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Studies on the physiological characteristics of *Colletotrichum truncatum* the causal organism of greengram anthracnose

Sunil Kulkarni and Raja

Abstract
Studies on the physiological characteristics of *Colletotrichum truncatum* causal agent of anthracnose of greengram. Temperature requirement of the fungus was found to 30^0 c where good growth was observed. The fungus exposed to alternate cycles of light and darkness produced maximum growth and sporulation when compared to continuous light and continuous darkness.

Keywords: *Colletotrichum truncatum*, temperature, light and darkness

Introduction
Greengram (*Vogna radiate* L.) is one of the important pulse crops of India. It is quite versatile crop grown for seeds, greengram manure and forage and it is also considered as "Golden Bean". Presently in India greengram is cultivated over an area of 32.99 lakh hectare with a production of 13.74 lakh tones (Rajendra Prasad, 2006). The Hyderabad Karnataka area particularly Bidar and Gulbarga districts has an extensive cultivated area of greengram, pigeonpea and chickpea hence this region are called as "Pulse Bowl" of Karnataka. In Karnataka anthracnose caused by *Colletotrivhum truncatum* (Schw.). Andrus and More is one of the major diseases of greengram. Anthracnose of greengram caused by *Colletotrichum truncatum* has been reported from all regions of India in mild to severe from. It causes considerable damage by reducing seed quality and yield. The fungus derive food and energy from the substrate upon which they grow in nature, in order to culture the fungus in the laboratory, there is no universal substrate or artificial medium upon which all the fungus can grow and reproduce. Further, temperature and light were playing important role in disease development. Therefore studies were conducted in different suitable media to identify surface medium for growth and also an effort was made to know the optimum temperature and light requirements for growth and sporulation of *C. truncatum.*

Materials and Methods
Selection of basal medium for growth and sporulation of the fungus was done by using potato dextrose agar, at meal agar, host extract agar, Czapek's agar, malt extract agar, Sabouraud's yeast extract agar, Richard's agar, potato carrot agar and corn meal agar.

Physiological studies-Effect of temperature on the growth and sporulation of *C. truncatum*
The growth of *C. truncatum* was tested at 10, 15, 20, 25, 30, 35 and 40^0C. Potato dextrose agar was poured into 90 mm diameter petriplates. After solidification, 5 mm disc from actively growing cultures were cut and inoculated to solidified petriplates and incubated for 15 days in the incubators adjusted to required temperature levels. Each treatment was replicated thrice. After incubation period, radial growth and sporulation from solid media were recorded as described earlier.

Effect of relative humidity (RH) on growth and sporulation of *C. truncatum*
Five mm discs of ten-day old culture of *C. truncatum* of greengram were placed at the centre of petridish containing PDA media under aseptic condition and petridish were exposed to 65, 75, 85, 95 and 100 per cent relative humidity levels maintained in desicators. Different levels of relative humidity were created by using different concentration solutions of H_2SO_4. The desicators were kept at 27 $\pm$ 1^0C with four replications. Observations of colony diameter and sporulation were recorded 13 days after incubation.

Effect of different pH on growth and sporulation of *C. truncatum*

Colletotrichum truncatum was grown on 30 ml potato dextrose broth with selected pH range of 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. The pH levels were adjusted by adding 1 N alkali (NaOH) or acid (HCl). A seven-day old five mm mycelial disc from actively growing culture were inoculated separately into conical flasks containing 30 ml medium at different pH levels. Three replications were maintained for each pH level. These flasks were incubated at 27 $\pm$ 1^{0}C for 10 days. The mycelial growth was harvested and dried in hot air oven and the dry weights were recorded by using electronic digital balance and sporulation was recorded as described earlier.

Effect of light intensities on growth and sporulation of *C. truncatum*

The effect of light intensities on the growth and sporulation of *C. truncatum* was studied by exposing the culture to following treatments.

1) Alternate period of 12 hr light under day light tubes and 12 hr darkness.
2) Continuous light under day light tubes.
3) Continuous darkness.

Twenty-one petridishes were prepared with 20 ml of potato dextrose agar medium in each. The petridishes were inoculated aseptically with 5 mm mycelial disc from ten days old culture. Seven replications were maintained for each treatment. The petridishes of each treatment were exposed to alternate period of 12 hr light under day light tubes and 12 hr of darkness, continuous light under day light tubes and continuous darkness for eight days. Observations on fungal growth and sporulation were recorded.

Result and Discussion

Physiological studies- Effect of temperature on the growth and sporulation of *C. truncatum*

The fungus *C. truncatum* was grown on potato dextrose agar medium at different temperatures *viz.*, 10, 15, 20, 25, 30, 35 and 40^{0}C to know the suitable temperature requirement for their maximum radial growth and sporulation (Table 1).

Data from Table 1 revealed that, maximum mean colony diameter of fungus at temperatures of 30^{0}C (80.17 mm) and 25^{0}C (79.77 mm) was significantly superior over all other temperatures and these were on par with each other. Lowest mean colony diameter was obtained at temperatures of 10^{0}C (23.47 mm) and 40^{0}C (21.30 mm) which were on par with each other (Plate 1).

Plate 1: Effect of temperature (%) on mycolial growth of C. frun Caturn

Excellent sporulation was observed at both 25 and 30^{0}C. Further, good sporulation was at 20^{0}C and fair at 35^{0}C, while in 10, 15 and 40^{0}C temperatures poor sporulation was seen.

Table 1: Effect of temperature on growth and sporulation of *Colletotrichum truncatum*

Sl. No.	Temperature (°c)	Mean colony diameter (mm)	Sporulation
1	10	23.47	+
2	15	35.30	+
3	20	50.33	+ + +
4	25	79.77	+ + + +
5	30	80.17	+ + + +
6	35	60.23	+ +
7	40	21.30	+
S.Em±		0.53	
CD at 1%		2.29	

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

Effect of relative humidity (RH) on the growth and sporulation of *C. truncatum*

The data pertaining to effects of relative humidity levels for the growth and sporulation are presented in Table 2.

The radial growth and sporulation of the fungus was observed maximum at 95.00 per cent relative humidity (85.05 mm), which was on par with 85.00 per cent relative humidity (83.68 mm). Lowest colony diameter was obtained at RH level of 65.00 per cent. Relative humidity levels of 85 and 95 per cent were found to be favourable for the excellent sporulation of fungus followed by good sporulation at 75 per cent RH (Plate 2).

Plato 2: Effect of relative humidity on mycelia! growth of C *truncatum*

Table 2: Effect of relative humidity % on growth and sporulation of *Colletotrichum truncatum*

Sl. No.	Relative Humidity (%)	Mean colony diameter (mm)	Sporulation
1	65	60.78	+ +
2	75	74.13	+ + +
3	85	83.68	+ + + +
4	95	85.05	+ + + +
5	100	64.15	+ +
S.Em±		0.59	
CD at 1%		2.56	

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

Effect of hydrogen ion concentration (pH) on the growth and sporulation of *C. truncatum*

The fungus was grown in Richard's medium at different pH levels and observations on dry mycelial weight and sporulation was taken as described in "Material and Methods" and data are presented in Table 3.

Data from Table 3 revealed that, *C. truncatum* grew well at 6.5 pH with maximum dry mycelial weight of 103.27 mg and was significantly superior over other pH levels followed by pH level of 6.0 (95.53 mg). At pH 5.5 and 7.0, it was on par with each other. The least mycelial weight was obtained at pH of 8.0 (49.50 mg). The fungus responded with excellent sporulation at 6.0 and 6.5 pH levels. Whereas, at 5.5 and 7.0 pH levels, it showed good sporulation. Poor sporulation was noticed at pH levels of 4.0, 4.5 and 8.0 (Plate 3).

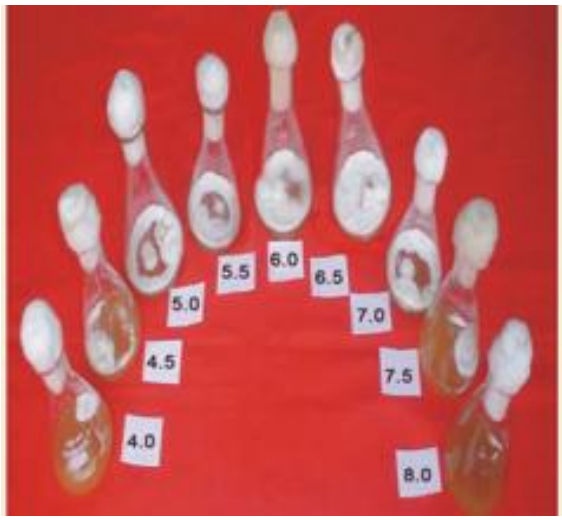

Plate 3: Effect of pH on dry mycelia' weight of *C truncatum*

Table 3: Effect of pH on dry mycelial weight and sporulation of *Colletotrichum truncatum*

Sl. No.	pH level	Mean mycelial dry weight (mg)	Sporulation
1	4.0	52.47	+
2	4.5	73.33	+
3	5.0	80.37	+ +
4	5.5	92.38	+ + +
5	6.0	95.53	+ + + +
6	6.5	103.27	+ + + +
7	7.0	91.30	+ + +
8	7.5	78.40	+ +
9	8.0	49.50	+
S.Em±		0.56	
CD at 1%		2.33	

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

Table 4: Effect of light on the growth and sporulation of *Colletotrichum truncatum*

S. No.	Light duration	Mean colony diameter (mm)	Sporulation
1	Continuous dark	70.27	+ + + +
2	Continuous light	61.97	+ + +
3	Alternate cycles of light & dark	82.50	+ + + +
S.Em±		0.40	
CD at 1%		1.73	

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

Effect of light intensities on the growth and sporulation of *C. truncatum*

The effect of light on the fungal growth and sporulation was studied. The results are presented in Table 4.

The data presented in Table 4 indicated that exposure of petridishes to alternate cycle of 12 hr light under day light tubes and 12 hr darkness resulted in maximum mycelial growth of the fungus to the tune of 82.50 mm as well as excellent sporulation. Similarly, exposure of petridishes to continuous darkness resulted in production of mean colony diameter of 70.27 mm with excellent sporulation. The least mean colony diameter of 61.97 mm was observed in the petridishes which was exposed to continuous light with good sporulation. All the treatments differed significantly (Plate 4).

Temperature plays an important role in infection and disease development. An effort was made to know the optimum temperature for the growth and sporulation. In the present study, it was observed that maximum fungal growth and sporulation was recorded at 30^0C followed by 25^0C and it was least at 40^0C. The present results are in agreement with the results obtained by Chowdhury (1957) [2] and Wong *et al.* (1983) [10]. Similarly, Singh and Shukla (1986) [8] and Laxman (2006) [4] reported that optimum temperature for growth and sporulation of *C. truncatum* was 25^0C to 30^0C.

In the present study, the excellent fungal growth and sporulation was observed at 30^0C followed by 25^0C. Hence, the temperature range of 25 to 30^0C can be recommended to obtain excellent fungal growth and sporulation of *C. truncatum*.

Relative humidity is another important epidemiological factor for influencing fungus as well as the outbreak of the disease. It plays a vital role in development of the disease into an epidemic form. The maximum growth of fungus was noticed at 95 per cent RH (85.05 mm) whereas the minimum mycelial growth was found at 65 per cent RH (60.78 mm). The sporulation was excellent at 85 and 95 per cent RH levels,

which play a key role during the disease initiation and dispersal of disease. Similarly, Shirshikar (1995) [6], Varaprasad (2000) [9] and Laxman (2006) [4] reported that maximum growth of fungus was observed at 85 and 95 per cent RH levels.

Any living organism requires a particular medium for the growth and development. The pH of the media should be optimum for growth. A wide range of pH supported the growth of *C. truncatum*. Good growth was found at a range of 5.5 to 7.0 pH. The sporulation was also influenced by the pH and is known to play a crucial role. In present investigation, the excellent sporulation was found in 6.0 and 6.5 pH and good sporulation was noticed at 5.5 and 7.0 pH. The optimum pH range was obtained towards acidic pH side and sudden decline were observed towards basic pH side which indicated that fungus was acid tolerant. Cochrane (1958) and Bilgrami and Verma (1978) [1] also opined that in contrast to bacteria and actinomycetes fungi are relatively more tolerant to acid ions (H^+) than basic ions (OH^-). The observations are in agreement with those of Singh and Shukla (1986) [8], Shirshikar (1995) [6], Mesta (1996) [5] and Laxman (2006) [4].

In the present study, the maximum dry weight was recorded at 6.5 pH. However, optimum growth of fungus was also observed at pH of 5.5 to 7.0. Hence, it can be recommended that to obtain good growth of *C. truncatum*, the pH level from 5.5 to 7.0 can be maintained in the culture.

Light is also playing an important role in disease development. In the present study, the results revealed that alternate cycles of light and darkness, as well as the continuous darkness supported maximum growth and excellent sporulation of the pathogen. However, maximum fungal growth was recorded in the treatment of alternate cycles of light and darkness. The present findings are in agreement with the studies conducted by Wong *et al.* (1983) [10], Sinclair (1988), Shirshikar (1995), Mesta (1996) [5] and Varaprasad (2000) [9]. Based on the findings, it can be recommended that for obtaining maximum fungal growth and sporulation, *C. truncatum* culture should be exposed to alternate cycles of 12 hr day light under day light tubes and 12 hr darkness.

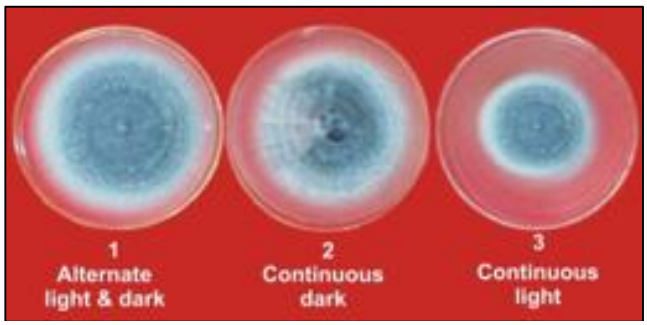

Plate 4: Effect of light on mycelia' Grouth of C truncatum

Conclusions

The optimum range of temperature and pH levels for the fungus were 25^0C to 30^0C and 5.5 to 7.0, respectively. However, maximum growth and sporulation of fungus was recorded at 30^0C temperature and 6.5 pH. In studies on relative humidity and light requirements for growth and sporulation of *C. truncatum* in culture, it was found that relative humidity of 85 to 95 per cent with alternate cycles of 12 hr day light under day light tubes and 12 hr darkness supported good growth and sporulation. The aerobiological studies on effect of weather factors on the development of spore load of *C. truncatum* indicated that more conidial counts were observed during last week of July and first week of August, which coincided with the critical stages of infection.

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