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Manoj Kumar Prajapati
Department of Plant Pathology, GBPUAT College of Agriculture, Pantnagar, Udham Singh Nagar Uttarakhand, India

Dr. Shilpi Rawat
Department of Plant Pathology, GBPUAT College of Agriculture, Pantnagar, Udham Singh Nagar Uttarakhand, India

Priya Singh
Department of Plant Pathology, GBPUAT College of Agriculture, Pantnagar, Udham Singh Nagar Uttarakhand, India

Kripa Shankar
Department of Fruit Science, College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh, India

Corresponding Author:
Manoj Kumar Prajapati
Department of Plant Pathology, GBPUAT College of Agriculture, Pantnagar, Udham Singh Nagar Uttarakhand, India

Cultural and morphological characterization of *Colletotrichum capsici* causing anthracnose of chilli (*Capsicum anum* L.)

Manoj Kumar Prajapati, Dr. Shilpi Rawat, Priya Singh and Kripa Shankar

Abstract
Anthracnose of chilli, incited by *Colletotrichum capsici*, is a major concern for the farmers in various parts of the country. An experiment was undertaken to study the cultural and morphological characterization of the pathogen through *in vitro* studies. Results revealed that OMA was found to be best for maximum radial growth (90.00 mm) of the test pathogen followed by CFDA (88.67 mm) whereas minimum radial growth (69.34 mm) was observed in RSA. Among five different temperature, maximum radial growth (89.87mm) of the pathogen was recorded at 30 ºC followed by 25 ºC (84.83mm) whereas at 40 ºC, no radial growth was found. Among the five different pH tested, pH 7.0 was found to be best suited for the radial growth of the pathogen (83.67 mm) followed by pH 8.0 (81.00 mm) whereas minimum radial growth was recorded at pH 4.0 (74.33mm). Colony culture of the fungus showed grayish to white colony colour with fluffy texture and smooth margin in OMA, PDA, CDA, RSA and MEA whereas brown to black with thin scanty mycelium was observed in CFDA. Microscopic observation (40X) revealed that mycelium was dense, filamentous and septate, acervuli with brown coloured and rounded. Setae were brown to black in colour long needle like, swollen at base and tapering towards apex having size of 110-272µm ×4-6 µm with 2-5 septa per setae. Conidia were hyaline, single celled, sickle shaped, presence of oil globules with size 18-27 µm × 2.1-4.1 µm.

Keywords: Chilli, *Colletotrichum capsici*, characterization, media, temperature, pH

1. Introduction
Chilli *(Capsicum annum* L.) is renowned and illustrious all over the world for its spicy taste. It is an important annual spice as well as vegetable crop belonging to *Solanaceae* family. The origin of chilli is considered as Southern American tropics and is currently being cultivated throughout the world including the tropical, subtropical and temperate regions (Pickersgill, 1997) [15]. Chilli, an important monetary crop worldwide (Poulos, 1992) [16] and sustainability of chilli production is threatened by various types of biotic factors including fungi, bacteria, viruses and other pests involving root-knot nematodes, aphids, thrips and weeds and abiotic factors involve light, temperature, rainfall, herbicides, pesticides which cause directly or indirectly significant yield losses in chilli production. The diseases like anthracnose, bacterial wilt, chilli mosaic, leaf curl and several insect pests have been reported to reduce the crop productivity (Issac, 1992; Anand *et al.*, 2010) [8, 5]. But it is severely exaggerated by anthracnose disease which is one of the major economic constraints to chilli production worldwide, especially in tropical and sub-tropical regions (Than *et al.*, 2008) [20] which may cause yield losses up to 50 per cent (Pakdeevaraporn *et al*., 2005) [14] and 10–80% loss due to pre and post harvest disease (Than *et al*., 2008) [20]. *Colletotrichum capsici* produces colony colour with white to grey having dark green centre. Initially mycelium is hyaline, richly-branched, dense, filamentous, septate later on become dark at maturity (Than *et al.*, 2008) [20]. The acervuli are saucer-shaped and surrounded by firm, black, unbranched hairs like structure typically referred to as setae. Conidia are colourless, one- celled and shape is varying from sickle shaped, ovoid, cylindrical size of conidia is around to 17-18 x 3-4 µm. (Agrios, 2005) [2]. Anthracnose is characterized by circular or angular, depressed, sunken lesions and concentric rings of acervuli and producing pink to orange conidial masses (Isaac, 1992 and Oo *et al.*, 2016) [8, 13]. Generally, warm, wet climate (rainy weather) along with temperature approx 27 °C, RH 75 to 80% and soil pH 5 to 6 favours the disease development. (Roberts *et al.*, 2001 and Rashid *et al.*, 2015) [19, 8]. Keeping in view the importance of the crop losses caused by this devastating pathogen the present investigation has been carried out on cultural and morphological characterization of the *Colletotrichum capsici* causing anthracnose of chilli.

2. Materials and Methods

2.1 Collection, isolation and identification

2.1.1. Isolation of test pathogen

Experiments were conducted during the crop season 2018-2019 for cultural and morphological characterization. Laboratory experiments were carried out in Department of Plant Pathology G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar (Uttarakhand). The pathogen was isolated from the freshly infected fruits through standard tissue isolation technique.The pure culture of the pathogen was obtained by single hyphal tip isolation technique. Pure cultures were maintained on PDA slants at 4ºC in refrigerator and sub cultured on petri plates containing potato dextrose agar medium for further experiments.

2.1.2. Effect of different media, temperature and pH on radial growth and colony characterization of test pathogen

The *in vitro* studies were laid out in completely randomized design with three replication each. Observation was recorded daily for 9 days. Six different media, three synthetic, i.e. Richard's synthetic agar media, Malt extract and Czapek's-dox, and three semisynthetic, i.e. Potato Dextrose Agar, Oat Meal Agar Media and Chilli Fruit Decoction Agar Media, were used to study the radial growth of the test pathogen as well as colony characteristics of *C. capsici* such as colour, texture and margin. The characteristics were compared with the colour chart (Akhtar and Singh, 2007). Similarly, the effect of temperature on the radial growth of the pathogen was studied at different temperature ranges viz. 20, 25, 30, 35 and 40 ºC and pH levels viz., 4.0, 5.0, 6.0, 7.0 and 8.0 which were adjusted with the help of digital pH meter using 0.1N NaOH or 0.1N HCl before autoclaving for maintaining the pH constant. The test fungus was grown on autoclaved, molten and cooled medium were poured aseptically on to sterilized petri plates and allowed for solidification of the media, and then each plates were centrally inoculated with 5 mm culture disc of pathogen cut from the margins of 7 to 10 days old culture by using sterilized cork borer under aseptic condition then incubated at 28±2 ºC in BOD incubator. Pathogen was identified for its morphological structures such as acervuli, presence and absence of setae septation and conidia shape and size and presence of oil globules were examined under stereo-binocular microscope as well as electron microscope.

3. Statistical Analysis

The data analysis was performed by STPR software.

4. Results and Discussion

4.1 Colony characters of the test pathogen on different media

The data presented in table 1 revealed that, in Potato Dextrose Agar media, fungus showed grayish to white colony colour with thin, scanty texture and smooth margin whereas in Chilli Fruit Decoction Agar media, colony colour was dark brown to black, circular with thin scanty texture and smooth margin. In Oat Meal Agar media, dull white to grey colony colour was observed having compact texture with smooth margin and prominent zonation. In Czapedox Agar media, buff whitish colony colour along with fluffy texture and smooth margin was observed. Dull white to gray colony colour, depression at the centre with slightly fluffy growth at the outer side and smooth margin was observed in Richards Synthetic Agar Media. White to greyish colony colour with slightly fluffy texture and serrete type of margin was observed in Malt Extract Agar media (Plate 1).

Table 1: Colony characteristics of the *C. capsici* on different media

S. No	Media	Colony character		
		Color	Texture	Margin
1	Potato Dextrose Agar	Grayish white	Thin scanty	Smooth
2	Oat Meal Agar	Dull white to grey	Compact	Smooth
3	Chilli Fruit Decoction Agar	Dark brown to black	Thin scanty	Smooth
4	Czapek's dox Agar	Buff whitish	Fluffy	Smooth
5	Richard's Synthetic Agar	Dull white to grey	Slightly fluffy	Smooth
6	Malt Extract Agar	White to greyish	Slightly fluffy	Serrete

4.2 Morphological characteristics of the test pathogen

Morphological characteristics under stereo-binocular microscope (40X) revealed that mycelium was dense, filamentous and septate, acervuli were dark brown, rounded, elongated. Setae were dark brown to black in colour long needle like structure swollen at base and narrow at the end with 110-272 µm in length and 4-6 µm in width with 2-5 septa per setae. Profuse thick walled conidia which were hyaline, uninucleate, falcate shape (sickle shaped) slightly tapering or rounded towards end with presence of oil globules in centre and measured 18-27 µm × 1.8-4.1 µm in size (mean 21.64×2.85 µm). As observed under electron microscopic pictograph acervuli measured approximately 39.84 µm in diameter at 550X. Long needle like structure of setae emerge out from the ruptured acervuli. Setaes were swollen at the base and tapering towards apex having size of 107.74 µm to 345.17 µm in length and width of 2.82 -5.74 µm with 2-5 septa per setae. Conidia are sickle shaped with narrower end and broad centre, size varying between 6.17 -6.7 µm ×1.6-1.78 µm at 2000X with a depression at the centre (Plate 2). These research findings are in accordance with Kulshrestha *et al.* (1976) [11] and Akhtar and Singh (2007) [3] they reported that the mycelium of *Colletotrichum capsici* was fine, shiny or whitish pink in colour. They described *Colletotrichum capsici* based on relative size of the setae and shape of conidia in relation to the conidial mass in acervuli. Acervuli were single or in groups. Setae numerous, blackish brown to dark black, longer than the conidial mass. Conidial mass was white to dull white, pale orange or bright orange. Conidia hyaline, fusoid, ends rounded or slightly tapering and having 15-27 x 2-5 µm in size. Setae have 0-9 septa and 48-468 x 2-7 µm in size.

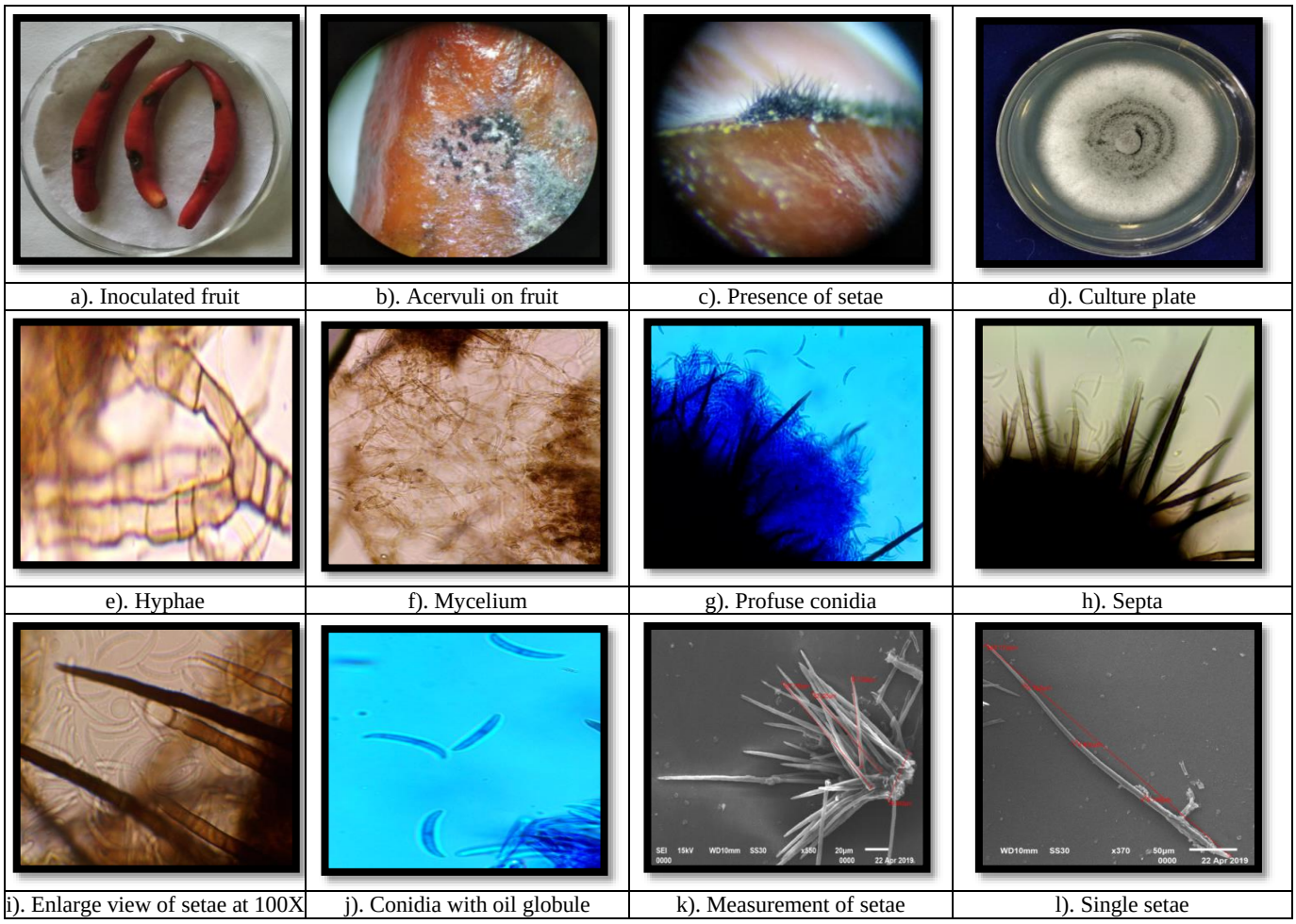


Fig 1: Morphological characteristics of the *C. capsici*

4.3 Effect of different media on radial growth of *C. capsici*

The data presented in table 2 revealed that maximum radial growth (90.00 mm) was observed on 9 days in OMA followed by CFDA (88.67 mm) which were statistically at par to each other and differ significantly with rest of media tested. Other media's viz. PDA showed radial growth of 82.67 mm followed by CDA with 80.16 mm, RSA with 69.34 mm where in PDA and CDA were found to be statistically at par with each other. Minimum radial growth of 53.33 mm was observed in RSA. (Figure2).These results are in accordance with the findings of Javed (2014) [9]. who reported that maximum radial growth of colony of *C. capsici* was in Oat Meal Agar and Corn Meal Agar, followed by PDA and Richard's agar whereas Admassie *et al.* (2015) [1] reported that maximum radial growth of *C. capsici* was observed on pepper dextrose agar (prepared from leaves, stems and fruits of pepper) and potato dextrose agar medium. However, these research findings are contradictory with research finding of Akhtar and Singh (2007) [3] who reported that PDA was found best suited media for mycelial growth of *Colletotrichum capsici* and full plate growth (90.00 mm) observed within 9 days followed by YEPD, CEA, WEA and MEA. The reason may be difference in the isolates of *C. capsici* and its selectivity for the media. Growth of mycelium and sporulation are affected by different types of media. Media contains carbohydrate, lipid, protein and elements are basic requirements and needed by the microorganisms to provide energy for biosynthesis and cell maintenance and production of biomass in fungi and growth-associated products requires nutrient-balanced media (Hilton, 1999) [7]. In OMA, presence of high amount of carbohydrate, proteins and lipids as compared to other media which is responsible for fast radial growth of the fungus. Vega *et al.* (2003) [22] reported that some dimorphic fungi require optimal nutrition to produce high biomass, but for sporulation require nutritionally poor media which trigger differentiation of conidia from vegetative growth. In spite of containing parts or chemical substances from the fungus, natural host and natural media do not always elicit the best sporulation and similar results were also observed by Pria *et al.* (1997) [17] and Hanada *et al.* (2002) [6].

Fig 2: Colony characters and radial growth of *C. capsici* on different media.

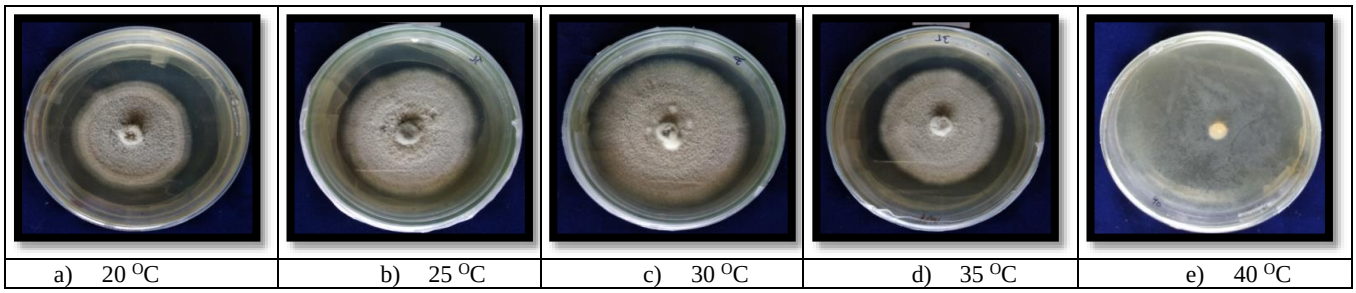
Table 2: Effect of different media on radial growth of *C. capsici*

T.N.	Media	Colony diameter (mm)*
T1	Potato Dextrose Agar Medium (PDA)	82.67
T2	Oat Meal Agar Medium (OMA)	90.00
T3	Chilli Fruit Decoction Agar (CFDA)	88.67
T4	Czapek's Dox Agar Medium (CZPX)	80.16
T5	Malt Extract Agar Medium (MEA)	53.33
T6	Richard's Synthetic Agar Medium (RSA)	69.34
SEm± = 1.11 CD at 5% = 3.42 CV = 2.48		

* represents average of three replication

4.4 Effect of temperature on radial growth of *C. capsici*

The growth rate of the pathogen was recorded at five different temperatures viz; 20, 25, 30, 35 and 40 ºC for 9 days of incubation. The data presented in table 3 revealed that maximum mycelial growth was recorded at 30 ºC (89.87 mm) followed by 25 ºC with 84.83 mm which differ significantly. At 35 ºC mycelial growth of 79.67 mm and at 20 ºC mycelial growth was observed 75.33 mm whereas at 40 ºC, no radial growth was found after 9 days of incubation. All the temperatures were statically significantly different with each other and temperature range of 25-35 ºC is optimum for the mycelial growth of the fungus (Figure 3). These results are confirmative with research findings of Admassie *et al.* (2015) [1], Tripathi *et al*. (2016) [21], Kommula *et al.* (2017) [10] and Akhtar *et al.* (2018) [4] they reported that the maximum mycelial growth of *C. capsici* was found at temperature range between 25-30 °C. Majority of the fungi require optimum temperature ranges of 25 to 30 ºC for their mycelial growth, whereas at high temperature (40 ºC) disintegration of cell wall, protein and enzymes lysis may occur.



a) 20 ^{O}C b) 25 ^{O}C c) 30 ^{O}C d) 35 ^{O}C e) 40 ^{O}C

Fig 3: Effect of different temperature on radial growth of *C. capsici*

Table 3: Effect of different temperature on radial growth of *C. capsici*

Tr. No.	Temperature (ºC)	Colony Diameter (mm)*
T1	20	75.33
T2	25	84.83
T3	30	89.87
T4	35	79.67
T5	40	0.00
SEm± = 0.07 CD at 5% = 0.23 CV = 0.19		

* represents the average of three replication

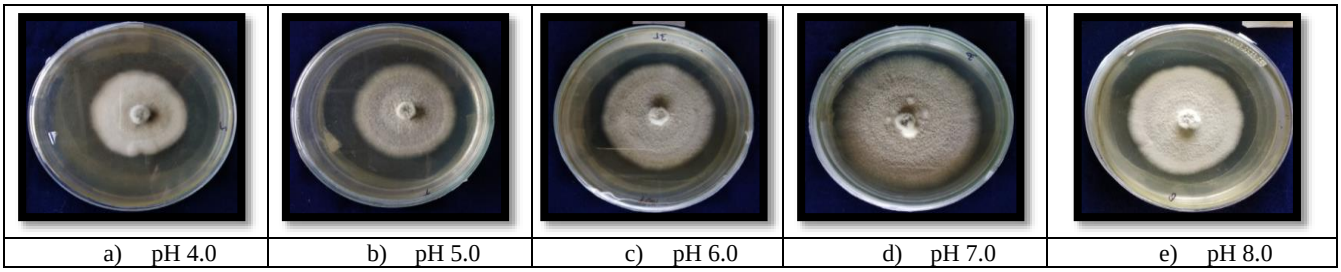
4.5 Effect of pH on the radial growth of the *C. capsici*

In vitro evaluation of five different pH levels viz., 4.0, 5.0, 6.0, 7.0 and 8.0 to find out suitable pH for the radial growth of the pathogen and observation were taken daily for 9 days of incubation. The data presented in table 4 revealed that pH 7.0 was found to be best suited for the growth of the pathogen with a significant maximum colony diameter of 83.67 mm followed by radial growth of 81.00 mm, 78.70 mm and 76.50 mm with pH of 8.0, 6.0 and 5.0 respectively. Least radial growth was recorded at pH 4.0 with 74.33 mm only. Among all pH, the radial growth of the pathogen was found to be statistically significant with each other (Plate 4).The research finding of Akhtar *et al.* (2018) [4] who reported that maximum radial growth (90.00 mm) of pathogen was observed at pH 7.0 followed by 8.0, 6.0, 5.0 and 4.0. Kommula *et al.* (2017) [10] studied the effect of different pH levels viz. 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 and found that maximum growth of the pathogen was observed at pH range of 6.5-7.0 and minimum growth was recorded at pH 3.0. Thus it can be concluded that cultural and morphological characterization of the pathogen provide better understanding about the pathogen biology and etiology through which integrated approach for disease management can be effort.

Table 4: Effect of pH on radial growth of the *C. capsici*

Tr. No	pH	Colony diameter (mm)*
T1	4.0	74.33
T2	5.0	76.50
T3	6.0	78.70
T4	7.0	83.67
T5	8.0	81.00
SEm = 0.15 CD at 5% = 0.48 CV = 0.32		

*represents the average of three replication



a) pH 4.0 b) pH 5.0 c) pH 6.0 d) pH 7.0 e) pH 8.0

Fig 4: Effect of pH on radial growth of *C. capsici*

5. Summary and Conclusion

The present experiment was conducted to figure out the suitability of culture medium, pH and temperature for the mycelial growth of the pathogen *Colletotrichum capsici* and to study its morphological character. The silent features of experimental findings are summarized here under:

Oat Meal Agar medium (OMA) was found very suitable for mycelia growth of *Colletotrichum capsici* resulting full plate growth (9cm) within 9 days followed by Potato Dextrose Agar medium, Chilli fruit decoction Agar medium, Czapedox Agar Medium, Malt Extract Agar medium and Richard's Synthetic Agar Medium. The growth of *Colletotrichum capsici* was found to be more vigorous at the temperature 30 °C where maximum growth of the mycelium (89.87mm) was found within 9 days followed by 25 ºC, 35 ºC and 20 ºC. Among the different pH maximum radial growth (83.67mm) was recorded at pH7 within 9 days followed by 8, 6, 5 and 4. pH4 was found least suitable for the growth of *Colletotrichum capsici.*

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