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***In vitro* inhibitory effect of botanicals and fungicides on mycelial growth of *Peziotrichum corticolum* (Masse) Subramanian causing Black banded disease of mango**

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Abstract

India is the largest producer of mango (*Mangifera indica* L.) in the world accounting for 52-63 per cent of total production. One of the constraints in mango production is black banded disease caused by *Peziotrichum corticolum* (Masse) Subramanian which is during previous four to five years has been on the rise under south Gujarat ecosystem. The black velvety growth of fungus can be found on the midribs and veins of leaves, twigs and branches. Therefore, an attempt was made with different botanicals and fungicides in managing the causal agent of black banded of mango. This investigation suggests leaf extract of *Azadirachta indica* and mancozeb, difenoconazole, propiconazole, carbendazim + mancozeb and hexaconazole + zineb as the most effective fungicides in combating mycelial growth of the fungus under *in vitro* conditions.

Keywords: Mango, black banded, *Peziotrichum corticolum*, botanicals, fungicides

Introduction

Mango (*Mangifera indica* L.) is one of the 73 genera of the family Anacardiaceae and order Sapindales (Ahmed and Mohamed, 2015) ^[1] which is one of the most versatile and widely grown fruit crops of tropical and subtropical regions (Vasugi *et al.*, 2012) ^[19]. It is known as king of fruits or super fruits due to its excellent aroma, delicious taste and high nutritive value (Ullah *et al.*, 2010), that makes it as a prominent horticultural crop of India. It is believed to be originated within a large area including North-Western Myanmar, Bangladesh and North-Eastern India. India has a rich wealth of mango germplasm with more than 1000 mango varieties grown throughout the length and breadth of the country. However, only about 21 of them are commercially cultivated in different mango growing regions (Yadav, 1997) ^[21].

Over 140 fungi (70 diseases), about 12 nematodes and a dozen of phanerogamic parasites and epiphytes are associated with this fruit (Prakash and Srivastava, 1987) ^[11]. Mango is subject to a number of diseases *viz*: powdery mildew, anthracnose, die-back, scab, sooty mould or sooty blotch, phoma blight, root rot and damping off, bacterial canker *etc* at all stages of its development. Black banded disease caused by *Peziotrichum corticolum* (Masse) Subramanian is one among them occurring on mango. Although, earlier this disease was considered as a minor disease and very scanty research work has been done on this but for the last few years it becoming severe in all the mango growing regions and fetching an emerging threat to mango orchards. This disease has also been recorded from Goa, West Bengal, Karnataka, Maharashtra, Tamil Nadu (Prakash and Srivastava, 1987) ^[11] and Gujarat State (Vala *et al.*, 1985) ^[18]. The occurrence of disease on mango was first reported by Masse from Poona (Saccardo, 1906) ^[13].

During the past four to five years, the incidence of black banded on mango in south Gujarat ecosystem particularly in the coastal belt of the region has been on the rise. The damage caused by this disease to the plants is a gradual process but it consequently leads to death of the plants and that too in their maximum bearing phase. The disease commonly occurs in monsoon season especially on the matured branches of the plants. The causal agent of disease, *Peziotrichum corticolum* (Masse) Subramanian grows superficially on the bark of trees and

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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) [10]. As the disease is becoming severe day by day and negligible information available on the management of the disease by means of botanicals and fungicides, Therefore, the present study has been carried out to investigate the efficacy of various botanicals and fungicides against *Peziotrichum corticolum* under *in vitro* conditions.

Materials and methods

The present research on *Peziotrichum corticolum* under *in vitro* conditions was conducted during 2018-2019 at the Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari which is situated in South Gujarat Heavy Rainfall Agro-climatic Zone of Gujarat state at 20°95'N latitude, 72°93'E longitude and at an altitude of 9.0 m above mean sea level. The material used and methodology adopted during the course of investigations are presented here.

In vitro effect of botanicals against *Peziotrichum corticolum*

The present investigation was carried out to test the extracts of nine plant species viz; *Datura stramonium* (Datura), *Calotropis procera* (Aak), *Azadirachta indica* (Neem), *Ocimum tenuiflorum* (Tulsi), *Nicotiana tabacum* (Tobacco), *Lantana camera* (Lantana), *Tagetes erecta* (Maigold), *Annona reticulata* (Custard apple) and *Casuarina equisetifolia* (Casuarina) to know the presence of fungitoxicant properties against *P. corticolum*.

Preparation of plant-based products

100 g fresh healthy leaves of plants as mentioned above 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 per cent, which was further diluted to required concentrations of 5 and 10 per cent.

In vitro effect of fungicides against *Peziotrichum corticolum*

In present investigation the efficacy of three contact fungicides, copper oxychloride (Blitox 50% WP), Mancozeb (Indofil M-45 75% WP), Chlorothalonil (Kavach 75% WP), three systemic fungicides, azoxystrobin (Amistar 23% EC), difenoconazole (Score 25% EC), propiconazole (Tilt 25% EC) and three ready mix combi- products, carbendazim 12% + mancozeb 63% WP (Saaf 75% WP), hexaconazole 4% + zineb 68% WP (Avatar 72% WP) and captan 70% +

hexaconazole 5% WP (Taqat 75% WP) were tested against *P. corticolum*.

Poisoned food technique

The botanicals and fungicides in two different concentrations were tested against *P. corticolum* on the PDA employing poisoned food technique as described by Nene and Thapliyal (1997) [9]. For this, Potato dextrose agar (PDA) medium was prepared and 250ml PDA medium was filled in 500ml conical flask, the flask was then sterilized in autoclave at 15lbs pressure for 20min. The calculated quantity of test botanicals and fungicides was added aseptically in autoclaved PDA medium in conical flask. Then the flask containing botanicals and fungicides was mixed well in media. Afterward, 20ml of the medium with respective botanicals and fungicides was poured in previously sterilized 90 mm diameter labelled petri plate and allowed them to solidify. The petri plate containing PDA media without addition of botanicals and fungicides was served as control. The fungal mycelial bit of 5mm diameter was taken from 12days old culture of *P. corticolum* with the help of sterilized cork borer and placed aseptically in centre of the petri plate and each treatment was repeated thrice. All the treatments were incubated at room temperature (27±1°C) and after 15days the radial growth of the pathogen was measured. The per cent inhibition of growth of test fungus was calculated using the formula given by Vincent (1947) [20].

$$\text{PGI} = \frac{\text{DC}-\text{DT}}{\text{DC}} \times 100$$

Where,

PGI = Per cent growth inhibition; DC = Average diameter of mycelial colony from control set (mm); DT = Average diameter of mycelial colony from treated set (mm)

Results and discussion

In vitro effect of botanicals against *Peziotrichum corticolum*

An experiment was conducted to assess the antifungal activity of nine plant extracts as per described under material and methods and the results are presented in table 1. The effect of plant extracts on the percent inhibition of mycelial growth of *P. corticolum* at two concentrations differed significantly. Among the nine plant extracts, maximum per cent inhibition of mycelial growth was recorded in leaf extract of *Azadirachta indica* (45.56%) with 49mm colony diameter followed by lantana leaf extract of *Lantana camera* (42.59%) with 51.67mm colony diameter. Least inhibition was recorded in eaf extract of *Tagetes erecta* (4.81%) with 85.67mm colony diameter at 5% concentration.

Table 1: *In vitro* effect of botanicals against mycelial growth of *Peziotrichum corticolum*

S. No.	Plant name	Colony diameter (mm) at		Mean colony diameter (mm)	Per cent growth inhibition at		Mean Per cent growth inhibition
		5% Conc.	10% Conc.		5% Conc.	10% Conc.	
1.	<i>Datura stramonium</i> (Datura)	53.33 [#] (7.34) *	41.33 (8.67)	47.33 (8.01)	40.74	54.07	47.41
2.	<i>Calotropis procera</i> (Aak)	74.67 (7.04)	68.33 (7.42)	71.50 (7.23)	17.04	24.07	20.56
3.	<i>Azadirachta indica</i> (Neem)	49.00 (7.90)	36.00 (7.22)	42.50 (7.56)	45.56	60.00	52.78
4.	<i>Ocimum tenuiflorum</i> (Tulsi)	54.67 (9.28)	45.00 (7.71)	49.84 (8.50)	39.26	50.00	44.63
5.	<i>Nicotiana tabacum</i> (Tobacco)	62.00 (9.10)	59.00 (6.47)	60.50 (7.79)	31.11	34.44	32.78
6.	<i>Lantana camera</i> (Lantana)	51.67 (8.29)	40.33 (6.04)	46.00 (7.17)	42.59	55.19	48.89
7.	<i>Tagetes erecta</i> (Maigold)	85.67 (6.75)	78.33 (7.71)	82.00 (7.23)	4.81	12.96	8.89
8.	<i>Annona reticulata</i> (Custard apple)	59.00 (6.39)	45.67 (8.88)	52.34 (7.64)	34.44	49.26	41.85
9.	<i>Casuarina equisetifolia</i> (Casuarina)	82.33 (6.79)	76.67 (8.78)	79.50 (7.79)	8.52	14.81	11.67

Source	Botanicals (B)	Concentration (C)	B × C		
S. Em±	0.07	0.03	0.10		
CD at 5%	0.20	NS	0.29		
CV%	-	-	2.28		

= Mean of three repetition * = \sqrt{x} transformed value

Similar trend was also observed at 10 per cent concentration, where maximum per cent inhibition of mycelial growth was recorded in leaf extract of *Azadirachta indica* (60.00%) with 36mm colony diameter followed by lantana leaf extract of *Lantana camera* (55.19%) with 40.33mm colony diameter. Least inhibition was recorded in leaf extract of *Tagetes erecta* (12.96%) with 78.33mm colony diameter.

Shinde (2018) [15] reported 55.55 per cent inhibition of *P. corticolum* by using neem leaf extract. Although, the findings of Gautam *et al.* (2017) [6] are remarkably diverging from the results of this study as they revealed only 11.11 per cent inhibition of pathogen by using neem leaf extract. This dissimilarity may be due to geographical location of host and pathogen, their interaction and botanical properties of plants from which extracts were prepared. The present results are in confirmation with the finding by other workers on different pathogens and plants (Tewari and Nayek, 1991; Al-Abed *et al.*, 1993, Qasem *et al.*, 1996;

Amadioha 1998; Amadioha 2003; and Shinde 2018) [17, 3, 12, 4, 5, 15]. Further, Shivapuri *et al.*, (1997) [16] noticed *Azadirachta indica*, as more fungitoxic among ten plant extracts. The effectiveness of leaf extract of *Azadirachta indica* as a pesticide may be due to phyto-constituents like alkaloids, glycosides, flavonoids and saponins which are antimicrobial in nature.

In vitro* effect of fungicides against *Pezizotrichum corticolum

Data with respect to inhibition of mycelial growth of *P. corticolum* at two concentrations (1000 & 2000 ppm) of three contact, three systemic and three combi-products fungicides were recorded and results are presented in table 2. It was observed that, fungicides, concentrations and their interaction differed significantly with respect to inhibition of the mycelial growth of *P. corticolum*.

Table 2: *In vitro* effect of fungicides against mycelial growth of *Pezizotrichum corticolum*

S. No.	Common name	Trade name	Colony diameter (mm) at		Mean colony diameter (mm)	Per cent growth inhibition at		Mean Per cent growth inhibition
			1000 ppm	2000 ppm		1000 ppm	2000 ppm	
Contact fungicides								
1.	Copper oxychloride	Blitox 50% WP	31.33 [#] (5.64) [*]	22.67 (4.81)	27.00 (5.22)	65.19	74.81	70.00
2.	Mancozeb	Indofil M-45 75% WP	1.67 (1.46)	0.00 (0.71)	0.83 (1.08)	98.15	100.00	99.08
3.	Chlorothalonil	Kavach 75% WP	18.67 (4.38)	13.33 (3.72)	16.00 (4.05)	79.26	85.19	82.23
Systemic fungicides								
4.	Azoxystrobin	Amistar 23% EC	26.67 (5.21)	23.33 (4.88)	25.00 (5.05)	70.37	74.07	72.22
5.	Difenoconazole	Score 25% EC	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	100.00	100.00	100.00
6.	Propiconazole	Tilt 25% EC	7.67 (2.86)	0.00 (0.71)	3.83 (1.78)	91.48	100.00	95.74
Combi-products								
7.	Carbendazim 12% + Mancozeb 63% WP	Saaf 75% WP	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	100.00	100.00	100.00
8.	Hexaconazole 4% + Zineb 68% WP	Avatar 72% WP	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	100.00	100.00	100.00
9.	Captan 70% + Hexaconazole 5% WP	Taqat 75% WP	23.33 (4.78)	19.67 (4.49)	21.05 (4.63)	75.19	78.15	76.67
	Source	Fungicides (F)	Concentration (C)	F × C				
	S. Em±	0.03	0.01	0.04				
	CD at 5%	0.08	0.04	0.11				
	CV%	-	-	2.56				

= Mean of three repetition * = \sqrt{x} transformed value

Among all nine tested fungicides at 1000 ppm, no mycelial growth with cent per cent inhibition of *P. corticolum* was recorded for difenoconazole (100%), carbendazim + mancozeb (100%) and hexaconazole + zineb (100%) with 0mm colony diameter, respectively, which were significantly superior to all other fungicides followed by mancozeb (98.15%) and propiconazole (91.48%) with 1.67mm and 7.67mm colony diameter, respectively. Least per cent inhibition was noticed in copper oxychloride (65.19% with 31.33mm colony diameter) at 1000ppm concentration.

Whereas, at 2000ppm concentration, cent per cent of inhibition with no mycelial growth of the fungus was recorded in mancozeb, difenoconazole, propiconazole, carbendazim + mancozeb and hexaconazole + zineb which inhibited cent per cent mycelial growth, followed by chlorothalonil (85.19%) with 13.33mm colony diameter.

Least per cent inhibition was found in azoxystrobin (74.07%) with 23.33mm colony diameter.

At higher concentration most of the fungicides inhibited maximum mycelial growth but decreased with reduced concentration. The present findings are in agreement with the results obtained by Shinde (2018) [15] with respect to mancozeb and difenoconazole. Gautam *et al.* (2017) [6] also reported that, Mancozeb and two combi products namely carbendazim + mancozeb and hexaconazole + zineb recorded 100% inhibition of mycelial growth of *P. corticolum*. Several workers have reported efficacy of different fungicides *viz.*, carbendazim, thiophanate methyl, chlorothalonil and mancozeb (Sharma and Verma 2007) [14], mancozeb (Lingaraj, 1969) [8], carbendazim and zineb (Jadeja and Vaibhav, 1980) [7], mancozeb (Ahmed *et al.*, 1991) [2] against pathogen of mango *in vivo* and *in vitro* conditions.

Present research provides some new combi-product fungicidal molecules which have been found effective in controlling *Peziotrichum corticolum* causing black banded of mango. Nevertheless, further evaluation of these effective fungicides is needed in field for better recommendation for management of black banded of mango.

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