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Evaluation of fungicides against *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. the incitant of mango anthracnose

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Abstract
Mango is one of the most important fruit crop of our country and anthracnose is the most important disease causing both pre and post-harvest losses. To manage the disease under field condition we need to use a combination of different fungicides. Hence, in the present study we investigated the efficacy of 14 different fungicides in inhibiting the growth of the *Colletotrichum gloeosporioides* under *in vitro* conditions. The result of the study indicated that, out of the 14 fungicides tested, a combi product Flurilazole + Carbendizim 37.5% WP and systemic fungicides Propiconazole 25% WP showed significantly superior inhibition over other fungicides with complete 100 per cent mycelial inhibition and absence of sporulation in all the three concentration *i.e*, 0.1%, 0.2%, 0.3% tested. Myclobutanil 10% WP showed inhibition of 97.2% followed by Tebuconazole + Trifloxystrobin 75% WG with 94.86% inhibition. Systemic fungicides Difenconazole 25% EC with 85.50% inhibition was on par with combi product Zineb 68% +Hexaconazole 100% WP with 85.06% inhibition.

Keywords: Fungicides, *Colletotrichum gloeosporioides*, mango anthracnose

1. Introduction
Mango (*Mangifera indica* L.) the National fruit of India is a commercial fruit crop with its captivating and attractive fragrance, flavour, excellent taste, sweetness and beautiful colour. India ranks first accounting for about 50 per cent of the world's mango production. Uttar Pradesh stands first in area and production followed by Andhra Pradesh and Karnataka. Although India is the largest producer of mango, the productivity is low and it is due to a wide range of biotic and abiotic stresses caused by fungi, bacteria, algae *etc.* Among the biotic stresses, fungal diseases are the major group of pathogens responsible for field and transit losses. Diseases like powdery mildew, anthracnose and stem end rot are economically important among fungal diseases. Among them, *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is the fungal pathogen causing anthracnose, the most serious disease in all mango growing regions of the world and the major constraint for export of mango. Anthracnose caused by *Colletotrichum spp.* reduces marketable yield from 10 to 80 per cent in developing countries (Poonpolgul and Kumphai, 2007 [1]; Kumar *et al.*, 2010 [2]). Primary symptoms of the disease appear on leaves as small and irregular yellow, brown, dark-brown, or black spots. The spots can expand and coalesce to cover the whole affected area. As it ages the colour of the infected parts get darkens. The disease produces cankers on petioles and on stems which causes severe defoliation and rotting of roots and fruits. Infected fruit has small, circular sunken spots with water soaked lesions that may increase in size up to 1.2 cm in diameter. The center of an older spot becomes blackish and pink spore masses can also be seen. Cup-shaped fruiting bodies *i.e.*, acervuli, whose fertile hyphae form a palisade on the surface of the conidiomata can be observed on diseased plant surface. Reproduction takes place as sticky mass of conidia are produced from acervuli during moist condition. Management of anthracnose of mango has been a challenge for both the scientists and the farming community because of non-availability of resistant varieties, effective fungicides and botanicals to manage disease. Hence, it is essential to find out the effective fungicides and plant based products against this disease.

2. Material and methods
2.1Isolation of the pathogen
The mango leaves infected with anthracnose were initially collected from mango orchard and used for isolation of the fungus. The infected portions along with some healthy parts were cut and surface sterilized using one per cent sodium hypochlorite solution for 60 seconds. These

bits were thoroughly washed in sterile distilled water for three times to remove the traces of sodium hypochlorite if any and then transferred to sterilized Petri plates (3 leaf bits per Petri plates) containing Potato Dextrose Agar (PDA) under aseptic condition under laminar air flow and incubated at room temperature (27±1 ºC). After 72 hr, colonies which developed from the bits, were transferred into fresh PDA medium. Colonies which developed from such culture was periodically observed for mycelia growth and sporulation under microscopic. Mycelial and spore character was used as a means for identification of the pathogen.

2.1 Identification of the fungus

The pathogen was identified based on its mycelial and spore characters described by Barnett and Hunter (1972) [3]. After identification they were transferred to new PDA slants and incubated at 27±1 ºC for further use. The fungus was sub cultured on PDA slants and allowed to grow at 27 ± 1°C for 7 days. Such slants were preserved in refrigerator at 5°C and maintained. Sub culturing was done once in a month, such cultures were used throughout the study.

2.2 *In vitro* evaluation of fungicides

The efficacy of three contact, three combi-products, seven systemic fungicides and one strobulin compound were assessed by Poison food technique. The pathogen *C. gloeosporioides* of mango was grown on PDA in Petri plates for seven days prior to setting the experiment. To obtain the desired concentration on the basis of active ingredient and whole product present in the chemical, fungicide suspension was prepared in PDA by adding required quantity of fungicide. Fifteen ml of poisoned medium was poured in each of the sterilized Petri plates and each treatment was replicated thrice with three different concentrations. Mycelial disc of 0.5 cm was taken from the periphery of seven day old culture and placed in the centre of Petri plate containing poisoned media and incubated at 27±1 °C till growth of the fungus reached the peripheries in the control plate. Suitable checks were also maintained without addition of any fungicide. The diameter of the colony was measured in three directions and average was worked out. Those Petri plate were also observed for presence or absence of sporulation. The list of chemicals used for the *in vitro* evaluation is listed in Table 1. The per cent inhibition of growth was calculated by using the formula given by Vincent (1947) [4].

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

Where,
I = Per cent inhibition of mycelium
C = Growth of mycelium in control
T = Growth of mycelium in treatment

Table 1: List of chemicals used for the *in vitro* evaluation against *Colletotrichum gloeosporioides*

Common name	Trade name	Concentrations (%)		
Contact fungicides				
Chlolorothalonil 75% WP	Kavach	0.1	0.2	0.3
Mancozeb 75% WP	Indofil M- 45	0.1	0.2	0.3
Copper oxychloride 50% WP	Blitox	0.1	0.2	0.3
Systemic fungicides				
Difenconazole 25% EC	Score	0.1	0.2	0.3
Hexaconzole 5% EC	Force	0.1	0.2	0.3
Tetraconazole 3.8% EC	Domex	0.1	0.2	0.3
Propiconazole 25% EC	Tilt	0.1	0.2	0.3
Thiophanate methyle 75% WP	Roko	0.1	0.2	0.3
Myclobutanil 10% WP	Boon	0.1	0.2	0.3
Captan 50% WP	Meriman	0.1	0.2	0.3
Strobulin compound				
Azoxystrobin	Mirador	0.1	0.2	0.3
Combi-products				
Zineb 68% + Hexaconazole 100%WP	Avatar	0.1	0.2	0.3
Flusilazole 12.5% + Carbendazim 37.5% WP	Lustre	0.1	0.2	0.3
Tebuconazole + Trifloxystrobin 75% WG	Native	0.1	0.2	0.3

3. Result and discussion

In the present investigation, 14 fungicides were evaluated for their antifungal activity (Table 2). Among the different fungicides tested, a combi product Flurilazole + Carbendizim 37.5% WP and systemic fungicides Propiconazole 25% WP showed significantly superior inhibition over other chemicals with complete 100 per cent mycelial inhibition and absence of sporulation in all the three concentration *i.e*, 0.1%, 0.2%, 0.3%. Myclobutanil 10% WP showed inhibition of 97.2% followed by Tebuconazole + Trifloxystrobin 75% WG with 94.86% inhibition. Systemic fungicides Difenconazole 25% EC with 85.50% inhibition was on par with combi product Zineb 68% +Hexaconazole 100% WP with 85.06% inhibition. The least mycelial inhibition was shown by strobulin compound Azoxystrobin with 35.70 per cent inhibition. Among the systemic fungicides tested, Propiconazole 25% WP showed complete 100 per cent mycelial inhibition and absence of sporulation followed by Myclobutanil 10% WP (97.21%) whereas, in contact fungicides tested chlorothalonil 75% WP showed maximum inhibition of 88.28% followed by Mancozeb 75% WP (76.83%). Similar results were reported by Prashanth *et al*., 2008 [5] who reported that among the tested fungicides, combiproduct carbendazim + mancozeb recorded highest per cent inhibition of mycelial growth (89.23%) of fungus at 0.1 per cent concentration. Patel (2009) [6] reported that, carbendazim and propiconazole showed 85% of effectiveness against *C. gloeosporioides*. Ekbote *et al*., 1996 [7] also found that mancozeb gave cent per cent inhibition at 0.3 per cent concentration and least inhibition by chlorothalonil. The effectiveness of the triazole fungicides like propiconazole may be attributed to their interference with the biosynthesis of fungal sterols and inhibit the ergosterol biosynthesis. In many fungi, ergosterol is required for the structure of cell wall and its absence cause irreparable damage

to cell wall leading to death of fungal cell. A similar study was reported for the effectiveness of triazoles, which inhibit the sterol biosynthesis pathway in fungi by Nene and Thapliyal (2002) [8]. At higher concentration most of the fungicides inhibited maximum mycelial growth but decreased with reduced concentration. These results are in agreement with that of Sudhakar (2000) [9].

Table 2: *In vitro* evaluation of fungicides against mycelial growth of *Colletotrichum gloeosporioides* causing anthracnose of mango

Fungicides		Inhibition (%)			Mean Inhibition (%)
Common name	Trade name	Concentration (%)			
		0.1	0.2	0.3	
Chlolorothalonil 75% WP	Kavach	64.84 (53.63)	100.00 (90.00)	100.00 (90.00)	88.28 (69.98)
Mancozeb 75% WP	Indofil M- 45	71.98 (58.04)	79.90 (63.36)	78.62 (62.46)	76.83 (61.23)
Copper oxy chloride 50% WP	Blitox	52.31 (46.32)	100.00 (90.00)	100.00 (90.00)	84.10 (66.50)
Difenconazole 25% EC	Score	83.22 (65.82)	86.68 (68.49)	86.60 (68.53)	85.50 (67.62)
Hexaconzole 5% EC	Force	84.11 (66.51)	86.61 (68.54)	86.61 (68.54)	85.77 (67.84)
Tetraconazole 3.8% EC	Domex	81.17 (64.28)	87.01 68.87)	79.90 (63.36)	82.69 (65.41)
Propiconazole 25% EC	Tilt	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Thiophanate methyle 75% WP	Roko	47.27 (43.44)	54.76 (47.73)	64.85 (53.64)	55.62 (48.23)
Myclobutanil 10% WP	Boon	91.65 (73.20)	100.00 (90.00)	100.00 (90.00)	97.21 (80.58)
Captan 50% WP	Meriman	56.06 (48.48)	78.62 (62.46)	54.76 (47.73)	63.14 (52.62)
Azoxystrobin	Mirador	31.78 (34.31)	37.66 (37.86)	37.66 (37.86)	35.70 (36.69)
Zineb 68% + Hexaconazole 100%WP	Avatar	81.17 (64.28)	87.01 (68.87)	87.01 (68.87)	85.06 (67.26)
Flusilazole + Carbendazim 37.5% WP	Luster	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Tebuconazole + Trifloxystrobin 75% WG	Native	92.05 73.62)	96.27 (78.86)	96.27 (78.86)	94.86 (76.90)
Control	-	***0.00***	0.00	0.00	-
Mean		76.86	79.64	78.15	-
S. Em±		1.29	2.46	1.17	-
CD @ 1%		3.75	7.14	3.40	-

4. Conclusion

In the present investigation we evaluated the efficacy of 14 fungicides in inhibiting the growth of the anthracnose causing pathogen, *Colletotrichum gloeosporioides.* Among the different systemic fungicides tested, Propiconazole 25% WP showed complete mycelial inhibition and absence of sporulation followed by Myclobutanil 10% WP with 97.21% inhibition whereas, among contact fungicides, chlorothalonil 75% WP showed maximum inhibition of 88.28% followed by Mancozeb 75% WP (76.83%). The result implicates that one of these fungicides or a combination of all these can be used to manage the anthracnose disease under field conditions.

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