Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

**E-ISSN:** 2278-4136
**P-ISSN:** 2349-8234
JPP 2019; 8(2): 2366-2369
Received: 07-01-2019
Accepted: 09-02-2019

**Sunil Kulkarni**
Professor and Head, Department of Plant Pathology, Agricultural Research Station, Bidar, University of Agricultural Sciences Raichur, Karnataka, India

**Raja**
Research Associate, Department of Plant Pathology, Agricultural Research Station, Bidar, University of Agricultural Sciences Raichur, Karnataka, India

**Correspondence**
**Sunil Kulkarni**
Professor and Head, Department of Plant Pathology, Agricultural Research Station, Bidar, University of Agricultural Sciences Raichur, Karnataka, India

# Studies on the cultural characteristics of *Colletotrichum truncatum* the causal organism of green gram anthracnose

**Sunil Kulkarni and Raja**

**Abstract**
Cultural studies of *Colletotrichum truncatum* causal agent of anthracnose of greengram on different solid media showed that the potato dextrose agar was best followed by oat meal agar for the growth and excellent sporulation. Among the various liquid media used maximum dry mycelial weight and excellent sporulation was observed in Richard's medium followed by Czapeck's medium. The growth phase study revealed that there was increase in the dry mycelial weight of fungus upto 15 days of incubation and there was decrease in growth for 17$^{th}$ day onwards.

**Keywords:** *Colletotrichum truncatum*, Solid media, Liquid media and mycelial

## 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

## 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, Sabauraud's yeast extract agar, Richard's agar, potato carrot agar and corn meal agar.

### Cultural studies -Growth phase of *Colletotrichum truncatum* on liquid media
Thirty ml of potato dextrose broth (PDB) was added into each of 150 ml conical flasks and sterilized. The growth of the fungus was studied at 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21 days of inoculation. The flasks were then inoculated with 5 mm disc of *Colletotrichum truncatum* from actively growing culture and incubated at 27 $\pm$ 1$^0$C. Each treatment was replicated three times. Three flasks were harvested separately at a time, starting from the third day onwards upto 19$^{th}$ day by leaving a gap of 48 h between the two successive harvests. The cultures were filtered through previously weighed Whatman No. 42 filter paper of 12.5 cm diameter, which were dried to a constant weight at 60$^0$C in an electric oven prior to filtration. The mycelial mat on the filter paper was thoroughly washed with sterile distilled water to get rid of the salts likely to be associated with the mycelial mass.
The filter paper along with the mycelial mat were dried to a constant weight at 60$^0$C for 48 h, cooled in a desiccator and weighed immediately on an analytical balance. The difference between final and initial weight of filter discs were taken as the weight of the mycelia. The data were analysed statistically.

**Growth of *Colletotrichum truncatum* on different solid media**

The cultural characters of the *C. truncatum* were studied on the following ten different solid media (Potato dextrose agar, Oat meal agar, Host extract agar, Czapek's agar, Malt extract agar, Sabouraud's agar, Yeast extract agar, Richard's agar, Potato carrot agar and Corn meal agar) and the best media for the fungus growth was identified.

The composition and preparation of the above mentioned synthetic and non-synthetic media were obtained from Ainsworth and Bisby's Dictionary of the fungi (Hawksworth *et al.*, 1983) [5]. The composition of the media is given below.

All the above ingredients, except potassium dihydrogen phosphate and agar were dissolved in 450 ml of distilled water. Agar was melted separately in 500 ml of distilled water and was mixed with the above solution. The volume was made upto 950 ml. Potassium dihydrogen phosphate was dissolved in 50 ml of distilled water. The two solutions were autoclaved and subsequently mixed together.

Twenty ml of each medium listed above was poured into 90 mm diameter Petri plates. After solidification, 5 mm discs of *Colletotrichum truncatum* from actively growing culture were cut using a cork borer and a single disc was placed upside down at the centre of Petri dish. Each set of experiment was replicated thrice and the plates were incubated at 27 $\pm$ $1^0$C. The measurements of the colony diameter was taken when the maximum growth was attained in any one of the media tested. Then, cultural characters such as colony diameter, colony colour, type of margin and sporulation were also recorded. The sporulation was graded as follows.

**Sporulation grade**

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

**Growth of *C. truncatum* on different liquid media**

The composition and preparation of different liquid media used were the same as that of solid media except that agar was not added. Twenty ml of different liquid media were added into each of 100 ml conical flasks. These flasks were then sterilized at 1.1 kg per $cm^2$ pressure for 20 min. The flasks were inoculated with 5 mm mycelial discs obtained from periphery of 10 days old culture and incubated at 28 $\pm$ $1^0$C for 15 days. Each treatment was replicated thrice. Dry mycelial weight and sporulation (under high power) in each treatment were recorded as described earlier.

**Result and Discussion**

**Cultural studies - Growth of *C. truncatum* on potato dextrose broth at different incubation periods**

The experiment was conducted to ascertain the number of days required for maximum growth of the fungus by monitoring the dry mycelial weight. The results are presented in Table 1.

There was significant difference among the incubation periods. The dry mycelial weight of *C. truncatum* gradually increased from third day (94.41 mg) and reached maximum on 15th day (214.13 mg) and was significantly superior over all other treatments. It showed declining trend from 17th day onwards.

**Growth and sporulation of *C. truncatum* on different solid media**

The diversity in cultural and morphological characters of *C. truncatum* was studied on ten solid media at room temperature (28 $\pm$ $1^0$C). The radial growth of the fungus and sporulation were recorded when it attained the maximum growth in all the media tested. Observations on various colony characters were recorded. The data were presented in Table 2. The fungus recorded maximum growth on potato dextrose agar (90.00 mm) and was found superior over other media. Next to potato dextrose agar, oat meal agar (85.20 mm) and Czapek's agar (84.23 mm), which were on par with each other. The minimum growth was observed in potato carrot agar (41.29 mm) (Plate 1).

The maximum sporulation was found in potato dextrose agar and oat meal agar. Poor sporulation was noticed in yeast extract agar and potato carrot agar. With respect to the mycelial colour, it varied from dull white to grey. The growth varied from flat to fluffy with smooth to irregular margins. The fungus showed light grey coloured with smooth margin mycelia on potato dextrose agar with excellent sporulation. Mycelial growth on Czapek's dox agar was light grey in colour and rough margin having good sporulation. Mycelial growth on host extract agar showed dark grey colour and margin was smooth with fair sporulation. Yeast extract and Sabouraud's agar media produced dull white colour mycelia with slight flat to fluffy growth having poor to fair sporulation. In case of potato carrot agar, malt extract agar and corn meal agar, the fungus showed dirty white coloured with slight flat to irregular margin mycelium with poor to fair sporulation.

**Growth and sporulation of *C. truncatum* in different liquid media**

Average dry mycelial weight and sporulation of *C. truncatum* grown on ten different liquid media after 15 days of incubation was recorded as descried in "Material and Methods" and data are presented in Table 3.

Data from the Table 3 indicated that, there was significant difference between the liquid media. Richard's medium supported the maximum growth (203.17 mg) and was significantly superior over other media. Next to Richard's medium Czapek's medium (168.30 mg), potato dextrose broth (162.30 mg) and Sabouraud's media (159.17 mg) were found best and differed significantly with each other. The minimum growth was observed in potato carrot medium (34.43 mg) (Plate 2).

Excellent sporulation was observed in Richard's and potato dextrose medium, while fair sporulation was noticed in Czapek's, host extract, Sabouraud's, malt extract and corn meal medium. Poor sporulation was seen in oat meal, yeast extract and potato carrot medium.

The growth phase of *C. truncatum* was studied by inoculating the fungus on potato dextrose broth and harvesting the growth starting from third day onwards upto 21st day. The findings of the experiment revealed that there was increase in the dry mycelial weight of fungus from third day onwards upto 15th day. Thereafter, there was a decline in the dry mycelial weight upto 21st day. The maximum dry mycelial weight of 214.13 mg was recorded on the 15th day and it was considered as the optimum period for growth of the fungus. The increase in the mycelial dry weight from third day to 15th day can be

attributed to presence of nutrients in the medium and the fungus showed gradual increase in the weight by utilizing them to the maximum extent. The decrease in the dry mycelial weight from 17th day onwards may probably be due to autolysis of the mycelium and exhaustion of nutrients in the medium. This remark is not an exception to the investigation made by Singh and Shukla (1986) [11] and Laxman (2006) [7].

In the present study, the maximum mycelial dry weight was recorded on 15th day. Hence, to obtain maximum fungal growth of *C. truncatum*, 15 days of incubation appears to be optimum.

Among the various media used for growth and sporulation of *C. truncatum* potato dextrose agar proved to be the best for good growth followed by oat meal agar. Excellent sporulation was seen on both these media. Semi-synthetic media supported better growth due to the presence of some vitamins, which are essential for growth and development of organism (Mathur *et al.*, 1950) [8]. Similar types of observations were also made by earlier workers (Kenchaiah, 1975; Mesta, 1996; Ekbote *et al.*, 1997 and Laxman, 2006) [6, 7, 4, 9]. Fungus made the least growth and poor sporulation in potato carrot agar which may be attributed to quality of sugar present in them. Agnihotri and Prasad (1971) [1] reported that sugar supplemented media supported good growth of *C. capsici* than the minimal media used as control.

In the present study, the potato dextrose agar recorded maximum growth and excellent sporulation. Hence, this medium can very well be used for obtaining maximum fungal growth as well as for the excellent sporulation of *C. truncatum*.

Several liquid media were evaluated for growth and sporulation of *C. truncatum.* It was found that Richard's medium supported maximum mycelial dry weight (203.17 mg) followed by Czapeck's medium (168.30 mg) and the excellent sporulation was observed in Richard's medium and potato dextrose broth. The ability of a fungus to grow more on Richard's medium indicated the requirement of nutrients present in that medium for *C. truncatum*. Similarly, Ekbote (1994) [3], Mesta (1996) [9], Shirshikar (1995) [10], Angadi (1999) [2] and Varaprasad (2000) [12] reported that maximum growth and sporulation was observed on Richard's medium.

In the present study, the Richard's medium showed maximum growth and excellent sporulation of the fungus. Hence, Richard's medium was used for obtaining maximum growth and sporulation of *C. truncatum*.

**Table 1:** Effect of incubation period on growth of *Colletotrichum truncatum* on potato dextrose broth

| S. No. | Days after inoculation | Dry mycelial weight (mg) |
|---|---|---|
| 1. | 3 | 94.41 |
| 2. | 5 | 122.40 |
| 3. | 7 | 126.47 |
| 4. | 9 | 146.37 |
| 5. | 11 | 161.22 |
| 6. | 13 | 184.38 |
| 7. | 15 | 214.13 |
| 8. | 17 | 197.18 |
| 9. | 19 | 173.38 |
| 10. | 21 | 122.37 |
| S.E m ± | | 0.58 |
| CD at 1 % | | 2.35 |

**Table 2:** Effects of different solid media on radial growth and sporulation of *Colletotrichum truncatum*

| S. No. | Media | Growth characters | Radial growth (mm) | Sporulation |
|---|---|---|---|---|
| 1. | Oat meal agar | Good growth with smooth margin and mycelium light grey in colour | 85.20 | + + + + |
| 2. | Potato dextrose agar | Good and fast growth mycelium is light grey in colour | 90.00 | + + + + |
| 3. | Czapek's agar | Mycelium is light grey in color with rough margin and good growth | 84.23 | + + + |
| 4. | Yeast extract agar | Mycelium is dull white in colour slight flat, smooth slight irregular | 58.30 | + |
| 5. | Sabouraud's agar | Mycelium is dull white with fluffy growth and margin is irregular | 65.27 | + + |
| 6. | Richard's agar | Good growth mycelium dark grey in colour with rough margin | 81.23 | + + + |
| 7. | Host extract agar | Good growth with dark grey mycelium, margin is smooth | 77.28 | + + |
| 8. | Potato carrot agar | Poor growth, irregular margin mycelium dirty white | 41.29 | + |
| 9. | Malt extract agar | Mycelium dirty white, slight flat medium irregular and poor growth | 56.30 | + + |
| 10. | Corn meal agar | Poor growth, dirty white with irregular margin | 50.33 | ++ |
| S.E m ± | | | 0.49 | |
| CD at 1 % | | | 2.01 | |

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

**Table 3:** Effect of different liquid media on dry mycelial weight and sporulation of *Colletotrichum truncatum*

| S. No. | Media | Dry mycelial weight (mg) | Sporulation |
|---|---|---|---|
| 1 | Czapek`s medium | 168.30 | + + |
| 2 | Richard`s medium | 203.17 | + + + + |
| 3 | Potato dextrose broth | 162.30 | + + + + |
| 4 | Host extract medium | 144.23 | + + |
| 5 | Sabouraud`s medium | 159.17 | + + |
| 6 | Oat meal medium | 131.23 | + |
| 7 | Yeast extract medium | 105.24 | + |
| 8 | Potato carrot medium | 34.43 | + |
| 9 | Malt extract medium | 88.30 | + + |
| 10 | Corn meal medium | 64.22 | + + |
| S.Em± | | 0.66 | |
| CD at 1% | | 2.70 | |

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

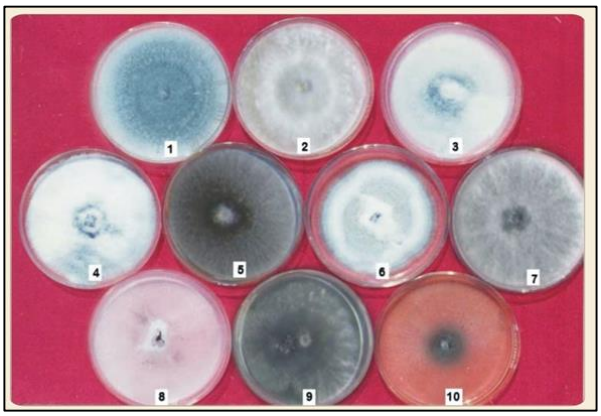


**Plate 1:** Effect of different solid media on growth and development

1. Potato dextrose agar
2. Yeast extract agar
3. S. Host extract agar
4. Czapek's agar
5. Richard's agar
6. Com meal agar
7. *Sabouraud's agar*
8. Corn meal agar
9. Malt extract agar
10. Potato carrot agar

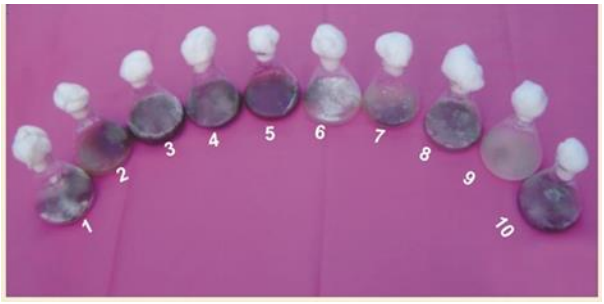


**Plate 2:** Effect of different liquid media on dry mycelia! weight of *C. truncaturn*

1. Richard's medium
2. Potatodextrose broth
3. Malt extract medium
4. Potato carrot medium
5. Corn meal medium
6. Host extract medium
7. Oat meal medium
8. Yeast extract medium
9. Sabouraud's medium
10. Gaspers medium

## Conclusions

The growth phase study on *C. truncatum* revealed that the fungus produced maximum dry mycelial weight of 214.13 mg on 15$^{th}$ day in potato dextrose broth, beyond which autolysis occurred.

Cultural studies conducted revealed that among the solid media, potato dextrose agar was best followed by oat meal agar for the growth and excellent sporulation of the *C. truncatum*. Among the various liquid media used, maximum dry mycelial weight and excellent sporulation was observed in Richard's medium followed by Czapeck's medium.

## References

1. Agnihotri JP, Prasad N. Production of protopectinase by *Colletotrichum capsici* f. *cyamopsicola*. Indian J Mycol. Pl. Path. 1971; 1:51-55.
2. Angadi HD. Studies on anthracnose of chilli (*Capsicum annum*) and its management. M. Sc. (Agri.) Thesis, Univ. Agric. Sci, Bangalore, Karnataka, India, 1999.
3. Ekbote SD. Studies on anthracnose of mango (*Mangifera indica* L.) caused by *Colletotrichum gloeosporoioides* (Penz) Penz. and Sacc. M. Sc. (Agri.) *Thesis*, Univ. Agric. Sci, Dharwad, Karnataka, India, 1994.
4. Ekbote S, Padaganur GM, Patil MS, Chattannavar SN. Studies on the cultural and nutritional aspects of *Colletotrichum gloeosporioides*, the causal organism of mango anthracnose. J Mycol. Pl. Path. 1997; 27(2):227-230.
5. Hawksworth DL, Sutton BS, Ainsworth GC., Ainsworth and Bisbay's dictionary of fungi, VII Eds. Commonwealth Mycological institution, Kew, surrey, England, 1983, 445.
6. Kenchaiah, Studies on chilli anthracnose caused by *Colletotrichum capsici* (Syd) Butler and Bisby, in Karnataka. M. Sc. (Agri.) Thesis, Univ. Agric. Sci., Bangalore, Karnataka, India, 1975.
7. Laxman R. Studies on leaf spot of greengram caused by *Colletotrichum truncatum* (Schw.) Andrus and Moore. M. Sc. (Agri.) Thesis, Univ. Agric. Sci., Dharwad, Karnataka, India, 2006.
8. Mathur RS, Barnett HL, Lilly VG. Sporulation of *Colletotrichum* lindemuthianum in culture. Phytopathology. 1950; 40:104-114.
9. Mesta RK. Studies on fruit rot of chilli (*Capsicum annum* L.) caused by *Colletotrichum capsici* (Sydow) Butler and Bisby. M. Sc. (Agri.) Thesis, Univ. Agric. Sci., Dharwad, Karnataka, India, 1996
10. Shirshikar SP. Studies on seed borne nature and cultural aspects of *Colletotrichum truncatum* (Schw.) Andrus and Moore; incitant of anthracnose disease of soybean (*Glycine max* (L.) Merrill). Ph. D. Thesis, Univ. Agric. Sci., Bangalore, Karnataka, India, 1995.
11. Singh RR, Shukla P. Cultural Studies on *Colletotrichum truncatum* causing anthracnose of blackgram. Indian J Mycol. Pl. Path. 1986; 16(2):172-174.
12. Varaprasad CH. Studies on blight disease of chickpea caused by *Colletotrichum dematium* (Pers. Ex. Fr.) Grove. M. Sc. (Agri.) Thesis, Univ. Agric. Sci., Dharwad, Karnataka, India, 2000.