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Comparative studies on isolation, identification and purification of *Colletotrichum capsici* causing anthracnose disease of chilli

Veerendra Gupta, Arvinder Kaur, Pramod K Fatehpuria and Himanshu Shekhar Garg

Abstract
Chilli (*Capsicum annuum*) is valued for its diverse commercial uses. It is one of the major vegetable crops that are grown throughout the world especially in tropical and subtropical regions. India is a major producer, exporter and consumer of chilli. In India, the major chillies growing states are Andhra Pradesh, Karnataka, Maharashtra, Orissa, Tamil Nadu, Madhya Pradesh and Rajasthan. In Madhya Pradesh chilli is grown under an area of 54.41 thousand ha with production of 93.57 thousand MT and productivity 0.98 ton per ha. Anthracnose disease can occur on leaves, stems, and both pre- and post-harvest fruits. *Colletotrichum spp*. are among the most important plant pathogen worldwide, causing the economically important disease anthracnose (die back or fruit rot, leaf spot, wilt, damping off, etc) in a wide range of hosts, including cereals, legumes, vegetables and tree fruits. Anthracnose of chilli (*Capsicum annuum* L.) caused by *Colletotrichum capsici* (Syd.) Butler and Bisby, is one of the major and devastating diseases of chilli causes severe losses (10-60%) both in yield and quality of the chilli depending upon the varieties. The present investigation was aimed to make comparative studies on Isolation, identification and purification of *Colletotrichum capsici* causing anthracnose of chilli. The present investigation was conducted at Department of Plant Pathology, college of Agriculture, RVSKVV Gwalior (M.P.) using Completely Randomized Block Design (CRD) during kharif 2015-16. Findings of the present investigation revealed that after purification of *Colletotrichum capsici* were identified on the basis of colony colour, pattern of growth and pattern of acervuli formation on PDA. 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 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 niddle like septate setae with several septations and pointed brown tips. After the pathogenicity test of *Colletotrichum capsici* (test fungus) the fungus was produced same symptoms on the crop.

Keywords: *Capsicum annuum*, *Colletotrichum capsici,* Isolation of Pathogen, Pathogenicity test

Introduction
Chilli (*Capsicum annuum*) is an important spice as well as vegetable crop cultivated worldwide. It is used in many cuisines but also found to have many medicinal properties. India accounts for 1.2 million tones of production annually, and it is the largest producer in terms of international trade, exporting 25 per cent of its total production (NHB 2010). In India it is grown in 775 thousand ha area with production of 1.4 metric MT and productivity of 1.9 ton per ha. In Madhya Pradesh chilli is grown under an area of 54.41thousand ha with production of 93.57 thousand MT and productivity 0.98 ton per ha (NHB 2013-14) [3]. The occurrence of diseases caused by fungi, bacteria and viruses greatly hampers the production of chilli. This crop suffers from about more than 40 fungal diseases (Rangaswami, 1979). *Colletotrichum spp*. are among the most important plant pathogen worldwide, causing the economically important disease anthracnose (die back or fruit rot, leaf spot, wilt, damping off, etc) in a wide range of hosts, including cereals, legumes, vegetables and tree fruits (Bailey and Jeger, 1992) [6]. Among these hosts, chilli (*Capsicum spp.*) is an important economic crop worldwide (Poulos, 1992) [20], is severely infected by anthracnose disease which may cause yield losses of up to 50% (Pakdeevaraporn *et al*., 2005) [21]. Anthracnose of chilli (*Capsicum annuum* L.)

caused by *Colletotrichum capsici* (Syd.) Butler and Bisby, is one of the major and devastating diseases of chilli causes severe losses (10-60%) both in yield and quality of the chilli depending upon the varieties (Bansal and Grover, 1969) [7]. On the leaves, small-circular spots appear. Severely infected leaves fall off leading to defoliation. The infection of growing tips lead necrosis of branches which progresses backward on the diseased branches (Dieback Stage).The die back may kill the whole plant (Kumar M and Bhaskaran, 2007) [18]. Black dots (acervuli) are formed all over the necrotic surface of the affected twigs later on. On fruits, dark brown to black sunken spots, circular or angular shape with concentric rings of acervuli that are often wet and produce pink to orange conidial (Spores) masses are evident. This disease is a severe problem on mature fruits, causing severe losses in field, transportation and storage due to both pre- and post-harvest fruit decay (Hadden and Black, 1989, Bosland and Votava, 2003) [14, 8]. The pathogen is seed, soil, and air borne. The disease is prevalent in almost all major chilli growing areas and it is reported to cause 25–48% loss in different parts of India (Datar, 1995; Fugro, 2000, Ekbote, 2001 and Rathore, 2006) [11, 13, 12, 23]. Traditionally, Identification and characterization of *Colletotrichum* spp. was based on morphological characters such as size and shape of conidia and appressoria, existence of setae or presence of a teleomorph, and cultural characters such as colony colour, growth rate and texture (Smith and Black, 1990) [24].

Material and Methods

The present investigation was carried out in the Department of Plant Pathology, college of Agriculture, RVSKVV Gwalior (M.P.) using Completely Randomized Block Design (CRD) during kharif 2015-16. In the present course of investigation, different fields of chilli growing area of Gwalior district were visited and samples of diseased fruits were collected. A critical study was made on symptoms produced by the pathogen on different plant parts of chilli like leaf, stem and fruits in the year 2015-16. The fruit samples of chilli showing typical fruit rot symptoms were collected in perforated polythene bags from chilli growing fields and brought to the laboratory for isolation, identification and purification of the mycoflora, associated with disease. For the isolation of *Colletotrichum capsici* Potato dextrose agar media (Peeled and sliced potato - 200 g, Dextrose-20.0 g, Agar-agar -20.0 g, Distilled water - 1000 ml) is used during course of investigation. The experimental study was conducted using Borosil make glassware were used throughout the experiment. They were cleaned with liquid detergent. After cleaning the glassware with the detergent, they were thoroughly washed with tap water, rinsed with water and dried before use. Petri plates were sterilized in hot air oven at 180 ℃ for two hours. Potato dextrose agar (PDA) medium and distilled water used during this experimental study were sterilized at 15psi (121.6 ℃) for 15-20 minutes in an autoclave. The inoculation needle and other metallic instruments were sterilized by dipping them in alcohol and heating over a flame. Surface sterilization of plant parts and diseased materials were done by dipping them in 0.1 percent mercuric chloride for 30 seconds followed by three washings in sterilized distilled water. And also the compound microscope (Olympus 10x, 40 x magnifications) was used for observing fungi. Hot air oven and autoclave were used for sterilization of glassware, Petri-plates and media respectively. Incubators were used for incubating test materials/cultures at 25±2 ℃ temperature.

Methodology

Preparation of Potato Dextrose Agar Media: - For the preparation of PDA, potato slice were boiled into 500 ml of water until they become soft. The supernatant was filtered. The supernatant and melted agar was then mixed, measured and the volume was restored to 1000 ml with hot water. It was again boiled for 5 minutes and then dextrose was added. The medium was poured into flask and culture tubes. The mouth of flask and culture tubes was plugged with cotton plug and sterilized in an autoclave at the pressure of 15 pound/square inch (1.05 kg/cm2) for 15 minutes and temperature goes up to 121.6 ℃ which are sufficient for proper sterilization.

Isolation of Pathogen

Small pieces of infected tissue (2-3mm in length) were cut at the junction of diseased and healthy portion with the help of disinfected blade after surface sterilizing the sample with alcohol. These bits were surface sterilized in 0.1 per cent mercuric chloride solution ($HgCl_2$) for 30 seconds followed by three washing with sterilized distilled water in Petri-plates under aseptic conditions using laminar air flow. These bits were air dried by placing on sterilized blotting paper. Five bits were transferred aseptically to the Petri-plates containing sterile potato dextrose agar (PDA) medium. Inoculated Petri plates were incubated at 25 ± 2 ℃ for five to seven days and examined at frequent intervals to see the growth of the fungus/conidia developing from different pieces. (Ahmad *et al.,* 2013) [1].

Purification of Pathogen

The fungus was further purified by single hyphal tip method. The fungus was grown by inoculating in the centre of a plain agar plate. The fungus spread out with its hyphal strands in search of nutrients. These hyphal strands could be located under low power of the microscope in the inverted Petri-plate and the isolated hyphal tips marked with marker. These tips were carefully transferred to potato dextrose agar slants and maintained at normally 25 ± 2 ℃ for 10 days.

Identification of Pathogen

Morphological and cultural characters of isolated fungi were recorded and compared with standard text for establishing their identity (Singh, 1978; Booth and Sutton, 1984; Brayford, 1993; Chowdhry, *et al*., 2000) [25, 4, 5, 9].

Pathogenicity Test

Pathogenicity of purified fungus was tested by employing semi ripe chilli fruits puncher method under laboratory condition. Different treatments followed under this methods, included distilled water inoculation, without injury inoculation, injury+inoculation. The spore suspensions of fungus was prepared by adding 10 ml of sterilized distilled water with 10 day of old culture grown on PDA in 20 ml test petriplate and maintaining the spore concentration of 10^6 spores / ml. The chilli fruits of different variety (JM-218) collected from the field were surface sterilized with 0.1 per cent mercuric chloride solution for 30 seconds and washed thrice with sterilized distilled water. These chilli fruits were air dried by placing on sterilized blotting paper and subsequently inoculated with spore suspension of purified fungus *C. capsici* respectively. The inoculated fruits were placed in moist chambers maintained under plastic tray box using wet blotting papers in four layers and incubated at 25±2 ^{0}C. The disease development was recorded after 5, 7, and 10 days of inoculation using with different treatments under

inoculation method and also calculated percent disease intensity on fruits in different cultivars/germplasm.

Result and Discussion

Results revealed that after purification of *Colletotrichum capsici* were identified on the basis of colony color, pattern of growth and pattern of acervuli formation on PDA. The fungus produced fairly white to light mouse grey, circular, fluffy mycelium with black coloured acervuli which were scattered all over the colony growth against light with the naked eyes and later 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, fusiform curved with narrow ends. The average dimensions of conidia which possessed large oil globule in the centre, Acervuli contained abundant dark brown niddle like septate setae with several septations and pointed brown tips. After the pathogenicity test of *Colletotrichum capsici* (test fungus), the fungus was produced same symptoms on the crop. Present findings are conformity with the findings of Desai and Prasad (1955) [10], Sutton (1973) [26], and Kulshrestha *et al.* (1976) [15]. *Colletotrichum capsici* (Sydow) Butler and Bisby is the well distributed, adopted and known nomenclature assigned to the fungus causing anthracnose of chilli. However, after critical examination, Kulshrestha and co-workers (1976) [15] have been listed and considered at as a synonym of *Colletotrichum dematium* (Persoon, ex. Fries) Groove based on the curved spores. The concept of Kulshrestha and co-workers (1976) [15], Sutton (1973) [26], Desai and Prasad (1955) [10] has been adopted. This nomenclature has been used by several other workers (Kaur *et al.,* 1985; Kumar and Mahmood, 1986; Lee *et al.,* 1986 and Siddiqui *et al.,* 1977) [16, 17, 19, 27].

Conclusion

Anthracnose disease can occur on leaves, stems, and both pre- and post-harvest fruits. Typical fruit 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. The acervuli of *Colletotrichum capsici* were disc shaped, waxy, sub epidermal, typically with dark needle like septate setae, conidiophore short, simple, conidia hyaline, single celled, ovoid or oblong.

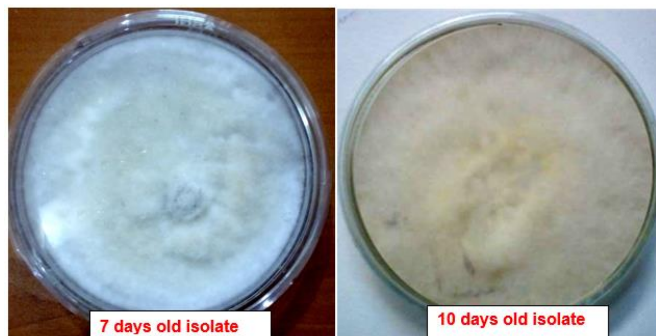

Plate 2: Pure culture of *Colletotrichum capsici*

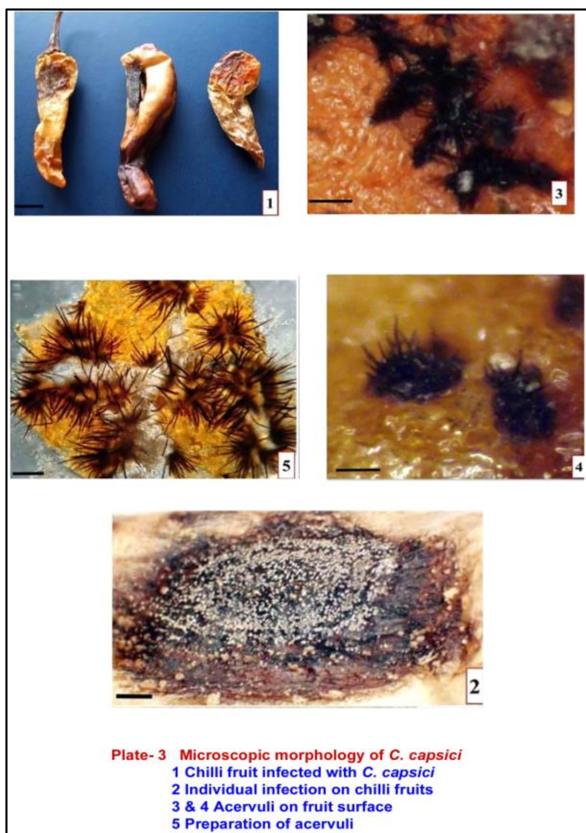

Plate- 3 Microscopic morphology of *C. capsici*
1 Chilli fruit infected with *C. capsici*
2 Individual infection on chilli fruits
3 & 4 Acervuli on fruit surface
5 Preparation of acervuli

Plate:4 Conidia and Setae of *C. capsici*

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