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Microbial Bioformulations for Suppression of Major Insect Pests and Diseases and Enhanced Biochemical Properties of Tea Crop

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A B S T R A C T

Four different microbial bioformulations (*Biosona*, *Biotime*, *Biogreen-5* and *Biogreen-L*) were explored under a biointensive strategy for management of major insect pests (Tea mosquito bug, Looper caterpillar, Red spider mites and Tea aphid) and disease (grey blight) of tea (*Camellia sinensis*). Analytical assays were also done to record biochemical changes in tea leaves due to application of the bioformulations following standard protocol. The bioformulations were applied as foliar spray at an interval of 15 days during May to September of 2016. Significantly highest reduction of tea mosquito bug (89.06%), red spider mite (92.04%), looper caterpillar (91.47%), aphid (92.53%) and grey blight (91.20%) was recorded due to application of Biogreen-5 (*Trichoderma viride, Pseudomonas fluorescens, Beauveria bassiana, Metarhizium anisopliae* and *Bacillus thuringiensis* based consortia bioformulation). Application of Biogreen-5 also showed enhanced level of caffeine (4.11%) and polyphenols (22.77%) along with increase in the green leaf yield (7.80 kg/plot)

Introduction

Tea (*Camellia sinensis* (L.) O. Kuntze) belonging to the family *Theaceae* and tribe *Gordonaceae* is a commercially grown, industry oriented evergreen crop. The crop is extensively cultivated in 13 states of India out of which Assam, West Bengal, Tamil Nadu and Kerala are the largest producers. Assam is the main tea growing state of India, mostly sub-Himalayan part of Assam from time immemorial. The total production of tea in Assam is 652.95 million kg per annum, which is more than 50 percent of India's total tea production (Anonymous, 2016). Tea is regarded as rich source of many beneficial properties such as stimulant, diuretic, digestive, anti aging, anti dysenteric, anti diarrhoea, slimming and as "summer heat and dampness". The chemical constituents present in tea includes, polyphenols, methylxanthines (caffeine, theophylline and theobromine), vitamins, amino acids, carbohydrates, proteins, chlorophyll, volatile compounds, fluoride, minerals, trace elements, and other undefined compounds (Cabrera *et al.*, 2003). Altogether, 1034 species of arthropods, 82 species of nematodes, 1 algal disease and 350 fungal diseases are associated with tea plants resulting in significant yield loss (Chen and Chen, 1989), and only, 167 species have been recorded from North-East India (Das, 1965), resulting 11 - 30 per cent annual yield loss in general. Amongst the insect pests, tea mosquito bug [*Helopeltistheivora* (Waterhouse) (Hemiptera: Miridae)] has assumed the status of a major pest in several tea growing areas in West Bengal and Assam (Somchowdhury *et al*., 1993). The red spider mite, [*Oligonychus coffeae* (Nietner) (Acarina: Tetranychidae)] causes considerable crop losses in South India (Muraleedharan *et al*., 2005) and at Terai, Dooars and Assam regions (Sharmah *et al*., 2009). Tea looper caterpillar, [*Biston suppressaria* (Guenee) (Lepidoptera: Geometridiae)] is a destructive and major defoliating pest of tea, shade trees and green crops (Ahmed and Mamun, 2010). Tea aphid, [*Toxoptera aurantii* (Hemiptera: Aphididae)] infestation also lead to poor crop productivity (Sohail *et al*., 2012). Among diseases, the grey blight disease of tea caused by *Pestalotiopsis* spp. has been reported from all tea-growing countries of the world (Venkata Ram, 1983).

In our present study attempts were made to explore some fungal and bacterial biocontrol agents with their consortium package for developing a biointensive management strategy against the major insect pests and diseases of tea.

Materials and Methods

Tea plants (TV-23 clone) from the Experimental Garden of Plantation crops, Assam Agricultural University, Jorhat, were used to study the efficacy of microbial bioformulation against the insect pests and disease (grey blight) during May to September of 2016. Four microbial bioformulations, *viz*., Biosona (*Beauveria bassiana*), Biotime (*Metarhizium anisopliae, Pseudomonas fluorescens* and *Trichoderma harzianum*), Biogreen-5 (powder formulation of *T. viride*, *P. fluorescens*, *Bacillus thuringiensis*, *B. bassiana* and *M. anisopliae*) and Biogreen-L (Liquid formulation of *T. viride*, *P. fluorescens*, *B. thuringiensis*, *B. bassiana* and *M. anisopliae*) and three chemicals *viz*., Spiromesifen, Clothianidin and Hexaconazole were used to evaluate their efficacy under field conditions. The bioformulations were collected from the Biocontrol Laboratory of Department of Plant Pathology, AAU, Jorhat and the chemicals were collected from the Experimental Garden of Plantation Crops, AAU, Jorhat.

The whole experimental area was laid out in RBD with five replications, five plots along with a control plot in each replication. Individual plots measured 5m x 3m. Between each replication, 1.5m of distance was maintained.

Each plot approximately contained 60 numbers of tea bushes. The treatment combinations compared were, foliar spray of *Biosona* (2.0%), *Biotime* (2.0%), *Biogreen-5* (2.0%), *Biogreen-L* (2.0%), three chemicals, *viz*., Oberon (Spiromesifen 22.9% EC) @ 0.05%, Dantotsu (Clothianidin 50% WDG) @ 0.25% and Sitara (Hexaconazole 5% EC) @ 0.25%. The control plants were sprayed with distilled water only. Altogether 10 sprays were done at an interval of 15 days from May-September, 2016 with manually operated hydraulic Knapsack sprayer.

For record of Tea mosquito bug incidence, 10 plants were randomly selected and thirty leaf samples were collected (3 samples/plant) from each plot. The incidence of tea mosquito bug was recorded in 0 to 4 scale of Jalgaonkar *et al*., (2009). The data so obtained was converted into per cent mosquito bug incidence.

For record of red spider mites, looper and aphid, the infested leaves and total leaves from 10 randomly selected plants were recorded in each treated and untreated plot and percentage incidence was calculated.

Grey blight disease incidence was recorded directly on the bush canopy using a quadrate of 1 sq. ft. size placing over the plucking table at random bushes. The number of infected and uninfected intact leaves, cut leaves, bare stalks and young shoots in 1 sq. ft. area were counted and the percentage were individually calculated. The disease reduction was calculated with the following standard formula: Disease reduction (%) = 100 – Disease Incidence.

Biochemical analysis

Caffeine content of leaf was estimated by the method of Yao *et al.*, 2006. The caffeine per cent in leaves were calculated by using the formula:

Caffeine (%) = E/1000 xV_0 x (100/V_1) x (50/25)/W = 0.2EV_0 /V_1/W; where E is mg of caffeine from the standard curve against the reading of the spectrophotometer, and E/1000 is to convert 'mg' into 'g'; V_0 is the total volume of the tea solution (250 ml); V_1 is the volume used for the measurement (10 ml), and 100/V_1 indicates 10 ml tea solution that were diluted to 100 ml, while 50/25 shows that another dilution from 25 ml tea filtrate madeto 50 ml in the measurement. 'W' is the dry weight of the tea sample.

Total polyphenol was estimated by Folin-ciocalteu method (Bray and Thorpe, 1954). For yield assessment, weight of green leaves harvested in each plucking round was recorded for individual plot and the monthly total was calculated.

For statistical analysis, the data of per cent values were transformed into corresponding angular values or log values wherever necessary. To compare the different treatments among themselves, critical differences were calculated out.

Results and Discussion

Efficacy of different microbial bioformulations in reduction of major insect pests and diseases of tea

All the treatments showed different degree of efficacy in controlling the insect pest as well as disease infestation as compared to control. The incidence of the pest was higher during the early part of May and as such, first spray was made during 1st week of May'2016 (1st May' 2016). The remaining nine sprays were made at an interval of 15 days following the 1st spray. The last spray was made on 15th Sept' 2016. The effect of standard chemical check seems to be highest but was significantly *at par* with the effect of bioformulation *Biogreen-5*. Lowest incidence of Tea mosquito bug (7.24%), Red spider mite (6.17%), Looper caterpillar (6.16%), Aphid (0.0%) and Grey blight (4.86%) was recorded after application of 10 sprays of *Biogreen-5* (2.0%). Other bioformulation treatments, i.e., *Biosona, Biotime, Biogreen-L* could control the insect pest and disease damage but their efficacy was lower as compared to *Biogreen-5*. Effect of different microbial bioformulations on incidence of tea mosquito bug, Red spider mite, Looper caterpillar, aphid, grey blight is presented in table 1.

PGPM formulation has been earlier tried for controlling various fungal and bacterial pathogens (Nandakumar *et al.,* 2001) which has provided an important clue of the commercial potential of the PGPR strains as well as commercialization of many microbial biocontrol products. Ramamoorthy *et al.*, (2001) has reported that several crop diseases and insect pests could be managed by use of talc-based microbial bioformulation. In addition to disease management, the PGPR strains has also been proved to increase plant growth and yield of various crops *viz.*, rice, potato, radish, mango and sugarbeet (Vivekananthan *et al.*, 2004). Our record showed that consortia of *P. fluorescens*, *T. viride*, *B. thuringiensis*, *B. bassiana* and *M. anisopliae* could significantly decrease pest and pathogen of tea along with increase in the yield. Reddy *et al*., (2001), observed that foliar spray of powder formulations of PGPR strains could promote the plant growth besides effectively controlling tomato bacterial spot (*Xanthomonas axonopodis* pv. *vesicatoria*), cucumber angular leaf spot (*Pseudomonas syringae* pv. *lachrymans*), tobacco blue mold (*Peronospora tabacina*) and wild fire (*P. syringae* pv. *tabaci).* In China, *B. bassiana* was used to control pests of tea, brown weevil (*Myllocerus aurolineatus*) successfully (Wu and Sun, 1994). Wu *et al*., (1995) sprayed 15-30 kg of the fungus @ 1-2 $x10^8$ spores/ml suspension and recorded 80 per cent control of the weevil in the field. Formulation of this fungus was prepared in order to apply it in combination with synthetic pyrethroid and organophosphorous insecticides for controlling *M. aurolineatus* during October - December, a period when adults emerge from the soil (Sun *et al*., 1993). Barua (1983) reported the occurrence of *Aspergillus* sp. on dead aphids collected from the tea plantation. The potentiality of utilizing *Verticillium lecanii*, *B. bassiana*, *Aegertia weberri* and *Entomophthora* sp. against scale insects was also reported by Hazarika *et al*., (1994). They sprayed water extract of plant products along with *Metarhizium anisopliae* and obtained good control of red spider mite.

Efficacy of different microbial bioformulations on yield (kg/plot) of green tea leaves

The data on green leaf yield of tea as influenced by different bioformulation treatments are also presented in table 1. There was a significant increase in the yield of green leaf in all the treatments as compared to the untreated control. The highest yield (7.80 kg/plot) was recorded in the plot treated with *Biogreen-5*. Among all treatments, lowest yield was recorded in the plot treated with *Biogreen-L* (3.73 kg/plot).

The decrease in the pest incidence and increase in the yield of tea might be due to suppression of the pest population by application of microbial bioformulation of different bioactive micro-organism. The present study also shows that there was significant reduction in insect pest and grey blight incidence in tea plants. Similar findings were also reported by Sravanakumar *et al.* (2007) that bioformulation of *P. fluorescens* and *B. thuringiensis* at weekly spray intervals consistently reduced the blister blight as well as grey blight disease incidence and increased the yield in tea plants.

Effect of different microbial bioformulations in caffeine and polyphenol content of tea leaves

The data of biochemical analysis (caffeine and polyphenol) are presented in table 2. There was a significant increase in the caffeine and polyphenols content of tea leaves in the treated plots as compared to the control (Fig. 1). Highest caffeine (4.11%) and polyphenols (22.77%) content was assayed in the leaves of plots treated with *Biogreen-5*, which was comparatively more than the standard chemical check with caffeine (3.74%) and polyphenol (19.20%) respectively. Assay of tea leaves treated with other bioformulation treatments, i.e., *Biosona*, *Biotime, Biogreen-L* showed standard concentration of caffeine as well as polyphenol, but was comparatively lower than treatment with *Biogreen-5*. Sudhakaran *et al.*, (2000) studied the influence of tea mosquito bug infestation on biochemical constituents of green leaf and quality parameters of black tea. Biochemical constituents such as chlorophyll, carotenoids, polyphenols, total sugars were very much reduced when the plants are severely affected. Kumaravadivelu *et al*., (1996) also reported a reduction in biochemical constituents of tea leaves due to flush worm infestation. Sanjay *et al.*, 2007 reported that biochemical constituents like polyphenols and catechins were highly reduced due to *Pestalotiopsis* infection in tea leaves. Mamun *et al*., (2016), reported that there was a reduction in chlorophyll as well as polyphenol contents of tea leaves due to infestation of red spider mites. In our study we also recorded a reduction of biochemical constituents (polyphenols and caffeine) of the young tea leaves due to insect pest and disease infestation.

Table.1 Effect of different microbial bioformulations on incidence of tea mosquito bug, red spider mite, looper caterpillar, aphid, grey blight and yield (kg/plot) of tea

Treatment	**Percent incidence of insect pests, disease and yield of tea plant after 10 sprays of bioformulations at 15 days interval**					
	Tea mosquito bug	**Red spider mite**	**Looper caterpillar**	**Aphid**	**Grey blight**	**Yield (Kg/plot)**
Biosona (2%)	35.85 (36.75)^{b}	34.11 (35.73)^{b}	30.23 (33.34)^{b}	10.36 (18.81)^{b}	79.54 (63.05)^{b}	4.77 (12.66)^{b}
Biotime (2%)	13.82 (21.81)^{c}	21.33 (27.49)^{c}	14.61 (22.46)^{c}	7.82 (16.22)^{c}	21.03 (27.27)^{c}	6.32 (14.54)^{c}
Biogreen-5(2%)	7.24 (15.56)^{d}	6.17 (14.42)^{d}	6.16 (14.42)^{cd}	0.00 (0.00)^{d}	4.86 (12.79)^{d}	7.80 (16.22)^{d}
Biogreen-L (2%)	47.44 (43.51)^{e}	58.57 (49.95)^{e}	48.25 (44.03)^{e}	22.53 (28.32)^{e}	33.94 (35.67)^{ce}	3.73 (11.09)^{e}
Clothianidin (0.05%), Spiromesifen (0.25%) & Hexaconazole (0.25%)	3.62 (10.94)^{df}	3.29 (10.47)^{df}	5.11 (13.05)^{df}	0.00 (0.00)^{df}	4.29 (11.97)^{df}	7.04 (15.34)^{cdf}
Control	96.30 (78.91)^{a}	98.21 (82.29)^{a}	97.63 (81.09)^{a}	92.53 (74.11)^{a}	96.06 (78.46)^{a}	0.63 (4.44)^{a}
SEd	2.71	3.90	4.41	0.51	4.30	0.51
CD (0.05)	5.64	8.15	9.21	1.06	8.98	1.06

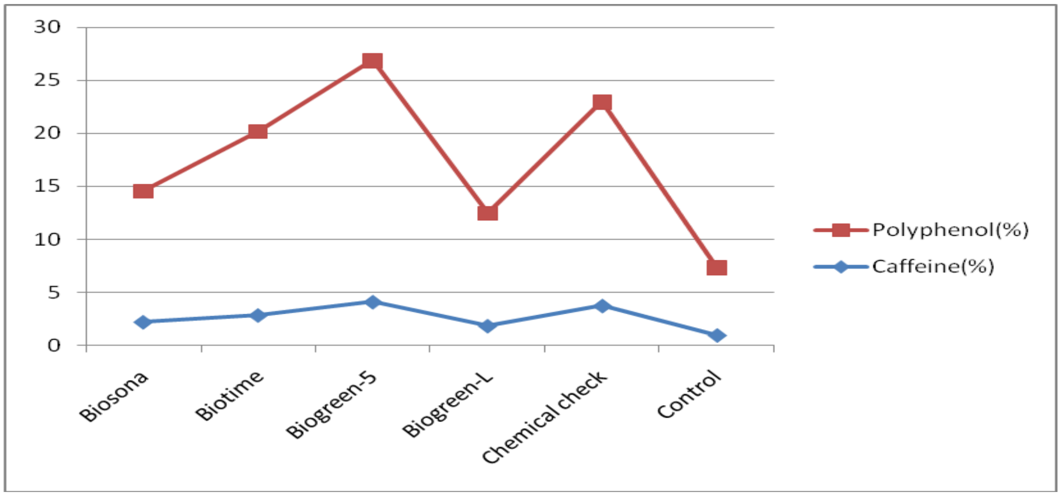
Values in the parenthesis are angular transformed values
Values superscripted with same letters are not significantly different (P=0.05)
Observations are mean of five replications

Table.2 Effect of different microbial bioformulations in caffeine (%) and polyphenol(%) content of green tea leaf

Treatment	Biochemical parameters of tea leaves treated with microbial bioformulation	
	Caffeine (%)	**Polyphenols (%)**
Biosona (2%)	2.20 (8.53)^{b}	12.35 (20.62)^{b}
Biotime (2%)	2.84 (9.63)^{c}	17.34 (24.58)^{c}
Biogreen-5 (2%)	4.11 (11.68)^{d}	22.77 (28.52)^{d}
Biogreen-L (2%)	1.82 (7.71)^{e}	10.62 (19.00)^{b}
Clothianidin (0.05%), Spiromesifen (0.25%) & Hexaconazole (0.25%)	3.74 (11.09)^{df}	19.20 (25.99)^{cde}
Control	0.94 (5.44)^{a}	6.36 (14.65)^{a}
SEd	0.35	1.69
CD (0.05)	0.72	3.41

Values in the parenthesis are angular transformed values
Values superscripted with same letters are not significantly different (P=0.05)
Observations are mean of five replications

Fig.1 Caffeine and Polyphenol content in tea (TV 23) clones treated with different microbial formulation



However, there was a significant decrease in the pest and disease infestation along with increase in the level of these biochemical constituents when the crop was treated with different microbial bioformulations, depicting that major foliar diseases and insect pests of tea can be controlled up to a desired level by application of microbial bioformulations. Application of microbial bioformulations as foliar spray can also help in minimizing the chemical pesticides load on tea. As tea consumption is gaining popularity throughout the world, more emphasis should be given in the production of quality tea with standard biochemical compounds as well as better agronomical characteristics for successful cultivation and commercialization.

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