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Integrated Approach to Management of Brown Root Rot Disease of Tea (*Camellia sinensis* (L)O.Kuntze).

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Abstract

Brown root rot disease caused by *Fomes lamoensis* (Murr.) Sacc. and Trott has been identified as the primary root disease of tea. Indiscriminate use of chemicals in tea garden area has an adverse effect on the environment. For management of this root disease, integrated approach is required for sustainability in tea cultivation. The present study involves the use of five systemic fungicides, which were screened and tested against the pathogen. Total five fungicides used, among the fungicides propiconazole and hexaconazole was inhibit 98.51% and 100% growth of pathogen @ 100mg/l concentration. However bavistin, roko and ektino, showed in12.58%, 5.18% and1.48% inhibition respectively. *Bacillus cereus* and *Trichoderma harzianum* were also evaluated and efficiently inhibited F. *lamoensis in vitro*. The zone of inhibition varied from 10 to 15 mm in case of B. *cereus* and 10 to 16 mm in case of T. *harzianum*. The nursery experiment exhibited that tea plants at 120 days after the treatment (DAT) with the pathogen showed 76.66% disease incidence. The reduction in disease incidence (23.33% and 13.25% respectively) was observed when the plants were treated with biocontrol agents i.e.B. cereus and T. *harzianum*. In case of chemically treated plants only 10% disease incidence was observed. The results of the nursery experiment showed that both the chemicals and biocontrol agents significantly affected incidence of disease and promoted growth of tea measured in terms of shoot height, root length, number of new leaves, lateral branches, biomass of shoot and root over the control.

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Introduction

One of the most popular and cheapest beverages in the world is tea (*Camellia sinensis* (L) O. Kuntze) which it is grown in more than 50 countries of the world. The ideal condition for these plant is a warm, humid climate with well distributed rainfall and long sunshine hours. But, along with it the conditions are also favourable for the growth of many pests and pathogens. This results in serious damages affecting productivity and quality. One of the primary root diseases of tea has been identified as brown root rot disease. There is the presence of brown mycellium on root surface to which soil, sand and stone particles remain encrusted.[1] Development of the disease is rapid and spreads to other plants through roots. The fungus is pathogenic to as young as one year old to one hundred years old plants.[2] The pathogen survives in debris remaining in the soil for more than ten years even after the removal of the diseased plants.[3] Since the last several decades synthetic chemical fungicides like hexaconaxole, propiconazole, bavistin etc have been used to control the root diseases of tea. But, certain drawbacks like fungicide resistance, effect on the environment etc have been seen. So, there is a necessity of a safer, ecologically tenable practice for disease management. Under the biological control, here is a concept of using plant growth promoting rhizobacteria (PGPR) and plant growth promoting fungi (PGPF) to control the diseases.[4] As part of an integrated management strategy to control root diseases, there have been efforts to adopt low concentration of chemicals/ non chemicals and bio- agents. The present study is designed to control brown root rot disease of tea by use of bio control agents against the tea root pathogen (F.*lamoensis*) and their plant growth promotion properties.

Materials and Methods

F. *lamoensis* isolated was the most dominant root pathogen of tea as it was isolated from most of the tea gardens. F. *lamoensis* (ITCC 4140) was also procured from the Indian Type Culture Collections (ITCC) of Indian Agricultural Research Institute (IARI), New Delhi for comparison with the new isolate to confirm its identification.

Bacillus cereus and *Trichoderma harzianum* isolated from the tea rhizosphere were used for the present study.

Isolation of Pathogen

Infected roots were collected from various tea gardens of Barak Valley of Assam, N.E. India. They were washed with tap water and cut into small pieces. These pieces were surface sterilized with 1% sodium hypochloride ($NaOCl_2$) for 20 to 40 seconds. Five pieces were placed in each petridishes containing Potato Dextrose Agar (PDA) medium and incubated at 28±2°C for 7 days. Mycelium grown from inoculated pieces were purified by repeated subculturing and finally transferred to PDA slants and preserved for further study.

Isolation of Rhizsophere Microbes

Rhizosphere microbes were isolated by serial dilution agar plate technique by using Nutrient Agar (NA) medium. Morphologically dissimilar colonies were purified and stored at 4^0 Celsius for further studies.

In vitro antagonistic efficacy of *B.cereus* and *T. harzianum*

Actively -growing mycelial discs (6 mm) of F. *lamoensis* were placed in the centre of the Petri plate containing Potato Dextrose Agar medium. A loopful of the *B. cereus* was spot-inoculated on the periphery of the Petri plate opposite and equidistant from the pathogen placed and incubated at $28±2^0$C. Inhibition zones were measured as distance (in mm) between the respective bacterial test antagonist and the fungal pathogen, F. *lamoensis* after 7 days of incubation.

Actively growing 4mm disc of T. *harzianum* were placed in petridishes containing sterile potato dextrose agar medium at 2cm apart from F. *lamoensis*. The controls sets were made without the T. *harzianum*. Plates were incubated at $25±1^0$C for 6 days and the growth of the pathogen against the test fungi was measured. The antagonistic ability of the test organism was studied by the method developed by Skidmore and Dickinson.[5]

In vitro Efficacy of Fungicides

The fungicides namely hexaconazole (Xantho 5% E.C), propiconazole (TILT 25% E.C) bavistin (Bavistin 50% W/P), Roko (Thiophanate methyl 70% WP) and Ektino were used in the present experiment. Three different concentrations i.e.10, 50 and 100 ppm (100 mg/l) of these fungicides were used to test their efficacy against F. *lamoensis* using the poison food technique.[6] Different concentrations of fungicides were prepared by dissolving the required quantity of each fungicide in warm PDA before autoclaving. The autoclaved media was poured into petridishes and were allowed to cool. A total of 45 plates of different fungicides at different concentrations were prepared. Seven day old actively growing culture of F. *lamoensis* was cut using cork borer into 5 mm diameter discs. The discswere placed at the centre of petridishes. Each treatment was maintained in triplicate. Media without fungicide served as the control. The plates were incubated at 25°C ±2 for 7 days. On the 8th day radial growth of the colony was recorded and the percentage inhibition of growth was calculated using the following formula.

$I = 100\times (C-T)/C$

I= percent inhibition

C= Control

T= Growth of fungus in the treatment.

Efficacy of Fungicides and Biological Control Agent in Growth Promotion and Disease Suppression under Nursery Conditions

One year old tea plants were planted in polythene bags filled up with sterilized garden soil and farm yard manure (FYM) in a ratio of 3:1. Ten days old actively growing culture of F. *lamoensis* in Potato Dextrose Broth (PDB) was homogenized in sterile distilled water using mortar and pestle. One plant was placed in one bag where these bags were inoculated with homogenized fungal suspension @ 50 ml per plant. Fungicides namely hexaconazole, propiconazole and bavistin were used for treating the plants. At the concentration of 100 ppm the fungicides were applied @ 25 ml per plants. The bags treated only with F. *lamoensis* served as control. The experiment was laid out in Completely Randomized Design (CRD) with five replications.

B. cereus and T. *harzianum* were grown separately in Nutrient broth and PDB medium for 48hours respectively. The broth was diluted to 40% and then sprayed over tea plants growing in polythene bags @25 ml per plant near the collar region. Both the treated and control plants were maintained under nursery conditions. Therewere four sets, the first tea plants treated with respective BCA alone, the second set contained tea plants treated with respective BCA + 20 ml of a well homogenized mycelial mixture of the pathogen F. *lamoensis*. The third setinoculated plants with only 50 ml of well homogenized mycelial mixture of F. *lamoensis* in sterilized water, whereas the fourth setinoculated tea plants weretreated neither with BCA nor with the pathogen,which served as control. The experiment was laid out in Completely Randomized Design (CRD) with five replications.

Drying and shredding of leaves and drying of entire plants were used for the assessment of disease suppression. Growth improvement of tea was measured in terms of numbers of new leaves, shoot height, root length, dry weight of shoots and dry weight of roots

Statistical Analysis

All the data are expressed as mean ± standard error. Data obtained were subjected to statistical analysis as given by Panse and Sukhtame[7] and Gomez and Gomez.[8] Significant differences between the treatments were calculated by analysis of variance (ANOVA).

Results and Discussion

In vitro* screening of fungicides and BCA against F. *lamoensis

The fungicides, hexaconazole, propiconazole and bavistin inhibited the radial growth of the pathogen at different concentrations (i.e. 1, 10, 50 and 100 ppm) with the corresponding days of observation *in vitro*. Fungicides at 1 and 10 ppm concentrations were less effective and the growth of the pathogen decreased with increase in concentration. Out of the three fungicides tested, no growth of pathogen was recorded in plates treated with 100 ppm hexaconazole during all the days of observation (Fig. 1). In case of plates with 100 ppm propiconazole a slight radial growth of the pathogen was observed (Fig.2). Bavistin treated plates though were initially effective against the pathogen but the effectivity declined with time (Fig. 3 plate 1).

Effect of fungicides on the percent inhibition of the radial growth of F. *lamoensis* is presented in Figure 4. Hexaconazole completely (100%) inhibited the *in vitro* radial growth of F. *lamoensis*, followed by propiconazole 98.51%, Bavistin 12.58%, Roko 5.18% and Ektino 1.48%. It was also observed that hexaconazole at all the concentrations significantly inhibited the growth of thetest pathogen irrespective of the concentrations used (Fig. 4).

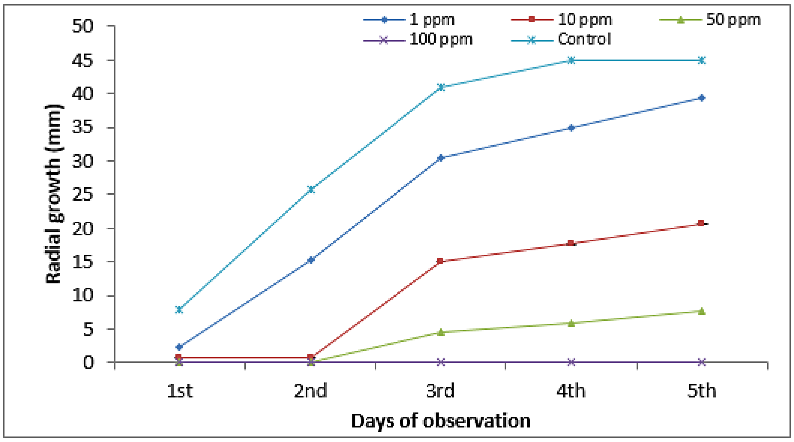


Fig. 1: Effect of different concentrations of hexaconazole on *in vitro* radial growth of *Fomes lamoensis*

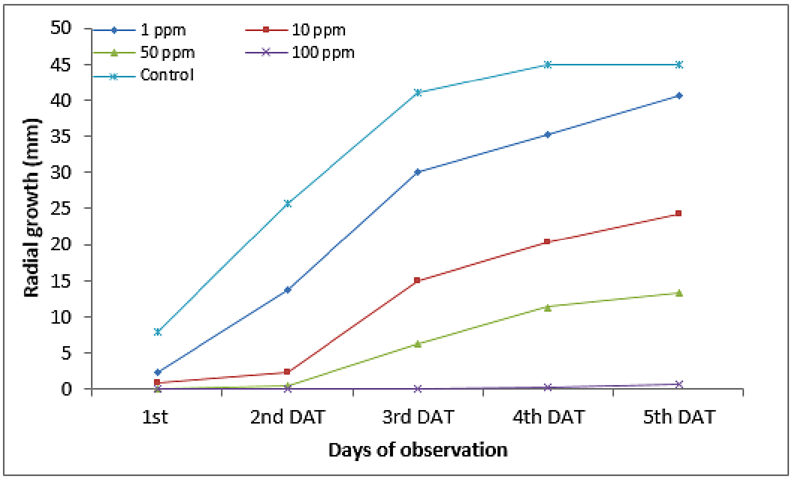


Fig. 2: Effect of different concentrations of propiconazole on *in vitro* radial growth of F. *lamoensis.*

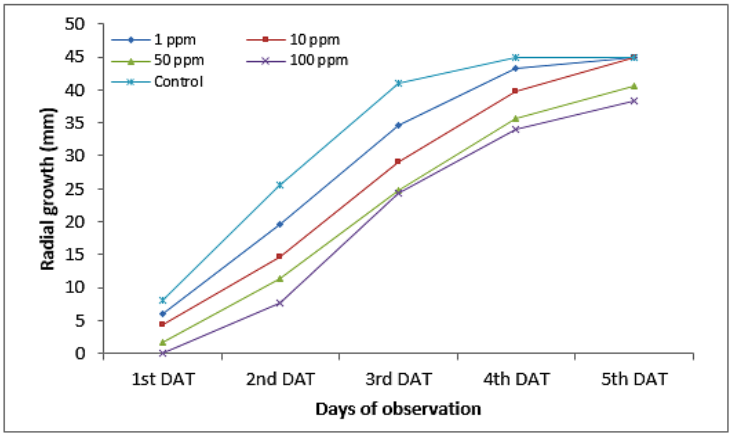


Fig. 3: Effect of different concentrations of bavistin on *in vitro* radial growth of F. *lamoensis*

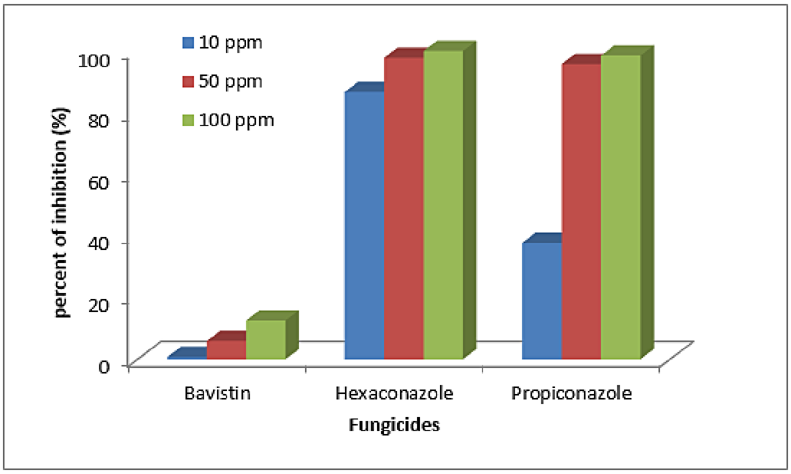


Fig. 4: Effect of different concentrations of fungicides on percent inhibition of *F. lamoensis* under *in vitro* conditions.

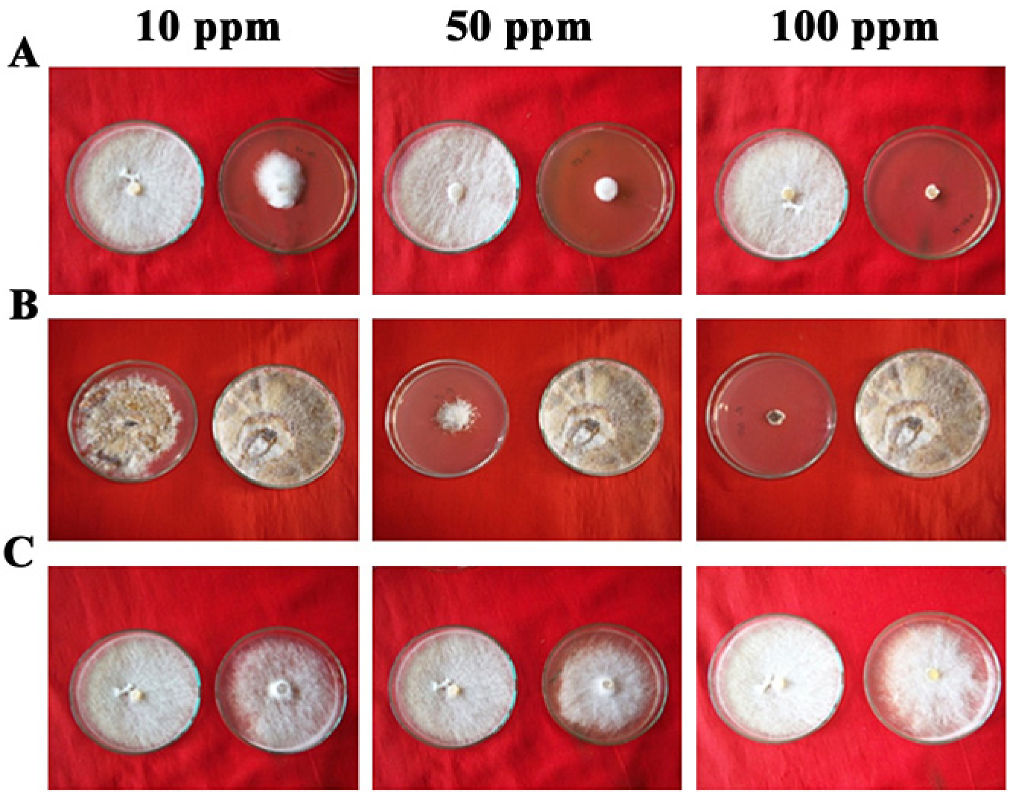
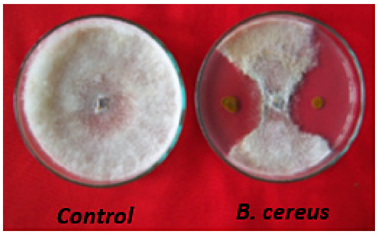
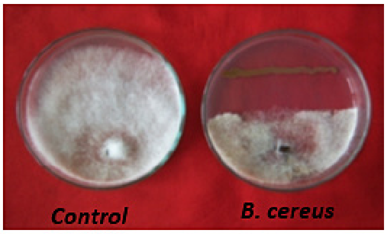


Plate 1: Effect of fungicides (A-Hexaconazole, B-Propiconazole and C-Bavistin) at different concentrations on *in vitro* growth of *F. lamoensis*.

In dual culture, the inhibitory ability of *B. cereus* differed in both spot and line inoculation. The zone of inhibition varied from 10 to 14 mm in Potato Dextrose Agar (PDA). Highest inhibition zone was recorded in line inoculation in PDA medium. On the other hand, T. *harzianum* also effectively inhibited the pathogen. According to the classification of Skidmore and Dickinson T. *harzianum* showed 'F' type of colony interaction, which meant the pathogen was overgrown by the antagonist (Plate 2).



Spot inoculation



Line inoculation

'F' type of colony interaction.

Plate 2: *In vitro* antagonism of *Trichoderma harzianum* and *Bacillus cereus* against *F. lamoensis*

Table 1: Effect of fungicides on the development of brown root rot symptoms in 120 days old tea plants infected by *F. lamoensis*

Treatment (s)	Leaf Drying			Leaf shedding			Drying of tea plants
	1 leaf	2 leaf	More than 2 leaves	1 leaf	2 leaf	More than 2 leaves	
Control (*F. lamoensisalone*)	28^{th} Day	31^{st} Day	36^{th} Day	30^{th} Day	39^{th} Day	48^{th} Day	90^{th} Days
Hexaconazole + *F. lamoensis*	49^{th} Day	56^{th} Day	-	54^{th} Day	65^{th} Day	-	-
Propiconazole + *F. lamoensis*	47^{th} Day	58^{th} Day	-	52^{nd} Day	-	-	-
Bavistin + *F. lamoensis*	39^{th} Day	47^{th} Day	64^{th} Day	44^{th} Day	57^{th} Day	66^{th} Day	-
B. cereus + *F. lamoensis*	65^{th} Day	-	-	77^{th} Day	-	-	-
T. harzianum+ *F. lamoensis*	65^{th} Day	78^{th} Day	-	90^{th} Day	-	-	-

Growth Promotion and Disease Suppression in Tea Plants Under Nursery Conditions

Initial symptoms of the disease on tea plants appeared on 30th days after inoculation and remained prominent up to the 90thday. The leaves started drying followed by shedding of leaves and finally the whole plants died and the roots showed complete rotting. Plants treated with the pathogen along with one of these fungicides i.e. hexaconazole, propiconazole and bavistin initially showed slight leaf drying respectively on 39, 47 and 49th day. On the other hand, the plants treated with pathogen together with *B.cereus* and T. *harzianum* showed initial leaf drying followed by shredding on 65th day but, the death of plants, however was not recorded till the 120th days of observation (Table1).

The treatment with both fungicides and biocontrol agents showed significant growth of tea plants. In the nursery conditions the shoot height, root length, number of leaves and lateral branches showed improvement over the control. Plants treated with hexaconazole and F. *lamoensis* produced more lateral branches (404.54%) followed by plants treated with 100 ppm propiconazole and F. *lamoensis* (303%) and bavistin+ F. *lamoensis* (203.02%) (Fig. 5 and 6). The number of lateral branches, however, did not vary when plants were treated with hexaconazole and propiconazole alone. The plants treated with 100 ppm bavistin produced less number of lateral branches which significantly differed from other treatments including untreated control (203.02%). Hexaconazole treated tea plants produced more new leaves (201.20%) as compared to plants treated with propiconazole (140.96%) and bavistin (60.24%) (Fig.5).

On the other hand, highest per cent increase in shoot height (64.10%) and root length (59.78%) over control was recorded in plants treated with propiconazole + F. *lamoensis* followed by hexaconazole + F. *lamoensis* (55.13% and 48.47%). Plants treated with propiconazole (54.57%, 36.16%) and hexaconazole applied alone showed significant shoot height and root length over the control (23.38%, 38.92%) (Fig. 7 and 8). Least percent increase in both shoot and root height was observed in the plants treated with Bavistin + F. *lamoensis* (0.06%, 0.02%) and Bavistin alone (0.06%, 0.05%). Pathogen infested control tea plants showed poor shoot height and root length as compared to the fungicide treatments (Plate 3).

The plants treated with biocontrol agents like *B. cereus* and T. *harzianum*, with pathogen F. *lamoensis* have showed increase in the number of new leaves (145.46%) and (8.3%) over the control. The number of lateral branches also increased by 178.57% and 57.51% in both bio control agents. It was also observed that shoot height (98.34%) and root length (127.52%) of plants treated with *B. cereus* significantly increased over control.

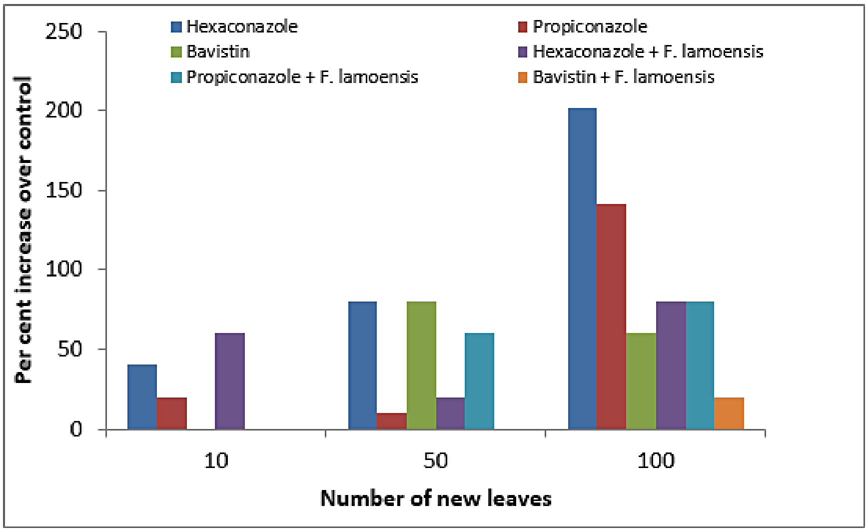


Fig. 5: Effect of different treatments on the development of new leaves 120 days after treatment

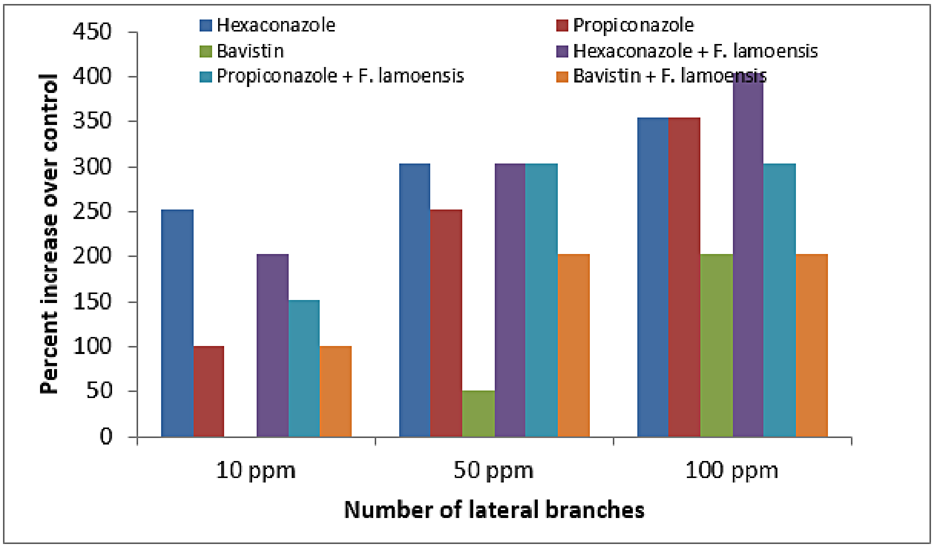


Fig. 6: Effect of different treatments on the development of number of lateral branches 120 days after treatment

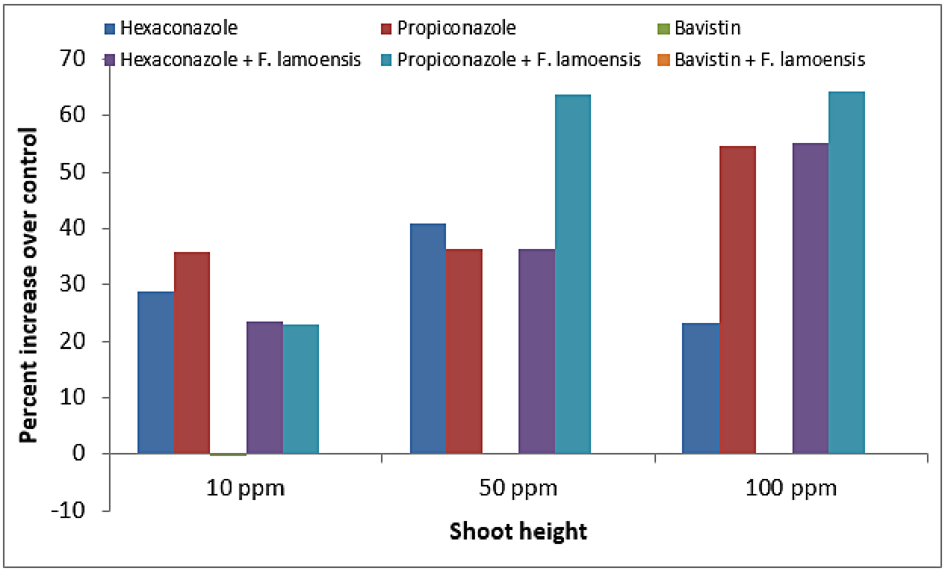


Fig. 7: Effect of different treatments on the development of shoot height 120 days after treatment

Effect of Different Fungicides and BCA on Shoot and Root Biomass

Both shoot and root dry weight of plants treated with fungicides increased significantly over the untreated control. Highest percent increase in fresh and dry shoot weight (36.95% and 69.04%) was recorded in plants treated with hexaconazole and F. *lamoensis*. Least increase was recorded in case of plants treated with bavistin (fig 9 & 10).

Highest per-cent increase in fresh and dry root biomass (274.78% and 244.68%) was recorded in case of plants treated with propiconazole and F. *lamoensis*. Lowest was observed in plants treated with bavistin. It was also found that fungicides hexaconazole, propiconazole and bavistin alone treated tea plants showed significantly increase of biomass over the control (Fig. 11 & 12).

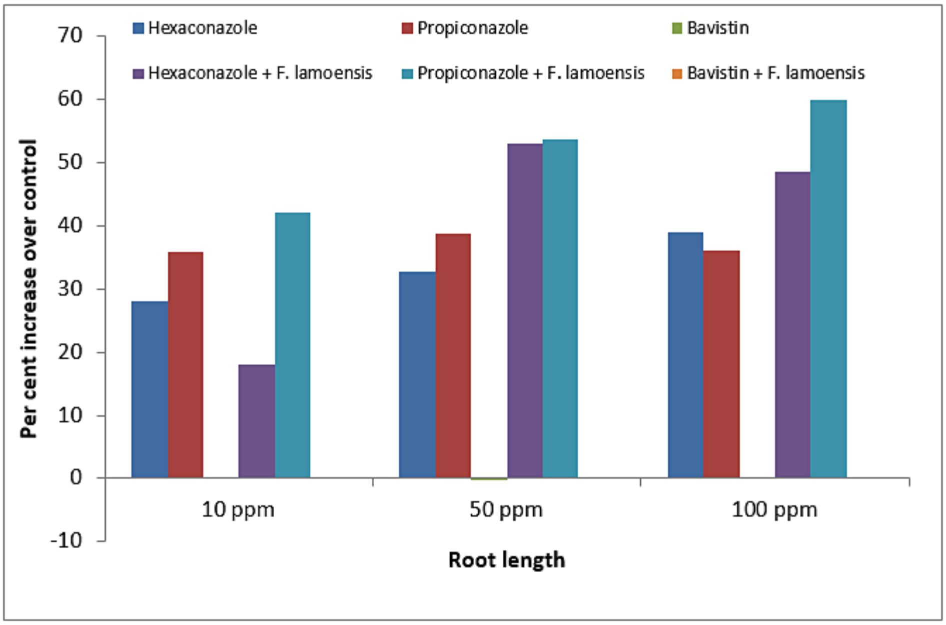


Fig. 8: Effect of different treatments on root length120 days after treatment

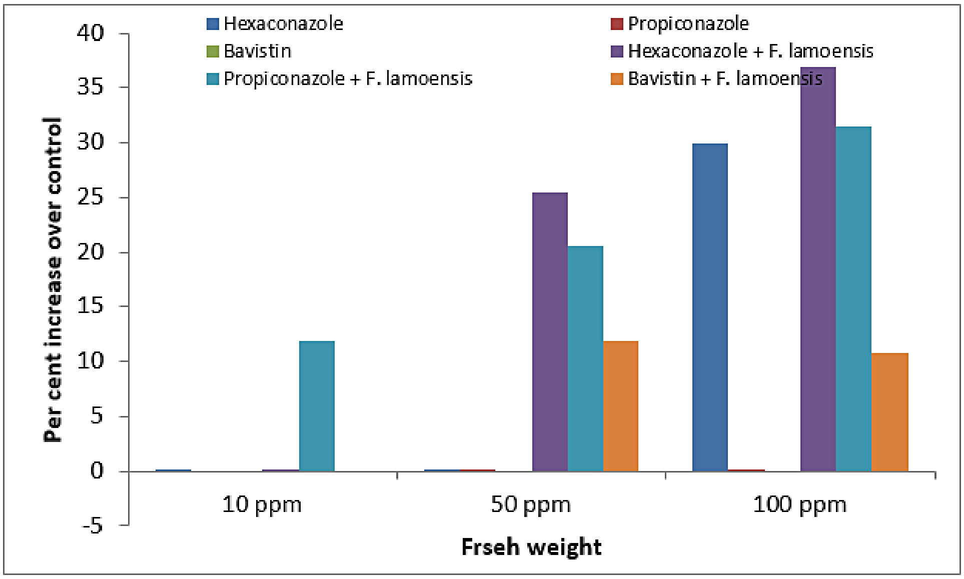


Fig. 9: Effect of different treatments on shoot fresh weight 120 days after treatment

On the other hand, in biocontrol agent treated tea plants both shoot and root biomass also increase significantly over the control. Highest percent in fresh weight of shoot and root was recorded in *B. cereus* with F. *lamoensis* treated plants (193.9%, 77.46%) whereas, T. *harzianum* with pathogen was recorded shoot (19.52%) and root (60.85%) over the control.

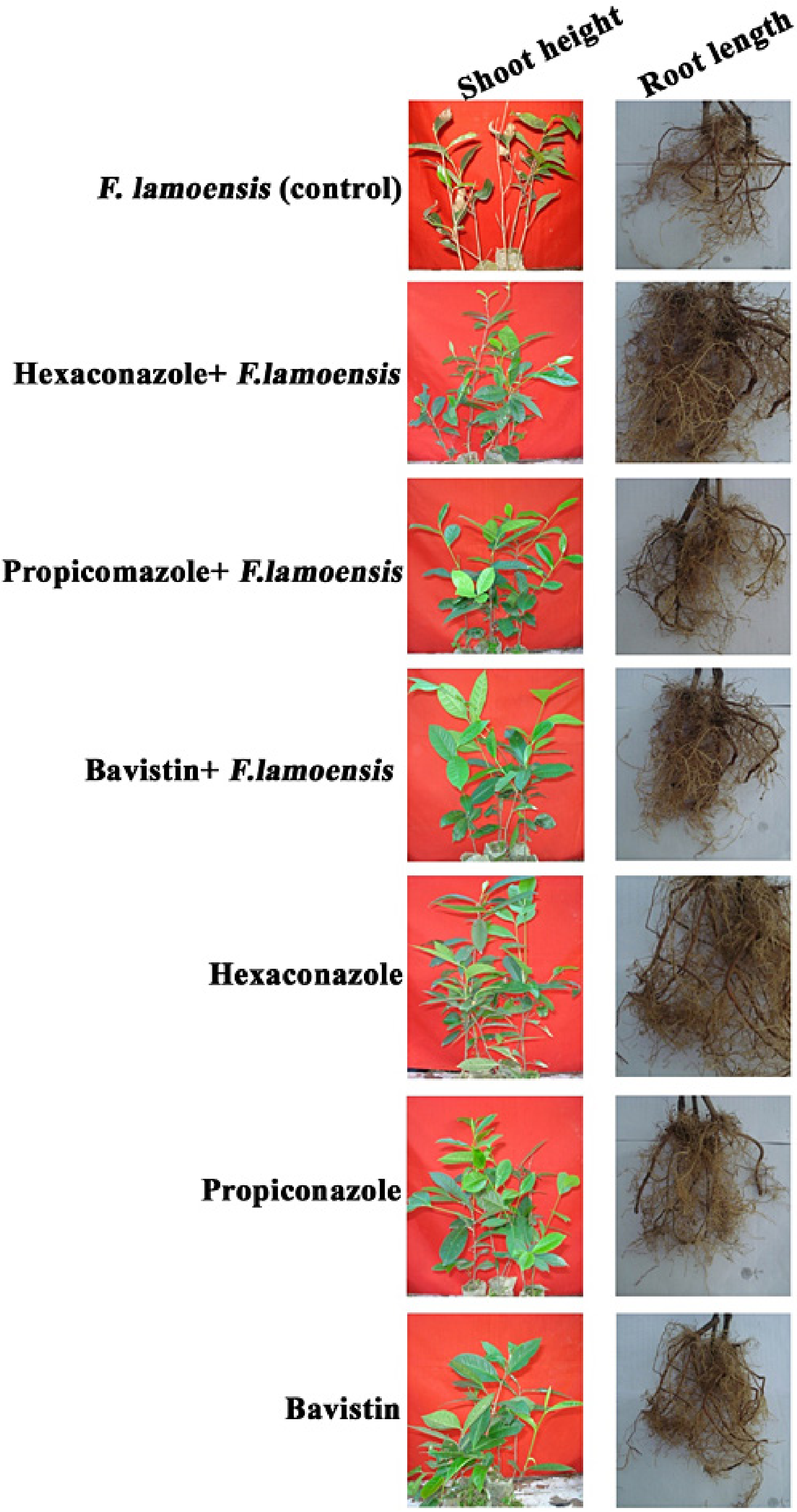


Plate. 3: Effect of different fungicides (100 ppm) on the shoot height and root length of 120 days old tea plants with or without *F. lamoensis* under nursery conditions

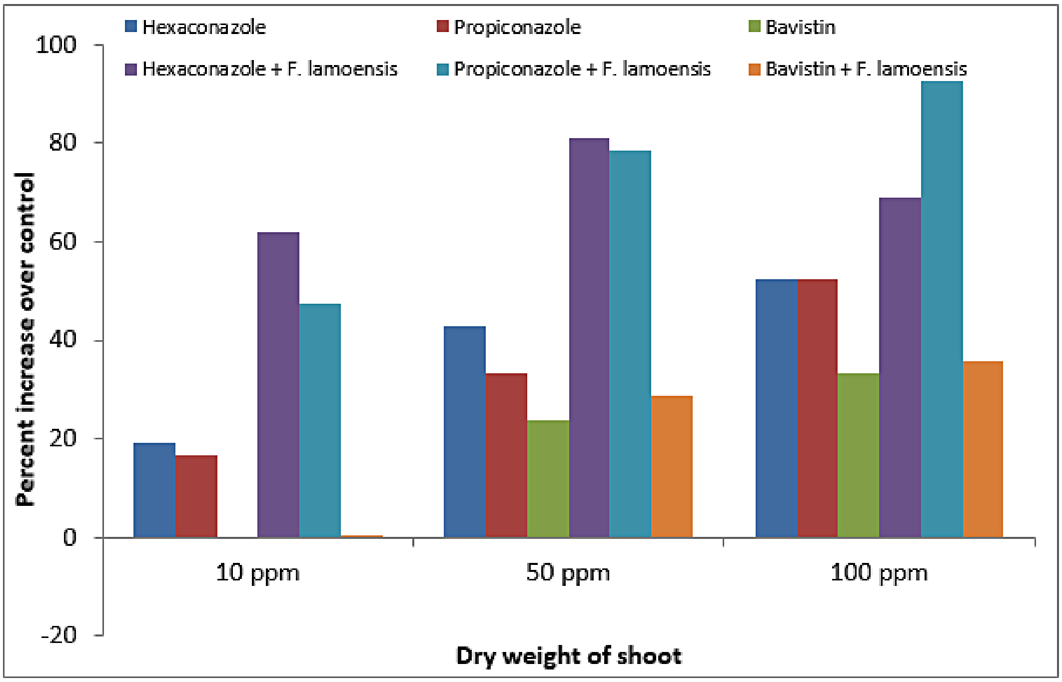


Fig. 10: Effect of different treatments on shootdry weight 120 days after treatment

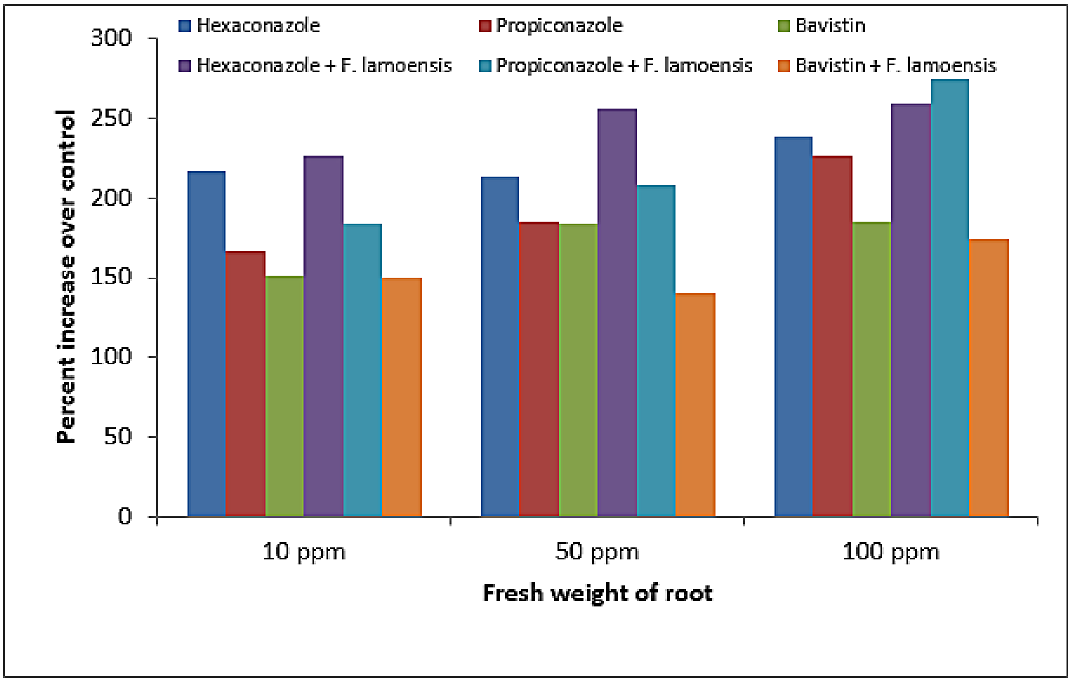


Fig. 11: Effect of different treatments on root fresh weight 120 days after treatment

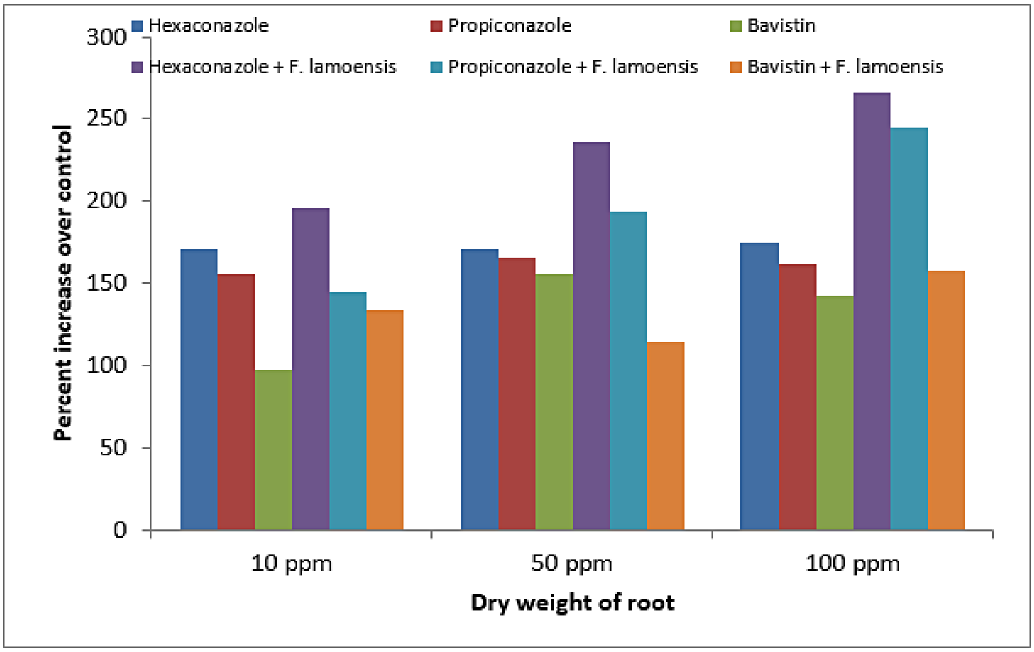


Fig. 12: Effect of different treatments on rootdry weight 120 days after treatment

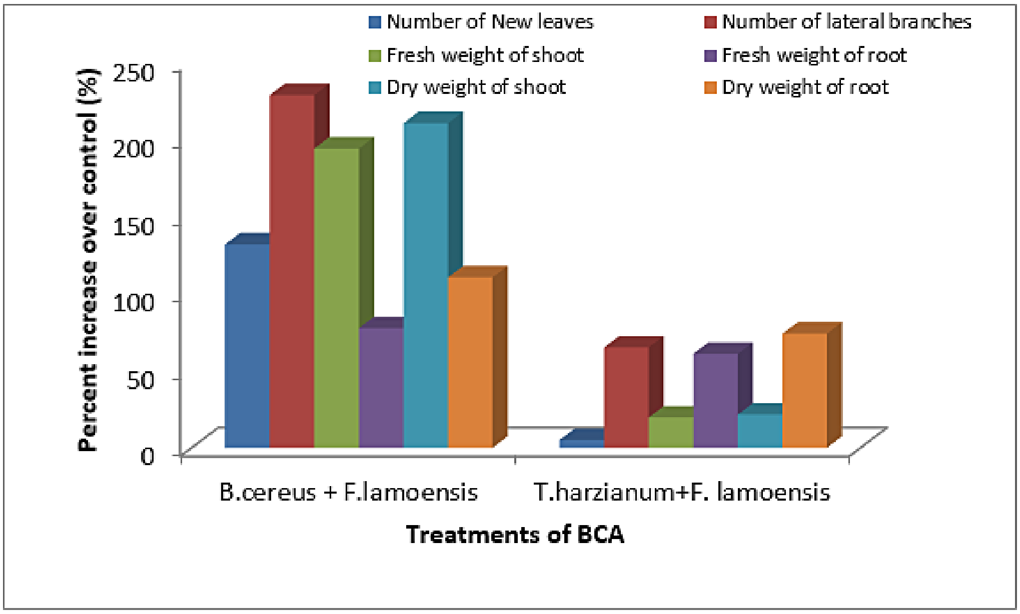


Fig. 13: Effect of different Biocontrol agent (BCA) treatments on growth parameter in tea plants 120 days after treatment

The antifungal activity of systemic fungicides has been attributed to their ability to inhibit ergosterol bio- synthesis in fungi. [9,10] It has also been reported that hexaconazole and propiconazole are more effective even at low concentrations against fungal pathogen.[11] The antifungal acitivity of triflumizole was observed (which act as a selective inhibitor of dimethylation) during the egosterol biosynthesis.[12]

The increased percent inhibition of the radial growth of the pathogen (F. *lamoensis*) in hexaconazole and propiconazole treated plants with the increase in concentration. On the other hand, no increase of inhibition was observed in case of bavistin, roko and ektino treatment with the increase in their concentration. Among the systemic fungicides, hexaconazole was found to be highly effective in inhibiting the growth of the pathogen even at the lowest concentration of 10 ppm (86.66%), followed by propiconazole (37.77%). Similar results were also observed with other tea pathogens like *Hypoxylon serpens*,[13] *Colletotrichum gloeosporoides*,[14] *Pestalotia theae*, *Fomes lamoensis*[15] and *Phomopsis theae*.[16] Systemic fungicides such as bitertanol has been reported to be effective in controlling grey blight of tea in Korea.[17] Evaluation of the number of triazole fungicides in the recent years revealed that the formulation containing cyproconazole, hexaconazole, propiconazole and tebuconazole have been excellent in controlling blister blight of tea.[18,19,20] The efficacy was improved when triazole were used in combination with copper oxychloride. Strobilurin fungicides were reported to be effective against various pathogen at very low concentrations, (12-20 ppm)[21,22,23] found that propiconazole was very effective compared to mancozeb against stripe rust of barley.

In hexaconaxole, propiconazole, and bavistin treated plants shoot height and root length increased with increase in their concentrations used (10,50 and 100 ppm). The fresh and dry weight of shoot and root were also found to be highest in the fungicide treated tea plants as compared to only F. *lamoensis* inoculated control. The results obtained in the present work are similar to those of Dimond,[24] who reported that the chemotherapeutic compounds help to the plants rid get of the established infection. Dutta[25] reported that use of fungicides increased the plant height as well as the yield of tomato. According to Dutta[26] the higher yield of the fungicides treated plants is attributed to the change in metabolism of the fungicides treated plants. The increase in yield could also be due to phyto-tonic effect of fungicides. Singh[27] reported that foliar application of propiconazole (tilt 25 EC) @ 0.1% reduced the disease incidence and also gave higher yield over untreated control of barley. Recently, Selvakuma[28] reported that application of systemic fungicides (propiconazole, tebuconazole, mancozeb) in barley stripe rust disease not only controlled the disease severity but also increased the yield.

The control of soil borne plant pathogen with the help of antagonists has gained momentum in recent years. From the results of *in vitro* antagonism study, it was observed that the biological control agents, *B.cereus*and T. *harzianum*, inhibited the radial growth of F. *lamoensis*. *In vitro* screening of organisms is a valuable tool to select the potential strains of bio-control agents.[29,30] This has been reported by several workers[31,32,33,34,35] Many rhizobacteria including Fluorescent Pseudomonas sp. secrete a variety of antifungal molecules under *in vitro* and in situ conditions.[30] Johri[36] have also reported the *in vitro* antagonism of pathogenic fungi and field performance by bacteria isolated from the rhizosphere of crop plants in India.

In the biocontrol disease suppression and growth promotion study in the nursery condition, it was observed that the tea plants treated with the pathogen Fomes *lamoensis* had shown the highest disease incidence, whereas minimum disease incidence was observed in the antagonistic bacteria and fungi treated tea plants. Besides controlling the diseases, antagonistic fungal and bacterial are also known to enhance plant growth promotion. In the antagonistic biocontrol agents treated tea plants number of new leaves, number of lateral branches, shoot height, root length, fresh weight of shoot and root and dry weight of shoot and root significantly increased in all the treatments. Various plant diseases have been successfully controlled by the application of bacterial and fungal antagonists.[37] Most of the early works on biocontrol of plant pathogen by *Trichoderma* spp. involved direct ability of the fungi to interact with the soil pathogens.[38] The results obtained in the present work are identical to the works of Barazani,[39,40, 41,42,43] who also showed that application of *Trichoderma* and *Sebacimales* induced root and shoot growth in Piper *dilatatum*.

Conclusion

In the present study fungicides hexaconazole and propiconazole at the 100 ppm concentration controlled the brown root rot disease of tea both in *in vitro* and in nursery conditions. *B. cereus* and T. *harzianum* not only showed strong antagonistic activity against the pathogen but also significantly influenced growth of tea plants. Thus it can be proposed as an integrated disease management (IDM) strategy which helps to control tea root pathogen F. *lamoensis* for the benefits of tea growers at large.

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Conflict of Interest

The authors do not have any conflict of interest.

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