

BIOCONTROL STUDIES ON RHIZOSPHERIC MICROORGANISMS AGAINST BLACK ROT DISEASE OF TEA CAUSED BY *CORTICIUM THEAE* BERNARD

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Abstract

Assessment of plant growth promoter and biocontrol properties of plant growth promoting rhizomicroorganisms (PGPR) from tea soil against black rot disease agent of tea caused by *Corticium theae* Bernard in Bangladesh was done. The antagonistic microorganisms were isolated from rhizosphere soils of tea fields and cultured on different nutrient media. The isolates were screened for their antagonism against *Corticium theae* by dual culture technique. The microbial strains were inoculated with tea nursery soils by mixing with 50 g of decomposed cowdung. In tea plantations, the microbial strains were sprayed on diseased plants two times at 15 days intervals. Four different species of PGPR strains such as *Bacillus*, *Pseudomonas*, *Streptomyces*, *Trichoderma* were isolated from rhizospheric soil of tea. These PGPR strains enhanced plant growth in nursery and had a positive effect on the rate of increased in number of leaves, height of plants and girth of plants by 33, 43 and 3%, respectively. Lowest severity of black rot was found in plants treated with *Trichoderma* followed by *Bacillus*, *Pseudomonas* and *Streptomyces* strains. *Trichoderma* and *Bacillus* caused 16 and 14% reduction of disease severity while both *Pseudomonas* and *Streptomyces* strains reduced disease severity by 10%. All the PGPR's have a great influence in reducing disease severity by 19% with optimistic relations. Radial mycelial growth of *C. theae* was also inhibited in similar trends. The biofertilizer showed comparatively lower response in reducing disease severity (8%) in comparison to PGPR's. It can be concluded that *Bacillus*, *Pseudomonas*, *Streptomyces* and *Trichoderma* isolated from tea soil have their growth enhance capacity as well as decrease the disease severity of black rot in tea.

Introduction

Camellia sinensis (L.) O. Kunze known as tea, is one of the oldest, nonalcoholic, beverage yielding perennial crop widely consumed all over the world. It is one of the largest agro-based industries in the country. There are 169 tea estates having about 59.61 thousand hectares of land under tea plantation producing about 63.86 million kg of made tea with average yield of 1,239 kg/ha during 2014 (PDU 2015). The intensive mono culture of a perennial crop like tea over an extensive and contiguous area in apparently isolated ecological zones in Bangladesh has formed virtually a stable ecosystem which provided unlimited opportunity for perpetuation and spread of endemic and introduced diseases (Alam 1999). The architecture of tea plantation, variability of plant types and the systemic interaction of various agro-techniques, intercultural operations etc. impose a significant impact on development of diseases. Diseases are one of the most common barriers for the production of tea. More than 400 pathogens cause various diseases in tea (Thoudam and Dutta 2012) viz., foliage, stem and root infections. The loss of tea in Bangladesh tea due to various pests, diseases and weeds has been estimated to be about 10-15% (Sana 1989). Black rot is a most destructive leaf disease of tea caused by *Corticium theae* Bernard. (Ali 1992).

The microbial diversity of tea soil is extremely diverse (Xue *et al.* 2006). There are approximately 1.1×10^{10} prokaryotes residing in tea soil, which can thrive in a variety of soil

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habitats including those with extremes of temperature, pH, water and salt stress (Chen *et al.* 2006). The fact that microbes are essential for the entire tea ecosystem since they perform numerous important functions like maintenance of biogeochemical cycles, promotion of plant growth (Phukan *et al.* 2012) inhibition of destructive tea pest and pathogens etc. has spurred keen interest in scientists for the exploration of tea soil microbial diversity (Wright 2003, Barthakur *et al.* 2004, Sarmah *et al.* 2005, Balamurugan *et al.* 2011). Growth promoting substances are produced in large quantities by the beneficial microorganisms that influence indirectly on the overall morphology of the plants.

The rhizosphere of established tea bushes has some specific characteristics, which are associated with long lived nature of tea plants *viz.* negative rhizospheric effect, lowering of soil pH, antagonistic activities among microbial communities and dominance of certain species (Sood *et al.* 2007). The overall interactions amongst tea roots, microbes and environmental conditions prevailing in the tea rhizosphere seems to favor the growth of microbes, which are known to produce strong antibiotics with potential biocontrol agents.

Use of naturally occurring free-living microbial strains can protect and promote plant growth. Their colonizing and multiplying along the root surface of the inoculated plants is said to be a safe and suitable alternative to the use of chemicals (Mishra *et al.* 2005). The organisms that establish positive interaction with plant roots and show observable benefits to the plant roots are collectively called as plant growth promoting rhizomicroorganisms (PGPR) (Kloepper and Schroth 1978). These organisms also provide protection to plants against diseases by suppressing deleterious and pathogenic microorganisms (Mishra *et al.* 2005). Beneficial effects of PGPR have been reported by various workers in a wide range of crops including cereals, pulses, vegetables, oil seeds and plantation crops (Alagawadi and Gaur 1992, Bashan and Holquin 1997, Riggs *et al.* 2001). The rhizosphere of tea is a good habitat for PGPR strains, represented by *Bacillus* and *Pseudomonas* spp., inhibitory to phytopathogenic fungi *in vitro* (Kumar and Bezbaruah 1996). However, scientific information on the use of PGPR in tea plantations is meager; their uses might be having many benefits as indicated above. The prospect of manipulating crop rhizosphere microbial populations by inoculation of beneficial bacteria to increase plant growth has shown considerable promise in laboratory and greenhouse studies but responses have been variable in the field (Bowen and Rovira 1999). Therefore, an effort was made to explore these rhizospheric microorganisms as potential PGPR which could be useful in developing bio-inoculants for enhancement of growth and yield of tea plants in experimental and commercial fields. Beside, the study was also conducted to explore PGPR strains to provide environment friendly management of plants against pathogenic fungal diseases.

Materials and Methods

The composite soils were collected from the rhizosphere of a tea plant. One g of rhizosphere soil was aseptically transferred to a 250 ml conical flask containing 100 ml of sterile distilled water. The flasks were kept in shaking condition at 200 rpm for 15 min and isolation of microbes was made following the serial dilution plate technique (Islam and Ali 2013). Microorganisms were isolated in different specific media, *viz.* Nutrient agar for bacteria and Potato Dextrose Agar (PDA) for fungi. One ml aliquot from 10^4 dilutions was taken for fungi and while for bacteria, 1 ml of aliquot was taken from 10^5 dilutions. The plates were incubated at $28 \pm 2^\circ\text{C}$ and observed after 48 hrs and 5-15 days for bacteria and others, respectively. All the isolated dominant colonies were purified and maintained in the laboratory for further evaluation. To exploit the biocontrol properties, colonies showing antagonistic behaviour against the pathogen were also subjected to laboratory as well as field experimentations.

The isolated bacterial colonies were screened for their antagonism against *C. theae* following dual culture technique (Modupe *et al.* 2007). The mycelial plug of four-day-old, actively growing *C. theae* was grinded and spread uniformly in plates containing PDA medium with the help of a sterilized spatula. These plates were spot inoculated with 24 hrs culture of isolated bacterial strains. Plates were incubated at $30 \pm 2^{\circ}\text{C}$ for 3-5 days. Antagonism was graded by observing the zone of inhibition produced around the bacterial colonies the radial growth of the pathogen was recorded every 24 hrs.

Nursery trials were initiated to study the effect of PGPR on the growth and development of tea rooted cuttings. The polybags used for transferring tea rooted cuttings were inoculated with the four isolated microbial strains and one collected bio-fertilizer by mixing with 50 g of decomposed cowdung. The responses to these microbes in context of the number of leaves, height of plants and girth of stems were recorded after 9 months. A field trial was laid out at Bangladesh Tea Research Institute during 2015 - 16 to study the effect of some isolated PGPR and one collected bio-fertilizer as foliar spray. Treatments were imposed in randomized block design with three replications for each treatment and 30 bushes in each plot. Two rounds of foliar sprays were given at 15 days interval at 10^4 dilutions concentrations. Data were recorded on the severity of the diseases by observing the typical symptom. These were done by using the following 0 - 5 scoring scale like no infection = 0, 1 - 20% infection = 1, 21 - 40% infection = 2, 41 - 60% infection = 3, 61 - 80% infection = 4 and 81 - 100% infection = 5 (Islam and Ali 2011). The severity of the disease was expressed in Per cent Disease Index (PDI), which was computed following a standard formula as described below (Singh 2000).

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{Maximum disease grade}} \times 100$$

Data were subjected to analysis of variance by MSTAT computer programme. Mean separation was done by Duncan's Multiple Rang Test (DMRT).

Results and Discussion

Four rhizospheric microorganisms namely *Bacillus*, *Pseudomonas*, *Streptomyces* and *Trichoderma* were recorded. These microbes and one collected bio-fertilizer enhanced plant growth identically ($p = 0.05$) in nursery regarding increasing number of leaves, plant height and stem girth (Table 1). The microbes have a positive impact on the rate of increase in number of leaves, height of plants and girth of plants by 33, 43 and 3%, respectively with encouraging relations (Figs 1, 2, 3 and 4). In case of disease severity, the lowest severity (21.00) was found with *Trichoderma* followed by *Bacillus*, *Pseudomonas* and *Streptomyces* with identical values (Table 2). *Trichoderma* and *Bacillus* caused 16 and 14% reduction of disease severity while both *Pseudomonas* and *Streptomyces* cause 10%. All the microbes have a great impact in reducing disease severity by 19% with optimistic relations (Fig. 4). Radial colony growth (mm) of *C. theae* was also in similar trends. Collected biofertilizer showed comparatively lower response in reducing disease severity.

Tea plantations usually resemble to "single species forest" (Daniels 2003, Madhab *et al.* 2009). Although tea soil microbiology was initially explored in 1901, while the studies on occurrence and functionality of mycorrhizae on tea roots was reported later (Webster 1953, Madhab *et al.* 2009). Since then, several bacterial and fungal strains like *Aspergillus*, *Azotobacter*, *Azospirillum*, *Fusarium*, *Penicillium*, *Trichoderma* and phosphate solubilizers like *Bacillus* and *Pseudomonas* have been reported in tea soil (Bezbaruah and Baruah 1985).

Table 1. The plant growth parameter against different growth promoting microbes in nursery trial.

Treatments	No. of leaves	Height of plant (mm)	Girth of stem (mm)
Control	16.67 b	466.7 b	1.53 b
<i>Bacillus</i>	19.67 a	548.0 a	1.87 a
<i>Pseudomonas</i>	19.67 a	553.7 a	1.80 a
<i>Streptomyces</i>	20.33 a	554.3 a	1.93 a
<i>Trichoderma</i>	19.67 a	553.7 a	1.63 b
Bio-fertilizer	19.33 a	549.0 a	1.53 b

Same letter(s) followed by values in column is/are not statistically different from each other.

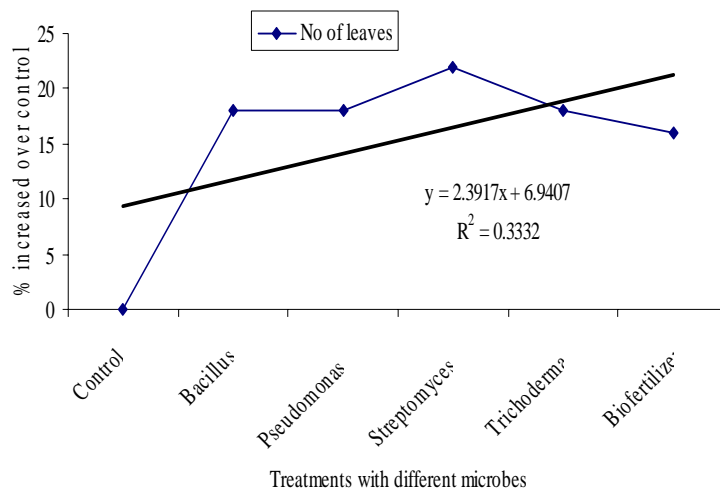


Fig.1. Rate of increased in number of leaves over the control.

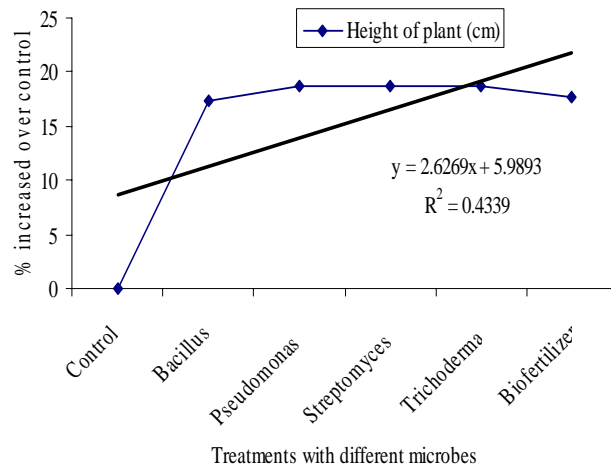


Fig. 2. Rate of increased in height of plants over the control.

Table 2. *In vitro* radial growth (mm) of *Corticium theae* after different periods (hour) of incubation and *in vivo* disease severity against different growth promoting microbes.

Treatments	Radial growth (mm) after different periods (hour) of incubation					Disease severity
	48	72	96	108	120	
Control	51.33 a	68.67 a	81.33 a	89.67 a	90.00 a	25.00 a
<i>Bacillus</i>	42.67 d	60.67 c	72.67 c	83.33 b	89.67 ab	21.50 cd
<i>Pseudomonas</i>	42.67 d	61.67 c	69.67 d	81.33 c	88.67 abc	22.50 bc
<i>Streptomyces</i>	43.67 c	61.67 c	68.67 e	79.67 d	87.67 c	22.50 bc
<i>Trichoderma</i>	27.67 e	42.67 d	56.67 f	67.33 e	72.67 d	21.00 d
Bio-fertilizer	44.67 b	67.67 b	77.67 b	83.67 b	88.33 bc	23.00 b

Same letter(s) followed by values in column is/are not statistically different from each other.

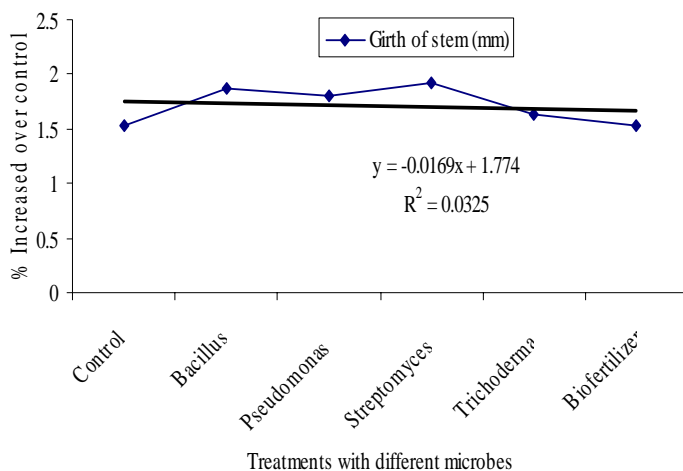


Fig. 3. Rate of increased in girth of stem over the control.

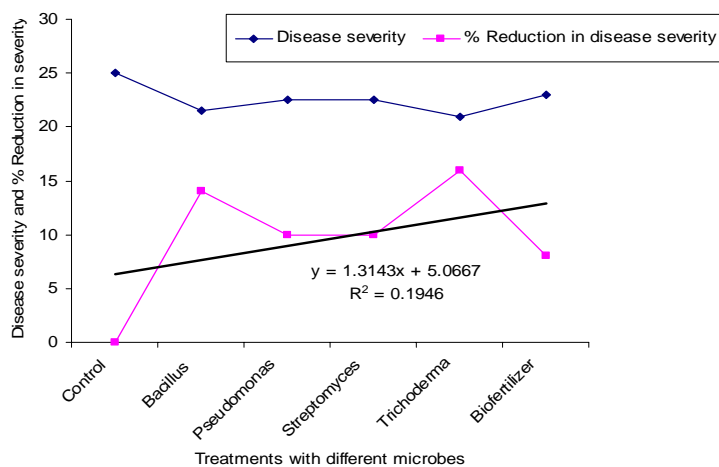


Fig. 4. Rate of reduced in disease severity over the control.

The overall interactions among the tea roots, microbes and environmental conditions prevailing in tea rhizosphere seems to favor the growth of antagonistic microbes (Pandey and Palni 1996, Sood *et al.* 2007, Pandey *et al.* 2013) which are known to produce strong antibiotics with potential biocontrol activities. The antagonistic behaviour of *Bacillus subtilis* against *Corticium invisum*, the causal agent of black rot disease of tea was established *in vitro* (Barthakur *et al.* 2004). *Trichoderma viride* and *T. harzianum* showed their efficiency in controlling charcoal stump rot, brown root rot and Poria branch canker diseases of tea.

From the result it may be suggested that *Bacillus*, *Pseudomonas*, *Streptomyces* and *Trichoderma* isolated from tea soil have their growth enhance capacity as well as decrease the disease severity of Black rot in tea fields.

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