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Isolation, Purification and Morpho-Cultural Characteristics of Colletotrichum capsici Causing Anthracnose of Chilli

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Abstract

Chilli (*Capsicum annum* L.) is a vital vegetable and spice crop from the Solanaceae family, renowned for its culinary and medicinal uses. The most important disease observed on chilli is anthracnose (*Colletotrichum capsici*). Diseased lesions produce pink to orange conidial masses. The fruit initially shows water-soaked patches. Isolation, cultural and morphological characteristics of test pathogen were studied during 2022-24 at Department of Plant Pathology and Microbiology section, College of Agriculture, Pune-05. Microscopic examination of fruit sections shows the presence of sickle-shaped conidia.Fungi from infected chilli fruit section was isolated using the tissue isolation method. The pathogen was then purified through hyphal tip isolation, followed by periodic sub-culturing on PDA medium using the single-spore isolation technique. A detailed analysis, incorporating symptomatology, microscopic observation and assessments of morphological and cultural characteristics, identified the fungal pathogen as *Colletotrichum capsici*, in alignment with descriptions from existing literature. The mycelium of *C. capsici* exhibited a white to grey colouration with smooth edges and showed septate and branched formations. The conidia, measuring 16 to 25 µm in length and 3.6 to 4.5 µm in width, were single-celled, hyalineand falcate, with a sharp apex and a narrow, truncated base, resembling the shape of a sickle. *C. capsici* growth on various media, with PDA showing the highest radial growth and significant differences in colony morphology and pigmentation across media.

Key words : *C. capsici*, Chilli, Conidia, PDA media, hyphal tip isolation, morphological characteristics, symptomatology.

Chilli (*Capsicum annuum* L.), an essential vegetable and spice crop in the Solanaceae family, is globally recognized for its culinary and medicinal value. The Capsicum genus includes 20-25 species, with C. annuum, C. frutescens, C. baccatumand C. pubescens being widely cultivated. The term "chilli" commonly refers to the hotter varieties of Capsicum. Originating from the American tropics, chillies were first cultivated in Mexico (Sahityaet al., 2014). According to statistical report 2023, Maharashtra produced approximately 18,990 metric tons of red chilli. The state is one of the significant contributors to India's overall chilli production, although it ranks lower, as compared to states like Andhra Pradesh and Telangana.Anthracnose, a fungal infection affects seeds, soil and spreads through the air, significantly impacting seed germination and vigour (Ahmad, 1982; Peraneand Mesta, 1996; Asalmol et al., 2001). The pathogen spreads through seeds in the form of microsclerotia and acervuli (Pernznyet al., 2003) and it remains viable on other leguminous or solanaceous crops, plant residues and decaying chilli fruits in agricultural settings. Even after harvest, during drying and storage, the disease persists, diminishing the nutritional and commercial value of affected fruits and resulting in the production of an inferior quality seeds (Pring et al., 1995). Kim et al. (2004) found that various species are associated with diseases affecting different parts of the chilli plant. These diseases develop in three stages: seedling blight or damping off, leaf spot and die back and anthracnose or fruit rot. Chilli anthracnose results in a reduction in the

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fruit's dry weight and in the levels of capsaicin and oleoresin (Mistry et al., 2008). This disease predominantly impacts ripe fruits. Anthracnose, known as fruit rot of mature chilli, it is caused by the fungus *C. capsici* (Sydow) Butler and Bisby. This disease is the most significant threat to chilli farming in India, resulting in substantial economic losses due to lower fruit quality and reduced marketability (Saxena et al., 2014).

Anthracnose, a term originating from the Greek word "coal," describes a common disease characterized by deeply sunken, dark lesions filled with fungal spores (Issac 1992). In India, this disease has led to yield losses ranging from 10% to 54%. It is widespread across the country, with particularly severe occurrences in regions like Assam, Bihar, Andhra Pradesh and Uttar Pradesh. An infection index reaching up to 5% suggests that extensive distribution of anthracnose in India. Moreover, the disease has been found in seed samples, with infections confirmed in seeds. This disease exhibits initial symptoms of small, circular lesions on leaves, progressing to severe leaf loss and eventual plant death. On fruits, angular or circular dark brown to black patterns appear submerged in the tissue. These areas are identified by dense clusters of moist acervuli, ranging from pink to orange in colour, where conidial spores accumulate. The pathogen generates septate, colourless mycelium that forms networks both inside and between cells, accompanied by dark brown setae tipped in light brown, which develop into acervuli. At the tip of the conidiophore, single, sickle-shaped conidia are produced, transparent and unicellular. Conidiophores are microscopic, unicellular, club-shaped and without branches.

Material and Methods

Present investigations on Laboratory experiments were carried out during 2022–2024 at Department of Plant Pathology and Agriculture Microbiology, College of Agriculture Pune-05,

Methodology

Symptomatology and Microscopic Examination : The Chilli fruits showing symptoms of Anthracnose were collected for the investigation fungal pathogen who associate with disease. For microscopic studies, sections of Chilli fruit showing typical symptoms of diseases were prepared and observed. These sections were cut carefully using sharp and sterilized blade and carefully cut using a sharp, then gently placed onto clean glass slide in a drop of water. Cover slip was placed to secure the sections in place. Initially, these prepared slide was mounted beneath the low-power objective lens of the research microscope and then observed under high power objective lens. This allowed for the observation of various pathogenic structures, such as mycelium, spores, conidiophores, fruiting bodies, acervuli, setae, conidiaand any other related elements. Additionally, samples were gently collected using a needle to explore the fungus growth present on chilli fruits. These samples were then carefully positioned on another clean glass slide in a droplet of distilled water and covered with a cover slip. This setup enabled inspection of the collected material under the research microscope.

Isolation : Diseased symptoms showing chilli fruits were collected from local market. The affected specimens were carefully rinsed under a running tap water, then dried with blotting paper. From sample fruits along with healthy portions 5 pieces of infected tissues were taken (5 x 5 mm^2) These pieces then surface sterilized with mercuric chloride 0.1 per cent solution for one minute and rinsed with sterile distilled water about three times in Petri plates to remove traces of mercuric chloride and blot dried. The pieces were subsequently transferred under

aseptic conditions onto sterilized Petri plates, each containing 20 ml of previously autoclaved potato dextrose agar solidified medium, under aseptic condition in laminar air flow. The PDA medium had been sterilized in an autoclave for 15 minutes at a pressure of 1.05 kg cm^{-2} and a temperature of 121.5°C. At temperature 27±2°C these Petri dishes were then placed in a BOD incubator. Within a week of incubation, the plates showed the fungal mycelial growth. The colour and sporulation of fungal colonies were observed, different colour and sporulation of colonies were separated by using the hyphal-tip technique. Those test isolates of the pathogen were aseptically sub-cultured, purifiedand maintain as distinct pure cultures on agar slants separately. The resulting single spore cultures were preserved on PDA slants at 27 ± 2°C for further investigations.

Identification of the pathogens : Identification of fungus followed by comparison of obtained cultures with the original fungal descriptions in previous literature. Several important characteristics were taken into consideration, such as size and shape of conidia, features of asexual fruiting bodies and various cultural characters like growth of colony, colony colour and texture. To accurate identification offungus, a microscopic examination of conidia was conducted using 40X magnification.Spores were carefully mixed with lactophenol to ensure a uniform distributionand a cover slip was gently placed for microscopic analysis. The length and width of the conidia were then measured using a stage and an ocular micrometer. The responsible fungi were identified through the evaluation of symptomatology, cultural and morphological traits, pathogenicity tests and a close examination of microscopic features, including colony characteristics, pigmentation, shapeand conidia.

Maintenance of the pure cultures : The fungal pathogens were sub-cultured onto PDA slants and then incubated at a temperature of 27 ± 2°C for a period of ten days. These slants were subsequently stored in a refrigerator at 5°C. To maintain viability, a regular renewal schedule is set for once in every 30 days.

Proving pathogenicity : Following purification, the pathogens isolated from the diseased fruits were tested its pathogenicity to confirm Koch's postulates by using detached fruit inoculation technique. Chilli fruits were cleansed with sterile distilled water. Subsequently, the fruits surface sterilization with 1:1000 mercuric chloride solution for two minutes. This process was followed by three consecutive rinses with sterilized water and the fruits were left to air dry individually. Pin prick method is used for testing the pathogenicity of the test fungi. The surface of the sterilized fruits was consciously punctured using a sterile needle. Sterilized cotton swabs were soaked in a spore suspension (1 x 10^5 spores ml-1) of the test fungi separately and gently applied to the wounded areas on the fruits. The inoculated fruits were then placed in a moist chamber along with a cotton plug to ensure the maintenance of adequate humidity. A portion of sterilized damp absorbent cotton was placed within a moist chamber and inoculated fruits were incubated at room temperature (24-28°C) within an incubation room to the development of symptoms. Regular observations were conducted to monitor the progression of symptoms. Following the development of symptoms, fungi were re-isolated from the artificially infected fruits. Symptoms produced were recorded and compared with the description in earlier literature. The morphological and cultural characters of re-isolated fungi were compared with original cultures to prove their pathogenicity.

Characterization of the Test Fungi

Morphological Characters : The morphological characteristics of the pathogens,

Colletotrichum capsici were studied using PDA (Potato Dextrose Agar) as the base culture medium. For this, 90 mm sterilized glass Petri-plates were filled with autoclaved and cooled PDA medium (volume of 20 ml per plate), which was then allowed to solidify at room temperature. After solidification under aseptic condition inoculation was performed on these PDA plates by placing a 5 mm culture disc from actively growing pure cultures of the test isolates in the centre of each plate. The plates were subsequently incubated at a temperature of 28 ± 2°C. Three sets of PDA plates were maintained for each test isolate to ensure reproducibility and reliability of the results. The temporary mounts on glass slide of the pure culture (10-12 days old) of Colletotrichum capsici test isolates were prepared separately, covered with cover slip and observed under research microscope to study their morphological characters. Different fungal structures viz., mycelium, acervuli, setae and conidia were observed under light microscope at magnification of 40X and 10X. Measurements of conidia and acervuli were recorded by using ocular micrometer calibrated with stage micrometer and noted mean dimensions.

Cultural Characters : Cultural characteristics, including colony growth, colony colour, colony margin, etc., were observed and recorded 7 days after incubation. Sporulation data were recorded between 10 to 12 days after incubation. The cultural characters of Colletotrichum capsici was studied on ten different media *viz*. Potato Dextrose Agar, Czapek's dox agar, Richard's Synthetic Agar, Malt Extract Agar, Oat Meal Agar, Chilli fruit Extract Agar, Sabouraud'sAgar,Asthma, Hawker's Agar,Conn's Agar and Nutrient Agar.All the media were sterilized at 1.1 kg cm-2 pressure for 15 min. To carry out the study, 20ml of each medium was poured in 90 mmPetri plates. Such Petri plates were inoculatedwith 5 mm disc cut from periphery of activelygrowing culture and incubated at 27±1°C. Eachtreatment was replicated thrice. Observationswere taken when the fungus covered completePetri plate in any one of the media. The colonydiameter was recorded. The fungus colony growth, colour and margin were alsorecorded. The data on radial growth wasanalysed statistically. Twenty ml of each medium was poured aseptically in to 90 mm diameter Petri plates. After solidification, five mm discs of the Colletotrichum capsici were selected from actively growing culture using a cork borer and a single disc placed at the center of petri dish. Each set of experiment replicated thrice and they were incubated at 27 ± 1°C for 7 days. The cultural characters viz., colony diameter, growth pattern, mycelial colourand observations on radial mycelial growth were recorded in all the replicated treatments. Per cent inhibition of the growth of the test pathogen was calculated after comparison with the control by applying the formula given by Vincent (1927).

$$I = \frac{(C-T)}{C} \times 100$$

Where, I = Per cent growth inhibition, C = Growth (mm) in control after seven days and T = Growth (mm) in treatment after seven days

Results and Discussion

Symptomatology and Microscopic Examination : The infected chilli fruits were inspected for the presence of various pathogenic structures such as spores, mycelium, conidiophores, fruiting bodies, acervuli, setae, conidiaand other related elements. This involved initially conducting a visual examination of the diseased fruit samples, followed by the preparation of slides from the fungal growth on the affected areas for microscopic analysis.

Both pre and post-harvest, anthracnose

disease affects leaves, stemsand fruits of Chilli. Ripe fruits and the plant's aerial portions are the main areas affected by the fungus. Tiny circular lesions appear on the leaf surfaces above, which frequently lead to defoliation as the diseased leaves drop off. When the disease infects the developing tips, it leads to branch necrosis, which gradually spreads downward. Several little spots indicate the presence of acervuli on the afflicted branches. Dieback symptoms can sometimes get so severe that they could destroy the entire plant. This type of symptoms are recorded by Hadden and Black, 1989; Bosland and Votava, (2003).

The primarily red ripe fruits were infected. When setae and sclerotia emerge, the brown necrotic lesions with concentric acervuli turn black.

Chilli fruits show typical symptoms often consist of deep lesions that were circular or angular in shape and had wet concentric rings of acervuli, resulting in pink to orange conidial masses. The lesions merged in cases of severe diseased condition. On the lesions, conidial aggregates were also seen to be dispersed or forming concentric circles. These similar symptoms were observed by Than et al. (2008).

Lesions on the leaf blades that were black, wet and slightly depressed were among the symptoms of the chilli plant. The symptoms appeared as water-soaked patches on the fruits, which later turned tan, mushy and slightly sunken. These spores spread over the fruit's surface, frequently combining to create several areas. On the seeds of contaminated fruits, there may also be little black dots. On the lesions, there were many black specks that were called acervuli.

Light brown septate mycelium was visible upon microscopic inspection of temporary mounts made from infected fruit tissues and pure cultures of *C. capsici*. The conidia were spindle-shaped, hyaline and curved. At the terminals of conidiophores, they developed singly and were single-celled. Round and elongated acervuli were present on fruits. The setae were inflated at the base, tapering to a whiter, pointed apex.

Anthracnose can damage leaves and stems before and after harvest, this type of symptoms were observed by Isaac (1992). Srinivas et al. (2006) recorded the similar symptoms as mentioned above of chilli anthracnose. Singh et al. (2003), Than et al. (2008) and Rahman et al. (2011) also found that exact symptoms of chilli anthracnose.

Isolation, Identification, Pathogenecity test of Pathogen

Isolation of pathogen : The pathogen was isolated from the diseased sections of chilli fruits using the tissue isolation method on PDA medium represented in (Fig. 1). The pathogen was subsequently purified using the hyphal tip separation method. To maintain the cultures for ongoing research, they were regularly sub cultured on PDA medium through the single spore isolation method. The fungal pathogen was isolated using above mentioned methods .

The morphological and cultural traits of the fungal isolates, such as colour, texture and mycelial development patterns, were investigated. Along with, the size, shape, colour and traits of asexual fruiting bodies of conidia were examined under a microscope. The

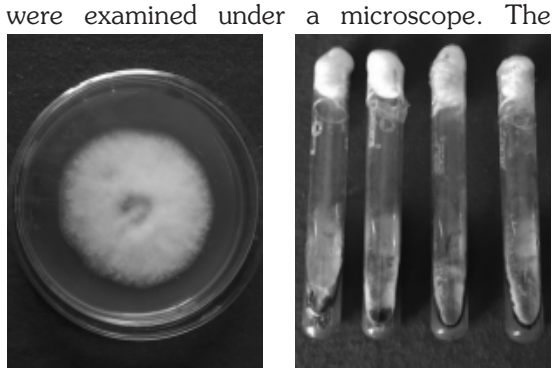


Fig 1. Pure cultures of isolated fungi

following fungal pathogen was found through a comparative analysis of the morphological and cultural features, microscopic findings and symptomatology of the fungal isolates with previously published descriptions in the literature.

The pathogen that causes chilli anthracnose and die back first identified by Sydow (1913) in the Madras Presidency.

Pathogenicity Test : Data presented in (Fig. 2) the detached fruit method was used to perform the pathogenicity test. Using the pin prick injury technique, the isolated fungal pathogens were injected into healthy chilli fruits (Gupta et al., 2017). The manifestation of common symptoms on the fruits verified the pathogenicity of fugal pathogen to differing extentsand the symptoms showed by those infections are described below.

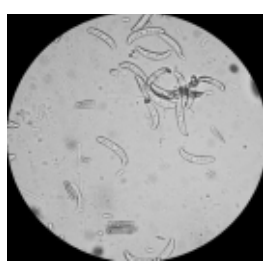
The study conducted by Raj et al. (2013) revealed that the Pinprick and Spore suspension spray technique of inoculation was the most effective in causing fruit and leaf infection in chillies. The mean percentage of infection was determined to be 60.0 and 50.0 respectively.

Reisolation : The artificially inoculated chilli fruits that exhibited the characteristic signs of the dark, sunken necrotic tissues with concentric ring of acervuli were used to reisolate the fungus and this produced a fungal infection that was identical to the original one.

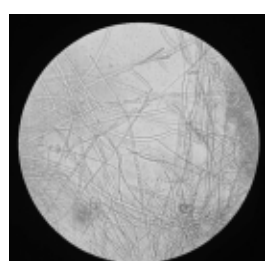
Fig 2. Pathogenicity test of isolated fungi

Identification of Pathogen : After comprehensively studying of various morphological and cultural characteristics, it observed that fungi associated with disease was *Colletotrichum capsici* which causes anthracnose disease. After being incubated at 27 ± 1°C for 7-8 days, the fungus started to grow, and 10 days after plating, the colonies reached their maximum size. The colony colour varied from dull white to grey at the centre and the culture had a cottony texture (PLATE 1). Using a 40x research microscope, a sample was placed on a glass slide coated with lactophenol cotton blue to observe the sporulated culture of the test pathogen. Fungus produced conidia which was hyaline, sickle shaped having size 16 to 25 µm in length and 3.6 to 4.5 µm in width. Based on above mentioned morphological and cultural characteristics, isolated pathogen identified as Colletotrichum capsicishowed in (Fig. 3 and Table 1).

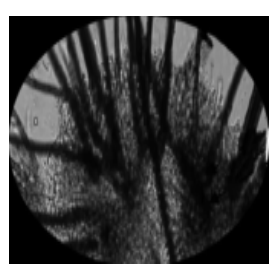
Anbhukumar and Kannabiran (2001) found that causal agent of chilli anthracnose is *Colletotrichum capsici*. Sinha et al. (2002) observed that *Colletotrichum capsici* can cause



Conidia of *C. capsici*



Mycelium of *C. capsici*



Acervuli of *C. capsici*

Fig 3. Microphotographs of isolated fungi

Table 1. Morphological and Cultural characteristics of test fungi

Characterization	*Colletotrichum capsici*
Morphological Characters	
Mycelium	Septate, hyaline, shape of mass myceliumirregular and non- uniform
Conidia	Hyaline, falcate with acute apex andnarrow truncate base (sickle shaped), aseptate, uninucleate.
Size of conidia	16 to 25 µm in length and 3.6 to 4.5 µm in width
Setae	Present
Acervuli	Fruiting body formed
Cultural Characters	
Colony growth	Dense, filamentous and cottony, fluffy or suppressed colony growth appearance
Colony colour	Initially white growth and then turned grey to pinkish-white colour. The aerial mycelium forms light to dark grey centre.
Colony margin	Smooth circular margin

anthracnose in chilli. Rajamanickam and Sethuraman (2014) demonstrated the virulence activity of *Colletotrichum capsici*. Sharma et al. (2017) noticed that 100 per cent disease incidence found due to Colletotrichum isolates. Akhtar et al. (2016) noticed that association of *Colletotrichum capsici* with anthracnose disease.

Characterization of Test Fungi

Morphological and Cultural Characterization of Colletotrichumcapsic

Growth characters on different solid media

Effect of culture media : The study of *C. capsici* on ten different culture media at room temperature (28 ± 2°C) revealed that various cultural characteristics. Radial growth was recorded after the fungus had fully developed on all tested media. Observations included cultural traits such as colony color, shape, margin type and colony diameter on the media plates.

Mycelial growth : The observations represented in (Fig. 4 and Table 2) indicated that all the tested media supported better growth of C. capsici. The average colony growth across the media ranged from 49.16 mm (Malt extract agar) to 89.63 mm (Potato dextrose agar). However, the mean radial growth of *C. capsici* was highest on Potato dextrose agar (89.63 mm) which was significantly highest over all other media. The second, third and fourth best media observed were Czapeck's dox agar (88.32 mm), Chilli fruit extract agar (85.39 mm), Oat meal agar (83.17 mm)but,Czapeck's dox agar was on par with Potato dextrose agar. This was followed by Nutrient agar (80.24 mm), Asthma and Hawker's agar (79.67 mm), Sabouraud's Agar (71.42 mm), Richard's Synthetic Agar (63.45 mm).Conn's Agar (59.41 mm). The least mycelial growth observed on malt extract agar (49.16 mm).

Growth characteristics : There was great

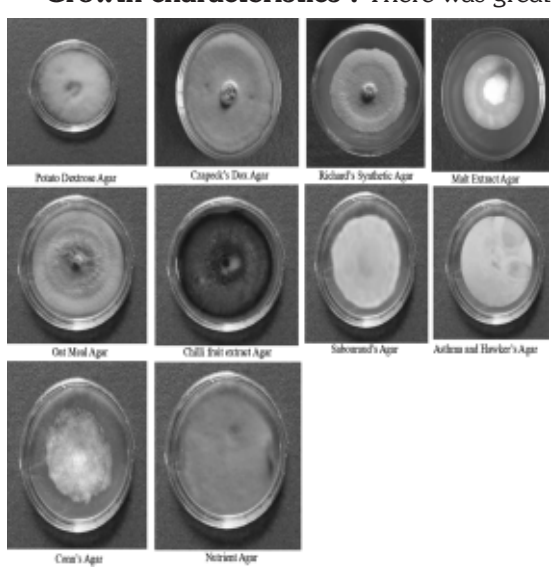


Fig 4. Effect of various culture media on mycelial growth, cultural characteristics of *C. capsici* A)PDA, B)CDA C)RSA D)MEA E)OMA F)CFA G)SA H)AHA I)CA J)NA

Table 2. Effect of various culture media on mycelial growth and cultural characteristics of *C. capsici*

Tr. No.	Media	Colony diameter* (mm)	Colony character		
			Colour	Texture	Margin
T_1	Potato Dextrose Agar	89.63	Greyish white	Thin scanty	Smooth
T_2	Czapek's dox agar	88.32	Buff whitish	Fluffy	Smooth
T_3	Richard's Synthetic Agar	63.45	Creamy white to dull white	Slightly fluffy	Smooth
T_4	Malt Extract Agar	49.16	White to greyish	Slightly fluffy	Serrete
T_5	Oat Meal Agar	83.17	Fair cottony growth	Compact	Smooth
T_6	Chilli fruit Extract Agar	85.39	Blackish white colour	Thin scanty	Entire
T_7	Sabouraud's Agar	71.42	Light grey with blackish white centre	Slightly raised	Entire
T_8	Asthma and Hawker's Agar	79.67	Milky white	Fluffy, raised	Entire
T_9	Conn's Agar	59.41	Cottony growth, light black in centre	Compact	Wavy
T_{10}	Nutrient Agar	80.24	Creamy white	Sparse, flattened	Filiform
SE(m)±	0.68				
CD @ 1%	2.74				
CV (%)	1.08				

*Mean of three repetitions.

variation in colony growth and pigmentation across all the studied culture media. On every medium, the mycelial development took on a variety of shapes and sizes, exhibiting loose to thick cottony structures as well as irregular ones. Across the various media, there were noticeable variations in the colonies colour and topology. Apart from Conn's agar which produced colonies with distinctive wavy margins, most culture media generated colonies with flat to elevated margins. The colony colour of *C. capsici* was white greyish at centre whereas colonies produced varied from fairy cottony growth, creamy white, milky white.

Conclusion

The present study concluded that, the anthracnose symptoms caused by Colletotrichum capsici and the pathogen was found during isolation from infected chilli fruits on PDA medium produced white to greyish white centre. While confirmation the isolated pathogen was identified as white to greyish white centre on the basis of morphological and cultural characteristics and Koch's postulates was fulfilled. As per different medium tested Potato dextrose agar, Czapek's dox agar and Chilli fruit Extract Agar medium were found best for mycelial growth and cultural characteristics of the pathogen.

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