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Isolation, identification and cross infectivity of *Colletotrichum* spp.

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Abstract
In the current study, the isolation of pathogenic fungi was carried out from the diseased tissues of Chilli, Soybean, and Mungbean plants. The samples were carefully collected from symptomatic regions exhibiting characteristic disease symptoms such as necrosis, lesions, or wilted tissue. To ensure the purity of the isolates, re-isolation was performed using Potato Dextrose Agar (PDA) plates, which served as a suitable nutrient medium for fungal growth. The re-isolated cultures were then subjected to detailed identification based on both cultural and morphological characteristics observed under a microscope. Three different species *i.e., Colletotrichum capsici, Colletotrichum truncatum,* and *Colletotrichum lindemuthianum* were identified. The Mycelial growth of *Colletotrichum capsici* was ashy grey to black color, *C. truncatum* was Light grey to white color, *Colletotrichum lindemuthianum* was light brown to whitish yellow color. The *C. truncatum and C. lindemuthianum* shows the symptoms on both Soyabean and green gram where as the rest only seen in chilli causing typical anthracnose symptoms such as dark lesions, fruit rot, and leaf blight. The manifestation of symptoms varied depending on the host and the specific *Colletotrichum* species involved.

Keywords: *Colletotrichum*, anthracnose, morphological, pathogenicity

Introduction
The word Anthracnose is derived from a Greek word that means ‘‘coal”, reflecting the dark coloration of the lesions it causes. This disease manifests as dark sunken lesions (Issac, 1992) [10], that can appear on various parts of the plant, particularly those above the ground such as leaves, stems, and fruits. The lesions typically start as small, water-soaked spots that gradually enlarge and deepen, leading to necrosis and tissue death. Anthracnose disease occurs in almost all parts of the plant above the ground. On seedlings dark brown, sunken lesions develop and it led to die back, sometimes causing death of the seedlings. The pathogen responsible for anthracnose has a remarkable ability to survive and persist in the environment. It can overwinter in soil, especially on infected plant debris and fallen leaves, where it remains dormant until favourable conditions arise for infection. Additionally, the pathogen can be present on seeds, serving as a primary source of infection for new crops. In the advanced stages of infection, the disease becomes more conspicuous due to the production of black fruiting bodies known as acervuli (Faske *et al.*, 2014) [8]. These structures are responsible for releasing spores that spread the disease further. The acervuli produce minute black spots called setae, which are often visible on the infected tissues, giving the disease a characteristic black speckled appearance. The serious leaf spotting leads to holes and defoliation.

Materials and Methods
Collection of plant disease samples and Studies on Symptomatology
Diseased samples which were showing the symptoms of anthracnose like black sunken lesions on the fruits, leaves, pods of chilli, soyabean and mungbean crops respectively were collected from the research fields of College of Agriculture, JNKVV, Jabalpur and brought to laboratory for study of symptomatology and isolation of associated organism.

Isolation, purification and identification of *Colletotrichum* spp. Isolation

The diseased samples collected during the season from fields were used for isolation in the laboratory. Small (2-5 mm) pieces of infected tissues were cut from the edge of the lesions on the collected samples using sterilized sharp scalpel and surface sterilized with 1 per cent Sodium Hypochlorite solution for 1-2 minutes. The pieces were then given three washings in sterile water, tissues were then wiped dry with sterilized filter paper and transferred into petri plates containing PDA. In each plate three pieces were kept in three replications. The plates were incubated at 25±2 °C and examined for the fungal growth after 10 days. The associated fungi were re-isolated, purified and identified.

Purification and Maintenance of culture

The fungus was purified by Hyphal tip method. The fungus spread out with its hyphal strands in search of nutrients. These hyphal strands were seen under microscope in the inverted Petric-plate and the isolated hyphal tips marked with marker. The fungus was sub cultured on PDA slants and allowed to grow at 25±2 °C for one week. The slants were preserved in refrigerator at 4 °C and renewed once in one month. The preserved culture was used for further studies.

Identification of pathogen

Cultural characters:

For cultural characters the pathogens were studies on PDA medium. The 5 mm disc of pure culture of each isolate was inoculated at the centres of the poured petri plates from ten days old actively growing culture. All inoculated plates were incubated at 25±2 °C in BOD incubator. The growth habit and color of the colonies were visually observed after 10 days of incubation.

Morphological characters

Ten days old fungus was stained and observed under the microscope. Observations on shape of conidia, Size of conidia, acervuli, setae and appressoria of the pathogen were recorded and measured with the help of ocular and stage micro meter.

Observations

Radial growth: Radial growth was recorded by measuring the diameter of colony cross wise and then average mean was taken.

Frequency of conidia: For measuring of frequency of conidia, the fungi were cultured on PDA for 4-18 days at 20-25 °C. Conidial suspension of each *Colletotrichum* species were harvested from 10 days of old culture on PDA. These *Colletotrichum* species was flooded with sterilized water and the conidia were gently scraped from the culture plates. The number of viable spores/ml of suspension was counted using a hemocytometer under a microscope at least 10 microscopic fields/slide were observed Therefore observation of sporulation was recorded after 10 days and categorised under following groups (Kumara and Rawal, 2008) [13].

- = No sporulation (0 spore/microscopic field)

+ = Poor (1-25 spores/microscopic field)

++ = Moderate (26-55 spores/microscopic field)

+++ = Good (56-75 spores/microscopic field)

++++ = Excellent (more than 75 spores/microscopic field)

Pathogenicity and Cross infectivity of the pathogen

Spore suspension and Disc inoculation method: Seedlings of chilli, soybean and mungbean were raised in earthen pots The seedlings were inoculated with spore suspension (as mentioned above) by pin pricked and unpin pricked methods. This pricked area was inoculated with one drop spore suspension. The control plants were sprayed with sterilized water and the seedlings were covered with polythene bags and were kept for some days for successful penetration. Similarly, method was used in unpin pricked method without pricking the leaves. The spores were calculated and adjusted to $1x10^5$ conidia/ml.

Result and Discussion

Isolation, Identification and cross infectivity of *Colletotrichum* spp.

Collection of diseased sample and symptomology

Diseased samples which were showing the symptoms of anthracnose like black sunken lesions on the fruits, pods and leaves of chilli, soybean and mungbean crops respectively were collected from the fields of College of Agriculture, JNKVV, Jabalpur and brought to laboratory for isolation of pathogen.

Symptoms of chilli anthracnose sample had black dots to sunken spots on fruits. These sunken spots are covered with pinkish mass of fungal spores. The symptoms of soybean anthracnose sample had Irregular brown lesions present on leaves, stems and pods. These spots were sunken and covered with black fruiting bodies and the seeds become mouldy brown and shriveled.

Mungbean sample had anthracnose symptoms like angular, brown to black spots on leaves. Sooty appearing spots on leaves. These brown spots dry and fall off made into shot hole.

Isolation and Purification of *Colletotrichum* spp.

The isolation of pathogen was done from the fruits, pods and leaves of chilli, soybean and mungbean crops. Small pieces of infected tissue were to be cut from the edges of lesion on the collected infected plant parts using sterilized sharp scalpel. The surface sterilization was done by wiping with the 70% ethanol and sterilized pieces were placed on PDA. The purification of pathogen was done by hyphal tip method. Pure culture was maintained by sub-culturing on PDA slants and used in remaining part of research.

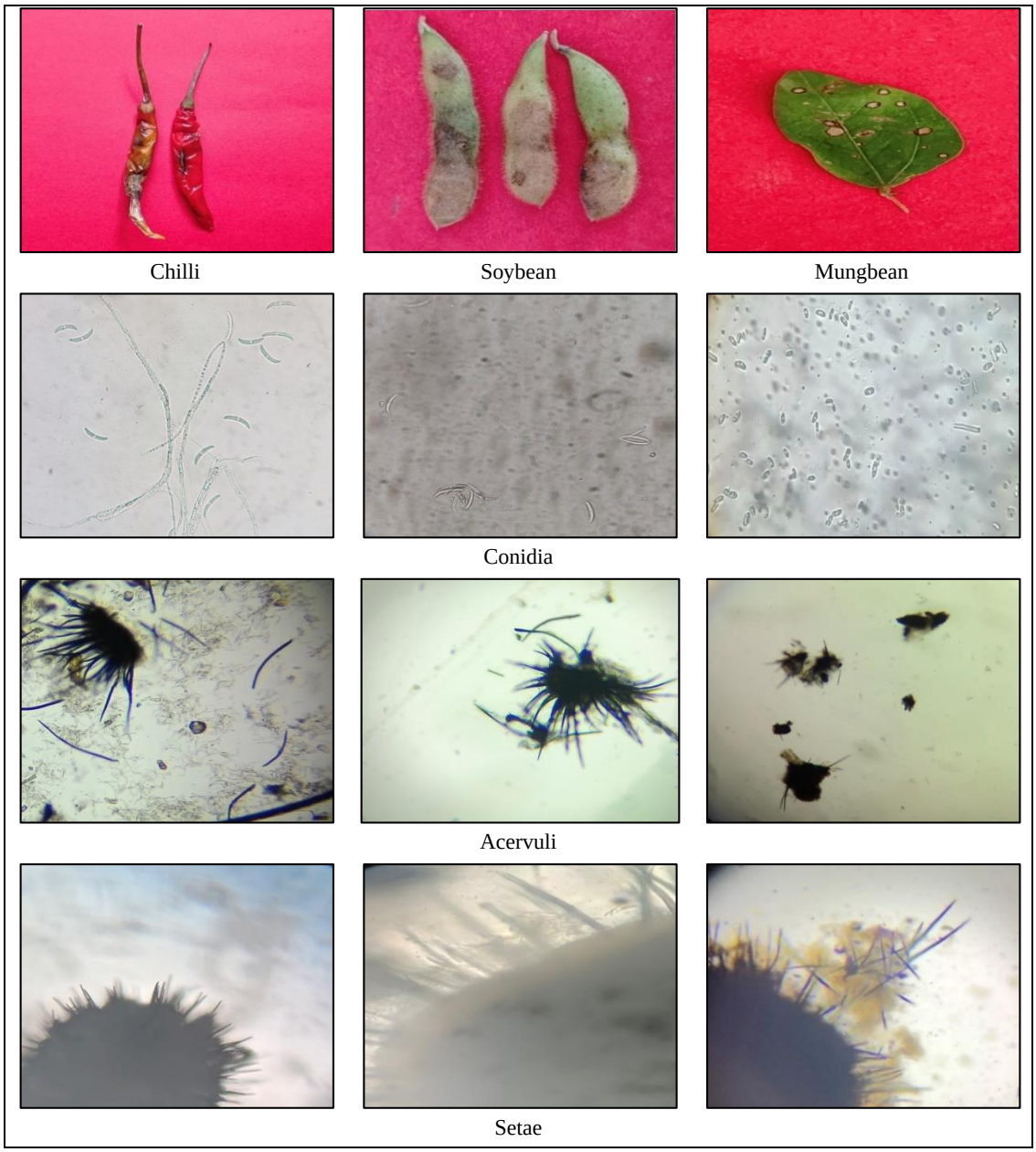


Plate 1: Symptoms and identification of anthracnose of Chilli, Soybean and Mungbean samples.

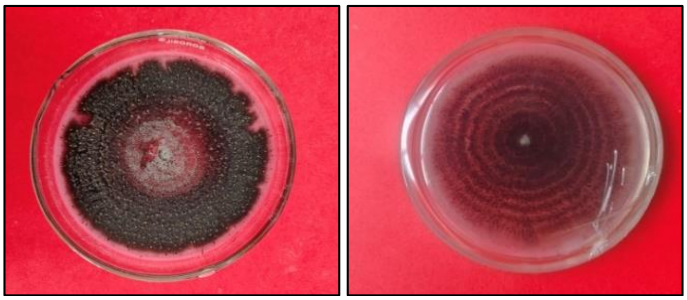
Identification of *Colletotrichum* spp.

The fungus was isolated and identified by morphological characteristics. The slides were prepared by teasing the small bit of mycelium and observed under the microscope.

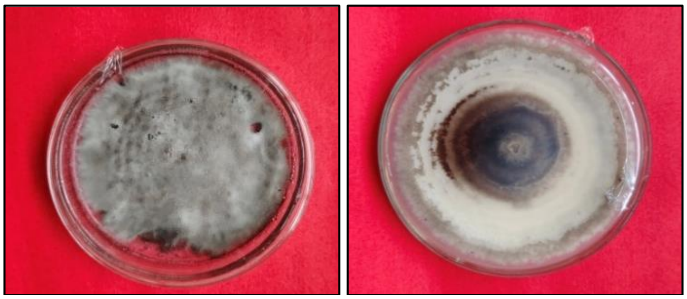
Chilli isolate showed the characteristics like septate mycelium, conidiophores were single, hyaline, aseptate and conidia were fusiform, hyaline, aseptate with one oil globule. The mycelial growth is Ashy grey to black with flat texture and serrated margin. The length of conidia was measured 22-24 x 3.5-5.0 µm. The acervuli size was 122-200 µm. The setae size was 120-270 x 4-5 µm. Black color with club shape appressoria. On the basis of above characters and with the help of available literature, this isolate was identified as *Colletotrichum capsici*.

Soybean isolate showed the characteristics like septate mycelium, conidiophores were single, hyaline, aseptate and conidia were fusiform, hyaline, aseptate with one oil globule. The mycelial growth is light grey to white color with flat texture and smooth margin. The length of conidia was measured 13-22 x 3.2-5.1 µm. The acervuli size was 150-250 µm. The setae size was 45-150 x 3-4 µm. Dark brown color with club shape appressoria. On the basis of above characters and with the help of available literature, this isolate was identified as *Colletotrichum truncatum*.

Mungbean isolate showed the characteristics like septate mycelium, conidiophores were single, hyaline, septate and conidia were aseptate rounded or blunt ends and oblong in shape, hyaline, aseptate with two or three oil globules. The mycelial growth is light brown to whitish yellow with fluffy texture and serrated margin. The conidia length was measured 12-16 ×3.5-5 µm. The acervuli size was 146-212 µm. The setae size was 48-160 x 3-4 µm. Black color with club shape appressoria. On the basis of above characters and with the help of available literature, this isolate was identified as *Colletotrichum lindemuthianum.*



C. capsici



C. truncatum

C. lindemuthianum

Plate 2: Pure culture of *Colletotrichum* spp.

Pathogenicity test and cross infectivity test:

The pathogenicity test of three *Colletotrichum* spp. was performed by spore suspension and disc inoculation method on the potted plant leaves. The leaves of the mungbean, soybean and chilli were injured by pricking the leaves with the help of sterilize needle. One ml spore suspension (1 x 10^5 spores/ml) or mycelial disc (five mm) of test fungi were inoculated on respective hosts. Similarly, un pricked leaves was also inoculated. The cross infectivity of *Colletotrichum* spp. on chilli, soybean and mungbean were also performed in the similar manner. The chilli plants were inoculated with *C. lindemuthianum* of mungbean and *C. truncatum* of soybean. The soyabean plants were inoculated with *C. lindemuthianum* of mungbean and *C. capsici* of chilli. The green gram plants were inoculated with *C. truncatum* of soyabean and *C. capsici* of chilli. The pots of the control will spray with sterile water. After inoculation the plants were be covered with polythene bags to facilitate air circulation and maintain humid conditions for infection process. The usual symptoms were recorded when the lesions were appeared in table 4.1. Symptoms on plants were seen minute to large spots.

Chilli

Soybean

Mungbean

Plate 3: Symptoms development in Chilli, Soybean and Mungbean plants.

Table 4.1: Efficiency of methods for pathogenicity and cross infectivity of *Colletotrichum* spp.

S. No	Method of inoculation		Symptoms occur on the surface of the leaves in days Pathogenicity and Cross infectivity					
			Chilli		Soybean		Mungbean	
			Initiation	Full Development (2-3 mm)	Initiation	Full Development (2-3 mm)	Initiation	Full Development (2-3 mm)
1	Spore suspension (*Colletotrichum capsici*)	Pin prickled leaves	4	5	-	-	-	-
		Unpin prickled leaves	6	7	-	-	-	-
2	Mycelial disc (*Colletotrichum capsici*)	Pin prickled leaves	5	6	-	-	-	-
		Unpin prickled leaves	6	7	-	-	-	-
3	Spore suspension (*Colletotrichum truncatum*)	Pin prickled leaves	-	-	3	6	3	4
		Unpin prickled leaves	-	-	4	7	4	6
4	Mycelial disc (*Colletotrichum truncatum*)	Pin prickled leaves	-	-	5	7	4	5
		Unpin prickled leaves	-	-	7	8	6	7
5	Spore suspension (*Colletotrichum lindemuthianum*)	Pin prickled leaves	-	-	4	7	4	5
		Unpin prickled leaves	-	-	5	8	5	7
6	Mycelial disc (*Colletotrichum lindemuthianum*)	Pin prickled leaves	-	-	5	8	6	8
		Unpin prickled leaves	-	-	6	9	7	9
7	Control	Pin prickled leaves	-	-	-	-	-	-
		Unpin prickled leaves	-	-	-	-	-	-

Conclusion

This study successfully isolated and identified *Colletotrichum capsici*, *C. truncatum*, and *C. lindemuthianum* from chilli, soybean, and mungbean based on cultural and morphological characters. Pathogenicity and cross-infectivity tests confirmed their ability to cause typical anthracnose symptoms on both original and alternate hosts, indicating their role in disease spread. The variation in symptom development among crops highlights the importance of host-pathogen interactions. Overall, the findings emphasize the need for accurate pathogen identification and timely management practices to reduce anthracnose losses and improve crop productivity.

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