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A. Balamurugan
Manager, Product Promotion,
Export Division, T.Stanes
Company & Ltd, Coimbatore
641 018, Tamilnadu.

B.bagyalakshmi
Department of Biotechnology, K.
S. Rangasamy College of
Technology, Tiruchengode - 637
215, Namakkal District,
TamiNadu.

P.Nepolean
Plant Pathology Division,
UPASI Tea Research Institute,
Nirar Dam B.P.O. Valparai
642127, Coimbatore District,
Tamilnadu.

T. Kuberan
Plant Pathology Division,
UPASI Tea Research Institute,
Nirar Dam B.P.O. Valparai
642127, Coimbatore District,
Tamilnadu.

J.Mareeswaran
Plant Pathology Division,
UPASI Tea Research Institute,
Nirar Dam B.P.O. Valparai
642127, Coimbatore District,
Tamilnadu.

R.Jayanthi
Plant Pathology Division,
UPASI Tea Research Institute,
Nirar Dam B.P.O. Valparai
642127, Coimbatore District,
Tamilnadu.

T.Princy
Plant Pathology Division,
UPASI Tea Research Institute,
Nirar Dam B.P.O. Valparai
642127, Coimbatore District,
Tamilnadu.

Correspondence:
A Balamurugan
Manager, Product Promotion,
Export Division, T.Stanes
Company & Ltd, Coimbatore
641 018, Tamilnadu.

Exploitation of *Pseudomonas* sp. for disease control and productivity in tea

**A. Balamurugan, B.Bagyalakshmi, P.Nepolean, T. Kuberan,
J.Mareeswaran, R.Jayanthi, T.Princy**

Abstract

A study was undertaken on exploitation of indigenous *Pseudomonas* strains isolated from tea rhizosphere for disease control and productivity in tea. This included *in vitro* studies on plant growth promotion and biocontrol properties like production of phytohormones, siderophore, hydrogen cyanide, phosphate solubilization, and resistance to agrochemicals. The ability of *Pseudomonas* sp. was determined by production of antimicrobial substances. Moreover, *Pseudomonas* sp. expressed synergistic activity with other beneficial bioinoculants which shows the way to make consortium. *Pseudomonas* sp. improved the soil nutrient status when applied integrately with 50% reduction of rock phosphate to provide higher yield of tea. The proposed study would provide the information on exploiting the *Pseudomonas* sp. as an ecofriendly and sustainable alternative to the existing chemicals for growth promotion and management of tea diseases.

Keywords: Disease control, Productivity, *Pseudomonas*, tea, etc.

1. Introduction

Tea is the most popular beverage produced from the shoots of commercially cultivated tea plants (*Camellia* spp.). In India, tea is being cultivated in the north eastern region particularly in Assam and West Bengal, besides in certain pockets in Himachal Pradesh, Uttar Pradesh and in the southern India regions of Tamilnadu, Kerala and Karnataka. Therefore foliar, stem and root diseases are of great concern in tea production and crop loss. The tea bush facilitates annual outbreak of the foliar and stem diseases and their development during the growing season. Chemical control measures are effective in controlling tea diseases (Premkumar and Baby, 2005). Moreover, food safety of tea beverage became major aspect due to pesticide residuals on tea leaves. The novel source of disease control may be of biological control which has been developed as an alternative to synthetic pesticide treatment. A variety of microbial antagonists and their metabolites have been reported to control several pathogens on various fruit and vegetables, but only little information is available on the biological control of tea foliar diseases. Moreover, chemical fertilizers are applied in tea soils to increase the maximum crop yield by providing major elements that are essentials for plant growth and productivity. Tea plantation solely depend the inorganic source of fertilizers so far in order to get sustainable yield and productivity. In present day context, organic farming implies that non chemical source of fertilizers to provide higher yield and better quality. Apart from this, the integration of biological, cultural and natural inputs to create an ecological balance. In other words a holistic and human centered approach which in turn ensure not only the soil health and it provides pollution free environment as well (Verma, 1998). With high productivity index, tea required enormous balanced macro and micro nutrients. Besides soil applications of fertilizers, nutrients were foliar applied to mitigate the deficiency symptoms. Fertilizer requirement vary in response to soil fertility and productivity of the tea crop. Fertilizers are broadcasted in splits between pre- to post monsoon periods. Continuous use of chemical fertilizers/foliar chemicals may adversely affect the phyllosphere and resulted in the decline in organic matter content of the soil leading to depletion of beneficial organisms in agricultural soil. In this context, non-chemical disease control strategies are gaining importance and which can be achieved by reducing chemical fertilizers and supplementing with organic sources of inputs and bioinoculants.

Microorganisms offer a biological system having evolved the mineral phosphate solubilizing traits to acquire sufficient phosphorus. Several soil bacteria, particularly those belonging to the genera *Pseudomonas* and *Bacillus* possess the ability to convert the insoluble phosphate and potassium into soluble/available forms by secreting organic acids then they were able to synthesis of plant growth hormones resulting in improvement of growth and yield of crop. However, concerns on environmental safety, pesticide residues in made tea, and escalating cost of fungicides, an alternative to chemical control, is the need of the hour. Being ecofriendly, economical and sustainable, biological control can be an alternative to fungicides or they can form an important component in integrated management of diseases where the chemical application is done when it is absolutely necessary. In case of nutrient supplement, alternate and ecofriendly source of nutrient supplement is of plant growth promoting rhizobacterial organisms (PGPRs) which can continuously provide nutrients to the plant and retain the soil fertility too. Microorganisms play a major role in the natural P and K cycle and there are considerable populations of P- or K-solubilizing bacteria in the soils and in plant rhizospheres (Sperberg 1958). Though PGPR'S has been successfully exploited in a wide range of cropping systems, their role in a plantation crop like tea is very much limited. Among the PGPRS, exploited, *Pseudomonas* spp. have been regarded as efficient in view of their rhizosphere abundance, excellent root colonizing ability, and their capacity to produce a diverse array of antifungal compounds. In the area of biological control, *Pseudomonas* spp. have emerged as the largest and most promising group of PGPRS. There are many reports available in various crops that they are capable of secreting antibiotics, PR enzymes, vitamins, growth promoting hormones and biological active substances. They lead the plant growth by producing growth hormones and minimize the fungal pathogens whichever invades the tea phylloplane and rhizosphere. The present study was attempted to evaluate biocontrol activity and plant growth promotion traits of indigenous *Pseudomonas* sp. collected from various tea growing districts of southern India.

2. Material and Methods

2.1 Isolation of *Pseudomonas* spp. from tea rhizosphere soils

Rhizosphere soil samples at 0-9" depth were collected from tea plantations of southern India. Further, potassium solubilizing bacteria (KSB) was isolated in Aleksandrov medium (Aleksandrov *et al.* 1967) incorporated with muriate of potash (MOP) and sulphate of potash (SOP) by following dilution plate technique. KSB colonies were identified by the formation of clear/transparent zone around the bacterial colonies due to the solubilization of K sources and selected for phosphate solubilizing study by using Pikovskaya's medium (Pikovskaya, 1948) supplemented with rock phosphate and single super phosphate. A clear zone around the bacterial colony indicated solubilization efficiency of phosphate sources.

2.2 Biocontrol activity and production of cell wall degrading enzymes

The biocontrol potential was performed by following dual inoculation technique using Potato dextrose medium (Saktivel and Gnanamanikam, 1987) against tea foliar pathogens, *viz.* *Pestalotiopsis theae*, *Glomerella cingulata*, stem pathogens *viz.* *Phomopsis theae*, *Hypoxyton serpens* and root pathogens *viz.* *Poria hypolateritia*, *Botryodiplodia theobromae*. The mineral salts medium was incorporated with various enzyme substrate sources in order to studying the production of cell wall degrading enzymes such as chitinase, cellulase and pectinase by selected strain. The substrates used were chitin, pectin, carboxy methyl cellulose and were individually added at 0.1% concentration in the basal medium. The strain was inoculated by simple streaking on the medium with suitable substrates and incubated at room temperature for 72 hours and to observe their growth. Utilization of the substrates by organism was designated as positive (+) and negative (-).

2.3 Estimation of plant growth promoters

The production of plant growth hormones such as indole acetic acid (IAA) and gibberellins (GA₃) by the bacterial strain was estimated in the basal medium supplemented with commercial IAA and GA₃ substrates by the method of Tien *et al.* (1979) and Mahadevan and Sridhar (1982) respectively. The production of siderophores, exopolysaccharide and hydrogen cyanide was also estimated by following the method of Cappuccino and Sherman (1992).

2.4 Resistance of bacterial strain to various agrochemicals

The pesticides such as Propargite, fenpyroximate, hexythiazox, thiamethoxam and deltamethrin, fungicides such as Carbendazim, Mancozeb, tridemorph, benomyl, tebuconazole and copper oxychloride were taken for the present study. They were prepared at the concentration of 0.5% recommended dose using sterile water and filter sterilized. They were added in glucose yeast extract medium and thereby *Pseudomonas* sp. inoculated and incubated at room temperature wherein, resistance of such organisms was observed by measuring OD at 560 nm using UV- Vis spectrophotometer.

2.5 Interactions among bioinoculants

An *in vitro* pot experiment was undertaken to study the interaction among nitrogen fixer, phosphobacteria and arbuscular mycorrhizae fungus (AMF). The nitrogen fixer, phosphobacteria and AMF are *Azospirillum lipoferum*, *Pseudomonas putida* and *Glomus intraradices* respectively. *A.lipoferum* and *P. putida* at each of 1x10⁷ cfu / ml and AMF at 60 infection propagules (IP) per gram were mixed with sterilized soil and taken in earthen pot. It was maintained at room temperature with keeping 40% moisture and sampling was done periodically till 90 days after incubation and was subjected to enumerate the population level of incorporated bioinoculants.

2.6 Field evaluation of *Pseudomonas* on productivity in existing tea plantation

Field experiments were conducted with selected strains of *A.lipoferum*, *P.putida* and AMF with various objectives to prove their potential in supplementing nutrients to tea. The biofertilizers were incorporated to the soil with composted coir pith as the carrier material. As a consortium of *Azospirillum*, Phosphobacteria and AMF bioformulations mixed with rock phosphate were incorporated near to the root zone by punching holes with a crowbar. The inorganic fertilizers, Urea/Diammoniumphosphate, Rock phosphate and Muriate of potash (5 splits/year) and organic manure (compost, neem cake and castor cake) @ 5 tonnes /ha. were used. The jat, "Jessie" - Assam seedlings were used and the experiment was initiated during 2008 and rate and time of bioinoculant applied at 10 g / plant during immediately after premonsoon showers (initial dose) followed by a booster dose prior to cessation of monsoon (2 splits every year). Chemical fertilizers were broadcasted (5 splits/year) according to the yield potential of the field. The green leaf yield was recorded at every plucking rounds and converted the yield to made tea (kg) per hectare by following formula,

| | |
|-----------------------------------|---|
| Made Tea Yield = (Kg per hectare) | Green leaf yield (kg) x Bush population per hectare x 0.225 |
| | Number of bushes in the experimental plot |

0.225 - conversion factor for green leaf to made tea (out turn)

2.7 Soil analysis

All the soil analysis was carried out in air dried soil samples. Methods and principles of the analyses are explained below. AR grade reagents were used for all analyses. Water used in the analysis was double distilled.

2.8 Determination of soil pH

About 30 g of air – dried soil which had already been passed through 2 mm sieve was taken in a 100 ml beaker. Then 60 ml of distilled water was added and stirred well with a glass rod thoroughly (Schofield and Taylor, 1955). After thirty minutes the previously calibrated electrodes were inserted in to the beaker containing the soil water suspension with constant stirring. The pH of the solution measured using the pH meter (Elico LI 120).

2.9 Determination of electrical conductivity

The soil samples were analysed for electrical conductivity (Mason and Obenshain, 1939) using the soil water suspension of 1:2 ratio prepared for the determination of pH. The soil suspension was allowed to settle in the beaker for about half an hour. The electrical conductivity of the soil was measured using conductivity meter (Systronics 304). The unit of electrical conductivity is dSm^{-1}

2.10 Estimation of soil organic matter

The soil samples were analysed for organic carbon content using Walkley and black method (1934). The principle involves the oxidation of organic matter present in the known quantity of soil by potassium dichromate and sulphuric acid

mixture. The excess dichromate was determined by titration with standard ammonium sulphate using ferroin indicator.

2.11 Estimation of Nitrogen

About 0.5 - 1 g. of ground leaf sample/soil sample/ made tea was taken in a Kjeldahl tube and the analysis was carried out using Kjeldahl method (Page *et al.* 1982). The principle involves the conversion of all forms of nitrogen into ammonium form by digesting with sulphuric acid, potassium sulphate, copper sulphate and sodium thiosulphate at 450 °C. Distillation was carried out in Gerhardt - Kjeltec instruments. The distillate was collected in a conical flask, which contains a known quantity of 0.05 N hydrochloric acid. The excess hydrochloric acid was then titrated against standard sodium hydroxide solution using digital burette.

2.12 Determination of available phosphorus in soil

The soil was extracted by solution containing 0.03 N ammonium fluoride and 0.1 N hydrochloric acid, which dissolves primarily Fe-P and Al-P and the extracted phosphorus was measured colorimetrically (Bray and Kurtz, 1945). The method is based on the reduction of $(\text{NH}_3)\text{PO}_4 \cdot 12\text{MoO}_3$ complex by ascorbic acid in the presence of antimony potassium tartrate.

2.13 Determination of exchangeable potassium in soil

The soil was extracted by 1 N ammonium acetate (pH = 7.0) according to the method given by Hanway and Heidal (1952). The intensity of potassium was directly measured in a flame photometer.

3. Results and Discussion

3.1 Biocontrol activity of bioinoculants against tea pathogens

A large number of plant diseases are successfully controlled by bacterial and fungal antagonists next to chemical sources of fungicides. Bacterial biocontrol agents are advantageous due to rapid growth, aggressive colonization, easy handling and better survival in the target environment. In this study, *Pseudomonas* sp. showed higher antagonism by producing inhibition zone more 2 cm against the tested pathogens (Plate 1) and it was able to antagonize the tea fungal pathogens to varied extent which may be due to production of certain antifungal compounds such as siderophore (35.6 μ moles / ml) and hydrogen cyanide (+++ grade level) and results were tabulated in Table 1. Similarly, the effectiveness of fluorescent *pseudomonads* against multiple pathogens have been reported earlier (Shivakumar, 2007). Similar biocontrol activity was observed by fluorescent *Pseudomonas* spp. against multiple pathogens in tea plants (Muralidharan *et al.* (2004). Our present studies revealed that KSB strain was found to produce siderophores, exopolysaccharide, and hydrogen cyanide belongs to volatile and non volatile compounds of bioactive compounds. Similar reports on production of siderophores, antifungal volatiles and antimicrobial metabolites by antagonistic fluorescent *Pseudomonas* spp. Siderophores play a vital role in the suppression of plant pathogens by chelation of ferric iron thereby creating competition for iron (Loper and Henckels 1999). Similar observation on the production of

polysaccharides by KSB has been made by several workers (Sheng and He 2006). The bacterial action on the formation of mucilaginous capsule consists of exopolysaccharide and organic acids were reported by Vijayabaskar *et al.* (2011). The secretion of polysaccharide by bacteria can bind metals and release potassium from insoluble minerals. When nutrient solubilization studies carried out, phosphate and potassium were solubilized by producing halo zones around the bacterial colonies in Pikovskayas and Aleksandrovs medium respectively (Plate 2). Because of phosphatase enzyme production by PSB, they are able to solubilize the rock phosphate and unavailable forms of other phosphorus in to available form in order to be taken up by plants. It could be very much helpful for minimizing the dose of inorganic fertilizers in field application and higher yield can be achieved. Production of plant growth promoting substances by fluorescent Pseudomonads have been widely studied with respect to biocontrol potential (Suneesh, 2004). Findings of Rashmi (2004) reported that biocontrol potential of P-solubilizing *Serratia* and *Pseudomonas* sp. besides producing plant growth promoting substances like IAA and GA₃ which very much supported the results of present studies. The mechanism of solubilization may be due to the production of various organic acids such as acetic, formic, gluconic, oxalic and succinic acids reported by several research works.

3.2 *In vitro* production of growth hormones and enzymes by bioinoculants

The bioinoculant, *Pseudomonas putida* was able to produce plant growth hormones *in vitro* at IAA (40.90 ppm) higher when GA₃ (16.81 ppm) (Table 1). These results indicated microbial inoculation can change the levels of IAA in the rice root environment (Raja *et al.* 2006). Similar observations on N₂ fixation, P-solubilization and production of IAA and GA₃ by *Azospirillum* and *Beijernickia* have been made earlier (Kulanthavel *et al.* 2006). With this view, attention was being focused on the use of organic manures and biofertilizers (*Azospirillum lipoferum* + phosphate solubilizing bacteria (Satter and Guar, 1987) are capable of producing growth promoting substances. Many plant growth promoting bacteria, which stimulate the growth of roots, can produce small amounts of auxin (Pattern and Glick, 1996).] Plant growth by PGPR bacteria includes the bacterial synthesis of plant hormones indole-3-acetic acid (IAA), cytokinin, gibberellin and breakdown of plant produced ethylene by bacterial production of 1-amino cyclopropane-1-carboxylate deaminase (Glick, 1995) and this might account for plant growth promotion in their study.

3.3 Resistance to agrochemicals

Agrochemicals especially pesticides, fungicides and herbicides could be used for minimizing pest, diseases and weeds respectively in tea plantation. They proved their adverse effect on growth of beneficial bioinoculants when mixed in the *in vitro* medium. In this present study, the selected bacterial biocontrol agent was tested for their compatibility and / or tolerance level with chemical pesticides and fungicides. Results obtained showed that, *Pseudomonas* sp. was tolerant to selected pesticides (propargite 57 EC, fenpyroximate 5 EC, hexythiazox 5.45

and thiamethoxam) whereas they were sensitive to deltamethin 28 EC. (Fig.1) But this bacterial biocontrol agent was highly tolerant to Fenpyroximate 5 EC. This finding was supported by Soumik *et al.* 2010.] In case of fungicides tolerance, *Pseudomonas* sp. was more compatible with all selected fungicides (Table 2). The organism was not much inhibited and strain also able to survive with all tested fungicides and it showed 5.0 x 10⁷ and 5.0 x 10⁷ cfu / ml when tebuconazole and benomyl fungicides amended respectively in the nutrient medium. Earlier reports suggested that biocontrol agents that can tolerate a certain level of fungicides which when mixed with agrochemicals resulted in eradication of diseases. This may be because of biodegradation and bioremediation properties of those beneficial biological organisms to break up the hazardous chemicals whichever reside in soil and consume energy from the agrochemicals. Like, when they meet in the soil environment and they got seriously affected with agrochemicals whichever used for pest and disease control and it was evidenced with the report of Gadkari (1987),] due to the incorporation of insecticides in the growth media causing either cell disruption or formation of cyst-like bacteria. The pesticides generally reduced the microbial population counts and inhibitory effect varied with different pesticides in tea soils (Bezbaruah, 1999). Antagonistic ability of *Pseudomonas* sp. and *Trichoderma* sp. combined with carbendazim strengthens the integrated approach of grey blight disease management in tea (Premkumar *et al.* 2011).

3.4 Interactions among bioinoculants under *in vitro*

The present study revealed that co-inoculation of nitrogen fixer, phosphobacteria (*Pseudomonas* sp.) and arbuscular mycorrhizae fungus (AMF) strains synergistically react with themselves. Moreover they solubilized rock P and other minerals which were added into the soil and made them much more available for uptake by plant roots. Results were documented and it was revealed that bioinoculants were compatible with each other and they are present at higher rate up to one month thereafter gradually reduced which may be due to lack of nutrients required by bioinoculants (Table 3). These three strains, showed good growth due to their mutualism for making consortium when they were mixed together and it implies to make a single consortium for field application in order to sustain higher yield, minimize synthetic fertilizers prevents pathogen population and retain soil health. Similar findings reported by Muthukumar *et al.* (2001) mentioned that combined inoculation of *G. intraradices*, *G. geosporum*, PSB and *A. brasilense* increased plant growth of carrot. The increase in phosphorus uptake by plants has been made possible by combining the abilities of the phosphorus solubilizing microorganisms and the efficient P uptake mechanisms of the VAM fungi (Dwivedi *et al.* 2003). VAM fungi produce obligate symbiotic association with plant roots and increase P availability by mobilizing the P with the help of their extrametrical hyphae, particularly in soil with less available P (Mosse, 1973).

3.5 Effect of INM on yield in existing tea plantation

The green leaf yield was recorded at every harvesting and it was expressed as made tea per hectare. Results revealed that

half recommended dose of inorganic fertilizers integrated with bioinoculants proved significantly higher yield (3145 Kg MT) next to standard practice (3290 Kg MT) (Table 4) when worked out the mean yield of 2010 and 2011. In the present study, introduction of BF resulted in 50% reduction in chemical fertilizers and BF treated plants performed better than un-inoculated control in terms of yield. Concurrent increase in the population of BF in rhizosphere of tea soils can improve the fertilizer use efficiency which ultimately leads to further reduction in application of chemical fertilizers and increase in nutrient reserve in soil over other treatments which may be due to proper exploitation of the introduced bioinoculants and compatible with the inorganics, thus resulted in improved performance of the treatment. Thamizh and Nanjan (1998) stated that the combined application of *Azospirillum*, phosphobacteria and VAM with 75% of recommended NPK (90:90:90 kg/ha) recorded higher yield (14.96 t/ha) which was 21 per cent higher than uninoculated control (11.93 t/ha) in potato. The yield and uptake of nitrogen and phosphorus was substantially increased due to use of bioinoculants. Premkumar *et al* (2012) observed that INM experiment conducted in Nilgiris and Central Travancore regions, 75% IOF + BF, 100% IOF+BF and conventional practice registered higher crop and they are significantly different at five per cent probability over untreated control and their report supported the result of the present study. Incorporation of beneficial PGPR consortium with reduced rate of IOF resulted in a progressive improvement in the yield. Rout *et al.* (2001) reported that biofertilizers along with inorganic N fertilizers can substitute up to 20% N fertilizers and can increase the maize yield. In the present study, introduction of BF resulted in 25% reduction in chemical fertilizers and BF treated plants performed better than un-inoculated control in terms of yield. Results were comparable with recommended dose of inorganic fertilizers and combination of rhizobium and PSB + 75% recommended dose of fertilizers + organics in soybean (More *et al.* 2009). The use of plant growth promoting rhizobacteria (PGPR), including phosphate and potassium solubilizing bacteria (PSB and KSB) as biofertilizers, was suggested as a sustainable solution to improve plant nutrient and production (Vessey 2003). Increase in N,P and K uptake generally occurred in the combined treatment i.e., soil coinoculated of N,P and K uptake in shoot and in the root. It is interesting to note that combined together bacterial inoculation and rock material fertilizer application also increased the N uptake by the plants (Han and Lee 2005).

3.6 Influence of INM on nutrient status of soil of tea plantation

In tea soil, the elements are continuously removed at each plucking round and thus in a year, considerable amount is removed from the soil which requires to be replenished to maintain the sustainable productivity of tea. Chemical fertilizers with instant ability to renovate deleted nutrients in necessary quantities and forms have come to be recognized as a key component of the soil fertility management and sustainable productivity (Karthikeyini, 2002). In the present study, the soil samples were subjected to analyse soil

parameters which indicated that pH was maintained as acidic wherever bioinoculants were incorporated (Table 5). The EC values were also recorded within favourable limits by the influence of bioinoculants. Even though organic matter was higher in organic manure applied plots, the integrated approach brought the soil organic matter high which is comparable to that of standard practice. The total nitrogen content was noticed higher in 50% IOF + BF (82%) next to standard practice (92%) during end of the experiment when compared to soil collected prior to start of the experiment. In case of available phosphorus and exchangeable potassium contents noticed significantly higher in the soil treated with 50% IOF + BF followed by half recommended dose of organic with biofertilizers. Productivity indicated by yield and harvest index and soil fertility indicated by available NPK in soil after harvest were significantly increased with the application of biofertilizer in combination with 50% N through organic manure (FYM or VC) and rest of 50% N and 100% PK through chemical fertilizer (Jayathilake *et al.* 2006). An integrated application of rock P and K materials with co-inoculation of bacteria that solubilize them might provide faster and continuous supply of P and K for optimal plant growth (Han *et al.* 2006). The enhanced uptake of P in AM-fungi-inoculated seedlings may be due to an increase in the number of uptake sites per unit area of roots and a greater ability of these roots to exploit the soil nutrients (Bolan, 1991).

4. Conclusion

The indigenous bacterial strain of *Pseudomonas* taken from soil of tea plantation ensure could be helpful to enhance nutrients use efficiency, enrichment of soil beneficial microbes, biocontrol of soil borne pests and pathogens, enhancement of soil fertility and organic matter, maintain acidic pH environment, improve soil fertility, minimize leaching process of nutrients, scope for possible reduction in NPK fertilizers under integrated approach in nutrient management in tea plantations. The enormous crop loss caused by pests and diseases requires implementation of efficient, eco-friendly control strategies alternative to chemical control. Since, combination of synthetic pesticides at reduced level and biocontrol agents for developing an integrated approach for pest and disease management on tea plantations are limited, the present findings will provide base-level data in this context. Development of integrated pest and disease management (IPDM) program will surely be helpful in reducing the usage of synthetic chemicals which in turn will have an impact on the environment by reducing the unwanted contamination of the tea soils apart from the health benefits of the workers who are directly handling the toxic chemicals used for the control of pest and diseases in tea plantation.

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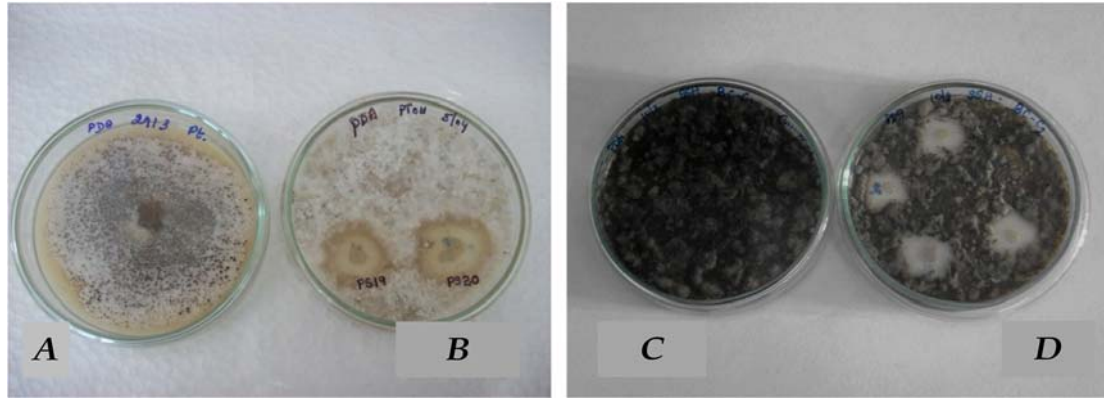


Plate 1: Biocontrol activity of *Pseudomonas* against tea fungal pathogens

A & C. Untreated control plates of *Pestalotiopsis* and *Macrophoma* sp. respectively

B & D. *Pseudomonas* sp antagonized the growth of *Pestalotiopsis* and *Macrophoma* sp. by producing inhibition zones

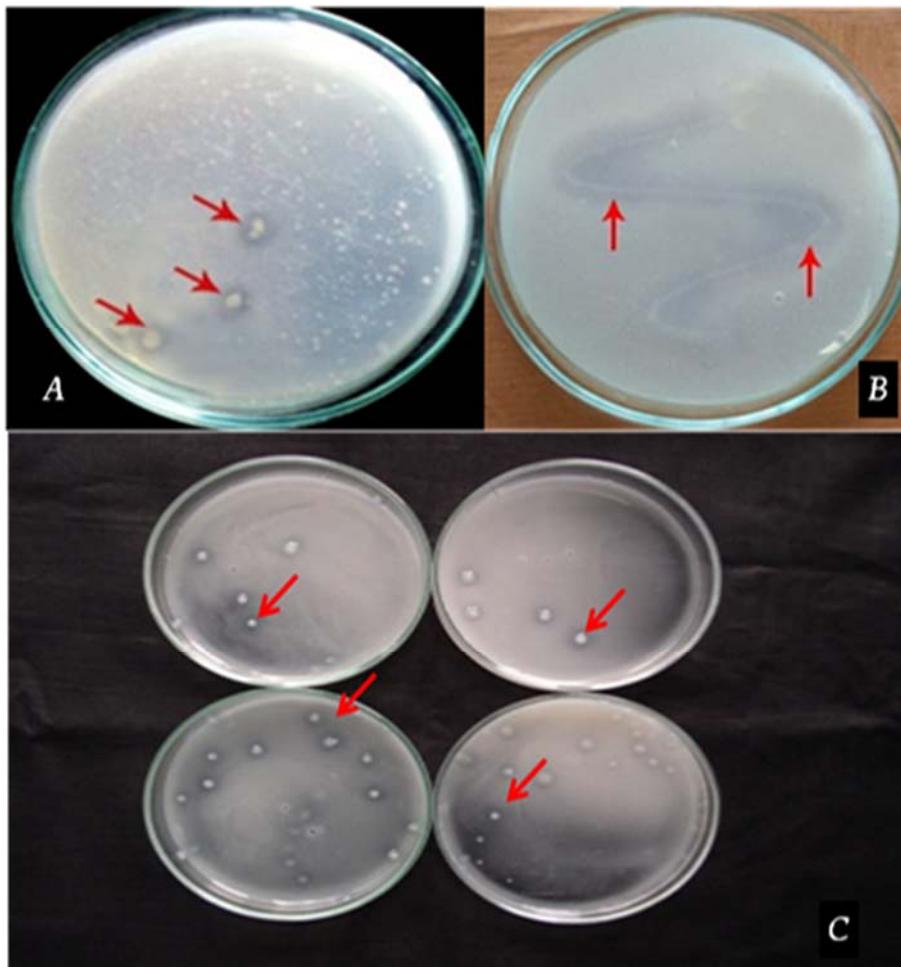


Plate 2: Growth of *Pseudomonas* sp in Aleksandrov agar (A & B) and Pikovskayas medium (C) - arrows indicate P & K solubilizing halo-zone around the bacterial colonies.

Table 1: *In vitro* production of *Pseudomonas* sp.

| Parameters | Production by <i>Pseudomonas</i> sp. |
|---------------------------------------|--------------------------------------|
| IAA (ppm) | 40.90 |
| GA ₃ (ppm) | 16.81 |
| Siderophore production (μ moles / ml) | 35.6 |
| Hydrogen cyanide | +++ |
| EPS (exopolysaccharides - ppm) | 43.8 |

Table 2: Resistance of *Pseudomonas* against fungicides

| Fungicides (0.5 %) | Carbendazim | Mancozeb | Tridemorph | Benomyl | Tebuconazole | COC | Control |
|---|-------------|----------|------------|---------|--------------|-----|---------|
| <i>Pseudomonas</i> (10 ⁷ cfu / ml) | 4.0 | 2.1 | 4.4 | 5.0 | 5.6 | 2.6 | 7.3 |

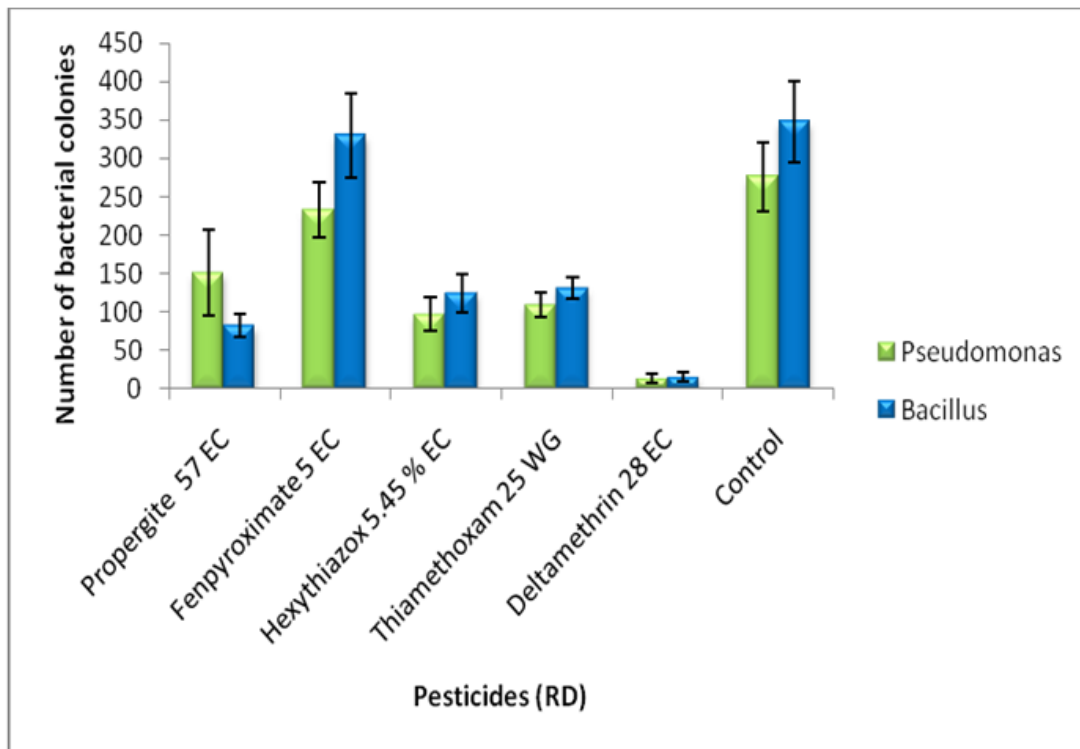


Fig 1: Sensitivity of bacterial biocontrol agents to pesticides used in tea

Table 3: Synergism among bioinoculants

| Incubation periods (days) | <i>A. lipoferum</i> (x 10 ⁷ cfu/g) | <i>P. putida</i> (x 10 ⁷ cfu/g) | AMF (%) |
|---------------------------|---|--|---------|
| Initial | 78.0 | 17.3 | 60 |
| 7 th | 125.0 | 42.0 | 65 |
| 15 th | 42.3 | 62.4 | 83 |
| 30 th | 42.0 | 93.6 | 105 |
| 45 th | 36.5 | 40.6 | 92 |
| 60 th | 19.3 | 26.8 | 66 |
| 75 th | 6.2 | 30.0 | 15 |
| 90 th | 7.2 | 5.0 | 0 |

Table 4: Field evaluation of *Pseudomonas* on productivity in tea plantation

| Treatment | Yield (made tea per hectare in Kg) | | | |
|--------------------|------------------------------------|------------|------------|------------|
| | 2009 | 2010 | 2011 | Mean |
| 100% IOF | 3018 | 3930 | 2920 | 3290 |
| 100% OM | 2580 | 3258 | 2708 | 2849 |
| 100% BF | 2382 | 3219 | 2544 | 2715 |
| 50% IOF + OM | 2714 | 3322 | 2623 | 2886 |
| 50% OM + BF | 2467 | 3292 | 2626 | 2795 |
| 50% IOF + BF | 2949 | 3731 | 2756 | 3145 |
| 33% IOF+OM+BF | 2669 | 3429 | 2768 | 2955 |
| Control | 2351 | 3188 | 2495 | 2678 |
| CD @ = 0.05 | 288 | 304 | 236 | 276 |

Table 5: Influence of *Pseudomonas* on nutrient status of tea plantation soil

| Treatment | Initial | | | Final | | |
|--------------------|--------------------|------------------------------|--------------------------------|--------------------|------------------------------|--------------------------------|
| | Total nitrogen (%) | Available phosphorus (mg/kg) | Exchangeable potassium (mg/kg) | Total nitrogen (%) | Available phosphorus (mg/kg) | Exchangeable potassium (mg/kg) |
| 100% IOF | 78 | 45 | 154 | 92 | 57 | 245 |
| 100% OM | 55 | 71 | 196 | 68 | 46 | 220 |
| 100% BF | 72 | 54 | 310 | 75 | 78 | 272 |
| 50% IOF + OM | 60 | 48 | 274 | 66 | 95 | 346 |
| 50% OM + BF | 64 | 62 | 236 | 59 | 112 | 325 |
| 50% IOF + BF | 65 | 86 | 207 | 82 | 128 | 386 |
| 33% IOF+OM+BF | 69 | 78 | 184 | 78 | 96 | 215 |
| Control | 74 | 59 | 176 | 60 | 45 | 211 |
| CD @ = 0.05 | 14 | 9 | 37 | 10 | 22 | 68 |

BF – biofertilizers containing N₂ fixer, *Pseudomonas* and AMF

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