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Effect of microbial bioagents in controlling certain pests and diseases of tea

B. K. BARTHAKUR, S. R. SARMAH, P. K. DUTTA AND KARAN SINGH
Plant Protection Department, Tocklai Experimental Station, Tea Research Association, Jorhat 785008

In an attempt to minimize the hard toxic pesticides for controlling pest and diseases of tea, numbers of eco-friendly indigenous miicrobial bioagents have been experimented as plant protection tools during the recent years. Among the different microbial bioagents tested in tea growing areas of N. E. India *Trichoderma viride, T. harzianum*, have proved their efficiency in controlling root diseases like Charcoal stump rot, Brown root rot and stem disease, namely *Poria* branch canker. The indigenous bacterial bioagent *B. subtillis* has yielded around 55% control of blister blight and around 70% control of black rot—the two major leaf diseases of tea. *Beauveria bassiana*, a native entomopathogen could also provide more than 50% control of *Helopeltis theivora*, a major sucking pest of tea leaves in N. E. Indian tea plantation.

Key words : Microbial bioagents, pests, diseases, tea

INTRODUCTION

Tea *Camellia sinensis* L. (O.) Kuntze, a revolutionary perennial crop has been facing serious threat from pesticide residue toxicity during the recent years due to injudicious use of various toxic health hazardous chemicals for controllig different pests and diseases. To escape from this problem ecofriendly biological agents are being experimented as plant protection tool in controlling the major pests and diseases of tea throughout the world including the richest tea growing region of India i.e. Assam and West Bengal.

Considering the need of the day the Plant protection Department of Tocklai Experimental Station, Jorhat has also been giving more priority on biological control of pests and diseases at present. As a result of our constant effort we could isolate several indigenous microorganisms from the tea ecosystem and some of them were found effective in controlling numbers of pests and pathogens causing serious damage of tea plantation. Among these bioagents fungi like *T. viride, T. harzianum, B. bassiana, Verticillium lecanii, Paecilomyces lilacinus* and bacteria like *B. subtilis* and another native bacterium isolated from dead caterpillar pests were tested extensively to control various pest and diseases of tea.

Since early seventies numbers of eco-friendly microbial bioagents are identified and successfully used to control various pests and diseases of different crops (Dennis 1971, Papavizas *et al.*, 1980, 1985, Mukhopadhyay and Chandra 1986). In tea research also considerable success has obtained by utilizing numbers of antagonistic/ hyperparasitic microbes in controlling certain pests and diseases of tea (Mulder, 1961, Barthakur *et al.*, 1992, 1993, 1994, Chandramouli, 1993, Barua *et al.*, 1989 ; Das and Barua, 1990).

Tea mosquito bug, *Helopeltis theivora*, a major sucking pest of tea for controlling of which toxic pesticides are used extensively by the tea growers of N. E. India. However, such tea growers have a constant demand for an alternative of the toxic chemicals and a result of which numbers of indigenous predators, parasitoids and entomopathogens are being experimented at present. The present investigation includes some of the findings of our continuous attempt to control the major pests and diseases of tea of N. E. India by using biological tools.

MATERIALS AND METHODS

1. Laboratory study

Isolation of the bioagents : During the course of investigation, a phylloplane bacterium *B. subtilis* was isolated from tea leaves by dilution plate method in pure culture and that culture was maintained in PDA medium during the entire experimentation.

A few dominant Rhizosphere mycoflora including *T. viride* and *T. harzinum* were isolated on PDA medium from rhizosphere soil of tea growing area by soil dilution plate method and kept in pure culture for experimentation.

B. bassiana, an entomopathogenic fungus was also isolated from the body of naturally infected insects of tea plantation and maintained in nutrient medium for further use.

Isolation of the test pathogens : The causal organisms of Black rot i.e. *Corticium invisum,* Charcoal stump rot i.e.*Ustulina zonata,* Brown root rot i.e. *Fomes lamaoensis* and Branch canker i.e. *Poria hypobrunnea* were isolated from the infected plant parts and had maintained in PDA for experimentation. The second responsible pathogen for Black root disease i.e. *C. theae* and the causal organism of Blister blight i.e. *Exobasidium vexas* could not be islated in pure culture.

Antagonistic screening : A series of antagonism tests was conducted in agar plates to monitor the efficacy of the test microbials i.e. the bacterium *B. subtilis* and fungi, *Trichodrma viride* and *T. harzianum* for controlling black rot, blister blight, primary root rots and branch canker pathogens of tea individually.

Mass culture for field aplication : For mass culture and growth of these microorganesms, 10% potato broth supplemented with 0.2% glucose was used. Medium was prepared in 1000 ml conical flasks and sterilized in autoclave at 121°C temp. and 15 lb vpsi for 20 minutes.

Inoculation of the medium was done with previously grown 72 hours old cultures of *T. viride, T. harzianum* and *B. bassiana* and 48 hours old cultures of *B. subtilis* respectively. These cultures were incubated for 240 hours for *Trichoderma* species and *B. bassiana* and 72 hours for *B. subtilis.*Then the organisms were harvested ; finely macerated, homogenized and immediately applied in the field. CFU load of the *Trichoderma* spp. and *B. bassiana* were maintained at 4.5×10^7ml and 2.4 $\times 10^7$/ml respectively.

2. Field study

(1) Application of *B. subtilis* for control of Black rot and Blister blight : Several field experiments were conducted to study the efficacy of *B. subtilis* in controlling black rot and blister blight diseases of tea caused by *C. theae/C. invisum* and *Exobasidium vexans* respectively.

Black rot : Two field trials were laid out in two different commercial tea gardens having climatic variations with uniform infection of black rot disease. In both the trials four treatments were replicted for five times in small blocks having 50 plants in each. Two monthly rounds of treatments were applied with high volume sprayer during the months of May-June. Care was taken to spray the undersurface of the leaves and small stems thoroughly while spraying.

The post-treatment disease severity was assessed after two months of the last spray and results are presented in Table 1 by recording the disease incidence on each bush in the 0-4 scale of severity (0=Nil, 1=1-24%, 2=29-49%, 3=50-74%, 4= 75-100%). In the trials copper oxychloride (COC) was incorporated as a standard fungicide for comparison.

Table 1 : Effect of *B. subtilis* on control of black rot caused by *C. theae* and *C. invisum*

Treatments	Trial I *C. theae*		Trial II *C. invisum*	
	Mean disease severity	% reduction over control	Mean disease severity	% reduction over control
B. subtilis (10%)	7.6	66.6	9.9	79.3
COC (1 : 400)	6.5	71.7	11.6	75.8
Trichoderma suspension (10%)	11.8	48.7	36.4	24.1
Untreated control	23.0	—	48.0	—

Blister blight : 10% water suspension of 72 hours old *B. subtillis* broth was sprayed on the blister blight infested bushes. Special care was taken in drenching the upper surfaces of the leaves and small stems while spraying the blister prone/ affected sections. Four rounds of treatments were imposed at 7 days interval. Copper oxychloride (50% WP) was kept as a standard fungicide in all these field trials too.

Post-treatment disease assessment was made after two weeks from the last day of treatment imposition on per cent shoot infestation and number of blister recorded on the third leaf. The results are presented in the Table 2 below.

Table 2 : Effect of *B.subtilis* treatments in controlling blister blight disease caused by *E. vexans*

Treatments	On infected shoots		On numbers blisters	
	Mean infected shoots	% reduction over control	Mean number of blisters	% reduction over control
B. subtilis (10%)	39.6	40.5	82.6	55.9
COC (1 : 400)	10.6	84.0	15.3	91.8
Untreated control	66.6	—	187.3	—

(2) *Trichoderma* for control of *Poria* branch canker and Primary root disease : Based on the laboratory findings on hyperparasitism/antagonism trials *Trichoderma* bioagents were evaluated in the field for controlling *Poria* branch canker and Primary root rots of tea.

***Poria* Branch canker :** In this field trial *T. viride* and *T. harzianum* were applied on the pruning cuts along with the use of conventional indopasting for controlling *Poria hypobrunnea* the causal organism of the disease. The post-treatment assessment on infection was measured upto 36 months and the data are presented in the Table 3 below. In this trial

Table 3 : Percentage of *P. hypobrunnea* infection in different treatments

Puring cuts treated with	Percentage infection		
	1st year	2nd year	3rd year
T. viride	3.53	5.8	7.5
T. harzianum	4.16	12.5	14.7
P. hypobrunnea	77.5	85.8	89.5
T. viride followed by *P. hypobrunnea*	7.5	9.2	11.6
T. harzianum followed by *P. hypobrunnea*	12.5	14.2	17.5
Indopaste	8.33	37.5	46.6
Untreated control	29.6	51.7	66.6

Poria hypobrunnea was aritificially inoculated on the pruning surfaces, to see the establishment of the disease for comparison. All the treatmtnts were replicated for 3 times at random.

Primary root diseases : Three field trials of varied scales were conducted under this study to establish *Trichoderma* bioagents as an effective tool to control charcoal stump rot and Brown root rot diseases of tea caused by *U. zonata* and *F. lamaoensis* respectively. 10 days old *Trichoderma* broth immediately after harvesting was mixed thoroughly with well rotten cattle manure/ concentrated organic manure/compost @ 30 litres in 3000 kg. 200 g of this mixture was applied in each planting pit mixing thoroughly with the excavated soil during planting both in the replanted and infilled sections. No rehabilitation was done in any case of the planting. However, sub-soiling was done upto 8-12 inches in both the cases.

In these trials treatments were imposed in blocks as detailed in Table 4. Untreated control plot was kept in each trial for comparison. Annual observations were made in all these trials on the cause of mortality of the plants and the results are presented in Table 4.

Table 4 : Effect of different treatments on control of primary root disease of tea.

Trials	*Tricho-derma*	Tride-morph	Man-cozeb	Carben-dizim	Untreated control
Trail I (infilled area)					
Total plants per plot	40	40	40	40	40
No. of plants died	9	16	27	24	34
Death due to primary root rot	1	4	6	6	12
Death due to other causes	8	12	21	18	22
Trail II (replanted area)					
Total plants per plot	540	540	540	540	540
No. of plants died	46	73	81	75	140
Death due to primary root rot	0	6	5	9	24
Death due to other causes	46	67	76	66	125
Trail III (replanted area)					
Total plants per plot	22666		22666		22666
No. of plants died	15	—	25	—	32
Death due to primary root rot	Nil	—	11	—	13
Death due to other causes	15	—	14	—	19

Trial 1 = After 7 years of planting (infilled area)
Trial 2 = After 8 years of planting (without rehabilitation)
Trial 3 = After 4 years of planting (without rehabilitation)

Population dynamics of *Trichoderma* spp. in tea soil : Sufficient numbers of top soil samples were pooled from each of the experimental areas, homogenized individually and assayed for population of *Trichoderma* spp. before and after imposition of treatments. Data are presented in the Table 5 below.

Table 5 : Population of *Trichoderma* spp. in the experimental tea areas

Field trials	*Trichoderma* population per gm of top soil		
	Pre-treatment	Post-treatment	
Trial I (infilled area)			
Trichoderma	1.2×10^4	3.8×10^4	after 7
Untreated control	-do-	1.8×10^4	years of planting
Trial II (Replanted area)			
Trichoderma	1.3×10^4	3.6×10^5	after 8
Untreated control	-do-	1.7×10^4	years of planting
Trial III (Replanted area)			
Trichoderma	1.5×10^4	4.6×10^6	after 4
Untreated control	-do-	2.4×10^4	years of planting

(3) *Beauveria bassiana* for control of *Helopeltis theivora* : For mass culture of *B. bassiana* Wheat bran and Sabouraus's dextrose broth were used as optimum media where 7.9×10^7 / gm and 8.4×10^7/ ml cfu were recorded in haemacytometer counting. The mass culture was subjected in the field triss at 10% cfu concentration for control of *Helopeltis*. The field trials with *B. bassiana* formulation were conducted one in Assam and two in Darjeeling and the findings were presented in the Table 6 below where Endosulfan @ 1:400 and Neem formulations @ 1 : 1500 were kept as standard ones for comparison.

Table 6 : Effect of *B. bassiana* on control of *Helopeltis theivora*

Treatment	Assam trial % control *Helopeltis*	Darjeeling trial % control of *Helopeltis*	
		Trial-I	Trial-II
B. bassinana 10% broth	26.6	48.6	52.2
Neem 1 : 1500	54.6	51.7	60.3
Endosulfan 1 : 400	90.4	82.2	84.6

RESULTS AND DISCUSSION

A. Bacillus subtilis for black rot control

Antagonistic tests conducted in the laboratory have shown the positive effects of *B. subtilis* culture in inhibiting the growth of *C.invisum*. The bacterium had restricted the growth of the *Corticium* colony by forming a clear antagonistic zone. Total inhibition of *Corticium* growth was observed in broth culture of the test bacterium.

The data collected from different field trials showed the efficacy of *B. subtilis* in controlling black rot caused by both the species or *Corticium*. However, the effect was found to be more pronounced against *Corticium invisum*. Table 1 reveals that the treatment of *Bacillus subtilis* could produce 66 and 79 percent reduction of the disease cauded by *C. theae* and *C. invisum* respectively. While the standard Copper oxychloride formulation yielded more than 70% control of the disease in both the cases.

B. B. subtilis for blister blight control

The data presented in the Table 2 showed that 40.5 and 55.9% reduction of the disease over control in terms of infected shoots and numbers of blisters respectively when four rounds of *B. subtilis* broth was applied at 7 days interval. On the other hand the standard COC formulation provided 84 and 91.8% reduction of the disease after application of 4 rounds of COC at weekly interval. The results are considered as quite satisfactory as the bioagent tested was very safe, ecofriendly and a native one of the tea plantation which can be multiplied easily. Particularly the organic farmers will be benefitted much as this bioagent does not have any risk of in-organic health hazard.

C. Trichoderma for control of Poria branch canker

The results presented in the Table 3 on the effect of *Trichoderma* biocides in controlling branch canker diseases of tea. From the Table it can be inferred that the bioagent is highly effective in preventing the infection of *Poria hypobrunnea*. *T viride* was found to be slightly superior to *T. harzianum*. *Trichoderma* have been isolated from the wood beats of pruning cuts even after third year of application thus confirming the total establishment of the bioagents without causing harm to the tea bushes. It is also observed that the effectiveness of indopaste gradually declines with the passage of time and subsequently exposes the cut surfaces to the invasion by *Poria*.

D. Trichoderma for primary root disease control

The long term effects of *Trichoderma* application for controlling primary root diseases were recorded in Table 4 where it is seen that no mortality of tea plant has yet been recorded in the *Trichoderma* treated plots in the replanted areas done without rehabilitation. In the infilled areas only one plant was died due to primary root disease in the *Trichoderma* treatment. However. considerable mortality was recorded in plots of chemical treatment and untreated control ones. All these experiments will be continued up to a period of ten years to achieve a final conclusion.

E. Population dynamics of Trichoderma

The data presented in the Table 5 showed the population of *Trichoderma* in three different tea growing areas. *Trichoderma* population was found to be more in the treated soils than the untreated ones. However, population of *Trichoderma* of the pre-treated soil was found to be less which might be due to insufficient organic matter content in the soil. Population of *Trichoderma* was enhanced because of incorporation of organic matters in both plots as well as in untreated control ones.

F. B. bassiana for Helopeltis control

The experiments conducted with *B. bassiana* in the commercial gardens revealed about 26.6 to 52.0% control of Tea Mosquito Bug after spraying two fortnightly rounds of the bioagents in Assam and Darjeeling respecrively. This difference of per cent control was due to the influences of temperature which is generally low and more suitable for the growth of *B. bassiana* in Darjeeling conditions (Table 6).

Conclusion

Based on these findings of the investigation it can be concluded that

1. *B. subtilis* will be a potent bioagent to control Black rot and Blister blight diseases of tea. The bacterium does not have any deleterious effect on made tea, which gives us ample scope in commercial utilization of the bioagent to control the said diseases.
2. *Poria* branch canker can be successfully prevented by application of *Trichoderma* on the pruning cuts which are the common entry point for *Poria.*
3. Control of charcoal stump rot and brown root rot diseases of tea are found possible by using bioagents like *T.viride* and *T. harzianum.* This can be considered as the most remarkable achievement as no chemical are available in the market to control these diseases at present.
4. *B. bassiana* can be successfully utilized as a component of IPM to control Tea Mosquito bug, *Helopeltis theivora,* a very serious pest of tea.
5. The problem of Pesticide residue toxicity of made tea can be avoided by incorporating these bioagents in pest and disease control schedules in tea.

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