RESEARCH ARTICLE

Use of Rhizospheric Microbes for Developing Plant Growth in Tea Nursery and Controlling Red Rust Disease of Tea Caused by *Ceplaleuros parasiticus* Karst, in Bangladesh

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Abstract

Assessment of plant growth promoter and biocontrol properties of plant growth promoting rhizomicroorganisms (PGPR) from tea soil against red rust disease of tea caused by *Cephaleuros parasiticus* Karst, in Bangladesh was conducted. The microbes were isolated from soils of tea fields and cultured on different nutrient media. The microbial strains were inoculated with tea nursery soils by mixing with 50 g of decomposed cow dung. In tea plantations, the microbial strains were sprayed on diseased plants two times at 15 days intervals. Four PGPR strains *Bacillus, Pseudomonas, Streptomyces, Trichoderma* were isolated from the rhizospheric soil of tea. *Streptomyces* significantly responded to increasing 21.95% leaves, 18.77% plant height, and 26.14% stem girth over the control followed by *Pseudomonas* and *Trichoderma*. These PGPR strains enhanced plant growth in nursery and positively affected the rate of increased number of leaves, plants' height and plants' girth by 33, 43 and 3%, respectively. The lowest severity (21.00) was found with *Trichoderma* after 35 days after spraying followed by *Bacillus, Pseudomonas* and *Streptomyces* with identical values. *Trichoderma* and *Bacillus* cause 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. The biofertilizer showed a comparatively lower response in reducing disease severity. It can be decided that *Bacillus, Pseudomonas, Streptomyces* and *Trichoderma* isolated from tea soil have their growth capacity as well as decrease the disease severity of Red rust disease of tea fields.

Keywords: PGPR, Severity, Red rust, Tea.

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INTRODUCTION

Tea (*Camellia sinensis* (L.) O. Kunze) 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. Tea, being a perennial monocultural crop consists of a stable microclimate for a number of pests and diseases. Crop loss (loss in the yield) in tea due to pests, diseases and weeds recorded as high as 43%.[1] Tea ecosystem is a complex agro-ecosystem. It comprises tea, shade trees, green crops, forests etc. The intensive monoculture of a perennial crop like tea over an extensive and contiguous area in apparently isolated ecological zones in Bangladesh has formed a virtually stable ecosystem, providing unlimited opportunity for the perpetuation and spread of endemic and introduced diseases.[2] The architecture of tea plantations, the variability of plant types, the systemic interaction of various agro-techniques, intercultural operations, etc., significantly impact disease development. Diseases are one of the most common barriers for the production of tea. The loss of tea in Bangladesh tea due to various pests, diseases and weeds has been estimated to be about 10 to 15%.[3] The evergreen and long-lived (over 100 years of cultivation) tea plantations are genetically diverse, providing a steady microclimate and food supply for various plant pests and pathogens.[4]

The perennial habit of the tea plant, unique cultural conditions and warm, humid climate of the tea growing areas are highly favorable for disease infection. A number of diseases have been recorded in tea of which the majority is of fungal origin except red rust disease caused by an algal pathogen *Cephaleuros parasiticus* Karst. and *C. virescens.* Among the tea diseases, leaf diseases are very important because tea plants are cultivated for its young succulent leaves for manufacturing tea.[5] Of the leaf diseases, red rust is an important disease prevalent in both young and mature tea fields under adverse soil and climate conditions. The algae can infect the branches at any stage of growth. When tea plants are affected by parasitic red rust disease, the leaves become variegated (yellow or white) and the whole coverage of spots in the leaf due to epiphytic red rust. In extreme cases, red rust causes severe damage to young tea plants by killing stem tissues in patches. Once the branch is infected, the algae remain latent for a year. In the following year, fruiting bodies of the pathogen are produced depending on the first rain.[6]

The microbial diversity of tea soil is extremely diverse.[7] There are approximately 1.1 x 1010 prokaryotes residing in tea soil, which can thrive in a variety of soil habitats, including those with extremes of temperature, pH, water and salt stress.[8] 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, inhibition of destructive tea pest and pathogens etc.,[9,10] has spurred keen interest in scientists for the exploration of tea soil microbial diversity.[11-15] Growth promoting

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substances are produced in large quantities by the beneficial microorganisms that indirectly influence the plants' overall morphology.

The rhizosphere of established tea bushes has some specific characteristics which are associated with the lived nature of tea plants, viz. negative rhizospheric effect, lowering of soil pH, antagonistic activities among microbial communities and dominance of certain species.[16,17] The overall interactions amongst tea roots, microbes and environmental conditions prevailing in the tea rhizosphere seem to favor microbes' growth, which are known to produce strong antibiotics with potential bio-control agents. The 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.[18] 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).[19] Various workers have reported the beneficial effects of PGPR in various crops, including cereals, pulses, vegetables, oil seeds and plantation crops.[20-22] The rhizosphere of tea is a good habitat for PGPR strains, represented by *Bacillus* and *Pseudomonas* spp., inhibitory to phytopathogenic fungi *in-vitro*.[23,24] 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. 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. Besides, the study was also conducted to explore PGPR strains to provide protection plants against red rust disease of tea.

MATERIALS AND METHODS

Field and nursery trial were laid out at Bilashcherra Experimental Farm of Bangladesh Tea Research Institute during 2017- 2018 to study the effect of some isolated PGP microbes and one collected bio-fertilizer as foliar spray. The composite soils were collected from the rhizosphere soil near the root surface of a tea plant. One gram 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 minutes and microbes were isolated following the serial dilution plate technique.[25] 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 ± 2°C and observed after 48 hours and 5 to 15 days for bacteria and others, respectively. All the isolated dominant colonies were purified and maintained in the laboratory for further evaluation.

Nursery trials were initiated to study the effect of PGP microbes on tea-rooted cuttings' growth and development. 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 cow dung. The responses to these microbes in the context of the number of leaves, plants' height and stems' girth were recorded after 9 months. In the field experiment, treatments were imposed in a 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 intervals at 10^{-4} dilution concentrations. Data were recorded on the severity of the diseases by observing the typical symptoms. These were done using the following 0–5 scoring scale:[26] no infection = 0, 1–20% infection = 1, 21–40% infection = 2, 41–60% infection = 3, 61–80% infection = 4 and 81–100% infection = 5. The severity of the disease was expressed in the percent disease index (PDI), which was computed following a standard formula as described below.[27]

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{maximum disease grade}} \times 100$$

Data subjected to analysis of variance by MSTAT computer program. Mean separation was done by Duncan's multiple rang test (DMRT).

RESULTS AND DISCUSSION

There are four microbes, like *Bacillus, Pseudomonas, Streptomyces,* and *Trichoderma* were recorded. These microbes and one collected biofertilizer enhance plant growth identically (p = 0.05) in the nursery regarding the increasing number of leaves, plant height and stem girth. *Streptomyces* significantly responded to increasing 21.95% leaves, 18.77% plant height, and 26.14% stem girth over the control, followed by *Pseudomonas* and *Trichoderma* (Table 1). The microbes positively impact the rate of the increased number of leaves, plants' height and plants girth by 33, 43 and 3%, respectively, with encouraging relations (Figures 1, 2 and 3).

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

In case of disease severity, the lowest severity (21.00) was found with *Trichoderma* after 35 days after spraying, followed by *Bacillus, Pseudomonas* and *Streptomyces* with identical values. *Trichoderma* and *Bacillus* cause 16 and 14% reduction of disease severity while both *Pseudomonas* and *Streptomyces* cause 10% (Table 2). All the

Table 1: Showing the plant growth parameter against different growth-promoting microbes in tea nursery

	Leaves		*Plant height (mm)*		*Girth of stem (mm)*	
Treatments	*Number*	*%increase over control*	*Plant height*	*%increase over control*	*Stem girth*	*%increase over control*
Control	16.67 b	00	466.7 b	00	1.53 b	00
Bacillus	19.67 a	17.99	548.0 a	17.42	1.87 a	22.22
Pseudomonas	19.67 a	17.99	553.7 a	18.64	1.80 a	17.64
Streptomyces	20.33 a	21.95	554.3 a	18.77	1.93 a	26.14
Trichoderma	19.67 a	17.99	553.7 a	18.64	1.63 b	6.53
Biofertilizer	19.33 a	15.95	549.0 a	17.63	1.53 b	00

Table 2: Severity of red rust disease at different periods after application of microbes

Treatments	Severity of red rust					Rate of reduction of disease severity over the control
	7 DAS	14 DAS	21 DAS	28 DAS	35 DAS	
Control	45.5	40.495	32.76	28.665	25.00 a	00
Bacillus	39.13	34.8257	28.1736	24.6519	21.50 cd	14
Pseudomonas	40.95	36.4455	29.484	25.7985	22.50 bc	10
Streptomyces	40.95	36.4455	29.484	25.7985	22.50 bc	10
Trichoderma	38.22	34.0158	27.5184	24.0786	21.00 d	16
Biofertilizer	41.86	37.2554	30.1392	26.3718	23.00 b	8

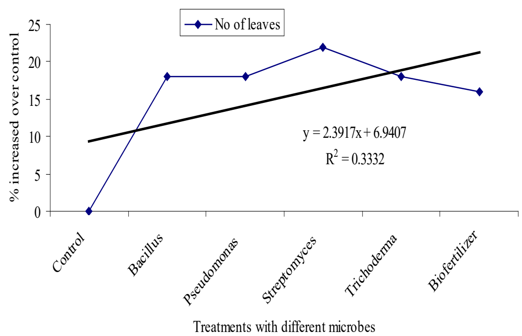

Figure 1: Rate of increase in the number of leaves over the control

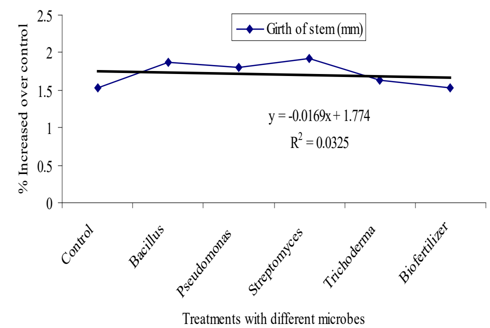

Figure 3: Rate of increase in girth of stem over the control

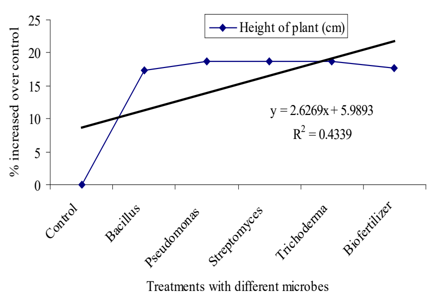

Figure 2: Rate of increase in height of plants over the control

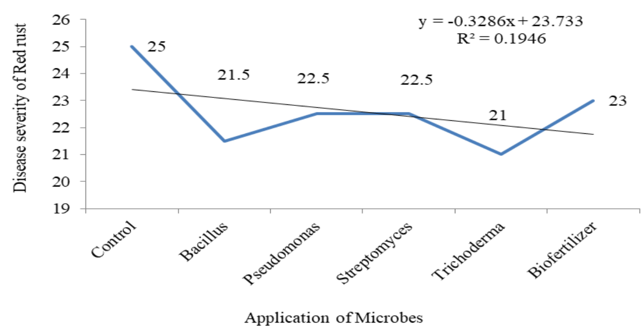

Figure 4: Rate of reduced in disease severity over the control

microbes greatly reduce disease severity by 19% with optimistic relations (Figure 4). Collected biofertilizer showed a comparatively lower response in reducing disease severity.

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

Although tea soil microbiology was initially explored in 1901, the studies on the occurrence and functionality of mycorrhizae on tea roots were reported later.[28,29] 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.[30] *Bacillus, Pseudomonas, Streptomyces* and *Trichoderma* isolated from tea soil has their growth capacity as well as decrease the disease severity of Black rot disease of tea.[31] The overall interactions among the tea roots, microbes and environmental conditions prevailing in tea rhizosphere seem to favor the growth of antagonistic microbes[32-34] 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.*[35] *Trichoderma viride* and *T. harzianum* showed their efficiency in controlling charcoal stump rot, brown root rot and Poria branch canker diseases of tea.

From this result it can be decided that *Bacillus, Pseudomonas, Streptomyces* and *Trichoderma* isolated from tea soil have their growth capacity as well as decrease the disease severity of Red rust disease of tea fields.

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