Original Research Article

A study to test the compatibility of fungal antagonists of isolated phylloplane micro-flora againstmango anthracnose with recommended fungicide – an *invitro* study

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Abstract:

India is the major mango *(Mangifera indica L.)* producer in the world, however, several infectious diseases caused by many phytopathogens are deteriorating mango quality and quantity. Disease control always remains a challenge for the farmers to get optimum production especially due to pesticide resistance. Hence, the present study was conducted to assess the compatibility of fungal antagonists of isolated phylloplane micro-florawith recommendedfungicide.The compatibility of antagonists of *C. gloeosporioides*(*Nigrosporaspharica (Sacc.)* E.W.Mason, *Gliocladiumroseum*Bainier and *Aspergillus spp.*)with different fungicides was tested. The data obtained in all experiments were statistically analyzed using methods Completely Randomized Design (CRD) was used for dual culture and FCRD for poisoned food technique.Two systemic fungicides *viz.*, carbendazim (0.1% -1 g/L), hexaconazole (0.05 % -1/2 ml/L) and one non-systemic fungicides *viz*., Sulphur (0.2% - 2g /L) were evaluated for their compatibility with potential antagonists by poisoned food technique. In this it was revealed that, the antagonist *Nigrosporasphaerica*(95.56 %) and *Aspergillus spp.* (91.11%) were most compatible with sulphur whereas*Gliocladiumroseum*, was more compatible with Hexaconazol (73.11 %).

Keywords:*Aspergillus spp.; C. gloeosporioides*; *Gliocladiumroseum*Bainier; *Nigrosporaspharica (Sacc.)* E.W.Mason

Introduction

India is the major mango *(Mangifera indica L.)* producer in the world, with an area of 2.218 million hectares and the annual production of 18.832 million tones with productivity of 8.49

MT/ha. India contributes about 64 per cent of the world mango production.[1]However, several infectious diseases caused by many phytopathogens are deteriorating mango quality and quantity. Disease control always remains a challenge for the farmers to get optimum production especially due to pesticide resistance. Resistance to fungicide in current days is a major threat to plant disease management.[2]

The surface of aerial plant part provides a habitat for epiphytic micro-organism, many of which are capable of influencing the growth of pathogens. Phylloplane is the leaf surface which serves as a habitat for a variety of microorganisms including pathogens and saprophytes. The term phylloplane was given by 'Kerling' in 1964. Kerling (1964) used the term 'phylloplane' while referring to the actual leaf surface and 'phyllosphere' to the zone near leaves.[3]A number of saprophytic microorganisms on the phylloplane, antagonistic to pathogen have been reported to produce antibiotics. Phylloplane micro-flora comprises a group of different microbes such as bacteria, mycelium forming fungi, yeasts etc. which are the inhabitants of the plant foliage.[4]Considering the importance of above mentioned points the present study was conducted to assess the compatibility of fungal antagonists of isolated phylloplane micro-flora against mango anthracnose fungus *Colletotrichum gloeosporioides (C.gloeosporioides).* with recommended fungicide.

Materials and method

The present study on use of phylloplane micro-flora of mango against mango anthracnose were carried out in the Department of Plant Pathology, College of Agriculture, Dr. B.S.K.K.V., Dapoli as described by Narware et al. (2018)[5].

Isolation and identification of phylloplane micro-flora:

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Isolation and Identification:

The tender, healthy leaves of mango were collected from the mango orchard in paper bags and brought to the laboratory.

Repeated isolations of phylloplane micro-flora of mango were done as described by Narwareet al.(2018)[5], revealed the presence of three fungi. In present study, other phylloplane organisms such as bacteria and yeasts were not found to be associated with mango leaves. All the three fungal antagonistswere observed under microscope.Among them two unidentified cultures were sent for identification to The Chief Mycologist, Agharkar Research Institute, Pune.

Compatibility of potential antagonists with different fungicides:

This experiment was conducted to test the compatibility of potential antagonists of *C. gloeosporioides* with the fungicides recommended against the pathogen. Two systemic fungicides *viz.*, carbendazim (0.1% -1 g/L), hexaconazole (0.05 % -1/2 ml/L) and one non-systemic fungicides *viz*., Sulphur (0.2% - 2g /L) were evaluated for their compatibility with potential antagonists by poisoned food technique (Nene and Thapliyal, 1997).[6]

Tr. No.	Treatments	Trade name	Conc. (%)
T_1	Sulphur	Sulpho	0.2 %
T_2	Hexaconazole 25 EC	Topper	0.05%
T_3	Carbendiazim	Bavistin	0.1%
T_4	Control	-	-

Fungicidal solution of required concentration was prepared and it was poured in to 100 ml PDA in measured quantity to get the desired concentration. Poisoned medium (15 ml) was poured in sterile Petri plates and allowed to solidify. A 5 mm mycelial disc of seven days old culture of each antagonist was inoculated separately at the center of each Petri plate and incubated at 26±1°C and maintained for ten days. A control was maintained without fungicide. Three replications were maintained per treatment. Per cent reduction in radial growth was compared with growth in control plates and per cent compatibility was calculated by the following formula:

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

Where,

I = Per cent compatibility.

C = Radial growth (cm) in control.

T = Radial growth (cm) in treatment.

The total colony area in all the treatments was plotted on graph paper as mentioned earlier.

Statistical Analysis

The data obtained in all experiments were statistically analyzed using methods Completely Randomized Design (CRD) was used for dual culture and FCRD for poisoned food technique.

Results

Isolation and Identification:

The colony of one of the isolated fungi was pink in colour. The growth of this fungus on PDA was very slow at ambient temperature. The colony of the second fungus was creamy white and slightly sticky. The third isolated fungus formed dark black colony on PDA and its growth was fast as it reached to the rim of the Petri plate within four days.Under microscopic observations one of the three fungal antagonists was confirmed as *Aspergillus* on the basis of morphological characters such as septate mycelium, collumela formed in apical region of the conidiophores and round black-coloured spores.The fungus forming pink colony was identified as *GliocladiumroseaumBainier* and the fungus with creamy white mycelium was identified as *Nigrosporasphaerica* (Sacc.) E. W. Mason. As per The Chief Mycologist, Agharkar Research Institute, Pune.

Compatibility of the antagonists with different fungicides by poisoned food technique

Table1:*Nigrosporaspharica (Sacc.)* E.W.Mason

Treatments	Mean colony dia.	% Inhibition	% Compatibility

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	(cm)		
T_1 Sulphur	8.60	4.44	95.56
T_2Hexaconazol	2.28	74.67	25.33
T_3 Carbendazim	7.20	20.00	80.00
T_4 Control	9.00		
SEm ±	**0.12**		
CD @1%	**0.49**		

The results in table1indicate that the antagonist *Nigrosporaspharica* was the most compatible with sulphur (95.56%) followed by Carbendazim (80.00%). Hexaconazole (25.33 %) was found to be slightly detrimental for the mycelial growth of the fungus.

Table 2:*Gliocladiumroseum*Bainier

Treatments	Mean colony dia. (cm)	% Inhibition	% Compatibility
T_1 Sulphur	**3.72**	58.67	41.33
T_2Hexaconazol	**6.58**	26.89	73.11
T_3 Carbendazim	**1.22**	86.44	13.56
T_4 Control	**9.00**		
S.Em±	**0.13**		
CD @1%	**0.53**		

Table 2 revealed that the antagonist was more compatible with Hexaconazole (73.11%) followed by sulphur (41.33%). carbendazim was found to be detrimental for the mycelial growth of the fungus.

Table 3: *Aspergillus spp.*

Treatments	Mean colonydia. (cm)	% Inhibition	% Compatibility
T_1 Sulphur	8.20	8.89	91.11
T_2Hexaconazol	6.60	26.67	73.33

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T_3 Carbendazim	8.80	2.22	97.78
T_4 Control	9.00		
SEm±	**0.10**		
CD @1%	**0.41**		

The resultsoftable 3 indicate that the antagonist was compatible with all the three fungicides but the most compatible with carbendazim (97.78 %) followed by sulphur (91.11%) and Hexaconazole (73.33%).

Discussion

The anthracnose infected leaves were collected from the naturally maintained, unsprayed mango orchard and pathogen *C. gloeosporioides* was isolated on Potato Dextrose Agar (PDA) medium. Soytong *et al.* (2005),[7]Evueh and Ogbebor (2008),[8]Ismet *et al.* (2012)[9]and Kuberan *et.al* (2012),[10] also reported that PDA is the best medium both for mycelial growth as well as spore formation of *Colletotrichum gloeosporioides.*

In the present study, isolation of phylloplane micro-flora was done by using leaf impression method where, both the leaf surfaces, dorsal and ventral, were pressed against the solid culture medium as per the method described by Anejaet al,[11] for isolation of phylloplane micro-flora.

In the present study the compatibility of fungal antagonists i.e., *Nigrosporasphaerica* (Sacc.), *Gliocladiumroseaum* and *Aspergillus* specieswhich are isolates of phylloplane micro-flora against mango anthracnose fungus *C. gloeosporioides*. was assessed with recommended fungicide.

Mathews et al[12] studied the compatibility of four phylloplane *Trichoderma* isolates used as antagonists against C.*gloeosporioides* with various fungicides at different concentrations. Among the four isolates, the isolates T_1 and T_7 were 100 per cent compatible with Mancozeb. The isolate T_7 was also compatible with Thiram but Thiram had inhibitory effect on T_1. The results of the present study are in similitude with them. The compatibility of three phylloplane antagonists with different fungicide was not similar. Out of the three antagonists two were (*Nigrosporaspharica* and *Aspergillus* spp.) more compatible with sulphur. It may be due to the fact that, sulphur plays vital role in growth and reproduction of many fungi and also acts as a

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component of sulphur containing amino acid in protein synthesis. The inhibitory effect of sulphur on *Gliocladiumroseum* may be due to the difference chitin content and chitin synthesis process of this fungus.

In case of Hexaconazole, it was found that, *Aspergillus* and *Gliocladiumroseum* were more compatible (above 73 %) while *Nigrosporaspharica* was the least compatible (25.33 %).

In respect of Carbendazim, *Aspergillus* and *Nigrospora* were compatible with it to the tune of 97.78 per cent and 80 per cent but it was harmful for *Gliocladiumroseum.*

Conclusion

In the present study, compatibility of isolated fungal antagonists with three fungicides was assessed by poisoned food technique. In this it was revealed that, the antagonist *Nigrosporasphaerica*(95.56 %) and *Aspergillus spp.* (91.11%) were most compatible with sulphur whereas*Gliocladiumroseum*, was more compatible with Hexaconazol (73.11 %).

The results of present study are quite encouraging for the eco-friendly management of the mango anthracnose but some more potential phylloplane fungal as well as bacterial antagonists may be present at different locations in the region. There is a need to isolate all such antagonists and study their interactions with each other to formulate consortium of synergistic microbes for the better management of the disease and thereby provide a pollution free technology for disease management to the farming community.

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