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In vitro and *In vivo* Evaluation of Fungicides against *Colletotrichum gloeosporioides* (Penz. & Sacc) in Mango

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Six fungicides were evaluated against the anthracnose pathogen *Colletotrichum gloeosporioides* (Penz. & Sacc) through poison food technique under *in vitro* conditions and through foliar sprays under field conditions. Under *In vitro* conditions carbendazim (50%) WP @ 0.1% completely inhibited mycelial growth while carbendazim (12%) + mancozeb (63%) WP @ 0.1% was found most effective in terms of Percent Diseases Index(27.55%), Per cent Disease Control (60%) and maximum yield (122.61 kg/tree) under field conditions.

Keywords: Colletotrichum gloeosporioides; mango; anthracnose; fungicide; management.

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1. INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most versatile and widely grown fruit crops of tropical and subtropical regions [1] and known as the "apple of the tropic". It is believed to have originated from South East Asia and more than 1000 varieties have been identified all over the world [2]. It is cultivated extensively as a commercial fruit crop in India, China, Indonesia, Thailand and Mexico. In India the crop is cultivated in 2263 M hectare area with 19687 M tones production. The major states in India cultivating mango include Utter Pradesh, Andhra Pradesh, Karnataka, Bihar, Gujarat, Tamilnadu, Orissa, West Bengal, Jharkhand, Kerala and Maharashtra.

One of the most severe mango diseases in many developing countries is anthracnose. The disease, caused by *Colletotrichum gloeosporioides* Penz. [*Glomerella cingulata* (Stons.) Spauld & Schrenk] was first reported from Puerto Rico in 1903 and later confirmed by Dodd et al. [3] from most of the regions of the world. Mango anthracnose disease is one of the world's leading mango fruit pre-harvest and post-harvest diseases and *Colletotrichum gloeosporioides* in Bangladesh [4, 5, 6] In India, it was first reported by McRae [7].This disease which can spread with rainfall, causes 25 to 30 percent yield loss in mango. Anthracnose also known as blossom blight, leaf spot or fruit rot is a destructive and widespread disease in all mango growing states of India. The disease is severe both in field and storage. Losses due to anthracnose have been estimated from 2-39 per cent. The sexual stage (teleomorph) and the asexual stage (anamorph) are known as *Glomerella cingulata* and *C. gloeosporioides* respectively [8].

2. MATERIALS AND METHODS

The experiments were conducted during 2019-20 in the Department of Plant Pathology, College of Agriculture, Jawaharlal Nehru Krishi Vishwa vidyalaya, and Jabalpur (M.P.) to assess the management strategies for anthracnose in mango.

2.1 Isolation and Identification of *C. gloeosporioides*

Mango leaves infected with anthracnose were collected from experimental site and different locations during survey and used for isolation of the fungus *in vitro*. Leaves infected with anthracnose were cut and surface sterilized by using 1 percent sodium hypochlorite (NaClO) solution for 60 seconds. These cut parts were thoroughly washed in sterile distilled water to remove the traces of sodium hypochlorite if any and then aseptically transferred to sterile potato dextrose agar (PDA) slants. Inoculated slants were incubated at room temperature (27±1^{o}C).One ml of diluted spore suspension from 10-12 days old culture slant was spread uniformly on 2% water agar plate. Plates were incubated at 27±1^{o} C for 12 hrs and observed for conidial germination. The growing hyphal tip portion of a single conidium was transferred to fresh PDA slants with the help of cork borer under aseptic conditions and incubated at 27 ± 1^{o} C. The pure culture thus obtained were used for further studies. The identity of the pathogen was confirmed through colony morphology as well as microscopical observations on the length and breadth of conidia and fruiting body (Plate 1).

2.2 Pathogenicity

The pathogenicity of *C. gloeosporioides* proved under field condition through spray spore suspension method. Healthy leaves were selected from experimental sites. Spore suspension of *C. gloeosporioides* (10^6 cfu/ml) was sprayed on the healthy leaves. Inoculated leaves were covered with transparent polythene and tagged. Development of symptoms was observed on 5, 7 and 10th days after inoculation.

2.3 Evaluation of Fungicides Against *C. gloeosporiodes in vitro*

Six fungicides namely Azoxystrobin 23SC (Amister®) at 2%, Chlorothalonil 33.1SC (Kavach®) at 0.2%, Mancozeb 75 WP (Dithene M-45®) at 0.1%, Hexaconazole 5EC (Contaf®) at 0.05%, Carbendazim(12)+ Mancozeb (63) WP (Saaf®) at 0.15% and Carbendazim 50 WP (Bavistin®) at 0.1% were tested against *C. gloeosporiodes* through poison food technique [9]. Fungicide suspension was prepared in PDA by adding required quantity of fungicide to obtain the desired concentration on the basis of active ingredient present in each commercial formulation. Twenty ml of poisoned medium was poured in each of the sterilized petri plates. Suitable checks also maintained without addition of any fungicide. Mycelial disc of 0.5 cm was drawn from the periphery of seven-day old culture and placed in the center of petri plate with

media and incubated at 27±1ºC till the fungal growth in control plates reached the periphery of plates. The seven treatments were replicated thrice in completely randomized design (CRD). The diameter of the colony was measured in three angles and mean colony growth was worked out. The colonies were also observed for presence or absence of sporulation. The per cent growth inhibition was calculated by using the formula given by Vincent [10].

$$I = C - T / C \times 100$$

Where,

I = Per cent inhibition of mycelium
C = Growth of mycelium in control (mm)
T = Growth of mycelium in treatment (mm)

2.4 Field Evaluation of Fungicides Against *C. gloeosporiodes*

The efficacy of fungicides (mentioned in 2.3) was also evaluated under field conditions under Randomized Block Design against anthracnose in mango (*var.* Langra) at Fruit Research Station, Imalia, Jabalpur during 2019-20. A total of seven treatments including control were replicated thrice. Foliar spray of fungicides was carried out twice at the interval of ten days on plants affected by anthracnose. Observations on disease severity were made prior to the start of the experiment as well as seven days after the sprays and scored through a 0-5 scale [11,12,13].

List 1. Percentage of leaf infection with rating

Rating	Percent leaf infected
0	0
1	1 – 10
2	11 – 20
3	21 – 30
4	31 – 50
5	>50

The per cent disease index (PDI) as calculated as below and per cent disease control (PDC) were also assessed.

Percent disease index = (Sum of all numerical ratings / Number of observations x Maximum Disease grade) x 100

3. RESULTS AND DISCUSSION

All the fungicides caused significant reduction in mycelial growth of pathogen *in vitro*, compared to control. Complete inhibition (100 per cent) of radial growth by carbendazim at 120 hrs while minimum colony growth was observed on carbendazim + mancozeb (8.50 mm and 10.33 mm) and mancozeb (7.33 mm and 10.83 mm) at 120 and 168 hrs. Azoxystrobin was least effective with maximum mycelial growth (22.50 mm and 36.50 mm) of *C. gloeosporioides* at 120 and 168 hrs. The colony growth of the pathogen in control plates was 60 mm at 120 hrs and 90 mm at 168 hrs(Table 1).

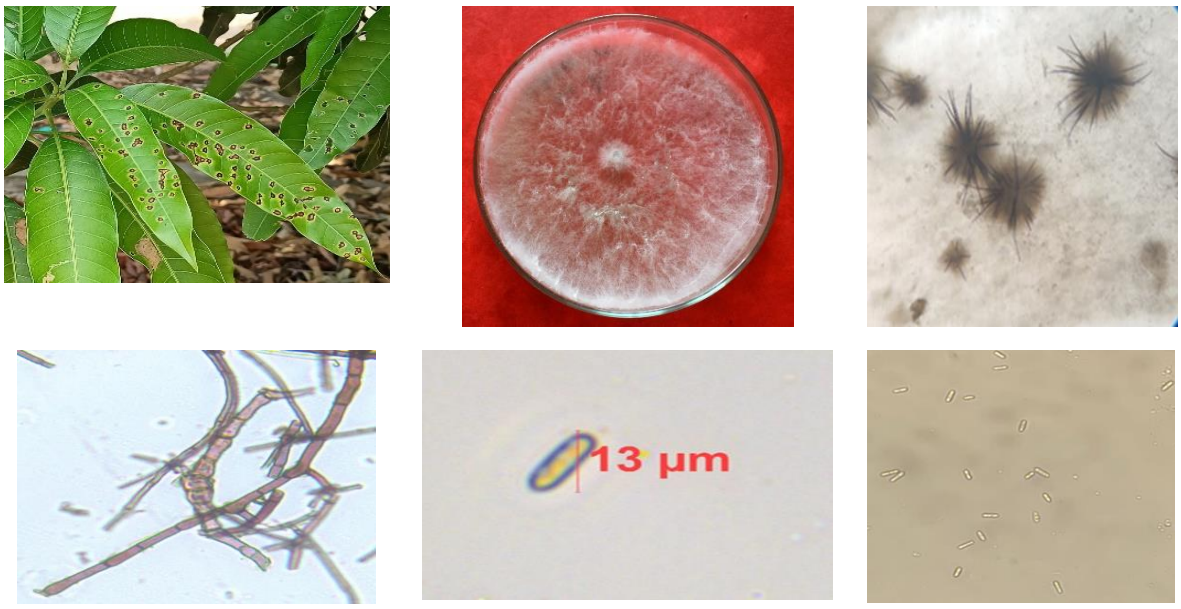

Plate 1. Collection, isolation and identification of *Colletotrichum gloeosporioides*

Table 1. *In vitro* evaluation of fungicides against *Colletotrichum gloeosporioides*

Fungicide	Dose%	Colony growth (mm) after 120 hrs	Colony growth (mm) after 168 hrs	Percent growth inhibition at 168hrs
Azoxystrobin	2	22.50	36.50	59.44
Chlorothalonil	0.2	15.33	20.33	77.41
Mancozeb	0.1	7.33	10.83	87.96
Hexaconazole	0.05	20.50	25.33	71.85
Carbendazim+ mancozeb	0.15	8.50	10.33	88.52
Carbendazim	0.1	0.00	0.00	100
Control	-	60.00	90.00	-
SE(±m)		0.19	0.26	
CD@5%		0.61	0.81	

Table 2. *In vivo* evaluation of fungicides against *Colletotrichum gloeosporioides* of mango

Detail of treatment	Doses (%)	Percent Diseases Index			Per cent Disease Control (PDC)	Yield kg / tree
		Pre - treatment	After First spray	After second spray		
Azoxystrobin 23% SC	2	28.01	20.15	17.95	32.45	107.65
Chlorothalonil 33.1% SC	0.2	27.66	26.24	25.48	4.13	83.5
Mancozeb 75% WP	0.1	27.69	19.38	15.28	42.51	116.82
Hexaconazole 5%EC	0.05	28.25	23.29	21.79	18.02	95.02
Carbendazim (12%) + Mancozeb (63%) WP	0.15	27.55	15.25	10.63	60	122.61
Carbendazim 50% WP	0.1	28.58	21.23	19.54	26.48	91.23
Control	-	27.58	28.21	26.58	-	75.17
CD@5%		N/A	0.73	0.58	-	2.97
SE(±m)		0.39	0.73	0.18	-	0.95

Among all the fungicides carbendazim showed maximum growth inhibition (100%) of *C. gloeosporioides* followed by carbendazim + mancozeb (88.52%), and mancozeb (87.96%). The least growth inhibition of 59.44% was recorded in azoxystrobin plates(Table 1).

In the *in vivo* evaluations **,** pretreatment infection levels were insignificant. All the fungicides showed significant reduction of *C. gloeosporioides* infection compared to control, seven days after first spray. Minimum percent disease index (PDI) of 15.25 per cent was recorded in carbendazim 12% WP+ mancozeb 63% WP , followed by 19.38 per cent in mancozeb 75% WP (Table 2). Maximum PDI of 28.21 per cent was observed in control. Apart from control maximum PDI of 26.24 per cent was recorded in chlorothalonil 33.1% SC . Similar trend of fungicidal effect was observed seven days after the second spray. The PDI values further declined indicating the cumulative impact of the treatments. Minimum PDI was recorded in carbendazim 12% WP+ mancozeb 63% WP followed by mancozeb 75% WP . Maximum percent disease index was recorded in control. Apart from control maximum percent disease index was recorded from chlorothalonil 33.1% SC. Percent Diseases Index Per cent Disease Control.

4. CONCLUSION

The pathogen isolated from mango (*Mangifera indica* L.) leaves having leaf spot disease or anthracnose was identified as *Colletotrichum gloeosporioides.* The pathogen mostly infect leaf and fruits causing brown to dark brown, circular depressed spots on fruit and violet to black spots on leaf. Among all fungicides tested against *C. gloeosporioides* carbendazim (50%) WP @ 0.1% was found most effective in controlling the disease under laboratory conditions. However,

under field conditions, among all fungicides tested against *Colletotrichum gloeosporioides* carbendazim (12%) + mancozeb (63%) WP @ 0.1% was found to be most effective in managing the disease and increasing yield under field conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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