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To isolate, identify and prove pathogenicity of *Colletotrichum capsici* on chilli

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Abstract
Chilli is a universal spice crop of India grown in almost all the states of the country. Anthracnose diseases occur on leaves, stems, and both pre and post-harvest fruits. On the basis of the characteristic symptoms of anthracnose disease, exhibited on naturally and artificially diseased foliage of chilli and cultural characteristics of the test pathogen isolated from naturally and artificially diseased foliage of chilli were found exactly similar. During the survey on incidence of anthracnose of chilli, minimum pooled mean disease incidence was found in Parbhani Tejas (16.61%) and maximum recorded with Local variety (31.84%). Varieties recorded mean pooled disease incidence was Green Gold (25.78%), Amulya (28.55%), Garima (22.90%), KSP 1194 (21.60%), Jwala (19.00%). Pathogenicity of *Colletotrichum capsici* was successfully proved on chilli plants under screen house condition.

Keywords: Colletotrichum capsici, pathogenicity, anthracnose

Introduction
Chilli (*Capsicum annuum* L.) is a very important vegetable as well spice crop and is being grown in almost all parts of tropical and subtropical regions of the world. It belongs to the genus *Capsicum* and *Solanaceae* family and commonly called as red pepper. Chilli has its origin from Mexico where it was cultivated around 6000 years ago and was brought to Asia during the 16th century by Portuguese navigators. Chilli contains 1.29 mg protein, 11 mg calcium, 870 I.U. 17.5 mg ascorbic acid, 0.06 mg thiamin, 0.03 mg riboflavin and 0.55 mg niacin per 100 g edible green fruit. It is a rich source of Vitamin C, A and B. Besides traditional use of chilli such as vegetables, spices, condiments, sauces and pickles; it is being used in pharmaceutics, cosmetics and beverages. Consumption of chilli protects against the risk of cancer and diabetes. The strong spicy taste comes due to the presence of active alkaloid compounds *capsaicin*, *capsanthin*, *capsorubin*.
In India, total area under chilli cultivation is 46 (‘000 ha) and production is 327 (‘000 MT) and productivity is 2.25 (MT/ha). (FAOSTAT, 2018-19). Chilli is a universal spice crop of India grown in almost all the states of the country. Apart from being a large consumer and producer of chilli, India is also largest exporter of the crop. Over 30% of the chilli produced in India is exported to countries of West Asia, East Asia, USA, Sri Lanka, and Bangladesh, most commonly in dried form (FAOSTAT, 2016). Karnataka ranks first in area and production of chilli and In Maharashtra, total area under chilli cultivation on 30.99 (‘000 ha) and production is 359.77 (‘000 MT) and productivity is 2.90 (MT /ha) cultivated in all districts as Nagpur ranks first among them. (FAOSTAT, 2017-18).

Hense, the present study was conducted with the following objectives
- To Survey the incidence of anthracnose of chilli in major growing areas of Parbhani district.
- To isolate, identify and prove pathogenicity of *Colletotrichum capsici*on chilli.

Material and Methods
Isolation -For the purpose, the diseased specimens were washed gently in running tap water; blot dried and cut with sharp sterilized blade into small bits (5mm), keeping half healthy and half diseased portion intact. Such leaf and fruit bits were surface sterilized with 0.1%

aqueous solution of sodium hypochlorite in glass petriplates for two minutes, washed by giving three sequential changes with sterile distilled water in petriplates to remove traces of sodium hypochlorite, blot dried and separately inoculated aseptically these bits on autoclaved and cooled Potato dextrose agar (PDA) medium in Petri plates, under of Laminar-air-flow cabinet and incubated in BOD incubator at 27+ 2^0C temperature. Within a week of incubation, profuse fungal mycelial growth was obtained. Applying hyphal-tip technique, the test isolates of the test pathogen were aseptically sub-cultured, purified and maintained the pure cultures separately on agar slant tubes in refrigerator for further studies.

Pathogenicity test: Seeds of chilli Cv. Parbhani Tejas susceptible to anthracnose disease (*Colletotrichum capsici*) were surface sterilized with 0.1% $HgCl_2$ and sown (@ 10 seeds / pot) in the earthen pots (30 cm dia.) filled with steam sterilized potting mixture of soil: sand: FYM (2: 1: 1). After a week, two healthy growing chilli seedlings per pot were maintained, watered regularly and kept in the screen house for further development. The test pathogen (*Colletotrichum capsici*) was mass multiplied on the basal culture medium PDA in Petri dishes. Spore-cum mycelial suspension of *Colletotrichum capsici* was prepared from seven days old culture in plates by flooding with 5-10 ml sterile distilled water. The resultant spore-cum- mycelial suspension was suitably diluted with sterile distilled water to get inoculum concentration of 2 x 10^6 spores / ml. Forty five days old seedlings of chilli Var. Parbhani Tejas grown in earthen pots were artificially spray inoculated (automizer) with the spore-cum-mycelial suspension (2 x 10^6 spores / ml) of the test pathogen. Chilli Var. Parbhani Tejas seedlings grown in earthen pots and sprayed with sterile water (without inoculum) were maintained as uninoculated suitable control. Pots (both inoculated and uninoculated) were kept in the screen house, covered with polythene bags during evening hours overnight and watered regularly to create optimum relative humidity and temperature required for development of the disease.

From the anthracnose symptomatic foliage on the chilli seedlings which were artificially inoculated and diseased, the pathogen was reisolated aseptically on PDA medium and incubated at 27 ± 2^0C. After a week of incubation the cultural and morphological characteristics of the test fungus developed were observed and compared with the characteristics (cultural and morphological) of the original fungus isolated from naturally anthracnose diseased foliage of chilli.

Identification of the pathogen: Based on symptomatology, cultural and morphological characteristics, microscope observations of pathogenicity test and pathogenic variability, the pathogen under study was identified as *Colletotrichum capsici* (Syd.) Butler and Bisby. The isolates of *Colletotrichum capsici* obtained assigned the nomenclature.

Results and Discussion

Occurrence and distribution of anthracnose disease of chilli

The cultivated fields of Parbhani district were surveyed to find out the incidence of anthracnose of chilli (Fig.1) for such survey chilli growing nine tehsils of Parbhani district were randomly selected and from each village of that tehsils diffferent fields were randomly selected. In each field the incidence of anthracnose of chilli was recorded on five $1m^2$ patches by counting the infected plant and total number of plants. The data is summarized in Table. 1.

Table 1: Tehsil-wise anthracnose disease incidence in Parbhani district

Sr. No.	District	Tehsils	2018-19		2019-20		
			No. of Fields	Average Incidence (%)	No. of Fields	Average Incidence (%)	Average incidence of two years
1	Parbhani	Parbhani	20	34.00	20	37.22	35.61
2		Manwath	25	38.25	25	24.24	31.24
3		Jintur	19	25.63	19	27.35	26.49
4		Sailu	17	47.22	17	38.24	42.73
5		Gangakhed	19	45.00	19	46.52	45.76
6		Purna	18	38.57	18	36.54	37.55
7		Pathari	24	35.52	24	27.25	31.38
8		Sonpeth	28	28.23	28	17.36	22.79
9		Palam	29	41.52	29	40.35	40.93
Overall total			199	333.94	199	295.07	314.48
Average			22.11	37.10	22.11	32.78	34.94

The results (Table1) indicated a wide range of anthracnose disease incidence, during both the years of survey and it was ranged from 25.63% (Jintur) to 47.22% (Sailu tehsil) and 24.24% (Manwat tehsil) to 46.52% (Gangakhed tehsil), during the year 2018-19 and 2019-20, respectively. However, during the year, 2018-19, maximum anthracnose disease incidence was recorded in Sailu tehsil (47.22%) followed by Gangakhed tehsil (45.00%), Palam tehsil (41.52%), Purna tehsil (38.57%), Manwat tehsil (38.25%), Pathari tehsil (35.52%), Parbhani tehsil (34.00%) and Sonpeth tehsil (28.23%). Whereas, minimum disease incidence was recorded in Jintur tehsil (25.63%).

Plate I

Plate 1: Typical symptoms of *Colletotrichum capsici* on Chilli leaves and fruits

Identification

In Potato Dextrose Agar culture, isolated isolate (*Colletotrichum capsici)* produced cottony colonies with a colour of grayish- white to dark grey on the ventral surface whereas the reverse of the colonies was mainly black. Sporulation of this species was sparse. The grey-whitish mycelium of *C. capsici* gradually developed from the isolates from the second day of culture. The spore measurement of this species was 13.21- 16.21µm length and 1.79-3.28µm width. Identification was done with specific characteristics which are listed below

1. Acervuli

Acervuli were disc shaped, waxy, sub epidermal, typically with dark needle like septate setae (Plate 3 A).

2. Setae

Setae were dark brown to black in colour erect and tapering towards the apex.

3. Conidia

Conidia were sickle shaped, single celled, hyline, smooth walled with a central oil globule.

The observations of identification of present investigations are observed that the colonies of *Colletotrichum capsici* on PDA were whitish cream to blakish gray with thin mat of mycelium without ariel growth while reverse side were brownish gray to black. Hyphae were initially hyaline and turning dark at maturity. Acervuli were brown to black with longer setae. Conidia were falcate to fusiform with acute apices and measured about 13.41-31.71 × 1.22-6.1 µm.

Plate II

Plate 2(A): Pure culture of *Colletotrichum capsici*

Plate 2(B): PDA slant of *Colletotrichum capsici* (pure culture)

Plate 2(C): Mass multiplication of *Colletotrichum capsici* on Potato dextrose broth

Plate III

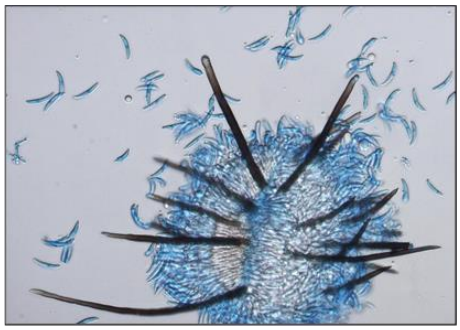

Plate 3(A): Conidia and Acervulii of *Colletotrichum capsici* (400x)

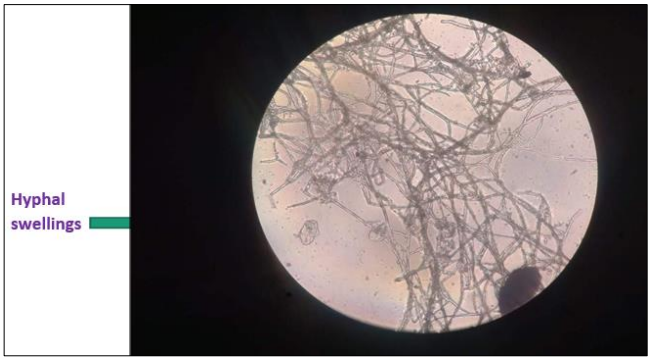

Plate (B): Hyphal swellings on mycelium of *Colletotrichum capsici*

The aforesaid isolation and identification of *Colletotrichum orbiculare* attempted in the present study are in consonance with the earlier reports of several workers.

Symptomatology

Anthracnose diseases occur on leaves, stems, and both pre and post-harvest fruits. The fungus infects the leaves, branches, green as well as ripe fruit. On the leaves, small circular spots appear. Severely infected leaves fall off leading to defoliation. The infection of growing tips leads necrosis of branches which progress back ward on the diseased branches, a large no. of dots represent the presence of acervuli. The die back symptom is severe and it may kill the whole plant. (Plate-1)

A. On leaves

The symptoms were produced in the form of numerous necrotic spots on leaves. The spots at first are small and dark or water soaked. As they enlarge, they become irregular in shape, variable in size and give a scorched appearance. Leaves develop small black circular spots that were markedly delimited by a thick and sharp black outline enclosing a lighter black or straw coloured area. The infected foliage becomes dark brown to black particularly during the period of continuous rains and cloudy weather. (Plate-4 A)

B. On stem

Dark brown cankerous spots or lesions produced on stem. The twigs was water soaked to brown, becoming grayish white or straw coloured, in advance stage of the disease. Large numbers of acervuli are found scattered all over the necrotic surface of the affected twigs.

C. On fruit

On ripe fruit there are small, sunken circular depressions up to 30 mm in diameter. The center of the lesions becomes tan in color while the tissue beneath the lesion is lighter-colored and dotted with many dark- colored fruiting bodies of the fungus that form concentric rings in the lesion. The salmon-colored areas on the surface in the central portions of the lesions consist of large masses of fungus spores. Green fruit may also be infected but symptoms will not appear until the fruit ripens at harvest time.

It was observed that small, minute, circular, dark brown, necrotic spots with thick and sharp outline on leaves and twigs of the chilli plants and reported that in severe infections, necrotic spots enlarged and coalesced each other into large typical anthracnose lesions leading to premature drying and dropping off of leaves. Diseased twigs and branches showed water soaked, brown black, circular, concentric, sunken spots causing necrosis of tender twigs and branches from the tip downward and finally withering of entire plants. Profuse white mycelial growth along with numerous acervuli scattered on necrotic surfaces of the twigs / branches were also observed.

Pathogenicity test

The pathogenicity of *C. capsici* was tested on the young and healthy plants of susceptible chilli Var. Parbhani Tejas in earthen pots under controlled conditions in screen house (PLATE 4 B). The inoculum of the test pathogen was prepared as outlined earlier. Healthy plants of chilli grown in pots were spray inoculated with spore suspension (5×10^6 spores/ml) by the atomizer and incubated in the screen house, where high humidity (80 to90%) and optimum temperature ($27+1^0$C) were maintained for further development of anthracnose disease symptoms. The plants treated with sterile distilled water were maintained as uninoculated control.

Plate IV (A)

Plate 4(a): Symptoms on leaves (inoculated)

Diseased Healthy

Plate 4(b): Pathogenicity test (symptoms) of *Colletotrichum capsici* on chilli plant

Plate IV (B)

Healthy Diseased

Plate 4(B): Pathogenicity test (symptoms) of *Colletotrichum capsici* on chilli plant

The symptoms of the disease developed within one week of inoculation on the leaves and twigs of the inoculated plants were of typical anthracnose symptoms those observed in the naturally infected plants under field conditions. The inoculated plants also dried prematurely and failed to regenerate. Control plants were free from infection and produced good healthy growth.

Reisolation from artificially inoculated chilli plant parts was done on PDA medium under aseptic conditions and incubated at 27+1 ^{0}C for a week. One week after incubation morphological and cultural characteristics of the test pathogen grown on PDA were observed which found similar to that of the test pathogen, isolated from naturally infected chilli plants. Thus, pathogenicity of the test pathogen was proved applying Koch's postulates. Hence, association of *Colletotrichum capsici* with the anthracnose and fruit rot of chillies was confirmed. Thus, on the basis of the characteristic symptoms of anthracnose disease, exhibited on naturally and artificially diseased foliage of chilli and cultural characteristics of the test pathogen isolated from naturally and artificially diseased foliage of chilli were found exactly similar.

Summary and Conclusions

During 2018-19 and 2019-20 an extensive roving survey carried outin the nine tehsils of Parbhani district for checking the incidence of anthracnose of chilli, and recorded overall disease intensity was 34.94% and Gangakhed tehsil (45.76%) recorded maximum disease intensity followed by Sailu tehsil (42.73%), Palam tehsil (40.93%), Purna tehsil (37.55%), Parbhani tehsil (35.61%), Pathri tehsil (31.38%) Manwat tehsil (31.24%), Jintur tehsil (26.49%), and whereas, minimum disease incidence was recorded in Sonpeth tehsil (22.79%). While during survey on different varieties of chilli, minimum anthracnose pooled mean disease incidence was found in variety Parbhani Tejas (16.61%) and maximum with Local variety (31.84%) while other varieties recorded mean pooled disease incidence was Green Gold (25.78%), Amulya (28.55%), Garima (22.90%), KSP 1194 (21.60%), Jwala (19.00%).

- *Colletotrichum capsici* (Syd.) Butler and Bisby is one of the major constraints in the production of chilli, causing quantitative losses.
- Survey studies indicated that anthracnose disease of chilli (*Colletotrichum capsici*) is of common occurrence and widely distributed in all the Parbhani districts of Maharashtra.
- During the survey on incidence of anthracnose of chilli, minimum pooled mean disease incidence was found in Parbhani Tejas (16.61%) and maximum recorded with Local variety (31.84%). Varieties recorded mean pooled disease incidence was Green Gold (25.78%), Amulya (28.55%), Garima (22.90%), KSP 1194 (21.60%), Jwala (19.00%).
- The pathogen, *Colletotrichum capsici* was successfully isolated on PDA from naturally diseased foliage / fruit specimens of chilli collected during survey one each representative of the nine tehsils of Parbhani district surveyed were obtained, purified and maintained for further studies.
- Pathogenicity of *Colletotrichum capsici* was successfully proved on chilli plants under screen house condition.

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