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Isolation and characterization of *Colletotrichum* spp. from infected chilli plant

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ABSTRACT: Chilli is an important commercial and vegetable as well as spice crop. Anthracnose, derived from a Greek word meaning 'coal', is the common name for plant diseases characterized by dark, sunken lesions, containing spores. The disease causes extensive pre- and post-harvest damage to chilli fruits. The disease symptoms appears on leaves, stem and also on the fruits. In nurseries, most common symptom is damping-off or seedling blight. At various stages of growth die-back and leaf spotting occurs in plant. Since the pathogen causes extensive yield losses at all the stages of growth of plant. There was a need to isolate and characterize the pathogen in order to take proper management practices. The pathogen from the ripened fruits showing typical symptoms was isolated using tissue isolation technique and cultural and morphological characters were studied. The pathogen produced whitish cottony growth on PDA and black coloured bodies representing acervuli. The pathogen produced septate mycelium and acervulus with sharp pointed septate setae containing unbranched conidiophores bearing aseptate, hyaline, falcate shaped conidia tapering at one end with single oil globule at the centre. The setae were commonly smooth, septate and light brown to dark brown in colour, with cylindrical to conical base and sometimes slightly inflated and the tips were acute to roundish.

Keywords: Chilli anthracnose, *Colletotrichum capsici*, cultural, morphological characters.

INTRODUCTION

Chilli (*Capsicum annuum* L.) is an important commercial and vegetable as well as spice crop in India, belongs to the family Solanaceae. It was introduced to India by Portuguese in Goa during middle of 17th century. Since then, it has been rapidly spread throughout the country and now became an essential part of our Indian cuisine, valued for its characteristic pungency, colour and aroma. India is the world's largest producer, consumer and exporter of chillies in the world followed by China, Pakistan, Thailand, Ethiopia and Indonesia. Indian chilli is considered to be the world famous for two important commercial qualities *i.e*., colour and pungency levels. Being an important spice crop grown worldwide, the decreased production is due to biotic and abiotic constraints, which significantly lead to the yield loss and seed production. Some of the biotic constraints are plant diseases which play a major role in crop losses. The plant diseases are caused by fungi, bacteria, viruses or nematodes which affect the chilli production in many parts of the world (Saxena *et al*., 2016).

Anthracnose, derived from a Greek word meaning 'coal', is the common name for plant diseases characterized by dark, sunken lesions, containing spores (Isaac, 1992). Anthracnose of chilli was first reported from New Jersey, USA, by Halsted (1890) in 1890, who described the causal agents as *Gloeopsorium piperatum* and *Colletotrichum nigrum*. These taxa were then considered as synonyms of *C. gloeosporioides* by Von Arx (1957). In India the disease was first time reported in Coimbatore of Madras Presidency – India (Sydow, 1913).

The disease causes extensive pre- and post-harvest damage to chilli fruits. The disease symptoms appears on leaves, stem and also on the fruits. In nurseries, most common symptom is damping-off or seedling blight. At various stages of growth die-back and leaf spotting occurs in plant. Die-back symptom begins from the tip of the plants branches and finally reach downwards results in the progressive death of the branch. Rotting and fruit spotting occurs in ripe chilli with formation of acervuli in concentric rings, sunken necrotic tissues and coalesced lesion (Siddiqui *et al*., 1977). Typical fruit rot symptoms are circular or angular sunken lesions, with concentric rings of acervuli that are often wet and produce pink to orange conidial masses. Under severe disease pressure, lesions may coalesce. Conidial masses may also occur scatteredly or in concentric rings on the lesions and also reduces dry weight of fruit, and quantity of capsaicin and oleoresin.

Leaves and stems are damaged by different species *viz*., *C. coccodes* and *C. dematium* whereas *C. acutatum* and *C. gloeosporioides* infect chilli fruits. There is an enormous variation among species of *Colletotrichum* causing anthracnose disease. Between the species already present, *C. capsici* / *C. truncatum* is a cosmopolitan fungus. In India *C. capsici* is most prevalent in ripen chilli whereas *C. acutatum* and *C. gloeosporioides* induce disease in green and red chilli (Than *et al*., 2008).

MATERIAL AND METHODS

Collection of infected plant samples from field. The chilli plant showing typical symptoms of matured/ripened fruits were collected from nearby farmer's chilli field.

Isolation and maintenance of culture of the pathogen *Colletotrichum* spp. To isolate and get the pure culture of *Colletotrichum* spp. causing infection on different parts of the plant, the infected plant parts were thoroughly washed under running tap water. The small fragments of tissue (0.5 - 1 cm) had been cut from the border of the healthy and diseased tissue using sterile razor blade. The surface was subsequently sterilized in 1 per cent sodium hypochlorite solution for a minute and three rinses with sterile distilled water. These sterilized fragments were inoculated on Potato Dextrose Agar (PDA) and the inoculated plates were then incubated at 28 ± 1 °C for five to seven days. The hyphal tips growing from infected fragments were observed for the characteristic features of pathogen under light microscope and then transferred to the PDA slants with the help of sterile needle. Thus, the four isolates of the pathogen were obtained from different parts. The isolates were purified by using hyphal tip technique (Rangaswami, 1972) and culture tubes were preserved in a refrigerator at 4 °C for the further use.

Identification of pathogen isolate

Cultural and morphological identification. The fungal pathogens are primarily identified by cultural and conidial characteristics up to the genus level or even up to species level. In order to identify the isolated pathogen, isolates were re-cultured on PDA media followed by the incubation for 10 days. Further, the plates were observed for the colony characters of the different isolates *viz*., type of growth, colour of the mycelium, radial growth, texture, margin of the colony and formation of asexual fruiting bodies. The conidia were harvested from each isolate and mounted in lactophenol and observed for their shape, size and presence of oil globules under light microscope. The production of asexual fruiting body, presence and absence of setae, septation were also recorded. For the appressorium formation, the conidial suspension (10^4 conidia/ ml) was prepared in sterile distilled water and then 10 µl of suspension was taken in cavity slide and placed with cover slip. The slides were kept for incubation under humid moist conditions then observed for the presence of appressorium at 24 h intervals.

Disease symptomatology. Under field conditions, anthracnose disease was manifested on different plant parts of chilli *viz*., leaf, stem, fresh green fruits and red ripened fruits. The typical symptoms were first seen on lower leaves as circular to irregular brown spots surrounded by the dark margin with grayish centre covered by yellow hallow. Under severe infestation, minute black dot like fruiting bodies called acervuli were found to develop on the spot. The collapse and shredding of necrotic tissue in the centre of the spot lead to the shot hole symptom (Fig. 1).

The typical symptoms on stem included, infection of growing tips leading to necrosis of branches from tip downwards. Necrotic tissues appeared as grayish white containing black dot like acervuli in the center of the spot were observed at branching point and stem surface. Shredding of flowers was noticed due to the infection at pedicel and tips of branches (Fig. 1).

The characteristic symptoms on green and ripened fruits were observed initially as water soaked lesions, which later found to develop as small, circular, yellowish to pinkish sunken spots on the fruit surface. The spots were found to increase along with fruit length attaining elliptical shape. Severe infection resulted in shrivelling and drying of fruits. Tissues around the lesion on the fruits were bleached and turned white or greyish in colour and found to lose their pungency. On the surface of these lesions minute black dot like fruiting bodies called acervuli were observed in concentric rings and infected fruits were discoloured with straw colour. Orange conidial masses were produced in acervuli on the surface that is wet and slimy. Lesions may coalesce under severe disease pressure leading to shredding of fruits prematurely. The seeds filled in severely infected fruits were found discoloured and covered with minute black dots indicating acervuli (Fig. 1).

The symptoms of chilli anthracnose were described by Kumar and Bhaskaran (2007) as small-circular spots, which increase in size with the progress of the disease leading to defoliation under severe infection condition. The infection of growing tips lead to necrosis of branches, which progresses back on the diseased branches (Die-back Stage). The die-back may lead to the death of whole plant. Later on black dots (acervuli) were formed all over the necrotic surface of the affected twigs. On the fruits, dark brown to black sunken spots, circular or angular shape with concentric rings of acervuli that were often wet and produce pink to orange conidial (spores) masses were evident.

Similar symptomatology of the disease was also observed by previous workers, Dastur (1922); Bosland and Votava (2003).

Isolation and identification of pathogen. The plants showing typical symptoms of anthracnose were collected from the infected field and the pathogen was isolated from different parts of the plant as explained in Material and Methods. After four days of incubation the pathogen produced the mycelium from an infected tissue. The identification of pathogen was done looking into its mycelial and conidial characteristics with the help of standard mycological keys (Barnett and Hunter 1972). The pathogenic fungi produced different morphological structures like mycelium, asexual fruiting structures and conidia, the conidium acts as disseminating structures which spreads the disease from

one place to other. The conidia found to germinate by producing germ tube which further developed into melanized appressorium, an anchoring organ of the pathogen with the host surface, which penetrate the host cell. These characters are used to identify and classify the pathogenic fungi.

Cultural and morphological identification of pathogen. The pathogen isolated from ripened red chilli fruit appeared initially as whitish cottony growth which later on turned grey to black in colour (Fig. 2). The growth rate was 8.33 mm/day at 27 °C on PDA. The black coloured asexual fruiting bodies were formed in the concentric rings. The pathogen produced septate mycelium and acervulus with sharp pointed septate setae containing unbranched conidiophores bearing aseptate, hyaline, falcate shaped conidia tapering at one end with single oil globule at the centre. The setae were commonly smooth, septate and light brown to dark brown in colour, with cylindrical to conical base and sometimes slightly inflated and the tips were acute to roundish (Fig. 2). The size of the conidia was 14.292 µm in length and 6.367 µm in width. Black colored appressorium with entire margin or slightly irregularly lobed measuring 14.292× 6.367 µm size. The length of germ tube ranged between 39.360 - 175.865 µm with many upright branches. The fungus was found to produce circular sclerotial bodies. The sexual fruiting bodies *i. e*., perithecia were formed at later stages with unicellular, hyaline slight sickle shaped ascospores measuring 18.934 × 4.573 µm (Fig. 2).

The morphological features of *Colletotrichum* spp., which were observed in the present study, can be well comparable with the results published by Veerendra *et al*. (2017). They observed that the fungus produced fairly white to light mouse grey, circular, fluffy mycelium with black colour acervuli which were scattered all over the colony growth against light with the naked eyes and later on confirmed with the help of microscope. Microscopic examination of different isolates revealed that, the mycelium was septate bearing aseptate unbranched conidiophores. Conidia were sickle shaped, hyaline, unicellular and fusiform curved with narrow ends. The average dimensions of conidia which possessed large oil globule in the centre, Acervuli contained abundant dark brown needle like septate setae with several septations and pointed brown tips.

As per the observations by Liu *et al*. (2016), the isolates from *Colletotrichum gloeosporioides*, *C. siamense* group produced pale yellowish colonies with sparse white aerial mycelia. The inverse side of the colonies was white and many bright orange conidial masses near to the point of inoculum. The colonies produced by *C. truncatum* group isolates varied from pale grey to dark grey, with dense pale grey aerial mycelia and small black granules over the entire surface. The inverse side of the colony was dark brown and a few pale yellow conidial masses were observed near the inoculum point. The mycelial appressoria produced by the isolates of *C. gloeosporioides* and *C. siamense* varied from ovoid, clavate and slightly irregular to irregular, smooth or slightly lobed and they were light brown to brown in colour. The appressoria of *C. truncatum* ranged from ovate, ellipsoidal or slightly irregular to irregular in shape, and they were smooth or lobate and brown to dark brown.

Fig. 1. Sympatology of chilli anthracnose.

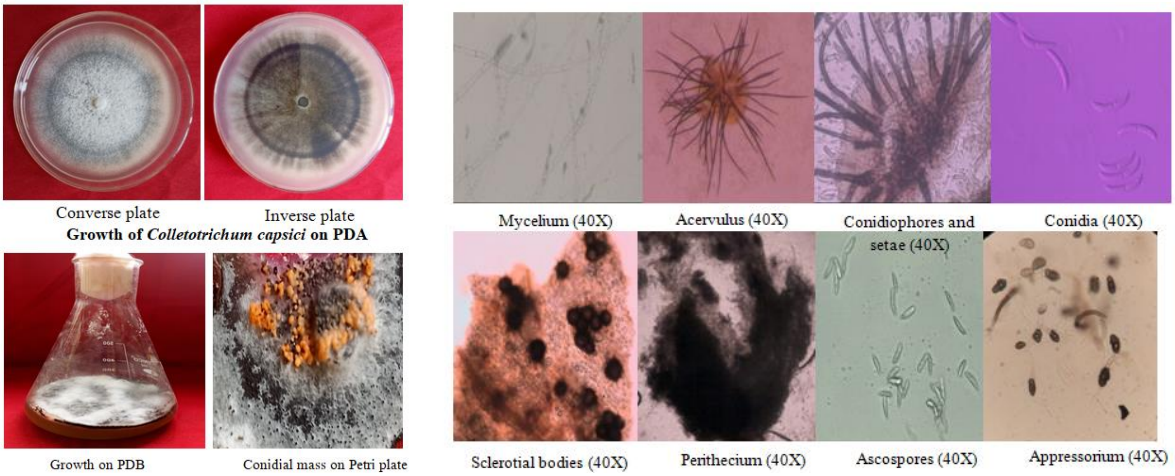

Fig. 2. Cultural and morphological features of *Colletotrichum capsici.*

CONCLUSIONS

The symptoms of chilli anthracnose were typical observed under field conditions. The ripened fruits showing the sunken ulcer like lesions with black fruiting bodies were selected and *Colletotrichum* sp. was isolated. The cultural and molecular characters have proven that the pathogen is *Colletotrichum capsica*.

FUTURE SCOPE

The study was conducted with the aim to isolate *Colletotrichum capsici* from ripened chilli. In future there is a scope to isolate pathogen from different parts of the diseases plant and study the ecology of *Colletotrichum* spp. associated with chilli

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Conflict of Interest. None.

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