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Management of Grey Leaf spot of mango with fungicides, botanicals and bioagents *in vitro* and *in vivo* condition in Manipur

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Roving survey of Grey Leaf spot of mango were done at different valley districts of Manipur during September- November, 2012 which showed maximum disease incidence in Imphal West (50.61%) and lowest in Thoubal (24.65%). *In vitro* evaluation of seven fungicides (Mancozeb, Tricyclazole, Propiconazole, Thiophanate methyl, Hexaconazole, Copper oxychloride and Carbendazim) showed complete inhibition by Hexaconazole and Propiconazole @ 0.05% and minimum by Mancozeb @ 0.1% (62.22%). Among seven botanicals (garlic, ginger, chinese chasetree, Indian woodworm, sweet flag, derek and turmeric) garlic clove extract @ 6% gave maximum inhibition (89.22%) followed by turmeric rhizome extract @10% (51.11%) whereas minimum inhibition in derek leaf extract @ 10% (18.88%). Among the bioagents (*Trichoderma viride*, *T. harzianum*, *T. hamatum*, *Penicillium citrinum* and *P. glabum*) *T. harzianum* and *T. hamatum* completely overgrowth the pathogen after 7 days of inoculation. *In vivo* condition showed, Thiophanate methyl (0.5%) was found most effective in reducing disease intensity to 19.40% which was closely followed by Carbendazim (15.40%), garlic (17.20%) and *T. harzianum* (16.76%) as compared to control.

Key words : Fungicides, botanicals, bioagents, antifungal properties, Grey leaf spot

INTRODUCTION

The mango is a fleshy stone fruit belonging to the genus *Mangifera*, consisting of numerous tropical fruiting trees in flowering plant family Anacardiaceae and order Sapinales. It is native to South Asia, from where it is distributed worldwide to become one of the most cultivated fruits in tropics. Mango trees grow up to 10-40 m tall, evergreen with symmetrical, rounded canopy ranging from low and dense to up right and open. Mostly distributed below 300 m but can occur at 600-1900 m above sea level. The species found as scattered individuals in tropical low land rain forest on well drained soil. India ranks first in production accounting for about 40.48 per cent of world's production (FAO, 2010). Mango is grown almost in all states

of India. Uttar Pradesh tops the list of mango producing states. Other major producing states are Andhra Pradesh, Maharashtra, Karnataka, Bihar, Gujarat, Tamil Nadu and Odisha (Anon. 2011). Rest of the states are quite less in production. Mango is an excellent overall nutritional source rich in dietary fibre and carbohydrates. The antioxidant, vitamins A, C and E; vitamins B₆ and K are present. It has various medicinal values. Mango suffers from several diseases at all stages of its life. All the parts of the plant, namely, trunk, branch, twig, leaf petiole, flower, and fruit are attacked by a number of pathogens including fungi, bacteria, and algae. They cause several kinds of Rot, Dieback, Anthracnose, Scab, Necrosis, Blotch, Spots and Mildews etc.

MATERIALS AND METHODS

Accurate quantity of each fungicide was added to

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sterilized 50 ml melted PDA to prepare desired concentrations and shaken well to mix thoroughly. The medium was poured into sterilized Petri dishes. Seven locally available botanicals namely turmeric (*Cucurma longa*), sweet flag (*Acorus calamus*), ginger (*Zingiber officinale*), Chinese chasetree (*Vitex trifolia* L.), Indian woodworm (*Artemisia nilagrica*), derek (*Melia azadirachta*) and garlic (*Allium sativum*) were evaluated for their efficacy on the growth of the fungus *in vitro*.

From plant, parts were collected, washed in running tap water and then washed with sterile distilled water. These were air dried over a blotting paper. The air dried plant parts were crushed separately in sterilized mortar and pestle with sterile distilled water at ratio 1:1 (w/v). These extracts were filtered through 2 fold muslin cloth and filtrate was centrifuged at 1500 rpm for 15 minutes and the supernatants were collected. These extracts thus prepared were considered as 100 per cent concentration. The medium without fungicides/ botanicals served as control.

Several 3mm diameter mycelial discs of fungus were cut with the help of sterilized cork borer from the periphery of the actively growing colony of 3 days old fungal culture and were plated in inverted position at the centre of fungicides/botanicals treated and untreated plates. The seeded plates were incubated at $28 \pm 1^\circ\text{C}$ and radial growth of fungus in each fungicide was recorded at 24 hr intervals till the control plates were covered fully with fungal mycelium. Each treatment was replicated 3 times. Per cent inhibition on growth was calculated as, $I = \frac{C-T}{C} \times 100$, where, C = radial growth of the fungus in control, T = radial growth of the fungus in treatment, for both fungicides and plant extracts.

Five biocontrol agents namely, *Trichoderma harzianum*, *T. hamatum*, *T. viride*, *Penicillium citrinum* and *P. glabrum* were evaluated for their antagonistic activities against the growth of the fungus associated with the Grey Leaf spot disease. The antagonistic activities was recorded by following dual culture technique using modified Bell's scale described by Bell *et al.* (1982) using PDA as basal medium. The plates were incubated at $28 \pm 1^\circ\text{C}$ until mycelium of the test pathogen covered the whole control plates.

RESULTS AND DISCUSSION

Effect of fungicides on the growth and sporulation of the fungus in vitro

Among the fungicides tested, Propiconazole, Hexaconazole and Thiophanate methyl at 0.05 per cent concentration completely inhibited the growth of pathogens.. However, carbendazim (0.05%), Tricyclazole (0.05%) and Copper oxychloride (0.1%) showed 97.77, 97.77 and 77.77 per cent inhibition over control). Mancozeb (0.1%) showed least inhibition (62.22%) on radial growth of the fungus (Table1).

Effect aqueous plant extract on growth and sporulation of the fungus in vitro

Among seven botanicals (garlic, ginger, chinese chasetree, Indian woodworm, sweet flag, derek and turmeric) garlic clove extract @ 6% gave maximum inhibition (89.22%) followed by turmeric rhizome extract @10% (51.11%) whereas minimum inhibition in derek leaf extract @ 10% (18.8%). This result indicates that botanical extracts have antifungal properties which inhibit the growth and sporulation of the fungus.

Effect of biocontrol agents on growth and sporulation of fungus in vitro

Among the *antagonists*, *T.hargianum* could come in contact with the fungus after 2 days of incubation. The growth of *P. mangiferae* was completely overgrown (100%, Class I) by the antagonist *T. harzianum* and *T. hamatum* while *T. viride* remained locked with the fungus at the point of contact (Class IV). Antagonists *P. citrinum* and *P. glabrum* could not overgrow the fungus but formed 0.3 cm and 1.1cm inhibition zones (ClassVI).

Effect of fungicides, plant extracts and biocontrol agents in vivo conditions

In vivo condition, it was observed that the least disease incidence was found in Thiophanate methyl(19.40%) sprayed plants followed by Carbendazim (15.40%). It was further observed that garlic (4%) and *T. harzianum* could reduce the incidence of disease to 17.20 and 16.44 per cent as compare to control.

Table 1 : Effect of fungicides on the growth and sporulation of the fungus *in vitro*

Treatments	Concentrations (%)	*Growth (cm)	Inhibition(%) over control	Sporulation cfu/ml	Per cent inhibition on sporulation
Mancozeb	0.10	3.40 (1.94)	62.22	-	100
Tricyclazole	0.05	0.20 (0.84)	97.77	-	100
Propiconazole	0.05	0.00 (0.71)	100.00	-	100
Thiophanate methyl	0.05	0.00 (0.71)	100.00	-	100
Hexaconazole	0.05	0.00 (0.71)	100.00	-	100
Copper oxychloride	0.10	2.00 (1.58)	77.77	-	100
Carbendazim	0.05	0.20 (0.84)	97.77	-	100
Control		9.00 (3.08)	0.00	2x10 ⁷	0.0
SE (d) ⁺		0.09			
CD _(0.05)		0.19			

*Mean of 3 replications, Figures in parenthesis are (x+0.5) transformed values, - No sporulation

Table 2 : Effect aqueous plant extract on growth and sporulation of the fungus *in vitro*

Treatments	Plant parts used	Concentration (%)	*Growth (cm)	Inhibition(%) over control	Sporulation cfu/ml	Percent inhibition on sporulation
Garlic	Clove	6	0.97 (1.21)	89.22	-	100
Chinese Chasetree	Leaf	10	4.93 (2.33)	45.20	-	100
Ginger	Rhizome	10	5.00 (2.35)	44.44	-	100
Indian woodworm	Leaf	10	6.97 (2.73)	22.55	-	100
Derek	Leaf	10	7.30 (2.79)	18.88	-	100
Sweet flag	Rhizome	10	5.93 (2.54)	34.00	-	100
Turmeric	Rhizome	10	4.40 (2.21)	51.11	-	100
Control			9.00 (3.08)	0.00	2x10 ⁷	0.0
SE (d) [±]			0.02			
CD _(0.05)			0.04			

*Mean of 3 replications, Figures in parenthesis are $\sqrt{(x+0.5)}$ transformed values, - No sporulation

Table 3 : Effect of biocontrol agents on growth and sporulation of fungus *in vitro*

Biocontrol Agents	Duration for point of contact (days)	Bell's scale	Sporulation cfu/ml	Per cent inhibition on sporulation
<i>Trichoderma viride</i>	2	Class – IV	-	100
<i>T. hamatum</i>	2	Class – I	-	100
<i>T. harzianum</i>	2	Class – I	-	100
<i>Penicillium citrinum</i>	-	Class – VI	-	100
<i>P. glabrum</i>	-	Class – VI	-	100
<i>Pestalotiopsis mangiferae</i> (control)			2×10 ⁷	0

Bell's scale (Bell *et al.* 1982) with slight modification: Class – I -100% overgrowth, Class – II (75% overgrowth), Class – III (50% overgrowth), Class – IV (the pathogen and the antagonist locked at the point of contact), Class – V(the pathogen overgrew the mycoparasite) Class - VI (formation of inhibition zone between pathogen and antagonist) and - (No spore formation)

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