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Evaluation of plant products and antagonistic microbes against grey blight (*Pestalotiopsis theae),* a devastating pathogen of tea

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The present study was carried out to evaluate the effect of different oils (lemongrass, neem, karanj, zinger, eucalyptus and patchouli oils), different plant product extracts (garlic, zinger, false ashoka and datura) and different antagonistic microorganisms (different species of *Trichoderma* spp., *Bacillus* sp. and *Pseudomonas* sp.) on grey blight of tea, *Pestalotiopsis theae*. Results reveal that eucalyptus oil and neem oil (0.05%) showed 98.1 and 94.3% inhibition of mycelial growth over the control, respectively. Although, both of them at 0.1% showed 100% inhibition for the pathogen. Similarly, plant extract garlic and datura showed 98.2 and 95.4% inhibition of mycelial growth over control. Among the different antagonistic agents, *Trichoderma viride* showed 74.3% inhibition of mycellial growth over the control. Among chemical fungicide, bavistin showed 100% inhibition over control. The various antifungal extracts showed inhibitory/fungicidal effect against grey blight of tea. These could serve as sources for development of new antifungal agent.

Key words: Plant products, antagonistic microorganism, grey blight of tea, mycelial growth.

INTRODUCTION

Many farmers do not use synthetic pesticides, and some consumers will only buy organic produce. Many plant species produce substances that protect them by killing or repelling the insects that feed on them (Pelegrini et al., 2006). Plant based products has been used for many centuries among limited resource farmers in developing countries to control insect pests of both field crops and stored produce, but their potential was initially limited and ignored (Chowdhury et al., 2003). Nowadays, scientists are exploring a lot of plant based products which are significantly used for controlling various diseases. Essential oils are steam volatile, aromatic oils different from fatty oils and the oils are obtained by enzymatic action (Nariman et al., 2009). Recently, essential oils are used

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for controlling various pests. Essential oils are natural anti-depressants, antibacterial fighters and immune strengtheners. It is a major group of agro-based industry products. Insect-pests like plants, can be infected by disease-causing organisms such as bacteria, viruses and fungi. Under some conditions, these naturally occurring organisms multiply quickly to cause disease outbreaks or epizootics that can decimate an insect population. These are called antagonistic microorganisms. Many microbial antagonists have been reported to posses antagonistic activities against plant fungal pathogens (Dabur et al., 2004).

Tea is an economically important crop which is cultivated extensively throughout north-east India. Several fungal pathogens produce foliar diseases of tea. Tea leaves are harvested from the young twigs but the lower leaves are not harvested but they are important for maintenance of the tea plants (Almada-Rui et al., 2003). One of the important leaf disease caused by *Pestalotiopsis theae* frequently attack tea plants in the sub-Himalayan West Bengal. Till date, the disease is controlled by chemical fungicides in most of the tea estates situated in the region. Use of chemical fungicides is becoming unpopular due to all round awareness of their hazardous effect on the environment (Bansod and Rai, 2008; Fukai et al., 2003). The application of broad-spectrum chemical fungicides is the common practice in most of the horticultural crops for controlling fungal diseases. The fungicides are extremely hazardous to our health and environment (Rocha et al., 2004). Therefore, it is essential to adopt eco-friendly methods to control fungal diseases of our vegetable crops. Numerous plant extraction, essential oil and antagonistic possessing potential pest-controlling properties under controlled condition, that is, in laboratory, but the step from the laboratory to the field eliminates many contenders, even when judged only on their efficacy against target pests under realistic field conditions. Keeping the export value of organic tea in foreign country, that is, chemical pesticide free fresh organic tea, *in vitro* study was carried to evaluate different plant products against grey blight (*P. theae)*, a pathogen of tea plant. It is likely that the results will broaden the scientific base upon which total control of the fungal diseases of tea may be established through eco-friendly disease management programmes.

MATERIALS AND METHODS

Pathogen

Infected tea plants (variety TV-9) were collected from tea nurseries of Kharibari, Siliguri, West Bengal, India and used in this experiment. Fungal pathogens (*P. theae*) were isolated from the infected tea plant leaves and grown in potato dextrose agar medium and maintained in the laboratory. The pathogens were subjected to Koch's postulates for verification of the diseases.

Thereafter, they were incubated at 4°C. After seven days of incubation, the hyphal tip of the fungus radiating from the infected tissue was transferred onto PDA slants. Freshly prepared sterile PDA slants were used for the maintenance of the fungal cultures by sub-culturing periodically. Pathogens grown on sterile PDA media were stored in two different conditions, viz. at low temperature in refrigerator (at 4°C) and in incubator at 27±1°C. At the interval of one week, subculture was done taking sample from incubator at 27±1°C for preparation of inoculums for different experiments. To avoid loss of virulence, fresh isolations were made as at when required and the pathogenicity of the isolates on tea plant leaves was ascertained.

Plant oils

Different plant oil: lemongrass oil (0.05 and 0.1%), neem oil (0.05 and 0.1%), karanja oil (3%), zinger oil (1%) and eucalyptus oil (0.01 and 0.1%) were obtained from local market. The oil cakes were soaked in sterile distilled water individually at 2 g/ml and kept overnight for extraction by microwave assisted hydrodistillation as described by Rodriguez et al. (2012).

Plant extract

To obtain alcohol extract from fresh plant parts, fresh plant parts were washed thoroughly with sterile distilled water and surface water was soaked by blotting paper at room temperature. The materials (3 g) were ground in a 'mortar and pestle' with 6 ml 50% ethanol with autoclaved water to make 0.5 g/ml concentration. The extracts were filtered through double-layered muslin cloth and centrifuged at 10,000 g for 30 min. The supernatant of alcohol extracts were collected in plastic vials and all extracts were stored at 4°C until used for bioassay.

Bioagents

Antagonistic microorganisms viz., *Trichoderma viride*, *Trichoderma virens*, *Trichoderma harzianum*, *Trichoderma longibrachiatum*, *Trichoderma reesi*, *Bacillus subtilis, Pseudomonas fluorescens* isolate 1 and *P. fluorescens* isolate 2 were purchased from New Agriculture, Kolkata, India. These were tested against the growth of *P. theae in vitro* by dual culture technique.

Efficacy of different oils against *P. theae*

Experiment (pre standardized) was carried out with different concentration for different plant oils. Then the concentration of plant oil for testing the efficacy was finalized. After emulsifying with teepol at 1 ml/L, appropriate concentration of plant oil (eucalyptus oil 0.05 and 0.1%, Neem oil 0.05 and 0.1%, Karanj oil 0.1%, lemongrass oil 1%, zinger oil 1%, Patchouli oil 1%) mixed with sterilized potato dextrose agar medium (PDA). Then they were thoroughly mixed just before plating to get specified concentration of the plant oils. Then 20 ml of this mixture was poured into a sterilized petri dish (10 cm diameter) in three replications to allow solidification. Culture disc of the pathogen *P. theae* having 6 mm in size was taken and placed onto the centre of the medium. The plates were incubated at 27±1°C. When the control plate showed full growth, then radial growth of the colony in each plate was measured.

In vivo antifungal activity of plant extracts

Antifungal activity of ethanolic extracts (1.0 mg/ml) of garlic (*Allium*

Table 1. Effect of different plant oils on disease occurrence of grey-blight (*Pestalotiopsis theae*) in tea cut shoot.

Plant oil	Mycelial growth (cm)	Per cent inhibition over control
Eucalyptus oil (0.05%)	0.03	98.1
Eucalyptus oil (0.1%)	0.00	100
Neem oil (0.05%)	0.08	94.3
Neem oil (0.1%)	0.00	100
Karanj oil (1%)	1.02	85.4
Lemongrass oil (1%)	1.31	78.4
Zinger oil (1%)	1.71	71.3
Patchouli oil (1%)	2.13	62.7
control	8.87	0.00
CD (*P=0.05*)	1.15	

sativum) bulbs, datura (*D. stramonium*) and false ashoka (*Polyalthia longifolia*) leaves and zinger (*Zingiber officinale*) rhizome was tested *in vivo* by the cut shoot technique. Tea twigs (10-12 cm long) were collected from plants of susceptible varieties grown in the experimental garden and placed into the holes of a Styrofoam board and floated on Hoagland and Knop's solution in a glass chamber. The extracts were sprayed to leaves of tea-twig until runoff, 24 h prior to inoculation with the pathogens. In all cases, 0.05% Tween-20 was added to all spraying solutions as wetting agent. Control plants were sprayed with distilled water. Disease index was computed on the basis of visual observations of lesions following the technique of Sinha and Das (1972).

Efficacy of antagonistic bacterial microbes against *P. theae*

To carry out this experiment, a 6 mm actively growing PDA culture disc of the pathogen was placed on a PDA petri dish at one side, 1.5 cm away from the edge of the plate. This was then incubated at 27±1°C. After 48 h, actively growing cultures of the respective test bacteria (*Bacillus* and *Pseudomonas*) were separately streaked onto the medium at the opposite side of the plate, 1.5 cm away from the edge. The plates were incubated at at 27±1°C. Three replications were maintained for each antagonistic bacteria. The radial growth of the pathogen was measured four days after inoculation. The results were expressed as per cent growth inhibition over control. The plates inoculated with the pathogen alone served as control.

Efficacy of antagonistic fungal microbes against *P. theae*

Culture disc having 6 mm size of actively growing *P. theae* was placed onto sterilized petri dish containing previously plated and solidified *Czapek's Dox* medium which are at 1.5 cm away from the edge of the plate. Another 8 mm fresh culture disc of antagonistic organism was placed opposite to *P. theae* disc. For each test three replications were maintained. The plates were incubated at 27±1°C. After full growth of the control sample, the radial growth of pathogen in each plate was measured. *Czapek's Dox* medium which is inoculated with pathogen alone served as control. The results were expressed as % inhibition of the mycelial growth of pathogen over control.

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

Where, I = percent inhibition over control; C = growth of pathogen in control; T = growth of pathogen in treatment.

Evaluation of the fungicides against *P. theae*

In 100 ml Erlenmeyer flask containing 20 ml sterilized and melted potato dextrose agar medium (PDA), 0.2% of each fungicide formulation was weighed and added. Then they were thoroughly mixed by gentle swirling, poured into Petri dish and allowed to solidify. A 6 mm actively growing mycelia disc of the pathogen was placed onto the medium. The plates were incubated at 27±1°C. Three replications were maintained for each test. The potato dextrose agar (PDA) medium without incorporating the fungicides and inoculated with the pathogen served as control. When the control plate showed full growth, the radial growth of pathogen in each plate was measured. The data obtained from this experiment were subjected to statistical analysis using SAS 9.3 software.

RESULTS AND DISCUSSION

In vitro assay of plant oils against *P. theae*

Among the six different plant oils tested against *P. theae,* eucalyptus and neem oil at 0.05 and 0.1 resulted 98.1 and 94.3% inhibition of mycelia growth of the pathogen than control followed by karanj, lemongrass, zinger and patchouli oil produced 85.4, 78.4, 71.3 and 62.7% inhibition of mycelia growth (Table 1). Although, eucalyptus and neem oil at 0.1% causes 100% inhibition over control. Use of different plant oils, lemongrass, neem, karanj, zinger, eucalyptus and patchouli oil for the management of *P. theae* could restrict the growth of fungus significantly. Similar type of results has been showed by Jadeja (2003), Mdee et al. (2009) and Nariman et al. (2009).

Effect of plant extract against *P. theae*

For studying the effect of plant extracts *in vivo*, susceptible

Table 2. Effect of plant extract on disease occurrence of grey-blight (*P. theae*) in tea cut shoot.

Plant species tested	Mean disease index/shoot			
	24 h	48 h	72 h	96 h
Garlic (*Allium sativum*)	0.00	0.04	0.06	0.11
Zinger (*Zingiber officinalis*)	0.00	0.05	0.08	0.15
False ashoka (*Polyalthia longifolia*)	0.00	0.03	0.06	0.14
Datura (*Datura stramonium*)	0.00	0.04	0.08	0.12
Control	1.12	1.43	2.83	2.90

Table 3. Effect of antagonistic microbes on the growth of *P. theae*.

Antagonistic organisms	Mycelial growth (cm)	Per cent inhibition over control
Trichoderma viride	2.15	72.4
T. Reesi	2.79	65.2
T. Virens	3.14	55.4
T. longibrachiatum	3.27	50.7
Bacillus subtilis	2.91	59.7
Pseudomonas fluorescens isolate 1	6.78	27.8
P. fluorescens isolate 2	5.78	35.4
Control		0.00
CD (*P=0.05*)	0.53	

plant tea was treated with selected plant extracts. Following the treatment, the plant was subjected to challenge inoculation pathogens *P. theae*. Garlic and datura (1.0 mg/ml in ethanol supplemented with 0.05% Tween-20) reduced the occurrence of diseases caused by *P. theae* in the susceptible inoculated plants in comparison to control followed by false ashoka and zinger. Mean disease index was lowest in garlic (0.11) followed by 0.12, 0.14 and 0.15 in datura, false ashoka and zinger, respectively (Table 2). All these plant extracts are bio-products which may be used for field application. Similar type of results has earlier been shown by Jadeja (2003), Baka (2010) and Hadizadeh et al. (2009).

In vitro assay of bio-control agents against *P. theae*

Among the bio-control agents *T. viride* and *T. reesi* showed significantly highest mycelia inhibition of *P. theae*. The mycelia inhibitions in the above plates were 72.4 and 65.2% respectively, over control. The bacterial antagonist *B. subtilis* was the next best antagonists in inhibiting 59.7% growth of *P. theae.* Isolate *T. virens* and *T. longibrachiatum* were the other two antagonists, which inhibited the pathogen growth to 55.4 and 50.7%, respectively. The antagonist *P. fluorescens* isolates 1 and 2 were identified as poor inhibitor against *P. theae* with the growth inhibition of only 27.8 and 35.4%, respectively over control (Table 3). *T. viride* was most effective in controlling *P. theae* in tea under *in vitro* condition. Our study on bio control agents and their application will broaden the knowledge base on which a total control of these diseases may be established. Similar type of results has been shown by Sangeetha et al. (2011) against *Colletotrichum capsici* causing fruit rot of chilli and also by Raghavendra et al. (2009).

In vitro assay of fungicides against *P. theae*

Among all the treatments, bavistin recorded absolute inhibition of mycelial growth of the pathogen followed by captaf that produced 82.4%, nystatin resulted in 72.4%, ziram resulted in 65.7%, copper oxy chloride in 55.7%, thiram in 41.2% and mancozeb 35.7%, respectively. Mancozeb showed the maximum mycelial growth of 6.82 cm and least percent inhibition over control (35.7%). Bavistin showed 100% inhibition of the pathogen in poison food technique. Two-three spray of bavistin recorded best control of *P. theae* (Table 4). Thus, bavistin could be used as a chemical agent for controlling the disease.

Conclusions

The result of this study suggests that locally available

Table 4. *In vitro* assay of chemical fungicides (0.2%) against *P. theae.*

Fungicide	Mycelial growth (cm)	Per cent inhibition over control
Bavistin	0.00	100
Captaf	1.78	82.4
Nystatin	2.14	72.4
Ziram	3.02	65.7
Copper oxy chloride	4.51	55.7
Thiram	5.73	41.2
Mancozeb	6.82	35.7
Control	8.87	0.00
CD (*P=0.05*)	0.62	

plant products extract and antagonistic microbes may play a great role in controlling grey blight of tea disease. This may encourage the farmers to produce organic tea to generate more revenue.

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