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Efficacy of different fungicides by *in vitro* against *Colletotrichum gloeosporioides*, the causal agent of mango anthracnose

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Abstract

The cultivation of mango is found to be increased due to its high income and nutritive value. The crop is widely grown throughout the world. Productivity of mango is decreased due to various biotic and abiotic stresses. Among biotic, anthracnose is the fungal disease infecting in both field and storage with an economical loss to the tune of 15-20 per cent so that management through fungicides is the necessary practice to reduce the loss. In laboratory condition different fungicides were tested against *Colletotrichum gloeosporioides* by using poison food technique. Among systemic fungicides viz., difenconazole 25% EC, propiconazole 25% EC and tebuconazole 25% EC completely inhibited the growth of fungus while in non-systemic fungicides propieb 70% WP significantly inhibited the fungus and among combi-fungicides tryfloxystrobin 25% + tebuconazole 50% was successful by completely inhibiting the growth of *C. gloeosporioides* at all the three tested concentrations.

Keywords: Mango, anthracnose, fungicides, per cent inhibition, *Colletotrichum gloeosporioides*

Introduction

Mango (*Mangifera indica* L.) is grown throughout the tropics and subtropics of the world it belongs to the family Anacardiaceae. It is considered as the king of fruits due to its wide ecological range, delicious taste, excellent flavor, high nutritive and medicinal value with great religion historical significance (Lakshmi *et al.*, 2011) [6]. Mango is ranked second only to banana both in quantity and value and fifth in total production among the major fruit crops worldwide with estimated production of 26 million tons per annum (Anon, 2016) [1], India ranks first in production by contributing 52 per cent of the world production.

In India, the crop is grown in an area of 2.2 million hectares with a production of 21.02 million tonnes and productivity per hectare is 8.17 tonnes. The major growing states in India are Uttar Pradesh, Andhra Pradesh, Karnataka, Bihar, Gujarat and Tamil Nadu. The crop in Karnataka occupied an area of 0.192 million hectares with a production of 1.829 million tonnes and productivity of 9.5 tonnes per hectare (Anon, 2018) [2].

Although mango is considered to be an hardy plant and India is the largest producer of mango. The productivity is low mainly due to a wide range of biotic and abiotic stresses. Among the biotic stresses, fungal diseases are the major once responsible for field and transit losses. Diseases like powdery mildew (*Oidium mangiferae* Barathet), anthracnose [*Colletotrichum gloeosporioides* (Penz.) Penz and Sacc], stem end rot (*Botryodiplodia theobromae* Pat) and black mould (*Meliola mangifera* Earle) are economically important among the fungal diseases. Butler (1918) [3] reported *Colletotrichum gloeosporioides* for the first time in India as a causal organism of coffee leaf spot and in 1924 McRae [7] reported it as causal organism of mango anthracnose.

Colletotrichum gloeosporioides is an asexual or anomorph (imperfect) state of the pathogen belongs to the family Phyllachoraceae of the division Ascomycota. It is a facultative parasite. The sexual or teleomorph (perfect) state is *Glomerella cingulata*. The fungus occurs on a broad range of host species producing acervuli within the host tissue during asexual (mitotic) phase of its life cycle.

Anthraco-nose is a fungal disease infecting mango in both field and storage. It reduces marketable yield from 10 to 80 per cent in developing countries (Poonpolgul and Kumphai, 2007 and Kumar *et al.*, 2010) [11] under field condition and also being post-harvest disease, causing an economical loss to the tune of 15-20 per cent (Ploetz and Prakash, 1997) [10]. Keeping the point in view, laboratory studies were under leaved to evaluate different group of fungicides by *in vitro* against the growth of the pathogen.

Materials and Methods

Collection of disease samples and isolation of the pathogen

The leaves, twigs and floral parts of mango which were infected by anthracnose were collected from the farmer fields and were used for isolation of the pathogen. The process of isolation of pathogen is explained below.

Isolation of the pathogen

The pathogen was isolated from mango leaves showing typical anthracnose symptoms by tissue segment method (Rangaswami and Mahadevan, 1999) [12] on potato dextrose agar medium (PDA). Small bits measuring about 3 mm size were cut off from the leaves showing lesions in such a way that, it contained both infected and healthy parts. Then these bits were surface sterilized in 0.1 per cent mercuric chloride (HgCl₂) solution for 30 seconds followed by three washings in sterilized distilled water. The bits were dried by transferring on to sterilized discs of blotting paper, and then subsequently transferred on to potato dextrose agar (PDA) medium in Petriplates under aseptic conditions. The inoculated plates were incubated at 27 ± 1 °C for seven days for the growth of the pathogen.

In vitro evaluation of fungicides

Poisoned food technique was followed to test the efficacy of different fungicides against the growth of *Colletotrichum gloeosporioides*. List of fungicides along with their concentration evaluated is furnished in Table 1. Suspension of each of the fungicide was prepared in PDA by adding required quantity of respective fungicide to obtain the desired concentrations based on the active ingredient present in each chemical. Fifteen ml of poisoned medium of each fungicide was poured separately into sterilized Petri plates and then allowed to solidify.

Mycelial disc of the fungus measuring 0.5 cm was taken from the periphery of seven days old culture and was placed in the center of the plates. Inoculated plates were incubated at 27 ± 1 °C. Control was maintained without addition of any fungicide. Three replications were maintained for each treatment. Observations were drawn for the growth of the pathogen in all the treated plates, whenever the growth of the fungus touched the periphery in control plate, the colony growth of the pathogen in each treated plate was measured in four directions

and average was worked out. The per cent inhibition of growth by the fungicide was calculated by using below mentioned formula given by Vincent (1927) [16]. And data were analyzed statistically using Completely Randomized Block Design with Factorial concept.

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

Where

I= Per cent inhibition

C= Radial growth of the fungus in control plate

T= Radial growth of fungus in treated plate

Details of fungicides used for *in vitro* evaluation against *C. gloeosporioides*

Sl. No.	Common name	Concentration
Systemic fungicides		
1	Difenconazole 25% EC	
2	Hexaconazole 5% EC	
3	Propiconazole 25% EC	A = 0.05
4	Tebuconazole 25% EC	B = 0.1
5	Azoxystrobin 23% SC	C = 0.15
6	Carbendazim 50% WP	
7	Thiophenate methyl 70% WP	
Non systemic fungicides		
1	Chlorothalonil 75% WP	
2	Copper oxy chloride 50% WP	A = 0.1
3	Copper hydroxide 50% WP	B = 0.2
4	Propineb 70% WP	C = 0.3
5	Mancozeb 75% WP	
6	Zineb 75% WP	
Combi – fungicides		
1	Carbendazim 12% + mancozeb 63%	
2	Pyraclostrobin 5% + metiram 55.5%	
3	Tryfloxystrobin 25% + tebuconazole 50%	A = 0.1
4	Azoxystrobin 23% SC + tebuconazole 25%	B = 0.2
5	Tricyclazole 18% + mancozeb 62%	C = 0.3
6	Captan 70% + hexaconazole 5%	
7	Iprodione 25% + carbendazim 25%	
8	Hexaconazole 5% + zineb 75%	

Results and Discussion

Efficacy of systemic fungicides against *C. gloeosporioides*

The efficacy of seven systemic fungicides was tested under *in vitro* against the growth of *C. gloeosporioides*. Among the seven systemic chemicals evaluated (Table -1 and Plate -1) significantly complete mycelial inhibition (100%) was noticed in the plates treated with *viz.*, difenconazole, tebuconazole and propiconazole at all the three concentrations followed by hexaconazole with 85.41 per cent inhibition. Thiophenate methyl was found to be significantly least effective (28.49%) whereas carbendazim recorded an inhibition zone of 44.26 per cent.

Table 1: *In vitro* efficacy of systemic fungicides against *C. gloeosporioides* of mango

Sl. No.	Fungicides	Per cent inhibition			
		Concentrations (%)			
		0.05	0.10	0.15	Mean
1	Difenconazole 25% EC	100.00 (90.00)*	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
2	Hexaconazole 5% EC	83.60 (66.11)	86.04 (68.06)	86.59 (68.52)	85.41 (67.55)
3	Propiconazole 25% EC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
4	Tebuconazole 25% EC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
5	Carbendazim 50% WP	41.00 (39.82)	45.08 (42.17)	46.70 (43.11)	44.26 (41.70)
6	Azoxystrobin 23% SC	32.76 (34.92)	36.83 (37.37)	37.67 (37.86)	35.75 (36.72)
7	Thiophenate methyl 70% WP	16.55 (24.01)	29.89 (33.14)	39.04 (38.67)	28.49 (32.26)

		S. Em \pm	C. D at 1%
	Fungicides (F)	0.30	1.15
	Concentration ©	0.20	0.75
	F×C	0.52	1.99

*Figures in parentheses indicate angular transformed values



Plate 1: *In vitro* efficacy of systemic fungicides against *C. gloeosporioides* of mango

Similar results were obtained by Ranjitha *et al.* (2019)^[13] that

propiconazole, difenconazole and hexaconazole were effective against *C. gloeosporioides* by recording an inhibition of 100, 85.50 and 85.77 per cent respectively, while thiophenate methyl recorded the least inhibition of 55.62 per cent. The effectiveness of triazole fungicides like propiconazole, tebuconazole and difenconazole was attributed to their interference with biosynthesis of fungal sterol and inhibition of ergo sterol biosynthesis. In many fungi ergo sterol is the structural component of the cell wall and its absence cause irreparable damage to cell wall leading to the death of fungal cell. Inhibition of sterol biosynthesis pathway in fungi by triazole fungicides was studied by Nene and Thapliyal (2002)^[9]. Most of the fungicides at higher concentrations usually exhibit superior efficacy as reported by Sudhakar (2000)^[15] and Shivakumar (2015)^[14].

Efficacy of non-systemic fungicides against *C. gloeosporioides* under *in vitro*

Among the six non systemic fungicides tested (Table -2 and Plate -2) propineb 70% WP was found to be significantly superior with the record of 100 per cent growth inhibition of pathogen at lowest concentration of 0.1 per cent followed by the next best effective fungicide as mancozeb 75% WP (84.09%). The other fungicides *viz.*, Chlorothalonil 75% WP and copper oxychloride 50% WP were found to be moderate effective. Copper hydroxide 50% WP recorded as significantly least effective (40.06%) fungicide. The results obtained were in accordance with the report of Ranjitha *et al.* (2019)^[13], who reported that, mancozeb is highly effective against *C. gloeosporioides* with 76.83 per cent mycelial inhibition. The effective inhibition of the pathogen by propineb was mainly due to its mode of action, that is alteration in the lipid metabolism of pathogen (Golakiya *et al.*, 2020)^[5] and similar results were obtained by Moreira *et al.* (2017)^[8] and Ekbote *et al.* (1994)^[4].

Table 2: *In vitro* efficacy of non-systemic fungicides against *C. gloeosporioides* of mango

Sl. No.	Fungicides	Per cent inhibition			
		Concentration (%)			
		0.10	0.20	0.30	Mean
1	Chlorothalonil 75% WP	54.85 (47.79)*	86.67 (68.58)	86.67 (68.58)	76.06 (60.71)
2	Copper oxychloride 50% WP	65.22 (53.86)	72.52 (58.38)	76.56 (61.04)	71.43 (57.69)
3	Copper hydroxide 50% WP	30.67 (33.63)	33.96 (35.64)	55.56 (48.19)	40.06 (39.27)
4	Propineb 70% WP	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
5	Mancozeb 75% WP	62.74 (52.38)	89.52 (71.11)	100.00 (90.00)	84.09 (66.49)
6	Zineb 75% WP	32.96 (35.04)	39.15 (38.73)	58.30 (49.78)	43.47 (41.25)
		S. Em \pm		C. D at 1%	
	Fungicides (F)	0.18		0.68	
	Concentration ©	0.12		0.48	
	F×C	0.31		1.18	

*Figures in parentheses indicate angular transformed values

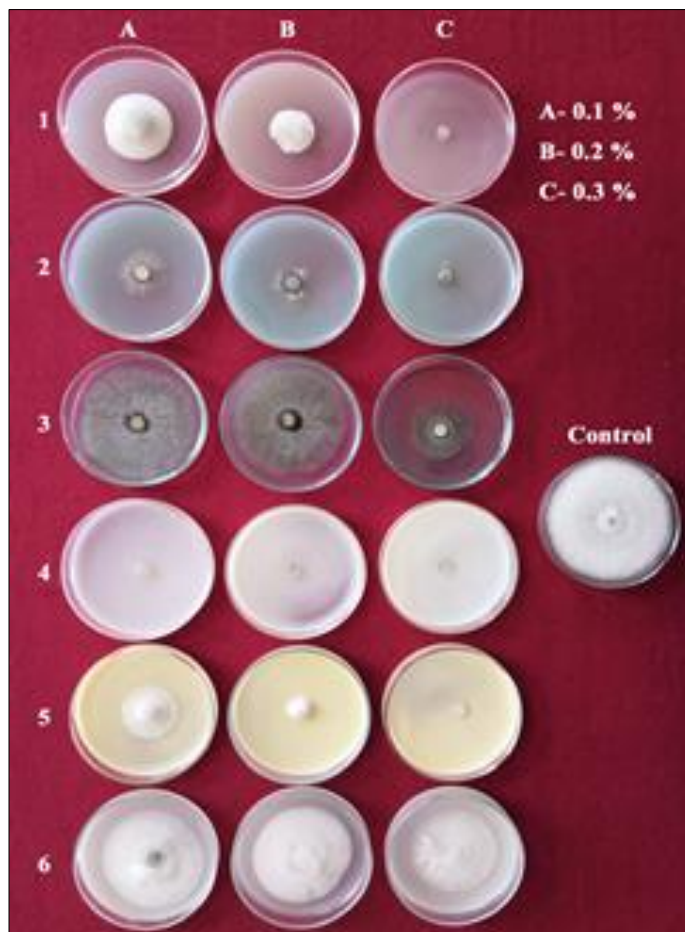


Plate 2: *In vitro* efficacy of non-systemic fungicides against *C. gloeosporioides* of mango

Effectivity of combi – fungicides against *C. gloeosporioides* under *in vitro*

Among the eight combi fungicides tested against the pathogen under *in vitro*, the tryfloxystrobin 25%+ tebuconazole 50% (Table -3 and Plate -3) showed 100 per cent inhibition of mycelial growth of the pathogen even at the lowest concentration of 0.1 per cent followed by pyraclostrobin 5% + metiram 55.5% with 95.86 per cent inhibition and captan 70% + hexaconazole 5% with 94.95 per cent mycelial inhibition and significantly least mycelial inhibition was recorded by carbendazim 12% + mancozeb 63% (45.18%) of mycelial inhibition. There is a significance difference in respect of efficacy between each of the fungicide. Ranjitha *et al.* (2019) [13] reported that, tryfloxystrobin 25% + tebuconazole 50%

was an effective fungicide against *C. gloeosporioides* with 94.86 per cent inhibition. Further 89.19 per cent inhibition of *C. gloeosporioides* with the same fungicide was obtained by Golakiya *et al.* (2020) [5]. From the results, it is confirmed that, triazole group of fungicides either alone or in combination with other fungicides were more effective.

There was positive correlation between the concentration and inhibition in growth of the pathogen. It is also observed that with the increase in concentration of any fungicide, there will be a corresponding inhibition in growth of the pathogen. Except some fungicides *viz.*, difenconazole, propiconazole, tebuconazole, propineb and tryfloxystrobin 25%+ tebuconazole 50% which resulted hundred per cent inhibition even at lowest concentration.

Table 3: *In vitro* efficacy of combi – fungicides against *C. gloeosporioides* of mango

Sl. No.	Fungicides	Per cent inhibition			
		Concentration (%)			
		0.10	0.20	0.30	Mean
1	Pyraclostrobin 5% + metiram 55.5%	87.59 (69.37) *	100.00 (90.00)	100.00 (90.00)	95.86 (78.26)
2	Tryfloxystrobin 25% + tebuconazole 50%	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
3	Tricyclazole 18% + mancozeb 62%	41.00 (39.82)	54.63 (47.66)	72.67 (58.48)	56.10 (48.50)
4	Carbendazim 12% + Mancozeb 63%	39.52 (38.95)	41.59 (40.16)	54.44 (47.55)	45.18 (42.24)
5	Azoxystrobin 23% + tebuconazole 50%	80.67 (63.92)	91.89 (73.45)	100.00 (90.00)	90.85 (72.39)
6	Captan 70% + hexaconazole 5%	84.85 (67.10)	100.00 (90.00)	100.00 (90.00)	94.95 (77.01)
7	Iprodione 25%+ carbendazim 25%	82.67 (65.40)	86.66 (68.58)	88.68 (70.34)	86.00 (68.03)
8	Hexaconazole 5% + zineb 75% WP	81.67 (64.65)	83.59 (66.10)	83.85 (66.31)	83.04 (65.68)
		S. Em ±		C. D at 1%	
	Fungicides (F)	0.26		0.99	
	Concentration ©	0.16		0.61	
	F×C	0.45		1.71	

*Figures in parentheses indicate angular transformed values



Plate 3: *In vitro* efficacy of combi – fungicides against *C. gloeosporioides* of mango

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