



Evaluation of germplasm, fungicides and biocontrol agents against anthracnose (*Colletotrichum gloeosporioides*) in mango (*Mangifera indica*) nursery

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ABSTRACT

Mango anthracnose caused by *Colletotrichum gloeosporioides* Penz. is economically important disease of mango (*Mangifera indica* L.). The experiments were conducted to evaluate the germplasm and efficacy of fungicides, biocontrol agents against anthracnose in mango nursery at Punjab Agricultural University, Ludhiana, University Seed Farm, Ludhiana and MS Randhawa Fruit Research Station, Gangian, Hoshiarpur during 2017-18. Twenty five mango cultivars were screened both under laboratory conditions by using detached leaf method and under field conditions. All the cultivars had shown susceptible reaction under laboratory and field conditions with highly susceptible reaction of Malika under laboratory conditions. The fungicides (carbendazim, thiophanate methyl, azoxystrobin, propiconazole, difenoconazole, copper oxychloride, mancozeb) and two biocontrol agents (*Trichoderma harzianum* and *Pseudomonas fluorescens*) were evaluated *in vitro* through poisoned food technique and dual culture technique, respectively. Azoxystrobin and propiconazole were found to be highly effective in inhibiting the mycelial growth (100%) of *C. gloeosporioides* at 50 µg/ml and 100 µg/ml and at 100 µg/ml, respectively. All the fungicides and biocontrol agents, tested *in vitro* were also evaluated under field conditions for management of mango anthracnose. Azoxystrobin and propiconazole were significantly effective in reducing per cent disease index and providing disease control at Ladhawal, Ludhiana and Gangian, Hoshiarpur.

Key words: Anthracnose, Biocontrol agents, *Colletotrichum gloeosporioides*, Mango, Screening

Mango (*Mangifera indica* L.) belonging to family Anacardiaceae is one of the world's most important and esteemed fruit of the tropical and subtropical regions. It is presumably originated in Indo-Burma region and has been cultivated for the last 4000 years with the existence of more than 1000 varieties in Indian subcontinent (Yadav and Singh 2017). India ranks first in mango production in the world and shares about 18.77 million metric tons to world's total production. In India, it is cultivated extensively as commercial fruit crop over an area of 2262.77 thousand ha with productivity 8.71 metric tons per ha (Anonymous 2018). In Punjab state, mango is grown over an area of 6.85 thousand ha with total production of 113.69 thousand metric tons and productivity is about 16.84 metric tons/ha (Anonymous 2018). The crop suffers from many diseases, among which anthracnose caused by *Colletotrichum gloeosporioides* Penz., implicits considerable losses in mango growing areas. This disease is severe in most of the places and more severe wherever continuous rainfall, high temperature and high humidity prevail. It is recognised as

the most important field and post-harvest disease because it causes economic loss of 15-39% (Prabakar *et al.* 2005). Therefore, in present studies different tactics, viz. host resistance, biocontrol agents and chemical control were used in integrated manner to manage the disease under *in vitro* and *in vivo* conditions.

MATERIALS AND METHODS

In vitro screening of mango cultivars against Colletotrichum gloeosporioides: To identify the sources of resistance against anthracnose disease, 25 mango cultivars were evaluated under laboratory conditions by using detached leaf method at Punjab Agricultural University (PAU), Ludhiana, during 2018. For conducting this experiment isolation was done from mango leaves showing typical symptoms of anthracnose and the pathogen was identified as *Colletotrichum gloeosporioides* (Identification Number: 11,038.19, ITCC, IARI, New Delhi). In this method one month old leaves were taken from the mango seedlings. They were washed with running tap water followed by washing with distilled water. To observe lesion size five leaves of each cultivar were pin pricked with clean and sterilized needle. Then these leaves were inoculated by placing a drop of spore suspension of 15 days old culture

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of the pathogen isolated from diseased leaves on the pricked areas. To calculate Percent Disease Index (PDI), five leaves of each cultivar were pricked and the spore suspension (2×10^5 spores/ml) was sprayed on these leaves. These leaves were placed in between two layers of cotton kept in a tray and incubated at $25 \pm 1^\circ\text{C}$ in BOD incubator. The humidity was maintained by moistening the tray with water at 12 hours interval. The leaves were incubated for seven days to observe the reaction of the cultivar to the pathogen. The size of the spots was measured on leaves of each cultivar. The PDI on the leaves of each cultivar was calculated by using 0-5 scale, where 0= Leaves free from infection, 1= Spots covering less than 5% leaf area, 2= Spots covering 5.1-10% leaf area, 3=Spots covering 10.1-25% leaf area, 4=Spots covering 25.1-50% leaf area and 5=Spots covering more than 50% leaf area. The PDI was calculated by using the formula described by Wheeler (1969).

In vivo screening of mango cultivars against anthracnose: Mango cultivars were also evaluated under natural environmental conditions for their reaction to the disease at M S Randhawa Fruit Research Station (FRS), Gangian, Hoshiarpur during monsoon season of 2017 and 2018. On the basis of PDI calculated, the cultivars (Sangeetha 2003) were categorized for their reaction against mango anthracnose as under:

Percent disease index	Reaction
0	Immune (I)
Traces-10	Resistant (R)
10.1-25	Moderately resistant (MR)
25.1-50	Susceptible (S)
>50	Highly susceptible (HS)

In vitro efficacy of fungicides and biocontrol agents against Colletotrichum gloeosporioides: Five systemic fungicides, viz. carbendazim (50 WP), azoxystrobin (23 SC), thiophanate methyl (70 WP), difenoconazole (25 EC) and propiconazole (25 EC), two non-systemic fungicides, viz. mancozeb (75 WP) and copper oxychloride (50 WP) were evaluated under *in vitro* conditions by Poisoned food technique and two bioagents, viz. *Trichoderma harzianum* and *Pseudomonas fluorescens* were evaluated for their efficacy under *in vitro* conditions by using dual culture technique at PAU, Ludhiana. Non-systemic fungicides were tested at a series of concentrations, viz. 10, 50, 100, 200, 500 $\mu\text{g/ml}$, whereas systemic fungicides were tested at different concentrations, viz. 5, 10, 25, 50 and 100 $\mu\text{g/ml}$. Required quantity of the test chemical was mixed with 100 ml of PDA medium and the poisoned medium was poured into Petri dishes (90 mm diameter) under aseptic conditions. Circular bits (5 mm) of the actively growing culture of fungus were placed aseptically in the center of each Petri dish and each concentration was replicated four times. The Petri dishes having PDA medium without fungicide were served as control. After inoculation Petri dishes were incubated at $25 \pm 1^\circ\text{C}$. The radial colony growth of pathogen was recorded when the growth in untreated Petri dish (control) was full (i.e. 90 mm). Per cent inhibition in colony growth was calculated by using formula devised by Vincent (1947). The

ED_{50} and ED_{90} values were also determined by plotting per cent growth inhibition against different concentrations. The data obtained was analysed by using CPCS1 software to determine the efficacy of fungicides.

In vivo efficacy of fungicides and biocontrol agents against mango anthracnose: These five systemic fungicides, viz. thiophanate methyl (0.1%), azoxystrobin (0.1%), carbendazim (0.1%), difenoconazole (0.1%) and propiconazole (0.1%), and two non-systemic fungicides, viz. copper oxychloride (0.3%) and mancozeb (0.25%) and two bioagents *T. harzianum* (10 g/l water) and *P. fluorescens* (10 g/litre water) were further evaluated as foliar spray against mango anthracnose on Dusehri cultivar of mango at University Seed Farm (USF), Ladhawal, Ludhiana and FRS, Gangian, Hoshiarpur during 2018. Each treatment was replicated thrice and 10 nursery plants were kept for each replication at both locations in Randomized Block Design (RBD). Five sprays of each fungicide and bioagent were given at 20 days interval starting from end June to end September with spray of water on plants kept as control. At the end of October, the PDI was recorded by using formula as described by Wheeler (1969). The data obtained was analysed by using CPCS1 software to determine the efficacy of fungicides and biocontrol agents under natural environmental conditions.

RESULTS AND DISCUSSION

In vitro screening of mango cultivars against Colletotrichum gloeosporioides: To identify the sources of resistance against anthracnose disease, 25 mango cultivars were screened using detached leaves under laboratory conditions. The data (Table 1) revealed the response of these cultivars against anthracnose. The mango cultivars were categorized on the basis of their reaction to the disease. The cultivar Malika had shown highly susceptible reaction to the pathogen as maximum lesion size of 7.38 mm and PDI of 50.32 was observed in this cultivar. Minimum lesion size (4.56 mm) and PDI (30.53%) was recorded in the cultivar Baramasi. All other cultivars exhibited susceptible reaction to the disease. None of the cultivars screened were found immune or resistant to the disease.

The results were in accordance with Sharma (2003) who evaluated 16 mango cultivars against mango anthracnose under *in vitro* conditions by using detached leaves and reported that Malika cultivar was highly susceptible. Pandey *et al.* (2012) also screened 73 mango cultivars under *in vitro* conditions. None of the cultivars was immune to *C. gloeosporioides*. The cultivars CISH-H-2035 and CISH-H-1734 were shown resistant response and CISH-H-1718, CISH-H-1719 and CISH-H-1886 were moderately resistant to the disease.

In vivo screening of mango cultivars against anthracnose: The cultivars were also ranked on the basis of their reaction to the disease under natural environmental conditions (Table 1). All the 25 cultivars had shown susceptible response to the disease. The cultivar Malika had shown maximum average PDI of 45.85%. The minimum

Table 1 *In vitro* and *in vivo* screening of mango cultivars against anthracnose

Cultivar	<i>In vitro</i> screening			<i>In vivo</i> screening			
	Mean lesion size (mm)	PDI*	Disease reaction	PDI*		Mean	Disease reaction
				2017	2018		
Alphonso	4.91	32.80	S	27.82	32.77	30.29	S
Rataul	7.02	48.50	S	36.60	48.33	42.46	S
Malika	7.38	50.32	HS	41.75	49.96	45.85	S
Maldha	5.67	38.96	S	32.68	39.00	35.84	S
Langra	5.46	37.65	S	28.60	38.44	33.52	S
Fazli	5.75	41.59	S	33.47	41.89	37.68	S
Dusehri	5.50	38.19	S	32.60	38.87	35.73	S
Baramasi	4.56	30.53	S	22.20	30.33	26.26	S
Amrapali	6.36	45.39	S	39.85	46.00	42.92	S
Chausa	6.39	46.09	S	40.30	47.25	43.77	S
Kishan Bhog	5.19	34.13	S	29.48	35.00	32.24	S
Ram Kela	6.71	46.83	S	35.80	47.97	41.88	S
Tikayo	7.20	48.96	S	41.40	49.66	45.53	S
Totapuri	5.25	34.28	S	31.15	35.66	33.40	S
GN-1	6.15	44.20	S	32.55	44.69	38.62	S
GN-2	5.09	33.98	S	25.48	34.61	30.04	S
GN-3	4.72	31.75	S	24.60	31.29	27.94	S
GN-4	5.39	36.80	S	29.50	37.95	33.72	S
GN-5	6.19	44.78	S	30.25	45.96	38.10	S
GN-6	5.33	36.07	S	27.80	37.40	32.60	S
GN-12	5.27	34.79	S	30.18	35.76	32.97	S
GN-15	6.01	43.15	S	35.62	42.35	38.98	S
GN-21	5.70	40.48	S	31.75	41.66	36.70	S
GN-51	5.82	42.38	S	30.20	41.92	36.06	S
GN-58	6.07	43.82	S	36.55	43.80	40.17	S

PDI* - Percent disease index

average PDI of 26.26% was recorded in cultivar Baramasi. These results are in agreement with the findings of Ghosh *et al.* (2010) who screened 14 mango varieties against *C. gloeosporioides* under natural conditions and categorized Alphonso and Farnand in varieties as susceptible. Similarly, Sharma (2003) evaluated 16 mango cultivars against mango anthracnose under *in vivo* conditions and reported that five cultivars, viz. Arka Anmol, Neelum, Amrapali, Arka Aruna and Totapuri Red Small were susceptible while all the other cultivars were highly susceptible against anthracnose.

In vitro efficacy of fungicides against Colletotrichum gloeosporioides: The data (Table 2) revealed that among the systemic and non-systemic fungicides evaluated against *C. gloeosporioides* azoxystrobin was found to be the most effective in inhibiting the growth of the pathogen up to 100% at 50 µg/ml and 100 µg/ml concentration followed by propiconazole with 100% inhibition at 100

µg/ml. Thiophanate methyl and carbendazim inhibited the growth of pathogen up to 98.23 and 96.07% at 100 µg/ml concentration, respectively. It was followed by difenoconazole with percent mycelial growth inhibition of 92.35 at 100 µg/ml concentration. Among the non-systemic fungicides copper oxychloride inhibited the growth of the pathogen up to 65.78% at 500 µg/ml. The fungicide mancozeb was the least effective fungicide as it inhibited only 32.89% growth of the pathogen at 500 µg/ml concentration. The results indicated that the ED₅₀ value of all the tested systemic fungicides was less than 5 µg/ml. Azoxystrobin and propiconazole were found to be highly effective having ED₉₀ values of more than 10 µg/ml and less than 25 µg/ml. The ED₅₀ value of copper oxychloride was more than 200 but less than 500 µl/ml. The ED₅₀ value of mancozeb was more than 500 µg/ml.

These findings corroborate with Sundravandna *et al.* (2007) who reported significant reduction in mycelial growth over control by azoxystrobin at all (0.25, 0.5, 1.0, 2.0 and 4.0 µl/ml) concentrations. Gud and Raut (2008) reported that, thiophanate methyl and propiconazole were most effective against mango anthracnose followed by carbendazim. Prabakar *et al.* (2008) reported that mancozeb (0.2%) was less effective against the mycelial growth inhibition of *C. gloeosporioides*.

In vitro efficacy of biocontrol agents against Colletotrichum gloeosporioides: Two biocontrol agents, viz. *Trichoderma harzianum* and *Pseudomonas fluorescens* were evaluated against *C. gloeosporioides* by dual culture technique. In dual culture with *T. harzianum* against *C. gloeosporioides*, the radial growth of the pathogen was 2.61 cm as compared to control (6.12 cm) and the radial growth of the antagonist was 3.63 cm (Table 2). Per cent growth inhibition of *C. gloeosporioides* in the presence of *T. harzianum* was 57.35 %. Similarly, *P. fluorescens* produced inhibition zone of 0.35 cm in dual culture. These results are in conformity with those of Patil *et al.* (2009) who reported that *T. harzianum* significantly reduced the mycelial growth (58.06%) of *C. gloeosporioides*. Galindez *et al.* (2017) also reported 59.16% mycelial growth inhibition of *C. gloeosporioides* by *T. harzianum*. Similar results were reported by Sudha and Narendrappa (2015) who reported 47-57.40% mycelial growth inhibition of *C. gloeosporioides* by *P. fluorescens*.

In vivo efficacy of fungicides and biocontrol agents against mango anthracnose: It is evident from the results (Table 2) that all the fungicides and biocontrol agents resulted into reduction in disease index when compared to control. The minimum PDI of 7.64 and 8.82% was recorded in azoxystrobin at Ladhawal and Gangian, respectively. This chemical was highly effective in controlling the disease upto 80.15 and 82.06% at Gangian and Ladhawal, respectively. These results corroborate the findings of Sundravandna *et al.* (2007) who reported the efficacy of azoxystrobin with 100 % control at all concentrations (0.25 ml/l - 4 ml/l). Pandey *et al.* (2016) also reported that azoxystrobin was highly effective against mango anthracnose as minimum PDI (upto 14.7 %)

Table 2 *In vitro* and *in vivo* efficacy of fungicides and biocontrol agents against mango anthracnose

Treatment	<i>In vitro</i> efficacy							<i>In vivo</i> efficacy					
	Per cent mycelial growth inhibition							Dose (g or ml/litre water)	USF, Ludhiana		FRS, Hoshiarpur		
	Concentration ($\mu\text{g/ml}$)								PDI	Disease control	PDI	Disease control	
Fungicide	5	10	25	50	100	ED ₅₀	ED ₉₀						
Carbendazim	70.97 (57.38)	73.72 (59.14)	78.43 (62.31)	85.29 (67.43)	96.07 (78.73)	<5	>50 and <100	1.0	11.72 (19.81)	72.49	13.20 (20.33)	70.30	
Thiophanate methyl	69.01 (56.15)	73.33 (58.89)	87.25 (69.05)	93.52 (75.23)	98.23 (82.39)	<5	>25 and <50	1.0	10.38 (18.55)	75.63	12.44 (20.38)	72.01	
Azoxystrobin	83.22 (65.80)	89.26 (70.86)	94.42 (76.33)	100 (89.96)	100 (89.96)	<5	>10 and <25	1.0	7.64 (15.46)	82.06	8.82 (16.67)	80.15	
Propiconazole	81.76 (64.69)	88.23 (69.92)	93.92 (75.71)	97.44 (80.86)	100 (89.96)	<5	>10 and <25	1.0	8.72 (16.63)	79.53	10.58 (18.25)	76.19	
Difenoconazole	69.40 (56.39)	73.91 (59.26)	78.03 (62.04)	82.74 (65.44)	92.35 (73.92)	<5	>50 and <100	1.0	11.88 (20.72)	72.11	13.36 (21.86)	69.94	
CD (P=0.05): Fungicides = 1.08; Concentrations = 1.02; Fungicides \times Concentrations = 2.41													
Non systemic	Concentration ($\mu\text{g/ml}$)												
	10	50	100	200	500	ED ₅₀	ED ₉₀						
Copper oxychloride	4.85 (17.75)	11.34 (21.91)	19.56 (25.20)	30.61 (30.22)	65.78 (34.98)	>200 and <500	>500	3.0	15.96 (23.72)	62.54	17.24 (24.56)	61.21	
Mancozeb	9.31 (12.65)	13.94 (19.66)	18.15 (26.23)	25.36 (33.58)	32.89 (54.28)	>500	>500	2.5	20.80 (26.93)	51.18	22.10 (27.94)	50.28	
CD (P=0.05): Fungicides=0.48; Concentrations=0.76; Fungicides \times Concentrations=1.07													
BCA	PG (cm)	AG (cm)	IZ (cm)	Pi (%)	C (cm)								
<i>Trichoderma harzianum</i>	2.61	3.63	-	57.35	6.12	10	25.08 (27.23)	41.14	27.48 (31.63)	38.17			
<i>Pseudomonas fluorescens</i>	5.38	-	0.35	-	6.08	10	23.76 (25.95)	44.23	25.82 (29.12)	41.91			
						Control	42.61 (40.98)	-	44.45 (40.61)	-			
						CD (P=0.05)	3.88	-	3.08	-			

BCA, Biocontrol Agent; PG, Pathogen growth; AG, Antagonist growth; IZ, Inhibition zone; Pi, Growth inhibition of pathogen; C, Control. Figures in parentheses are arc sine transformed values

was recorded. Propiconazole was also highly effective as PDI 8.72 and 10.58% were recorded at Ladhawal and Gangian, respectively. This treatment provided disease control up to 79.53 and 76.19% at Ladhawal and Gangian, respectively. These findings provide an evidence that azoxystrobin and propiconazole were statistically at par with each other for their efficacy against mango anthracnose. Sayiprathap *et al.* (2018) reported similar results as propiconazole was highly effective in controlling mango anthracnose with PDI 10.72 over control (PDI 32.23).

Thiophanate methyl and carbendazim also reduced the disease significantly up to 72.01% and 70.30% at Gangian and 75.63 and 72.49% at Ladhawal, respectively. It was followed by difenoconazole with reduction in disease up to 69.04% and 72.11% at Gangian and Ladhawal, respectively. The results revealed that these three fungicides, namely thiophanate methyl, carbendazim and difenoconazole did

not differ among themselves significantly in respect of their effectiveness in managing mango anthracnose. Mancozeb proved the least effective among all the fungicides as it showed maximum PDI upto 22.10 and 20.80 at Gangian and Ladhawal, respectively. The results were in accordance with Sayiprathap *et al.* (2018) who reported that all the systemic fungicides significantly reduced the PDI over control and the contact fungicide mancozeb was less effective with maximum PDI 24.96 over control (PDI 32.23).

The biocontrol agents, viz. *T. harzianum* and *P. fluorescens* were less effective in controlling the disease under field conditions. *P. fluorescens* was effective in reducing the disease up to 44.23 and 41.91% at Ladhawal and Gangian, respectively. Similarly Bhagwat *et al.* (2016) reported that *P. fluorescens* was effective in reducing mango anthracnose upto 43.79% under field conditions. The maximum PDI of 25.08 and 27.48 % was recorded in case

of *T. harzianum* at Ladhowal and Gangian, respectively. It reduced the disease upto 41.14 and 38.17% at Ladhowal and Gangian, respectively. Similar results were reported by Patel (2017) that different isolates of *T. harzianum* reduced chilli anthracnose up to 40%.

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