INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

ISOLATION AND BIOCONTROL POTENTIAL OF PHYLLOPLANE *MICROFLORA* AGAINST *COLLETOTRICUM* IN MANGO

[a]Jeetu Narware, [b]Borkar, P.G. & [b]Joshi, M.
[a]Department of Mycology and Plant pathology, Institute of Agricultural science, Banaras Hindu University
[b]Department of plant pathology, College of Agriculture, DBSKKV, Dapoli

ABSTRACT
Three phylloplane microflora antagonist isolates were collected from mango phylloplane from different orchards of DBSKKV Dapoli. Their cultural morphology and antagonistic potential against Antracnose pathogen *Colletotricum gleosporiodies* were studied and results were documented. The growth rate of antagonist was quite fast and their cultural morphology was alike on potato dextrose agar medium. This study revealed that, the antagonists were highly effective to control all isolates of *Colletotricum* based on results obtained.

KEY WORDS: Colletotricum, antagonist, biocontrol, plylloplane microflora.

INTRODUCTION

Mango *(Mangifera indica* L.) is known as "king of fruits" grown throughout the tropics and subtropics worldwide. India is the world's largest producer of mango. It is commercially grown in more than 111 countries but nowhere it is as greatly valued as in India where 40 % of area under fruit crops is only under mango. Diseases not only reduce yield but also greatly impair the quality and stability of production which consequently affect the agricultural sustainability. Mango is affected by a number of diseases. Among these, diseases caused by fungal pathogens are responsible for major crop losses. One of the important fungal diseases which affect mango is anthracnose of mango. Anthracnose symptoms occur on leaves, twigs, petioles, panicles and fruits. Under field conditions, the initial appearance of the disease is manifested in the form of minute, sunken brown lesions on the tender foliage which enlarge into conspicuous brown spots within a week or so. Though, The disease can effectively controlled by an array of chemical fungicides available in the market, the long term effects of the residual toxicity of these chemicals is a matter great concern. With these view in mind, it was thought to take up initial work on phylloplane micro-flora of mango which may in turn, be the most tactical way for eco-friendly management of the disease in future. 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.

MATERIALS & METHODS

The various experiments in 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. BSKKV Dapoli, during 2016 -2017 and the leaf samples were collected from mango orchards of college premises. Potato Dextrose Agar (PDA) medium was used for isolation of the causal organism from infected leaf samples

Visual observation

Visual observations on symptoms were recorded in field to know the development of the disease in a plant population under natural condition.

Microscopic examination

Fresh disease sample showing typical symptoms of anthracnose were collected and brought to the laboratory. These samples were then washed under tap water to remove extraneous material. Temporary mounts prepared from the diseased specimens in lacto phenol cotton blue and examined under microscope.

Isolation of causal organism

Collected sample were washed with running tap water to remove extraneous material. Small bits of desired size were cut by taking care that each bit contained half infected and half healthy portion. Such bits were then disinfected with 1 per cent sodium hypochlorite solution for 30 seconds followed by three washings in distilled sterile water to remove the traces of sodium hypochlorite. These bits were then placed on sterilized blotters for drying. Properly dried bits were transferred aseptically in sterilized Petri plates containing sterilized, solidified PDA medium. The plates were incubated in BOD incubator at 26 ±1°C till the fungal mycelium fully covered the surface of the medium. The bits of fully developed fungal growth were cut with sterilized cork borer and transferred to PDA slants and maintained as stock culture for further studies. The culture was maintained by periodical transfer.

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. PDA was prepared by standard procedure and poured in sterilized Petri plates in laminar air flow chamber. Each leaf sample was pressed from dorsal as well as ventral surface separately against the sterilized semi liquid agar medium. The procedure was repeated twice to confirm whether specific phylloplane micro-flora

is constantly associated with mango leaves. The plates were incubated at 26 $\pm 1^0$ C for seven days. After seven days, individual isolates with profuse mycelial growth were selected and were transferred to PDA in sterilized Petri plates. Pure cultures were maintained by periodic transfer PDA slants. Spores are the unique feature of each fungus which confirms their morphological identification. Some fungi sporulate easily in culture while others require specific treatment. Light is essential for sporulation of most of the fungi but some fungi sporulate better in diffused light while others sporulate in alternate light and dark conditions. In order to confirm sporulation of the isolated fugal antagonists, the culture plates were exposed to continuous light, continuous darkness and alternate light and dark conditions for a week period. The cultures were observed under microscope for confirmation after 7 days.

Identification

The pure cultures of the phylloplane micro-flora obtained on isolation were observed under microscope by preparing temporary mounts. The cultures were tentatively identified by comparing morphological and colony characters with the information available in the reviewed literature as well as on the standard websites for fungal identification. Two cultures of the isolated unknown fungi were sent to the Chief Mycologist, Agharkar Research Institute, Pune, for confirmation up to species level.

Testing the antagonistic ability of potential phylloplane antagonists against *C. gloeosporioides* by dual culture technique

The antagonistic ability of the pure isolates against *C. gloeosporioides* was determined by dual culture technique under *in vitro* conditions. Mycelial discs of 6 mm diameter of seven day old cultures of the isolated fungal antagonists and *C. gloeosporioides* were placed equidistantly from the centre of Petri plates containing PDA medium. The Petri plates were then incubated at 26 $\pm 1^0$ C. Three replications were maintained in each treatment. The plates with mycelial bits of the pathogen alone served as control. The observations on growth of both the organisms were recorded on 7th day after inoculation. Inhibition percentage of mycelial growth of the pathogen was calculated by the formula:

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

Where,
I = Per cent inhibition
C = Radial growth (mm) in control
T = Radial growth (mm) in treatment.

In order to record the antagonistic effect in dual culture, the total colony area of isolated phylloplane micro-flora as well as the pathogen (*C. gloeosporioides)* was plotted on graph paper to measure per cent inhibition by phylloplane micro-flora. On confirming the antagonistic activity of phylloplane micro-flora against the *C. gloeosporioides*, the cultures were identified by comparing morphological and colony characters with the information available in the reviewed literature as well as on the standard websites for fungal identification .Two cultures of the isolated unknown fungi were sent to the Chief Mycologist, Agharkar Research Institute, Pune, for confirmation up to species level while the culture of *Aspergillus* was identified up to genus level.

Evaluation of isolated phylloplane micro-flora against *C. gloeosporioides in vivo*

Evaluation of the antagonistic ability of phylloplane micro- flora under field conditions is possible by spraying the suspension of the organism on host surface. In case of fungal antagonists, it is necessary to spray the mycelial suspension containing spores as the spores have inherent ability to adhere, establish and germinate on the host. In order to get a suspension, the cultures of all the antagonists were transferred to potato dextrose broth in flasks and incubated at ambient temperature up to 7 days. When a thick mycelial mat of the antagonists was formed on broth medium, it was strained through muslin cloth to harvest the mycelium. The harvested mycelial mat along with spores was ground separately in a kitchen grinder by adding required quantity of distilled sterile water in order to avoid chocking of spray nozzle. The mycelial suspension thus obtained was used for spraying. Disease free, healthy, 25 days old mango seedlings with coppery red foliage were used for spraying mycelial suspension. The leaves of the seedlings were washed with distilled sterile water to ensure the elimination of any other phylloplane organism as well as dust particles if any. When the foliage of the seedlings was air dried, the suspension of each antagonist was sprayed separately on the seedlings. Five seedlings were maintained for suspension of each antagonist. The seedlings were then transferred to a muslin cloth moist chamber to provide required humidity. The antagonists were allowed to establish on the foliage for a period of 7 days. Required humidity was maintained by frequently moistening the muslin cloth with water. The spore suspension of *C. gloeosporioides* was sprayed with an atomizer on each treated seedling. Total leaf area was calculated by leaf area meter and per cent disease incidence was recorded by plotting infected area on graph paper.

RESULTS & DISCUSSION

The leaf samples collected from the naturally maintained, unsprayed mango orchard revealed the typical symptoms of anthracnose of mango leaves. The microscopic examination of infected tissues revealed the presence of rod shaped spores with two oil globules. The asexual spore fruit of *C. gloeosporioides* -acervulus was also observed under microscope. The pathogen *C. gloeosporioides* grew profusely on PDA. Soytong *et al.* (2005), Evueh and Ogbebor (2008), and Kuberan *et al.* (2012), also reported that PDA is the best medium both for mycelial growth as well as spore formation of *Colletotrichum gloeosporioides.* The colony was white initially which gradually turned grey in colour. The mycelium completely covered the surface of the medium within 7 days. The bits of fully grown mycelium were cut with a sterile cork borer and transferred to PDA slants to use as stock culture for further studies. Repeated isolations of phylloplane micro-flora of mango 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. The colony of one of the isolated fungus was pink in colour. The growth of this fungus on PDA was very slow at ambient temperature.

TABLE 1. Testing the antagonistic ability of isolated phylloplane fungi against *C. gloeosporiodes* by dual culture

Treatments	Mean colony diameter of the antagonist	Mean colony diameter of *C. gloeosporioides*	% inhibition
T_1-*Gliocladium*	6.56	2.44	27.11
T_2-*Nigrospora*	5.54	3.46	38.44
T_3-*Aspergillus*	6.48	2.52	72.00
T_4- control	9.00	9.00	-
SE (M)±	0.05		
CD @1%	0.19		

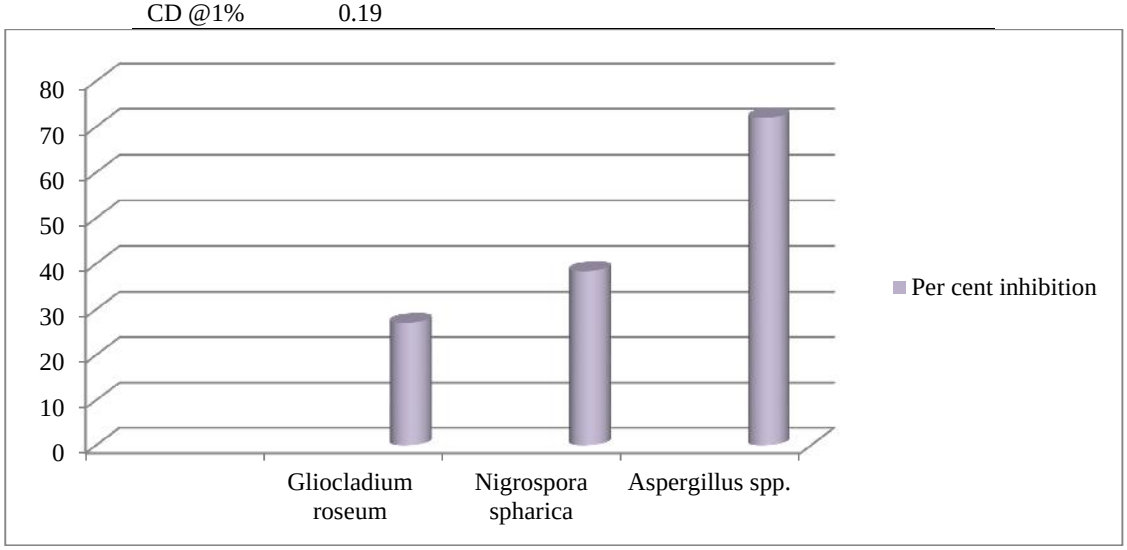

FIGURE 1 (a) Efficacy of antagonistic fungi against C. *gloeosporioides* in dual culture

TABLE 2 . Evaluation of isolated phylloplane antagonists against *C. gloeosporioides in vivo*

Treatments	Total Mean healthy leaf area of the seedling (Cm)	Total mean diseased leaf area of the seedling (cm)	Per cent diseased area (cm)
T_1 : *Nigrospora*	96.40	14.00	14.52
T_2 : *Gliocladium*	107.04	21.5	20.08
T_3 : *Aspergillus*	115.33	25.6	22.19
T_4: Control	86.98	36.91	42.43

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. 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 (2003), for isolation of phylloplane micro-flora. The experiment on antagonistic effect of phylloplane fungi revealed that all the three isolated fungal antagonists inhibited the growth of *C. gloeosporioides* at varying degree in dual culture. Among the three phylloplane fungal antagonists, *Aspergillus* was the best with highest per cent of inhibition followed by *Nigrospora sphaerica* (Sacc.) E.W. Mason and *Gliocladium roseum.* It may either due to competition for space, due to secretion of volatile chemical which is toxic to the pathogen or due to hyper parasitism. Similar results were also reported by Mathews *et al.* (2010) who isolated *Trichoderma* from phylloplane of mango in Andhra Pradesh and it was found to be a potential antagonist against the anthracnose pathogen *in vitro*. Kuberan *et al* (2012) also reported similar results. Antagonistic ability of *Gliocladium* spp and *Aspergillus spp.* against *C. gloeosporioides* was reported by Evueh *et al.* (2008).They concluded that the antagonists coagulate the cytoplasmic contents of the pathogen which consequently results in lysis of apical tip of the mycelium of the pathogen and leads to its death. In the present study also *Gliocladium roseaum* and *Aspergillus* species were isolated from mango phylloplane and were effective as antagonists against C. *gloeosporioides*. Hence the results are in concurrence with those of Evueh *et al.* (2008).

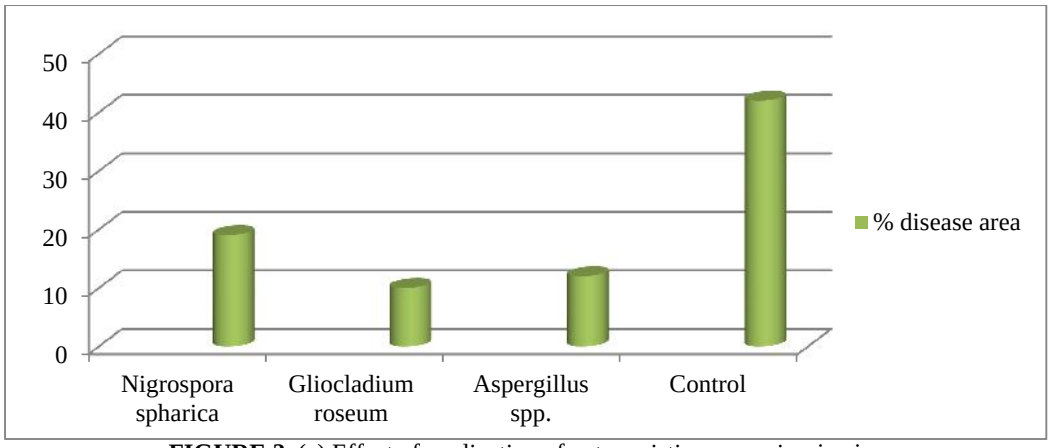

FIGURE 2. (a) Effect of application of antagonistic suspension *in vivo*

It is revealed from the data presented in Table 2 that *Nigrospora sphaerica* was the best phylloplane antagonist as application of its suspension on the foliage resulted in results of the experiment on application of mycelial suspension of the antagonists in the field revealed that, all the three antagonists were effective against the anthracnose pathogen. Among the three antagonists, *Nigrospora* was the most effective with 85.48 per cent control (14.52% disease incidence) followed by Gliocladium (79.92 % control – 20.08% disease incidence) and Aspergillus (77.81 % and 22.19 % disease incidence in *in vivo* condition.

CONCLUSION

The pathogenic fungus was isolated on potato dextrose agar medium from anthracnose infected mango leaf. The pathogen was identified as *C. gloeosporioides* on the basis of microscopic observations, cultural characteristics and symptoms observed in field. Isolation of phylloplane antagonists fungi was done by leaf impression method on semi liquid potato dextrose agar medium. In the in vitro experiment of testing the antagonistic ability of isolated phylloplane fungi against *C. gloeosporioides* by dual culture it was revealed that, all the three fungal antagonists were effective against the pathogen but maximum per cent inhibition (72%) was recorded in *Aspergillus* .

In the evaluation of isolated phylloplane antagonists against *C. gloeosporioides in vivo* condition, it was revealed that *Nigrospora sphaerica* was the best phylloplane antagonist.

REFERENCES

Aneja, K.R. (2003) Experiments in microbiology, plant pathology and biotechnology (4th edition), New Age International (p) Limited New Delhi: 35-56

Mathews, A.A., Basha, S.T. and Eswara Reddy, N.P. (2010) Mode of action of native potential antagonist, *Trichoderma fasciculatum* against *Colletotrichum gloeosporioides* causing mango anthracnose, *International Journal of Plant Protection*, **3** (1) : 34-39

Evueh, G.A. and Ogbebor, N.O. (2008) Use of phylloplane fungi as biocontrol agent against *Colletotrichum* leaf disease of rubber (*Hevea brasiliensis* Muell. Arg.) , *African Journal of Biotechnology*, 7 **(15)** : 2569-2572

Kuberan , T., Sharma, R.V., Angusamy B., Nepolean, P., Jayanthi, R. & Robert, P.K. (2012) Isolation and biocontrol potential of *Trichoderma* against *Glomerella cingulata* in tea, *Journal of Phylloplane Agricultural Technology* , ***8***(3): 1039-1050.

Soytong, K., Srinon, W., Rattanacherdchai, K., Kanok medhakul, S. and Kanokmedhakul, K. (2005) Application of antagonistic fungi to control anthracnose disease of grape. *Journal of Agricultural Biotechnology*, 1: 33-41.