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Isolation of Pesticide Resistant Bacteria with Biofertilizer Potential from Agricultural Soil of Erutheyampathy, Palakkad, Kerala

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Abstract: ***Soil loaded with millions of microorganisms forms the most important medium for the survival, growth and multiplication of microorganisms along with plant growth promotion. Understanding the importance of soil micro flora will enable us to learn deeply about soil ecosystem which could be effectively utilized in agricultural practices. The quantity of production is increased gradually by treating large variety of chemical pesticides, which also inhibits the useful microorganisms present in the soil.***

Chemical pesticides kills the useful bacteria, nematodes, earthworms also causing health effects to people. In this study the soil samples were collected from tomato farms of Erutheympathy where chemical pesticides is used. Total heterotopic population were determined and predominant bacteria were identified as genus Bacillus, Pseudomonas, Serratia, Micrococcus and Enterococcus. After studying pesticide resistance property Pseudomonas found to be resistant to pesticide followed by Serratia. After analysing the bio fertilizer potential for NPK of isolated bacteria Bacillus and Pseudomonas found to solubilize Phosphorus and Potassium. Bacillus, Serratia, Micrococcus and Enterococcus found to have role in conversion of nitrogen which can be absorbed by plants. Unique combination of the soil bacteria with pesticide resistance and bio fertilizer property is a boon to farmers since soil fertility can be retained even after application of chemical pesticide. Key Words: Tomato, Pesticides, Bio fertilizers

I. INTRODUCTION

The tomato (Solanum lycopersicum) is one of the most widely grown vegetable in the world (Ganeshan and Chethana et al., 2009). India is the second largest tomato growing country after China in area (8.65 lakh ha) and production (168.26 lakh tons). The tomato is known for its nutritive value and combined in many different dishes and eaten in different ways as a fresh vegetable or made into soup or sauce. Eating tomatoes has been promoted as helping to prevent human diseases and improving the immune system response (Ntonifor et al., 2013).

Varieties of tomatoes include Meghadoot, Santo, Shivam, Ahalya etc. Erunthieanpathy (10.7503° N, 76.8779° E) which is near to Palakkad, is one of the main district of Kerala where tomato is widely grown.. Tomato is of great importance to the small holders in the district in terms of income generation and local consumption. (G.Keshavareddy et al.,2018). In order to maintain the huge amount of agricultural production, with the application of high-yielding-seeds and proper fertilizers, huge amount of quality pesticides have also been used. The excessive application of pesticides also leads to the accumulation of huge amounts of pesticide in soil and water affecting food chain and drinking water. Various studies suggested that a wide range of microorganisms are capable of degrading pesticides (Rani, M. S. et al.,2008.). Organophosphorous insecticides such as parathion, methamidophos and chlorpyrifos are a group of highly toxic agricultural chemicals widely used in plant protection.

The contamination has been found up to about 24 km away from the point of use. If pesticides are not degraded or detoxified rapidly, the risk of their off-site migration may pose a health risk to humans and other beneficial soil microorganisms.Some of the microorganism have the ability to degrade pesticides includes Pseudomonas can resist or degrade the chlopyrifos. Biofertilizer is a product that contains living microorganisms, which exert direct or indirect beneficial effects on plant growth and crop yield through different mechanisms.

Most of the Pseudomonas sp shows pesticide resistance and biofertilizer potentials ,these charecteristics of Pseudomonas sp enhance the production of agriculture field These bacteria are great interest as they have been proposed as inoculants for agriculture.

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II. MATERIAL AND METHODS

A. Sample Collection

1) *Sampling site:* The soil samples for the study were collected from a tomato farm where tomato is cultivating for more than 20 years at Villoni, Eruthempathy village, Chittur taluka of Palakkad district in Kerala, India (10.7503^0 N and 76.8779^0 E). The village have a total agricultural area is 1052 hectors sharing borders with Tamilnadu.
2) *Collection of soil samples:* The soil samples were collected in sterile polythene bags and transferred aseptically to the microbiology laboratory for further procedures.

B. Determination Of Physiochemical Properties Of Soil

Physiochemical properties of fresh soil samples pH, EC, available nutrients (NPK kg/ha) were determined at soil testing laboratory, Kerala Forest Research Institute, Peechi, Thrissur, Kerala.

C. Enumeration Of Bacteria From Soil

The soil bacteria were isolated and enumerated by serial dilution technique and spread plate technique on nutrient agar medium .One gram of soil sample were suspended in 9ml of distilled water and mixed well for 15 minutes. Suspension was serially diluted from 10^{-2} to 10^{-6}.From the diluted sample.0.1 ml was pipetted out on to sterile nutrient agar plates and spread with a sterile glass L rod. Plates were incubated at 37 ^{0} C for 24 hours. The number of colony forming units were counted.Colonies with different morphologies were counted and subcultured. The isolated colonies were maintained at 4^0C for further studies (Murugalatha et al.,2018).

D. Determination Of Pesticide Resitant Bacteria

Effect of pesticide on growth of microbes and their interaction

Pesticide chlorpyrifos was selected since tomato farmers were using this chlorpyrifos pesticide in tomato fields continuously. Effect of different concentrations of pesticide on isolated soil bacteria was determined by disc diffusion technique.(Bauer *et al*., 1966) (Mallik and Tesfai *et al.*, 1983)(Martensson *et al.*, 1992). Chlorpyrifos insecticide was obtained from the market. Various concentrations of pesticide were prepared using sterile distilled water (25,50,75,100,200µl/ml). The sterilized discs were dipped in different concentrations of pesticides and kept on sterile Muller Hinton agar plates swabbed with 24 hour old cultures of isolated soil bacteria. For control antibiotic disc and a sterile disc dipped in sterile distilled water was kept on Muller Hinton agar plates inoculated with isolated soil bacteria. The plates were then incubated at 30 °C for 48 hours. After 48 hours, the plated were observed for zones of inhibition around the discs .(Jay Prakash Verma *et al.*,2016)

E. Determination Of Bio Fertilizer Potential Of Isolated Bacteria

1) Determination Of Phosphate-Solubilizing Property Of Isolated Soil Bacteria

The isolated soil bacteria were inoculated into the Pikovskaya's agar plates. The plates were incubated at 30 °c for 7 days. The strains with clear zone surrounding the colony indicates the phosphate solubilisation. The diameter of the solubilisation zone is measured and tabulated. (Nautiyal 1999, Wang, Y.*et al* ;2020).

2) Determination of Potassium solubilising property of isolated soil bacteria

The isolated soil bacteria were inoculated in Aleksandrov medium. The plates were incubated at 30 °c for 7 days. The colonies surrounded with clear zones indicating Potassium solubilisation were selected and diameter of the solubilisation zone is measured and tabulated. (Hassan Etesami *et al;*2017).

3) Determination of Nitrification property of isolated soil bacteria

The nitrate reduction test is a qualitative procedure for determining the ability of bacteria to reduce nitrate into nitrite. Bacteria were cultured overnight in nitrate broth (HiMedia Labs, Mumbai, India) at 37 °C and then tested for the presence of nitrite. Nitrite detection is a two-step process whereby the reduction of nitrate to nitrite is determined by the addition of nitrate reagent A (sulfanilic acid) followed by reagent B (alpha-napthylamine). If a red color develops, this then confirms the presence of nitrite. If there is no color change, then either nitrate remains unreduced and is determined by the addition of zinc powder which is a strong reducer that will reduce nitrate to nitrite to turn the culture red, confirming there was unreduced nitrate in the tube and then further reduced to other nitrogen compounds. (Arjun Bhusal *et al*;2021).

III. RESULT

The collected soil were having a texture of sandy clay loam and light brown in colour.After analysing the Physiochemical properties of soil samples pH is found to be 6.7, Electro Chemical Conductivity 0.00039 (dS/m). and available nutrients Nitrogen is 317.36 kg/ha, Phosphorous is 452.45 kg/ha and Pottasium is 498.40 kg/ha. The total hetero tropic bacteria from soil was 150 X 10^3 per gram of soil with 5 different colonies . The predominant bacteria present in the soil of tomato farm were identified as genus *Bacillus, Pseudomonas, Serratia, Micrococcus and Enterococcus.*We have tested the effect of this pesticide on different concentration on soil bacteria *Pseudomonas, Bacillus, Enterococcus, Serratia* , Micrococcus by disc diffusion technique. The growth inhibition test was performed at different concentrations of pesticide 25,50,75,100,200 µl/ml. The bacteria Micrococcus, Serratia, Bacillus, and Enterococcus shows zone of inhibition and Pseudomonas does not shows the zone of inhibition indicating it can grow in the presence of pesticide(Table 1). Bacillus, Serratia, Micrococcus, Enterobacter shows the zone of inhibition in different concentrations. Micrococcus found to be highly sensitive to pesticide.After analysing the biofertilizer potential of five isolated bacteria all the bacteria found to possess at least one biofertilizer potential(Table 2). *Bacillus* found to solubilize Phosphorus and Pottasium along with conversion of nitrate to nitrite. *Enterococcus*, *Micrococcus* and *Serratia* found to have role in nitrogen cycle. *Pseudomnas* found to solubilize Phosphorus and Pottasium.(Figure:1)

Table 1 – Response of growth of isolated bacteria in various concentrations of pesticide

Pesticide Concentration/Bacteria	Zone of Inhibition (mm)				
	Bacillus sp	*Enterococcus sp*	*Micrococcus sp*	*Pseudomonas sp.*	*Serratia sp*
25 µl/ml	0.9	0.7	1	0	0
50 µl/ml	1	0.9	1.4	0	0.2
75 µl/ml	1.1	1	1.6	0	0.5
100 µl/ml	1.2	1.2	1.7	0	0.7
200 µl/ml	1.4	1.4	1.8	0	0.9

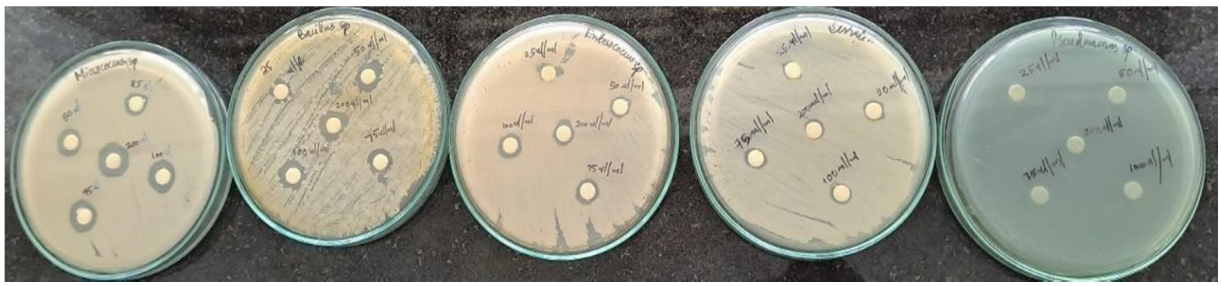


Figure 1 :Growth of isolated Bacteria in different concentrations of pesticide

Table 2: Biofertilizer Potential of Isolated Bacteria

	Bacillus sp	*Enterococcus sp*	*Micrococcus sp*	*Pseudomonas sp.*	*Serratia sp*
Nitrogen	Positive	Positive	Positive	Negative	Positive
Phosphorus	Positive	Negative	Negative	Positive	Negative
Potassium	Positive	Negative	Negative	Positive	Negative

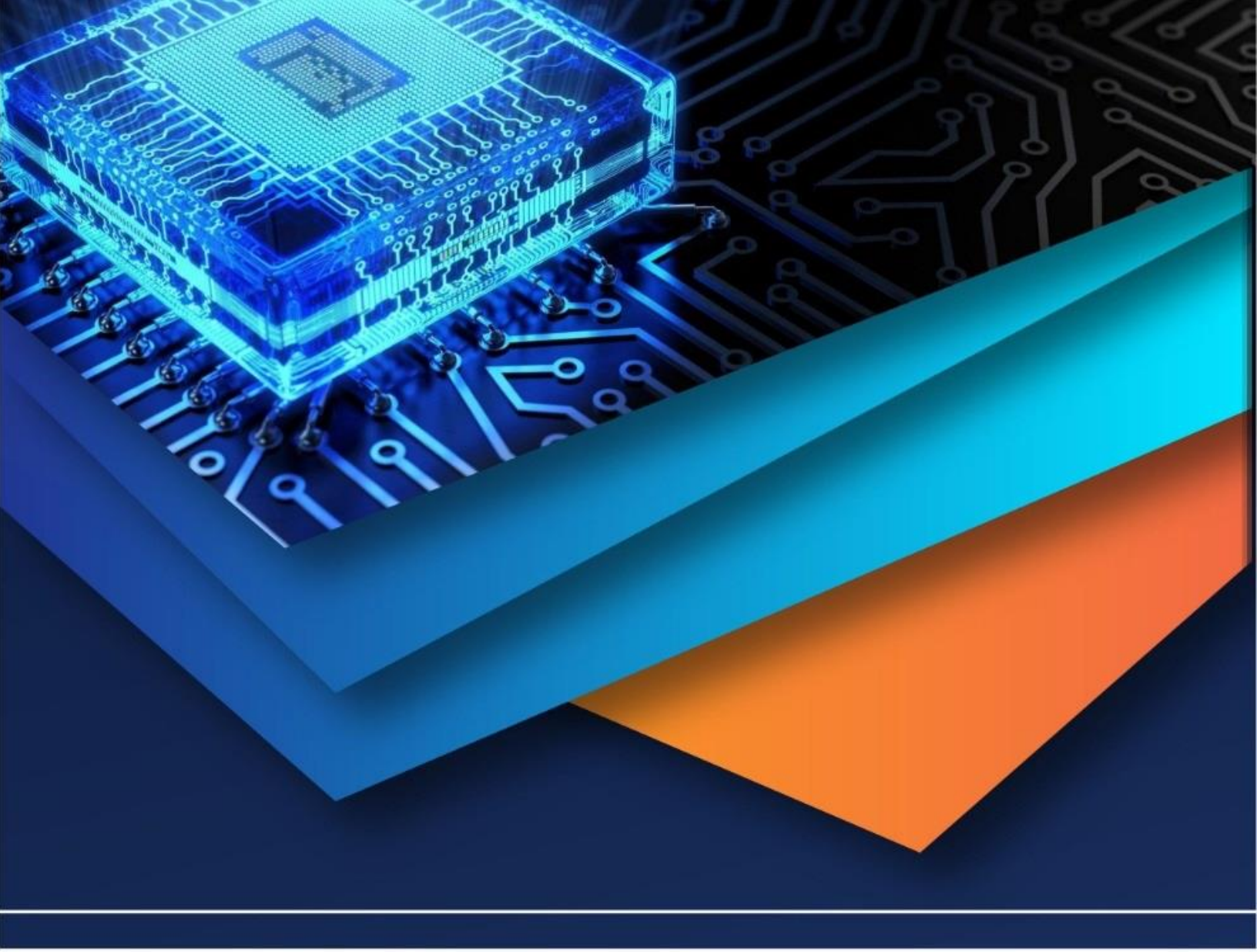
IV. DISCUSSION

Comparing these five organism *Pseudomonas* sp and Bacillus sp both of them are the best biofertilizer properties. Pseudomonas also found to be resistant to higher concentration of pesticide. This unique property of Pseudomonas with pesticide resistance and biofertilizer potential makes the organism farmer friendly and can be used widely.

The present study reports the identification of bacterium Pseudomonas, which is capable of utilizing chloropyrifos as a source of carbon. Utilisation of xenobiotic compounds by soil microorganisms is a crucial phenomenon by which these compounds are removed from the environment, thus preventing environmental pollution. Results from the present study suggest that the isolated Pseudomonas sp is able to grow in medium in presence of added pesticide and may therefore be used for bioremediation of pesticide contaminated soil. Based on our work, bio fertilizers can be developed using species of Pseudomonas sp ,Serratia sp,Micrococcus sp,Enterococcus sp, Bacillus sp were isolated from soil .Pseudomonas sp and Bacillus sp are phosphate and potassium solibilising bacteria. Serratia sp,Micrococcus sp,Enterococcus sp are nitrate reducing bacteria.These study indicate a significant increment in the productivity of plants treated with newly developed biofertilizers.It is necessary to continue researching in this field as it has the potential to be highly profitable for farmer as well as provide a way to more suitable future.

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