Isolation and *in vitro* compatibility of fungal antagonists of mango anthracnose with fungicides

Abstract:

The compatibility of antagonists of *Colletotrichumgloeosporioides*(*Nigrosporaspharica (Sacc.)*E.W.Mason, *Gliocladiumroseum*Bainier and *Aspergillussp.*)in mango, with different fungicides was testedthrough dual culture and poisoned food technique.Two systemic fungicides *viz.*, carbendazim (0.1% -1 g/L), hexaconazole (0.05 % -1/2 ml/L) and anon-systemic fungicide *viz*., Sulphur (0.2% - 2g /L) were evaluated for their compatibility with potential antagonists by poisoned food technique. The antagonist *Nigrosporasphaerica*(95.56 %) and *Aspergillussp*..(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.

Keywords:Antagonists, mango anthracnose; *Aspergillus*sp.sp.;*Colletotrichumgloeosporioides*; *Gliocladiumroseum*; *Nigrosporaspharica*

Introduction

India contributes about 64 per cent of the world mango(*Mangiferaindica*L.)production.[1]However, several infectious diseases deterioratethe quality and production of mango. Mango anthracnose disease (MAD) is a widespread and highly destructive ailment that affects mango trees both before and after harvest. It leads to substantial harm to infected mango trees, resulting in reduced fruit yield and quality. In conditions favorable for the disease's spread, such as poorly maintained orchards, it can lead to 100% loss of crop yield[2,3,4].Disease control always remains a challenge for the farmers to get optimum production especially due to pesticide resistance.[5]

Kerling (1964) used the term 'phylloplane' while referring to the actual leaf surface and 'phyllosphere' to the zone near leaves.[6]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.[7]Thephylloplanemicroflora, with its diverse microbial community, plays a vital role in antagonizing potential plant pathogens and protecting the plant's foliage from disease. In the realm of agricultural research, numerous studies have delved into the fascinating world of phylloplanemicroflora and unveiled its remarkable antagonistic abilities[8,9,10].The present study was conducted to assess the compatibility of fungal antagonists of isolated phylloplane micro-flora against mango anthracnose fungus *Colletotrichumgloeosporioides*(Penz.) Penz.andSacc.with recommended fungicides to develop a sound strategy for management of anthracnose.

Materials and methods

The present study on use of phylloplane micro-flora of mango against anthracnose were carried out in the Department of Plant Pathology, College of Agriculture, Dr. B.S.K.K.V., Dapoli.

Isolation and identification of phylloplane micro-flora:

The tender, healthy leaves of mango were collected from the mango orchard in paper bags and brought to the laboratory.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 Aneja et al,[11] for isolation of phylloplane micro-flora.After islolation of the microbes, the antagonistic ability of phylloplanemicroflora was described by Narwareet al.(2018)[12], 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).[13]

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:

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

Where,

I = Per cent compatibility.

C = Radial growth (cm) in control.

T = Radial growth (cm) in treatment.

Statistical Analysis

The data obtained in all experiments were statistically analyzed using methods Completely Randomized Design (CRD) and FCRD.

Results andDiscussion

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(26 ± 1 ^{O}C). 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(Figure1).Under microscopic observations one of the three fungal antagonists was confirmed to be*Aspergillus*sp.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 *Gliocladiumroseaum*Bainier and the fungus with creamy white mycelium was identified as *Nigrosporasphaerica* (Sacc.) E. W. Mason(Figure1).The identification of all these fungi were made byThe Chief Mycologist, Agharkar Research Institute, Pune.

NigrosporasphaericaGliocladiumroseumAspergillus sp.

Figure 1.Isolated Phylloplane Fungal Antagonists of Mango

Compatibility tests through poison food techniqueindicated that the antagonist *N.spharica* 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 1).

Table1:Compatibility of phylloplane fugal antagonists with different fungicides

Treatments	**Mean colony dia. (cm)**			**% Inhibition**		
	Nigrosporasp harica	*Gliocladiumr oseum*	*Aspergi llus sp.*	*Nigrosporasp harica*	*Gliocladiumr oseum*	*Aspergi llus sp.*
T1: Sulphur	8.60	3.72	8.20	4.44	58.67	8.89
T2: Hexaconazol	2.28	6.58	6.60	74.67	26.89	26.67
T3: Carbendazim	7.20	1.22	8.80	20.00	86.44	2.22
T4: Control	9.00	9.00	9.00	0.00	0.00	0.00
SEm ±	0.12	0.13	0.10			
CD @1%	0.49	0.53	0.41			

The antagonist *G. roseum*was most compatible with Hexaconazole (73.11%) followed by sulphur (41.33%). Carbendazim was found to be detrimental for the mycelial growth of the fungus(Table 1).

The antagonist *Aspergillus*sp.was compatible with all the three fungicides but wasmost compatible with carbendazim (97.78 %) followed by sulphur (91.11%) and Hexaconazole (73.33%)(Table 1).

Mathews et al[9] studied the compatibility of four phylloplane*Trichoderma* isolates used as antagonists against C.*gloeosporioides* with various fungicides at different concentrations. Among them, 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. In the present study, out of the three antagonists two were (*N.spharica* and *Aspergillus* sp.) 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 component of sulphur containing amino acid in protein synthesis. The inhibitory effect of sulphur on *Gliocladiumroseum* may be due to the differences in chitin content and chitin synthesis process of this fungus.One of the initial hypotheses suggested that fungi can reduce elemental sulfur to generate hydrogen sulfide (H_2S), which is harmful to cells[15,16,17].

It was found that *Aspergillus* and *G.roseum* were more compatible (above 73 %) with Hexaconazolewhile *N.spharica* was the least compatible (25.33 %). Carbendazim was compatible with *Aspergillus* and *Nigrospora*but harmfulto *G.roseum.* 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 same 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.

Conclusion

In the present study, compatibility of fungal antagonists of mango anthracnose isolated from phylloplanewith three fungicides was assessed by poisoned food technique. In this it was revealed that, the antagonist *Nigrosporasphaerica*(95.56 %) and *Aspergillussp.*(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.

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