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## **Compatibility Study of Indigenous Plant Growth Promoting Rhizobacteria with Inorganic and Organic Fertilizers used in Tea (*Camellia sinensis*)**

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### **ABSTRACT**

An *in vitro* study was undertaken to evaluate the compatibility of indigenous plant growth promoting rhizobacteria (PGPR) with commonly used inorganic and organic sources of fertilizers in tea plantations. The nitrogenous, phosphatic and potash fertilizers used for this study were urea, rock phosphate and muriate of potash, respectively. The organic sources of fertilizers neem cake, composted coir pith and vermicompost were also used. PGPRs such as nitrogen fixer; *Azospirillum lipoferum*, Phosphate Solubilizing Bacteria (PSB); *Pseudomonas putida*, Potassium Solubilizing Bacteria (KSB); *Burkholderia cepacia* and *Pseudomonas putida* were used for compatibility study. Results were indicated that PGPRs preferred the coir pith and they proved their higher colony establishment in the formulation except *Azospirillum* spp. that preferred vermicompost for their establishment. The optimum dose of neem cake powder supported the PGPR incidence when compared to lower and higher doses. As the concentration of rock phosphate increased, the population of PGPR was also increased whereas medium dose of MOP supported their growth and in the case of urea even at lower dose totally retarded the growth of bioinoculants. This study would give a prior knowledge on PGPR sensitivity with synthetic and organic manures in order to suggest the field recommendation on combined application of organic/inorganic/biofertilizers for improving the tea yield and also to protect soil health under integrated nutrient management approach.

**Key words:** PGPR, tea, compatibility, organic fertilizers, inorganic fertilizers, INM

### **INTRODUCTION**

Tea is the most preferable beverages in the world and being cultivated in more than 50 countries. Its habitat could be hilly regions with humid environment and favoured moderate-high rainfall. The soil condition is restricted to acidic pH environment and medium to high organic matter content. The primary nutrients for plant growth are nitrogen, phosphorus and potassium (NPK). The absolute or relative absence of any one of these nutrients can hamper plant growth; alternatively, too high of concentration can be toxic to the plant or to humans. The soil productivity depends on other than plant nutrients. Effects of low nitrogen on growth of plants were already reported (Ngaboyisonga *et al.*, 2009). The physical, biological and chemical

characteristics of soil viz., its organic matter content, acidity, texture, depth and water-retention capacity all influences fertility of the soil. Good management of soil includes usage of biofertilizers and soil conservation measures; addition of organic matter to the soil and judicious use of chemical fertilizers, pesticides, etc. (Gruhn *et al.*, 2005). There are reports available for the effect of organic fertilizers and NPK rates on different crops (Priya *et al.*, 2009; Affendy *et al.*, 2011).

The selection of manure for application and treatment options depends on environmental regulations to prevent pollution. Thus, the importance of organic manure in present agriculture is increasing day by day, because of its utility not only improving the physical, chemical and biological properties of soil but also maintaining the soil health without pollution (Kondapa-Naidu *et al.*, 2009).

Tea plantations mainly depend on inorganic fertilizers in order to manuring the crop for higher yield. In recent years of practice, tea plantations intensively started to use alternate sources of nutrient supplement due to huge shortage, demand and high cost of production of synthetic fertilizers. Earlier reports evidenced that the response of plants to nitrogen fertilizer during intercropping and also in maize (Onasanya *et al.*, 2009; Undie *et al.*, 2012). In this scenario, the ecofriendly and environment safely alternative to synthetic fertilizers is of both biological and organic manures. These two alone cannot fulfill the nutrient support for yield and development of tea crop. Balanced use of manures and fertilizers is very essential. Chaudhry and Sarwar (1999) have optimized the application of nitrogen fertilizer in cotton. Neither inorganic fertilizers nor organic manures can achieve the sustainability with stable soil fertility where nutrient turn over in the soil plant system is faster and larger (Paul *et al.*, 2005). But such a combination of biological and organic fertilizers with reduced consumption of synthetic fertilizers could be perfect way to bring sustainable yield and quality of crop. Before implementing the same under field condition a prior knowledge should be needed on response of PGPR biofertilizers to organic and inorganic sources of fertilizers.

The application of beneficial microorganism to the soil can enhance plant resistance to adverse environmental stress, water and nutrient deficiency and heavy metal contamination (Wu *et al.*, 2005). Plant growth promoting rhizobacteria (PGPR) are beneficial to plants due to the increased acquisition, biocontrol (Walsh *et al.*, 2001) plant hormone production and induction of resistance (Van Loon *et al.*, 1998).

The balanced nutrition can be supplied by using chemical and biological agents together in integrated nutrient management system. Application of organic manures in general improves the availability of micronutrients like zinc, iron, manganese and copper. A balanced application of both organic, inorganic and biofertilizers appear to be an ideal suggestion to meet nutrient requirements of dry land crops rather than single application (Kondapa-Naidu *et al.*, 2009). Hence, a study was undertaken to investigate the compatibility of bioinoculants to commonly used and recommended inorganic and organic sources of fertilizers *in vitro*.

## **MATERIALS AND METHODS**

**Colony establishment of PGPRs in vermicompost and coir pith:** Native plant growth promoting rhizobacteria such as nitrogen fixer; *A. brasilense*, Phosphate solubilizing bacteria (PSB)-*P. putida*, Potassium solubilizing bacteria (KSB)-*B. cepacia* and *P. putida* were isolated from tea soil and selected to study for their higher colony formation in two different organic carrier materials such as vermicompost and composted coir pith. Three days old cultures of PGPRs were mixed with both carrier materials at the ratio of 1:2 (500 mL inoculum in 1000 g of organic carrier)

and they were maintained 40% moisture and kept for incubation. The inoculated carrier was kept away from direct heat and sunlight. Sampling was done at different day's intervals and was subjected to enumerate the population by dilution plate technique with a LB medium (Luria Bertani) for about three months. Three replicates were maintained in all the experiments.

**Compatibility between neem cake extract and plant growth promoting rhizobacteria (PGPR):** Neem cake powder extracts were prepared by mixing it with sterile distilled water and incubated for overnight. Then it was filtered through blotting papers and passed it via micro filtration unit for getting filter sterilized neem extract. Then the neem cake extract was incorporated at a concentration of 25, 50 and 75% in Luria Bertani (LB) media. Filter sterilized neem extracts were incorporated in to the growth medium was being inoculated with selected PGPR organisms and kept for incubation at  $28\pm 2^{\circ}\text{C}$  for 48 h. The neem uninoculated LB media was taken as control for comparison. After incubation period it was subjected to measure the growth or turbidity at 560 nm of wavelength using UV-visible spectrophotometer. Three replicates were maintained in all the experiments.

**Influence of inorganic nutrients (MOP, urea and RP) on PGPRs:** As mentioned above, extracts of inorganic fertilizers such as Urea, Muriate of Potash (MOP) and Rock Phosphate (RP) were prepared. The lower, higher and recommended doses of all three (NPK) minerals were amended in LB medium. PGPRs were inoculated in minerals extract amended LB broth and kept for inoculation at  $28\pm 2^{\circ}\text{C}$  for 48 h. The above extract of uninoculated LB media was kept as control for comparison for all four individual organisms. After incubation the growth or turbidity was measured at 560 nm of wavelength using UV-visible spectrophotometer. Three replicates were maintained in all the experiments.

**Statistical analysis:** All the data analysed using SPSS 14.0 version of statistical software package (SPSS, Inc., Chicago, IL). Data obtained were subjected to analysis of variance (ANOVA) and the significant means were segregated by Critical Difference (CD) at various levels of significance. The Standard Error (SE) and Coefficient of Variance (CV) were also calculated (Gomez and Gomez, 1984).

## RESULTS AND DISCUSSION

**Compatibility of vermicompost and coir pith on colonization of plant growth promoting rhizobacteria (PGPR):** The present study aimed to find out the compatibility of PGPRs with organic and inorganic nutrients practiced in tea plantation. Colony establishment of PGPRs in vermicompost and coir pith results were presented in Table 1 and 2. Among all the four PGPRs studied except *A. brasilense* were colonized well in coir pith formulation when compared to vermicompost. During incubation period, PGPR organisms started to establish in both carrier materials gradually and they reached maximum population at 30th day of incubation. After that population of PGPRs was gradually reduced as the incubation period increased. *Azospirillum* spp., showed more compatibility with vermicompost than coir pith. On 30th day of incubation they showed  $39.8\times 10^7$  CFU  $\text{g}^{-1}$  in vermicompost but  $23\times 10^7$  CFU  $\text{g}^{-1}$  in coir pith formulation. Highest population at 30th day was observed in vermicompost bioformulation by *P. putida* (KSB) than other PGPRs. But coir pith bioformulation supported the growth of *B. cepacia* (KSB) among all the four studied. Linu *et al.* (2009) have studied the phosphate solubilizing bacteria *Burkholderia* sp., on cow pea. Table 3 presents growth of bioinoculants in Urea amended LB media.

Table 1: Colony establishment of PGPRs in coir pith

PGPRs	Population of PGPRs in coir pith formulation ( $\times 10^7$ CFU $g^{-1}$ )						
	7th day	15th day	30th day	45th day	60th day	75th day	90th day
<i>Azospirillum</i> spp.	4.8 $\pm$ 0.2 <sup>a</sup>	5.0 $\pm$ 1.2 <sup>a</sup>	27.0 $\pm$ 0.1 <sup>a</sup>	14.0 $\pm$ 0.1 <sup>b</sup>	6.6 $\pm$ 0.2 <sup>a</sup>	6.3 $\pm$ 0.2 <sup>a</sup>	2.3 $\pm$ 0.4 <sup>a</sup>
<i>P. putida</i> (PSB)	22.0 $\pm$ 0.1 <sup>b</sup>	33.0 $\pm$ 0.8 <sup>b</sup>	36.0 $\pm$ 0.7 <sup>b</sup>	21.0 $\pm$ 0.4 <sup>d</sup>	16.0 $\pm$ 0.2 <sup>c</sup>	15.0 $\pm$ 0.4 <sup>d</sup>	5.5 $\pm$ 0.1 <sup>c</sup>
<i>B. cepacia</i> (KSB)	20.0 $\pm$ 1.2 <sup>c</sup>	43.0 $\pm$ 0.6 <sup>c</sup>	58.0 $\pm$ 0.5 <sup>d</sup>	17.0 $\pm$ 0.4 <sup>e</sup>	10.0 $\pm$ 0.4 <sup>b</sup>	8.6 $\pm$ 0.6 <sup>b</sup>	3.1 $\pm$ 0.1 <sup>b</sup>
<i>P. putida</i> (KSB)	51.0 $\pm$ 0.2 <sup>d</sup>	58.0 $\pm$ 0.2 <sup>d</sup>	38.0 $\pm$ 0.3 <sup>c</sup>	13.0 $\pm$ 0.2 <sup>a</sup>	10.0 $\pm$ 0.6 <sup>d</sup>	10.0 $\pm$ 0.1 <sup>c</sup>	7.4 $\pm$ 0.2 <sup>d</sup>
SE $\pm$	0.83	0.44	0.87	0.63	0.78	0.68	0.43
CD at p = 0.05	1.23	1.00	1.22	1.18	1.44	1.20	0.83

Values are mean of three replications, Standard deviations followed by the same letter are not significantly different (p<0.05) as determined by Duncan's multiple range test

Table 2: Colony establishment of PGPRs in vermicompost

PGPRs	Population of vermicompost formulation ( $\times 10^7$ CFU $g^{-1}$ )						
	7th day	15th day	30th day	45th day	60th day	75th day	90th day
<i>Azospirillum</i> spp.	132.0 $\pm$ 0.6 <sup>d</sup>	48.0 $\pm$ 0.5 <sup>e</sup>	32.0 $\pm$ 0.1 <sup>d</sup>	30.5 $\pm$ 0.9 <sup>d</sup>	9.7 $\pm$ 0.8 <sup>e</sup>	8.3 $\pm$ 1.0 <sup>e</sup>	5.0 $\pm$ 0.1 <sup>d</sup>
<i>P. putida</i> (PSB)	5.1 $\pm$ 0.4 <sup>b</sup>	7.5 $\pm$ 1.1 <sup>a</sup>	28.3 $\pm$ 0.2 <sup>b</sup>	17.7 $\pm$ 0.5 <sup>e</sup>	16.2 $\pm$ 0.6 <sup>d</sup>	16.3 $\pm$ 0.4 <sup>d</sup>	3.2 $\pm$ 0.2 <sup>c</sup>
<i>B. cepacia</i> (KSB)	5.0 $\pm$ 0.2 <sup>a</sup>	16.3 $\pm$ 1.5 <sup>b</sup>	30.9 $\pm$ 0.4 <sup>c</sup>	7.7 $\pm$ 1.8 <sup>a</sup>	5.0 $\pm$ 1.0 <sup>a</sup>	4.3 $\pm$ 0.4 <sup>a</sup>	0.9 $\pm$ 0.4 <sup>a</sup>
<i>P. putida</i> (KSB)	65.2 $\pm$ 0.3 <sup>c</sup>	51.3 $\pm$ 0.8 <sup>d</sup>	21.2 $\pm$ 0.1 <sup>a</sup>	8.2 $\pm$ 1.3 <sup>b</sup>	8.3 $\pm$ 0.2 <sup>b</sup>	6.6 $\pm$ 0.2 <sup>b</sup>	1.2 $\pm$ 0.1 <sup>b</sup>
SE $\pm$	0.61	0.52	0.67	0.41	0.65	0.46	0.52
CD at p = 0.05	1.11	1.04	1.02	0.84	0.92	1.00	1.21

Values are mean of three replications, Standard deviations followed by the same letter are not significantly different (p<0.05) as determined by Duncan's multiple range test

Table 3: Growth of bioinoculants in urea amended LB media

PGPRs	Concentration of urea in LB (%) OD at 560 nm			
	0	4	8	12
<i>Azospirillum</i> spp.	1.4138 $\pm$ 0.05 <sup>a</sup>	0.0409 $\pm$ 0.003 <sup>a</sup>	0.0121 $\pm$ 0.001 <sup>a</sup>	0.0
<i>P. putida</i> (PSB)	1.9691 $\pm$ 0.34 <sup>c</sup>	0.0465 $\pm$ 0.007 <sup>b</sup>	0.0223 $\pm$ 0.001 <sup>d</sup>	0.0
<i>P. putida</i> (KSB)	1.9718 $\pm$ 0.22 <sup>d</sup>	0.0677 $\pm$ 0.005 <sup>d</sup>	0.0137 $\pm$ 0.002 <sup>b</sup>	0.0
<i>B. cepacia</i> (KSB)	1.7376 $\pm$ 0.28 <sup>b</sup>	0.0646 $\pm$ 0.009 <sup>c</sup>	0.0145 $\pm$ 0.003 <sup>c</sup>	0.0
SE $\pm$	0.22	0.13	0.04	-
CD at p = 0.05	1.08	0.21	0.12	-

LB: Luria Bertani, Values are mean of three replications, Standard deviations followed by the same letter are not significantly different (p<0.05) as determined by Duncan's multiple range test

PGPRs strains may withstand themselves in organic materials either coir pith or vermicompost by observing the nutrients from the carrier materials and made available the inbuilt nutrients of such organic materials to the plants well. In order to field application of PGPRs through proper bridge material and to deliver the organisms and nutrients to soil and plant should be in need of better organic carrier material (Balamurugan *et al.*, 2011). Besides plant growth promotion, PGPR can induce resistance in plants to various pests and diseases by activating latent defense related compounds or enzymes (Mansour and Shaaban, 2007). Incorporation of organic matter in soil enhances the organic carbon level and also improve the soil fertility (Prakash *et al.*, 2007). The organic manure (FYM or VC) increasing the mineral nutrients, growth hormones, vitamins and improving other physical characters in soil (Islam *et al.*, 1998) might have significant influence on

microbial population. Those organic materials, vermicompost and coir pith play dual role in supplementing nutrients to plants meanwhile to be as shelter to PGPRs. Moreover the moisture retaining capacity of vermicompost was poorer than in coir pith which may be reason for retaining higher colony forming units of PGPRs in it (Balamurugan *et al.*, 2011). The bioinoculants may help in increasing crop productivity by increasing biological N fixation, availability or uptake of nutrients through solubilization or increasing absorption, stimulation of plant growth through hormonal action or antibiosis or by decomposition of organic residues (Mansour and Shaaban, 2007; Wani and Lee, 1995).

**Compatibility between neem cake extract and plant growth promoting rhizobacteria (PGPR):** Compatibility between Neem cake extract and Plant growth promoting rhizobacteria results were presented in Fig. 1. The growth pattern of bioinoculants were higher in 25% neem extract added LB when compared to control and higher concentrations (50 and 75%) of neem amended LB media. Maximum growth at 25% of neem extract was obtained from *Azospirillum* sp., followed by *P. putida* (KSB) and *B. cenocepacia* (KSB).

**Influence of inorganic nutrients (MOP, urea and RP) on growth of PGPRs:** Influences of inorganic nutrients (MOP, Urea and RP) with PGPRs were studied. In the case of MOP on growth of PGPRs revealed that 3% of MOP showed optimum concentration. Among all *Azospirillum* spp., showed maximum growth (2.5 OD at 560 nm) at 3% of MOP amended LB followed by PSB (2.0 OD at 560 nm) and KSB (1.7 OD at 560 nm). When concentration of MOP increased to 6 and 9% decline in growth of PGPRs was observed (Fig. 2). In the cases of urea the growth of bioinoculants were less at lowest concentration (4%). There was no growth observed at 8 and 12% urea concentration. Among different concentration of RP tested (3.5, 7.0 and 10.5%) growth of bioinoculants were enhanced while concentration increased. Potassium and phosphate solubilizing bacteria showed maximum growth during the increase of concentration of RP (Fig. 3).

The lowest population of these bacteria in the medium supplied with chemical fertilizers in our findings which may be due to the absence of organic media and no simulative effect to increase the bacterial population. Similarly, the occurrence of natural nitrogen fixing bacteria i.e., *Azotobacter* and *Azospirillum* in uninoculated organic treatments also showed the significantly higher value over the inorganic treatments. This is in conformity with the findings of Bhavalker (1991) and

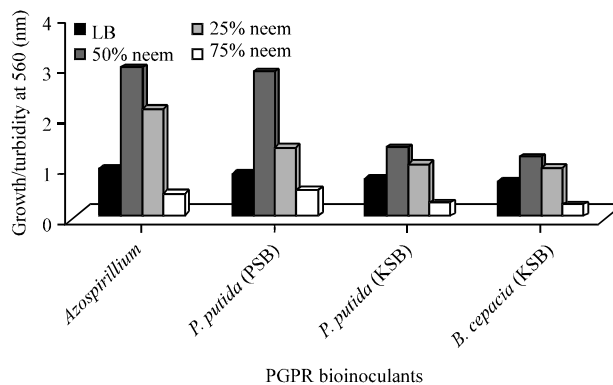


Fig. 1: Compatibility between neem cake extract and bioinoculants (PGPR), LB: Luria Bertani medium

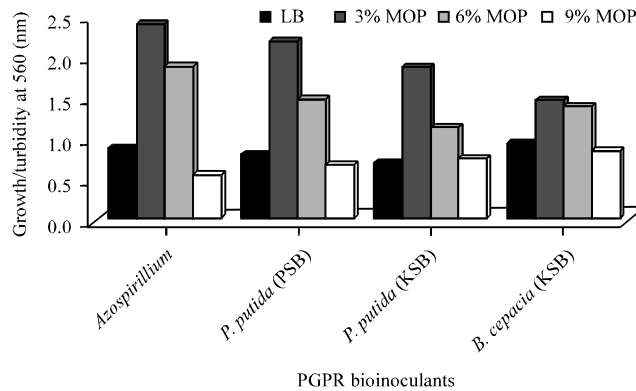


Fig. 2: Growth pattern of bioinoculants in MOP amended LB media, MOP: Muriate of potash, LB: Luria Bertani medium

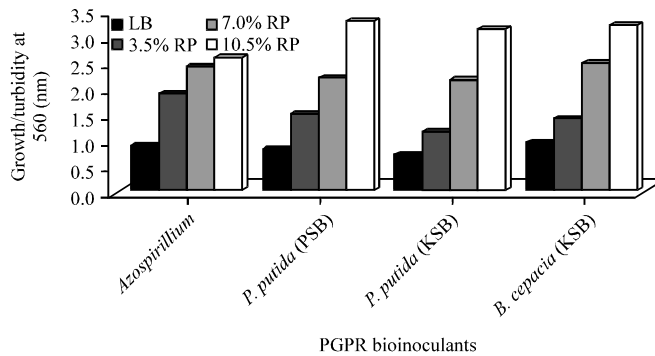


Fig. 3: Growth pattern of bioinoculants in RP amended LB media, RP: Rock phosphate, LB: Luria Bertani medium

Jayathilake *et al.* (2006). The bacteria themselves are stimulated to secrete additional enzymes, liberating more Ca, Fe and P until both the humic acid and bacterial population are satisfied (Mansour and Shaaban, 2007). Nawaz *et al.* (2003) reported that the increase application of nitrogen resulted in oil content of sunflower. In the same way, trace elements of organic and inorganic manures are also converted into forms more easily used by plants. Therefore, plants grown on soils which contain adequate nutrients produce higher yield and the nutritional quality of harvested foods and feeds are superior.

The soil environment may lose its health, microflora and nutrient status by receiving only inorganic sources of fertilizers. In this context, role of biofertilizers and organic manures are very important to revive the soil environment and bring back to healthy condition. Straight application of PGPRs will not be economical. However, when they are incorporated with any of the organic carrier materials such vermicompost and coir pith will be minimized the cost of application. Such organic nutrients as carrier materials not only provide shelter to PGPRs and they are having nutrient content too for supplying to the tea crop. The study might be useful and provide information on the better utilization of integrated source with organic materials and inorganic fertilizers. Integrated use of inorganic and organic fertilizers should be employed to maximize economic yield and to improve soil health (Ismail *et al.*, 2001; Ramanjavenyulu *et al.*, 2006). The importance of the use of organic sources of nutrients along with chemical fertilizers for maintaining soil health has been emphasized by Katyal (2000) and Singh *et al.* (2011).

## CONCLUSION

Intensive farming practices produces high yield and quality requires the extensive use of fertilizers that are costly and it create environmental problems. In this regard recent research moves towards the ecofriendly and sustainable agricultural practices. In our research findings the optimum tolerance level of PGPR organisms with neem cake and Muriate of potash will be useful to make integrated nutrient management approach and reduce manpower implementation by minimizing the application rounds. The higher compatible effect of organisms with Rock phosphate, vermicompost and composed coir pith will be immensely helpful to boost the tea plantations by balanced nutrient supply with minimized consumption of synthetic fertilizers consequently to improve the yield and quality too. The above study provide the authentic information on nutrient supply to the tea plantation through different sources of fertilizers under ecofriendly manner with minimized level of synthetic fertilizers for sustainable yield and quality with better soil health environment.

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