Original Research Article

In-vitro and *In-vivo* management of anthracnose of mango caused by *Colletotrichum gloeosporioides* (Penz. &Sacc).

Abstract

Mango (*Mangifera indica* L.), the King of the fruits, is the eighth-most cultivated fruit globally, producing more than 43 million tons in India, Bangladesh, Nepal, and many other tropical nations. It is a crucial component of nutrition in many developing nations since it offers vitamins and minerals and the demand is rising day by day**.**India is the largest producer of mango in the world. Uttar Pradesh ranks first in mango production with a share of 32.47% and highest productivity. Mango plants disease is a great barrier to produce enough fruits to meet the people demand. There are many diseases, such as Mango malformation, Anthracnose, Bacterial black spot, Red rust, Powdery mildew, Root rot, Damping off, Ganoderma, root rot, Dieback, Sooty molds and Stem canker etc.which affect the mango trees.The experiment was carried out through poison food technique under *in vitro* and through foliar sprayunder field conditions. Under *Invitro* carbendazim was completely inhibited mycelial growth up to 100 per cent and carbendazim (12%) + mancozeb (63%) WP @ 0.1% was found most effective and maximum yield (122.61 kg/tree) under field conditions.

Keywords: (*Mango, Anthracnose, Fungicide, Management)*

1. Introduction

Mango (*Mangifera indica* L.) is one of the most versatile and widely grown fruit crops of tropical and subtropical regions (Vasugi *et al*., 2012) and known as the “apple of the tropics. It is believed to have originated from South East Asia and more than 1000 varieties have been identified all over the world (Rymbai*et al*., 2014). It is cultivated extensively as a commercial fruit crop in India, China, Indonesia, Thailand and Mexico. By virtue of its wide range, delicious taste, superb flavour, very high nutritive and medicinal value as well as great religious-historical significance, it is called the “King of the fruits” (Hayes, 1953; Pandey *et al*., 2012).

The genus *Mangifera* belong to the order sapindales in the family Anacardiaceae which consists of 69 species and mostly restricted to tropical Asia. It is cultivated in 2263 M hectare area with 19687 M tones production in India. It is cultivated in the states of Utter Pradesh, Andhra Pradesh, Karnataka, Bihar, Gujarat, Tamilnadu, Orissa, West Bengal, Jharkhand, Karla and Maharashtra with annual production of 125.4 lakh tones from 2.30 million hectares of area.

Many fungal diseases in mango have been reported by several research workers. Well known fungal diseases of mango are Anthracnose (*Colletotrichum gloeosporioides*), Blossom blight (*Botrytis cinerea*), Crown rot (*Fusarium solani*), Crusty leaf spot (*Zimmermanniellatrispora*), Dieback (*Botryosphaeria disrupta*), Gall (*Fusarium decemcellare*), Leaf blight (*Bipolarishawaiiensis*), Leaf spot (*Curvularialunata*),

Fruit rot (*Alternaria alternata*), Macrophoma rot (*Macrophomamangiferae*), Phoma blight (*Phoma glomerata*), Pink disease (*Erythriciumsalmonicolor*), Powdery mildew (*Oidium mangiferae*), Root rot (*Pythium splendens*), Scab (*Elsinoemangiferae*), Sooty molds (*Capnodiumcitri*), Stem canker (*Phoma sp.*) etc. Bacterial, Nematode and other pathogenic diseases also play an important role in the destruction of mango orchards.

One of the most severe mango diseases in many developing countries is *Colletotrichum gloeosporioides*. Anthracnose and stem end red which can spread with rainfall, have reported about 25 to 30 percent loss of overall mango yield. Anthracnose caused by *Colletotrichum gloeosporioides* Penz. [*Glomerellacingulata*(Stons.) Spauld& Schrenk] was first reported from Puerto Rico in 1903 and later confirmed by (Dodd *et al.,*1997) 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 (Arriel *et al*., 2016; Bhanudas, 2020; Uddin *et al*., 2018) In India, it was first reported by McRae (1924).

Anthracnose is 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. *Glomerellacingulata* is the sexual stage (teleomorph) while the asexual stage (anamorph) is called *C. gloeosporioides*(Schrenk and Spaulding, 1903). *Colletotricumgloeosporioides* is belongs to kingdom Fungi, phylum Ascomycota, class Sordariomycetes, order Phyllachorales, family Phyllachoraceae (Gautam AK, 2014).

2. Material and Methods

An experiment was conducted during 2019-20 in the Department of Plant Pathology, College of Agriculture, Jawaharlal Nehru Krishi Vishwavidyalaya, and Jabalpur (M.P.). The details of the material used and methodologies adopted during the course of investigation are described below.

2.1 Isolation of pathogen

Mango leaves infected with anthracnose were collected from experimental site and different locations during survey and used for isolation of the fungus *in vitro*. The isolation of the fungus was made by using standard tissue isolation technique as described below.

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 (NaClO) if any and then aseptically transferred to sterile potato dextrose agar (PDA) slants. Inoculated slants were incubated at room temperature (27±1^{o}C).

2.2 Purification

2.2.1 Single spore isolation

Ten ml of clear filtered 2% water agar was poured into the sterile Petri-plates and allowed to solidify. Diluted spore suspension was prepared in sterilized distilled water from ten to twelve days old culture. One ml of such suspension was spread uniformly on water agar plate. Plates were incubated at 27±1^{o} C for 12 hrs and watched under compound microscope so as to locate germination of conidia. Single germinated conidium was marked with ink-marker on the lower surface of the plates. The growing hyphal tip portion was transferred to fresh PDA slants with the help of cork borer under aseptic conditions and incubated at 27 ± 1^{o} C, Obtained pure culture tubes were used for further studies.

2.3 Identification of *Colletotrichum gloeosporioides*

For identification of pathogen, morphological characters of the fungus such as mycelial and cultural characters, length and breadth of conidia, fruiting body were observed with the help of compound microscope.

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

2.4 Pathogenicity

To test the pathogenicity of *Colletotrichum gloeosporioides*spore suspension method was used.

2.4.1 Spore suspension method

The pathogenicity of *Colletotrichum gloeosporioides* proved under field condition through spray spore suspension method. Healthy leaves were selected from experimental sites. Spore suspension of *Colletotrichum gloeosporioides* was prepared in amount of 10^6cfu/ml and sprayed on the healthy leaves. Inoculated leaves were covered with transparent polythene and tagged. Observations were taken at 5, 7 and 10th days after inoculation for symptom development.

2.5 Management

List1 :List of fungicides used against *Colletotrichum gloeosporiodes*

S.No.	Common name	Trade name	Doses (%)
1	Azoxystrobin 23% SC	Amister	2
2	Chlorothalonil 33.1% SC	Kavach	0.2
3	Mancozeb 75% WP	Dithen M-45	0.1
4	Hexaconazole 5%EC	Contaf	0.05
5	Carbendazim (12%) + Mancozeb(63%) WP	Saaf	0.15
6	Carbendazim 50% WP	Bavistin	0.1

2.5.1*In vitro* evaluation of fungicides against *C. gloeosporiodes*

Efficacy of fungicides were assessed by using poison food technique (Nene and Thapliyal, 1993). The pathogen *C. gloeosporioides* of mango was grown on PDA in petri plates for seven days prior to setting the experiment. Fungicide suspension was prepared in PDA by adding required quantity of fungicide to obtain the desired concentration on the basis of active ingredient and whole product present in the chemical. Twenty ml of poisoned medium was poured in each of the sterilized petri plates. Mycelial disc of 0.5 cm was be taken from the periphery of seven day old culture and placed in the center of petri plate containing poison media and incubated at 27 ± 1^0 C till growth of the fungus touched the periphery in control plate. Suitable checks also maintained without addition of any fungicide, three replications were maintained for each treatment. The diameter of the colony was measured in three directions and average was worked out. Those petriplate were also observed for presence or absence of sporulation. The per cent inhibition of growth was calculated by using the formula given by Vincent (1947).

$$I = C – T/ C \times 100$$

Where, I = Per cent inhibition of mycelium

C = Growth of mycelium in control (mm)

T = Growth of mycelium in treatment (mm)

2.5.2*In-vivo* evaluation of fungicides against *C. gloeosporiodes*

To evaluate the fungicides for management of anthracnose, in mango *var.* Langra, all field experiments were conducted under *in vivo* conditions at Fruit Research Station, Imalia, Jabalpur during 2019-20 in three replications with Six treatments and one control.Foliar spray of fungicides was done two times at the interval of ten days in infected plants by anthracnose. Observations on disease severity were scored by using 0-5 scale.

List 2 : 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

$$\text{Percent disease index} = \frac{\text{Sum of all numerical rating}}{\text{No of observations X Maximum Disease grade}} \text{X } 100$$

3. Result and Discussion

The individual effect of six fungicides was evaluated against *Colletotrichum gloeosporioides* with control inexperiments. A total of seven treatments including one control were planted in CRD and RBD under lab and field condition. The result of experiments is mention under following heading.

3.1 *In vitro* evaluation of fungicide against *Colletotrichum gloeosporioides*

A total six fungicide were used to evaluate their efficiency against *Colletotrichum gloeosporioides*. The fungicides were screened under laboratory condition. Fungicides showed significant reduction in mycelial growth of pathogen when compared to control.

Among all fungicides carbendazim was completely inhibited radial growth up to 100 per cent at 120 and 168 hrs. followed by minimum radial 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. However, Azoxystrobin showed maximum radial growth (22.50 mm and 36.50 mm) of *C. gloeosporioides* at 120 and 168 hrs followed by hexaconazol (20.50 mm and 25.33 mm) and chlorothalonil (15.33 mm and 20.33 mm). The radial growth of control (60 mm) at 120 hrs and (90 mm) at 168 hrs were found. The data presented in Table 1.

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

Treatment	Fungicide	Dose%	Radial growth (mm) after 120 hrs	Radial growth (mm) after 168 hrs	Percent growth inhibition
T1	Azoxystrobin	2	22.50	36.50	59.44
T2	Chlorothalonil	0.2	15.33	20.33	77.41
T3	Mancozeb	0.1	7.33	10.83	87.96

T4	Hexaconazole	0.05	20.50	25.33	71.85
T5	Carbendazim+ mancozeb	0.15	8.50	10.33	88.52
T6	Carbendazim	0.1	0.00	0.00	100
T7	Control	-	60.00	90.00	-
	SE(±m)		**0.19**	**0.26**	
	CD@5%		**0.61**	**0.81**	

Among all the fungicides carbendazim showed maximum growth inhibition (100%) of *Colletotrichum gloeosporioides*at 0.1 per cent concentration followed by carbendazim + mancozeb (88.52%) at 0.2% concentration, mancozeb (87.96%) at 0.2 per cent, chlorothalonil (77.41%) at 0.2 per cent, hexaconazole (71.85%) at 0.2 per cent concentration. However, less growth inhibition was recorded in azoxystrobin (59.44%) at 2% concentration. The data presented in Table 1.

3.2*In vivo* evaluation of fungicide against *Colletotrichum gloeosporioides*

A total six fungicide were used to evaluate the efficiency against *Colletotrichum gloeosporioides*. Sprays of fungicide were conducted two times at the interval of ten days and observation were recorded after seven days of both spray. The per cent disease index (PDI) and per cent disease control (PDC) were calculated. The data presented in Table 2.

3.2. 1 Pre treatment

Before any spray of fungicides all the treatments showed non- significant effect in reducing growth of *Colletotrichum gloeosporioides*.

3.2. 2 After First spray

Observation were taken at the interval of seven days after first spray. All the treatment showed significantly effect in reducing *Colletotrichum gloeosporioides*. Minimum percent disease index was recorded in T_5 (carbendazim 12% WP+ mancozeb 63% WP @ 0.15%) 15.25 per cent, followed by T_3 (mancozeb 75% WP @ 0.1%) 19.38 per cent, T_1 (azoxystrobin 23% SC @ 2 %) 20.15 per cent, T_6 (carbendazim 50% WP @ 0.1 %) 21.23 per cent and T_4 (hexaconazol 5% EC @ 0.05 %) 23.29 per cent, respectively. Maximum percent disease index was recorded in T_7 28.21 per cent. Apart from control maximum percent disease index was recorded from T_2 (chlorothalonil 33.1% SC @ 0.2 %) 26.24 per cent.

3.3.3 After second spray

Observations were taken at the interval of seven days after second spray. All the treatment showed significantly effect in reducing *Colletotrichum gloeosporioides*. Minimum percent disease index was recorded in T_5 (carbendazim 12% WP+ mancozeb 63% WP @ 0.15%) 10.63 per cent, followed by T_3 (mancozeb 75% WP @ 0.1%) 15.28 per cent, T_1 (azoxystrobin 23% SC @ 2 %) 17.95 per cent, T_6 (carbendazim 50% WP @ 0.1 %) 19.54 per cent and T_4 (hexaconazol 5% EC @ 0.05 %) 21.79 per cent, respectively. Maximum percent disease index was recorded in T_7 26.58 per cent. Apart from control maximum percent disease index was recorded from T_2 (chlorothalonil 33.1% SC @ 0.2 %) 25.48 per cent.

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

			Percent Diseases Index	**Per cent**	

Treatment Symbol	Detail of treatment	Doses (%)	Pre - treatment	After First spray	After second spray	Disease Control (PDC)	Yield kg/tre e
T1	Azoxystrobin 23% SC	2	28.01	20.15	17.95	32.45	107.65
T2	Chlorothalonil 33.1% SC	0.2	27.66	26.24	25.48	4.13	83.5
T3	Mancozeb 75% WP	0.1	27.69	19.38	15.28	42.51	116.82
T4	Hexaconazole 5%EC	0.05	28.25	23.29	21.79	18.02	95.02
T5	Carbendazim (12%) + Mancozeb (63%) WP	0.15	27.55	15.25	10.63	60	122.61
T6	Carbendazim 50% WP	0.1	28.58	21.23	19.54	26.48	91.23
T7	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

4. Conclusion

The pathogen isolated from mango (*Mangifera indica* L.) leaves having leaf spot disease was identified as *Colletotrichum gloeosporioides.*

The pathogen *Colletotrichum gloeosporioides* 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 *Colletotrichum gloeosporioides* carbendazim (50%) WP @ 0.1% was found most effective in controlling the disease under laboratory conditions.

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

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