

Cultural and Physiological Requirements of *Colletotrichum truncatum* causing Soybean Pod Blight

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ABSTRACT: Fungi are heterotrophic organisms that rely on external sources of sustenance for their growth, reproduction, and survival. It is critical to provide all of the necessary nutrients in the form of media in order to grow fungus in a laboratory setting. Temperature and relative humidity are crucial external factors to consider. Temperature has an impact on practically every function of fungi, including growth and sporulation, and relative humidity is crucial in the progression of the disease into epidemic form. At the Department of Plant Pathology, UAS Raichur, an experiment was done to investigate the effects of different medium, temperature, and relative humidity on the growth of *Colletotrichum truncatum*, which causes soybean pod blight. Solid media PDA was found to be best for good growth subsequently oat meal agar with excellent sporulation. Among liquid media tested, Richard's broth was found significantly superior over other broths with dry mycelial weight of 427.67 mg, followed by potato dextrose broth. Temperature of 25 and 30° and relative humidity of 80, 85 and 95% were optimum for growth and sporulation of the pathogen.

Keywords: Soybean, pod blight, temperature, relative humidity, solid media, liquid media.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) also known as "Golden Nugget" a miracle crop, valued for its high protein and oil content. It has maximum protein content among leguminous crops belonging to the family leguminaceae. Soybean, occupies a premier position as a world crop because of its high and virtually unrivalled protein (36 %), carbohydrate (35 %) (17 % of which dietary fiber) with edible vegetable oil content (19 %), minerals (5 %) and several other components including vitamins (Ajay *et al.*, 2011). The protein quality of soybean is equivalent to that of meat, milk and eggs. It is cheapest source of proteins, hence called "poor man's meat". Soybean is renowned as the "Wonder Crop" because it is the richest, cheapest, and easiest source of high-quality protein and lipids, with a wide range of applications in food and industry. The crop is grown on 11.33 million hectares in India, with a production of 13.79 million tonnes and a productivity of 1217 kg/ha (Anon., 2019). Soybean is produced on 0.29 million hectares in Karnataka, with a production of 0.29 million tonnes and a productivity of roughly 1008 kg/ha (Anon., 2019).

Plant health is an important factor in ensuring a profitable soybean harvest. One of the greatest roadblocks to enhancing soybean productivity is its sensitivity to a wide range of fungal infections.

Anthraco-nose (pod blight) produced by *C. truncatum* has been described as the primary restriction in successful soybean cultivation among the major fungal diseases (Khan and Sinclair, 1992; Mittal *et al.*, 1993). In 1917, Korea was the first country to report the sickness.

Fungi are heterotrophic organisms that rely on external sources of sustenance for their growth, reproduction, and survival. It is critical to provide all of the necessary nutrients in the form of media in order to grow fungus in a laboratory setting. Temperature and relative humidity are crucial external factors to consider. Temperature has an impact on practically every function of fungi, including growth and sporulation, and relative humidity is crucial in the progression of the disease into epidemic form. At the Department of Plant Pathology, UAS Raichur, an experiment was done to investigate the effects of medium, temperature, and relative humidity on the growth of *Colletotrichum truncatum*, which causes soybean pod blight. The finest solid and liquid were discovered to be potato dextrose agar and Richard's broth.

C. truncatum can infect soybeans at any stage of development, but especially from bloom to pod fullness. Symptoms of the illness emerge as irregularly shaped brown lesions on the stem, pods, leaves, and petioles during the early reproductive stages (Sinclair and Backman, 1989). The pod blight phase, on the other

hand, is the most harmful (Vyas *et al.*, 1997). In comparison to non-affected plants, plants affected by anthracnose/pod blight disease are much shorter, have fewer pods and seeds, and have a lower seed weight. Brown discoloration is seen in infected seeds. *C. truncatum* seed infection was found in up to 70% of samples with up to 30% seed discoloration (Hepperly *et al.*, 1983).

Information on the cultural characterization and physiological requirements of the pathogen such as, temperature and relative humidity which are essential factors for the growth of any fungi. Hence, generating the information on these factors will aid in formulating the effective management strategy and also, aid in finding out the nutritional requirements and ideal environmental conditions for the growth of *C. truncatum*, the causal agent of soybean anthracnose.

MATERIAL AND METHODS

A. Isolation and identification of the pathogen

Samples of the pod blight disease were taken from a farmer's field (Bidar isolate). The pathogen was isolated using the standard tissue isolation technique. Furthermore, using the single spore isolation approach, a pure culture of the fungus was obtained. The pure culture was kept in test tubes on agar slants and stored in the refrigerator for further research.

B. Cultural studies

(i) **Growth characters of *C. truncatum* on solid and liquid media.** The growth, sporulation and cultural

Sr. No.	Score	Grade	Description (conidia/ microscopic field [10X])
1.	++++	Excellent sporulation	>150
2.	+++	Good sporulation	101-150
3.	++	Fair sporulation	51-100
4.	+	Poor sporulation	50
5.	-	No sporulation	-

(C) Physiological studies

(i) **Effect of temperature and relative humidity (RH) levels on growth and sporulation of *C. truncatum*.** *C. truncatum* was grown at 10, 15, 20, 25, 30, 35, and 40 degrees Celsius, with relative humidity values of 75, 80, 85, 90, and 95 percent maintained in desiccators. In Petri plates, PDA was poured. After solidification, a 5 mm disc from an actively growing 7- 9 day old culture was injected into solidified Petri plates and cultured for nine days in incubators set to the proper temperature ranges, with each treatment being reproduced three times. Using varied concentrations of H₂SO₄, different amounts of relative humidity were achieved. With four replications, the desiccators were kept at 27°C. Radial growth and sporulation from solids after the incubation period.

RESULTS AND DISCUSSION

A. Isolation and identification

The pathogen was isolated from pods (blighted) on potato dextrose agar (PDA) medium. The pathogen identification was carried out based on cultural and

characters of *C. truncatum* was studied on different synthetic and non-synthetic media. The media used are listed below

Synthetic media	Non synthetic media
Asthana and Hawker's agar	Carrot agar
Czapek's Dox agar	Carrot dextrose agar
Richard's agar	Host leaf extract agar
Sabourd's agar	Malt extract agar
V- 8 juice agar	Oat meal agar
Water sucrose agar	Potato dextrose agar

To conduct the study, all of the media were sterilised. In 90 mm Petri plates/100 ml conical flasks, 20 ml of each medium was poured. Under aseptic circumstances, 5 mm discs cut from the periphery of an actively growing fungal culture were injected into Petri plates/conical flasks and incubated at (27 1°C). Each treatment was tested three times. When fungus covered the entire Petri plate in any of the solid media, observations were taken. In liquid media, dry mycelial weight and sporulation in each treatment were recorded, as well as colony diameter (mm), colony colour, growth pattern, type of margin, colony form, colony texture, and sporulation. The radial growth and dried mycelial weight data were examined.

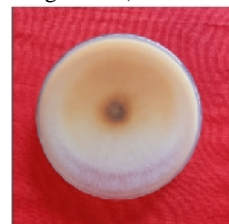
The composition and preparation of different liquid media used were same as that of solid media except that agar was not added.

Sporulation was graded as follows

morphological characters. The colonies of *C. truncatum* were whitish coloured on upper side of the Petri plate and orange coloured at underside with blackish center (Plate 1).



Mycelial growth (creamish white)



Orange pigmentation with blackish center (Inverse side)

Plate 1. Pure culture of *Colletotrichum truncatum*.

B. Cultural studies

(i) **Growth characters of *C. truncatum* on solid media and liquid media.** (Table 1) shows the results of mycelial growth and sporulation of the pathogen on various solid media. The highest growth was produced in PDA and V-8 juice agar with 90 mm of mycelial

growth, followed by oat meal agar and Sabour's agar with 86.83 mm of mycelial growth, which are significantly superior to all other treatments and on par with each other. Richard's agar showed the smallest radial development of 55.83 mm, while water sucrose agar showed no pathogen growth (Plate 2).

Table 1: Effect of different solid media on mycelial growth and sporulation of *C. truncatum*.

Sr. No.	Media	Mean colony diameter (mm)	Sporulation
1.	Asthana and Hawkers agar	63.67*	+++
2.	Carrot agar	82.50	++++
3.	Carrot dextrose agar	83.83	++++
4.	Czapek's dox agar	82.33	++
5.	Host leaf extract agar	83.67	++
6.	Malt extract agar	71.83	++++
7.	Oat meal agar	86.83	++++
8.	Potato dextrose agar	90.00	++++
9.	Richard's agar	55.83	++++
10.	Sabour's agar	86.83	+++
11.	V-8 juice agar	90.00	-
12.	Water sucrose agar	0.00	-
S. Em ±		1.19	
C.D at 1%		4.72	

* Mean of three replications

Sporulation: +++++ = Excellent; ++++ = Good ; +++ = Fair; ++ = Poor; - = No sporulation

Excellent sporulation of the pathogen was observed in carrot agar, carrot dextrose agar, malt extract agar, oat meal agar, potato dextrose agar and Richard's agar with more than 150 spores/ microscopic field (10X) followed by Asthana and Hawker's agar and Sabour's agar with good sporulation of 101-150 spores/ microscopic field (10X) and fair sporulation was found in Czapek's Dox agar and host leaf extract agar with 51- 100 spores/ microscopic field (10X) and there was no sporulation in V-8 juice agar.

Among the various media used for growth and sporulation *C. truncatum*, PDA was found to be best for good growth subsequently oat meal agar with excellent sporulation. Least growth was produced in water sucrose agar and there was no sporulation in V-8 juice agar.

The observations on cultural characters of *C. truncatum* studied on 12 different solid media is represented in Table 2 and Plate 2. The colony colour varied from dull white (carrot agar, carrot dextrose agar, host leaf extract agar and malt extract agar) to white (Asthana and Hawker's agar, Czapek's Dox agar, oat meal agar,

PDA, Richard's agar, Sabour's agar and V-8 juice agar) on different media tested. In respect of colony shape, the pathogen exhibited regular (carrot dextrose agar, Czapek's Dox agar, oat meal agar, potato dextrose agar, Richard's agar, Sabour's agar and V-8 juice agar) and irregular (Asthana and Hawker's agar, carrot agar, host leaf extract agar and malt extract agar) and on majority of media the pathogen showed feathery type colony growth pattern whereas circular type of colony growth pattern was produced in oat meal agar, PDA, Sabour's agar and V-8 juice agar. The colony margin was smooth in Czapek's Dox agar, oat meal agar, PDA, Sabour's agar and V-8 juice agar and the pathogen produced serrated type of margin in remaining media. The pathogen produced varied colony texture ranging from appressed and fluffy (Asthana and Hawker's agar, carrot agar and Richard's agar), appressed (carrot dextrose agar), fluffy (Czapek's Dox agar, oat meal agar, PDA, Sabour's agar and V-8 juice agar) and scanty (host leaf extract agar and malt extract agar).

Table 2: Cultural characters of *C. truncatum* on different solid media.

Sr. No.	Name of the media	Colony shape	Growth pattern	Colony colour	Colony margin	Colony texture/ type of margin
1.	Asthana and Hawkers agar	Irregular	Feathery	White	Serrated	Appressed and fluffy
2.	Carrot agar	Irregular	Feathery	Dull white	Serrated	Appressed and fluffy
3.	Carrot dextrose agar	Regular	Feathery	Dull white	Serrated	Appressed
4.	Czapeks dox agar	Regular	Feathery	White	Smooth	Fluffy
5.	Host leaf extract agar	Irregular	Feathery	Dull white	Serrated	Scanty
6.	Malt extract agar	Irregular	Feathery	Dull white	Serrated	Scanty
7.	Oat meal agar	Regular	Circular	White	Smooth	Fluffy
8.	Potato dextrose agar	Regular	Circular	White	Smooth	Fluffy
9.	Richards agar	Regular	Feathery	White	Serrated	Appressed and fluffy
10.	Sabourds agar	Regular	Circular	White	Smooth	Fluffy
11.	V-8 juice agar	Regular	Circular	White	Smooth	Fluffy
12.	Water sucrose agar	-	-	-	-	-

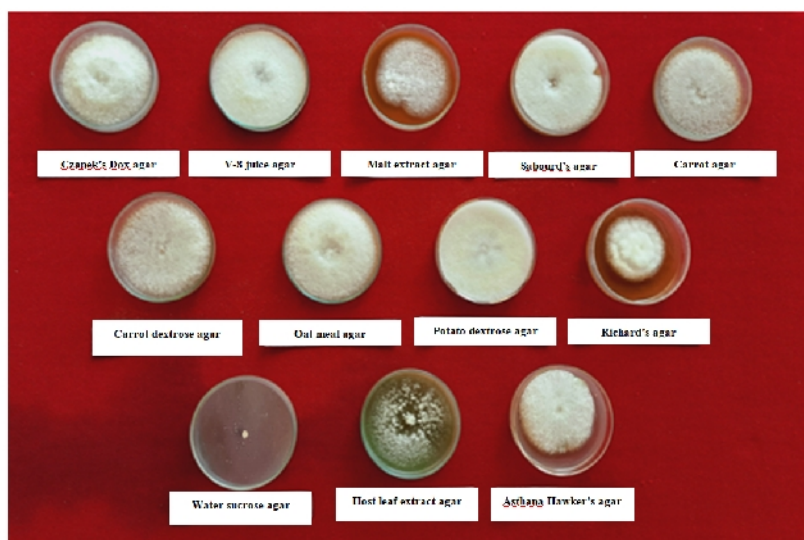


Plate 2. Performance of *Colletotrichum truncatum* on different solid media.

The findings of the study correspond with those of previous researchers that looked into the pathogen's cultural characteristics and produced similar observations. According to Kulkarni and Raja (2019), maximal growth was achieved on PDA and oat meal agars, with great sporulation, white colony colour, and fluffy colonies with smooth edges. PDA and oat meal agar yielded the best growth and sporulation, according to Marak *et al.*, (2019), and colony colour was white. Rajashree (2019) agreed that PDA yielded the best growth and sporulation, with colony colour ranging from dull whitish to white and fluffy to sparse growth with smooth borders. The presence of dextrose as a carbohydrate source, which serves as a growth stimulant, and potato infusion, which offers a nutritious base for luxuriant growth of *C. truncatum* on PDA, is ascribed to good growth of *C. truncatum* on PDA in the current study.

Among the different liquid media tested, Richard's broth with a dry mycelial weight of 427.67 mg, was shown to be significantly superior to other broths, followed by potato dextrose broth, with a dry mycelial weight of 321.67 mg. Malt extract broth and V-8 juice broth yielded significantly fair dry mycelial weights of 188 mg, which were comparable to Czapek's Dox broth (151 mg). With dry mycelial weights of 140.33 and 138.33 mg, carrot dextrose broth and oat meal broth were comparable respectively. Carrot broth and host leaf extract broth had dry mycelial weights of 111.00 and 111.67 mg, respectively, and were comparable. Water sucrose broth produced the lowest dry mycelial weight (11.17 mg), followed by Asthana and Hawker's broth with a dry mycelial weight of 40.50 mg (Table 3 and Plate 3).

Table 3: Influence of different liquid media on dry mycelial weight and sporulation of *C. truncatum*.

Sr. No.	Media	Dry mycelial weight (mg)	Sporulation
1.	Asthana and Hawkers broth	40.50*	-
2.	Carrot broth	111.00	-
3.	Carrot dextrose broth	140.33	-
4.	Czapek's dox broth	151.00	-
5.	Host leaf extract broth	111.67	-
6.	Malt extract broth	188.00	++++
7.	Oat meal broth	138.33	+++
8.	Potato dextrose broth	321.67	++++
9.	Richard's broth	427.67	-
10.	Sabour's broth	71.33	-
11.	V-8 juice broth	188.00	-
12.	Water sucrose broth	11.17	++
	S.Em±	0.98	
	CD at 1%	3.86	

* Mean of three replications

Sporulation: + + + + = Excellent; + + + = Good; + + = Fair; + = Poor; - = No sporulation

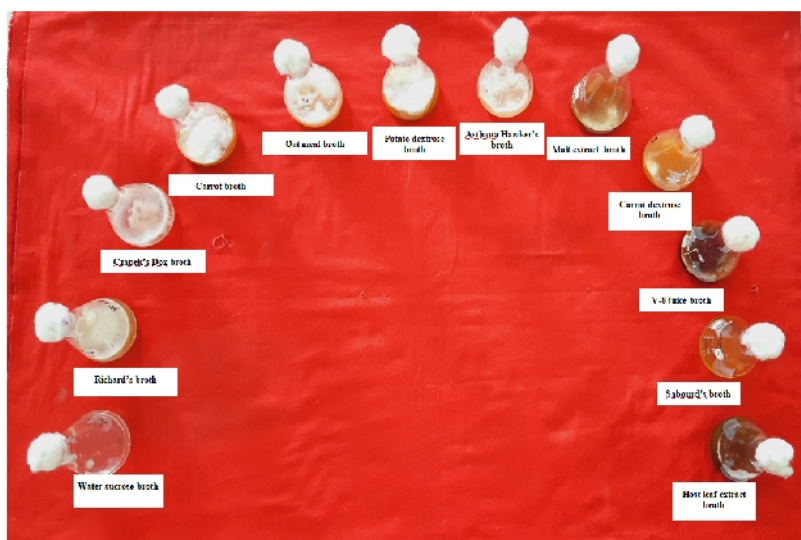


Plate 3. Performance of *Colletotrichum truncatum* on different liquid media.

In malt extract and potato dextrose broths, excellent sporulation was seen, followed by good sporulation in oat meal broth. The water sucrose broth showed good sporulation, while the other broths showed no sporulation.

The outcomes of the current study are in accordance with Kulkarni and Raja (2019) who reported that Richard's broth supported maximum mycelial dry weight (203.17 mg) followed by Czapek's Dox broth (168.30 mg). Ekbote (1994); Mesta (1996); Shirshikar (1995); Angadi (1999); Varaprasad (2000) also reported that maximum growth was observed in Richard's broth. Richard's broth media contains all three major compound for pathogen growth i.e. carbon, nitrogen, phosphate as well as presence of potassium, magnesium and sulphur elements in the media. These elements support the dry weight growth of the fungus.

C. Physiological studies

(i) Effect of temperature and relative humidity (RH) levels on growth and sporulation of *C. truncatum*.

The pathogen was grown on PDA medium at different temperature levels viz., 10, 15, 20, 25, 30, 35 and 40°C in order to know the optimum temperature requirement for its maximum mycelial growth and sporulation (Table 4).

Temperature has a significant impact on the fungi's vegetative and reproductive activity. The effect of temperature on pathogen mycelial development revealed that the pathogen's mycelial growth was maximum at 25°C with 90 mm, followed by 30°C with 87.67 mm, which are significantly superior to all other treatments and on par with each other. Significantly, the minimum mycelial growth of 12.25 mm was reported at 40°C, followed by mycelial growth of 14.33 mm at 35°C, both of which are comparable (Plate 4).

Table 4: Effect of temperature on growth and sporulation of *C. truncatum*.

Sr. No.	Temperature (°C)	Mean colony diameter (mm)	Sporulation
1.	10	64.17*	+
2.	15	73.83	++
3.	20	79.17	+++
4.	25	90.00	++++
5.	30	87.67	++++
6.	35	14.33	+
7.	40	12.25	+
S.Em ±		1.07	
C.D at 1%		4.49	

* Mean of three replications

Sporulation: + + + + = Excellent; + + + = Good; + + = Fair; + = Poor; - = No sporulation

Excellent sporulation was observed at 25 and 30°C and at 20°C there was a good sporulation. Further fair sporulation was observed at 15 °C and poor sporulation was noticed at 10, 35 and 40°C.

Infection and illness development are influenced by temperature. As a result, the research was carried out to determine the ideal temperature for pathogen growth and sporulation. The highest mycelial growth was

observed at 25°C, followed by 30°C, which had excellent sporulation, and the lowest mycelial growth was observed at 40°C. Laxman (2006); Nagaraj (2013); Kulkarni and Raja (2019) found that the optimal temperature for *C. truncatum* growth and sporulation was 25 to 30°C, and that the minimum growth temperature was 40°C.

Generally, an increase in temperature will increase enzyme activity but if temperature get too high enzyme activity will diminish and the protein (the enzyme) will denature. On the other hand, lowering temperature will decrease enzyme activity. Majority of the fungi require

optimum temperature ranges of 25 to 30°C for their mycelial growth, whereas at high temperature (40°C) disintegration of cell wall, protein and enzymes lysis may occur.

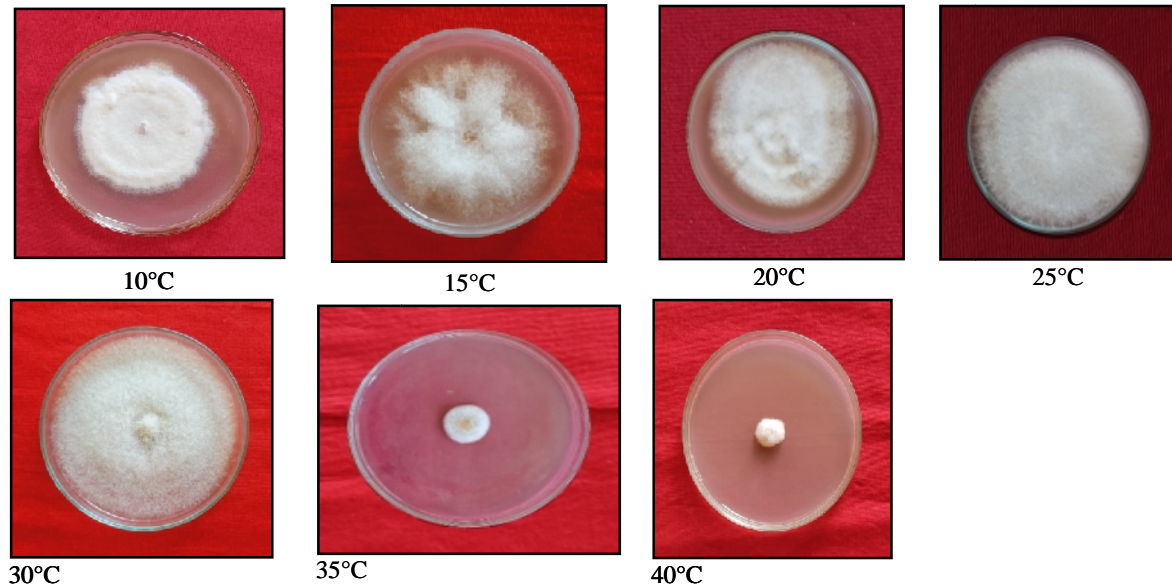


Plate 4. Effect of temperature levels on mycelial growth of *Colletotrichum truncatum*.

Table 5 shows that at 80 percent relative humidity, the highest radial mycelial growth of 90 mm was attained, followed by 90, 85, and 95 percent relative humidity with mycelial growth of 89 mm, 88.75 mm, and 87.12 mm, respectively, which are significantly superior and

on par with each other. At 75.5 percent relative humidity, the minimal mycelial growth of 75.5 mm was achieved, which was considerably different from other treatments (Plate 5).

Table 5: Growth and sporulation of *C. truncatum* at different relative humidity.

Sr. No.	Relative Humidity (%)	Mean colony diameter (mm)	Sporulation
1.	75	75.50*	+++
2.	80	90.00	+++
3.	85	88.75	++++
4.	90	89.00	++++
5.	95	87.12	++++
S.Em ±		1.11	
C.D at 1%		4.64	

* Mean of four replications

Sporulation: ++++ = Excellent; +++ = Good; ++ = Fair; + = Poor; - = No sporulation

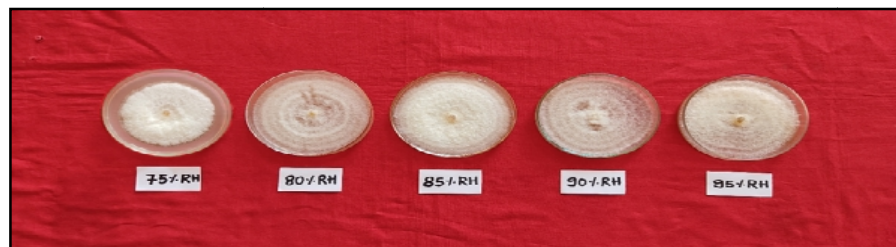


Plate 5. Effect of relative humidity levels on mycelial growth of *Colletotrichum truncatum*.

At 85, 90, and 95 percent relative humidity, excellent sporulation was seen, followed by good sporulation at 75 and 80 percent relative humidity. The relative humidity levels of 80, 85, 90, and 95 percent were shown to be ideal for *C. truncatum* growth and sporulation.

Relative humidity is a significant epidemiological determinant for the pathogen in terms of infection, transmission, and disease outbreak. It is crucial in the progression of the disease into epidemic proportions. The optimal relative humidity range for maximal development and sporulation of *C. truncatum* was

discovered to be 80 to 95 percent in the current study. These findings are nearly identical to those of previous researchers Laxman (2006); Kulkarni and Raja (2019); Rajashree (2019), who found that the optimum relative humidity range for maximal development and sporulation of *C. truncatum* was 85 to 95 percent.

CONCLUSION

According to the results of this study, potato dextrose agar and Richard's broth are the best solid and liquid media for cultivating fungus, respectively. Furthermore, a temperature of 25 °C and a relative humidity of 80% were optimal conditions for the pathogen growth.

Conflict of Interest. None.

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