



Evaluation of some botanicals and microbial bioformulations against grey blight disease of tea (*Camellia sinensis*)

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ABSTRACT

Grey blight disease of tea (*Camellia sinensis*) is considered as a major threat to tea plantation owing to adverse affect on yield and quality parameters in tea. In the present study, an effort was made to explore some botanicals and microbial formulations for management of grey blight disease under field condition in the pretext of growing demand of organic tea. Seven botanicals at three concentrations (5.0, 10.0 and 15.0%) and four microbial bioformulations were screened *in vitro* against *Pestalotiopsis theae*, the grey blight pathogen. Of the botanicals, significant inhibition was recorded by water extract of *Xanthium strumarium* and *Pongamia pinnata* at 15% concentration. Biogreen 5 (Formulation of *Trichoderma viride*, *Pseudomonas fluorescens*, *Bacillus thuringiensis*, *Beauveria bassiana* and *Metarhizium anisoplae*) and Bioveer (*T. viride*) among the bioformulations recorded highest reduction in mycelial growth of pathogen. The two most effective botanicals and bioformulations screened *in vitro* were further evaluated individually and in combination under field condition for two consecutive seasons. Lowest percent disease incidence (5.23%) was recorded with combination of Biogreen 5 and Bioveer followed by Bioveer alone (7.79%) at 150 days of spray. Foliar spray of *X. strumarium* and *P. pinnata* in combination also showed satisfactory reduction in grey blight incidence with PDI of 17.97%. The study revealed that both bioagents and botanicals could be an ecofriendly and sustainable solution for grey blight disease management under organic tea production system.

Key words: Botanical, Bioformulations, Grey blight, Organic production, Tea

Tea, (*Camellia sinensis* (L.) O., Kuntze) is commercially grown in state of Assam, West Bengal, Tamil Nadu and Kerala (Hazarika *et al.* 2009). India is globally a leading tea producer with an annual production of 1.31 million tons accounting for 22.4% of world tea basket (Anon. 2019a). Assam is the single largest contiguous tea growing area in the world with annual production of 652.95 million kg from an area of 3.19 lakh ha (Anno. 2019b). The grey blight disease of tea caused by *Pestalotiopsis* like species is reported as one of the most destructive diseases in all the tea-growing countries of the world (Chen *et al.* 2008), with 17% production loss in India (Joshi *et al.* 2009). The disease is characterized by appearance of small oval and pale-yellow symptoms first appearing on young leaves and later, turning grey brown, affecting the quality of tea leaves (Sanjay and Baby 2002). Application of fungicides is the most widely and frequently adopted strategy to combat the disease among the growers; however accumulation of fungicide residues has jeopardized the potential of tea to be exported from India, a major concern to tea industry (Das

et al. 2010, Bora 2020).

In northeastern region, the use of botanicals like *Azadirachta indica*, *Lantana camera*, *Acorus calamus* etc. is being practiced by many small tea growers for management of diseases (Hazarika *et al.* 2009). Botanical biopesticides have been reported to be highly effective against grey blight pathogen (Sarkar *et al.* 2009). The plant growth promoting microbes (PGPM), such as fluorescent pseudomonads and *Trichoderma* spp. are regarded as plant growth promote as well, as efficient biocontrol agents against many fungal pathogens (Bora *et al.* 2016a). Despite these fragmentary efforts, very limited success has been observed with botanicals and microbial bioagents against grey blight disease. With this background, we studied the efficacy of some important botanicals and microbial bioformulations against grey blight disease as a part of developing organic production system.

MATERIALS AND METHODS

Collection of botanicals and microbial formulations: Habitat specific surveys were carried out in the commercial tea growing areas of Assam state, viz. Dibrugarh, Tinisukia, Sonitpur, Jorhat and Udalguri districts to develop a repository of baseline information with regard to kind of botanicals used by the tea growers against grey blight disease management.

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Seven plant species *Xanthium strumarium* (Burweed), *Aegle marmelos* (Bael), *Acorus calamus* (Sweet flag), *Pongamia pinnata* (Karanj), *Lantana camara* (wild sage), *Annona squamosa* (Custard apple) and *Psidium guajava* (Guava) were shortlisted based on the past history and experience from the tea growers duly cross verified with the help of botanical descriptors developed by AAU, Jorhat.

Four microbial bioformulations developed at AAU, viz. Biogreen 5 (*Trichoderma viride*, *Metarhizium anisopliae*, *Beauveria bassiana*, *Bacillus thuringiensis* and *Pseudomonas fluorescens*), Biosona (*B. bassiana*), Biometa (*M. anisopliae*) and Bioveer (*T. viride*) were collected from Biocontrol Laboratory, Department of Plant Pathology, AAU, Jorhat for evaluation.

Isolation of pathogen: Diseased tea leaves showing typical symptoms of grey blight were collected in sealed polypropylene bags and brought to the laboratory for pathogen isolation. The diseased samples with healthy tissues were cut into small pieces and surface sterilized by dipping in 0.1% sodium hypochloride (NaOCl) solution for two minutes. The cut pieces were then placed onto sterilized potato dextrose agar (PDA) in petridishes and incubated at $27 \pm 2^\circ\text{C}$, until mycelium formation. Hyphal tips of the fungus were transferred onto PDA plate and incubated at $27 \pm 2^\circ\text{C}$ in order to obtain the pure culture (Burnett 1983).

In vitro evaluation of botanicals: The antifungal activity of plant extracts of shortlisted seven botanicals was evaluated by poison food technique (Nene and Thapliyal 1979). Plant extract of 100 % aqueous solution was prepared by grinding thoroughly washed and air dried plant parts with sterile water in sterile pestle and mortar at the rate of one ml/g (1: 1 w/v) (Chethana *et al.* 2012). In order to prepare 5, 10 and 15% plant extract a 5, 10 and 15 ml of stock solution was aseptically transferred and mixed with 95, 90 and 85 ml of sterilized molten potato dextrose agar medium, respectively. The medium was poured into sterilized petri dishes and after complete solidification, 5mm disc of seven-day-old culture of the pathogen was inoculated at the centre of the Petri dishes. The Petri dishes containing media devoid of the extracts served as control. The plates were incubated at $22 \pm 1^\circ\text{C}$ for seven days. All the treatments were replicated three times with completely randomized design (CRD). The antifungal activity of the extracts was calculated Vincent (1927) as:

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

where I, C and T stand for per cent inhibition of mycelial growth, colony diameter in control and colony diameter in treatment, respectively.

In vitro evaluation of bioformulations: The bioformulations at standardized concentration (5% Biogreen 5 and 2% each for other formulations) were initially screened against *P. theae* following poison food technique. Hundred percent stock solution of bioformulation was prepared by mixing each bioformulation with sterile water separately @ 1 g/ml (w/v) followed by centrifuging at 5000 rpm for 10 minutes and the supernatant was used for the study. The

concentration of 5% and 2% was prepared by method as described for botanicals. Petri dish with media was used as control. The experiment was designed in CRD with 4 replications. The results were expressed as per cent inhibition in mycelial growth over control (Vincent 1927).

Field experimentation: Field experiment was conducted during spring (March- April) of 2017–18 in the organic tea garden at Nakochari, Jorhat, Assam. Two botanicals and two bioformulations were selected based on the *in vitro* assay and evaluated for their efficacy against grey blight disease under field condition. All together 7 treatments were laid out and arranged in RBD, each replicated 4 times. Each plot had 50 tea bushes of TV23 clone susceptible to grey blight and naturally infected by the pathogen. Each plot was separated with two buffer rows. Foliar spray of two botanicals @ 15% alone and in combination (7.5% each), the two bioformulations alone and in combinations were given at 15 days interval from May to September with a total of 10 sprays. The percent disease incidence was recorded at 30, 60, 90, 120 and 150 days after the first spray (DAS) following the method as described by Sanjay *et al.* (2008). Per cent disease incidence (PDI) was calculated as:

$$\text{PDI} = (\text{IL} + \text{CL} + \text{BS} + \text{YS}) / 4$$

IL, disease incidence on intact leaves; CL, disease on cut leaves; BS, disease on bare stalk; YS, the disease on young shoot.

Statistical analysis: Data obtained were statistically analysed by one way analysis of variance (ANOVA) in SPSS programme. The significant difference were compared by using critical difference (CD) at $P=0.05$ significance level.

RESULTS AND DISCUSSION

In vitro evaluation of botanicals against Pestalotiopsis theae

All the botanicals evaluated against the pathogen showed inhibition of mycelial growth of test fungus at all the three test levels of 5 %, 10% and 15% with varying magnitude of response (Table 1). However, highest inhibition was caused by *X. strumarium* (71.33%) followed by *P. pinnata* (65.43%) at 15% concentration. Hence, 15% concentration of the two superior botanicals was used for field evaluation. Efficacy of *X. strumarium* was reported by Saha *et al.* (2012) against some tea pathogens, viz. *Colletotrichum camelliae*, *Curvularia eragrostidis* and *Rhizoctonia solani* and they identified the active antifungal compound as a sesquiterpene lactone “Xanthatin”. Similarly *P. pinnata* possess antifungal and antibacterial properties against many plant pathogens including *Sclerotium rolfsii*, (Shaheen *et al.* 2017). Leaf extract of *Pongamia* spp contain flavonoids which inhibit cytoplasmic membrane function, nucleic acid synthesis and also slow down energy metabolism and subsequently prevents the germination and mycelia growth of fungi (Tim and Lamba 2005).

In vitro evaluation of microbial bioformulations against P. theae: The *in vitro* screening revealed Biogreen -5 showed

Table 1 *In-vitro* screening of antifungal activity of botanicals against *P. theae*

Treatment	Percent inhibition at concentration		
	5%	10%	15%
<i>Pongamia pinnata</i>	19.53	40.36	65.43
<i>Lantana camara</i>	10.33	21.86	38.06
<i>Xanthium strumarium</i>	33.10	62.10	71.33
<i>Acorus calamus</i>	10.33	17.60	35.10
<i>Annonus squamosa</i>	19.93	34.86	53.90
<i>Psidium guajava</i>	9.30	14.80	22.43
<i>Aegle marmelos</i>	9.30	29.23	41.26
Absolute control	0.00	0.00	0.00
CD (P=0.05)	3.68	1.78	2.97

highest inhibition (94.05%) of the test fungus followed by Bioveer (65.17%). Barman *et al.* (2015) earlier reported 74.3% mycelia inhibition of *P. theae* by *T. viride* (Bora 2021). A comprehensive review on antagonistic potential of *P. fluorescens* and *T. viride* alone or in combination has been widely reported against many major plant pathogens (Bora *et al.* 2016b).

Khan *et al.* (2018) reported significant inhibition of *F. oxysporum* f. sp. *lactucae* isolated from hydroponically grown lettuce by combination of *P. fluorescens*, *T. viride* and *B. thuringiensis*. *Trichoderma* spp. inhibited the growth of plant pathogens through a number of mechanisms, viz. hyperparasitism, antibiotics, diffusible toxic substances and overcrowding the pathogens (Bora *et al.* 2013). Fluorescent pseudomonads antagonized plant pathogens by producing siderophores, antibiotics and other substances such as cyanide (Bora *et al.* 2019, 2020). Secondary metabolites from entomopathogen, *M. anisoplaea* can also inhibit the fungal plant pathogens (Ravindra *et al.* 2014, Sharma *et al.* 2020).

Field evaluation of botanicals and bioformulations: The minimum PDI was recorded in combined application of Biogreen @ 5% + Bioveer @ 2% (5.23%) followed by Biogreen alone (7.79%) treated plants at 150 days after foliar spray application (Table 2). Sanjay *et al.* (2008) report that *Trichoderma*, *Gliocladium* and *Pseudomonas* provided an effective control over grey blight disease in tea under field condition. Fluorescent pseudomonads and *Trichoderma* spp, known as Plant growth promoting microbes (PGPM) also suppress phytopathogens through enhancing plant growth as well as activating plant defense response (Bora and Bora 2008, Bora *et al.* 2013). Collective action of five plant beneficial microbes present in Biogreen 5 formulation resulted in proportionately higher magnitude of control of grey blight disease as compared to *T. viride* based Bioveer alone. Stockwell *et al.* (2011) reported that application of microbes in a consortia formulation could improve their efficacy, consistency and reliability under diverse soil and environmental conditions.

In our investigation, botanicals also showed satisfactory

Table 2 Field evaluation of selected botanicals and bioformulations against grey blight disease (Pooled data: 2017–18)

Treatment	Percent disease incidence (PDI) at different days after spray				
	30	60	90	120	150
<i>P. pinnata</i>	41.63	37.50	36.00	30.25	30.07
<i>X. strumarium</i>	36.68	30.41	26.7	22.00	22.17
<i>P. pinnata</i> + <i>X. strumarium</i>	32.73	26.75	21.25	19.75	17.97
Biogreen-5	27.58	21.17	13.75	10.60	7.79
Bioveer	42.98	37.35	32.2	29.25	28.12
Biogreen-5 + Bioveer	23.53	16.79	11.22	10.20	5.23
Untreated Control	76.30	83.95	86.80	88.50	95.05
CD (P=0.05)	1.80	2.18	2.16	2.14	2.39

control over grey blight disease. Foliar application of *X. strumarium* in combination with *P. pinnata* and *X. strumarium* alone showed satisfactory results reducing the incidence to grey blight to 17.97% and 22.17% respectively. Saha *et al.* (2012) recorded that field application of compounds purified from *X. strumarium* leaf extracts significantly reduced grey blight and brown blight incidence of tea plantlets. Raja and Sreenivasulu (2016) also highlighted fungitoxic effect of *P. pinnata* against *Alternaria solani* and *Helminthosporium turcicum*. Apart from plant secondary metabolites, disease reduction by botanicals is attributable to their ability to induce synthesis of defense related phytochemicals viz. polyphenol oxidases, super oxide dimutases etc (Hayat *et al.* 2018). Foliar application of *Azadirachta indica* also shows increased activity of defense related enzymes and accumulation of phenolics in cucumber and induces resistance against powdery mildew disease (Aboellil 2007). Such studies demonstrated that botanicals could be effectively used in control of grey blight diseases in an organic production system.

The study, hence, put forward a database evidence for strong role of bioinputs as a suitable option in grey blight management programme under present scenario of organic or biointensive tea cultivation practice.

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