Original Research Article

~~*In-vitro and In-vivo m*~~*In vitro* and *in vivo* evaluation of fungicides against~~anagementof anthracnose of mango caused by~~ *Colletotrichum gloeosporioides* (Penz. &Sacc) in mango.

Abstract

Six fungicides were evaluated against the anthracnose pathogen *Colletotrichumgloeosporioides* (Penz. &Sacc)~~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*conditions and through foliar sprays under field conditions. Under *In ~~vitro~~ vitro* conditions carbendazim (50%) WP @0.1% ~~was~~ completely inhibited mycelial growth ~~up to 100 per centand~~ whilecarbendazim (12%) + mancozeb (63%) WP @ 0.1% was found most effective in terms ofPercent Diseases Index(….),Per cent Disease Control(…) and maximum yield (122.61 kg/tree) under field conditions.

Keywords: *Colletotrichum gloeosporioides,(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 tropic”~~s~~. 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~~In India the crop is cultivated in 2263 M hectare area with

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19687 M tones production~~in India.. It is cultivated in the states of~~The major states in India cultivating mango include Utter Pradesh, Andhra Pradesh, Karnataka, Bihar, Gujarat, Tamilnadu, Orissa, West Bengal, Jharkhand, ~~Karla~~ Kerala 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 (*Zimmermanniella trispora*), Dieback (*Botryosphaeria disrupta*), Gall (*Fusarium decemcellare*), Leaf blight (*Bipolaris hawaiiensis*), Leaf spot (*Curvularia lunata*), Fruit rot (*Alternaria alternata*), Macrophoma rot (*Macrophoma mangiferae*), Phoma blight (*Phoma glomerata*), Pink disease (*Erythricium salmonicolor*), Powdery mildew (*Oidium mangiferae*), Root rot (*Pythium splendens*), Scab (*Elsinoe mangiferae*), Sooty molds (*Capnodium citri*), 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 anthracnose.~~Anthracnose~~ The disease, caused by *Colletotrichum gloeosporioides*Penz. [*Glomerellacingulata*(Stons.) ~~Spauld &~~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).

~~*Colletotrichum gloeosporioides*.Anthracnose and stem end red~~This disease which can spread with rainfall, ~~have reported about~~causes 25 to 30 percentyield loss in~~25 to 30 percent loss of overall~~ mango ~~yield~~. ~~Anthracnose 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.*,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,~~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 *Glomerellacingulata*~~is the sexual stage (teleomorph) while the asexual stage (anamorph) is~~ and~~called~~ *C. gloeosporioides*respectively(Schrenk and Spaulding, 1903). ~~*Colletotricum gloeosporioides* is belongs to kingdom Fungi, phylum Ascomycota, class Sordariomycetes, order Phyllachorales, family Phyllachoraceae (Gautam AK, 2014).~~

~~2.~~ Material and Methods

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An experiment wasThe experiments were conducted during 2019-20 in the Department of Plant Pathology, College of Agriculture, Jawaharlal Nehru Krishi Vishwavidyalaya, and Jabalpur (M.P.). .) to assess the management strategies for anthracnose in mango.The details of the material used and methodologies adopted during the course of investigation are described below.

2.1 Isolation and identification of *C.gloeosporioides*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 One ml of diluted spore suspension was prepared in sterilized distilled waterfrom from ten to twelve10-12 days old culture slant. One ml of such suspension was spread uniformly on 2% water agar plate. Plates were incubated at 27±1^{o} C for 12 hrs and watched observed for under compound microscope so as to locate germination of conidial germination. Single germinated conidium was marked with ink marker on the lower surface of the plates. 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. Obtained. The pure culture tubes thus obtainedwere used for further studies.

2.3 Identification of *Colletotrichum gloeosporioides*

For identification of pathogen,The identity of the pathogen was confirmed throughcolony morphological morphologycharacters of the fungus such as mycelial and cultural characters,as well as microscopical observations on the length and breadth of conidia and, fruiting body(Plate 1).ywere observed with the help of compound microscope.

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

~~2.5~~ Management

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~~List 1 : List of f~~Evaluation of fungicides~~ungicides used~~ against ~~*Colletotrichum*~~ *C.gloeosporiodes*~~*in vitro*~~

~~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~~

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 testedagainst *C. gloeosporiodes*

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

~~*Efficacy* of fungicides were assessedby using~~through 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~~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 ~~be taken~~drawn from the periphery of ~~seven day~~seven-day old culture and placed in the center of petri plate ~~containing poison~~with media and incubated at 27±1^0 C till thefungal growthin control plates reached~~ of the fungus touched~~ the periphery of plates~~ in control plate. Suitable checks also maintained without addition of any fungicide, three replications were maintained for each treatment.~~ The seven treatments were replicated ~~The~~thrice in completely randomized design (CRD). The diameter of the colony was measured in three ~~directions~~ anglesand mean colony ~~average~~ growthwas worked out. ~~Those~~ The ~~petriplate~~colonieswere also observed for presence or absence of sporulation. The per cent growth 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)

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

~~To evaluate t~~The efficacy of fungicides (mentioned in 2.3) was also evaluated under field conditions under Randomized Block Design ~~for management~~ against ~~of~~ anthracnose ~~,~~ in mango (*var.* Langra) ~~,all field experiments were conducted under *in vivo* conditions~~ at Fruit Research Station, Imalia, Jabalpur during 2019-20. ~~A total of seven treatments including control were~~ replicated thrice ~~in three replications with Six treatments and one control.~~ Foliar spray of fungicides was ~~done~~ carried out ~~two times~~ twice at the interval of ten days ~~in~~ on ~~infected~~ 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 ~~by using~~ through a 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

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

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

~~3.~~ Results 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~~ All the fungicides caused significant reduction in mycelial growth of pathogen *in vitro* ~~when~~ , compared to control.

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~~Among all fungicides c~~Complete inhibition (100 per cent) of ~~arbendazim was completely inhibited~~ radial growth ~~up to~~bycarbendazim~~100 per cent~~ at 120 ~~and 168~~ hrswhile ~~followed by~~ minimum ~~radial~~ colonygrowth 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~~ was least effective withmaximum ~~radial~~ mycelialgrowth (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~~ colony growth of the pathogen in control ~~(~~ plates was60 mm~~)~~ at 120 hrs and ~~(~~90 mm~~)~~ at 168 hrs ~~were found. The data presented in~~(Table 1).

Comment [GN13]: The data in table 1 shows the diameter of the colony and not the radial growth.

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

Fungicide	Dose%	~~Radial~~ Colony growth (mm) after 120 hrs	~~Radial~~ Colonygrowth (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**	

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Among all the fungicides ~~carbendazim showed~~carbendazim showed maximum growth inhibition (100%) of ~~*Colletotrichum*~~ *C.gloeosporioides*~~at 0.1 per cent concentration~~ followed by carbendazim + mancozeb

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(88.52%)~~at 0.2% concentration~~, and 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. T~~However, less~~he least growth inhibition of 59.44% was recorded in azoxystrobin ~~(59.44%) at 2% concentration~~plates~~. 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*.~~

In the *jn vivo*evaluations~~**3.2. 2 After First spray**~~

~~Observation were taken at the interval of seven days after first spray.~~ pretreatment infection levels were insignificant.~~All~~ All the ~~treatment fungicides~~showed significant~~ly effect in reducing~~ reduction of ~~*Colletotrichum*~~ *C.gloeosporioides*~~*gloeosporioide*~~sinfection compared to control, seven days after first spray. Minimum percent disease index (PDI) of 15.25 per centwas recorded in ~~T_5 (~~carbendazim 12% WP+ mancozeb 63% WP ~~@ 0.15%) 15.25 per cent~~, followed by 19.38 per centin ~~T_3 (~~mancozeb 75% WP ~~@ 0.1%) 19.38 per cent,~~ (Table 2)~~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~~PDI~~was recorded in T_7 28.21~~of 28.21 per cent was observed in~~,~~control. Apart from control maximum ~~percent disease index~~PDIof 26.24 per centwas recorded ~~from T_2 (~~in 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*.~~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 ~~percent disease index~~PDI 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~~control. Apart from control maximum percent disease index was

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recorded from ~~T_2 (~~chlorothalonil 33.1% SC ~~@ 0.2 %) 25.48 per cent.~~ **Percent Diseases IndexPer cent Disease Control**

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

~~4.~~ Conclusion

The pathogen isolated from mango (*Mangifera indica* L.) leaves having leaf spot disease or anthracnose 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* *C.*~~*gloeosporioides* carbendazim (50%) WP @ 0.1% was found most effective in controlling the disease under laboratory conditions. However, under field conditions,

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

References

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