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Impact of Inoculum type and Mode of Inoculation Favouring *Colletotrichum gloeosporioides* in causing anthracnose Disease in Mango Fruits

K. Jayaprakash[1*], K. Prabakar[2], G. Karthikeyan[3], R. Gnanam[3] and C. Kavitha[4]

[1]Ph.D. Scholar, Department of Plant Pathology, TNAU, Coimbatore (Tamil Nadu), India.
[2]Professor, Department of Plant Pathology, TNAU, Coimbatore (Tamil Nadu), India.
[3]Professor, Department of Bioinformatics, TNAU, Coimbatore (Tamil Nadu), India.
[4]Associate Professor, Department of Fruit Science, TNAU, Coimbatore (Tamil Nadu), India.

(Corresponding author: K. Jayaprakash)*
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ABSTRACT: Mango is a commercially cultivated in both tropical and sub tropical climatic zones. Mango anthracnose is caused by *Colletotrichum gloeosporioides.* The pathogen invades the fruit and sustains in inflorescence as quiescent infection and cause disease after the fruit reaches the harvesting stage. In leaves and fruit, it initially appears as black sunken spots later coalesce to form large necrotic lesions leading to death of tissues. In order to manage the disease, knowledge about survival, multiplication and spread of the pathogen is essential. Hence in this study, evaluation was done on the different method of inoculation and type of inoculation that involves in successful establishment and spread of disease. As a result of investigation it was found that fruit inoculated with spores alone was able to produce symptoms at early stage and causes rotting of fruits compared to other mode of inoculation and inoculum types. Early stage of infection leads to further production of secondary inoculum which tends to infect nearby healthy susceptible crops and host tissues.

Keywords: Method of inoculation, Mode of inoculation, spore suspension, inoculum.

INTRODUCTION

The mango, or *Mangifera indica* L., is a highly consumed fruit crop in tropical and sub-tropical regions worldwide, particularly in Asia. Mangos have been referred to, as the "King of fruits" in the tropical world is a clear indication of its commercial significance (Singh, 1996). India is the world's largest mango-growing nation, accounting for approximately 46.74 per cent of the world's total area in mago cultivation and 40.48 per cent of its total mango production. With a production of 151.88 lakh tonnes, it is primarily grown in Andhra Pradesh, Uttar Pradesh, Bihar, Karnataka, Tamil Nadu, West Bengal, Orissa, and Maharashtra states of India, which accounts for 20.28 per cent of all fruit produced in India (Kumar *et al*., 2011). Many diseases were reported to date has unbearable impact on mango production at any point in its growth, from the nursery to the harvest (Chou, 2002). But mango anthracnose is a notorious disease caused by *Colletotrichum gloeosporioides* that causes yield losses in field as well as in post-harvest stage (Ploetz, 2003). Mango anthracnose can cause yield loss upto 100% in an unmanaged field condition (Dofuor *et al*., 2023). Mango anthracnose is a major post harvest disease causes reduced fruit quality and shelf life (Kankam *et al.,* 2022). Anthracnose disease can infect all the parts of plants *viz.*, inflorescence, twig, leaves, panicles and premature fruits. In fruits, fungal infections initiated at advance stage before ripening of fruit as a latent infection and start to express symptoms as the fruit ripens. Initially it occurs as a black spot on epicarp of fruit, which increases in size as the days progresses and coalesce to form large sunken lesions leads to rotting of entire fruit. Lesions generally occurs on pericarp alone but in severe stage of infection invades pulp of fruit and cause untreatable decay of fruit (Arauz, 2000). Inoculum is any part of pathogen that can start infection, in fungi it may be spores, conidia, sclerotia (clump of mycelium) and fragment of mycelium (Agrios, 2005). For a successful infection and disease establishment appropriate inoculum is essential. It has been scientifically proved by Lin *et al.* (2002) in chilli and was supported by Giri *et al.* (2013). Conidia of *Colleotrichum gloeosporioides* in mango flowers initiate the disease and increases the disease severity within 48hrs from the time of inoculation as spores were produced appresoria for strong anchoring and disease initiation (Nor Dalila *et al.,* 2020). Mango seedlings inoculated with spore suspension showed high Percent disease incidence (Rwala *et al.,* 2022). In this study certain methods of inoculation were carried out to find out the suitable inoculum and mode of entry facilitates the successful infection and development of anthracnose disease in mango fruits after harvested.

MATERIALS AND METHODS

Isolation and morphological characterization. Mango fruits showed typical anthracnose lesions were collected for the isolation of *Colletotrichum gloeosporioides.* The infected tissue along with healthy portion was sectioned surface sterilized with 1%

sodium hypochlorite followed by rinsing with sterile distilled water, air dried and place in PDA (Potato Dextrose Agar) medium and kept in room temperature for incubation. When mycelium emerged from the isolated tissue the young growing mycelium is transferred to new petri dish containing PDA medium to maintain axenic culture of *C. gloeosporioides* (Guettia *et al.,* 2014). Morphological characterization was observed by colony colour, texture, sporulation character and formation of conidiomata. Macromorphology observed manually whereas micro morphological character were observed in phase contrast image analyzer at 40X resolution. Micro morphological characters include shape, size, mycelial character, conidiophores were observed and recorded (Phoulivong *et al.,* 2010).

Pathogenicity test. Agar plugged mycelial disc of 4 mm scooped from 15-day-old culture that was isolated from diseased specimen and cultured in PDA medium was inoculated in healthy susceptible Neelum cultivar of mango and observed for expression of symptoms. After the symptom expressed the pathogen recovered with same colony and morphology character that was re-isolated from the inoculated fruit (Shabi *et al*., 1997).

Molecular characterization

DNA extraction and PCR amplification. Genomic DNA extracted by CTAB (Cetyl Trimethyl Ammonium Bromide) method as constructed by Knapp and Chandlee (1996). The PCR reactions were carried out as 50 µl of reaction mixture containing 1.5 units of *Taq* DNA polymerase (Qiagen, Germany), 1x polymerase chain reaction (PCR) buffer, 200 µM each dNTP, 0.2 µM each primer ITS 1 (5'-TCCGTAGGTGGACCTGCGG-3'), ITS 4(5'TCCTCCGCTTATTGATATGC-3') and 100 ng of template DNA. Reaction mixtures were executed to PCR programme in (Nexus Eppendorf) begins with an initial denaturation step at 94°C for 2 mins, 40 cycles of amplification with denaturation at 94°C for 60 secs, annealing at 58°C for 60 secs min and end 72°C for 1 min for extension with final extension 72°C for 10 min as final step. Separation of PCR product was carried out in 2 µl of ethidium bromide stained 1.2% agarose gel and observed in gel documentation unit (BIO RAD, Gel Doc™ EZ Imager, Bio-Rad Laboratories Inc.) and the amplicon size were determined by molecular marker (100bp DNA ladder) (White *et al.,* 1990). sequencing of amplified Inter transcribed spacer regions in 18s rRNA by sangers dideoxy method done in Biokart India Pvt Ltd, Bangalore, India. Sequence obtained was compared with sequences deposited in NCBI database for further confirmation and accession number were brought from NCBI GenBank portal.

Preparation of inoculum and methods of inoculum used. Two types of inoculum were employed to determine the mode and type of inoculation. To evaluate the impact of mycelium on disease initiation 6 mm agar plugged mycelial disc were directly placed on fruits surface and pinpricked followed by mycelial disc inoculation were followed. To assess the spore intensity, spore suspension were collected from 15 days old broth and standardized to 1×10^1 conidia ml^{-1} of suspension (Hong and Hwang 1998). In spore suspension application, prepared suspension is directly inoculated on fruit by spraying. In spore suspension injection one ml of suspension is injected into fruit. In pinpricked accompanied spore suspension inoculation conidial suspension is inoculated on punctures made on fruit with sterilized section needle. After inoculation all the fruits were incubated for 15 days. At the 15th day of observation size of lesion and PDI were calculated to evaluate the type and mode of inoculation. Three replications were carried out for each treatments.

RESULT AND DISCUSSION

Macromorphology of *Colletotrichum gloeosporioides.*

C. gloeosporioides isolate recovered from infected tissues of fruits that showed anthracnose symptoms were sectioned and placed on PDA medium resulted in emergence of white mycelium. The emerged mycelium transferred to sterilized petri dishes containing fresh sterilized PDA medium and incubated until the colony reached periphery of petri dishes (Fig. 1A).

On incubation, fungal colony covers the entire petri dish with mycelium growth at 15th day of incubation. At 15th day of incubation colony exhibited white to salmon colour, smooth texture and orange pigmentation. It is also observed that after 15th DAI small sclerotia like structures were observed in scattered manner are found to be acervuli when examined under phase contrast microscope and it is inferred that colonies were initiated to produce conidia and conidiomata from 13th day of incubation. The results are confirmed with findings reported by Sayiprathap *et al.* (2018). All the colonies sporulation at 12th day of incubation and results are agreed with findings given by Vinita (2019).

Micromorphology of *Colletotrichum gloeosporioides.*

C. gloeosporioides isolate subjected to microscopic observation revealed the characteristic features of mycelium, size and shape of conidia and structure of conidiomata whether it is acervular or conidiogenous hyphae. *C. gloeosporioides* possessed septate, hyaline mycelium, acervuli without setae, produced condiogenous hyphae that facilitates the formation of conidia, produced cylindrical shaped conidia with round to narrow ended edges with presence of oil globules at mid region, which are emerged directly from condiogenous hyphae of colony or emerged from acervuli formed in culture plate (Fig. 1B & C). Conidial size of *C. gloeosporioides* is 19.8µm in length 2.8 µm in width. The conidial results were in agreement with the findings reported by Kamara *et al.* (2020 where the size ranges from 19.7 µm to 15.4 µm and 5.2 µm to 4.8 µm in length and width respectively. Conidial shape of *C. gloeosporioides* associated with mango was cylindrical oblong with oil globules with length and width, 12.5 µm and 3.75 µm respectively (Abera *et al.,* 2016).

Pathogenicity test. The isolate successfully establish the disease at 3rd day after inoculation and the lesion size enlarged as the day progressed. The lesion covers the whole fruit on 15th day of inoculation. The results were in agreement with Tovar *et al.* (2020) where the pathogenicity of mango anthracnose caused by

Colleotrichum asianum and *C. gloeosporioides* was proved.

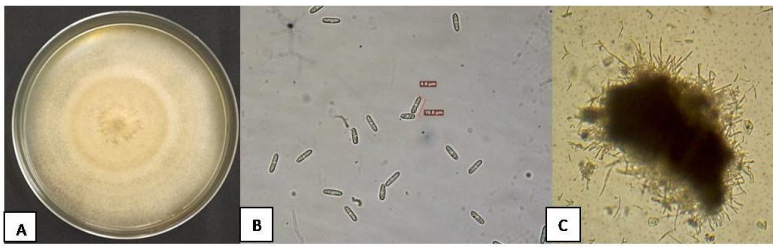
Molecular characterization and confirmation. The genomic DNA of *C. gloeosporioides* isolate produced amplicons at 560 bp when executed to ITS1 and ITS4 primer pair, visualized and recorded in gel documentation unit. ITS region is a noncoding region of 18S ribosomal RNA which is a conserved region found in all eukaryotic fungi. The ITS are specific and vary for all the genus of fungal domain at different base pair and loci. Hence the genus of pathogenic fungi can be identified by characterizing the ITS region of 18S rRNA through sequencing and resulted that sangers dideoxy method yielded sequences with 99.00 percent identity with sequences deposited in NCBI database and GenBank accession number was also provided as **OR717522**. ITS region of genomic DNA produces amplicons at 560 bp which can be ensured that isolates belong to genera *Colletotrichum.* The ITS region sizes were differed for different fungal pathogen from 500 to 800 bp. White *et al*. (1990) showed that the sequences obtained belonged to fungal domain. Further it is supported by Kamle *et al.* (2013) who reported that ITS region of *C. gloeosporioides* associated with mango anthracnose disease were amplified at 560 bp. Chowdappa *et al*. (2012) reported that *C. gloeosporioides* causing anthracnose disease in Orchid amplified at 560 bp.

Determination of mode of inoculation and inoculum type. Among the different methods of inoculation maximum lesion size and disease incidence was recorded in pin pricked followed by spore suspension spray with highest Per cent disease incidence of 77.77 and maximum lesion size of 71.2 mm (Fig. 2E) followed by spore suspension application through hypodermal injection (Fig. 2F, Table 1). Lowest percent disease incidence (38.91) and lesion size (10.5) was observed in mycelial disc inoculated after pin pricked method (Fig. 2C) where no symptom and lesion development was observed in fruits inoculated with mycelial disc alone (Fig. 2B, Table 1).

The results were agreed with findings reported by Pavitra *et al*. (2017) states that maximum disease incidence of 71.1 on mango fruits inoculation with pinprick + spore suspension assay. It is also supported by Baria *et al*. (2021) suggesting that fruit inoculation done with spore suspension on wounded area yields maximum disease incidence of 35.73%. Abraham (2017) reported that there is increase in disease severity and percent disease incidence in the mango leaves inoculated with spore suspension and low disease severity was found in leaves inoculated with mycelial contact alone.

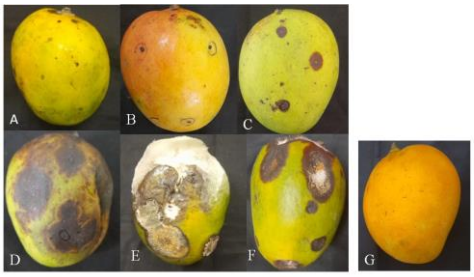
Table 1: Different modes of inoculation and inoculum.

Sr. No.	Inoculation Method	Percent Disease Incidence 15 DAI	Size of the lesion (mm) 15 DAI
1.	Mycelial disc without pin prick	0.00^{d}	0.0 ^{c}
2.	Mycelial disc and pin pricked	38.91 (38.40)^{bc}	10.5^{c} (18.8)
3.	Spore suspension spray	55.55 (48.24)^{ab}	40.1^{b} (39.3)
4.	Pin pricked and spore suspension spray	77.77 (62.65)^{a}	71.2^{a} (51.08)
5.	Spore injection	58.33 (49.98)^{ab}	60.5^{a} (57.63)
6.	Control pin prick alone	0.00^{d}	0.0^{c}
7.	Healthy control	0.00^{d}	0.0^{c}



A) Colony character B) Conidia C) Acervuli

Fig. 1.



A) Pin pricked control
B) Mycelial disc alone
C) Pin pricked + disc inoculation
D) Spore suspension spray
E) Pin pricked + spore suspension
F) Spore injection ; G) Uninoculated healthy conrtol

Fig. 2.

CONCLUSIONS

From the results, it can be concluded that next to natural openings present in plants and fruits, for a successful infection and disease establishment, openings caused by injuries plays major role in favoring entry of pathogen that can occur due to post harvest agricultural practices and exportation injuries. It was also observed that conidial suspension achieved highest percent disease incidence as well as maximum lesion size with in short period of time which also gives a lead to production of more secondary inoculum for the rapid spread of disease when compared to mycelium as a inoculation propagule. The finding not only applicable for disease establishment it totally describes about nature of fungal organisms which can be concluded that even biocontrol agent with high sporulation tendency can spread and survive in substrates in natural environment before the invasion of pathogen.

FUTURE SCOPE

This study enables to understand the viable disease causing source and favourable environment. It also encourages to take decision about evolutionary changes in handling of fruits and agricultural products in trading and exporting facilities, breeding for cultivars with morphology that facilitates in evading the resting of spore on fruit surface. This provides awareness about invention of biocontrol agent with high sporulation competency against pathogen utilizing mango fruit and plants as a substrate.

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Conflict of Interest. None.

REFERENCES

Abera, A., Lemessa, F., & Adunga, G. (2016). Morphological characteristics of *Colletotrichum* species associated with mango (*Mangifera indica* L.) in Southwest Ethiopia. *Food Science and Quality Management*, *48*, 106-115.

Agrios, G. N. (2005). *Plant pathology*. Elsevier.

Arauz, L. P. (2000). Mango anthracnose: Economic impact and current options for integrated management. *Plant Dis*., *84*, 600-611.

Baria, T. T., Rakholiya, K. B., & Chaudhari, A. K. (2021). Impact of Inoculation Methods and Fruit Maturity on Development of Fusarium Fruit Rot (*Fusarium musae*) Disease in Banana. *Indian J. Agric. Res.*, *1*, 4-7.

Chou, K. C. (2002). A new branch of proteomics: Prediction of protein cellular attributes. *In: Gene Cloning and Expression Technologies*. Eaton Publishing, Chapter 4, Westborough, MA, pp. 57-70.

Chowdappa, P., Chethana, C. S., Bharghavi, R., Sandhya, H., & Pant, R. P. (2012). Morphological and molecular characterization of *Colletotrichum gloeosporioides* (Penz) Sac. isolates causing anthracnose of orchids in India. *Biotechnol. Bioinf. Bioeng*, *2*(1), 567-572.

Dembele, D. D., Kamara, A., Grechi, I., Silué, N., N'goran, N. S., Yéo, Y. S., & Koné, D. (2020). Morphological characteristics and distribution of *Colletotrichum* isolates morphotypes infecting mango (*Mangifera indica* L.) in the north of Côte d'Ivoire. *African Journal of Food, Agriculture, Nutrition and Development*, *20*(3), 15837-15856.

Dofuor, A. K., Aidoo, O. F., Osabutey, A. F., Antwi-Agyakwa, A. K., Obenewa, B. B., Ablormeti, F. K., & Loh, S. K. (2023). Mango Anthracnose Disease: The Current Situation and Direction for Future Research. *Frontiers in Plant Science*, *14*, 1168203.

Guettia, M. Y. N., Kouassi, N., Diallo, H. A., & Kouakou, F. R. Y. Y. (2014). Evaluation of anthracnose disease of mango (*Mangifera indica* L.) fruits and characterization of causal agent in Côte d'Ivoire. *Int. J. Agric. Innov. Res*, *2*(6), 1008-1017.

Giri, P., Taj, G., & Kumar, A. (2013). Comparison of artificial inoculation methods for studying pathogenesis of *Alternaria brassicae* (Berk.) Sacc on *Brassica juncea* (L.) Czern. (Indian mustard). *African Journal of Biotechnology*, *12*(18).

Hong, J. K., & Hwang, B. K. (1998). Influence of inoculum density, wetness duration, plant age, inoculation method, and cultivar resistance on infection of pepper plants by *Colletotrichum coccodes*. *Plant Disease*, *82*(10), 1079-1083.

Kamle, M., Kumar, P., Gupta, V. K., Tiwari, A. K., Misra, A. K., & Pandey, B. K. (2013). Identification and phylogenetic correlation among *Colletotrichum gloeosporioides* pathogen of anthracnose for mango. *Biocatalysis and Agricultural Biotechnology*, *2*(3), 285-287.

Kankam, F., Larbi-Koranteng, S., Adomako, J., Kwodaga, J. K., Akpatsu, I. B., Danso, Y., & Sowley, E. N. K. (2022). Anthracnose Disease of Mango: Epidemiology, Impact and Management Options. In *Current and Emerging Challenges in the Diseases of Trees*. IntechOpen.

Knapp, J. E., & Chandlee, J. M. (1996). RNA/DNA mini-prep from a single sample of orchid tissue. *Biotechniques*, *21*(1), 54-56.

Kumar, B. (2011). Commodity wise status. Indian horticulture database 2011. National Horticulture Board. pp.90-99.

Kumari, P., Singh, R., & Punia, R. (2017). Evaluation of different inoculation methods for mango anthracnose disease development. *Int. J. Curr. Microbiol. App. Sci.*, *6*, 3028-3032.

Lin, Q., Kanchana-udomkarn, C., Jaunet, T., & Mongkolporn, O. (2002). Genetic analysis of resistance to pepper anthracnose caused by *Colletotrichum capsici*. *Thai Journal of Agricultural science*, *35*(3), 259-264.

Meshram, M. C., Guldekar, D. D., & Potdukhe, S. R. (2014). Morphological character of *Colletotrichum gloeosporioides* from various hosts. *Journal of Soils and Crops*, *24*(2), 275-280.

Monteon-Ojeda, A. (2018). An alternative inoculation technique of *Colletotrichum gloeosporioides* on mango for early anthracnose tolerance screening. *Agron. Mesoam*, 263-274.

Nor Dalila, N. D., Muhamad Hafiz, M. H., Nurul Fahima, M. A., & Nur Aisyah Anis, A. K. (2020). Evaluation of Different Inoculation Techniques for Blossom Blight Disease, *Colletotrichum gloeosporioides* of Mango Flower. *Transactions of the Malaysian Society of Plant Physiology*, 227.

Phoulivong, S., Cai, L., Chen, H., McKenzie, E. H., Abdelsalam, K., Chukeatirote, E., & Hyde, K. D. (2010). *Colletotrichum gloeosporioides* is not a common pathogen on tropical fruits. *Fungal Diversity*, *44*, 33-43.

Ploetz, R. C. (2003). Diseases of Mango. *In*: *Diseases of Tropical Fruit Crops*. CABI Publishing. Wallingford, UK. pp544.

Qambrani, R. A., & Khaskheli, M. I. (2022). Mango seedlings as affected by fungus *Colletotrichum gloeosporioides* through different inoculation methods. *JPAA*, *7*(1).

Sayiprathap, B. R., Ekabote, S. D., Adivappar, N., Narayanaswamy, H., & Ravindra, H. (2018). Cultural and morphological variability in *Colletotrichum gloeosporioides* inciting mango anthracnose. *IJCS*, *6*(4), 2227-2230.

Shabi, E., Katan, T., & Freeman, S. (1997). Characterization of *Glomerella cingulata* isolates from almond fruit using VCG, molecular, fungicide sensitivity and pathogenicity tests 1. *EPPO Bulletin*, *27*(4), 469-478.

Singh, R. N. (1996). Mango. New Delhi: ICAR

Tovar-Pedraza, J. M., Mora-Aguilera, J. A., Nava-Diaz, C., Lima, N. B., Michereff, S. J., Sandoval-Islas, J. S., ... & Leyva-Mir, S. G. (2020). Distribution and pathogenicity of *Colletotrichum* species associated with mango anthracnose in Mexico. *Plant disease*, *104*(1), 137-146.

White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications*, *18*(1), 315-322.

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