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Studies on Collection, Isolation, Purification and Maintenance of Culture of *Colletotrichum gloeosporioides*

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Abstract **– Mango anthracnose caused by *Colletotrichum gloeosporioides* is one of the most destructive diseases of mango in India particularly in Haryana. The disease can appear at all stages of the plant growth right from nursery to post harvest depending upon favourable conditions. *Colletotrichum gloeosporioides*are present in both tropical and subtropical regions of the world. The optimum temperature for growth of this pathogen is 25-28°C, and pH 5.8-6.5. It is generally inactive in dry season but during encouraging conditions it causes anthracnose disease to large number of economic crops amongst which mango anthracnose is important as far as losses caused by pathogen is concerned. The pathogen firstly establishes interaction with host by producing melanized appressorium and then penetrates the host cuticle. After the penetration, infection vesicles and primary hyphae are formed. Later, secondary hyphae developed and spread to kill the host cell. *Colletotrichum gloeosporioides* follows the hemibiotrophic mode of infection where, biotrophic and necrotrophic phases are sequentially occur. The pathogen produced lesions on leaves, fruit and other parts of plant. Finally these lesions become dark and form concentric ring pattern.**

Keywords **– Anthracnose, *Colletotrichum gloeosporioides*, Conidia, Mycelia growth.**

I. INTRODUCTION

Mango (*Mangifera indica* L.) is one of the world's most important fruit of the tropical and subtropical countries. It is cultivated extensively as a commercial fruit crop in India, China, Indonesia, Thailand and Mexico. By virtue of its wide adaptation, delicious taste, superb flavour, very high nutritive and medicinal value as well as its religio-historical significance, it is called the "King of the fruits" (Shad *et al.,* 2002; Pandey *et al.,* 2012). In India, it is cultivated in an area over 2163470 hectare with a production of 1852980 metric tonnes of fruit. However, in Haryana mango is cultivated in area over 9220 hectares with a production of 8872 metric tonnes (Anonymous, 2016). In India, the pathogen has been reported to infect wide range of cultivated crops, including the mango cultivar (Sharma and Kulshrestha, 2015). Various biotic and abiotic stresses cause immense loss to mango crop throughout the world and destructive disease of mango are those caused by fungi, bacteria, viruses and phytoplasma. Among biotic stresses, mango anthracnose is the most serious fungal disease that causes maximum damage in mango (Kumari *et al.,* 2017).*C. gloeosporioides* (Penz. and Sacc.), the causal agent of anthracnose of mango (*Mangifera indica* L.) is a devastating pre and post-harvest fungal disease which has wide occurrence and causing substantial yield losses. 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). 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. Under favourable conditions conidia are strewn and invade young twigs causing twig die back in some cases (Ploetz *et al.,* 1996). Relative humidity >95 per cent for at least 12 hours is essential for infection and development of *C. gloeosporioides* on mango fruit. Infection progresses faster in wounded tissues and in ripe fruits (Prakash, 1996). Various growth parameters of *C. gloeosporioides* were studied using solid media such as effect of concentration and composition of media, inoculums density and temperature on the spore carrying capacity and microcycle conidiation. Slade *et al.,* 1987, compared spore production of *C. gloeosporioides* on solid media with liquid media. *C. gloeosporioides* grow well on PDA (potato dextrose agar) and CWA (coconut watery endosperm) which contain appropriate amounts of carbohydrates, proteins, minerals and lipids (Santoso *et al*., 1996). The present study was carried out only to know the favourable conditions for infection of *C. gloeosporioides* on mango plant.

II. MATERIALS AND METHODS

Collection, Isolation, Purification and Maintenance of Culture of Colletotrichum gloeosporioides

Infected leaves of mango plant (cv. Dashehari) showing typical symptoms of anthracnose were collected from experimental orchard of Department of Horticulture in the month of March, 2016 and brought for further experimentation. Infected portions of leaves were cut into small pieces after microscopic examination. These pieces were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for 30 seconds and then rinsed 3-4 times in distilled sterilized water. The bits were then aseptically placed on potato dextrose agar (PDA) slants and incubated in BOD incubator at 25±1° C and observed daily for fungal growth. After five days of incubation, the small mycelia bits from actively growing margins of the fungal colony were aseptically transferred to fresh PDA media in Petri plates and purified by hyphal tip method (Riker and Riker, 1936). The cultures were maintained on PDA slants at 4±1° C in refrigerator and sub-cultured periodically.

Pathogenicity Test

Leaves of mango which showed the typical anthracnose symptoms were collected from experimental orchard of Department of Horticulture and the pathogen was isolated on the PDA. Mycelial growth was started after two days of inoculation and this fresh mycelial growth were homogenized in blender for approximately 1-2 minutes at lowest speed with 1000 ml of sterilized water. Leaves and fruits of highly susceptible variety Dashehari were artificially inoculated (Pinprick method) with conidia suspension of *C. gloeosporioides*, incubated at 25±1° C under laboratory conditions**.** The symptoms of the disease appeared after 4-5 days of inoculation. The infected leaves and fruits exhibited the brown necrotic spots on leaves and dark brown to black depressed necrotic areas on fruits measuring 20-25 mm in diameter as on leaves from which the pathogen was isolated. These diseased leaves and fruits were used for reconfirmation of the pathogen associated with this disease.

III. Results and Discussion

Collection, Isolation, Purification and Maintenance of Culture of C. gloeosporioides

Isolations from the mango anthracnose infected leaves collected from the experimental orchard on PDA resulted in the initiation of creamy white, regular and fluffy fungal growth after 24 hours of inoculation. Afterward, the fungal growth was fast and covered the entire Petri plate (90 mm diameter) within 6-7 days (*Fig 1*). The individual hypha was septate hyaline; conidia are hyaline (15-20 µm length and 5-7 µm width), one celled, ovoid to oblong, dumbbell shaped. After six to seven days of incubation, fungal mycelial growth was pinkish in culture. The mycelium of growing culture is hyaline, septate and branched. The results of experiment are agreement with Freeman *et al* 1998 where he reported that the conidiomata are acervular, separate, composed of hyaline to dark brown septate hyphae. The setae are long, brown and septate. The conidiogenous cells are enteroblastic, phialidic, hyaline and conidia are hyaline, one celled, straight, cylindrical and obtuse at apices. Variation in the dimension of conidiogenous cells is also observed in different studies. The fungus produces dumbbell shaped conidia, 10-15 µm (average) up to 20 µm in length and 5-7 µm in width.

Pathogenicity Test

The inoculated leaves and fruits produced disease symptoms after 3-4 days of inoculation. They exhibited mass brown or mummy brown necrotic areas measuring 20-25 mm in diameter when old leaves become ruptured and blighted. Thesymptoms observed were same as that of isolated leaves and fruits (*Fig. 2*).The infected leaves and fruits were subjected to re-isolation of the pathogen. The re-isolated cultures showed same symptoms *i.e.* brown necrotic spots on leaves and dark brown to black depressed necrotic areas on fruits measuring 20-25 mm in diameter as from the pathogen was isolated, hence proved the pathogenicity of *C. gloeosporioides*. Thus result obtained during the study was in accordance with Sattar and Malik (1939), where they reported that 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.

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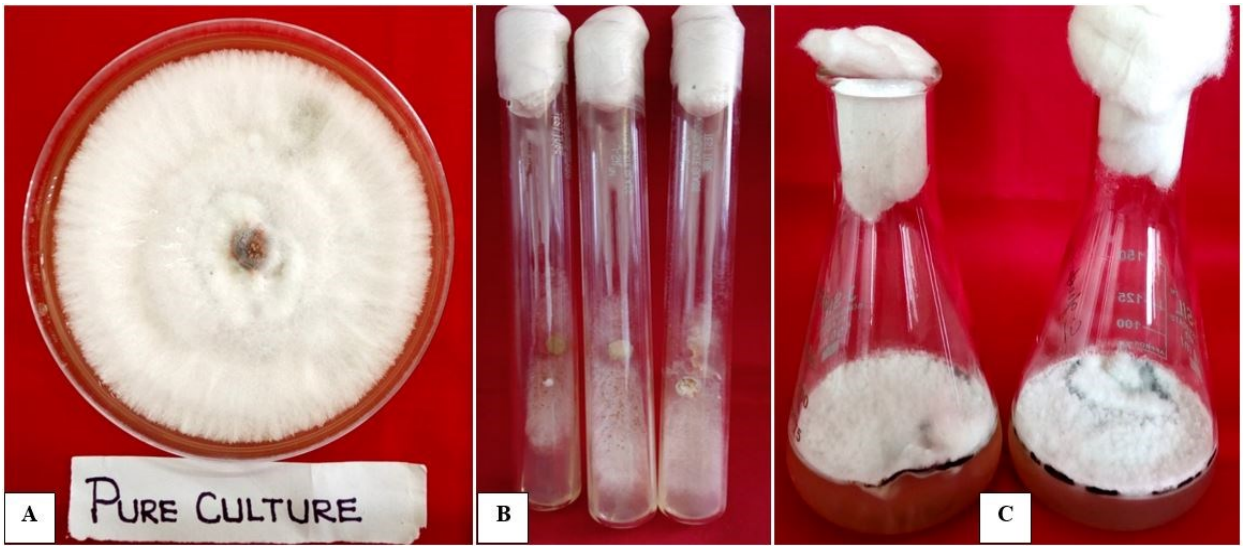

Fig.1. Pure culture of *C. gloeosporioides* in; A. Patri plates, B. Test-tubes, C. Conical flask

Fig. 2. *Anthracnose disease symptoms on A. Fruit, B. Leaves*