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Review article

STUDIES ON THE INCIDENT AND PATHOGENESIS OF *COLLETOTRICHUM GLOEOSPORIOIDES* PENZ. CAUSES ANTHRACNOSE OF MANGO

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ABSTRACT
Mango (*Mangifera indica* L.), an important fruit crop of subtropical countries and its production is drastically affected by *Colletotrichum gloeosporioides*, is one of the most damaging pathogen causes mango anthracnose. In this paper we review the studies on the incident and pathogenesis of fungus and its management. *C. gloeosporioides* was considered to be cylindrical with rounded ends and less than 4.5 µm in diameter. *In vitro* culture of pathogen glutamic acid and alanine supported maximum growth and sporulation. The range of temperature 20-30°C was found optimum for the growth and sporulation. From histopathological studies it revealed that mycelia were prominent after 120 hrs after invasion by the fungus (*C. gloeosporioides*). The variation of *C. gloeosporioides* was confirmed in several experiments through molecular polymorphism generated by RAPD. In order to management of pathogen through bioagents *Trichoderma* spp. has been found significant to check the radial growth of pathogen. With regard to botanicals for management of this disease, leaf extract of *Azadirachta indica* was found more effective in inhibiting the redial growth. Among the fungicides tested trichyclazole was found to be superior for controlling the incidence of pathogen while, cultivars *viz.* Edward, Mayaguazano and Elamandi were observed resistant to anthracnose.

KEY WORDS: *Anacardiaceae, Mangifera indica* L., Mango, Anthracnose, *Colletotrichum gloeosporioides*, Management.

INTRODUCTION

Mangoes (*Mangifera indica* L.) are universally considered as one of the choicest fruits in tropical and subtropical areas of the world (Shad *et al.,* 2002) are member of the family *Anacardiaceae* and also known as the cashew nut family (Nakasone and Paull, 1998). There are at least 62 species in the genus of which 15 bear edible fruit (Litz, 1994). It is grown in at least 87 countries but no where it is so greatly value as in India where 40 per cent of total fruits grown in India is only mango. Mango is affected by a number of diseases at all stages of the development right from nursery to post harvest including storage and transit. Hardly any plant organ is immune, almost every part *viz*., stems, branch twig, root, petiole, flower and fruit are affected by various pathogens. Mango anthracnose disease is manifested with symptoms *viz.,* rot, die back, mildew, necrosis scab, blotch, stem bleeding, wilt, spots, canker, sooty mould, malformation and leaf spot diseases.

The post harvest disease is the most damaging as to causes economical loss to a tune of 15-20 per cent (Ploetz and Prakash, 1997). It directly affects the marketable fruit rendering it worthless. This phase is directly linked to the field phase where initial infections usually starts on young twigs, leaves and later spreads to the flowers causing blossom blight, destroys the inflorescences and finally prevent fruit set.

Depending on the prevailing weather conditions blossom blight may vary in severity from slight to a heavy infection of the panicles. Black spots develop on panicles as well as on fruits. Severe infection destroys the entire inflorescence resulting in no setting of fruits. Young infected fruits develop black spots, shrivel and drop off. Fruits infected at mature stage carry the fungus into storage and cause considerable loss during storage, transit and marketing. The fungus perpetuates on twigs and leaves of mango or other hosts. Anthracnose caused by *C. gloeosporioides* is the most important and widespread form of decay affecting mature avocado and mango fruit worldwide (Jeffries *et al.,* 1990; Prusky and Keen, 1993). Varietals differences in susceptibility have been noted in India. In Kerala, maximum damage was observed on Neelum, whereas variety Edward was reported to be resistant.

Distribution of the mango anthracnose

Worldwide mango anthracnose is recognized as the most important field and post-harvest disease (Ploetz and Prakash, 1997). It was first reported from Puerto Rico (Collins, 1903) and later from Hawaii (Higgins, 1906), Florida (Fawcett, 1907), Cuba (Cardin, 1910), Philippines (Wester, 1911), Columbia (Taro, 1929), South Africa (Doidge, 1932), Brazil (Bitancounrt, 1938), United States (Traub and Robinson, 1938) and Pakistan (Sattar and Mallik, 1939). In India, this disease was reported by Stevens and Pierce (1933). It is a widely distributed in the entire mango growing states of the India causing huge economic loss.

Symptoms of anthracnose

The characteristic symptoms are numerous oval or irregular vinaceous brown or deep brownish spots of various sizes scattered all over the leaf surface under damp conditions. The fungus grows rapidly forming elongated

mass brown or mummy brown necrotic areas measuring 20-25 mm in diameter which when old become ruptured and blighted. They do not become much larger as the leaf grow but often become dry and fall out giving the older leaves a 'shot hole' appearance (Fig. 1).

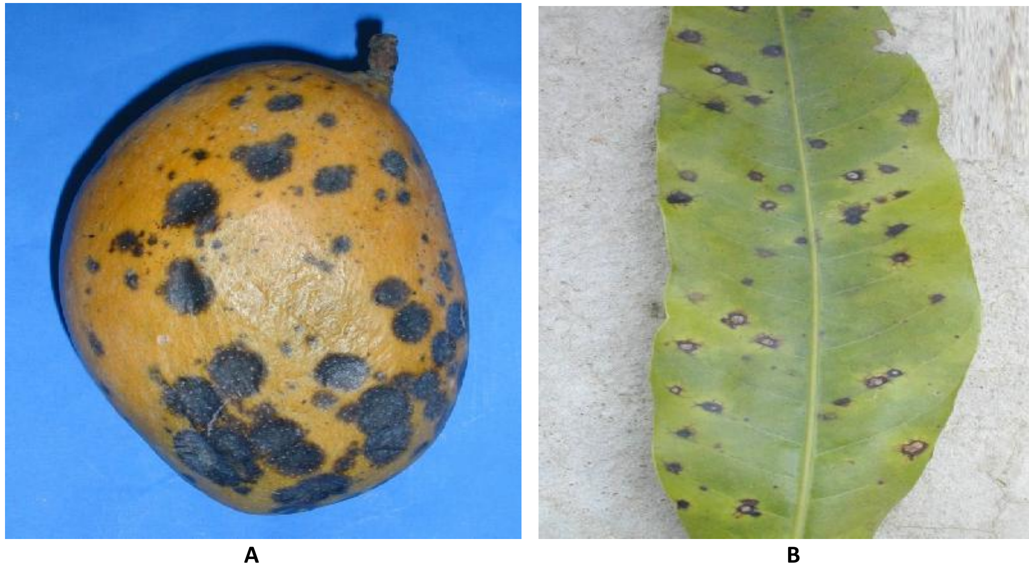

FIGURE 1. Symptom of anthracnose on mango fruit (A) and on mango leaf (B).

The petiole when affected turn grey or black and the leaves droop down, become dry and ultimately fall off having the black scar of the twig. Disease produces elongated black necrotic areas on the twigs. The tip of very young branches is attacked first and then twigs go on drying from the tip downwards. Under humid conditions all the branches as well as the main stem of young thin branches are also similarly affected on older plants but the big branches are not attacked (Sattar and Malik, 1939).

C. gloeosporioides infects mango and results in blossom blight (Jeffries *et al.,* 1990). The blossom blight and accompanying peduncle blight are the most destructive phase of this disease as it prevents the fruit from setting and this reduces the production. On the inflorescence an earliest recognizable symptom of the disease is the production of blackish brown specks on the peduncle and flowers. Small black spots appear in the open flower panicle which gradually enlarges and often coalesce to cause death of flower either directly or indirectly by invasion (drying up) of flower stalks. The loss may be small but under favorable conditions the whole flower stalk may become blackened blighted and set in fruit. The infected flowers fall off having the more persistent spikes on the peduncles. The severity of the disease varies according to prevailing weather condition (Prakash and Raoof, 1985).

Symptomology of *C. gloeosporioides* infection varies very little between different hosts and is characterized by dark, depressed lesions on ripe fruit often accompanied by pink, slimy spore masses which develop as acervuli mature (Jeffries *et al.,* 1990). Infections on stems, leaves and young inflorescences are manifested as sub circular or angular black lesions which enlarge and coalesce, frequently destroying leaf edges or entire inflorescences (Jeffries *et al.,* 1990).

Lesions often coalesce to form large necrotic areas frequently along the leaf margins severely affected leaves usually curl. Lesions develop primarily on young tissue and conidia are formed and can be observed in lesions of all ages. In older leaves lesions do not develop but latent infections are formed and the fungus remains dormant until the tissue senesces. Under favorable conditions conidia are dispersed and invade young twigs causing twig die back in some cases (Ploetz *et al.,* 1996). Relative humidity above 95 per cent for 12 hrs is essential for infection and development of *C. gloeosporioides* on mango fruit. Infection progresses faster in wounded tissues and in ripe fruits (Prakash, 1996).

Pathogenicity

All plants inoculated with *C. gloeosporioides* isolate were infected and exhibited lesions typical of those observed in the field described by others (U. S. Dept, 1963). Lesions began as dark brown, punctate, circular to irregular spots of < 1.5 mm in diameter, often with distinctly gray centers. In many cases infections were apparently initiated at phyllode tips, after which they progressed in a basipetal direction. Stem lesions sometimes completely girdled smaller stems. Reisolation of *C. gloeosporioides* from inoculated seedlings was consistent and confirmed the pathogenic role of this organism. Non inoculated checks remained symptom less. Cross-infection potential among different species of *Colletotrichum* has been well documented (Freeman and Shabi, 1996).

In addition some new species with different pathogenicity have been reported and segregated from *C. gloeosporioides* (Shivas *et al.,* 1998). There was no difference in pathogenicity between *C. acutatum* and *C. gloeosporioides* isolates (Hong *et al.,* 2008). *C. acutatum* has been differentiated from *C. gloeosporioides* based on phenotypic traits such as mycelial growth rate characters of conidia and appressoria, colony appearance and production of setae. It was reported that *C. acutatum* grew slower than *C. gloeosporioides* (Talhinhas *et al.,* 2002). Unlike *C. gloeosporioides* with cylindrical conidial shape, *C. acutatum* has fusiform or acuminate conidia at least on

one end. Fitzell (1979) noticed that *C. acutatum* caused mango anthracnose in New South Wales as it was isolated from leaves, panicles and fruits and its pathogenicity confirmed.

Cell wall degrading enzymes (CWDEs) are considered to play a role in the pathogenesis of bacteria and fungi on their hosts. Significant evidence on the role of pectolytic enzymes, in fungal pathogenicity have been reported (Have *et al.,* 1998).

Morphology of *Colletotrichum gloeosporioides*

Von Arx (1957) reported that *C. gloeosporioides* had more than 600 synonyms and showed many morphological and physiological variations. The morphology of the fungus was described by Palo (1932) where in the spore of fungus was found to be 8.3 to 27.4 µm in length and 2.0 to 6.6 µm in width (mean 14.2 x 4.4 µm). They were irregular and appear as brown to black dots. The acervuli when mature exude pink masses of conidia under moist conditions (Sattar and Malik, 1939). The acervuli measured 115-467 x 95-22 µm (Bose *et al.,* 1973) 80-250 µm (Sattar and Malik, 1939). The conidia were borne on distinct well developed hyaline conidiophores. The conidia were straight, cylindrical or oval 8-20 x 5-7 µm hyaline usually with two rarely one oil drops (Sattar and Malik, 1939). The size of conidia varied from 11-16 x 4-6 µm (Bose *et al.,* 1973). Simmonds (1965) reported conidia 11.9-17.0 x 3.6-5.8 µm (mean 13.8 x 4.8 µm) broad oblong with rounded ends 11.1-17.7 x 3.1-5.0 µm (mean 14.0 x 3.7 µm) for *C. gloeosporioides* var. minor.

Sutton (1992) described 7 formae speciales of *C. gloeosporioides* and recognized the species as a heterogeneous group with a great variation in morphology although some species were separated from *C. gloeosporioides*. The current method used for the detection and identification of *C. gloeosporioides* of *C. oleifera* depends on isolation of pure cultures on nutrient media followed by morphological examination of the isolates (Ji and Guo, 1992).

C. gloeosporioides aggregate has been defined using morphological methods by (Baxter *et al.,,* 1983) primarily conidial characteristics they were considered to be cylindrical with rounded ends and less than 4.5 µm in diameter (Fig. 2). Such features are not considered to be reliable as *Colletotrichum* species in culture frequently produce secondary conidia that are highly variable in size and shape.

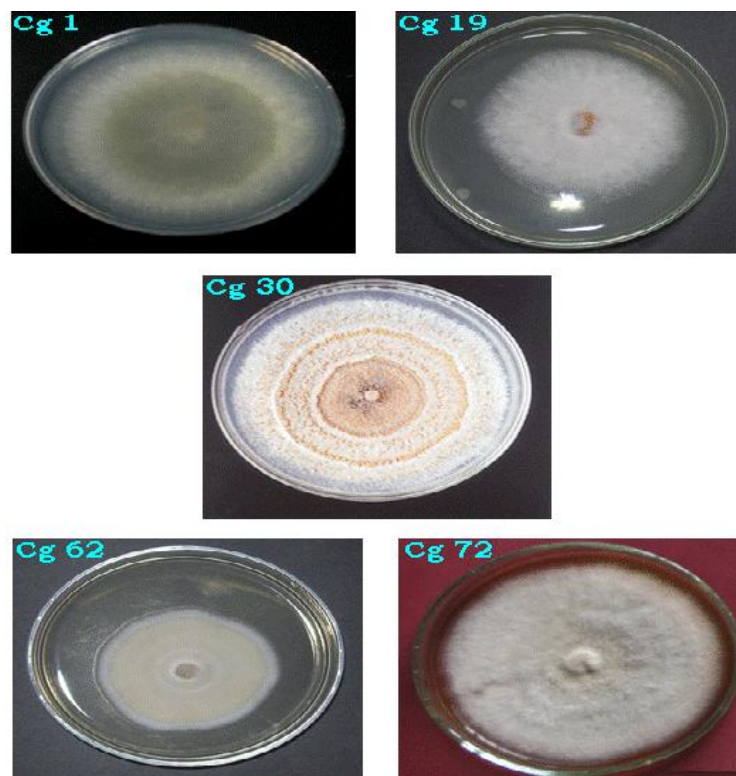

FIGURE 2. Cultural and morphological variability among the different isolates of Colletotrichum *gloeosporioides*

The majority of the conidia of *C. gloeosporioides* was oblong with obtuse ends and was generally shorter and broader than conidia of *C. fragariae* and *C. acutatum* (Gunnell and Gubler, 1992). In general, conidia of *C. acutatum* are elliptic to fusiform in shape, whereas conidia of *C. gloeosporioides* are oblong with obtuse ends (Freeman *et al.,* 1998).

It is a well-known phenomenon that the germination of *C. gloeosporioides* tends to be low *in vitro* due to the crowding effect or inhibitors (Bailey *et al.,* 1992). In a study, Weng and Chuang (1997) demonstrated that if spore concentration was above 106 spores/ml the germination percentage was reduced to 0 per cent over a 18 hr period whereas, the germination percentage reached 100 per cent when the spore concentration was adjusted to 105/ml. *Colletotrichum* (imperfect state of *Glomerella*) differs from *Gloeosporium* (conidial state of *Glomerella*) in having setae which may be absent in some cultures

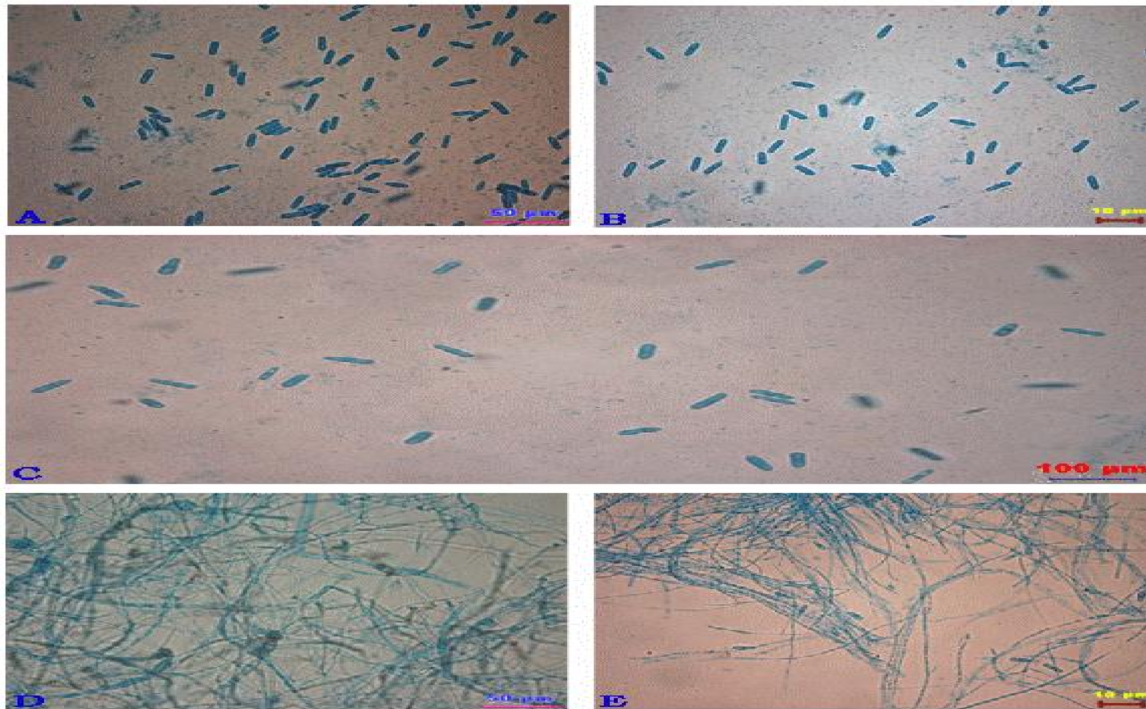

FIGURE 3. A, B and C spores of different isolates of *Colletotrichum gloeosporioides*; D mycelia; E spore with mycelia

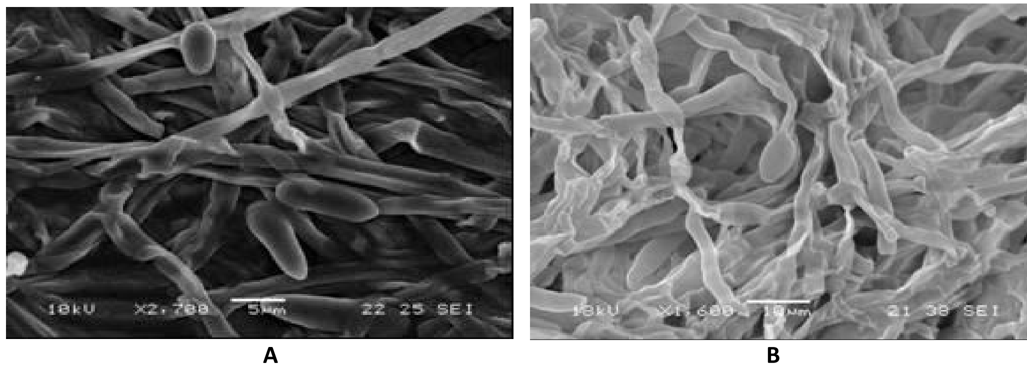

FIGURE 4. *Colletotrichum gloeosporioides* A & B – Scanning electron micrographs showing germinated conidia and a globose appresorium attaches to a germ tube (gt). (Source: Yun *et al.,* 2009)

According to Sutton (1992) pathogenic fungus *C. gloeosporioides* produced similar symptoms and the same conidia shape and size of conidia (6.0-10 × 2.0-2.5 µm) on PDA culture was slightly different compared to those previously found on white fleshed species in Okinawa Prefecture, Japan and Miami-Dade County the USA *i.e.* 9.0-24 × 3.0-4.5 µm on PDA culture (Valencia-Botin *et al.,* 2003) and 12.5- 17.5 × 3.8-7.5 µm on one-half strength acidified PDA culture (Palmateer *et al.,* 2007), respectively whereas the isolate infecting yellow species in Brazil had conidia 12.1-18.1× 3.6-8.2 µm in size on PDA culture (Takahashi *et al.,* 2008). Barhoom and Sharon (2004) showed that *C. gloeosporioides* uses two different germination strategies for pathogenic and saprophytic development.

The conidia of *C. gloeosporioides* were straight with obtuse apex, hyline, cylindrical to clavate and sometimes fusiform like *C. acutatum* (Fig. 3&4). Clavate and lobed appressoria were mainly present in matured colonies. The spore measurement of *C. gloeosporioides* varied between 3.94-12.14 µm long and 1.43-2.14 µm wide (Yun *et al.,* 2009).

In addition, some new species with different morphology and pathogenicity have been reported and segregated from *C. gloeosporioides* (Shivas *et al.,* 1998). However, these species are usually considered as synonym of *C. gloeosporioides* because the species was not clearly differentiated from *C. gloeosporioides* by morphological characteristics except by host range (Singh and Sharma, 1982).

In vitro* culture of *Colletotrichum gloeosporioides

The effects of inoculum density, medium composition, concentration and temperature on spore-carrying capacity (SCC) and microcycle conidiation by *C. gloeosporioides* were studied on solid media by several workers. For this fungus spore production on solid media was analogous in liquid media (Slade *et al.,* 1987). *C. gloeosporioides* grew well on PDA and CWA as the coconut watery endosperm

contains suitable amounts of lipids (1.26%), proteins (2.1%), carbohydrates and minerals (Santoso *et al.,* 1996).

The fungus produces aerial mycelium in Richard's and Brown's agar and profusely sporulates on oat meal and corn meal agar along with abundant development of acervuli in rings and few setae. The optimum growth was at pH range of 5.8 to 6.5 at 25°C but greater ceased beyond 35°C on starch and peptone containing medium. Glutamic acid and alanine supported maximum growth and sporulation. *C. gloeosporioides* grew at 15-35°C (opt. 25-30°C) and at 10°C the growth was completely inhibited. Light was unnecessary although it enhanced sporulation. Growth and sporulation occurred over a wide pH range (opt. pH 6) and germination was better on a more acidic medium. Growth was maximum on Czapek's and yeast extract agar medium.

Initial trials to recover the pathogen from artificially inoculated soil using potato dextrose agar amended with streptomycin sulphate did not give any positive result because the pathogen was often overgrown by fast growing micro organisms such as *Rhizoctonia* spp., *Rhizopus* spp., *Penicillium* spp. and bacteria which interfered with detection of the pathogen. It was therefore considered that a selective or semi selective medium would be helpful in the isolation of *C. gloeosporioides* from soil. In semi solid media it recorded that malt extract agar (MEA) medium was best suited for growth in terms of radial mycelial diameter for all the isolates (Pandey, 2011)

Role of temperature and relative humidity on growth and development of the pathogen

It was identified that *C. gloeosporioides* required free water or relative humidity above 95 per cent for conidial germination and aspersorium formation. Conidia however survived for up to 2 weeks at humidity as low as 62 per cent and germinated at high humidity. Optimum production of conidia was observed between 25 and 30°C when free moisture was available. In general, infection is favored at temperatures ranging from 20 to 30°C. Within this range there is considerable variation in the optional temperature requirements for germination and aspersorium formation.

Temperature and moisture requirements for infection have also been used to build forecasting systems for mango anthracnose a vital component for the disease management. Several workers have reported range of 20-30°C as the optimum temperature for the growth and sporulation (Davis *et al.,* 1987) of *C. gloeosporioides* on mango but not similar with those who noted range of RH from 95-100 per cent as the *in vitro* optimum RH for conidial production and germination as well as appressoria development of *C. gloeosporioides* on mango (Akem, 2006; Dodd *et al.,* 1991). In our study isolates, Cg 72 (from Maharashtra) showed more virulence and maximum sporulation (137.5×10^3 ml^{-1}) at 28°C and media pH 6. Maximum growth and virulence at 28°C was observed with Cg 62 isolate. However, media of pH 6 was found to be most suitable for the growth of respective isolates (s), but Cg 62 which was collected from Bihar found most virulent in this experiment (Pandey, 2011).

Epidemiology and host range

Simmonds (1965) determined the host range by artificial inoculation of seven species of *Colletotrichum.* Quimio and Quimio (1975) tested the pathogenicity of mango anthracnose (*C. gloeosporioides*) isolated from mango, citrus and papaya which proved to be cross pathogenic with varying degrees of crop specificity. *C. musae* only could infect banana, *Gloeosporium pasidii* could infect mango and banana as well as guava. Different strategies were used to study the epidemiology of anthracnose caused by *C. gloeosporioides* (*Glomerella cingulata*) in mango.

Generally, genetic and geographical data suggests that the mango population of *C. gloeosporioides* was disseminated throughout the world from a single source as an entophyte. An increased understanding of the origins and diversity of *C. gloeosporioides* on mango would have relevance to future research on host and chemical control strategies across regions and locations. Detached mango leaves inoculated with *Glomerella cingulata* was incubated at 26°C for 5 days to determine which developmental stage was most susceptible to anthracnose.

Histopathology

C. acutatum and *C. fragariae* underwent a very brief biotrophic phase (less than 12 h) before entering their extended necrotrophic phase. Representatives of both species of fungi were observed entering living host cells and developing a matrix comparable to that described in reported biotrophic systems (Brown, 1977; O'Connell, 1987; O'Connell *et al.,* 1986; O'Connell and Ride, 1990) but they killed the host cells within a few hours. Previous descriptions for other *Colletotrichum* species cite biotrophy at a minimum of "less than 24 h" (Wharton *et al.,* 2001). But a situation in which a living cell is entered and quickly killed might be considered a modification of necrotrophy, rather than biotrophy or hemibiotrophy. Both intracellular hemibiotrophic and subcuticular intramural *Colletotrichum* species avoid triggering resistance responses during the symptomless phase (Perfect *et al.,* 1999).

To understand the infection process of *C. gloeosporioides*, germination and penetration processes by the pathogen within the whole leaf were observed in our study. The first evidence of penetration into the whole leaf was observed 48 hrs after invasion. It also revealed that mycelia were prominent after 120 hrs after invasion by the fungus (*C. gloeosporioides*). Subcuticular infection by hyphae was present in transverse leaf sections (T.S.) of the diseased sample after 72 hrs. Also, both inter and intra-cellular hyphal invasion were observed after 72 hrs. Mesophyll cells were highly affected by fungal invasion and rapidly collapsed. Swelling of epidermal cell walls was also observed. After 96 hrs almost all the cells became necrotized (Nc). Necrotized mycelial mats (M) of *C. gloeosporioides* was observed after 120 hrs and all the invaded cells became necrotized (Nc) forming a spot which eventually the cells ruptured leaving a shot hole symptom. All these observations pertained to the cells of mesophyll tissue indicating that these are the regions of fungal invasion and host tissue damage resulting in the disease symptoms. Naturally infected and artificially inoculated (*in vitro*) presented no significant differences suggesting that the pathogen invasion and symptom

development process is similar in both the conditions (Pandey, 2011).

Molecular studies on *C. Gloeosporioides*

Selection neutral Random Amplified Polymorphic DNA (RAPD) markers have been used to demonstrate genetic variation in *C. gloeosporioides* population infecting *S. scabra* in Australia (Chakraborty *et al.,* 1999) and *S. guianensis* in Colombia (Kelemu *et al.,* 1999). Similar association between *S. guianensis*, *S. scabra* and *C. gloeosporioides* genotypes has been recorded earlier in Australia (Chakraborty *et al.,* 1999) and Colombia (Kelemu *et al.,* 1999) (Table 1). *Colletotrichum boninense* previously fell within the broad species concept of *C. gloeosporioides* but is differentiated from *C. gloeosporioides* by colony shape, conidial morphology and molecular phylogenetic analysis of internal transcribed spacer (ITS) sequences (Lu *et al.,* 2004). Molecular phylogenetic analysis on ITS sequences clearly distinguished the species from *C. gloeosporioides* as well as other similar *Colletotrichum* species such as *C. musae* and *C. fragariae*. Moriwaki *et al.,* (2003) presented that the interspecific DNA homologies with related taxa were 80.2 to 82.3 per cent for *C. gloeosporioides*. Phylogenetic analysis of the ITS sequences of fungus showed that the interspecific DNA homologies were low and ranged 88.7 to 93.9 per cent in *Colletotrichum* spp.

The identity of the pathogen was determined by culture morphology on PDA media and sequence homology of the ITS region to reference sequences of isolates of *C. gloeosporioides* available from NCBI database. Lee *et al.,* (2005) compared related species of *C. gloeosporioides*, *C. musae, C. fragariae*, *C. spinaciae* and *C. lindemuthianum* and found that the interspecific DNA homologies were 93.9, 91.7, 93.6, 92.6 and 88.7 per cent respectively. Molecular phylogenetic analysis on ITS sequences clearly distinguished the species from *C. gloeosporioides* as well as other similar species such as *C. musae* and *C. fragariae*. Molecular polymorphism generated by RAPD confirmed the variation in virulence of *C. gloeosporioides* the cause of mango anthracnose isolates collected from Agri Export Zone (AEZ) of Andhra Pradesh and from Tamil Nadu were grouped into three clusters (Shampatkumar *et al.,* 2007). *C. kahawae* was found to be very close to *C. gloeosporioides* on the basis of rDNA sequences (Sreenivasaprasad *et al.,* 1993).

The aggregate is now more objectively defined using molecular methods and sequences including rDNA-ITS, β-tubulin, MAT1-2 and GDPH (Than *et al.,* 2008a) may be used to assign strains to *C. gloeosporioides* in its currently defined sense. ITS analysis was not proved to be informative in distinguishing subgroups within the *C. gloeosporioides* aggregate although there are suggestions that a sequence dichotomy exists that are reflected in the gene's secondary structure (Bridge *et al.,* 2008b).

Eight random primers were tested by Gupta *et al.,* (2010) in the genome of *C. gloeosporioides* isolates collected from different locations generated distinct fingerprints. Primers OPA-1, OPA-3 and OPA-18 produced reproducible polymorphic major bands among the selected isolates. Amplicons of 2138, 1202 and 955 bp as generated by OPA-3, 2291 and 1995 bp was obtained with OPA-18 which was shared by all isolates except for isolates 7 and 8 as for the set of amplicons generated by primer OPA-1. Overall fingerprints obtained from *C. gloeosporioides* with the selected primers were unique and species-specific.

Pandey et al. (2010) reported that genomic DNA from 12 isolates of *C. gloeosporioides* belonging to different agro-climatic regions was amplified by PCR with *C. gloeosporioides* species-specific primer. All the isolates amplified a uniform DNA fragment of size 450 bp. PCR-RFLP using the restriction endonuclease *Alu*I*, Hae*III*, Msp*I*, Rsa*I and *Taq*I reliably reproduced unique restriction patterns specific for *C. gloeosporioides*.

Disease cycle of *Colletotrichum gloeosporioides*

Under field conditions beneath mango tree the disease can be found on the fallen leaves and the blighted peduncles frequently remain *in situ* for many weeks. These produce spores under favorable moisture conditions and serves as foci of infection for the succeeding bloom. Even after they have fallen to the ground they may continue to be a source of infection for some weeks. It was likely that the potential and possibilities for infection are very great at all times and a favorable season and moisture disease occurrence is in abundance. The optimum temperature for inflection by *C. gloeosporioides* was found to be 25°C. The injury done by the anthracnose disease is closely dependent on humidity the prevalence of rain, misty condition or heavy dews at the time of blossoming and greatly increased its severity (Doidge, 1932).

The latent infection is carried from the field and develops further. Healthy fruits develop infection after coming in contract with diseased fruit (Sohi *et al.,* 1973). The latent infection of the fungus does not begin to spread until ripening so the loss of fruit intended for local consumption is not very serious unless infection is severe. Moreover, it has been shown by Wardlaw and Leonard (1936) that fruit stored at a low temperature (40-45°F) and subsequently ripening of the fungus starts to develop over before edibility is reached.

C. gloeosporioides of mango utilized potassium nitrate more efficiently and ammonium nitrate less efficiently for growth and sporulation as reported by. Saxena (2002) reported that potassium nitrate proved to be better for growth and sporulation of *C. gloeosporioides* isolated from betel vine and pomegranate, respectively.

Management of Anthracnose

Control of post harvest anthracnose disease can be achieved from field management after harvest treatments or preferably a combination of both. Management strategies must be efficient and cost-effective as well as safe to consumer's agricultural workers and the environment.

TABLE 1. Selected molecular techniques recently used for differentiation of phytopathogenic fungi (*C. gloeosporioides*)

Pathogen	Host	Techniques used for differentiation	Target/visualization	Reference
C. gloeosporioides (Penz.) Penz. & Sacc. in Penz.	Various	Isozyme analysis	11 enzyme systems	Kaufman and Weideman, 1996
C. gloeosporioides	Tropical fruits	RFLPs (*Cla*I, *Eco*RI, *Hind*III), RAPDs (1 10-mer primer)	Probes targeting rDNA and mtDNA, genomic DNA	Alahakoon *et al.*, 1994
C. gloeosporioides	*Stylosanthes* spp.	RAPDs (58 10-mer primers)	Genomic DNA	Poplawski *et al.*, 1998
C. gloeosporioides	*Stylosanthes* spp.	RAPDs (20 10-mer primers), RFLPS (*Cfo*I, *Dde*I)	ITS regions of rDNA	Munaut *et al.*, 1998
C. gloeosporioides	Tropical fruits	RAPDs (7 10-mer primers)	Genomic DNA	Hayden *et al.*, 1994
C. gloeosporioides	*Stylosanthes* spp.	DNA probes specific to 1.2Mb mini-chromosome, RFLPs (*Eco*RI, *Hind*III)	1.2Mb mini-chromosome	He *et al.*, 1995
C. gloeosporioides	*Stylosanthes scabra*	RAPDs (6 10-mer primers), electrophoretic karyotyping	Genomic DNA	Chakraborty *et al.*, 1999
C. gloeosporioides	Tropical fruits	RFLPs (*BamH*I, *Eco*RI, *Pst*I, *Sma*I)	Probe targeting rDNA	Hodson *et al.*, 1993
C. gloeosporioides	Citrus	RFLPs (*Pst*I), DNA polymorphisms, electrophoretic separation of chromosomes	rDNA, genomic DNA	Liyanage *et al.*, 1992

Cultural practices

Diseased leaves, twigs and fruits, lying on the floor of the orchard, should be collected and all infected twigs from the tree should be pruned and burnt. Plant vigour plays an important role in keeping the plants free from twig infection. Therefore, proper irrigation and fertilizer application are essential to maintain the tree vigour.

BIOLOGICAL CONTROL

Management through bioagents

The addition of chitin or chitosan adjuvant to improve the efficacy of the antagonists and to induce systemic resistance in the plant either alone or in combination with other biocontrol agents has been successful against *C. gloeosporioides* in various crops (Commare et al. 2002). For the *in vitro* screening of the antagonistic activity of the biocontrol agents against *C. gloeosporioides* the biocontrol agents were streaked on one side of a Petri dish (1 cm from the edge of the dish) containing potato dextrose agar (PDA) and a mycelial disc (8 mm diameter) of a seven day old culture of the highly virulent *C. gloeosporioides* isolate was placed on the opposite side of the petri dish perpendicular to the bacterial streak (Nandakumar *et al.,* 2001).

Biological products formulated from *Trichoderma* in the forms of pellets and suspensions were tested in the field to control mango anthracnose caused by *C. gloeosporioides*. It revealed that with *Trichoderma*'s pellets could also significantly reduce the pathogen inoculum and incidence of anthracnose of 81.26 and 55.53 per cent, respectively (Noiaium and Soytong, 1999). A mycelial disc obtained from the periphery of a 7 day old culture of *C. gloeosporioides* and *B. theobromae* were tested for antagonism for *T. harzianum*. Patin-o-Vera *et al.* (2005) reported that post harvest biological control of mango anthracnose caused by *C. gloeosporioides* has been used but only to a very limited extent. In an investigation with a strain of *Bacillus* spp., as bioagent it was found that the latent infections could be controlled (De Jager *et al*., 2001).

Pandey *et al.* (2011) tested the efficacy of three species of *Trichoderma* namely *T. virens, T. harzianum* and *T. viride* as biocontrol agents against *C. gloeosporioides* in dual culture method *in vitro*. Results of the experiment revealed that all the three species have been found significant to check the radial growth of pathogen with a per cent inhibition ranging from 25.17–63.24 per cent. *T. harzianum* was found superior over *T. viride* and *T. virens*, showing 63.24 per cent growth inhibition. In another study, Pandey *et al.* (2010) found that the radial growth of pathogen with a per cent inhibition ranging from 27.56-58.10 per cent significant ($P<0.05$) was achieved. *T. harzianum* and *T. viride* exhibited the greatest 58.10 per cent growth inhibition. The radial growth inhibition was due to both antibiosis and mycoparasitism between *Trichoderma spp*. and *C. gloeosporioides.*

Management through botanicals

The essential oil of *H. cannabinus* rich in (E)-phytol (28.16%), (Z)-phytol (8.02%), n-nonanal (5.7%), benzene acetaldehyde (4.39%), (E)-2-hexanal (3.10%) and 5-methylfurfural (3%) had antifungal activity against *C. fragaria*, *C. gloeosporioides* and *C. acutatum* (Kobaisy *et al.,* 2001). Silva *et al.* (2008) studied several extracts from *A. eupatoria*, *Petiveria* sp. *D. lanata*, *P. lanceolata* and *S. rebaudiana* afforded very promising results to be used for the control of *C. gloeosporioides*. The most active extract was that from *O. manjorona* which inhibited 96 per cent of *C. gloeosporioides* spore germination. One member of the *Amaryllidaceae* family *Polianthes tuberosa* L. was evaluated against the mycelial growth of *C. gloeosporioides* on potato-dextrose-agar medium.

Pandey *et al.* (2009) observed that 17 plant extracts checked the radial growth of the pathogens, however leaf extract of *Azadirachta indica* was found more effective in inhibiting the redial growth of the pathogen followed by *Moras alba* which is equally effective in inhibiting the radial growth of *C. gloeosporioides.* Leaf extract of *Syzygium communi* and *Lantana camara* were comparatively less effective against all the isolates of *C. gloeosporioides* Penz. and Sacc.

CHEMICAL CONTROL MEASURES

Management through fungicides

Much attention and efforts on anthracnose control concentrated on the use of chemical fungicides. Since, anthracnose is difficult to control in wet seasons when blossom blight is serious (Pope, 1924). Fungicide application focuses on reducing damage to inflorescences and fruit. This practice stated a long time ago but unfortunate only few fungicides are presently approved for use on mango in importing countries. The choice of fungicides used therefore depends on the intended destination of the exported fruit.

Seven sprays of Captan (0.3%) or Captan with two sprays of Zineb (0.2%) at flowering gave maximum control on fruits (Aragaki and Ishii, 1960). Zineb (0.2%) or Bordeaux mixture (4:4:50) twice at flowering and then at 14 day intervals until harvest (Tandon and Singh, 1968a) or Bavistin (0.1%) at 15 day intervals.

Benomyl has been shown superior to other protectant fungicide during both flowering and fruit development (Mc Millian, 1973). The surfactant Nu Film-17 was shown to enhance the level of control with Copper and Benomyl (Fitzell, 1979). But others could not improve control with the surfactants Nu Film 17 Agral 60 or summer oil. Benzimidazoles like carbendazim, thiophanate-methyl and benomyl were most effective compared to non-systemic fungicides in controlling mango anthracnose (Mc Millan, 1984). Dithiocarbamate fungicides are highly effective for anthracnose control. Because ethylene thiourea (ETU) is a by-product of their degradation ethylene bisdithiocarbamates such as maneb or mancozeb are no longer labeled for mango shipped to the United States but they can be used on fruit shipped to some European countries. The methyl dithiocarbamate fungicides such as ferbam do not produce ETU and are an alternative for anthracnose control on fruit grown in or shipped to the United States.

Fungicides with after infection activity for mango anthracnose include benzimidazoles and the imidazole prochloraz. Benomyl has been used in calendar based schedules usually in a mix with protectant fungicides to delay the buildup of resistance in the pathogen population

(Jimenez *et al.,* 1989). It has also been applied as an eradicant spray following infection periods (Estrada *et al.,* 1996). Prochloraz has been used as a protectant or as an eradicant spray (Estrada *et al.,* 1996). Copper fungicides are recommended for control of mango anthracnose but their efficacy is lower than dithiocarbamate (mancozeb) under high pressure (Arauz, 2000). The mode of action of this fungicide was thought to be inhibition of the ergosterol biosynthesis pathway (Scheinpflug and Kuck, 1987) but has been used in anthracnose management in many countries (Jeffries *et al.,* 1990). Laboratory experiments conducted on the effect of 10 fungicides formation and hyphal growth of *C. gloeosporioides* (*Glomerella cingulata*) showed that the fungicides (Chlorothalonil, Thiram and Carbendazim) have distinct control effect against anthracnose.

Azoxystrobin is one among the strobilurin class of systemic fungicides which collapses mycelial sporulation which disrupts some vital stages of fungal development (Hsiang *et al.,* 2004). Sundravadana *et al.,* (2007) reported 100 per cent inhibition of mycelial growth of *C. gloeosporioides* the causal agent of mango anthracnose by azoxystrobin. Organic sulphur group fungicide mancozeb was found to be effective in controlling anthracnose but cannot be used because of ethylene produced as a by product. Moreover, *C. gloeosporioides* developed resistant to benomyl (0.1%) a benzimidazoles systemic fungicides for controlling of pre and post harvest development of anthracnose (Akthar *et al.,* 1998). Ahmed *et al.* (1991) evaluated eight fungicides and observed dithane M-45 (Suncozeb) to give the best control of anthracnose (*C. gloeosporioides* Penz.) followed by Bordeauxe mixture. Excessive use of Benomyl, Thiophanate-methyl and Thiobendazole as pre and post harvest sprays has led to a reduction in effectiveness in certain areas where pathogen resistance to fungicide has reported (Spalding, 1982). The number of persistent calyxes was used as a measure of efficacy since it is known to be directly associated with the disease PFD caused by *C. gloeosporioides* (Fagan, 1979).

Green (1994) recorded losses in excess of 85 per cent while (Mignucci *et al.,* 1988) reported losses exceeding 90 per cent. Farmers in the Caribbean can no longer rely on the hitherto effective benzimidazole fungicides due to the development of fungicide-resistant *C. gloeosporioides* strains (Bayart and Pallas, 1994).

Spraying of the chemical fungicides such as Carbendazim, Zineb, Maneb and Copper oxychloride showed that the pathogen inoculums and disease incidence could reduce only 23.83 and 50.16 per cent, respectively (Noiaium and Soytong, 1999). This differential reaction of *C. gloeosporioides* to benomyl is consistent and has been used from comparison of *C. gloeosporioides* isolates from different hosts (Freeman *et al.,* 1998). Several, fungicides are reported to reduce disease development, but are uneconomical and also cause environmental pollution (Misra and Pandey, 1999). In comparison with *C. gloeosporioides*, *C. acutatum* is less sensitive to Benomyl, captan, and propiconazole but more sensitive to myclobutanil and tebuconazole. Based on the, Benomyl sensitivity test is frequently applied to separate *C. acutatum* from *C. gloeosporioides* (Freeman *et al.,* 1998; Talhinhas *et al.,* 2002).

Host resistance

Among the various components of disease management strategies, use of resistant variety is one of the most important components. Several mango varieties have been documented for susceptibility to anthracnose but none of them known significant resistance to the disease. Kulkarni *et al.* (2009) suggested that development of resistant varieties is the most appropriate approach to control the disease.

Many mango varieties like Alphonso, Baramasi, Carabao, Carrie Early Gold, Keaw, Kent, Kishen Bhog, Rad, Saigon, Tommy Atkins and Van Dyke have been found to be more tolerant to anthracnose (Peterson, 1986; Dinh *et al.,* 2003). Varietal differences in susceptibility have been reported in India as well as in the world with maximum damage observed on Neelum in Kerala and Tamil Nadu (Prabakar *et al.,* 2005). Donald (2006) reported that varieties, Keitt and Kensington Pride, were classed as resistant, whereas R2E2, Nam Doc Mai, Kent, Calypso and Honey Gold were classed as susceptible. Whereas cultivars Edward, Mayaguazano and Elamandi were resistant against anthracnose (Sohi et at. 1973). Presently, none of these varieties is performing true resistances that significantly reduce the use of pre and post harvest fungicides.

CONCLUSION

Isolates of *C. gloeosporioides* have shown differential response for the parameters *viz*. media, media pH and temperature regimes in respect of growth and sporulation. The optimum temperatures for maximum growth of *C. gloeosporioides* were 28°C followed by 32°C with 6.0 media pH. Thus, the *C. gloeosporioides* pathogen can grow maximum under the temperature ranging 28 to 32°C with media pH of 5.5 to 6.0. Thus it may be concluded that the temperature and media pH are the critical factors for the growth of pathogen, which might be the main reason for the expression of mango anthracnose symptoms under field conditions in the Northern parts of India.

As far as reaction between pathogen and mango variables is concerned, it can be concluded that those hybrids have shown resistance in the experiment may further be subjected for the observation on resistance to anthracnose under field condition in different agro-climatic region of mango. The identification and development of new hybrid/varieties with resistance to the disease would be most effective alternative for disease management. Integrated management of postharvest mango anthracnose under tropical conditions requires knowledge of the biology of the pathosystem and the technologies available for control, their economical feasibility, and ecological acceptability.

A hot water treatment for anthracnose control may be necessary in fruit destined for the export market. The decision whether to apply a postharvest fungicide (tricyclazole) would depend on weather conditions, treatments applied during fruit development, and the market requirements (*i.e.* organic or conventional). *Trichoderma*

may be used to develop and ecofriendly low cost disease management module for control of mango anthracnose.

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ABBREVIATIONS: Cell wall degrading enzymes (CWDEs), Spore-carrying capacity (SCC), Coconut watery endosperm (CWA), Transverse leaf sections (TS), Internal transcribed spacer (ITS), National Center for Biotechnology Information (NCBI), Agri Export Zone (AEZ), Ethylene thiourea (ETU), Integrated management (IM).