

Research Article

Microbial biocides - Viable alternatives to chemicals for tea disease management

S. R. SARMAH¹*, P. N. BHATTACHARYYA¹ and A. K. BAROOAH²

¹Mycology and Microbiology department, Tocklai Tea Research Institute (TTRI), Tea Research Association (TRA), Jorhat - 785008, Assam, India

²Director, Tocklai Tea Research Institute (TTRI), Tea Research Association (TRA), Jorhat - 785008, Assam, India

*Corresponding author E-mail: s.sarmah@rediffmail.com; drsrsarmah@tocklai.net

ABSTRACT: Tea plantations in North east India are mostly rain fed and the cropping season needs moist climate with alternating wet and dry periods that eventually attracts diverse tea pathogens like *Fusarium solani*, *Cephaleuros* spp., *Corticium* spp. *Poria hypobrunnea*, *Ustilina zonata*, *Fomes lamaeensis* etc., known to cause several serious diseases in tea and thereby make enormous losses to crop yield and quality, influencing world tea market, adversely. Tea disease management using chemical fungicides alone is, however, prohibitive since the latter is known for destabilizing soil fertility and thereby directly affects the native microbial populations present in soil. Application of new fungicide molecules at its low doses along with exploitation of biological substances (more especially, microbial biocontrol agents) and adoption of Integrated Disease Management (IDM) schedule has been practiced in the present investigation for controlling certain devastating diseases in tea plantation of North East India. Multilocal field trials has been made throughout the investigations using locally isolated microorganisms such as *Azotobacter*, *Azospirillum*, *Bacillus*, *Pseudomonas*, *Streptomyces*, *Trichoderma* etc. (@ 2%, 5% and 10% spore concentrations), along with crude extracts of botanicals (@ 5% concentration) prepared from *Amphineuron opulentum*, *Cleome gynandra*, *Ipomea convolvulus*, *Polygonum hydropiper* etc. against dominant tea diseases such as black rot, red rust and *Fusarium* die back respectively. Maximum emphasis has been made to reduce the load of chemicals and move towards alternative strategies of tea disease management as to promote sustainable tea cultivation. Biological control measures (especially microbial) have potential to reduce the disease severity (up to 78.2% disease reduction) and thereby brought impending perspective for the use of effective biopesticides in tea ecosystem. The potential microbial strains were identified using morphological, biochemical as well as molecular characterization and deposited at NCBI GenBank database with accession numbers. The application technology of microbial biocides has also being popularized among the tea growers throughout the region to accelerate the use of these beneficial microorganisms in tea.

KEY WORDS: Alternative Biological approaches, biopesticide, fungicides, multilocal field trials, sustainable tea cultivation, tea disease management

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INTRODUCTION

Tea, “the queen of beverages” is an intensively managed, long-duration global monoculture crop, cultivated in diverse array of large and small-scale plantations (spreading over from Georgia (43° N) to Nelson (New Zealand, South Island; 42° S) including more than 50 countries across Asia, Africa, Latin America and Oceania (Hazarika *et al.*, 2009; Roy *et al.*, 2016) and from sea level to 2300 m above sea level (Roy *et al.*, 2014). A large number of tea pathogens are, thus, not surprising for their existence in tea monoculture across diverse topography, climate, planting material and cultural practices and thereby

leads to pathogenesis in tea. According to Lehmann-Danzinger (2000); Anita *et al.* (2012) and Ali *et al.* (2014) tea naturally encourage for the establishment of several tea pathogens as it mostly favours a stable microclimate with alternating wet and dry periods. Blister blight (*Exobasidium vexans*), black rot (*Corticium invisum* and *C. theae*), red rust (*Cephaleuros parasiticus* and *C. mycoidea*), charcoal stump rot (*Ustilina zonata*), brown root rot (*Fomes lamaeensis*), black root rot (*Rosellina arcuata*), *Fusarium* die back (*Fusarium solani*), brown blight (*Colletotrichum* sp.), grey blight (*Pestalotiopsis theae*), poria branch canker (*Poria hypobrunnea*), twig die back (*Macrophoma theicola*), red leaf spot (*Phoma*

theicola), violet root rot (*Sphaerostilbe repens*), leaf blight in tea (*Nigrospora sphaerica*), horse hair blight (*Marasmius crinis-equi*), bacterial canker (*Xanthomonas theae*) etc., are considered as the major depredating tea diseases during the last few decades known for posing real challenge to the tea planters throughout the world although the outbreak and magnitude of damage/crop loss for one or more of these diseases vary according to change in topography, climate and associated environmental conditions.

India is the second largest producer of tea (900,094 tonnes/year) in the world after China (1,000,130 tonnes/year) (Anonymous, 2015). North-eastern tea plantations in Indian subcontinent are characterized by evergreen and perennial plants, comprising genetically diverse tea cultivars with interplanted shade trees. Tea biosphere which is under direct influence of soil and climate (Banerjee, 1983) is likely to be infested by several major tea pathogens like *Exobasidium vexans*, *Corticium* spp., *Cephaleuros* spp., *Fusarium solani* etc., causing losses to the tea industry both in terms of productivity as well as quality. Inorganic chemical-based fertilizers are considered as the vital force since the time of its inception up to the last decade for tea disease management (Adesmyee and Kloepper, 2009), although overuse of pesticides are known to generate toxic environmental effect including soil quality deterioration, air and groundwater pollution, developing undesirable residues in made tea, resurgence of primary pests followed by outbreak of secondary pests and resistance development (Roy *et al.*, 2010; Saha and Mukhopadhyay, 2013; Ye *et al.*, 2014; Bhattacharyya and Sarmah, 2018). Improvement in the use of biological control measures have revolutionized the strategy to ameliorate the environmental pollution and health-hazard issues associated with overuse of synthetic pesticides (Chen and Chen, 1999). According to Ahkami *et al.* (2017) biological control measures have diverse beneficial effects on soil and plants including enhanced growth and crop production as well par excellence in improving soil health and productivity.

Screening the points under consideration, the present investigation has been aimed to enrich the nutritional requirement of tea plantations (*via* reducing the load of toxic chemicals) and improve bush health through the use of alternative biological approaches (microbes, in particular) along with adoption of Integrated Disease Management (IDM) approach and Good Agricultural Practices (GAP). Black rot, red rust (both stem and leaf) and *Fusarium* die-back are the tea diseases against which the present disease management schedule has been made. Some new molecules are tried along with biological disease management strategies, to observe their efficacy in disease reduction. As most of the organic

and inorganic substances are phytotoxic and responsible for disruption of normal growth and development of plants, an attempt, has, also been made to assess the phytotoxicity of the compounds. With the implementation of Plant Protection Code (PPC) by Tea Board, Ministry of Commerce and Industry, Govt. of India, in 2014, issues have been raised to maintain sustainability through GAP and thereby increased the dependency on alternative control strategies to gradually reduce the dependence on chemicals. The present investigation, thus, broadens the scope in exploiting the potential of beneficial microbial biocides against toxic chemicals for effective management of prominent tea diseases in N. E. India.

MATERIALS AND METHODS

Experimental plot selection, trial layout and treatment application

The experiments were conducted at different registered Tea Estates (TE) of TRA, located in Assam (latitude 24.28° N and longitude 90.96° E), North-East India. Altogether five different sites (site 1-site 5) that represent five different tea estates (site 1: Tocklai TE; site 2: Borting TE; site 3: Narayanpur TE; site 4: Latekoojan TE; site 5: Hoolungoorie TE) were considered in the present investigation for treatment application followed by field evaluation and data generation. Survey was made during April-May to assess the tea plants for disease infestation.

During the present investigation, treatment applications (exploitation of biological approaches (especially, microbials) along with new fungicide molecules) were made to control tea pathogens like *Corticium* spp., *Cephaleuros* spp. and *Fusarium solani* responsible in causing black rot, red rust (both stem and leaf) and *Fusarium* die-back diseases in tea, respectively. Table 1 represents site-wise description of the prevailing tea diseases against which experiments were

Table 1. Site-wise description of the tea diseases considered for the experiment

Sites	Tea estates	Diseases under scoring, evaluation and management		
		Black rot	Red rust (both stem and leaf)	<i>Fusarium</i> die back
Site 1	Tocklai TE	✓		
Site 2	Borting TE		✓	✓
Site 3	Narayanpur TE		✓	
Site 4	Latekoojan TE		✓	
Site 5	Hoolungoorie TE			✓

*The mark (✓) indicates the name of the diseases against which disease reduction evaluations were conducted.

conducted.

Randomized Block Design (RBD) with three replications was made along with a control plot in each replication. Between each replication, a standard gap (approx. 0.5 m distance) was maintained to differentiate the treatment applications. Each plot approximately contained 60-65 numbers of tea bushes. Efficacies of the microbial formulations along with other treatments (reduced doses of some chemical molecules and botanicals) to study the effect against selected tea pathogens was made during May to October. Some of the microbial strains under field exploration were identified using morphological, biochemical as well as molecular characterization and the data were submitted at NCBI GenBank database and obtained accession numbers. Foliar spray was made for different treatments along with their replications as designed accordingly. The control plants were sprayed with distilled water only. Two round of treatments were applied at 15 days interval followed by another two round at monthly intervals for controlling red rust disease, while for black rot control, treatments were applied for two rounds at 15 days interval and one round at monthly interval. Similarly, two rounds of treatments were applied in *Fusarium* infected shoots at 15 days intervals. Microbial biopesticides were used at different spore concentrations (SC) i.e., @ 2%, 5% and 10% with approx. $2.1-10.0 \times 10^8$ cfu/ml of the fluid; while fungicides were used at their recommended doses. Botanical extracts were prepared and used as per standard protocol (Fig. 1).

The spraying was done with manually operated hydraulic Knapsack sprayer (hollow cone NMD 60/450 nozzle, droplet diameter 1.6 mm, droplet size 140 μ m, discharge 450 ml/min at 40 psi pressure and distance between nozzle and target 30–45 cm) at 400 L/ha. Intensive care was taken to

thoroughly drench the entire bush specially the lower surface of the foliages.

Disease evaluation, identification and scoring under field conditions

Tea bushes were assessed for disease incidences like black rot, *Fusarium* die-back and red rust pathogens. Standard methodology (Sarmah, 1960a; 1960b) and cultivation-based approach was followed to identify and grow the tea pathogens *in vitro* (Gilman, 1957; Barnett and Hunter, 1972; Domsch *et al.*, 2007) after their collection from infected tea bushes/areas. The intensity of pathogenesis was measured in accordance with a modified 0-4 scale method (Sahni *et al.*, 2005), on the basis of percent area of infections/bush/leaves (0 = No infection, 1 = 1~25% infection, 2 = 25~50% infection, 3 = 50~75% infection and 4 = >75% infection) (Sharma and Singh, 2002). Scoring of individual bushes was calculated through direct field monitoring for disease incidence and by totalling every individual score, the final score per treatment was measured. Quadrate of approx. 1 sq. ft. size was used randomly over the plucking table in order to count the number of infected and uninfected intact leaves, cut leaves, bare stalks and young shoots over the area and the percentage were individually calculated. Post treatment disease severity was observed by scoring the individual bushes for disease severity. The efficacy of the treatments was assessed by calculating the per cent reduction of the disease over control using the following formula.

$$\% \text{ reduction over control} = \frac{\text{Control (C)} - \text{Treatment (T)}}{\text{Control (C)}} \times 100$$

Phytotoxicity screening and tainting test

Phytotoxicity symptoms like injury on leaf tips, injury on leaf surfaces, leaf wilting, necrosis, vein clearing, epinasty and hyponasty were observed up to 28 days after first spraying to record the toxic effect of applied compounds on plant growth. Phytotoxicity symptoms were calculated using a percentage scale as mentioned in Table 2.

Field experiment was conducted to study if the applied compounds have imparted any taint to black tea. Required dilutions were prepared to test the tainting effect. Spraying was made with manually operated Knapsack sprayer at 400 L/ha. Untreated control block was sprayed with water. Leaves were harvested on 7th and 14th day after spraying and processed separately in a mini CTC machine. The samples were analysed for accessing the tainting parameters.

Data analysis

Least Significant Difference (LSD), Standard Error Mean (SEM) among different treatments was calculated

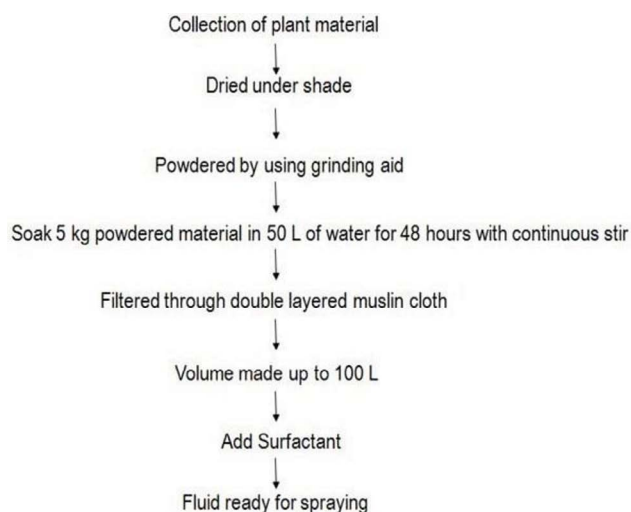


Fig. 1. Protocol for preparation of botanical extract for field evaluation

Table 2. Phytotoxicity screening scale

Percentage	Grade
0-10	01
11-20	02
21-30	03
31-40	04
41-50	05
51-60	06
61-70	07
71-80	08
81-90	09
91-100	10

in accordance with Gomez and Gomez (1984). Duncan's Multiple Range Test (DMRT) comparisons were made for different treatments to test the significance at 5% level using IBM SPSS Statistics. Values within the same column followed by different letters are significantly different ($P = 0.05$) from each other.

RESULTS AND DISCUSSION

Field evaluation for disease incidence and identification

As mentioned, experiments were conducted in Tea Estates where mature tea bushes were severally attacked by target tea diseases like black rot, red rust (both stem/leaf) and *Fusarium* die back. General symptoms of disease infestations noticed during field evaluation have been summarized in Table 3.

The present investigation emphasized for the exploitation of several Plant Growth Promoting (PGP) microbes as biofungicides over chemicals in tea as the former is eco-friendly and produces no toxicity after application as well have considerable level of efficacy in disease management. The microorganisms along with their identification code/GenBank accession number have been represented in table 4. 16S rDNA amplification of bacterial strains like *B. subtilis* and *B. pumilus* has been represented in Fig. 3.

Efficacy of biofungicides in reducing disease severity

Efficacy of microbial treatments (*Azotobacter chroococcum*, *A. brasilense*, *Pseudomonas corrugata*, *Bacillus subtilis*, *Streptomyces nojiriensis*) along with chemical fungicides for reduction of black rot disease (site 1) has been represented in Table 5-6. Application of microbial formulations showed significant reduction in disease severity (up to 70.6% reduction over control) which is, moreover, comparable to Copper Oxychloride (COC) in terms of per cent reduction of the disease.

Table 3. Major disease symptoms of tea under field evaluation

Tea diseases	Field observation and salient symptoms
Black rot	The infected bush shows symptoms of burning of leaves. Dead leaves remain suspended or attached to the branches. Brown, yellowish-brown and grey covering was observed on the upper surface of the infected leaf. The under surface was light brown and covered with a network of brown mycelium. Dark purplish brown thick cord of mycelium was noticed on the infected branches.
Red rust	Brick red or orange fructifications was noticed on the infected stems consisting of a dense growth of tiny orange colour hairs, bearing at their ends minute knobs (sporangia). The leaves of the infected branches variegate with yellow patches. The alga later penetrates deep into the epidermal cells and intercellular spaces and produces brownish colour dead spot.
<i>Fusarium</i> die-back	Blackening of the petioles was observed that gradually extend to the nodes and internodes, followed by wilting of the primaries. The surrounding tissues of the point of attack gradually die off and fail to regenerate the shoot.

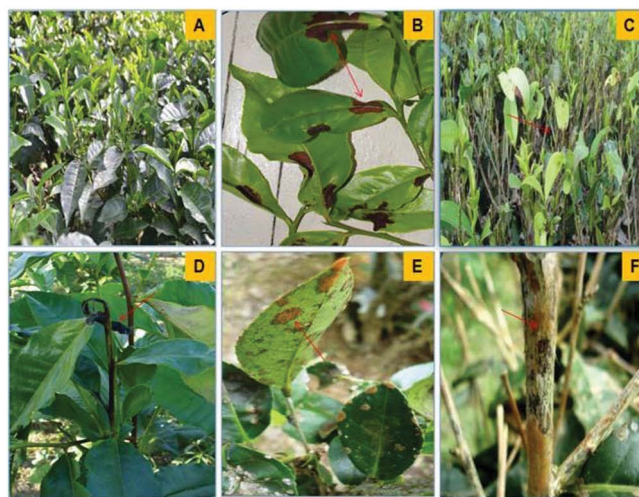


Fig. 2. Photographs of disease infestation in tea along with healthy bush canopy (overview). A. Canopy of healthy tea bushes; B. *Corticium invisum*; C. *Corticium theae*; D. *Fusarium solani*; E. *Cephaleuros mycoidea*; F. *Cephaleuros parasiticus*

Among the microbial treatments, *B. subtilis* @ 10% SC showed better efficacy in disease reduction which might be due to the presence of effective spore load should be replaced by more effective spore load. Commercial exploitation of Plant Growth Promoting (PGP) strains for fungal disease management have been experimented by Nandakumar *et al.* (2001) and thereby established the potential of beneficial microbial strains as biocontrol agent. The efficacy of standard chemical (COC, in present investigation) seems to be highest

Table 4. Identification of microorganisms

Identification	Identification code/ Accession number	Morphological/ biochemical/ Molecular characterization
<i>Bacillus subtilis</i>	MG519830	Molecular
<i>B. pumilus</i>	MG557973	Molecular
<i>Trichoderma viride</i>	MH030275	Molecular
<i>Azotobacter chroococcum</i>	MM/AZR/08	Morphological/ biochemical
<i>Azospirillum brasilense</i>	MM/AZM/10	Morphological/ biochemical
<i>Pseudomonas corrugata</i>	MM/PDS/01	Morphological/ biochemical
<i>Aspergillus niger</i>	MM/PSM/10	Morphological
<i>Streptomyces</i> spp.	MM/AC/01	Morphological/ biochemical

Table 5. Efficacy of different treatments (microbial, new fungicide molecules) in controlling black rot diseases of tea at site 1 (Expt. A)

Treatments and dose	MDI	Reduction over control (%)
Hexaconazole 5 % SC @ 10ml/10L	9e	71.0
Hexaconazole @5ml/10L	11d	64.5
<i>Pseudomonas fluorescens</i> 1% W.P @ 50g/10L	17bc	45.2
<i>Pseudomonas fluorescens</i> 1% W.P @ 25g/10L	19b	38.7
<i>Pseudomonas fluorescens</i> 1% W.P @12.5g/10L	20b	35.5
Myclobutanil 10%WP @10g/10L	13cd	58.1
Myclobutanil 10%WP @7.5g/10L	14c	54.8
COC 50% WP @ 25g/10L	7e	77.4
COC @ 12.5g/10L	12d	61.3
COC @ 1:400	7e	77.4
BST @ 2%SC	15c	51.6
BST @ 5%SC	12d	61.3
BST @ 10%SC	10de	67.7
AC 01 @ 5%SC	11de	64.5
Control	31a	-
SEM (±)		2.19
LSD ($p = 0.05$)		6.38
CV (%)		27.36

*MDI; Mean disease incidence; COC: Copper oxychloride; BST; *B. subtilis*; AC 01; *S. Nojiriensis*; Values within the same column followed by different letters are significantly different from each other ($P = 0.05$) by Duncan's Multiple Range tests (DMRT) comparisons

Table 6. IDM strategy against black rot disease of tea at site 1 (Expt. B)

Treatments and dose	MDI	Reduction over control (%)
Trifloxystrobin 25%+Tebuconazole 50% WP @ 1: 100	15d	57.1
Trifloxystrobin 25%+Tebuconazole 50% WP @ 1:100	12de	65.7
Tetraconazole 3.8% EW @ 500ml/ha	12de	64.9
Tetraconazole 3.8% EW @ 750ml/ha	11.6e	66.9
Copper 435 @ 600ml/ha	12.6de	64.0
Copper 435 @ 1000ml/ha	10.3e	70.6
Copper 435 @ 1500ml/ha	11e	68.6
Captan70%+Hexaconazole 5% WP @ 1000gm/ha	12.6de	64.0
Tagero @ 250gm/ha	18.6c	46.9
Tagero @ 500gm/ha	17.3c	50.6
COC @ 1:400	8.6e	75.4
AZR @ 10% SC	21.3b	39.1
AZM @ 10% SC	19.7bc	43.7
PDS @ 10% SC	18c	48.6
BST @ 10% SC	10.3de	70.6
Control	35a	-
SEM (±)		2.03
LSD ($p = 0.05$)		5.92
CV (%)		22.77

*MDI; Mean disease incidence; COC: Copper oxychloride; AZR; *A. chroococcum*; AZM; *A. brasilense*; PDS; *P. corrugata*; BST; *B. subtilis*; Values within the same column followed by different letters are significantly different from each other ($P = 0.05$) by Duncan's Multiple Range Tests (DMRT) comparisons

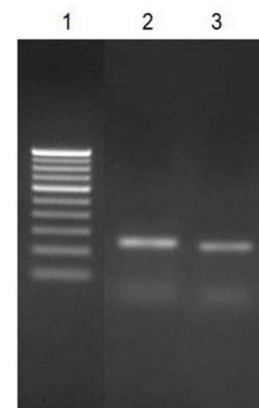
**Fig. 3. 16S rDNA amplification of bacterial strains. Lane 1; molar mass marker (100 bp); Lane 2; DNA sample of *Bacillus subtilis*; Lane 3; DNA sample of *B. pumilus***

Table 7. Microbial management of red rust disease in tea at site 2

Treatments and dose	MDI	Reduction over control (%)
AZR @ 10% SC	15.7c	48.9
AZM @ 10% SC	14d	54.4
PDS 01(@ 10% SC	16.3c	47.0
BST@ 10% SC	6.5d	78.9
PSM 10 @ 10% SC	19.7b	35.8
Control	30.7a	-
SEM (\pm)		0.739
LSD ($p = 0.05$)		2.15
CV (%)		7.47

*MDI; Mean disease incidence. AZR; *A. chroococcum*; AZM; *A. brasiliense*; PDS 01; *P. corrugata*; BST; *B. subtilis*; PSM 10; *A. Niger*; Values within the same column followed by different letters are significantly different from each other ($P = 0.05$) by Duncan's Multiple Range Tests (DMRT) comparisons.

but was significantly at par with the effect of certain microbial formulations @10% SC. Post-treatment data analysis for red rust disease reduction also indicates effectiveness of microbial strains in disease reduction (Table 7).

Among the biocontrol agents, *B. subtilis* @ 10% SC recorded maximum reduction (up to 78.9%) over untreated control. The bush health was also improved as there was increase in crop gain in the next plucking session. Exploitation of microbial formulations against tea disease management has been made by Deb *et al.* (2017) and thereby indicates the potential of microbial biocides for their use in tea. Vivekananthan *et al.*, (2004) observed significant enhancement in crop yield after application of microbial consortium.

Table (8-9) indicates evaluation of certain new fungicide molecules, botanicals and microbials that were tried against red rust disease in tea at site 3 and 4. The results exhibited reduction of red rust disease intensity ranging from 37.6-77.5% over control. Although, hexaconazole (1:1000) (Table 8) and COC + APSA 80 (1:400+6 ml) (Table 9) seemed to be superior to other treatments; the influence of alternative disease management approach (especially application of microbials) is no less non-significant. Among the microbial, *A. niger* @ 10% SC is recorded as the most effective that showed disease reduction up to 72.1%. Extracts of botanicals such as *A. opulentum*, *P. hydropiper*, *C. gynandra*, *I. convolvulus* also showed their efficacies in controlling the disease ranging from 37.6-74.8% over control. Post treatment observation for controlling red rust disease at site 4 (Table 9)

Table 8. Bio-efficacy of different treatments (microbial, botanical and new fungicide molecules) against red rust diseases of tea at site 3

Treatments and dose	MDI	Reduction over control (%)
COC @1:400	10d	65.5
Hexaconazole @1:1000	6.9e	76.2
Tetraconazole @1:1000	11.2c	61.4
Tetraconazole @1:500	12.9c	55.5
Captan 70%+ Hexaconazole 5% WP @1:100	15.5bc	46.6
Trifloxystrobin 25%+Tebuconazole 50% WP @ 1:100	10d	65.5
COC + Hexaconazole @ 1:800+1:2000	8.6de	70.3
<i>Amphineuron opulentum</i> @ 5% WE	16.1bc	44.5
<i>Polygonum hydropiper</i> @ 5% WE	15.4bc	46.9
<i>Cleome gynandra</i> @ 5% WE	11.7c	59.7
<i>Ipomea convolvulus</i> @ 5% WE	7.3e	74.8
<i>A. opulentum</i> + <i>P. hydropiper</i> @ 5% WE + 5% WE	8de	72.4
<i>C. gynandra</i> + <i>I. convolvulus</i> @ 5% + 5% WE	18.1b	37.6
PSM 10 @10% SC	8.1de	72.1
BST @ 10% SC	16.7bc	42.4
AZR @ 10% SC	10.5d	63.8
Control	29a	---
SEM (\pm)		1.87
LSD ($p = 0.05$)		5.43
CV (%)		25.44

*MDI; Mean disease incidence; PSM 10; *A. niger*; BST; *B. subtilis*; AZR; *A. chroococcum*; Values within the same column followed by different letters are significantly different from each other ($P = 0.05$) by Duncan's Multiple Range tests (DMRT) comparisons

indicates potential of *B. pumilus*, resulting 64.3% reduction of the disease over control.

Similarly, disease management approach against *Fusarium* die back using the microbial biopesticide as the sole applicant has been made in site 2 (Table 10). The microbial strains used in the experiment were *A. niger*, *Streptomyces nojiriensis*, *S. griseoluteus*, *S. somaliensis*, *P. corrugata*, *B. subtilis*, *A. chroococcum* and *A. brasiliense*. The microbes were used @ 10% SC. There was decrease in disease intensity ranging from 33.9-62.3% after application of the microbials over control. *B. subtilis* @ 10% SC showed maximum efficacy (up to 62.3%) among different treatments.

Table 9. IDM strategy for controlling red rust disease in tea at site 4

Treatments and dose	MDI	Reduction over control (%)
Myclobutanil 10% WP @ 1:1000	11.7bc	58.2
COC 50% WP @ 1:400	11c	60.7
Hexaconazole 5%SC @ 1:1000	12.3b	56.1
COC @ 1:400	7e	75.0
Cu Tribasic @30 ml/10 L	11c	60.7
Cu Tribasic @25 ml/ 10L	11.7bc	58.2
Kocide @1:400	12b	57.1
COC + APSA 80 (adjuvant) @ 1:400+6 ml	6.3e	77.5
COC + Dhanuvit (adjuvant) @ 1:400+6 ml	6.7e	76.1
Propiconazole 25%EC @ 1:1000	10d	64.3
BST @ 5% SC	9.7d	65.4
BST @ 10% SC	8.7de	68.9
AC 01 @ 5% SC	11c	60.7
Sesame oil 1.0% @ 100 ml/10L	12.7b	54.6
Sesame oil 1.5% @ 150 ml/10L	13.3b	52.5
Koranj oil 1% @ 100 ml/10L	12b	57.1
Koranj oil 1.5% @ 150 ml/10L	11.3c	59.6
KMB 08 @ 5% SC	10d	64.3
Control	28a	-
SEM (±)		1.91
LSD ($p = 0.05$)		6.13
CV (%)		26.73

*MDI: Mean disease incidence; BST; *B. subtilis*; AC 01; *S. nojiriensis*; KMB 08; *B. pumilus*; Values within the same column followed by different letters are significantly different from each other ($P = 0.05$) by Duncan's Multiple Range tests (DMRT) comparisons

Another disease management approach exploiting the potential of new fungicide molecules and microbial biopesticides such as *B. subtilis* and *T. viride* at different spore concentrations have been made at site 5 (Table 11). Microbial biocides like *B. subtilis* and *T. viride* when applied @ 2% and 5% spore concentrations could control the disease from 56.8-78.2%. Both the microbial, *B. subtilis* and *T. viride* @ 5% spore concentration recorded 65.7% and 78.2% reduction of the disease respectively, over control. The microbes were also found to be effective even at low doses (@2% SC) and thereby reducing the disease intensity from 56.8-69.9%. The efficacy of *T. viride* was, thus, comparable to hexaconazole in terms of reducing the disease severity (Table 11) although several other factors like soil pH, moisture content and nutrient

Table 10. Microbial management of *Fusarium* die back diseases in tea at site 2

Treatments and dose	MDI	Reduction over control (%)
PSM 10 @ 10% SC	14.3c	44.4
AC 01 @ 10% SC	12.7d	50.6
AC 02 @ 10% SC	17b	33.9
AC 03 @ 10% SC	10e	61.1
PDS 01 @ 10% SC	16.3b	36.6
BST @ 10% SC	9.7e	62.3
AZR @ 10% SC	12d	53.3
AZM @ 10% SC	14.7c	42.8
Control	25.7a	-
SEM (±)		2.72
LSD ($p = 0.05$)		7.92
CV%		96.03

*MDI: Mean disease incidence; PSM 10; *A. niger*; AC 01; *S. nojiriensis*; AC 02; *S. griseoluteus*; AC 03; *S. somaliensis*; PDS 01: *P. corrugata*; BST: *B. subtilis*; AZR: *A. chroococcum*; AZM: *A. brasilense*; Values within the same column followed by different letters are significantly different from each other ($P = 0.05$) by Duncan's Multiple Range tests (DMRT) comparisons

Table 11. Bio-efficacy of new fungicide molecules and microbial formulations against *Fusarium* die back diseases in tea at site 5

Treatments and dose	MDI	Reduction over control (%)
COC 1:400	8.4d	73.1
Myclobutanil @10g/10L	14.3b	54.2
Copper @1:400	11.4c	63.5
Hexaconazole @ 5.0 ml/10L	8.1d	74.0
Hexaconazole @ 10.0 ml/10L	6.8e	78.2
Propiconazole @ 10 ml/10L	8.8d	71.8
Tribasic 436 @ 30g/10L	10cd	68.0
BST @ 2% SC	13.5b	56.8
BST @ 5% SC	10.7c	65.7
TV @ 2% SC	9.4d	69.9
TV @ 5% SC	6.8e	78.2
COC+APSA 80 (adjuvant) @1:400+6ml/10L	7.7e	75.3
COC+Dhanuvit (adjuvant) @1:400+6ml/10L	8.2d	73.7
Control	31.2a	-
SEM (±)		1.03
LSD ($p = 0.05$)		2.99
CV (%)		16.08

*MDI: Mean disease incidence; BST: *B. subtilis*; TV: *T. viride*; Values within the same column followed by different letters are significantly different from each other ($P = 0.05$) by Duncan's Multiple Range tests (DMRT) comparisons

status of soil and topography might influence the potential of microbial action under field evaluation (Bhattacharyya and Jha, 2012).

The data so, obtained, was found statistically significant at $p < 0.05$ level. The approach of adopting alternative disease management practices including the foliar application of microbial biocides for controlling significant tea diseases is, thus, essential for minimizing the chemical load on tea and thereby assists to develop sustainable tea cultivation.

Analysis of phytotoxicity and tainting of compounds

The chemicals used in the present investigation has been analysed for phytotoxicity symptoms and tainting abilities with the assistance of Tea Taster. The compounds are not reported for causing any phytotoxicity symptoms and also observed as taint free.

Exploitation of microbial biocides as an alternative to chemical pesticides is a promising approach towards development of eco-friendly tea cultivation. Effective and consistent control of the target disease, however, largely dependent on the concentrations and quality of the microbial formulation under use. As tea consumption has attained its popularity throughout the globe, more emphasis should be given in the production of quality tea with minimal use of toxic plant protection chemicals and thereby encouraging the use of alternative approaches for disease management and quality control. Adoption of non-chemical, eco-friendly and safer approach, thus, necessitates high demands in world market to ensure healthy and non-toxic tea consumption.

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