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Efficacy of Systemic Fungicides Against Brown Root Rot (*Fomeslamoensis*) Disease of Tea (*Camellia sinensis* (L) O. Kuntze) *in vitro* and in Nursery Condition

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Abstract: The tea plant of commerce (*Camellia sinensis* (L) O. Kuntze) is grown in more than 50 countries lying between 43°N and 42°S latitudes and from sea level to 2300 m above mean sea level. These plants prefer warm humid climatic conditions, well-distributed rainfall and long sunshine hours. These conditions are also conducive for the growth of many pests and pathogens and further, the monoculture habitat provides a stable microclimate for their easy transmission and establishment. Brown root rot disease identified as the primary root disease of tea was isolated from naturally infected root of tea. The fungus is pathogenic to one year of tea plants, which exhibited brown root rot disease symptoms within one months of inoculation. Five systemic fungicides were screened and evaluated against the pathogen. Among the five fungicides used two namely, Propiconazole and Hexaconazole have shown 98.51 % and 100% inhibition properties against the pathogen at the concentration of 100mg/L, whereas Bavistin showed 12.58%, Roko 5.18 % and Ektino 1.48 % inhibition respectively. Therefore, the result of the present study suggest the potentials of use of Propiconazole and Hexaconazole at minimum concentration for maximum suppression inhibition of *Fomeslamoensis*. The results of the field observation have also been presented.

Key words: Brown root rot · Inhibition · Systemic fungicides · Tea (*Camellia sinensis* (L) O. Kuntze)

INTRODUCTION

Tea (*Camellia sinensis* (L) O. Kuntze) belongs to family *Theaceae*. It is herbaceous, dicotyledonous and perennial crop. It is the second most commonly consumed non alcoholic beverage in the world after water. Worlds total tea production in 2007 was 3.527 million tones and the total area under cultivation is around 5 million hectares [1]. The seven top tea producing countries are China, India, Kenya, Sri Lanka, Indonesia, Turkey and Vietnam, where total production occupies 80.56% of the world production [2]. Tea cultivation was initiated in India around 1886 with the opening of few gardens under two tea companies of Assam. Now, India is the largest producer (944.68 million Kg) as well as the consumer of tea in the world (786 million Kg). Assam is the leading producer of tea (479925 thousand Kg) with a total area of 3.128 lakh hectares under cultivation. Brown root rot diseasethe causal organism *Fomeslamoensis*is the primary root disease of tea is commonly prevalent in low elevation tea growing areas particularly in hills garden. Petch describes this disease as the earliest known root disease of tea. It is prevalent in all the tea growing areas of India but seldom causes any serious trouble. The progress of the disease is usually slow and, although the fungus produces external mycelium, it does not appear to spread to any appreciable distance through the soil. Apparently the disease passes from one bush to another along roots which happen to be in contact. The organism causing brown root rot gains entry through the roots. Infection also occur by infected material coming into contact with healthy plants [3]. The disease is more common on tea in sandy soil and characteristic feature of brown rot is the presence of brown mycelium on root surface to which soil,

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sand and stone particles remain encrusted [4]. Protactent fungicides are used as prophylactic chemicals, as they act outside the plant prior to infection by the pathogen. It is a treatment intended to prevent or protect the plant against infection. Protactent fungicides which may be applied to seeds, soil or to plant surfaces, cannot penetrate into plant tissue in effective amounts. By contrast systemic fungicides, which are taken up by the plant, cure established infection [5] and [6]. Keeping the above in view the present work has been taken up to observe the potentiality some systemic fungicides against the brown root rot pathogen *Fomeslamoensisin vitro* and nursery conditions.

MATERIALS AND METHODS

The methodology followed and the materials used as follows:

Plant: one years old tea (*Camellia sinensis* (L.) O. Kuntze) plants (Clone: TV 1) obtained from Roskandy Tea estate, Silchar

Fungicides: Following systemic fungicides are used

Funicides		
Trade Name	Chemical Name	Active ingredient
Bavistin	Carbendazim	50% W/P
Roko	Thiophenate methyl	70% W/P
Ektino	Tricyclazole	75% W/W
Xantho	Hexaconazole	5% EC
Tilt	Propiconazole	25% EC

Fungal Isolate: The fungal pathogens *Fomeslamoensis* (culture no. 4140) was procured from Indian Type Culture Collections (ITCC) of Indian Agricultural Research Institute (IARI), New Delhi. Culture was made from the brown root rot infected tea root system and compared with the isolate received from ITCC (IARI).

***In vitro* Managementstudy:** This was done according to Dhingra and Sinclair 1985 [7] using poison food technique. Systemic fungicides were tested at three different concentrations of 10, 50 and 100 mg/L for their efficacy *in vitro* against the pathogen *Fomeslamoensis*. Different concentration of fungicides were prepared by dissolving the requisite quantity of each fungicides in warm Potato Dextrose Agar (PDA) before autoclaving. The autoclaved media were poured into petri plates and allowed to cool. An actively growing 10 days old culture mycelial disc (approx. 6mm^2) using cork borer was placed in the centre of the petri plates incubated at 28±2°C for 7 days. Each treatment was having five replicates. Media without fungicides served as control. On the 8th day the radial growth of mycelia were recorded and the percentage growth inhibition was calculated using the following formula,

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

Whereas, I= Percentage inhibition
C= Control growth of fungus
T= Growth of fungus in the treatment

Disease Assessment Study under Nursery Condition: Brown root rot pathogen *Fomeslamoensis* was grown in Potato Dextrose Broth (PDB) and was prepared using a 10 -day-old actively growing mycelium of the culture, homogenized in sterile distilled water using mortar and pestle. One year old tea plants were planted in polythene bags and were inoculated the homogenized fungal suspension @50ml of the per plants. Simultaneously fungicides hexaconazole, propiconazole and bavistin at the concentration of 100mg/L (100ppm) were prepared followed to application at the collar region @10 ml each of the tea plants. Disease assessment was done in 15, 30, 45, 60 and 90 days of inoculation. There were five replicates having 10 plants in each replicate. Disease assessment was done using following parameters such as drying of leaves, leaf shedding and drying of tea plants. In nursery experiments were done with the following treatments throughout the entire period of investigation.

I) *Fomeslamoensis* alone, ii) Hexaconazole + *Fomeslamoensis*, iii) Propiconazole + *Fomeslamoensis,* iv) Bavistin + *Fomeslamoensis*, v) Hexaconazole, vi) Propiconazole and vii) Bavistin.

Statistical Analysis: Data were subjected to analysis of variance (ANOVA) and the significance was calculated at $p<0.05$. The experiment had five replications for each treatment.

RESULTS AND DISCUSSION

Isolation of Pathogen: Roots were washed in running tap water and thin transverse sections (T.S.) were made. These sections were observed under microscope for detection of presence of fungal mycelium associated with root section. Diseased root sections were surface

Fig. 1: Re isolated of the pathogen *Fomeslamoensis*

sterilized in 90% alcohol and placed in potato dextrose agar media amended with streptomycin sulphate (@100mg/l) for isolation of the fungus. Fungus isolated was again observed under microscope for identification and the confirmation was done from Indian Type Culture Collection, Indian Agricultural Research Institute, New Delhi. (Figure. 1).

***In vitro* Management Study:** The effect of fungicides on the radial growth of *Fomeslamoensis* is presented in Table 1. Among the systemic fungicides tested, hexaconazole was the most effective on the growth of *Fomeslamoensis*,followed by propiconazoleand Bavistin at the concentration (100ppm) tested (Figure 2, 3 and 4 respectively). At the 100 ppm concentration growth inhibition caused by hexaconazole, propiconazole, bavistin, roko and ektino amended Potato Dextrose Agar (PDA) medium was 100%, 98.51%, 12.58%, 5.18% and 1.48% respectively. (Figure 2)

Disease Assessment Study: Initial symptoms had occurred 30 days onward and became prominent up to 90 days. The leaves started drying followed to shedding and finally whole plants died and roots fully rotted (Figure 1 B). Here pathogen (*Fomeslamoensis*) alone treated drying of leaf followed to shedding occurred 28th Day to 30th Day and finally drying of whole plants occurred 90th Days onwards (Figure 5).

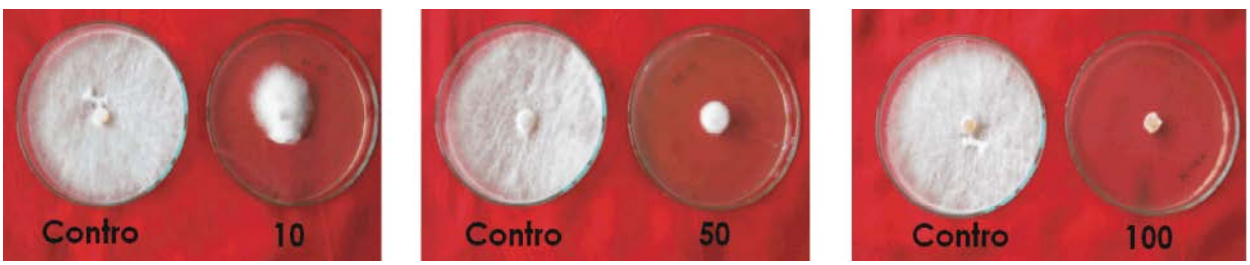

Fig. 2: Effect of Hexaconazole 5% EC at different concentration, 100ppm showing 100% inhibition against the pathogen Fomeslamoensis

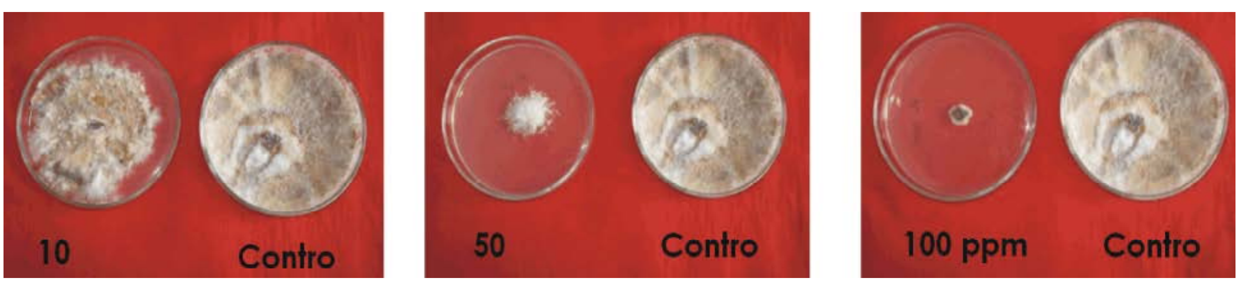

Fig. 3: Effect of propiconazole 25% EC at different concentration, 100mg/L showing 100% inhibition against the pathogenFomeslamoensis.

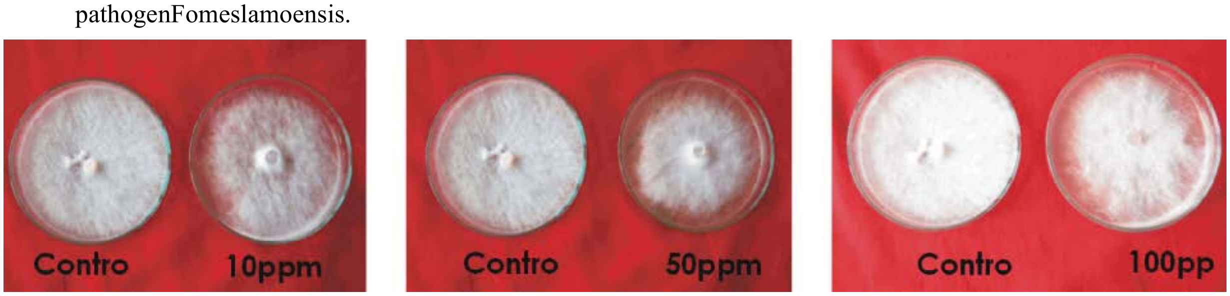

Fig. 4: Effect of Bavistin (Carbendazim 50%WP) at different concentration showing no inhibition.

Fig. 5: Test of pathogenicity showing drying of tea plant 90 Days after inoculation

Table 1: Effect of fungicides at different concentration on the growth of *Fomeslamoensis in vitro* 7th day after treatment

Treatment	Concentration (mg/L)	Fungal growth (in cm)	Growth inhibition (%)
Bavistin	10	4.46 ± 0.03^{a}	0.74 ± 0.74^{a}
	50	4.30 ± 0.11^{a}	5.92 ± 1.48^{b}
	100	3.93 ± 0.14^{b}	12.58 ± 3.22^{c}
Roko	10	4.50 ± 0.05^{a}	2.22 ± 1.28^{d}
	50	4.30 ± 0.05^{a}	4.44 ± 1.28^{e}
	100	4.50 ± 0.00^{a}	5.18 ± 0.74^{f}
Ektino	10	4.50 ± 0.10^{a}	2.22 ± 2.22^{d}
	50	4.40 ± 0.05^{a}	2.22 ± 1.28^{d}
	100	4.43 ± 0.06^{a}	1.48 ± 1.48^{g}
Hexaconazole	10	0.60 ± 0.06^{ac}	86.66 ± 1.28^{ah}
	50	0.10 ± 0.05^{d}	97.77 ± 1.20^{h}
	100	0.00 ± 0.00^{e}	100.00 ± 0.00^{i}
Propiconazole	10	2.80 ± 0.11^{af}	37.77 ± 2.56^{j}
	50	0.20 ± 0.05^{g}	95.77 ± 1.09^{k}
	100	0.00 ± 0.06^{h}	98.51 ± 1.48^{h}
Control	-----	4.50 ± 0.00^{a}	0.00 ± 0.00^{a}

Values are average from five sets each

The Values followed by the same letter(s) do not differ significantly ($p<0.05$).

Table 2: Occurrence of disease symptoms in nursery conditions

	Leaf Drying			Leaf shedding			
Treatment (at100ppm)	1 leaf	2 leaf	More than 2 leaves	1 leaf	2 leaf	More than 2 leaves	Drying of tea plants
Control (*F. lamoensis* alone)	28thDay	31stDay	36th Day	30th Day	39th Day	48th Day	90th Days
Hexaconazole+ *F. lamoensis*	49th Day	56th Day	--------	54th Day	65th Day	--------	------=
Propiconazole+ *F. lamoensis*	47th Day	58th Day	--------	52nd Day	--------	--------	-------
Bavistin + *F. lamoensis*	39th Day	47th Day	64th Day	44th Day	57th Day	66th Day	-------
Hexaconazole	64th Day	--------	---------	---------	--------	---------	-------
Propiconazole	59th Day	63rd Day	---------	---------	--------	---------	-------
Bavistin	50th Day	66th Day	---------	62nd Day	70th Day	---------	-------

------ = No symptoms

Whereas, Pathogen treated with Hexaconazole, propiconazole and bavistin initially leaf drying was occurred 49th Day, 47th Day and 39th Day but did not showed any drying symptoms of the plants till upto 90th Days of observation (Table 2).

Suppressive effect of various fungicides was observed on the radial growth of the pathogen *Fomeslamoensisin vitro.* The antifungal activity of systemic fungicides have been reported to be the result of their ability to inhibit ergosterol bio- synthesis in fungi [8], [9], [10], [11]. It was also reported that the antifungal acitivity of triflumizole which act as a selective inhibitor of dimethylation during egosterol biosynthesis [12]. Increase percent inhibition of the radial growth of the fungus was observed in both hexaconazole and propiconazole as the concentration increased, on the other hand, no increase of inhibition was found in Bvistin, roko and ektino as the concentration increased (Table1). Among the systemic fungicides, hexaconazole was highly effective in inhibiting the pathogen growth at the lowest concentration of 10 ppm (86.66%) followed by propiconazole (37.77%) (Table1). *Hypoxylonserpens* [13], *Colletotrichumgloeosporoides* [14] *Pestalotiatheae* [15] and *Phomopsistheae* [16] of these tea pathogen similar results were obtained. It was also reported that hexaconazole and propiconazole were found to be more effective even in low concentration against the fungus [17]. Systemic fungicides such as bitertanol were effective in controlling gray blight of tea in Korea [18]. The fungicides Carbendazim was the best in controlling thorny stem blight disease of tea and may be attributed to its ability in inhabiting the mitosis *in vitro* [19], [20]. Results from the nursery condition have also showed that hexaconazole and propiconazole at the concentration of 100 ppm have the efficacy to control brown root rot disease of tea.

Use of systemic fungicides to control the major diseases of tea is economically important. These fungicides are having both the curative and protective properties against the diseases. Therefore, from the present screening of the systemic fungicides, it has been found to be effective to control the disease for the benefit of the tea industry at a large.

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