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***In vitro* Evaluation of Fungicides, Botanicals and Bioagents against *Peziotrichum corticolum* causing Black Banded Disease of Mango**

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ABSTRACT

Keywords

Black banded disease, *Peziotrichum corticolum*, Fungicides, Plant extract, Bioagents.

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Among the tested five non-systemic, three combi- products, three systemic fungicides against *Peziotrichum corticolum* under laboratory condition, Bordeaux mixture, Copper oxychloride, Mancozeb, Carbendazim + Mancozeb, Hexaconazole + Zineb recorded maximum (100%) mean percent inhibition of *P. corticolum*, followed by Cuprous hydroxide (96.39%), Propiconazole (95.56%), Captan + Hexaconazole (86.39%), Difenconazole (80.74%), Hexaconazole (74.07%), Chlorothalonil (73.92%). Among the five plant extracts (botanicals), maximum mean percent inhibition of mycelial growth (22.78%) was recorded in neem kernel extract followed by *Parthenium* leaf extract (21.48%), *Lantana camara* leaf extract (13.70%), neem leaf extract (11.11%). Least inhibition was recorded in *Nerium* leaf extract (0.56%). Among all the tested bioagents, *Trichoderma harzianum* (77.22%) was found to be significantly superior in inhibiting the mycelial growth of *P. corticolum* followed by *Verticillium lecanii* (45.83%), *Pseudomonas fluorescens* (6.11%) and *Bacillus subtilis* (5.83%).

Introduction

Mango (*Mangifera indica* L.) commonly known as “king of fruits”, is one of the world’s most important and esteemed fruit (Basha *et al.*, 2010). It is in high demand because of its strong aroma, delicious taste and high nutritive value. It occupies an area of 2.51 mha having annual production of 18.43 mt with the productivity of 7.3 t/ha in India and contributes 20.7 % production share of major fruit crops in India. In Karnataka, the area under mango cultivation is 1.80 lakh ha with a production of 1.75 mt and productivity is 9.7 t/ha (National Horticultural Database, 2014). Mango suffers from a number of

diseases, because of diverse production conditions and the vast area grown. Some diseases take heavy toll on the crop and limit the production and productivity (Mamta and Singh, 2015).

Earlier black banded disease of mango was considered as a minor disease but for the last three to four years it is becoming severe in all the mango growing regions. The causal agent of black banded disease, *Peziotrichum corticolum* (Masse) Subramanian grows superficially on the bark of trees and forms large, dark black, irregular, girdle-like

infection patches. The black velvety growth of fungus can also be found on the midribs and veins of leaves (Pandey and Dinesh, 2010). This disease is becoming severe day by day and no information available on the management of the disease by using fungicides, botanicals and bioagents. Present study was carried out to assess the efficacy of various fungicides, botanicals and bioagents against *Peziotrichum corticolum* under *in vitro* conditions.

Materials and Methods

The present study on black banded disease of mango was conducted during 2014-2015 at the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad. Dharwad is situated in northern transitional zone (Zone 8) of Karnataka state at 15°15'N latitude, 75°7'N longitude and at an altitude of 774.0 m above mean sea level. The material used and methodology adopted during the course of investigations are presented here.

In vitro* evaluation of fungicides against *P. corticolum

The efficacy of five non-systemic, three combi-products and three systemic fungicides were tested against *P. corticolum* for growth inhibition on the potato dextrose agar media using poisoned food technique under *in vitro* conditions.

Poisoned food technique

Poisoned food technique (Shravelle, 1961) was followed to test the efficacy of the fungicides. The pathogen *P. corticolum* was grown on suitable solid medium in Petri plates for ten days prior to experiment. Fungicide suspension was prepared in that medium by adding requisite quantities of fungicides to obtain the desired concentration.

Poisoned medium was poured into sterilized petriplates. The plate without any fungicide served as control. Mycelial disc of 5 mm was taken with the help of sterilized cork borer, from the periphery of the ten days old culture and placed in the centre of petriplate and incubated at $28 \pm 2^\circ\text{C}$ till growth of the fungus reaches the periphery in the control plate. Three replications were maintained for each treatment. The colony diameter was measured in two directions and average was worked out. The percent inhibition of growth was calculated by using the formula given by Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Percent inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

In vitro* evaluation of botanicals against *P. corticolum

The present investigation was carried out to evaluate the extracts of five plant species (Table 1) to know the presence of fungitoxicant properties against *P. corticolum*.

Preparation of plant based products

Fresh healthy plant parts of 100 g (leaves or kernels) as indicated below were collected and washed with distilled water and air dried and followed by crushing in 100 ml of sterile water. The crushed product was tied in muslin cloth and filtrate was collected. The prepared solution gave 100%, which was further diluted to required concentrations of 5 and 10%. The extracts were tested against *P. corticolum* on the PDA using poisoned food technique under *in vitro* condition as described earlier. The percent inhibition of

growth of the test fungus was calculated by using the formula of Vincent (1947).

In vitro* evaluation of bioagents against *P. corticolum

The efficacy of four bioagents viz., *Trichoderma harzianum*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Verticillium lecanii* was tested against *P. corticolum* for radial growth inhibition on the potato dextrose agar media using dual culture technique under *in vitro* condition.

Dual culture test

Bioagents were evaluated for their efficacy following dual culture technique. The bioagents and the test fungus were inoculated side by side on a single Petridish containing solidified PDA medium. Five replications were maintained for each treatment with one control by maintaining only pathogen separately. They were incubated for 12 days. The diameter of the colony of both bioagents and the pathogen was measured in two directions and average was recorded. Percent inhibition of growth of the test fungus was calculated by using the formula of Vincent (1947).

Results and Discussion

In vitro* evaluation of fungicides against *P. corticolum

Screening of fungicides was done against *P. corticolum* under laboratory condition by following poisoned food technique as described in "Material and Methods". Five non-systemic fungicides, three combi products and three systemic fungicides were evaluated against *P. corticolum* in laboratory at four, four and three concentrations respectively by poisoned food technique.

In vitro* evaluation of non systemic and combi fungicides against *P. corticolum

Data with respect to inhibition of mycelial growth of *P. corticolum* at four concentrations of five non-systemic fungicides and three combi products were recorded and results are presented in table 2. Data from the table revealed that, the efficacy of different non-systemic fungicides, concentrations and their interaction on percent inhibition of mycelial growth of *P. corticolum* differed significantly.

Among all five non-systemic fungicides, maximum mean percent inhibition (100%) of *P. corticolum* was recorded for Bordeaux mixture, Copper oxychloride and Mancozeb which were significantly superior to all other fungicides followed by Cuprous hydroxide (96.39%). Least mean percent inhibition was noticed in Chlorothalonil (73.92%). Bordeaux mixture, Copper oxychloride and Mancozeb recorded 100% inhibition of mycelial growth irrespective of concentration. For Cuprous hydroxide maximum percent inhibition of mycelial growth (100%) was recorded at 0.25 and 0.3% concentration. Chlorothalonil showed maximum percent inhibition of mycelial growth (84.44%) at 0.3% concentration.

At 0.3 % concentration, Copper oxychloride, Cuprous hydroxide and Mancozeb recorded highest percent inhibition of mycelial growth (100%) of fungus which was significantly superior to other fungicides followed by Chlorothalonil (84.44%) which showed least inhibition of mycelial growth of fungus. At 0.15% concentration, maximum percent inhibition of mycelial growth (100%) of the fungus was recorded in Copper oxychloride and Mancozeb. The least percent inhibition (68.89%) of fungus was recorded for Chlorothalonil.

Among the three combi products, Carbendazim + Mancozeb, Hexaconazole + Zineb recorded cent percent inhibitions which were significantly superior to all other fungicides followed by Captan + Hexaconazole (86.39%) which was least among combi products.

Carbendazim + Mancozeb and Hexaconazole + Zineb recorded 100% inhibition of mycelial growth irrespective of concentration. Captan + Hexaconazole recorded (78.89%) inhibition of mycelial growth at 0.15% concentration and (92.22%) inhibition at 0.3% concentration.

In vitro* evaluation of systemic fungicides against *P. corticolum

Data with respect to inhibition of mycelial growth of *P. corticolum* at three concentrations of three systemic fungicides were recorded and results are presented in table 3. It was observed that, fungicides, concentrations and their interaction differed significantly with respect to inhibition of the mycelial growth of *P. corticolum*.

Among three systemic fungicides, maximum mean percent inhibition of growth of *P. corticolum* was observed in Propiconazole (95.56%) which was significantly superior to all other fungicides followed by Difenconazole (80.74%). The least mean percent inhibition of fungus was recorded in Hexaconazole (74.07%).

Among the tested three concentrations, 0.15% concentration of all fungicides was significantly found superior to 0.1 and 0.05 %. At 0.05 % concentration, maximum percent inhibition of mycelial growth (93.33%) of the fungus was recorded in Propiconazole, followed by Difenconazole (72.22%). The least percent inhibition of fungus was recorded in Hexaconazole

(68.89%). At 0.1 % concentration, maximum percent inhibition of mycelial growth (93.33%) of the fungus was recorded in Propiconazole, followed by Difenconazole (81.11%). The least percent inhibition of fungus was recorded in Hexaconazole (73.33%). At 0.15 % concentration, maximum percent inhibition of mycelial growth (100%) of the fungus was recorded in Propiconazole, followed by Difenconazole (88.89%). The least % inhibition of fungus was recorded in Hexaconazole (80%). At all the tested concentrations among systemic fungicides, Propiconazole recorded highest inhibition of mycelia growth.

Mycelial inhibition of *P. corticolum* by Propiconazole at 0.05 % (93.33%) and 0.1 % (93.33%) remained on par with each other. Further mycelial inhibition by Hexaconazole at 0.1 % concentration (73.33%) remained on par with Difenconazole at 0.05 % (72.22%). Further mycelial inhibition by Hexaconazole at 0.15 % concentration (80%) remained on par with Difenconazole at 0.1 % (81.11%).

In vitro* evaluation of botanicals against *P. corticolum

An experiment was conducted to assess the antifungal activity of five plant extracts as described under “Material and Methods” and the results are presented in table 4. The effect of plant extracts on the percent inhibition of mycelial growth of *P. corticolum* at three concentrations differed significantly.

Among the five plant extracts, maximum mean percent inhibition of mycelial growth (22.78%) was recorded in neem kernel extract which was significantly superior to other all treatments tested, followed by *Parthenium* leaf extract (21.48%), *Lantana camara* leaf extract (13.70%), neem leaf extract (11.11%). Least inhibition was recorded in *Nerium* leaf extract (0.56%).

At 10 % concentration of plant extracts, maximum of 30% inhibition of mycelial growth was recorded in neem kernel extract followed by *Parthenium* leaf extract (22.96%), neem leaf extract (17.78%) and *Lantana camara* leaf extract (15.19%). Least inhibition was recorded in *Nerium* leaf extract (10%). At 5 % concentration of plant extracts, maximum of 20 % inhibition of mycelial growth was recorded in *Parthenium* leaf extract followed by neem kernel extract (15.56%), *Lantana camara* leaf extract (12.22%) and neem leaf extract (4.44%). Least inhibition was recorded in *Nerium* leaf extract (3.33%).

Mycelial inhibition of *P. corticolum* by *Nerium* leaf extract at 5 % (3.33%) remained on par with neem leaf extract at 5 % (4.44%). Further at 10 %, mycelial inhibition by *Lantana camara* leaf extract (15.19%) remained on par with neem leaf extract (17.78%). Further mycelial inhibition by neem kernel extract at 5 % (15.56%) remained on par with *Lantana camara* leaf extract at 10 % (15.19%).

In vitro* evaluation of bioagents against *P. corticolum

Efficacy of bacterial and fungal bioagents was studied under *in vitro* condition by following dual culture method as described in “Material and Methods” and the results are presented in table 5. There were significant differences among all the tested bioagents.

Trichoderma harzianum (77.22%) was found to be significantly superior in inhibiting the mycelial growth of *P. corticolum* followed by *Verticillium lecanii* (45.83%) and *Pseudomonas fluorescens* (6.11%). The least inhibition of mycelial growth of *P. corticolum* was recorded in *Bacillus subtilis* (5.83%). Further *Pseudomonas fluorescens* and *Bacillus subtilis* remained on par with each other.

In vitro* evaluation of fungicides against *P. corticolum

Use of fungicides to manage different plant diseases is an old age practice. Fungicides are found as best option to manage plant disease in case of epidemic outbreak. These fungicides must be used judiciously according to the need and kind of organism involved. It is necessary to evaluate the fungicides under *in vitro* conditions to know their efficacy, and initiate spray schedule in field conditions.

Among all three groups of fungicides, non-systemic fungicides and combi products were found to be more effective than systemic fungicides. Three non systemic fungicides namely Bordeaux mixture, Copper oxychloride, Mancozeb and two combi products namely Carbendazim + Mancozeb and Hexaconazole + Zineb recorded 100 % inhibition of mycelial growth of *P. corticolum*. Least mean percent inhibition was noticed in Chlorothalonil (73.92%). Among three systemic fungicides, maximum mean percent inhibition of growth of *P. corticolum* was observed in Propiconazole (95.56%) and least mean percent inhibition of fungus was recorded in Hexaconazole (74.07%).

At higher concentration most of the fungicides inhibited maximum mycelial growth but decreased with reduced concentration. Naik and Hiremath (1986) reported the complete inhibition of mycelial growth of *Colletotrichum gloeosporioides* with Copper oxychloride (0.1, 0.2 and 0.3%). Ashoka (2005) reported that Carbendazim + Mancozeb recorded 100 % inhibition of mycelial growth of *Colletotrichum gloeosporioides*. Contrary to it, Venkataravanappa and Nargund (2002) reported tricyclazole as very effective under laboratory condition against *Colletotrichum gloeosporioides* among all the tested fungicides.

In vitro* evaluation of botanicals against *P.corticolum

Extensive use of fungicides has led to various environmental problems, human health and their persistence in the fruits. To sort out these problems botanicals were tested in laboratory against *P. corticolum*. Continuous use of chemical fungicides in the management of disease also brought new problems with them. Amongst them is pollution of air, water, soil, residual toxicity, development of resistance in the pathogen against chemicals there by the need to apply them more with their escalating prices and harmful effects on non target organisms. Consequently, fungicides have been alarming the development of harmful environment for human beings. Contrary to the problems associated with use of synthetic chemicals, botanicals are environmentally non-pollutive, renewable, in-exhaustible, indigenously available, thus readily biodegradable relatively cost effective and hence constitute as a suitable plant protection in the strategy of integrated disease management. Hence, screening of plant products for its effective antifungal activity against the pathogen is essentially required to minimize the use of fungicides and considered as one of the components in the integrated disease management (Khadar,

1999; Nagesh, 2000 and Jayalakshmi, 2010). At 10 % concentration of plant extracts, maximum of 30 % inhibition of mycelial growth was recorded in neem kernel extract and least inhibition was recorded in *Nerium* leaf extract (10%).

At 5 % concentration of plant extracts, maximum of 20 % inhibition of mycelial growth was recorded in *Parthenium* leaf extract followed by neem kernel extract (15.56%) and least inhibition was recorded in *Nerium* leaf extract (3.33%).

The effectiveness of Neem kernel extract as a pesticide may be due to phyto-constituents like alkaloids, glycosides, flavonoids and saponins which are antimicrobial in nature. Further, Shivapuri *et al.*, (1997) noticed *Azadirachta indica*, as more fungitoxic among ten plant extracts.

In vitro* evaluation of bioagents against *P.corticolum

Regular use of synthetic chemicals / fungicides in mango has been a norm in many orchards that brings with it many hazards such as pesticide residue, development of resistant strains among the pathogen, ecological consideration etc.

Table.1 Plant species and their parts used for *in vitro* evaluation

Sl. No.	Plants product/extract	Plant part used
1.	<i>Lantana camara</i>	Leaf
2.	Neem kernel extract	Kernel
3.	Neem leaf extract	Leaf
4.	<i>Nerium oleander</i>	Leaf
5.	<i>Parthenium hysterophorus</i>	Leaf

Table.2 *In vitro* evaluation of contact fungicides and combi -products against mycelial growth of *Peziotrichum corticolum*

Common name	Trade name	Inhibition (%)					Mean
		Concentrations (%)					
		0.15	0.20	0.25	0.30		
Contact fungicides							
Chlorothalonil	Kavach 75% WP	68.89 [#] (56.08)*	74.44 (59.61)	81.11 (64.21)	84.44 (66.74)	73.92 (61.66)	
Copper oxychloride	Blitox 50% WP	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	
Cuprous hydroxide	Kocide 77% WP	92.22 (73.78)	93.33 (75.01)	100.00 (89.96)	100.00 (89.96)	96.39 (82.18)	
Mancozeb	Indofil M-45 75% WP	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	
Combiproducs							
Carbendazim 12% + Mancozeb 63% WP	Saaf 75% WP	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	
Hexaconazole 4% + Zineb 68% WP	Avatar 72% WP	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	
Captan 70% + Hexaconazole 5% WP	Taqat 75% WP	78.89 (62.23)	85.56 (67.67)	88.89 (70.50)	92.22 (73.80)	86.39 (68.65)	
Source		Fungicides (F)		Concentration (C)		F × C	
S.Em. ±		0.13		0.10		0.26	
CD at 1%		0.49		0.37		0.98	
Bordeaux mixture	-	Concentrations (%)				Mean	
		0.50	0.75	1.00	1.25		
		100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	

* = Arcsine values

= Mean of three replications

Table.3 *In vitro* evaluation of systemic fungicides against mycelial growth of *Peziotrichum corticolum*

Common name	Trade name	Inhibition (%)			
		Concentration (%)			Mean
		0.05	0.10	0.15	
Difenconazole	Score 25% EC	72.22 [#] (58.17)*	81.11 (64.21)	88.89 (70.50)	80.74 (64.29)
Hexaconazole	Contaf 5% EC	68.89 (56.08)	73.33 (58.89)	80.00 (63.41)	74.07 (59.46)
Propiconazole	Tilt 25% EC	93.33 (75.01)	93.33 (75.01)	100.00 (89.96)	95.56 (79.99)
Source		S.Em±		CD at 1%	
Fungicides (F)		0.39		1.59	
Concentration (C)		0.39		1.59	
F × C		0.68		2.76	

*=Arcsine transformed values

[#]=Mean of five replications

Table.4 *In vitro* evaluation of botanicals/plant extracts against mycelial growth of *Peziotrichum corticolum*

Sl. No.	Name of plant	Plant part used	Per cent Inhibition		
			Concentration (%)		Mean
			5	10	
1.	<i>Lantana camara</i>	Leaf	12.22 [#] (20.44)*	15.19 (22.90)	13.70 (21.67)
2.	Neem kernel extract	Kernel	15.56 (23.21)	30.00 (33.20)	22.78 (28.20)
3.	Neem leaf extract	Leaf	4.44 (12.10)	17.78 (24.92)	11.11 (18.51)
4.	<i>Nerium oleander</i>	Leaf	3.33 (10.06)	10.00 (18.36)	6.67 (14.21)
5.	<i>Parthenium hysterophorus</i>	Leaf	20.00 (26.55)	22.96 (28.61)	21.48 (27.58)
Mean			11.11 (18.47)	19.19 (25.60)	15.15 (22.04)
Source			Botanicals (B)	Concentration (C)	B × C
SEm±			0.69	0.43	0.97
CD at 1%			2.76	1.75	3.90

[#]= Mean of five replications

*= Arcsine transformed values

Table.5 *In vitro* evaluation of bioagents against *Peziotrichum corticolum*

Sl. No.	Bioagent	Per cent inhibition
1.	<i>Bacillus subtilis</i>	5.83 [#] (13.82)*
2.	<i>Pseudomonas fluorescens</i>	6.11 (14.19)
3.	<i>Trichoderma harzianum</i>	77.22 (61.48)
4.	<i>Verticillium lecanii</i>	45.83 (42.58)
S.Em±		1.03
CD at 1%		4.29

[#] = Mean of five replications

* = Arcsine transformed values

Use of bioagents, nowadays, is best and has been most emphasized and widely accepted practice as it is environmentally safe and can overcome the residual problems associated with heavy use of fungicides for management disease. Hence, the present investigation was taken up to screen the bioagents for effective management of black banded disease of mango.

Among the bioagents tried during present investigation *Trichoderma harzianum* was found to be best in inhibiting mycelial growth of *P. corticolum* (77.22%) followed by *Verticillium lecanii* (45.83%) and *Pseudomonas fluorescens* (6.11%) and least percent inhibition of mycelial growth was observed in *Bacillus subtilis* (5.83%).

Present studies recorded significant mycoparasitism of *Trichoderma harzianum* on *P. corticolum* that caused lysis of the hyphae *in vitro*. Result of the experiment was further supported by Watve *et al.*, (2009), according to them; maximum inhibition of *Colletotrichum gloeosporioides* causing leaf

spot disease of jatropha was achieved by *Trichoderma harzianum*.

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