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Evaluation of Pesticide Tolerance Capability of Phosphate Solubilizing Bacterial (PSB) Species

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ABSTRACT:

The present investigation was carried out to study growth kinetics of Phosphate solubilizing bacterial species, collected from the pesticide treated soil sample. Isolation of bacteria was done by serial dilution and pour plate method. The pure bacterial culture was obtained after streaking and further biochemical tests were performed for the bacterial identification using Bergy's Manual which was found as Pseudomonas Spp. Then optimize was done to study the growth kinetics of bacterial species. Two fermentation media was prepared among which one media was kept as control (pesticide free) and 1% of pesticide was added to the other media. Then, bacterial sample was inoculated to the both media from the pure culture and finally the optical density was recorded of the samples of different time intervals and the growth kinetics was studied. It was found that at 72 hours the growth rate was maximum while at 96 hours the growth rate was decline.

Keywords: Pseudomonas Sp., DDT (Dichloro-diphenyl-trichloroethane), Biochemical Tests, Optical Density etc.

[I] NTRODUCTION

Phosphorus is a major essential macro element required for growth and development of plants. The bioavailability of soil inorganic phosphorus in the rhizosphere varies considerably with plant species, nutritional status of soil and ambient soil conditions. It is mostly deficient in soils as it is fixed as water insoluble iron and aluminum phosphates in acidic soils or calcium phosphate in alkaline soils [1]. Phosphorus plays a significant role in physiological and biochemical plant activities. But, due to different chemical reactions there is limited availability of this nutrient for plants especially in arid and semi-arid soils. Most of the essential plant nutrients remain in insoluble form in soil [2]. Approximately 95–99% of soil phosphorous is present in the form of insoluble phosphates and cannot be utilized by the plants [3]. A greater portion of inorganic phosphates applied to soil as fertilizer is rapidly immobilized after application therefore; it becomes unavailable to plant. Thus, the insoluble and fixed form of phosphorous is released in order to increase the soil phosphorous availability [4]. Seed or soil inoculation with phosphate solubilizing bacteria is known to improve solubilization of fixed soil phosphorus and applied phosphates resulting in

higher crop yields [5]. The best suitable pH for phosphorous uptake by plants is 6.5 this was indicated by [6]. Phosphorus plays a significant role in physiological and biochemical plant activities like photosynthesis, transformation of sugar to starch and transporting of the genetic traits [7]. Phosphorous also causes early ripening in plants, decreasing grain moisture, improving crop quality and is the most sensitive nutrient to soil pH. The advantage of feeding the plants with phosphorus creates deeper and more abundant roots [8]. PSB have been used to improve rock P value because they convert insoluble rock P into soluble forms available for plant growth [9]. This conversion is through acidification, chelation and exchange reactions [10]. Solubilization of inorganic insoluble phosphate by microorganisms leads to the production of organic acids and chelating oxoacids from sugars. Several mechanisms like lowering of pH by acid production, ion chelation and exchange reactions in the growth environment have been reported to play a role in phosphate solubilization by Phosphate solubilizing microorganisms (PSMs) [11]. In the present study the isolation and characterization of phosphate solubilizing bacteria, Qualitative estimation and determination of solubilized phosphate, concentration and evaluation of the potential of PSB as fertilizer were undertaken.

[II] MATERIALS AND METHODS

2.1. Sample collection

Soil sample was collected, treated with pesticide (DDT) and diluted upto 10^{-6} dilution for the isolation of bacterial species viz; spread plate technique on nutrient agar media with pH 7.5±0.25 at 37^{o}C.

2.2. Identification of bacterial species

Morphological and biochemical activity of isolated bacterial species was studied by gram's staining and various biochemical tests. Identification of bacterial species was done via; Bergy's manual.

2.3. Preparation of fermentation media for Phosphate solubilizing bacteria

Fermentation media for phosphate solubilizing bacteria was prepared having composition Glucose (10.00g), Ammonium Sulphate (0.50g), Magnesium Sulphate (0.10g), Yeast Extract (0.50g), Potassium Chloride (KCl) (0.20g), Sodium Chloride (NaCl) (0.20g), Manganous sulphate (0.002g) in two different conical flasks (100 ml each) in which one media was treated as control (without pesticide) while other media contain 1% pesticide to study the growth kinetics of phosphate solubilizing bacteria.

2.4 Study of Growth Kinetics

The fermentation media inoculated with bacterial species were kept at 37^{o}C for 7 days after that growth kinetics of bacterial was studied using double beam spectrophotometer at 660nm.

[III] RESULTS AND DISCUSSION

3.1 Isolation of Phosphate solubilizing Bacteria (PBS)

Isolation of phosphate solubilizing bacteria was done by serial dilution method in which pesticide was added to the soil sample. Serial dilution of soil sample was done upto 10^{-6} dilution. Nutrient agar media was prepared for the isolation of bacterial species viz; spread plate methods.

3.2 Identification of bacterial species

Bacterial species identified from the Bergey's Manual was found to be Gram-negative; Coccus shaped (Figure 1).

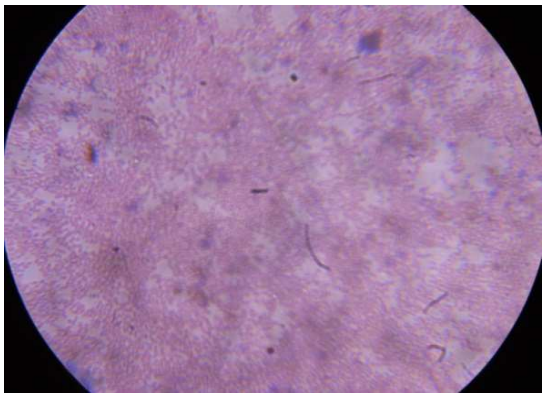


Fig 1: Gram's staining of Bacteria, (a) Gram's negative (b) Coccus shaped (c) Stains used: Crystal Violet (1min) &

Safranin (30sec) (d) Decolourizing Agent: 70% ethanol (30sec); (e) Mordant: Gram's Iodine (1min).

The bacterial species which was identified on basis of different biochemical test were named as *Pseudomonas spp* (Figure 2, 3 & 4).

Fig 2: Positive citrate test for *Pseudomonas spp.* (a) colour of the media was changed from green to blue. (b) Reagent used- Bromothymol blue; (c) Media used- Simmon citrate agar.

Fig 3: Negative indole test for *Pseudomonas spp.* (a) No red colour ring is formed. (b) Reagent used- KOVAX Reagent. (c) Media used- Tryptone broth

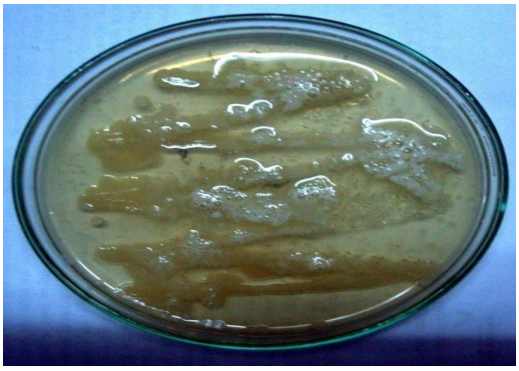


Fig 4: Positive result of catalase test is shown by *Pseudomonas spp.* (a) Production of bubbles after addition of H_2O_2 because of presence of catalase enzyme (b) Reagent used: Hydrogen peroxide

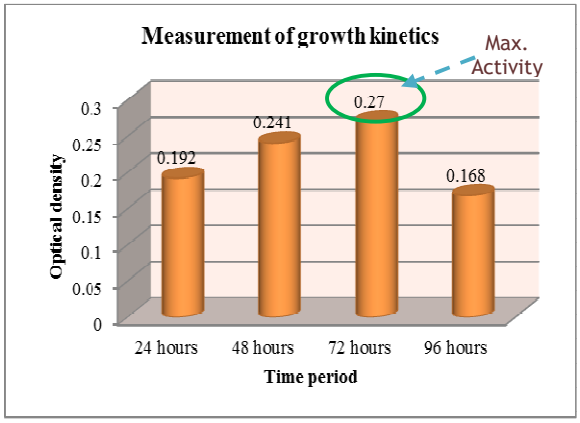
3.3 Growth kinetics of phosphate solubilizing bacteria

Growth kinetics of phosphate solubilizing bacteria was studied at different time interval i.e., 24 h, 48 h, 72h and 96 h at 660nm. The maximum growth was observed at 72 hours (0.22±0.04)*. (Table 1) (Graph 1)

Table 1: Measurement of growth kinetics (OD)

Sample No.	Day	Optical Density (660 nm)
1	24 hours	0.192
2	48 hours	0.241
3	72 hours	0.270
4	96 hours	0.168

(*Mean ± Standard deviation)



Graph 1: Measurement of Growth Kinetics (OD) of *Pseudomonas spp.*

[IV] CONCLUSION

The present investigation was carried out to study growth kinetics of Phosphate solubilizing bacterial species, collected from the pesticide treated soil sample. Isolation of bacteria was done by serial dilution and pour plate method. The pure bacterial culture was obtained after streaking and further biochemical tests were performed for the bacterial

identification using Bergy's Manual which was found as *Pseudomonas Spp.* Then optimize was done to study the growth kinetics of bacterial species. Two fermentation media was prepared among which one media was kept as control (pesticide free) and 1% of pesticide was added to the other media. Then, bacterial sample was inoculated to the both media from the pure culture and finally the optical density was recorded of the samples of different time intervals and the growth kinetics was studied. It was found that at 72 hours the growth rate was maximum (OD=0.270) and at 96 hours the growth rate was decline (OD=0.168).

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