Influence of different culture media on growth and sporulation of *Colletotrichum capsici*

Abstract

Aim: To know the best culture media for growth and development of Colletotrichum capsici causing chilli anthracnose.

Study design: Twelve different culture media were selected and three replications were maintained.

Place and duration of study: Department of Plant Pathology, University of Agricultural Sciences, Raichur, Karnatka, which was carried out in the year 2021-2023.

Methodology: The laboratory study was conducted to know the best nutrient media for the growth and sporulation of the pathogen. The pathogen was isolated from infected chilli fruit using tissue isolation technique and pure culture was maintained. Twelve different nutrient media were selected and tested for the growth and sporulation of the pathogen in three replications.

Results: Among twelve media tested, Red chilli dextrose agar have promoted the maximum growth of *C. capsici* (90.0 mm), which was followed by carrot dextrose agar and potato carrot agar (89.50 mm). Green chilli extract agar significantly promoted minimum growth of *C. capsici* (33.50 mm), which was followed by red chilli extract agar (48.00 mm) and Sarbouraud's agar (48.50 mm). The cultural characters were varied among the media.

Conclusion: Potato dextrose agar and carrot dextrose agar media were proved to be best media for growth and sporulation of *Colletotrichum capsici*.

Keywords: Chilli anthracnose, *Colletotrichum capsici*, culture media, growth, sporulation

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1. INTRODUCTION

As a member of the Solanaceae family, chilli (*Capsicum annuum* L.) is a significant commercial, vegetable, and spice crop in India. It goes by several names, such as cayenne pepper, red pepper, and hot pepper. In the middle of the 17th century, Portuguese settlers in Goa

brought it to India. With its distinctive pungency, color, and aroma, it has quickly spread across the nation and is now a crucial component of Indian cuisine. A major spice crop farmed all over the world, yield loss and seed production is largely affected by biotic and abiotic stresses, which have resulted in decreased production. Plant diseases are among the biotic limitations that significantly contribute to crop losses. In many places of the world, nematodes, bacteria, viruses, or fungi produce plant diseases that impact the output of chilies (Saxena et al., 2016[1]). "Chilli is usually affected by fungal diseases like anthracnose (dieback/fruit rot), Cercospora leaf spot, damping off, wilt, leaf spots, powdery mildew, bacterial diseases like bacterial wilt, soft rot and some viral diseases like *Cucumber Mosaic Virus* (CMV), *Tomato Spotted Wilt Virus* (TSWV), *Tobacco Mosaic Virus* (TMV), *etc"*. (Than et al., 2008[2]). "Among the fungal diseases, anthracnose/ die-back/ fruit-rot of chilli is an economically important disease causing serious losses in field, transit, transport and storage and cause the major share of crop loss" (Saxena et al., 2016[1]). "In India, a calculated loss of 58.6 per cent has been reported in fruit yield due to this disease" (Yadav et al., 2017[3]).

Plant diseases with black, sunken lesions that contain spores are commonly referred to by the term anthracnose, which is derived from a Greek word meaning "coal." (Isaac, 1992[4]). Anthracnose of chilli was first reported from New Jersey, USA, by Halsted (1890) [5] in 1890, who described the causal agents as *Gloeopsorium piperatum* and *Colletotrichum nigrum*. "These taxa were then considered as synonyms of *C. gloeosporioides"* by Von Arx (1957)[6]. "In India the disease was first time reported in Coimbatore of Madras Presidency – India" (Sydow, 1913[7]).

According to Kim et al. (2004[8]), "different species of chilli plants infected at different growth stages causing anthracnose lesions". "Even small anthracnose lesions on chilli fruits reduce their marketable value" (Manandhar et al., 1995[9]). "Leaves and stems are damaged by different species *viz*., *C. coccodes* and *C. dematium* whereas *C. acutatum* and *C. gloeosporioides* infect chilli fruits. There is an enormous variation among species of *Colletotrichum* causing anthracnose disease. Between the species already present, *C. capsici* / *C. truncatum* is a cosmopolitan fungus. In India *C. capsici* is most prevalent in ripen chilli whereas *C. acutatum* and *C. gloeosporioides* induce disease in green and red chilli" (Kim et al., 2004[8]; Than et al., 2008[2]).

Keeping in view the importance of the crop losses caused by this devastating pathogen the present investigation has been carried out on cultural characterization of the *Colletotrichum capsici* causing fruit rot in chilli.

2. Material and Methods

Isolation and maintenance of *Colletotrichum capsici*

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The diseased sample was collected from field and conducted an experiment in the year 2022-23 in the Department of Plant Pathology, University of Agricultural Sciences, Raichur. "To isolate and get the pure culture of *Colletotrichum* spp. causing infection on ripened chilli fruit, the infected plant parts were thoroughly washed under running tap water. The small fragments of tissue (0.5 - 1 cm) had been cut from the border of the healthy and diseased tissue using sterile razor blade. The surface was subsequently sterilized in 1 per cent sodium hypochlorite solution for a minute and three rinses with sterile distilled water. These sterilized fragments were inoculated on Potato Dextrose Agar (PDA) and the inoculated plates were then incubated at 28 ± 1 °C for five to seven days. The hyphal tips growing from infected fragments were observed for the characteristic features of pathogen under light microscope and then transferred to the PDA slants with the help of sterile needle. Thus, the four isolates of the pathogen were obtained from different parts. The isolates were purified by using hyphal tip technique and culture tubes were preserved in a refrigerator at 4 °C for the further use". (Rangaswami, 1972[10])

Cultural and morphological identification

"The fungal pathogens are primarily identified by cultural and conidial characteristics up to the genus level or even up to species level. In order to identify the isolated pathogen, isolates were re-cultured on PDA media followed by the incubation for 10 days. Further, the plates were observed for the colony characters of the different isolates *viz*., type of growth, colour of the mycelium, radial growth, texture, margin of the colony and formation of asexual fruiting bodies. The conidia were harvested from each isolate and mounted in lactophenol and observed for their shape, size and presence of oil globules under light microscope. The production of asexual fruiting body, presence and absence of setae, septation were also recorded. For the appressorium formation, the conidial suspension (10^4 conidia/ ml) was prepared in sterile distilled water and then 10 µl of suspension was taken in cavity slide and placed with cover slip. The slides were kept for incubation under humid moist conditions then observed for the presence of appressorium at 24 h intervals". [15]

Influence of different culture media on growth of *Colletotrichum capsici*

The growth characteristics of *Colletotrichum capsic* were studied using different synthetic, semi-synthetic and non-synthetic media were used. Asthana and Hawker's agar, Czapek's- Dox agar , Sabouraud's agar , Richards' agar medium, Potato Dextrose Agar , Potato Carrot Agar , Carrot Dextrose Agar , Nutrient Agar, Red Chilli Extract Agar, Red Chilli Dextrose Agar, Green Chilli Extract Agar and Green Chilli Dextrose Agar were selected for the study.

Twenty ml of each media was poured in to the sterilized Petri plates and allowed for solidification. Later, plates were inoculated with 5 mm disc, which was cut from the periphery of the actively growing culture and then incubated at 28 ± 1 ºC temperature for nine days. Each treatment was repeated thrice. The observations were drawn for the parameters such as colony diameter, colony colour, production of fruiting bodies and sporulation were recorded.

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Observations were taken when the fungus covered complete Petri plate in any one of the media. The data on radial growth was analyzed statistically using completely randomized design. The sporulation on different media was graded per microscopic field at 40X magnification, according to Purkayastha and Sengupta (1975) [11] as follows,

List 1: The sporulation on different media as per grade

(-)	No sporulation
(+)	Poor sporulation (0-50 conidia)
(++)	Moderate sporulation (51-100 conidia)
(+++)	Good sporulation (101-150 conidia)
(++++)	Excellent sporulation (>150 conidia)

3. Results and Discussion

Isolation and identification of *Colletotrichum capsici*

The pathogen isolated from ripened red chilli fruit appeared initially as whitish cottony growth which later on turned grey to black in colour (Fig. 1). "The growth rate was 8.33 mm/day at 27 °C on PDA. The black coloured asexual fruiting bodies were formed in the concentric rings. The pathogen produced septate mycelium and acervulus with sharp pointed septate setae containing unbranched conidiophores bearing aseptate, hyaline, falcate shaped conidia tapering at one end with single oil globule at the centre. The setae were commonly smooth, septate and light brown to dark brown in colour, with cylindrical to conical base and sometimes slightly inflated and the tips were acute to roundish" (Fig. 1). [15] Black colored appressorium with entire margin or slightly irregularly lobed were observed. The fungus was found to produce circular sclerotial bodies. The sexual fruiting bodies *i. e.*, perithecia were formed at later stages with unicellular, hyaline slight sickle shaped ascospores (Fig. 1).

Veerendra et al. (2017) [12]. They observed that "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 on 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

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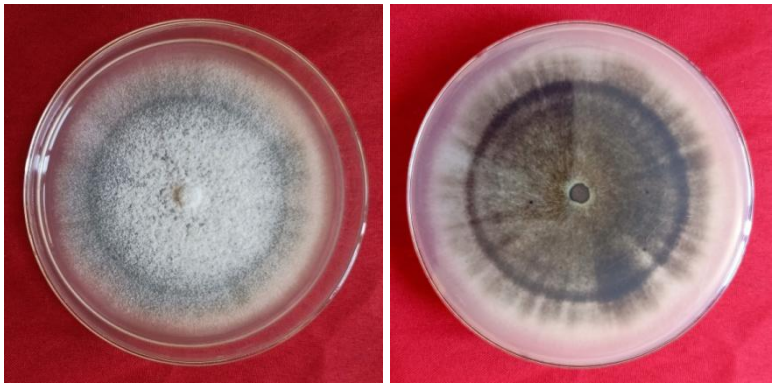
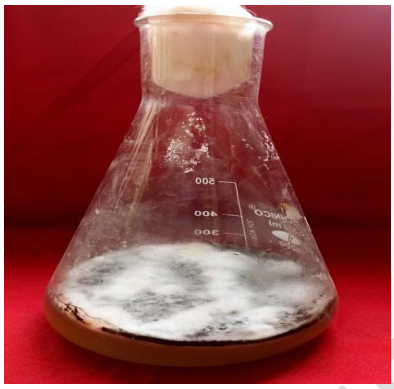
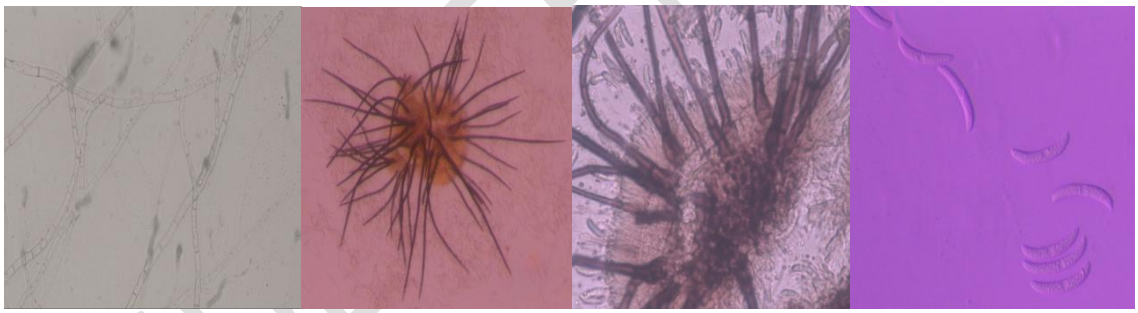


Growth of *Colletotrichum capsici* on PDA

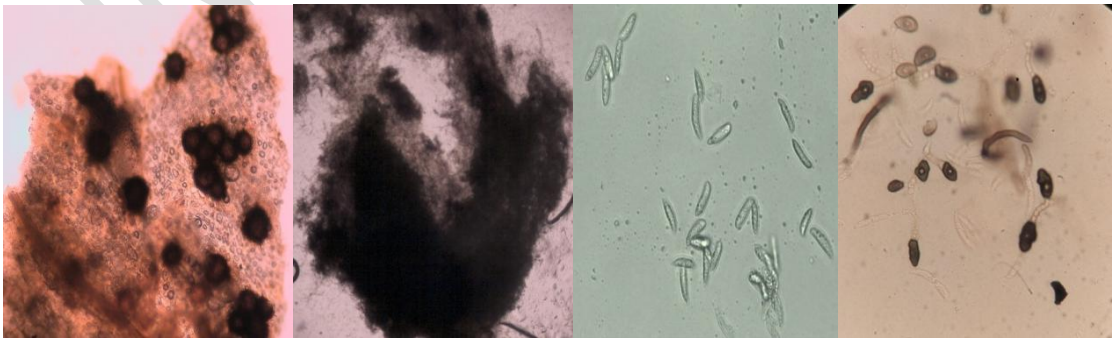


Growth on PDB

Conidial mass on Petri plate



Mycelium (40X) Acervulus (40X) Conidiophores and setae (40X) Conidia (40X)



Sclerotial bodies (40X) Perithecium (40X) Ascospores (40X) Appressorium (40X)

Fig. 1: Cultural and morphological features of *Colletotrichum capsici*

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possessed large oil globule in the centre, Acervuli contained abundant dark brown needle like septate setae with several septations and pointed brown tips.

Influence of different media on growth of *Colletotrichum capsici*

All the tested media have some influence on the radial growth of *C. capsici*, as it varied from 33.50 mm to 90.00 mm. Red chilli dextrose agar have promoted the maximum growth of *C. capsici* (90.0 mm), which was followed by carrot dextrose agar and potato carrot agar (89.50 mm). These media have no significant influence on the growth. Green chilli extract agar significantly promoted minimum growth of *C. capsici* (33.50 mm), which was followed by red chilli extract agar (48.00 mm) and Sarbouraud's agar (48.50 mm) which was on par with each other (Table 1). Red chilli dextrose agar well supported the growth of *C. capsici*, which was sparse, flattened with entire margin and appeared as grayish black in converse and black in inverse side of the Petri plate. It also supported the production of maximum number of acervuli which were arranged irregularly throughout the plate and enhanced the sporulation. Whereas in carrot dextrose agar and potato carrot agar, the cottony, flattened growth with entire margin showing grayish black colour in converse and black colour in inverse side of Petri plate was observed. These media also supported the maximum production of acervuli which were arranged irregularly throughout the plate having excellent sporulation. Similarly, there was a slight change in the growth of fungus in potato dextrose agar, which have shown diurnal zonations in the colony with entire margin and appeared as grayish white in the converse side and black colour in the inverse side. This medium also enhanced the excellent sporulation with moderate number of acervuli which were arranged in concentric circles. Whereas, the colony appeared as creamy white and creamy black in converse and inverse, respectively with sparse flattened growth having filiform margin in green chilli extract agar. This medium was not efficiently enhanced the production of acervuli with moderate sporulation (Table 1 and Fig. 2).

Similarly, Tripathi (2016) [13] used different media to test their efficacy on growth of *C. capsici*. Among which, potato dextrose agar supported significantly the maximum growth of the fungus followed by Richard's medium. Asthana and Hawker's and Czapek's Dox agar promoted fair growth of the fungus. According to Akhtar et al. (2018) [14] the growth of *C. capsici* was fast in PDA media attaining full growth of 9 cm in nine days followed by Chilli Extract Agar medium.

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Table 1: Colony characteristics of *Colletotrichum capsici* on different solid media

Sl . No.	Culture media	Growth (mm)	Colony colour		Type of growth	Colony margin	Arrangement of acervuli	Sporulation**
			Converse	Inverse				
1	Asthana's agar	87.50[ab]*	Milky white	Whitish black	Fluffy, raised	Entire	Irregularly in aggregation	+++
2	Czapek- Dox agar	86.00[bc]	Milky white	Whitish brown	Cottony, slightly raised	Entire	At the centre	++
3	Richard's agar	85.00[bc]	Grayish black	Black	Sparse	Entire	At the centre irregularly	+++
4	Sabouraud's agar	48.50[d]	Creamy white	Whitish brown	Cottony, flattened	Irregular	Very sparse at the centre	+
5	Potato dextrose agar	87.50[ab]	Grayish white	Black	Diurnal zonations	Entire	In concentric rings	++++
6	Carrot dextrose agar	89.50[a]	Grayish black	Black	Cottony, flattened colony	Entire	Irregularly throughout the plate and maximum at the margin	++++
7	Potato carrot agar	89.50[a]	Grayish black	Black	Cottony, flattened	Filiform	Throughout the plate	++++
8	Nutrient agar	84.50[c]	Creamy white	Creamy white	Sparse , flattened	Filiform	Irregularly at the centre	+
9	Green chilli extract agar	33.50[e]	Creamy white	Creamy black	Sparse, flattened	Filiform	In concentric rings at the centre	++
10	Green chilli dextrose agar	89.00[a]	Grayish black	Black	Cottony, flattened	Entire	Irregularly maximum at the centre	+
11	Red chilli extract agar	48.00[d]	White	Creamy	Sparse, flattened	Entire	Sparsely at the centre	+
12	Red chilli dextrose agar	90.00[a]	Grayish black	Black	Sparse, flattened	Entire	Irregularly throughout the plate	++++
S. Em ±		**0.67**						
CD at 1%		**2.67**						

**Mean of three replications; **(-) no sporulation, (+) poor sporulation, (++) moderate sporulation, (+++) good sporulation, (++++) excellent sporulation*

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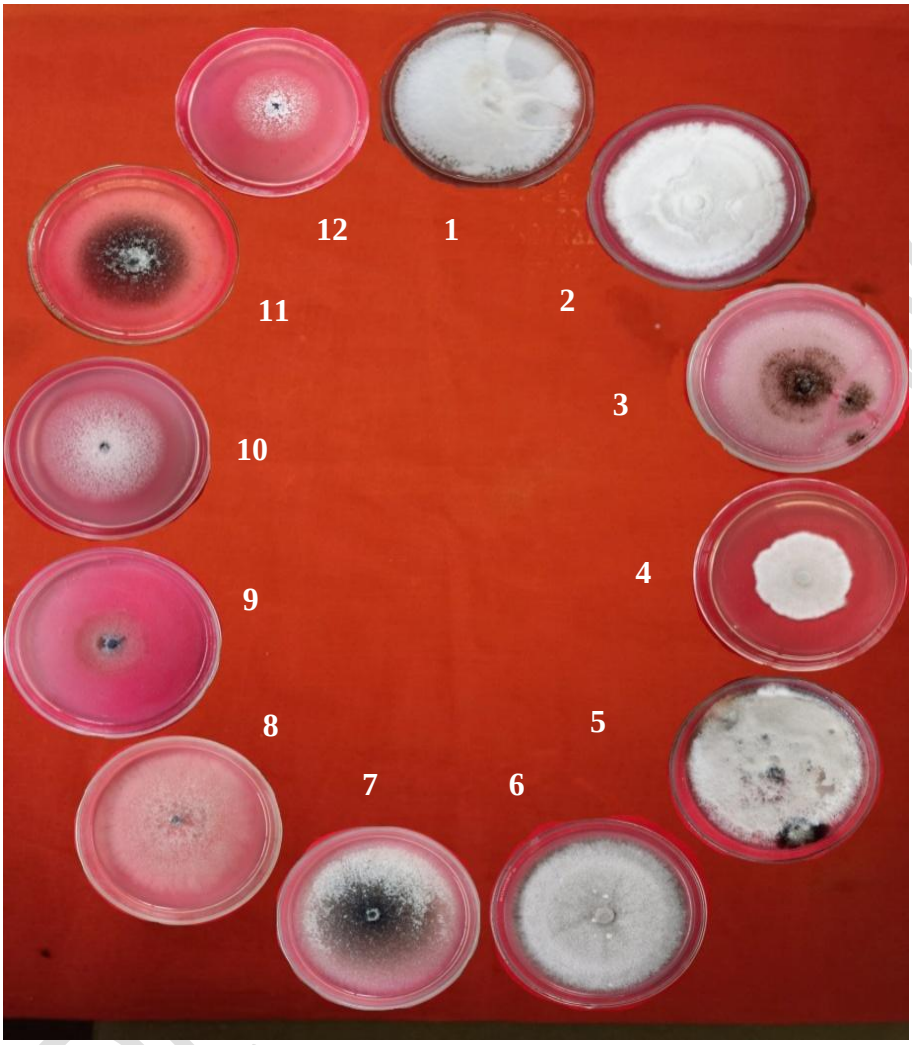

Fig. 2: Growth of *Colletotrichum capsici* on different solid media

1) Asthana's agar; 2) Czapek- Dox agar; 3) Richard's Agar; 4) Sabouraud's agar; 5) Potato dextrose agar; 6) Carrot dextrose agar; 7) Potato carrot agar; 8) Nutrient agar; 9) Green chilli extract agar; 10) Green chilli extract dextrose agar; 11) Red chilli extract agar; 12) Red chilli extract dextrose agar.

CONCLUSION

For the growth and development of any pathogen under laboratory conditions majorly depends on the nutrient media. *Colletotrichum capsici* has grown best in potato dextrose agar and carrot dextrose agar media. These two media were found to be the best for *C. capsici*.

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