# Influence of biofertilizers and vermicompost on soil health and Soil Microbiology

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## Abstract

Bio-fertilizers are the formulations containing active beneficial organism that are used for different purposes such as in increasing soil fertility and crop yield. Bio-fertilizers are environment-friendly and so they do not pose any harm to the environment or to the health of the human beings whereas chemical fertilizers cause many damages. Current strategies which are in regular practice for the management of soil involve chemical fertilizers and this is because of the rising of burden on the agricultural land for the production of food crops. The use of different microorganism as a biofertilizer is increasing in the sector of agriculture and forestry because of their various advantages. Field trials were conducted on sandy loam soil in Peanut at Edaiyarnatham, Mannargudi, Tamilnadu, India during summer seasons to study the effect of liquid biofertilizer and vermicompost on soil microbial population and soil characteristics.Among various combinations, combined inoculations of beneficial organism (T4- 30%RDF+30%Vermicompost+PGPR) significantly influence the microbial diversity and soil fertility. The present study revealed that the combined inoculations of liquid Plant Growth Promoting Rhizobacteria (PGPR) with vermicompost helps to better plant growth, yield and restore the soil health.

**Key wors:** Biofertilizers, Liquid PGPR, Microbial population, Soil health, Vermicompost

## INTRODUCTION

Intensive use of chemical fertilizer and pesticides for multiple crops in agriculture leads to poor soil fertility. Increased usage of chemicals affects the soil quality day by day. Use of agriculturally beneficial microbes in different combinations is the only solution to restore the soil health. In agro-ecosystem microorganisms play a vital role in fixing, solublizing, mobilizing nutrients. These beneficial microbes naturally present in the soil, but their populations are limited.

Liquid biofertilizers are special liquid preparations containing the desired microorganisms in liquid and suitable cell protectants or additives that vitalize the formation of cyst , spores for longer viability and tolerance to environmental conditions (Brar *et al*., 2012).

Vermicomposting is a composting process by which epigeic earthworm species used for the conversion of organic waste into vermicompost , excellent organic manure synthesized by earthworms by its consumption of waste as food. Vermicomposting is one of the solid waste management practices in which the solid wastes are considered as resources. Vermicomposts contain all the nutrients that enhances the plant growth.

The farming practices with the use of non-chemical substances enhance the soil biodiversity and confirms the safety of food (Morshedi et al., 2017). Organic farming relies on the use of soil microflora, which consists of various PGPR's (Plant Growth Promoting Rhizobacteria). The implication of biofertilizer retains the soil's micro and macronutrients with the help of solubilisation of phosphate or potassium, fixation of nitrogen, antibiotic production, the liberation of plant growth regulating substances and performing biodegradation of organic matter present in soil (Bharadwaj,2014). The implication of biofertilizers, allows the mycorrhizal hyphae to keep the soil masses together and thus consolidate the soil structure and decreases soil erosion (Rashid *et al.*,2016). When biofertilizers are applied as soil inoculant or seed, they proliferate and contributes to nutrient cycling and enhances crop yield (Ju,2018). The present investigation was aimed to determine the influence of liquid biofertilizer and vermicompost for soil microbial population and soil health in field trials.

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## MATERIALS AND METHODS

Field experiments were conducted in peanut crop at Edaiyarnatham ,Mannargudi , Thiruvarur district, Tamilnadu, India during summer season of 2018, using sprinkler irrigation system, to evaluate the effect of liquid biofertilizer and vermicompost(VC) with recommended dose of fertilizer ,on soil health under sandy loam soil conditions. Representative soil samples were collected from the top 20 cm layer of the experimental field, air-dried and sieved through a 2 mm screen. Organic fertrilizer – Vermicarnival (Vermicompost) used was kindly supplied by Biominin Laboratories, Sundarakkottai.

The following co-inoculation treatments were conducted

T1 – 100% of RDF (Recommended Dose of Fertilizer)

T2 –50% Vermicompost +50 %RDF

T3 – 50 % RDF + 40%( Vermicompost)VC+ *Rhizobium sp*

T4 – 30% RDF +30% VC+ Liquid *Rhizobium sp* + Liquid PGPR (*Azotobacter sp*, *Pseudomonassp* + *Bacillus sp and Enterobacter sp*)

T5 – UFUI ( Unfertilized and Uninoculated)

### Soil sample collection

Soil samples were collected from rhizospheric soil of peanut crop grown at the field of Edaiyarnatham, Mannargudi. The soil samples were collected at different time intervals- 0, 30, 60 and 90 DAS (Days After Sowing) and at harvest. The soil sampling was done from different treatments by collecting soil from 3-4 places in each plot with the aid of auger. Then the samples from same plots were mixed to get representative sample.

### Enumeration of microbes in soil samples (Cappucino and Shermann, 2008)

Enumeration of bacteria, fungi, actinomycetes, diazotrophs, in PGPR and Phosphate Solubilizing Bacteria (PSB) was done on Nutrient Agar medium, Glucose Yeast Extract medium, Trypticase Soy Agar (TSA) medium, Jensen's medium, King's B medium and National Botanical Research Institutes Phosphate growth (NBRIP) medium respectively, using serial dilution spread plate technique. The media were prepared and sterilized in an autoclave at 15 psi pressure and 121°C temperature for 20 minutes. Ten grams of the fresh soil was transferred to Erlenmeyer flask (150 ml) containing 90 ml sterile distilled water and was shaken at 120 rpm for 15 minutes to make homogenous solution. Serial dilutions (upto 10-8) were made by pipetting 1 ml of the soil suspension into 9 ml of sterile water blank. Finally, 0.1 ml aliquot of the diluted soil suspension was uniformly spread with the help of sterilized spreader on solidified petriplates with respective medium. The Petriplates were incubated for 2 to 6 days at 28±2°C in an inverted position. After incubation, the number of colonies appearing on dilution plates were counted to find the number of cells per gram of soil sample:

Colony forming Unit (CFU)/g soil = Number (average numberof 3 replicates) of colonies × Dilution factor

### Physico-chemical properties of soil

Physico chemical characteristics of the soil samples were determined by using standard analytical methods. Soil pH (Schofield and Taylor, 1955),Soil Texture (Toogood and Peters, 1953),Soil Electrical Conductivity (Richards, 1954)and available nitrogen, potassium, phosphorous were also determined.

## RESULTS AND DISCUSSION

In this experiment, the highest yield parameters ( number of pods per plant, shelling percentage, 100kernel weight pod yield and halum yield ) were recorded in T4 (30% RDF +40% VC+ *Rhizobium sp*+ PGPR) similar to T1 (100 % RDF). Almost fifty percent yield increased in combined inoculation of PGPR when compared with T5 (UFUI).Bacteria, especially Pseudomonads and bacilli found in the rhizosphere of various leguminous crops, have been found to assist in root colonization by rhizobia and in suppressing soilborne plant pathogens (Parmar and Dadarwal, 2000). Verma *et al* (2010) investigated co-inoculation of *Rhizobium* with PGPR, namely, *Azotobacter chrococcum, Pseudomonas flourescens* and *Bacillus sp* on chick pea and found to significantly increase the plant biomass growth and yield under field trial.

### Effect of liquid PGPR inoculants on microbial population in rhizospheric soil

### Soil characteristics before cultivation

The initial soil microbial population and physico-chemical characteristics were determined before cultivation of peanut i.e. before sowing. Microbial population calculated at zero day of the experiment included bacterial population (56 $\times 10^7$ CFU/g of soil), fungal population (9 $\times 10^3$ CFU/g of soil), actinomycetes (23 $\times 10^4$ CFU/g of soil), diazotrophs (15 $\times 10^5$ CFU/g of soil), PSB (10 $\times 10^4$ CFU/g of soil) and PGPR population (44 $\times 10^5$ CFU/g of soil). Physico-chemical properties of soil at initial stage of the experiment included soil pH (6.9), electrical conductivity (0.20 dSm-1), organic carbon content (0.23 %), available nitrogen (111.6 kg/ha), available phosphorus (20.3 kg/ha) and available potassium (110.4 kg/ha).

### Soil Microbial population

Soil microbial population was enumerated at different time intervals such as 30, 60, 90 DAS and at harvest in rhizospheric soil of peanut crop. Influence of different treatments on the growth of bacteria, fungi, actinomycetes, diazotrophs, PSB and PGPR were studied.

### Microbial population at 30 days after the seeds sown

At 30 DAS the microbial population significantly increased as compared to zero day. This may be associated with the colonization of microflora due to increased rhizospheric interaction on the growing crop. The microbial population at harvest decreased significantly from that at 90 days after sowing. This may be due to the environmental and nutrient deficient conditions of the soil at harvest.

### Bacteria

Highest bacterial population (148×$10^7$ CFU/g of soil) was recorded in treatment T4 (30% RDF +40% VC+ *Rhizobium sp*+ PGPR) at 60 days which was significantly higher than treatment T3 (50 %RDF + 40%VC+ *Rhizobium sp*) having bacterial population of 100 ×$10^7$ CFU/g of soil. (Fig. 1)

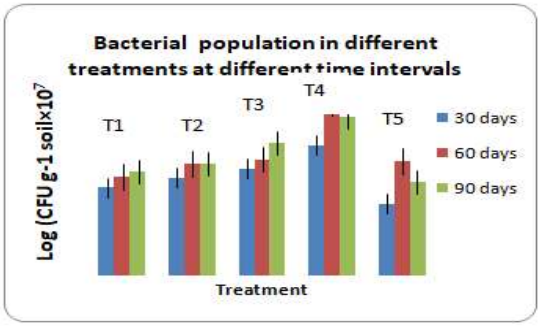


**Fig. 1**

### Fungi

Maximum fungal population (14 ×$10^3$ CFU/g of soil) was recorded in treatment (T4 -30% RDF +40% VC+ *Azotobacter sp*+ PGPR) which was statistically at par with fungal count (10 ×$10^3$ CFU/g of soil) in treatment T3(50 %RDF + 40%VC+ *Azotobacter sp*) as shown in Fig-2. The minimum fungal population (7×$10^3$ CFU/g of soil) was observed in treatment T1 (100% RDF) and T5 without any inoculation. There was a non significant increase in fungal count from zero days (7×103 CFU/g of soil).

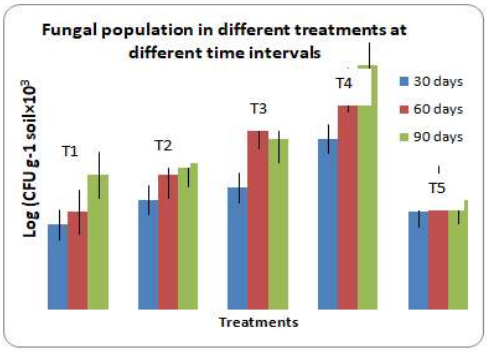


**Fig. 2**

### Actinomycetes

Higher actinomycetes population 58×$10^4$ CFU/g of soil) was noted in treatment T1 i.e. uninoculated control followed by treatment (Fig. 3). The actinomycetes count at 30 DAS increased significantly from that at zero days (23 ×$10^4$ CFU/g of soil).

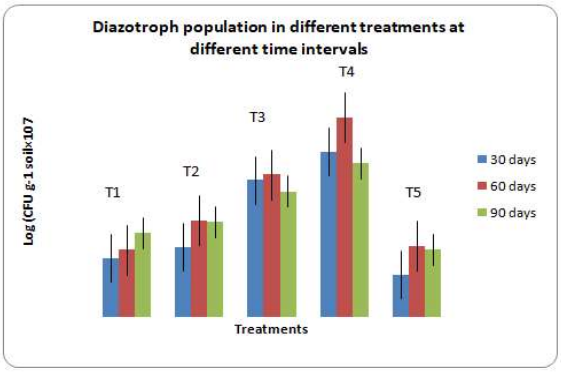


**Fig. 3**

### Diazotrophs

Maximum population of diazotrophs were observed (118 ×$10^5$ CFU/g of soil) in T4 treatment followed by diazotrophic population (98×$10^5$ CFU/g of soil) in treatment having 50 %RDF + 40%VC+ *Rhizobium sp* (Fig-4). The minimum count of diazotrophs (30 ×$10^5$ CFU/g of soil) was observed in soil samples without any inoculation i.e. control, which was significantly higher than diazotrophic population at zero day (11 ×$10^5$ CFU/g of soil).

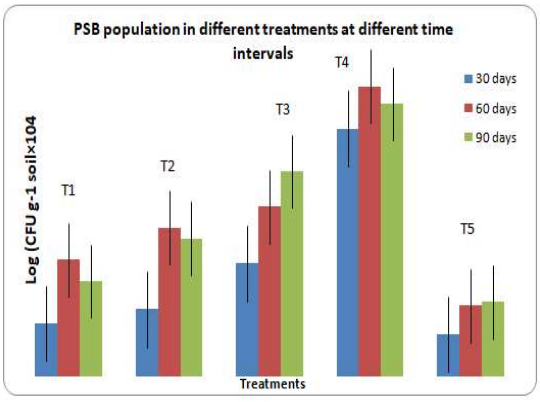


**Fig. 4**

### Phosphate Solubilizing Bacteria (PSB)

Maximum PSB population (70 ×$10^4$ CFU/g of soil) was recorded in treatment T4 followed by treatment T3 (50% Vermicopost + 50% recommended dose of nitrogen and phosphorus +*Rhizobium sp*) having PSB population

of 32 ×10⁴ CFU/g of soil as shown in Figure 5. The minimum PSB population (10 ×10⁴ CFU/g of soil) was observed in treatment without any inoculation i.e. treatment T5 control and T1 (100% RDF). The PSB population at zero days (9×10⁴ CFU/g of soil) was statistically at par with treatment T1 at 30 days, whereas it significantly increased in other treatments.

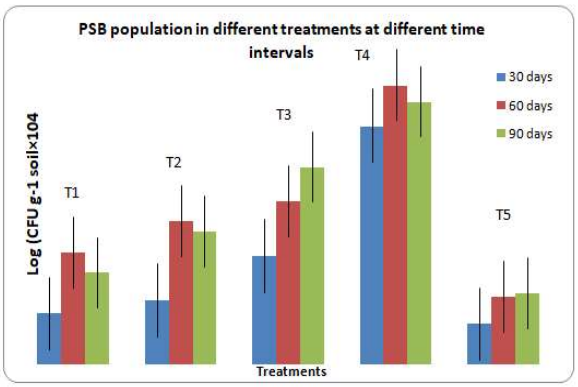


**Fig. 5**

**Plant Growth Promoting Rhizobacteria (PGPR)**

Maximum PGPR population count (162 ×10⁵ CFU/g of soil) was recorded in treatment T4 (– 30% RDF +40% VC+ *Rhizobium sp* + PGPR) which was followed by PGPR population (96×10⁵ CFU/g of soil) in soil samples having treatment T3 - 50 %RDF + 40%VC+ *Rhizobium sp* The minimum count of PGPR (52 ×10⁵ CFU/g of soil) was observed in treatment T5 (control) (Fig. 6). The PGPR population at 30 days was significantly higher than that at zero day o days (40 ×10⁵ CFU/g of soil)

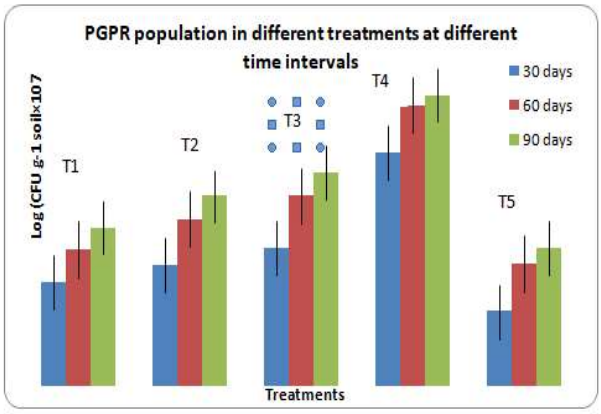


**Fig. 6**

After 60 days of sowing, the highest bacterial count (148 × 10⁷ CFU/g of soil) was noted in treatment having 30% RDF +40% vermicompost and liquid inoculants of PGPR followed by bacterial population (100 × 10⁷ CFU/g of soil) in treatment having 50% RDF +50% vermicompost (Table - 1). Bacterial population in treatment T4 (148 × 10⁷ CFU/g of soil) was remarkably higher than treatment T1 (100% RDF) having bacterial population of 98× 10⁷ CFU/g of soil. The minimum bacterial population (85 × 10⁷ CFU/g of soil) was observed in treatment T1 (control). The results indicated that bacterial population at 60 DAS increased significantly from bacterial population at 30 DAS . This can be associated with the changes in root exudates which resulted in rhizodeposition and availability of essential nutrients for the growing bacteria. The fungal population (18 ×10³ CFU/g of soil) was recorded to be the maximum in treatment T4 which was significantly higher than treatment T3 (50 %RDF + 40%VC+ *Rhizobium*sp) having fungal population of 15 ×10³ CFU/g of s (Table 1) Fungal population intreatment T4 was notably different with treatment T1 (100 % RDF) having fungal population of 8 ×103 CFU/g of soil. Highest actinomycetes population (50×10⁴ CFU/g of soil) was observed in uninoculated treatment (control) which was statistically at par with treatment T4 having actinomycetes population of 44 ×104 CFU/g of soil (Table - 1). Highest Diazotrophic population (143 ×10⁵ CFU/g of soil) was observed at 60 days in treatment T4 (– 30% RDF +40% VC+ *Rhizobium sp* + PGPR) followed by treatment T3 (50 %RDF + 40%VC+ *Rhizobium sp*) having diazotroph population of 102 ×10⁵ CFU/g of soil which was significantly higher than with treatment T1 (100% RDF ) having diazotroph population of 48 ×10⁵ CFU/g of soil (Fig. 2). Population of diazotrophs increased from 30 days to 60 days time interval as shown in table -1. The diazotroph count at

**Table 1.** Effect of liquid PGPR inoculants on microbial population in peanut rhizospheric soil at 60 days after sowing

| Treatments | Bacteria (CFU g-1 soil×10⁷ ) | Fungi (CFU g-1 soil×10³ ) | Actinomycetes (CFU g-1 soil×10⁴ ) | Diazotrophs (CFU g-1 soil×10⁵ ) | PSB (CFU g-1 soil×10⁴ ) | PGPR (CFU g-1 soil×10⁵) |
|---|---|---|---|---|---|---|
| T1 | 85 | 8 | 22 | 48 | 33 | 95 |
| T2 | 96 | 11 | 28 | 69 | 42 | 116 |
| T3 | 100 | 15 | 30 | 102 | 48 | 132 |
| T4 | 148 | 18 | 44 | 143 | 82 | 195 |
| T5 | 98 | 10 | 50 | 51 | 20 | 85 |

60 days showed similar growth pattern as compared to that at 30 days after sowing. At 60 days, it was observed that soil samples treated with vermicompost and liquid biofertilizers had significantly higher diazotrophic population. This increase may be due to the higher amount of organic matter content present in vermicompost increased carbon and energy supply favouring higher number of diazotrophs in the soil. Maximum PSB population (82 $\times 10^4$ CFU/g of soil) was observed in treatment T4 which was statistically at par with treatment T3 (50 %RDF + 40%VC+ *Rhizobium sp*) having PSB population of 78 $\times 10^4$ CFU/g of soil. The present results indicated that PSB population was remarkably higher in soil samples taken after 60 days of sowing as compared to PSB population in soil after 30 days of sowing as shown in Fig. 5. This could be attributed to the organic acid produced by some bacteria under low phosphorus conditions. Phosphorus sources applied through inorganic fertilizers have been efficiently utilized by the plants for their growth thereby initiating favourable environment for the multiplication of phosphate solubilizing bacteria.

Highest PGPR population 60 days after sowing (195 $\times 10^5$ CFU/g of soil) was recorded in treatment T4 (30% RDF +40% VC+ *Rhizobium sp* + PGPR) which was significantly higher than population (132 $\times 10^5$ CFU/ g of soil) in treatment T3 (50 %RDF + 40%VC+ *Rhizobium sp*) as given in Table 1. The PGPR population in treatment T4 was significantly higher than treatment T1 (100% RDF) having PGPR population of 95 $\times 10^5$ CFU/g of soil. Minimum PGPR population (85 $\times 10^5$ CFU/g of soil) was observed in treatment T5 which was the control treatment (Table 1).

**Microbial population at 90 days after sowing**

Microbial count at 90 days reduced remarkably as compared to that at 60 days after sowing. (Table 2)

The highest bacterial population (136 $\times 10^7$ CFU/g of soil) was recorded in treatment T4 at 90 DAS as shown in Table 2. Bacterial population in treatment T3 was followed by treatment T2 (50 %Vermicompost +50% RDF) having bacterial count of 96 $\times 10^7$ CFU/g of soil which was notably higher than bacterial population (89$\times 10^7$ CFU/g of soil) in treatment T1 (100%RDF). The minimum bacterial population (80 ×107 CFU/g of soil) was observed in treatment without any inoculation i.e. control. The results revealed that there was decreased in bacterial population from 60 DAS to 90 DAS as shown in Table - 2. This may be associated with the reduction in optimum temperature for bacterial growth which reduced from 60 days to 90 days after sowing. The reduction of temperature for bacterial growth can be accounted for lower population of bacteria at 90 days time interval.The minimum fungal count (10 $\times 10^3$ CFU/g of soil) was observed in treatment T1 (control).Maximum diazotrophic population (110 $\times 10^5$ CFU/g of soil) was observed in treatment T4 followed by treatment T3 (100% FYM + consortium) having diazotroph count of 90 $\times 10^5$ CFU/g . Minimum Diazotroph count (42 $\times 10^5$ CFU/g of soil) was observed in T5 treatment without any inoculation (Unfertilized and Uninoculated).

Results of the present study showed that diazotrophic population reduced significantly in all the treatments at 90 DAS as compared to 60 DAS as shown in Fig. . This could be due to the decreased in optimum temperature at 90 days (17æ%C) as compared to that at 60 days (23æ%C) which was not suitable for the growth of diazotrophs.

The results of the present study indicated that PSB population at 90 days after sowing was significantly lower than PSB population at 60 days time interval as depicted in Fig - 5 . This could be associated with reduction of temperature which inhibited the growth of phosphate solubilizing bacteria in the rhizosphere.Population of PGPR increased from 60 days time interval to 90 days time interval in all the treatments as shown in Fig- 6. This could be multiplication and colonization of bacteria in the rhizosphere. In addition to that, root exudates of rhizobacteria in promoting the growth of other

**Table 2.** Effect of liquid PGPR inoculants on microbial population in peanut rhizospheric soil at 90 days after sowing

| Treatment | Bacteria (CFU g-1 soil×$10^7$ ) | Fungi (CFU g-1 soil×$10^3$ ) | Actinomycetes (CFU g-1 soil×$10^4$ ) | Diazotrophs (CFU g-1 soil×$10^5$ ) | PSB (CFU g-1 soil×$10^4$ ) | PGPR (CFU g-1 soil×$10^5$) |
|---|---|---|---|---|---|---|
| T1 | 89 | 11 | 18 | 60 | 27 | 110 |
| T2 | 96 | 12 | 25 | 68 | 39 | 132 |
| T3 | 114 | 14 | 36 | 90 | 58 | 148 |
| T4 | 136 | 20 | 20 | 110 | 77 | 202 |
| T5 | 80 | 9 | 42 | 48 | 21 | 96 |

**Table 3: Physico chemical characteristics of Pre-sown and Post harvest soils**

| Treatments | Soil PH | Electrical conductivity | Soil organic carbon | Available Phosphorous | Available Potassium | Available Nitrogen |
|---|---|---|---|---|---|---|
| T1 – 100% of RDF | 7.9 | 3 | 0.2 | 21.75 | 160 | 276 |
| T2 – 50 %Vermicompost +50% RDF | 7.8 | 3.02 | 0.28 | 23.87 | 165 | 275 |
| T3 – 50 %RDF + 40%VC+ Rhizobium sp | 7.82 | 3.17 | 0.3 | 24.59 | 174 | 289 |
| T4 – 30% RDF +40% VC+ Rhizobium sp + PGPR | 7.23 | 3.62 | 0.39 | 27.5 | 181 | 332 |
| T5 – UFUI | 7.6 | 2.98 | 0.2 | 18.4 | 140 | 269 |
| Pre sown soil | 7.56 | 2.56 | 0.2 | 19 | 148 | 148 |

rhizobacteria in dangerous environmental conditions. Rhizodeposits contains carbohydrates and other growth promoting substances which may provide an excellent nutrient supplement for the proliferation of bacteria in the rhizosphere.

Neha Khipla (2017) reported that sustainable agriculture involves optimizing the benefits from biological and inorganic inputs through their interactive application for the maintenance of soil health and productivity. The author investigated the synergistic effect of inorganic fertilizers (75% and 100% of recommended dose of nitrogen and phosphorus fertilizer) and biofertilizers (*Azotobacter*, PSB Consortium) on microbial population and enzyme activities in the rhizospheric soil of Poplar under nursery conditions. The results indicated significantly higher bacterial, fungal and PGPR population under conjoint application of consortium biofertilizer with recommended dose of fertilizers. Mokhtar Dashadi *et al* (2011) studied the effects of native *Rhizobium legominosarumbvviciae* strain F46 and *Azotobacter chroococcom* strain AGO11 inoculation and nitrogen fertilizer on growth and growth indices of faba bean (*Vicia faba*L.) in water stress condition.

**Effect of liquid inoculants of PGPR on soil characteristics**

The present study found positive effects of liquid biofertilizer combined with vermicompost and recommended dose of fertilizer on soil parameters (Table 3). Similarly, Subashini *et al*., (2007) proved that there was a gradual increase in the efficiency of biofertilizer and its compatability with inorganic fertilizer. They found significant difference in the soil fertility status (available N, P and K) and the soil biota.

**Effect of liquid inoculants of PGPR on soil characteristics**

The results of the present study showed that soil electrical conductivity increased remarkably with the addition of organic fertilizer vermicompost and biofertilizers.Application of organic fertilizer vermicompost and liquid biofertilizers positively influence the availability of potassium of soil. Soil potassium (181 kg ha-1) recorded to be the maximum in treatment T4 (30% RDF + 40% vermicompost + liquid biofertilizers). The present study found positive effects of liquid biofertilizer combined with vermicompost and recommended dose of fertilizer on soil parameters. Simillary. Subshini*et al*., (2007) proved that there was a gradual increase in the efficiency of biofertilizer and its compatability with inorganic fertilizer. They found significant difference in the soil fertility status ( available N, P and K) and the soil biota.

The interactions between these PGPR and rhizobia may be synergistic or antagonistic and beneficial effects of such interaction may be exploited for enhancing the biological nitrogen fixation and crop yield (Dubey, 1996). There is also report of the presence of plant growth-promoting Bacillus strains in the root nodules of soybean plants (Yu Ming *et al*., 2002). Mathivanan*et al*., (2013) studied the effect of vermicompost on growth and yield parameters of groundnut. The present study revealed that co inoculation of *Rhizobium* and other PGPR liquid inoculants along with vermicompost suitable for the maintenance of soil microbial population. This new agronomic practice reduces the use of agrochemicals and helps to restore the soil health.

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