been reported inside the host. *Ptm* is reported to grow biotrophically, forming a vesicle intracellularly in epidermal cells before switching to intercellular necrotrophic growth, whereas, *Ptt* does not have the initial biotrophic growth phase (Hysing and Wiik 2013; Lightfoot and Able 2010).

Ascospores are considered to be the primary inoculum driving net blotch epidemics, and they may be aerially or splash dispersed to initiate infection (Mathre, 1982). Conidia, which also produced on infected tissue in stubble, may also serve as primary inoculum (Mathre 1982), although they usually serve as a source of secondary inoculum when they are produced on mature and senescent leaves in the later part of the growing season (Jordan 1981). Sporulation occurs on conidiophores formed on the surface of the primary lesions when the relative humidity is near 100% (Mathre 1982). Conidial sporulation is diurnal, with light promoting sporulation. An eighteen hour light period is generally enough to stimulate spore production from the conidiophores at temperatures between 15 °C and 25 °C (Mathre 1982). Necrosis and chlorosis can occur within a distance of 10 cells from the hyphae, which results in the characteristic necrotic net-like pattern in *Ptt* infected plants. The symptoms are believed to be caused at least partly by necrotrophic effectors secreted by the pathogen that induce programmed cell death. Sarpeleh et al. (2007) hypothesize that proteinaceous metabolites are responsible for the necrotic symptoms, while low molecular weight compounds produce the chlorosis. Neupane et al. (2015) attributed the high variability in symptoms caused by different isolates on the same host or by the same isolate on different hosts to different necrotrophic effectors and their effect of different host genotypes. At the end of the growing season, the fungus colonizes the senescent tissues and forms the sexual stage, which overwinters and initiates infection in the following year (McLean et al. 2009; Liu et al. 2011).

## IV. LIFE CYCLE OF BARLEY NET BLOTCH PATHOGEN

The life cycles of *Pyrenophora teres* f. *teres* and *Pyrenophora teres* f. *maculata* are almost identical and involve both asexual and sexual stages. Both *Ptt* and *Ptm* are residual borne pathogens that can overwinter as pseudothecia (sexual fruiting bodies)



or conidia on plant stubbles (Mathre, 1997). It takes up to six months to develop a fertile pseudothecia under field condition when temperatures range between 10-15 °C (Shipton et al., 1973). However, it takes about two months under laboratory conditions to form pseudothecia. Following the growing season, pseudothecia actively release ascospores as far as 35 cm into the air, which act as a primary source of inoculum (Jordan, 1981). Alternatively, the mycelia and conidia that overwinter on plant stubbles or infected seed may also serve as a primary source of inoculum (Shipton et al., 1973). The ascospores germinate under 95-100% relative humidity, form appressoria, and produce penetration pegs that directly penetrate host epidermal cells to initiate intracellular growth and colonization (Hargreaves and Keon, 1983). After successfully infecting the host, *Pyrenophora teres* produces conidia throughout the growing season in multiple cycles (polycyclic), which serves as a source of secondary inoculum. Conidia are often disseminated via rain splash and wind to neighbouring plants or fields (Mathre, 1997). Towards the end of the growing season, either pseudothecia are developed on the plant stubble, or conidia and mycelia overwinter on stubble or infected kernels, which serves as a primary source of inoculum for the next growing season. However, only *Ptt* has been shown to transfer across generations via infected seed to the subsequent growing seasons (Mathre, 1997).

## V. HOST RANGE OF BARLEY NET BLOTCH PATHOGEN

Barley (*Hordeum vulgare* (L.)) and its wild progenitor (*Hordeum spontaneum* L.), are the primary hosts of *P. teres*, although the pathogen may also infect a few wild relatives of barley including (but not limited to): *Hordeum marinum* (Hudson), *Hordeum murinum* (L.), *Hordeum brachyantherum* (Nevskii), and *Hordeum distichon* (L.). *P. teres* can also attack other more distantly related species, including *Avena sativa* (L.) (oats), *Avena fatua* (L.) (common wild oat) and *Triticum aestivum* (L.) (bread wheat) (Liu et al.2011).

## VI. GEOGRAPHIC DISTRIBUTION OF BARLEY NET BLOTCH DISEASE

Steffenson (1997) reported that net blotch of barley occurred in most barley-growing regions of the world, but was most severe in temperate regions of high rainfall and humidity. According to Steffenson (1997) *P. teres* is present in countries of Europe, Asia, Africa, Americas and Oceania, as follows: **Asia:** Afghanistan, Armenia, China, India, Iran, Iraq, Israel, Japan, Republic of Korea, Kyrgyzstan, Myanmar, Nepal, Pakistan, Turkey, Turkmenistan, Uzbekistan. **Europe:** Austria, Baltic States, Bulgaria, Cyprus, Denmark, Faroe Islands, Finland, Former USSR, Former Yugoslavia, France, Germany, Greece, Ireland, Italy, Malta, Moldova, Netherlands, Norway, Poland, Romania, Russia, Spain, Sweden and United Kingdom. **Africa:** Egypt, Ethiopia, Kenya, Libya, Morocco, Saint Helena, South Africa, Tanzania, Tunisia, Zambia. **North America:** Canada, Mexico, USA. **South America:** Argentina, Brazil, Colombia, Peru, Uruguay. **Oceania:** Australia, New Zealand.

## VII. BARLEY NET BLOTCH DISEASE MANAGEMENT

There are several methods to reduce yield losses due to foliar diseases such as barley net blotch disease. For example, crop rotation, fungicide application, and the deployment of resistant cultivars can be used to manage net blotch of barley (Turkington *et al.*, 2015). However, integrated pest management is one of the most important strategies that should be followed to reduce the effect of plant diseases in crops. A promising approach to achieve this aim while minimising use of pesticides is to apply and combine different agriculture practices that contribute to increasing crop yield by decreasing plant diseases directly or indirectly. For instance, combining good crop hygiene practices, the use of resistant cultivars and chemical control (both as seed dressing and foliar applications), is currently the most effective net blotch disease management strategy.

### 7.1 Cultural Control

There are three sources of primary inoculum for barley net blotch disease; infected seeds, crop debris, and straw residue. Therefore, the first step to control net blotch is the deletion of the primary inoculum of *P. teres* by sowing healthy seeds (Jalli, 2011). Use of certified clean seed is vital as seed-borne inoculum has the ability to contaminate straw on which it will produce abundant inoculum in the following year (Piening, 1968). It is well-established that seed-borne inoculum is a source of primary inoculum for both forms of the pathogen (i.e., NFNB & SFNB); conidia, perithecia and sclerotia of *P. teres* can be found on the surface of the seeds (Jordan, 1981). Although barley straw is a major source of inoculum, the seeds contribute to the introduction of the pathogen into plots which were previously free from disease (Youcef-Benkada *et al.*, 1994), highlighting the importance of using clean certified seed.

Cropping practices such as sowing date, application of nitrogen, the rogueing of diseased plants and the use of conventional tillage all affect net blotch disease development. Early sowing means a longer growing season with increased exposure to

carry-over inoculum. Late sowing results in an increase in yield, thousand grain weight and seed number per head when compared with early sowing (Delserone and Cole, 1987). Application of nitrogen fertilizer ensures high yield and quality; however it may favour disease development (Locke *et al.* 1981). Nitrogen application results in increased relative humidity within the crop canopy which favours the development of disease (Jordan and Hutcheon, 1999).

After harvesting barley kernels, debris and straw residues are other sources of primary inoculum. A study has demonstrated that amounts of residue infested can increase disease intensity and thus reduce the yield (Adee, 1989). Destruction of infested barley residues is often suggested as a means to eliminate potential sources of primary inoculum that initiate net blotch epidemics. In contrast, incidence of net blotch can increase due to retention of stubble (Jordan and Allen, 1984). This leads to a build-up of inoculum that is present on straw debris from previous crops (Jordan, 1981; Jordan and Allen, 1984). Removal of inoculum may be earned out in a number of ways; deep ploughing, shallow cultivations, burning, etc. Piening (1968) found that 42% net blotch infection resulted from a plot where the straw and stubble were lightly diced, compared to only 8% infection where the stubble had been ploughed under. Ascospores of the pathogen were responsible for half the net blotch lesions produced on volunteer barley plants (Piening, 1968). In the past, burning was an important and effective method for the eradication of inoculum sources; however it is not environmentally acceptable as it may be detrimental to nesting birds, cause smoke pollution and removes organic matter from the soil.

In addition to these measures to limit the sources of inoculum, preventive agronomic measures play an essential role in the management of net blotch. Crop rotation is beneficial to reduce the severity of the pathogen. Crop rotation can help minimize plant disease potential by reducing populations of disease organisms surviving on crop residues. Although crop rotation reduces the risk of many cereal diseases, it does not eliminate them. Crop rotation is essential for the control of this disease because mono-cropping encourages build-up of pathogen populations (Pusey, 1996). Crop rotation also benefits the soil by maintaining a balance of nutrients and improving soil structure (Pusey, 1996). A minimum of 2 years between barley crops is required to prevent net blotch (Duczek *et al.*, 1999).

## 7.2 Chemical Control

Fungicides are one of the most common and widely known methods used to minimise the effect of net blotch. However, fungicides can have adverse effects on the environment. In addition, the continual use of chemicals can lead to increases in resistant strains of pathogens (Vinale *et al.*, 2008a). The aim of fungicide application is to maximise the green leaf area of the top three leaves during grain filling; in barley it is the 2<sup>nd</sup> and 3<sup>rd</sup> leaves and flag 2) that are most important (Weppler and Hollaway, 2004).

Chemical control to manage net blotch has generally focused both on the application of foliar fungicides, and seed treatments (Hysing and Wiik 2013). Foliar application of fungicides to the upper leaves during grain filling provides effective chemical control. Whilst applications at early growth stages are generally not economically justifiable (Liu *et al.* 2011). Seed treatments have been found effective in reducing net blotch incidence (Hampton, 1980). However, seed treatments alone are not considered reliable for the management of net blotch (Hysing and Wiik 2013). The effectiveness of control using foliar fungicides varies depending on factors such as degree of disease pressure, mode of action of the active ingredient, application rate, timing and number of applications and the presence of reduced sensitivity or resistance in the pathogen population (Van den Berg and Rossnagel 1990).

One widely adopted strategy to manage barley net blotch disease is to apply foliar fungicide to the infected crop at predetermined plant growth stages to protect the flag leaf and the emerging ear from infection. This strategy aims to protect the photosynthetic potential of the top four leaves which can contribute 72% of the total yield (Paveley *et al.* 2000).

Fungicides of the quinone outside inhibitors (QoI), the succinate dehydrogenase inhibitor (SDHI), and azole or demethylase inhibitor (DMI) classes are used as site-specific systemic fungicides (Mair *et al.*, 2016). The foliar fungicide application effectiveness to control net blotch has been largely carried out (Sutton and Steele, 1983; Mclean *et al.*, 2009). First studies have shown that triazole-based fungicides by pulverization allowed to control net blotch (Sutton and Steele, 1983; Van Den Berg and Rossnagel, 1990). Triazoles, known as DMI (propiconazole and prothioconazole), inhibit dimethylation between substrates that are necessary for the biosynthesis of ergosterol in fungi. In addition, SDHIs are also used to reduce the disease severity.

The strobilurins, a new class of broad-spectrum fungicides, have been adopted recently for net blotch control (Bartlett *et al.*, 2002). Strobilurin fungicides were inspired by natural fungicidal derivatives of  $\beta$ -methoxyacrylic acid (Bartlett *et al.*, 2002). Belonging to QoI (pyraclostrobin and picoxystrobin), strobilurins are natural substances isolated mainly from fungi and more

specifically, Basidiomycetes. The strobilurin name is derived from the fungi genera *Strobilurus* (Balba, 2007). First introduced to the market in 1996, strobilurins inhibit mitochondrial respiration by blocking electron transfer at the level of cytochromes b and c (Gisi et al., 2002; Balba, 2007).

The antifungal efficacies depend also on the period of their application and how they are applied, as well as on the plant growth stage (Van Den Berg and Rossnagel, 1990). Seed treatments were successful if applied early in the season corresponding at Zadoks growth stage 23–24, but less at later growth stages (Martin, 1985). Barley seeds are considered as a source of inoculum for the *ascomycete* *P. teres*. The severity of the barley net blotch is reduced when a fungicide seed treatment is applied (Martin, 1985). Seed treatment effectiveness depends on fungal sensitivity, chemical fungitoxicity, and seed coverage quality. Iprodione is the fungicide providing the best control of dematiaceous fungi (*Bipolaris* and *Drechslera*) on seeds (Reis et al., 2012). Another study demonstrates the efficiency of one application of propiconazole at spike emergence for the management of net blotch (Sutton and Steele, 1983). Seed infected with *P. teres* f. *teres* can be treated with thiram to reduce carry over by inhibiting spore and mycelia growth. Unfortunately, no seed fungicide is currently available for management of SFNB (Wallwork 2011).

A correct application of fungicides before the emergence of the flag leaf and the ear aims to protect the photosynthetic potential of the top four leaves, which contribute to 72% of the total yield (McClean et al., 2009). A single application of propiconazole is not enough when the pathogen progresses quickly. A recent study demonstrates that two applications with the combination of pyraclostrobin and epoxiconazole improved net blotch control and increased the yield in two experimental years (Stepanovic et al., 2016). Belonging to QoI, metyltetraprole is a new fungicide, which is effective against important cereal diseases, including net blotch (Suemoto et al., 2019). Further, the metyltetraprole suppresses succinate-cytochrome c reductase activity in QoI susceptible *P. teres*. With time, resistant strains to these products have emerged (Jørgensen and Olsen, 2007). In Europe and in Australia, *P. teres* developed a resistance to DMI fungicides (Peever and Milgroom, 1993; Rehfus et al., 2016). Shortly after the first QoIs uses, resistant isolates to these antifungal products were detected in field populations (Gisi et al., 2002).

More specifically, in 2003, resistance to QoI fungicides in *P. teres* was detected in France, Sweden, and Denmark. The resistance mechanism to QoIs has been identified as mutations in the mitochondrial target gene, cytochromes b (Sierotzki et al., 2007). In *P. teres*, this mutation has been described as a substitution of phenylalanine to leucine at amino acid position 129 (Sierotzki et al., 2007). To conclude, the fungicide exerts a selection pressure, which leads to the selection of isolates, which have a mutation providing fungicide resistance, while susceptible isolates will be eliminated. There is a subsequent increase in the number of resistant individuals in the population. Successive rounds of fungicide use repeat the selection of resistant isolates, which leads to the increase of the resistance mutation in the population each time the fungicide is used. Eventually, the resistant isolates will dominate the population and the effectiveness of the fungicide will be reduced (Gisi et al., 2000). To reduce the risk of fungicide resistance development, the use of recommended fungicide rates is important as the application of low rate doses can increase the likelihood of fungicide resistance emerging in *P. teres* population. Also, the use of a mixture of fungicides (of different modes of action) to control net blotch disease is desirable, as it reduces the risk of fungicide resistance emerging in *P.teres* populations and the usage of fungicide mixtures can reduce the number of fungicide applications required throughout the growing season (Whitehead, 2004).

In addition to the resistance among several fungal species, azoles use has also been affected by a restriction with a wide range of significant toxicities, including hallucinations, hepatotoxicity, and QTc prolongation (Gintjee et al., 2020). Faced with these problems, varietal selection, preventive agronomic measures, and biocontrol agents might be considered as alternative solutions to fungicides products.

Generally, Present-day control of net blotch disease of barley relies on the use of seed dressing and foliar formulations of fungicides, with current chemical groupings available for control of net blotch including strobilurins, triazoles, benzimidazole, chlorothalonil, morpholine, chlorophenyl, anilinopyrimidine, guanidine, carboxamide and dithiocarbamates (Whitehead, 2004).

### 7.3 Biological Control

Biological control or 'biocontrol' is defined as a strategy for reducing disease incidence or severity by direct or indirect manipulation of microorganisms (Maloy, 1993). Use of non-pathogenic fungi and bacteria as biological control agents for management of pathogenic fungi are increasingly being investigated as alternatives to chemical foliar and seed fungicides (Whipps and McQuilken 2009). Although biocontrol offers a positive alternative to chemical pesticides, the overall

contribution of biocontrol represents about 1% of agricultural chemical sales (Lidert, 2001). There are currently 13 bacteria and 12 fungi registered with the US Environmental Protection Agency which can be sold as biocontrol agents against plant disease (Fravel, 2005).

While several researchers have investigated the potential of bacteria and fungi to control cereal diseases, presently, *Pseudomonas chlororaphis* strain MA 342 marketed as Cedomon is the only biocontrol agent commercially available for the control of net blotch disease of barley (Copping, 2004). This bacterium is formulated as a seed treatment containing  $1 \times 10^6$  colony forming units (cfu) per gram. *Pseudomonas chlororaphis* MA 342 competes with pathogens for nutrients and space, encourages the plant's natural defence system, promotes the development of roots and shoots (Copping, 2004) and suppresses fungal growth by producing the antifungal compound 2,3-deepoxy-2,3-didehydrorhizoxin (Hokeberg, 1998). This product is commercially available in the United States, Sweden, Norway, Finland and Austria. It is anticipated that more biological agents will be registered for the control of net blotch disease, especially for use in the growing organic cereal production tillage sector. An important criterion for the selection of such agents will be to ensure that they are adapted to the climate and soil in which they are to be used (Leyns *et al.*, 1990; de Bruyne *et al.*, 1991; Hokeberg *et al.*, 1997). The mechanisms of pathogen suppression by bacteria include the production of antimicrobial substances, induced resistance, competition between the biocontrol microorganism for nutrients and plant surface area (Weller, 1988; Pedersen *et al.*, 1999) and competition for iron through the production of siderophores (Whipps, 2001; Baaker *et al.*, 1993). These mechanisms need not be exclusive, and it is desirable that any new biocontrol agents developed for the control of net blotch disease possess as many of these attributes as possible. Also any biological control agent should be formulated so that it has a long and stable shelf life, is easy to apply and is active once applied and multiplies on the plant surface.

Ali-Haimoud *et al.* (1993) observed that mycelial suspensions and culture filtrates of several fungal isolates (strains of *Trichoderma koningii*, *T. viride*, *Ipseudokoningii* and of two unidentified fungi) and of the actinomycete *Micromonospora* spp. significantly inhibited the *in vitro* formation of sclerotoid organs on *P. teres* var. *teres*- and var. *mocw/ato*-inoculated barley straw. Sclerotoid organs are important survival and reproductive structures (Ali-Haimoud *et al.*, 1993). Both the mycelium and culture filtrate of *T. viride* and *T. pseudokoningii* also significantly inhibited sclerotoid organ germination on barley straw, whether applied pre- or post- *P. teres* inoculation. The spot form of the pathogen was generally more sensitive to the culture filtrates than was the net form. However, Amundsson and Hokeberg (1984) found that several known antagonists, including *Trichoderma* spp., *Serratia* spp. and various strains of *Pseudomonas fluorescens*, were not effective against *P. teres*. More recently, Hokeberg *et al.* (1997) found that *Pseudomonas chlororaphis* strain MA 342 seed treatment resulted in a > 98 % reduction in the incidence of *P. teres*-infected plants derived from pathogen-inoculated seed grown under field conditions. The disease control and yield increases resulting from this bacterial seed treatment were similar to those achieved by treating seed with the fungicide Panocrine Plus 400 (guazatine and imazalil). This bacterium also suppressed common bunt of wheat under field conditions, had a shelf life of up to six weeks and freezing did not influence its biocontrol efficacy.

#### 7.4 Host Plant Resistance

Disease resistance is an important agronomical trait in all crop plants and the use of resistant cultivars is often the most economically and environmentally friendly means to control a disease. The same holds true for barley net blotch disease control (Shipton *et al.*, 1973).

Research on net blotch resistance dates back to the 1920s when Geschele (1928) discovered that it followed Mendelian inheritance. By the end of the 1950, the presence of at least three genes conferring incomplete dominant resistance was known (Mode and Schaller 1958). The first resistance loci that could be localized in the genome were found by Bockelman *et al.* (1977) on chromosomes 1H, 2H and 3H in the cultivars Tifang, CI7584 and CI9819. Based on these early studies, net blotch resistance was mainly understood as a gene-for-gene relationship involving major-effect genes. In the late 1980s and early 1990s, a number of studies were conducted on adult plants, which found that resistance was quantitatively inherited under field conditions (Steffenson *et al.* 1996). With recent advances in molecular marker techniques, the location of resistance loci can be determined in a much more exact way, and we have learned that the mechanisms underlying this pathosystem are much more complex than initially thought. Today, resistance genes/QTL is known on all seven chromosomes, and many of them are specific to either *Ptt* or *Ptm* (Liu *et al.*, 2011 and McLean *et al.*, 2009). Many of these QTL have been projected onto consensus maps, which facilitate the comparison of loci across different studies and populations (Richards *et al.* 2017). The majority of the resistance QTL found in these mapping studies confer dominant resistance, but a number of recessive resistance genes have also been identified. Ho *et al.* (1996) showed that resistance to

two *Ptt* isolates in the Leger x CI9831 mapping population is conferred by one and three recessive resistance genes, respectively. Abu Qamar et al. (2008) detected two dominant susceptibility loci on chromosome 6H in the Rika x Kombar mapping population that are linked in repulsion and confer susceptibility to the *Ptt* isolates 15A and 6A, respectively. In a mapping population of the parental isolates 6A and 15A, Shjerve et al. (2014) identified four putative virulence genes, two of which confer virulence on Rika and two on Kombar, and hypothesized that the previously identified 6H region contains four closely linked susceptibility genes. The locus was subsequently fine-mapped to a 0.24 cM interval in the centromeric region of 6H (Richards et al. 2016).

Chromosome 6H is considered a hotspot for both major resistance genes and small-effect QTL, although the exact number of loci still remains to be determined (Abu Qamar et al. 2008; Friesen et al. 2006a; Steffenson et al. 1996). Some of the genes found on 6H are pathotype-specific (Abu Qamar et al. 2008; Friesen et al. 2006b). Chromosome 6H also harbors the first putative susceptibility gene to a *Ptt* NE (Liu et al. 2015). This QTL named SPN1, which was identified in the Hector x NDB112 mapping population after inoculation with the *Ptt* isolate 0-1, explained 31% of the phenotypic variation. The same QTL was also found after infection with five other globally collected *Ptt* isolates, indicating that isolates producing the corresponding NE may be found around the world. It remains to be elucidated whether other known dominant susceptibility genes also encode susceptibility to NEs. No NEs have been identified in *Ptm* yet, but it seems likely that this form also secretes them, most likely during later stages of infection. Both chromosomes 3H (Liu et al. 2015), and 7H are also considered hotspots for large-effect resistance QTL (König et al. 2014).

In the last years, it has become feasible to genotype large populations with thousands of SNP markers and GWAS has gained popularity in plant pathology. Currently, there are three GWA studies on *Ptm* resistance and one on *Ptt* resistance, reflecting the increasing importance of *Ptm* in many regions worldwide. The continuous distribution of disease severity in populations and the presence of between eight and 29 QTL per population underline the quantitative nature of resistance mechanisms in the patho-system (Burlakoti et al. 2016).

Most of these studies are performed on seedlings under controlled growth conditions, and more knowledge is required about how the resistance found in these studies holds up under field conditions (Williams et al. 2003), where genotype x environment effects may play a major role. Many studies found QTL that confer resistance consistently in both seedling and adult plants under field conditions (Cakir et al. 2003), but some of the resistance was specific to a developmental stage. In a GWA study on four Australian breeding populations, 75% of the QTL conferred resistance both in seedlings and adult plants, while 17% were only effective in adult plants and 7% in seedlings only.

Sato and Takeda (1997) identified *P. teres* resistance in many *Hordeum* species, especially in *H. spontaneum*, which thus constitutes an interesting source for improved resistance, provided that closely linked markers are available. Progress is currently made in characterizing the genomes of wild relatives of barley (Wendler et al. 2014), and a NAM population generated from a cross between *H. spontaneum* and *H. agriocrithon* and the cultivar Barke is currently being used in a GWA study to map resistance to *P. teres* (Vatter et al. 2016).

Apart from the aforementioned putative effect or Ptt NE1 and the putative virulence genes in the *Ptt* isolate 6A and 15A, little is known about genes conferring virulence or avirulence in the pathogen. Lai et al. (2007) identified the locus AvrHar conferring avirulence to the cultivars Tifang and Canadian Lake Shore in the isolate 15A and the loci AvrPra1 and AvrPra2 conferring virulence to the cultivar Prato in the isolate 0-1. AvrHar and AvrPra2 co-segregate, but it is currently not known if these loci are alleles of the same gene or two different genes.

#### **7.4.1 Types of host plant resistance**

The use of resistant cultivars is a very important means to control fungal pathogens and can have a direct impact on yield (Turkington et al. 2006; Østergård et al. 2008). Plant resistance is usually divided into two different forms.

##### **7.4.1.1 Race-specific resistance**

Race-specific resistance also termed monogenic, qualitative or vertical resistance, is effective against one or a few races of the same pathogen species (Van der Plank 1968). Our classical understanding of disease resistance follows the gene-for-gene model, according to which pathogens produce virulence gene products that interact with corresponding receptors in the plant (Flor 1956; Flor 1971). If the receptor is able to recognize the pathogen molecule, a defense response often involving a hypersensitive reaction will be elicited to ward off the pathogen (incompatible reaction). If no recognition occurs because one of the gene products is missing, the pathogen will be able to evade recognition by the immune system and infect the plant

(compatible reaction) (Jones and Dangl 2006). Whereas this type of defense is largely effective against biotrophic pathogens, some necrotrophic pathogens have evolved NEs to deliberately induce a hypersensitive response, so that the pathogen can thrive on the dead plant tissue (Tan et al. 2010). NEs have been extensively studied in pathogens related to *P. teres* such as *Parastagonospora nodorum* and *Pyrenophora tritici-repentis*, the causal agents of *Septoria nodorum* blotch and tan spot in wheat, respectively (McDonald et al. 2013)

#### 7.4.1.2 Race non-specific resistance

Race non-specific resistance also termed polygenic, quantitative or horizontal resistance, is usually effective against all races of a pathogen species and is usually governed by several genes, most of them with small effects (Clair 2010). These genes often encode pathogenesis-related (PR) proteins, phytoalexins, etc. (Golshani et al. 2015) or developmental and morphological features (Melotto et al. 2006). Genomic regions harboring loci that affect quantitative traits are termed quantitative trait loci (QTL).

Since quantitative resistance is conferred by a number of genes, it is usually more stable since many mutations in the pathogen population are required to overcome this resistance (McDonald and Linde 2002). Quantitative resistance is often dependent on environmental factors (genotype x environment effects), and often only effective in certain growth stages or plant tissues (Steffenson et al. 1996).

#### 7.4.2 Sources of host plant resistance

The use of genetic resistance is the most cost effective and environmentally desirable method of controlling yield and quality losses caused by barley net blotch disease; however, new and relevant sources of resistance are essential to make this strategy feasible. New sources of disease resistance are needed in developing barley (*Hordeum vulgare* L.) cultivars that are resistant to current pathotypes of the barley net blotch disease. The introduction of new sources of resistance will also increase genetic diversity and enhance the durability of resistance.

Studies of landraces for resistance to net blotch have been carried out by many scientists (Schaller and Wiebe, 1952; Buchannon and McDonald, 1965; Gaike, 1970; Smirnova and Trofimovskaya, 1985; Lukyanova, 1990; Faiad et al., 1996). Sato and Takeda (1994) studied the variation of host resistance of 2233 accessions of the barley world collection and found sources of resistance in accessions from Ethiopia, North Africa and Korea. New sources with resistance to up to eight races of *P. teres* were found among Peruvian landrace accessions (Afanassenko et al., 2000).

Thirteen sources of host resistance to *P. teres* f. *maculata* have been investigated in worldwide barley germplasm. Several feed barley varieties are resistant to SFNB because they have the *Ha4* allele for cereal cyst nematode resistance (Vanstone et al. 2008), which is associated with SFNB resistance (Arabi et al. 1992).

Breeding for resistance to SFNB will be challenging as resistance sources can have either major or minor effect and are usually conferred by multiple genes found on different chromosomes. Minor or 'partial' resistances are typically only effective for part of the crop's developmental stages and provide a moderate resistance, while major or 'complete' resistances are effective throughout the crop's life. Liu et al. (2010) have recently summarised the resistances that have been characterised for *P. teres* f. *maculata* and *P. teres* f. *teres*, noting that they are spread across several chromosomes.

In some cases resistance to both forms of *P. teres* were identified in the same regions of the barley genome (Manninen et al. 2006). However, the majority of resistances appear to be independent and appear in different barley lines which will mean considerable effort will be required to combine resistance to multiple pathogens in a single parental breeding line.

Several genes that confer partial resistance and are effective at seedling stages have been mapped in Australian barley germplasm. The first to be mapped was designated *Rpt4*, which is on the long arm of chromosome 7H in the variety Galleon (Williams et al. 1999). Since then, *Rpt4* has also been identified in breeding lines and varieties such as CI9214, Keel and Tilga (Williams et al. 1999; Williams et al. 2003). Breeding programs in Australia initially used *Rpt4* as a source of resistance to SFNB, however, this resistance has since been utilised less due to a lack of expression at adult stages of plant development (Williams et al. 1999). Other seedling resistance genes were tentatively identified in breeding lines by Williams et al. (2003) at various locations on chromosomes 7H, 4H and 2H. A resistance gene in variety Chebec was located at or near *Rpt4*. Weak association of seedling resistance was observed on chromosomes 1H and 3H in the breeding line VB9104 (Williams et al. 2003). Effective seedling resistance has been mapped to chromosomes 7H, 6H, 4H and 3H in two Canadian-derived barley varieties, TR250 and TR251 (Gupta et al. 2006). Friesen et al. (2006) also identified a major resistance gene on chromosome 4H in barley variety Q21861, which confers moderate resistance.

Genes that confer complete or adult resistance to SFNB have more recently been targeted by barley breeders and researchers in favour of seedling resistance (Williams 2003). One particularly useful complete resistance locus has been identified in association with the *Ha4* allele, originally derived from the Egyptian land race CI3576 (Arabi *et al.* 1992). This resistance locus is associated with resistance to cereal cyst nematode (*Heterodera avenae*) and has been mapped to the 5H chromosome in a Galleon/Haruna Nijo cross (Williams *et al.* 2003).

Sources of adult resistance have been identified on chromosome 7H in the breeding line VB9104 (Williams *et al.* 2003), while a Galleon/Haruna Nijo cross revealed interactions on chromosomes 7H, 5H and to a minor extent 4H (Williams *et al.* 2003). The variety Keel appears to possess alleles associated with adult plant resistance on chromosomes 5H, 7H, 1H, 2H and 4H (Arabi *et al.* 1992). Good levels of resistance have been identified in three Canadian six-rowed barley varieties, Leduc, Argyle and Bedford; this resistance is highly expressed at the seedling stage and at intermediate levels at adult stages (Tekauz 1990). These loci are not mapped and may correspond to those in other varieties. Multiple gene loci contribute to a seedling resistance and moderate level of adult resistance in the Canadian breeding lines TR250 and TR251 and the genes have been mapped to chromosomes 7H, 6H and 4H (Gupta *et al.* 2006). The resistance gene designated as *Rpt6* in the barley breeding line CI9819 on chromosome 5H was found to only be effective against limited pathotypes of *P. teres f. maculata* (Manninen *et al.* 2006). Ho *et al.* (1996) identified a potential dominant gene resistance in a doubled haploid population of crosses between varieties Leger and CI9831. However, further analysis showed that the resistance was actually due to two genes (Molnar *et al.* 2000). Other potential sources of resistance have been identified in European spring barley varieties Agneta, Clermont, Nordel, Arve, Tellus, Pamina, Albert and Birka (Jorgensen *et al.* 2000). However, the genes involved have not been identified.

Wild relatives can provide novel sources of resistance that are not present in adapted germplasm and may provide good resistance to multiple pathogens from a single line. Effective resistance toward the anamorph stage, *Dreschlera teres*, has been reported in *Hordeum vulgare* subsp. *spontaneum* accessions Gay and Leon 2004). For many of the wild relatives of barley known to possess resistance to *P. teres f. maculata*, the chromosomal locations of resistance loci are yet to be mapped and their responses to the worldwide population of *P. teres f. maculata* are yet to be investigated. These sources may yield some effective resistances, but efforts will need to be made to incorporate them into breeding lines with agronomically adapted backgrounds.

Using different molecular techniques, several studies have identified net blotch resistance genes or quantitative trait loci (QTL) on all seven barley chromosomes (Mode and Schaller, 1958; Steffenson *et al.*, 1996; Clare *et al.*, 2020). Major QTL have been identified on barley chromosomes 1H (Manninen *et al.*, 2006), 2H (Tamang *et al.*, 2019), 3H (Graner *et al.*, 1996), 4H (Islamovic *et al.*, 2017), 5H (Manninen *et al.*, 2006) 6H (Adawy *et al.*, 2013), and 7H (McClean *et al.*, 2009; Tamang *et al.*, 2019). Localized on chromosome 6H, the *Rpt5* locus has been reported by several studies and is considered to be essential in the *P. teres f. teres* – barley interaction (Clare *et al.*, 2020). According to several studies, the majority of the markers significantly associated with NFNB resistance localize to the centromeric region of chromosome 6H (Richards *et al.*, 2016). In the same way, the high-resolution mapping of a dominant susceptibility locus located in the centromeric region of barley chromosome 6H has been described using markers (Richards *et al.*, 2016). In addition, the *Rpt7* locus confers resistance to *P. teres f. teres* in barley on the chromosome 4H. Recently, 449 barley accessions were phenotyped for *P. teres f. teres* resistance in greenhouse trials. Using genome-wide association, the results identified 254 marker-trait associations corresponding to 15 QTLs. Four of these regions were new QTL not described in previous studies and are located on chromosome 3H at 233–350 Mpb, 5H at 579 Mbp, 6H at 406–410 Mpb and 7H at 5 Mbp, respectively (Novakazi *et al.*, 2019).

Initially, the genetics conferring resistance to *P. teres f. maculata* contained three major designated loci and therefore has been considered less complex to compare the *P. teres f. teres* – barley interaction (Clare *et al.*, 2020). Designated as *Rpt4*, *Rpt6*, and *Rpt8*, these three major loci confer in barley a resistance to *P. teres f. maculata*. The *Rpt4*, *Rpt6*, and *Rpt8* loci are localized on chromosome 7H, 5H, and 4H, respectively. Burlakoti *et al.* (2017) revealed the effect of two- and six-row barley, and concluded that the two-row barley (13%) resistant to *P. teres f. maculata* was less than the six-row barley (43%) tested.

## VIII. CONCLUSION

Net blotch of barley caused by the fungal pathogen *Pyrenophora teres* is a major foliar disease in major barley-growing regions throughout the world. It causes significant grain yield loss and reduces grain quality. Net blotch develops quickly when the environmental conditions are optimal including long periods of wet and cultural practice used.. It is the most

important constraint that limits productivity of barley and results in constantly low yield of barley in Ethiopia. The net blotch control provides a significant challenge now and in the future.

There are several methods to reduce yield losses due to barley net blotch disease. For example, crop rotation, fungicide application, and the deployment of resistant cultivars can be used to manage net blotch of barley. However, integrated disease management is one of the most important strategies that should be followed to reduce the effect of plant diseases in crops. A promising approach to achieve this aim while minimising use of pesticides is to apply and combine different agriculture practices that contribute to increasing crop yield by decreasing plant diseases directly or indirectly. For instance, combining good crop hygiene practices, the use of resistant cultivars and chemical control (both as seed dressing and foliar applications), is currently the most effective net blotch disease management strategy.

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