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Comparative Study of Pesticide Resistant Soil Microbes from Mango Farms of Pallam and Nenmeni Panchayath in Muthalamada, Palakkad

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Abstract: ***In the current study a comparison between the soil bacterial population of Mango farm polluted and non-polluted with pesticide were carried out. The study showed that different insecticides were used in each growing season and that farmers apply a lot of pesticides to control pests of mango. Pesticides are applied once every three or four days. The mixtures for insecticides and fungicides used and at least three types of variations with doses of 30–40 ml for each type. Excessive use of pesticides has been known to be hazardous to the environment, affect soil fertility as well may impart toxicity in living beings including beneficial bacteria. Major bacterial genera found in the soil includes; Bacillus, Pseudomonas, Micrococcus, Serratia Proteus, Enterobacter. Bacterial resistance towards the pesticides used in the mango farms were studied using pesticides Hamla, Larincare and Zipvin in various concentrations like (2 µl/ml) (4 µl/ml) (6 µl/ml).It has been found that all the four bacteria can grow in the presence of pesticides indicating all the four bacteria is resistant to all the tested pesticides.***
Keywords: Pesticides, Toxicity, Bacterial resistance

I. INTRODUCTION

Agriculture and its importance in human life is well known especially at present where population is increasing rapidly acrossthe world. The increase in world population led to food shortage ; to overcome this plight farmers were forced to use chemical fertilizers and synthetic pesticide to get more yield. Horticulture isone of the highest revenue generating fields through exporting and also high employment potential .

In agriculture pest is the main anti-protagonist it may be fungi ,bacteria, nematodes decrease in yield .To protect the agriculture from pest farmers were forced to use pesticides .Pesticides are thechemical compounds use to kill pests. The term pesticide covers a wide range of compounds including insecticides, fungicides, herbicides ,rodenticides, nematicides and plant growth regulators .

The capacity of the soil to filter, buffer, degrade, immobilize, anddetoxify pesticides is a function or quality of the soil. Soil quality also encompasses the impacts that soil use and management can have on water and air quality, and on human and animal health.

Muthalamada is called as the mangocity. But of late, Muthalamada Gramapanchayat has gained notoriety for excessive use of endosulphan in its mango orchards. Its effects is showing on people living there. Due to the excessive use of chemical pesticides the number of soil microbes are degrading day by day. This study mainly concentrate on how the pesticides affect the life of soil microbes in Pallam And Nenmeni Panchayath In Muthalamada, Palakkad.

II. MATERIALS AND METHODS

A. Sample Collection

All the soil sample needed for study were collected from mango farms of Muthalamada Panchayath ,Palakkad district,Kerala. Mango orchards spread in 10,000 hectares of land in this gram Panchayat sharing borders with Tamilnadu. Muthalamada is one of the biggest centers of Mango production in the country and first to reach global marketsmuch before the mangoes mature in the gardens of competitors.

1) Collection of Soil Sample

Two different soil samples were collected from mango farms of Muthalamada. The soil sample collected from Pallam is contaminated with pesticide and from Nenmeni is pesticide free soil. Samples were collected in sterile ziplock cover and were transferred aseptically to laboratory. Soil samples collected from three different sites in mango farms and are marked as sample 1, 2, 3, 4, 5, 6 accordingly.

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Sample 1,2,3 (pesticide contaminated soil)

Three soil samples were collected from 3 different location of Pallam-Palakkad District. Topsoil from the location were collectedin sterile ziplock bags with proper labels and sealed immediately and transferred to laboratory for enumeration bacteria. Use of pesticide over the year have been confirmed by the farmers andmango production was found to decrease over the year.

Sample 4,5,6 (soil with out pesticide application)

The soil samples without pesticide application were collected from different locations of Nenmeni-Palakkad district. No pesticides were used and no specific farming practices were adopted. The trees are growing naturally without any additives. The soil samples are collected in a Ziplock bag and sealed immediately and transfer to laboratory for the enumeration of Bacteria.

B. Isolation of Bacteria from Soil Sample

Soil sample is diluted by measuring a volume or weight of sample and adding it to volume of sterile water, making the resulting solution less concentrated than the original. We repeated this process for each new tube, making a series of more and more diluted samples and hence it is called serial dilution .

1) Serial Dilution

Each dilution blank contains 9 ml of sterile distilled water.1g of the soil sample is transferred to the 1st test tube containing 9ml sterile distilled water and it give 1g in total volume of 10ml .

C. Determination of Pesticide Resistant Bacteria

The isolated colonies were sub cultured in the nutrient agar medium using streak plate method**.** After incubation the bacterial growth on nutrient agar plates with various concentrations of pesticide were sub cultured in nutrient agar slants and stored at 4^0C.Pesticides used for the study were Hamla, Larincare and Zipvin

D. Identification of Pesticide Resistant Bacteria

1) Staining

a) Gram Staining: It was done to differentiate bacteria in to two groups such as grampositive and gram negative. Bacterial smears were prepared using 24 hours old culture and stained with crystal violet, fixed with grams iodine respectively for one minute, decolorized with alcohol for 15 seconds and counter stained with safranin for 1-2 minutes and observed under oil immersion.

b) Spore Staining: The smear was prepared on a clear glass slide, heat fixed, stained with malachite green for 15minutes under steam, washed and counterstained with safranin and observed under oil immersion.

2) Motility

Motile organisms were detected by hanging drop method using sterile technique, a loopful or fresh broth culture was added on to the centre of a cover slip with vaseline on its four sides.

3) Biochemical Tests for Organisms

a) Catalase Test: Hydrogen peroxide (one drop) was poured over the surface of an agar or broth culture and effervescence was observed.

b) Oxidase Test: Oxidase disc was taken and the colonies were rubbed on to it andobserved for colour change to purple.

c) Urease Test: Christensen's urease agar was prepared in a slant and the sample was inoculated in to it.

d) Carbohydrate Fermentation Test: Acid and gas production from fermentation of Phenol red broth with sugar glucose were prepared and Durham's tube were inserted, inoculated and incubated at 37 °C for 48hours and observed for acid and gas production

e) Indole Test: The culture was inoculated in to peptone broth, incubated at 37°C for 24-48hours. Following incubation 5-6 drops of Kovac's reagentwas added.

f) Methyl Red Test: The organisms were inoculated into MR-VP broth, inoculated at 37°C for 24-48 hours. Following incubation 5-6 drops of methylred reagent was added .

g) Voges Proskauer Test: The organisms were inoculated in to MIR-VP broth, incubated at 37°C for 24-48 hours. Following incubation, Barritt's reagent was added.

h) Citrate Utilization Test: The organism were inoculated into Simmon's citrate agar slants containing Bromothymol blue and incubated at 37°c for 24-48 hours.

i) Casein Hydrolysis: Skim milk agar plates were prepared and inoculated with the cultures as a single line streak. The plates were then incubated at37°c for 24-48 hours.

j) Gelatin Hydrolysis: Nutrient gelatin tubes were prepared, inoculated with the culture and then incubated at 37°c for 24-48 hours. Following incubation,the tubes were placed in are refrigerator at 4°C for 30 minutes.

k) Starch Hydrolysis: Starch pla /tes were prepared and inoculated with the cultures as a single line streak. The plates were then incubated at 37° for 24-48hours. Following incubation, the surface of plates were flooded with gram's iodine. The positive reaction was indicated by a clearzone surrounding the bacterial growth.

l) Urea Hydrolysis: Urea broth was prepared, dispensed in to test tubes, sterilized andinoculated with the culture. The test tubes were then incubated at 37°c for 24-48 hours.

III. RESULT AND DISCUSSION

Table:1 Total Heterotrophic Count of Bacteria from soil samples

Sl.No.	Sample No.	Sample Details	CFU/g in 10^{-1} Dilution	CFU/g in 10^{-2} Dilution	CFU/g in 10^{-3} Dilution	CFU/g in 10^{-4} Dilution
1	SS 1	Soil without Pesticide	200×10^{1}	148×10^{2}	100×10^{3}	30×10^{4}
2	SS2	Soil without Pesticide	TNTC	200×10^{2}	150×10^{3}	50×10^{4}
3	SS3	Soil without Pesticide	250×10^{1}	78×10^{2}	30×10^{3}	15×10^{4}
4	SS4	Pesticide Applied Soil	120×10^{1}	31×10^{2}	14×10^{3}	5×10^{4}
5	SS5	Pesticide Applied Soil	100×10^{1}	80×10^{2}	20×10^{3}	9×10^{4}
6	SS6	Pesticide Applied Soil	140×10^{1}	50×10^{2}	26×10^{3}	16×10^{4}

The Result clearly indicate the soil collected from the mango farm not applied pesticide is having more bacteria with 200×10^{2} cfu/g. The soil where pesticide applied have very less bacteria 31×10^{2} cfu/g.

From the above two sets of samples we selected the most predominant bacteria which is present in all samples. Bacterial colonies with 4 different colony morphology were selected, isolated and identified. Out of 4 selected bacteria 2 were gram positive rods and the other two are gram negative cocci.

Sl.No.	Tests	SB 1	SB 2	SB3	SB4
1	Gram Staining	Positive	Positive	Negative	Negative
2	Shape	Rods	Cocci	Rod	Rod
2	Spore Staining	Spore Former	NonSpore Former	NonSpore Former	NonSpore Former
3	Motility	Non-Motile	Non-Motile	Motile	Motile
3	Catalase Test	Positive	Positive	Positive	Positive
4	Oxidase Test	Negative	Negative	Positive	Positive
7	Carbohydrate Fermentation Test – (Glucose)	Positive	Negative	Negative	Positive
8	Indole Test	Negative	Negative	Negative	Negative
9	Methyl Red Test	Negative	Negative	Negative	Negative
10	VogesProskaeur Test	Negative	Positive	Negative	Positive
11	Citrate utilization	Negative	Negative	Positive	Positive
12	Casein Hydrolysis	Positive	Positive	Negative	Positive
13	Gelatin hydrolysis	Positive	Negative	Negative	Positive
14	Starch hydrolysis	Positive	Positive	Negative	Positive
15	Urea hydrolysis	Negative	Positive	Positive	Positive
Identified Bacteria		***Bacillus***	***Micrococcus***	***Pseudomonas***	***Serratia***

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From the above results based on staining ,motility and biochemical tests four different organisms were identified. The predominant bacteria present in the soil of mango farm were identified as Genus *Pseudomonas* (Gram Negative), *Serratia* (Gram Negative), *Micrococcus* (Gram Positive), *Bacillus* (Gram Positive).

Bacterial resistance towards the pesticides used in the mango farms were studied using pesticides Hamla, Larincare and Zipvin in various concentrations like (2 µl/ml) (4 µl/ml) (6 µl/ml).It has been found that all the four bacteria can grow in the presence of pesticides indicating all the four bacteria is resistant to all the tested pesticides.

In the current study a comparison between the soil bacterial population of Mango farm polluted and non-polluted with pesticide were carried out. The study showed that different insecticides were used in each growing season and that farmers apply a lot of pesticides to control pests of mango. Pesticides are applied once every three or four days. The mixtures for insecticides and fungicides used and at least three types of variations with doses of 30–40 ml for each type.

Excessive use of pesticides has been known to be hazardous to the environment, affect soil fertility as well may impart toxicity in living beings including beneficial bacteria. Major bacterial genera found in the soil includes; *Bacillus, Pseudomonas, Micrococcus, Serratia Proteus, Enterobacter.* Indiscriminate use of chemicals might work for a few years, but after a while, there aren't enough beneficial soil organisms to hold onto the nutrients" (Savonen, 1997). Plants depend on a variety of soil microorganisms to transform atmospheric nitrogen into nitrates, which plants can use.

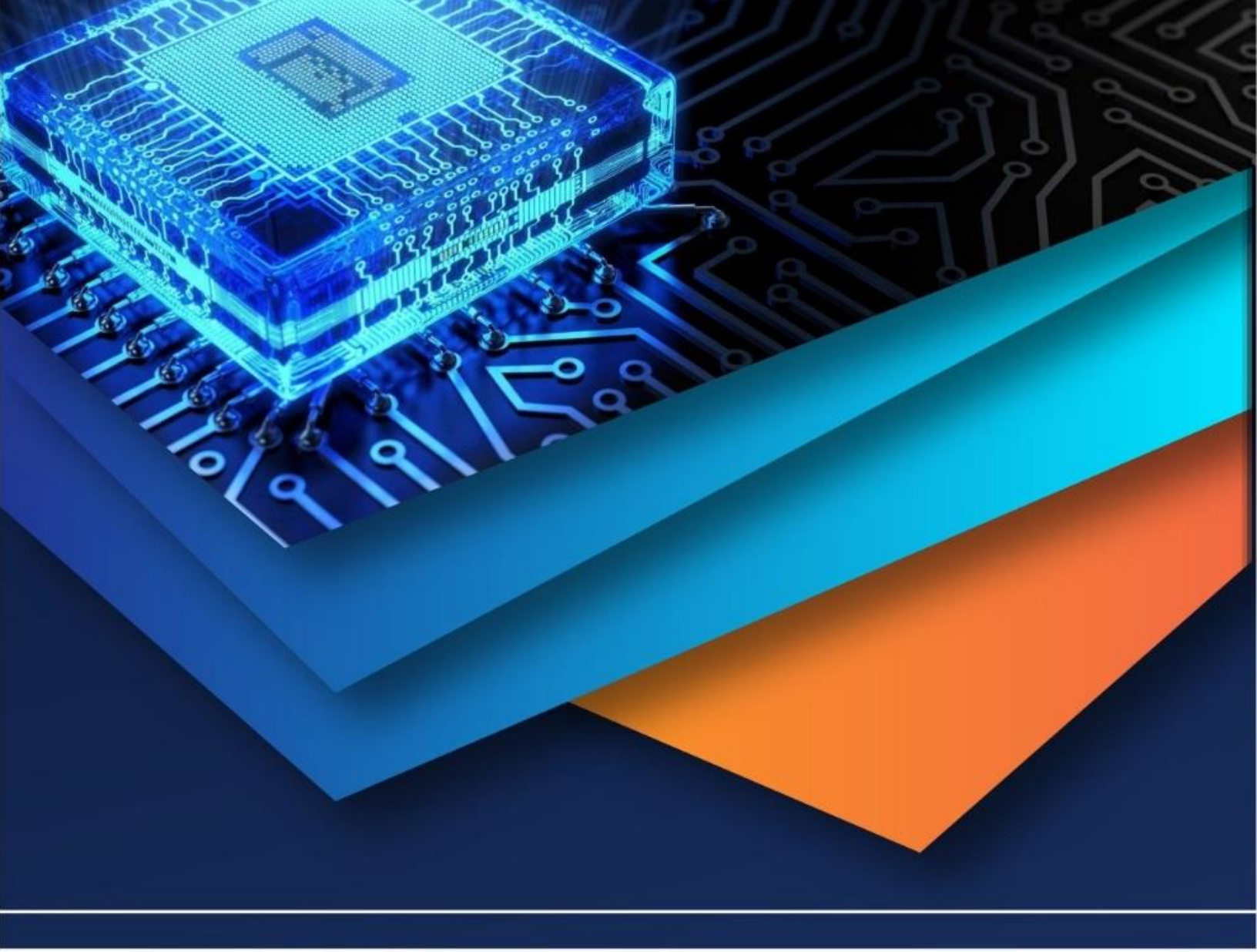
Common landscape herbicides disrupt this process: Triclopyr inhibits soil bacteria that transform ammonia into nitrite (Pell *et al*., 1998)

Pesticides are used to control unwanted and dangerous species of insects. These are the economic poisons employed to regulate the impact of various pests on our land, life and economy. Hamla 550, Zipvin and Larincare are most commonly used commercial insecticides. Out of four isolates, two was found to be a Gram negative and other two isolates were found to be Gram positive. It also reduces the growth of beneficial microflora in the (Santos and Flores, 1995) (Arias and Fabra, 1993).

Many bacteria have found to possess pesticide resistant property along with plant growth promoting and other traits. Usage of such bacteria in crop is a boon to farmers since they are having the dual property of growing in pesticide contaminated soil and helping plants to grow.(Shadab Ala metal.,2018). In our study the bacteria isolated and identified were having pesticide resistant property. Among the four isolates two bacterial genus *Pseudomonas* and *Bacillus* are biofertilizers having the potential for phosphate solubilization. (Postma-Blaauw et al., 2012).

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