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RESEARCH ARTICLE

PHYSICO-CHEMICAL PROPERTIES, SOIL ENZYME ACTIVITY AND POPULATION DENSITY OF TEA RHIZHOSPHERE MICROFLORA OF INM TREATED POT CULTURE SOILS

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ABSTRACT

In modern agricultural practices recommendations were made to apply synthetic fertilizers in tea plantations. Five splits of fertilizer applications were done every year coinciding with wet seasons in plantations. Plants did not utilise the applied fertilizers completely for their growth and development, the required amount of nutrients plants uptake and the rest of its requirement is met by the activity of population of soil microflora to solubilise the unavailable form. Continuous application of chemical fertilizers is harmful to the rhizosphere microorganisms. A pot culture experiment was carried out to document the impact of plant growth promoting rhizobacteria (PGPR) under integrated nutrient management (INM) system on soil fertility, soil enzymes and microbial population. The treatments includes 100% NPK, 100% NPK along with bioinoculants, 75% NPK + bioinoculants, 50% NPK + bioinoculants, bioinoculants alone and control. Results revealed that nutrient status and soil microflora was enhanced and reduction of 25% NPK combined with bioinoculants registered significant variation among the various treatments. Detailed results were presented and discussed.

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INTRODUCTION

Plant nutrition is closely associated with the activity of rhizosphere microflora, which in turn plays an important role in supplying nutrients as well as growth promoting substances to the plants. Indiscriminate usage of chemical fertilizers though increased the yield, depleted the *nutrient level of the soil and polluted the environment and ground water resources. Public* awareness on the adverse effect of the large scale use of chemical fertilizers emerged the use of bioinoculants as a possible alternative to intensive agriculture (Anonymous, 2015). Bioinoculants considerably supplement the nutrients; minimize nutrient leaching in the soil, leading to possible partial withdrawal of inorganic fertilizers from the existing fertiliser recommendations. Earlier a pot culture experiment was carried out to document the effect of PGPR strains on seed germination (Princy, 2017). Results indicated that PGPR strains inoculated along with seeds registered significantly higher rate of germination. However the study was confined only to the germination and there is no consolidated report about the soil enzymes, soil nutrient status and rhizosphere microflora. Hence the present study was conducted and the results were presented and discussed.

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MATERIALS AND METHODS

The study area: Present investigation was executed under the auspicious Department of Plant Physiology and Biotechnology, UPASI Tea Research Foundation, Tea Research Institute, Valparai 642127, Tamil Nadu. Native plant growth promoting rhizobacterial (PGPR) strains were screened, isolated and mass multiplied at Department of Pathology and Microbiology, UPASI Tea Research Foundation, Tea Research Institute, Valparai 642127, Tamil Nadu. Greenhouse assessments were undertaken at UPASI KVK and UPASI Tea Research Foundation, Coonoor 643 101, The Nilgiris, Tamil Nadu.

Source of bioinoculants: Bioinoculants used in the present study were isolated, identified, characterised and mass multiplied for large scale utility by the parental institution, UPASI Tea Research Foundation, Tea Research Institute, Valparai 642 127, Coimbatore District, Tamil Nadu. The native PGPR strains identified as N fixers, *Stenotrophomonas rhizophils* (NF1) and *Bacillus* sp. (NF2) were offered the accession numbers as KP004437 and EMPL-HF934967, respectively by NCBI Database, New Delhi, while NF 1 and NF 2 are the code provided by host institute. P solubilisers, PSB 5 and PSB 9 were identified as *B. thurungiensis* and *B. amyloliqueofaciens* and they were given the accession

numbers, KP004438 and KP004439, respectively, by NCBI Database. K mobilisers isolated from the tea ecosystem were recognized as *Pseudomonas nitroreducens* (KSB 2) and *Burkholderia cepacia* (KSB 9). Accession numbers provided by NCBI Database are JQ618330 and JR610569, respectively (Princy, 2017). Ability of rapid multiplication of bioinoculants was tested using various media composition and colonization was detected by observing the population level of bioinoculants in the serial dilution and plate count method[2]. The higher cfu level of organisms was used for preparation of bioformulations. Multiplied N fixers, PSB and KSB cultures were mixed with sterile carrier materials. The viable count in the inoculum was 2 x 10^9 cfu/ml prior to mixing with carrier material. Prepared biofertilizers maintained proper moisture level, not exceeded 40%. In nursery, INM experiment was conducted using "China" jat (UPASI-9) and the experiment was repeated two times between 2014 and 2015. Mud pots were filled with 10 kg of the soil and sand mixture and prior to filling the pots, the soil pH (4.8) and EC (0.05) were adjusted. Before planting, consortium of bioinoculants (3.0 g per pot as booster dose) comprised of nitrogen fixers, PSB and KSB mixture was applied. Each trial had six treatments which were replicated four times and every replicate had 25 plants each. The treatment details are as follows: T1 - recommended practice (100% NPK), T2 - 100% NPK + Bioinoculants (*N fixers,* PSB and KSB), T3 - 75% NPK fertilizers + bioinoculants, T4 - 50% NPK fertilizers + bioinoculants, T5 - Bioinoculants alone and T6 - control. Sleeve grown UPASI-9 plants (10 months old) were transferred in each pot where the shoot length measured about 30 cm height and the adventitious root registered between 24 and 27 cm in length. Pot transferred plants were maintained under nursery conditions where the relative humidity was maintained at 80 $\pm$ 5% and temperature at 25 $\pm$ 2^{o}C. Manuring of the pot cultured tea plants were done as per the treatments. Soluble tea nursery mixture contained ammonium phosphate (20:20) 60 parts by weight, potassium sulphate or muriate of potash 24 or 20 parts respectively and magnesium sulphate 16 parts. In other words above nutrients (30 g in 10 litres of water (3.0%) to cover about to 400 to 450 plants) were applied fortnightly as per the treatments (Durairaj, 2015). Initial dose of bioinoculants was incorporated by mixing them with soil prior to filling the pots before planting. After six months booster dose, bioinoculants was applied making a hole away from the main stem and filled with soil. Maintaining of soil moisture, plant protection measures and other operations were executed uniformly, irrespective of the treatments.

Soil sampling from pot culture: Prior to the start of the experiment (pre-treat samples) and at the end of the experiment (post-treat samples) were collected according to the treatments and subjected to soil enzymes, nutrient analysis and microbial enumeration.

Estimation of chemical properties and quantification of nutrients: Soil samples collected were subjected to the analysis of pH, EC, OM, available P, K, Ca and Mg by adopting standard procedures. Hydrogen ion concentration and electrical conductivity were estimated with soil:water (1:2) aqueous suspension. Organic carbon was estimated by adopting Walkley and Black method as suggested byJackson (Jackson, 1962). Soil OM computed with the OC values (OM contains 58% carbon, then percentage of OC multiplied by 1.724 will give the percentage of OM). Soil available phosphorus was estimated by extracting the sample with Bray II as reported by Bray and Kurtz (Bray, 1945) and quantification of P by ascorbic acid method using spectrophotometer as per Murphy and Riley (Murphy, 1962). The amount of available K was determined by extracting the soil with one molar ammonium acetate solution and estimating extracted K by flame photometric method (Jackson, 1962). Available Ca and Mg were estimated by titrimetric method (EDTA titration) after extracting the soil with one molar ammonium acetate solution (Walter, 1965).

Hydrolytic enzymes of tea soils: The native level of urease activity was determined using the method suggested by Tabatabai and Bremner (Tabatabai, 1972). Nitrate reductase activity was assayed as per the method reported by Tabatabai (Tabatabai, 1994). Acid phosphatase activity was assayed using p-nitro phenyl phosphate disodium as substrate (Tabatabai, 1994).

Microbial enumeration: Collected soil samples were used for the enumeration of microbes. Media used for enumeration of bacteria is nutrient agar (Gordon, 1973) while for fungi it was Rose Bengal agar medium (Martin, 1950). For the enumeration of *Actinomycetes*, starch casein agar medium (Kuster, 1964) was used and for *Azospirillum*, nitrogen free malic acid medium (Dobereiner, 1976) was utilised. Pikovskaya's medium (Pikovskaya, 1948) for PSB, *Trichoderma* selective medium for *Trichoderma* and King's B medium (King, 1954) for *Pseudomonas* were used. Number of colonies formed were counted and expressed as number of colonies per gram dry weight of soil sample (cfu/g).

RESULTS AND DISCUSSION

Tea plants flourishes well under acidic conditions (Barua, 1989). As the NPK 100% was applied to the plants, the soil pH registered extremely acidic pH while the pH value was on par with that of NPK 100% + bioinoculants. When the NPK mixture reduced from 100% to 75% the pH value was significantly different and it is on par with that of 50% NPK along with bioinoculants and bioinoculants alone (Table 1). In the case of extremely acidic conditions, along with NK magnesium sulphate was recommended for field planted young tea[3]. Since the pH values were monitored at the end of the experiment, inclusion of magnesium sulphate was not possible. It may be noted that prior to start of the experiment, the soil pH was adjusted to 4.8 (desirable range). With regard to EC of the soils there was no dramatic variation exhibited by the treatments, however, NPK fertilisers declined the EC significantly from the pre-treat value of 0.05, except 50% NPK + bioinoculants (Table 1). As the elevation of the Coonoor, the Nilgiris is higher than 1850 m above MSL, usually the organic matter content will be higher than that of mid elevation areas like the Anamallais in Coimbatore District and Vandiperiyar at Kerala. Organic matter content due to the treatments were significantly higher in INM when compared to that of NPK 100% while bioinoculants alone registered on par values with inorganic fertilisers alone. Since the OM results are computed for OC, similar trend was followed in the case of organic carbon content. Available P was on par with each other in the case of NPK 100% NPK and 100% NPK + bioinoculants. The values registered by the other treatments significantly reduced from the 100% NPK. Since the variation is higher the critical variation was registered higher value of 13.24. This may be due to the sampling error or due to the slow activation of the PSB included in the treatments under high elevation areas like the Nilgiris.

Table 1. Physico-chemical properties of soils (post treatment) of pot culture experiment on INM

Treatment	pH*	EC	OM	OC	P	K	Ca	Mg
100% NPK	4.08	0.05	8.70	5.06	39.17	308	675	142
100% NPK+ Bioinoculants	3.92	0.04	9.74	5.65	39.23	241	654	152
75% NPK + Bioinoculants	4.36	0.04	9.22	5.35	30.67	221	718	152
50%NPK + Bioinoculants	4.16	0.05	9.34	5.42	25.77	219	773	152
Bioinoculants alone	4.33	0.05	9.14	5.30	22.33	201	733	152
Control	4.32	0.05	8.65	5.02	16.09	184	612	142
Statistical significance at P = 0.05:								
S.E.	0.08	0.003	0.27	0.11	1.41	6.65	4.99	1.46
C.D.	0.16	0.006	0.52	0.21	2.76	13.03	9.77	2.86
C.V. (%)	5.96	10.25	8.62	7.18	13.4	8.38	4.82	8.76

pH*: soil pH; EC: electrical conductivity, dSm^{-1}; OM: organic matter in per cent; OC: organic carbon in per cent; P: available P, mg/kg; K: exchangeable K, mg/kg; Ca: exchangeable calcium, mg/kg and Mg: exchangeable magnesium, mg/kg

Table 2. Soil enzyme activity of pot culture experiment on INM

Treatment	Urease*	Acid phosphatase	NRA
100% NPK	52.3	85.7	0.47
100% NPK+ Bioinoculants	50.0	92.7	0.54
75% NPK + Bioinoculants	44.1	68.2	0.48
50%NPK + Bioinoculants	42.5	67.8	0.37
Bioinoculants alone	47.0	87.7	0.38
Control	39.7	65.2	0.33
Statistical significance at P = 0.05:			
S.E.	2.89	3.46	0.03
C.D.	5.66	6.78	0.07
C.V. (%)	18.4	12.9	22.1

Urease*: urease activity, µg of NH4-N formed per g of soil; Acidphosphatase, µg of p-nitro phenol released per g soil; NRA: nitrate reductase, µg of potassium nitrite formed per g of soil

Table 3. Microbial density in response to INM treatment in pot culture soils

Treatment*	Colony forming unit / gram of soil						
	Bact.	Fungi	Acti.	Tricho.	PSB	Azo	Pseu.
T1	6.55	6.9	2.30	1.03	4.04	1.42	0.95
T2	7.55	1.9	2.65	2.15	2.85	1.33	0.75
T3	6.85	2.95	2.30	1.25	3.35	1.05	1.05
T4	6.25	3.3	2.35	1.51	4.25	1.05	0.65
T5	4.85	3.3	2.70	1.95	3.35	1.12	0.34
T6	3.52	1.9	1.75	2.53	3.27	0.99	0.34

Treatment*: T1: 100% NPK, T2: 100% NPK + bioinoculants, T3: 75% NPK + bioinoculants, T4: 50% NPK + bioinoculants T5: bioinoculants alone and T6: control. Bact.: total bacteria, (x 10^6); Fungi: total fungi, (x 10^5); Acti.:Actinomycetes(x 10^5); Tricho.: *Trichoderma*, (x 10^4); PSB: phosphate solubilising bacteria, (x 10^5); Azo.: Azospirillum, (x 10^6); Pseu.: Pseudomonas, (x 10^5)

It may be noted that the same bioinoculants (PSB strains) performed desirable level at the Anamallai conditions (Princy, 2017). Here again, the applied inorganic phosphatic fertilisers not utilised properly by the plants, hence, the soil available P was high in 100% NPK and 100% NPK + bioinoculants. It has already reported that the P is slowly mobile nutrient from soil to plant continuum (Raj Kumar, 1991). Identical trend was observed with the values of exchangeable potassium. But soil available K was significantly lower in the bioinoculants alone incorporated soils. Calcium content in the soils are significantly varied but it was not followed any trend (Table 1). NPK 100% + bioinoculants registered very low value followed by 100% NPK. On the other hand, reduction of inorganic fertilisers and bioinoculants alone registered significantly higher values of Ca content in the soils. Magnesium content of treated soils showed significant variations, especially Mg content of the K 100% treated soil was significantly lower than that of other treatments. This may be due to incorporation of bioinoculants as INM or as organic fertilisers alone. Magnesium is yet another important nutrient even though a relatively small quantity is taken up by the plants. Apart from its presence in the chlorophyll molecule, Mg is required non-specifically by large number of enzymes involved in phosphate transfer (Raj Kumar, 1991). This line of research is further needed with respect to PGPRs performance.

With respect to soil enzymes, urease, acid phosphatase and nitrate reductase activity were assayed (Table 2). Urease activity was registered low by the treatments 50% NPK along with bioinoculants, 75% NPK + bioinoculants and bioinoculants alone registered the values statistically on par with each other. It is interesting to note that both 100% NPK alone showed higher values an increase in the activity of urease activity but it is on par with that of 100% NPK along with bioinoculants. As far as acid phosphatase activity was concerned the treatments 50% NPK along with bioinoculants and 75% NPK + bioinoculants recorded statistically on par values. NPK (100%) alone and bioinoculants alone exhibited statistically identical values in terms of acid phosphatase. However acid phosphatase activity recorded by 100% NPK along with bioinoculants registered higher values among the treatments. Both bioinoculants alone and 50% NPK along with bioinoculants registered significantly lower values in terms of nitrate reductase activity. However, other three treatments recorded statistically on par values with each other in terms of nitrate reductase activity. Soil microbes of pot cultured tea plants when considered, there was no identical trend in increase/decrease in their population density; it was irregular (Table 3). Bioinoculants showed lower values in terms of total bacterial count than that of NPK 100% along with bioinoculants, while NPK 100% + bioinoculants recorded

lower values than that of NPK 100% alone in terms of total fungi. *Actinomycetes* recorded in the present study revealed a different trend, where 75% NPK + bioinoculants and 100% NPK registered very low values than that of 100% NPK along with bioinoculants. The range of *Trichoderma* population varied from 1.00 to as high as 2.15. It is interesting to note that NPK combination inhibit the PSB population, even though it is compatible and the bioinoculants and inorganic fertilisers applied separately. The range of population density of *Azospirillum* was 1.05 to 1.40. The difference was very marginal. *Psuedomonas* level in bioinoculants applied treatments registered very low value when compared to that of 75% of NPK and bioinoculants. Phosphate solubilisation is mainly due to the production of microbial metabolites including organic acids which decreases the pH of the culture media (Shahid, 2012). Presence of P solubilising microbial population in the soils may be considered as positive indicator which utilising the microbes as biofertilisers. The study indicated that establishment of potential PGPR strains under given conditions influenced by number of factors which includes environmental, physico-chemical characteristics of rhizosphere and interaction of PGPRs with plants. Rhizosphere bacteria promote plant growth and yield either directly or indirectly (Glick, 1995). The direct growth promoting mechanisms are a) nitrogen fixation, b) solubilisation of P and K, c) sequestering of iron production of siderophores, d) production of phytohormones and e) lowering of ethylene concentration (Glick, 1999).

Moreover the bioinoculants are highly sensitive to N and K fertilizers. In case of P fertilizers, as the concentration of rock phosphate increased, the population of beneficial organisms increased concurrently. The compatible nature of tested PGPRs reflects the application of both of them as combined manner by which they solubilize the inorganic rock phosphate and make into available form to the plants (Premkumar, 2012). Decreasing inorganic fertilizers proportion from 75% to 25% declined the yield. Earlier study indicated that application of biofertilizers sustained the soil health in terms of soil micro flora and a reduction of 25% of NK fertilizers can be achieved when biofertilizers and/or vermicompost are included in the fertilizer schedule (Princy, 2017). As a result, savings on 25% of NPK fertilisers and mode and time of applications required reduced number of labour which in turn improves the benefit cost ratio when compared to that of conventional fertiliser schedule. It may be noted region specific (considering climatic and edhaphic factors) given prior importance.

Conclusion

Consolidated results obtained in the present study demonstrated that the possible utilisation of bioinoculants for sustenance of soil health in south Indian tea plantations especially under the Nilgiri conditions. There may be certain differences between pot culture studies and field experimentation. However, there will not be significant variations. Induction of biofertilisers in regular inorganic fertiliser schedule will reduce the cost of production to an extent under the field conditions.

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Key points

- Utility of bioinoculants in INM was established
- Inclusion of bioinoculants will improve the soil health
- Comprehensive data on soil nutrients, hydrolytic enzymes and microflora with pot cultured young tea
- Inclusion of bioinoculants in the fertilizer schedule will reduce the cost of production

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