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Mango (*Mangifera indica* L.) crop suffers from many diseases, among them anthracnose caused by *Colletotrichum gloeosporioides (*Penz.) Penz. and Sacc. is a major threat to mango production. Cultural characters of *C. gloeosporioides* were studied by growing the organism on seven different solid media, seven liquid broth and nitrogen sources. Observations on various cultural characters and sporulation and dry mycelial weight of *C. gloeosporioides* were taken after ten days of incubation. Among the seven solid media evaluated, maximum radial growth of *C. gloeosporioides* was observed on Richards's agar medium with colony diameter of (78.8 mm) followed by Malt extract agar (66.0 mm), Dextrose Asparagine agar (54.0 mm) and Sabourauds agar (37.8 mm). The colony growth and sporulation of the fungus was significantly superior and faster on Richards's agar. However, poor growth with colony diameter of 5.2 mm was observed in in Potato dextrose agar. Though the growth of *C.gloeosporioides*on Sabourud's (37.8mm) and Czapek's (15.8mm) solid media was significantly inferior to Richards's solid media, the dry mycelial weight in Richards's broth (151.6 mg), Sabourud's broth (141 mg) and Czapek's broth (123.2 mg) were on par among themselves. The least dry mycelial weight was recorded in Coons broth (13.4 mg). Maximum and significantly superior growth and sporulation of *C. gloeosporioides* was observed in Ammonium nitrate while the growth in Potassium nitrate (470 mg), L. proline (456 mg) and Aspartic acid (418 mg) were found to be on par with control (354 mg).

Key words: *Colletotrichum gloeosporioides*, mango anthracnose, media, nitrogen source

INTRODUCTION

Mango (*Mangifera indica* L.) is an important fruit crop of India and other tropical and subtropical countries of the world. Mango is affected by many biotic and abiotic stresses which affect its productivity greatly at all the stages of its development right from plant in nursery to the fruit in storage or transit. Mango is prone to fungal diseases like anthracnose, *Rhizopus* rot, stem end rot, *Penicillum* rot, black mould rot, *Mucor* rot, *Pestalotia* rot and powdery mildew, leading to heavy losses in yield (Ploetz, 2002).

Among these diseases, anthracnose is the major disease of mango as it occurs on all the growing parts including leaves, twigs, flowers, fruits except root and trunk throughout the year.

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Anthracnose caused by the fungus *Colletotrichum gloeosporioides* is also known by the name of its prefect stage as *Glomerella cingulata* is the most important disease of mango worldwide (Akem, 2006). Anthracnose is the major pre and post-harvest disease. The incidence of this disease can reach almost 100 per cent in fruit produced under wet or very humid conditions. Round, brown to black lesions with an illegible border on the fruit surface are the hallmark of postharvest anthracnose. Infection in larger fruit does not often develop into lesions. The fungus remains latent or dormant after initial colonization in the fruit until the fruit starts to mature. Dark depressed circular lesions initially grow on the ripening fruit, later quickly enlarge and in real severe situations, they may even completely cover the surface of the fruit. Lesions larger than 2 cm are rather typical on fruit that has been badly diseased. Lesions of various sizes can combine to cover large sections of the fruit, usually in the form of tear stains, as they

progress from the fruit's base to its distal end (Arauz, 2000). Majority of the time, lesions are limited to the peel, but in extreme situations, the fungus can even infiltrate into the fruit pulp. The fungus generates acervuli and profuse orange to salmon-pink masses of conidia on the lesions during advanced stages of infection.

This disease is one of the contributing factors for the low productivity as well as huge economic losses among the farmers. Therefore, the studies of cultural, and morphological characters of the pathogen are of immense use in understanding the nature and adaptation of the pathogen in different environmental and nutritional conditions. By this way these studies will also help in understanding of pathogenicity of the pathogen as well as adoption of better management practices.

MATERIALS AND METHODS

Collection of Diseased Samples

The diseased samples of mango fruit showing typical symptoms were collected from mango orchard of Department of Horticulture, College of Agriculture, University of Agricultural Sciences, GKVK.

Isolation, purification and identification of the Pathogen

The pathogen causing anthracnose disease in mango was isolated from diseased fruit samples. The infected tissue bits were separated with a sterile blade and surface sterilized with one per cent sodium hypochlorite solution for one min. and subsequently washed three times with sterile distilled water. Then they were transferred into a sterile Petri dish containing Potato Dextrose Agar (PDA) medium (Ainsworth, 1971) amended with streptomycin under laminar air flow. The plates were then incubated at room temperature (28 ± 2°C). The emerging colonies were sub cultured on to PDA slants. Single hyphal tip method was followed for making pure culture and maintained on PDA slants (Aneja, 2003). The identity of isolate was confirmed by microscopic observations based on morphological characteristics as per the key suggested by Barnett *et al.* (1972).

Cultural characteristics of *C. gloeosporioides*

Growth of *C. gloeosporioides* on different solid media

The cultural characters of *C. gloeosporioides* were studied on seven different media viz., Malt extract agar, Dextrose asparagine agar, Sabourauds agar, Czapecks (dox) agar, Coons agar, Potato dextrose agar and Richards's agar. The various solid media were prepared as per the procedure given by Mc Clean and Clock (1965) and Ainsworth (1971). The media were sterilized at 121°C for 20 min. Twenty-five ml. of each of the sterilized medium was poured into the Petriplates under aseptic conditions. The treatments were replicated five times. Inoculations were made with identical culture discs (5 mm) from seven-day old culture. The inoculated Petriplates were incubated at room temperature (27±1°C). Observations on the growth of the fungus, colony color, topography, margin of the colony and extent of sporulation was recorded in each replication. The data thus collected was compiled and statistically analyzed.

Growth of *C. gloeosporioides* on different liquid media

Seven different liquid broths were used to find out the most efficient media for growth and sporulation of *C. gloeosporioides.* Details of preparation and composition of various media used are the same as those of solid media except that agar is not added. Twenty-five ml of each of liquid media was poured separately to each 100ml flasks. Later these flasks were sterilized at 121°C for 20 min. Treatments were replicated five times. Fungal mycelial discs of 5 mm diameter from seven days old cultures were inoculated to each of the flasks under aseptic conditions. These flasks were plugged with non-absorbent cotton close to burner flame and incubated at room temperature (27±1°C) for ten days. At the end of the experiment, cultures were filtered through pre weighed Whatman No.42 filter paper disc of nine centimeter diameter, which were dried at a constant temperature of 60°c for 24h prior to filtration. Mycelial mat on the filter paper was washed thoroughly with distilled water to remove any salts likely to be associated with it. Filter paper along with the mycelial mat was dried at a constant temperature in an electric oven at 60°C, cooled to room temperature and weighed on electric balance. Growth in each treatment was calculated using the formula:

Dry mycelial weight (mg)=Total weight of filter paper along with mycelial mat - Initial weight of filter paper

Utilization of nitrogen source by C. gloeosporioides

The experiment was conducted to study how the fungus responds to different forms of nitrogen for growth and sporulation. Ammonium nitrate, aspartic acid, potassium nitrate and L-proline were used as different nitrogen sources and incorporated into Richard's liquid medium at 1385.5 grams of nitrogen per litre of the medium. In control, no nitrogen source was added. Sucrose was added as source of carbon in all the treatments. Twenty-five ml of each of the broth was added to 100ml flask, plugged with non-absorbent cotton and autoclaved at 121°C at 15 psi for 20 min. Each treatment was replicated 5 times. All the flasks were aseptically inoculated with 5mm fungal mycelial discs from 7days old cultures and incubated at room temperature (27±1°) for 10days. Mycelial mat was harvested and observation on growth in each treatment was recorded and subjected to statistical analysis. Growth in each treatment was calculated using the formula.

Dry mycelial weight (mg)=Total weight of filter paper along with mycelial mat -Initial weight of filter paper

RESULTS AND DISCUSSION

Collection of diseased samples

Diseased mango fruits showing typical anthracnose symptoms (Fig. 1) were collected from mango orchard of Department of Horticulture, University of Agricultural Sciences, GKVK. Symptoms initially started as small, round and depressed spots. Later on, these spots coalesced to cover large area and looked like rotten patches. Under humid conditions, pinkish spore masses were noticed which are acervuli of the fungus. Similar symptoms were reported by Shirshikar (2002).

Isolation ,purification and identification of the pathogen

The pathogen *C. gloeosporioides* was successfully isolated on PDA culture medium from the infected mango fruits showing typical anthracnose symptoms. Isolation was carried out at the,Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Bangalore. The fungal culture of the pathogen was purified and the pure culture was maintained for carrying out the investigation. The hyphal tip of isolate fungus growing on PDAwas cut and shifted to a slant containing PDAas basal medium and kept for incubation. After incubation the slant was transferred to refrigerator and sub-cultured at regular intervals of time.

The pathogen produced colonies with abundant whitish (Fig.2), septate and hyaline aerial mycelium, slimy pinkish spore masses, acervulate (Fig.3**).**The fungal culture isolated from diseased mango fruit was identified as *C. gloeosporioides* causing anthracnose disease on the basis of microscopic observation for morphological and conidial characteristics. Conidia were oblong or oval or cylindrical, straight, hyaline, non-septate with rounded ends, thin walled having oil globules in the centre**(**Fig. 2**).**Isolation and identification of the pathogen during the present investigation coordinated with view of following reports viz., Sharma and Verma (2007), Mukherjee *et al*. (2011) who have successfully isolated the pathogen-*C. gloeosporioides* from mango fruit responsible for anthracnose disease.

Growth of C. gloeosporioides on different solid media

Studies on cultural characteristics of *C. gloeosporioides* causative agent of anthracnose of mango was carried out on seven different solid media and is presented in Table 1, Figs. 4 and 5. The present investigation revealed that the colony characters and growth of *C. gloeosporioides* varied on different media. The colony diameter of *C. gloeosporioides* on different solid media evaluated ranged from 5.2 mm in Potato dextrose agar to 78.8mm in Richards agar. The colony diameter of *C. gloeosporioides* was maximum on Richards agar (78.8 mm) which was significantly superior over all other media tested followed by Malt extract agar (66.0 mm), Dextrose aspargine agar (54.0 mm), Sabourauds agar (37.8 mm),Czapek's (dox) agar (15.8 mm), Coons agar (10.6 mm) and Potato dextrose agar(5.2 mm). These results are in conformation with the reports of Akthar (2000); Reddy (2000); Ashoka (2005).

During the study, variation in colony characters of the pathogen was observed on different solid media with respect to colony colour, type of margin, colony

Fig.1: Typical Anthracnose symptoms on mango fruits

Fig. 2: Pure culture of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. on PDA

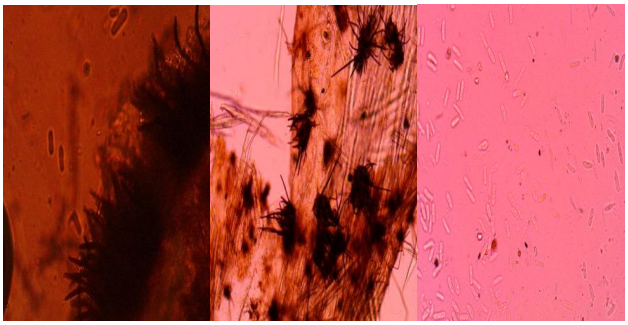

Fig.3: Mycelia, Conidiophore and Conidia (10X) of the pathogen

Fig. 4 : Growth of *Colletotrichum gloeosporioides* on different solid media

T1: Sabourauds dextrose agar, T2: Oat agar, T3: Czapecks dox agar, T4: Malt agar, T5: Glucose Asparagine medium, T6: Richards agar, T7: Potato dextrose agar

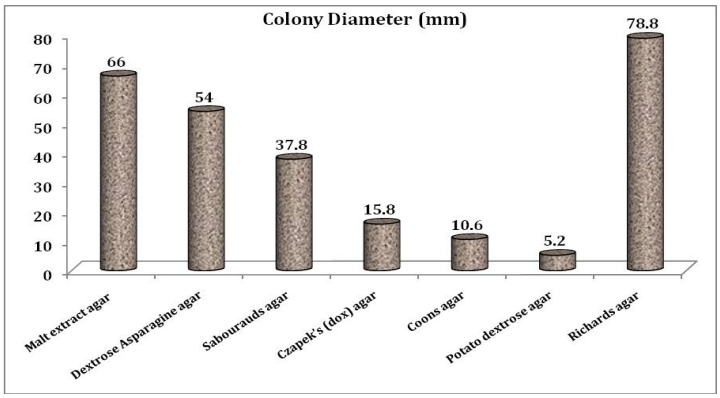

Fig.5: Effect of different solid media on growth of *C. gloeosporioides*

Table 1: Effect of different solid media on Colony characters, sporulation and growth of *C. gloeosporioides*

Solid media	Colony Diameter(mm)	Colony colour	Type of margin	Mycelial growth	Sporulation
Sabourauds dextrose agar	32.2	White	Smooth regular	Dense fluffy	+++
Oat agar	66.0	White	Smooth Regular	Dense fluffy	+++
Czapecks dox agar	44.0	White	Rough Irregular	Sparse	+++
Malt agar	22.8	White	Smooth Regular	Sparse	++
Glucose Asparagine medium	40.4	Light grey to white	Rough Irregular	Sparse	+
Richards agar	20.2	Light grey to white	Smooth Regular	Sparse	+
Potato dextrose agar	78.8	White	Rough Irregular	Dense fluffy	+++
Anova test		Significance			
S. Em ±	2.7				
CD @ 1%	8.2				

- : No sporulation, + : Poor sporulation, ++ : Moderate sporulation, +++ : Good sporulation

Table 2: Growth of *Colletotrichum gloeosporioides* on different liquid media

Liquid media	Dry mycelial weight (mg)	Sporulation
Potato broth liquid	123.2	++
Malt broth	141.0	+++
Richard's broth	151.6	+
Sabouraud's broth	133.8	-
Czapeck's dox broth	72.2	+
Coons broth	60.6	+
Dextrose asparagine broth	83.4	++
S.E.m. ±	15.69	
C.D. @1%	47.8	

Table 3: Effect of different nitrogen sources on growth and sporulation of *C.gloeosporioides*

Nitrogen sources	Dry mycelial weight (mg)	Sporulation
Aspartic acid	456	+
Potassium nitrate	382	++
Ammonium nitrate	786	++
L- proline	418	++
Control	470	++
S.Em. ±	58.4	
C.D. @1%	184.2	

+: Poor sporulation, ++: Moderate sporulation, +++: Good sporulation

Fig.6: Effect of different liquid media on the growth and sporulation of *Colletotrichum gloeosporioides*

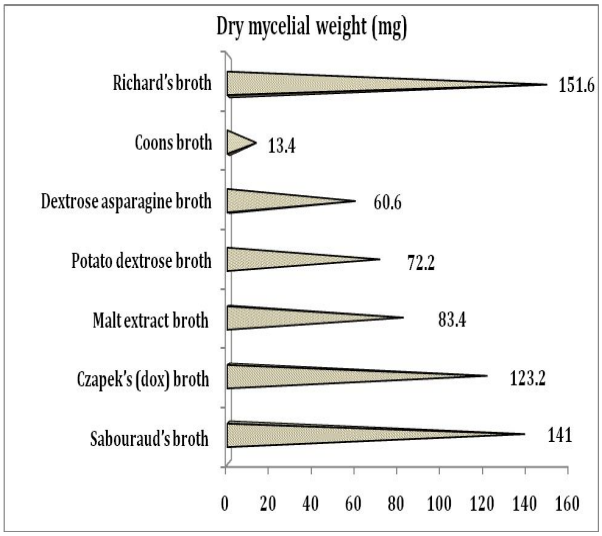

Fig.7: Growth of *C. gloeosporioides* on different liquid media

Fig. 8: Effect of different nitrogen sources on growth and sporulation of *C.gloeosporioides*
T_1: Ammonium nitrate, T_2: Aspartic acid, T_3: Potassium nitrate, T_4: L- proline, T_5: Control

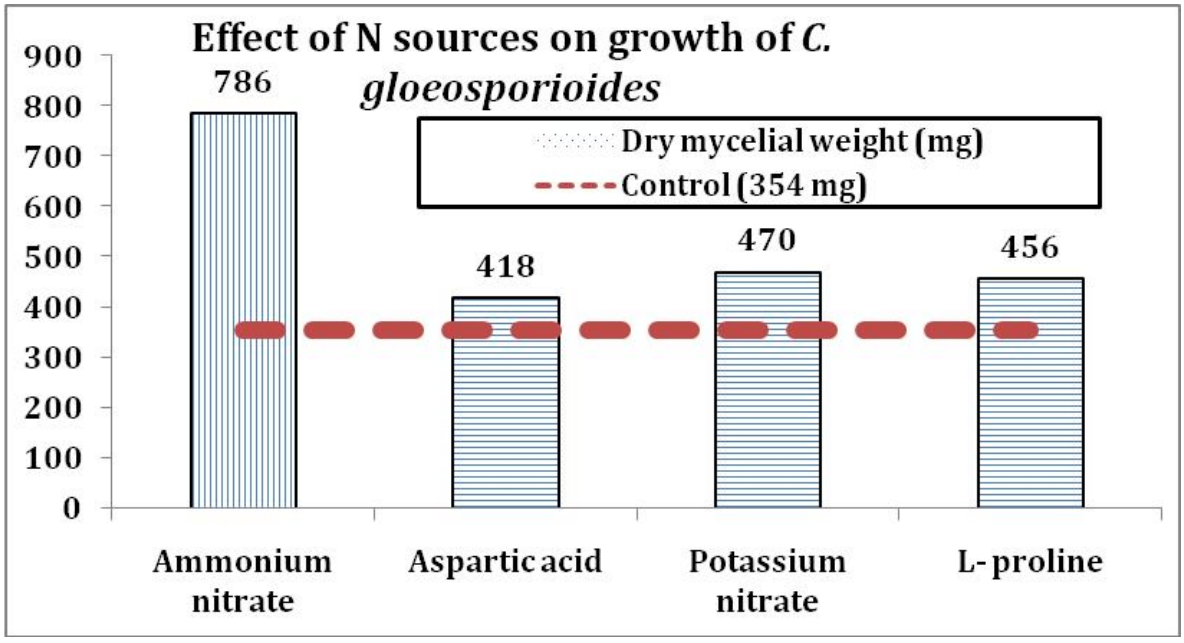

Fig. 9: Effect of nitrogen sources on growth of *C.gloeosporioides*

texture and growth nature and sporulation (Table 1). The colony colour varied from white to light gray coloured in all the media evaluated. The sporulation in Malt extract agar and Dextrose aspargine agar was poor, while, moderate sporulation was observed in Sabourauds agar and Good sporulation was observed in Czapek's (dox) agar, Coons agar, Potato dextrose agarand Richards agar.

Growthof C. gloeosporioides on different liquid media

An experiment was conducted to know the mycelial weight of *C. gloeosporioides* on seven different liquid broth. The mycelial growth was harvested after ten days as described in Material and Methods and the data thus recorded was subjected to statistical analysis is presented in Table 2, Fig. 6 and Fig. 7. The results indicated that maximum dry mycelial weight of fungus was in Richards's broth (151.6 mg), Sabourud's broth (141.0 mg) and Czapek's broth (123.2 mg) that were found to be significantly superior over other media. Dry mycelial weight recorded in Malt extract broth (83.4 mg) was on par with Potato dextrose broth (72.2 mg) and Dextrose asparagine broth (60.6 mg). The least dry mycelial weight was recorded in Coons broth (13.4mg). Sudhakar (2000) reported that in liquid media the maximum dry mycelial weight of *C. gloeosporioides*was recorded in Richards's broth (288.33 mg).

Sporulation of *C. gloeosporioides*was good on Czapeck's (dox) broth while moderate sporulation was noticed in Richard's broth, Malt extract broth and Sabourud's broth. The sporulation was poor in Potato Dextrose broth, Coons broth and Dextrose asparagine broth.

Effect of different nitrogen sources on growth and sporulation of Gloeosporioides

Nitrogen is an important component required for protein synthesis and other vital functions. Its requirements by *C. gloeosporioides*were studied using different sources and the results are presented in Table 3, Fig. 8 and Fig. 9.The study revealed that maximum growth of *C. gloeosporioides*was observed in ammonium nitrate (786 mg) over all other media. The growth of *C. gloeosporioides* in potassium nitrate (470 mg), L-proline (456 mg) and aspartic acid (418 mg) were found to be on par with control (354 mg). Reddy (2000) reported that in ammonium nitrate good sporulation and mycelial growth.Nitrates were better than ammonical compounds. The organism could not grow on nitrites of potassium or sodium and on media lacking nitrogen. The results also indicate that the sporulation in ammonium nitrate was good while potassiumnitrate, L- proline, and control recorded moderate sporulation and in aspartic acid the sporulation was poor.

CONCLUSION

Fungi usually obtain their nourishment from the substrate, on which they grow and multiply. In order to culture the fungi artificially in the laboratory, it is essential to provide all the basic nutritional elements in easily accessible form in the medium. The maximum mean colony diameter of the fungus, *C. gloeosporioides* was recorded in Potato Dextrose Agar (78.81mm) which was significantly superior over all other tested medium followed by oat agar (66.05mm). The results indicated that maximum dry mycelial weight of fungus was obtained in Richards's broth (151.6 mg) which was significantly superior to all other media which was followed by Malt dextrose broth (141.0 mg), Potato dextrose broth (123.2 mg) and Dextrose asparagine broth (83.4 mg). The growth of *C. gloeosporioides* in Richards's broth where potassium nitrate (786 mg) was used as the nitrogen source was maximum and significantly superior over all other treatments.

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