



Cultural characterization of *Colletotrichum truncatum* causing anthracnose of soybean on different media

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Abstract

Anthracnose caused by *Colletotrichum truncatum* is a major constraint in soybean (*Glycine max* (L.) Merrill) production. The understanding of the pathogen's cultural behaviour is essential for isolation and management protocols. The growth and cultural characteristics of pathogen were evaluated on 12 solid and corresponding liquid media, both synthetic and non synthetic media. Among the solid media, the maximum mycelial growth was recorded on potato dextrose agar (PDA) with a mean colony diameter of 90.00 mm, while Asthana and Hawker's agar recorded the minimum growth (60.00 mm). Excellent sporulation (+++++) was observed on malt extract agar, whereas poor sporulation (+) was observed on host leaf extract agar and no sporulation was observed on V-8 juice agar despite the medium supporting relatively good mycelial growth of 80.00 mm. In liquid media, V-8 juice broth produced the highest dry mycelial biomass (297.43 mg) and minimum dry mycelial weight was observed in water sucrose broth (11.25 mg). Excellent sporulation (+++++) was observed in malt extract broth and potato dextrose broth. No sporulation was observed in Asthana and Hawker's broth, carrot broth, carrot dextrose broth, Czapek Dox broth, host leaf extract broth, Richard's broth, Sabouraud's broth and V-8 juice broth. The results indicate that medium composition strongly influences both the vegetative and reproductive phases of the pathogen and that biomass accumulation and sporulation are not always favoured by the same medium offering a practical basis for selecting media for inoculum multiplication in future anthracnose research.

Keywords: Anthracnose, *Colletotrichum truncatum*, Cultural characterization, Liquid media, Solid media, Soybean

Introduction

Soybean (*Glycine max* (L.) Merrill), belonging to the family Leguminaceae (subfamily Papilionaceae), is native to China and was introduced into Indian agriculture during the 1970s - 80s (Andole, 1984) [2]. Recognized globally as a major oilseed and protein crop, it is valued for its high protein content (~38 per cent), oil (~18 per cent) and diverse industrial applications ranging from biodiesel and biodegradable plastics to soy based food products (Chauhan and Joshi, 2005; Shin *et al.*, 2015) [7, 19]. Despite its economic importance, soybean production is constrained by biotic stresses, particularly fungal diseases, which account for 6 - 8 per cent of the total 12 - 20 per cent yield loss attributed to biotic factors (Mittal *et al.*, 1993) [17]. Among these, anthracnose caused by *Colletotrichum truncatum*, is one of the most destructive, with yield losses ranging from 16 to 100 per cent (Sinclair, 1992) [12]. First reported from Korea in 1917, the disease affects the crop from flowering through pod - filling stages, causing brown lesions on stems, pods and petioles, premature drying and shriveled, discoloured seed with reduced germination (Khan and Sinclair, 1992; Sinclair and Backman, 1989 [12, 22]; Chandrasekaran and Rajappan, 2002) [6]. Seed infection levels of up to 70 per cent have been reported under favourable warm, humid conditions (Agam *et al.*, 2019) [1]. The genus *Colletotrichum* is taxonomically complex and morpho culturally variable, with growth rate, colony colour, texture, margin and sporulation capacity differing widely,

not only between species but also between isolates of the same species, depending on the nutritional and physical composition of the medium on which they are grown (Xiang *et al.*, 2014; Asalkar *et al.*, 2019) [4, 23]. Accurate characterization of these cultural attributes is fundamental to pathogen identification, isolate comparison and the standardization of protocols for the mass multiplication of inoculum required for pathogenicity assays, virulence screening and the evaluation of host resistance and management options (Kulkarni and Raja, 2019; Roja *et al.*, 2022) [15, 18]. Since the nutrient composition of a medium directly governs the mycelial growth and sporulation efficiency of the pathogen, identification of the most suitable medium is a prerequisite for raising healthy, sporulating cultures for further experimentation (Andrews and Nordheim, 1987; Harshitha *et al.*, 2022) [3, 9]. However, information on the comparative performance of *Colletotrichum* spp. across a wide range of synthetic and non synthetic solid and liquid media remains fragmented, with different studies reporting varying optimum media depending on the host and geographic origin of the isolate (Kumar *et al.*, 2022; Khare *et al.*, 2025) [13, 16]. Keeping this in view, the present investigation was undertaken to study the cultural characteristics and growth behaviour of *C. truncatum* on 12 different solid and liquid media, so as to identify the media best suited for optimum vegetative growth as well as sporulation of the pathogen.

Material and methods

1. Cultural characterization

1.1 Growth characteristics on solid media

The growth and cultural characters of *Colletotrichum truncatum* were studied on the following 12 different synthetic and non synthetic solid media.

List 1: Different solid media

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

The composition of the above media was obtained from the Ainsworth and Bisby's Dictionary of the Fungi by Hawksworth *et al.* (1983) [10]. All the media were sterilized to carry out the study. About 20 ml of each medium was poured into 90 mm Petri plates. Such Petri plates were inoculated with a 5 mm disc cut from the periphery of an actively growing 7 - 9 day old culture of the fungus under aseptic conditions and incubated at $27 \pm 1^\circ\text{C}$. Each treatment was replicated thrice and observations were taken once the fungus covered the complete Petri plate in any one of the media. The colony diameter (mm), colony colour, growth pattern, type of margin, colony shape, colony texture and sporulation were recorded and sporulation was graded as follows.

List 2: Scoring and grading of sporulation

Sl. 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	-

1.2 Growth characteristics on liquid media

The composition and preparation of the different liquid media were the same as for the solid media, except that agar was not added. About 20 ml of each liquid medium was added to 100 ml conical flasks, which were then sterilized at 1.10 kg / cm² pressure for 20 minutes. The flasks were inoculated with 5 mm mycelial discs obtained from the periphery of a 10 day old culture and incubated at $27 \pm 1^\circ\text{C}$ for 15 days. Each treatment was replicated three times. Dry mycelial weight and sporulation in each treatment were recorded as described earlier. The different synthetic and non synthetic liquid media used in the experiment are given below.

List 3: Different liquid media

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

Results and discussion

For cultural characterization of the pathogen, isolate C-1 was selected and cultured on 12 different solid and liquid media to assess its growth behaviour and cultural variability under different nutritional conditions. Observations were recorded for parameters such as colony growth, colour, texture, pigmentation, sporulation and other characteristic features on solid media, while dry mycelial weight and sporulation were noted in liquid media. The results of these evaluations are presented below.

1. Growth characteristics of *Colletotrichum truncatum* on solid media

The growth and sporulation of *C. truncatum* was evaluated on 12 different solid media and variation was observed in mean colony diameter and sporulation among the media tested. The maximum mycelial growth was recorded on potato dextrose agar (PDA) with a mean colony diameter of 90.00 mm, which was statistically superior to malt extract agar (80.33 mm) and statistically on par with carrot dextrose agar (80.00 mm) and V-8 juice agar (80.00 mm). The minimum colony diameter was recorded on Asthana and Hawker's agar (60.00 mm), followed by host leaf extract agar (62.33 mm) (Table 1, Fig. 1, Plate 1). Sporulation also varied markedly with the culture medium. Excellent sporulation (++++) was observed on malt extract agar, oat meal agar, PDA and Richard's agar. Good sporulation (+++) was recorded on Asthana and Hawker's agar, carrot agar, carrot dextrose agar, Sabouraud's agar and water sucrose agar. Czapek Dox agar supported moderate sporulation (++) , whereas poor sporulation (+) was observed on host leaf extract agar and no sporulation was observed on V-8 juice agar despite the medium supporting relatively good mycelial growth of 80.00 mm (Table 1).

The variation in radial growth of *Colletotrichum* isolates observed on different culture media in the present investigation was in agreement with Harshitha *et al.* (2022) [9], who reported considerable differences in the mycelial growth of *C. truncatum* on different solid media, with radial growth ranging from 38.00 to 90.00 mm, maximum growth of 90.00 mm on Czapek Dox agar and minimum growth of 38.00 mm on Czapek's malt agar. The present findings are also comparable with Kumar *et al.* (2022) [16], who evaluated eight solid media for the growth of *C. gloeosporioides* and recorded maximum radial growth of 90.00 mm on both Richard's synthetic agar and PDA, followed by Czapek Dox agar (89.00 mm) and oat meal agar (88.00 mm), whereas potato carrot agar recorded the minimum growth of 72.50 mm. Shivakumar *et al.* (2016) [20] demonstrated considerable variation in the sporulation of *C. gloeosporioides* on different solid media, with maximum conidial production recorded on Richard's agar (7.25×10^4 conidia ml⁻¹), followed by PDA (6.72×10^4 conidia ml⁻¹) and Asthana and Hawker's agar (3.90×10^4 conidia ml⁻¹), whereas host leaf extract agar recorded 2.62×10^4 conidia ml⁻¹. Asalkar *et al.* (2019) [4] found that Richard's agar supported maximum growth (90 mm) with excellent sporulation, followed by PDA (88.83 mm) and Czapek's Malt Agar (88.40 mm) and observed that colony colour and sporulation varied with the culture medium. The variation in mycelial growth of *Colletotrichum* spp. on different solid media indicated the influence of the nutritional composition of the medium on fungal growth. Potato dextrose agar recorded the maximum mycelial growth, with a mean colony diameter of 90.00 mm,

which may be attributed to the readily available nutrients and carbohydrate source present in the medium.

The cultural characteristics of the colonies also differed among the media. Colonies were predominantly white on Asthana and Hawker's agar, carrot agar, carrot dextrose agar, Czapek Dox agar, host leaf extract agar, malt extract agar, potato dextrose agar, Sabouraud's agar and water sucrose agar, whereas creamy white colonies were observed on oat meal agar, Richard's agar and V-8 juice agar. Regular colony shape with circular growth was the most common pattern, observed on Asthana and Hawker's agar, carrot dextrose agar, Czapek Dox agar, malt extract agar, oat meal agar, potato dextrose agar, Sabouraud's agar and V-8 juice agar, whereas irregular colonies with feathery growth were observed on carrot agar, host leaf extract agar, Richard's agar and water sucrose agar. Colony margins were mostly serrated, found on Asthana and Hawker's agar, carrot agar, carrot dextrose agar, Czapek Dox agar, host leaf extract agar, malt extract agar, Richard's agar, Sabouraud's agar, V-8 juice agar and water sucrose agar media, while smooth margins were observed on oat meal agar and PDA. Colony texture varied from appressed and fluffy to scanty depending on the medium, appressed growth was recorded on Asthana and Hawker's agar, carrot dextrose agar, malt extract agar, oat meal agar, Richard's agar, V-8 juice agar and water sucrose agar, fluffy growth was observed on carrot agar, Czapek Dox agar, PDA and Sabouraud's agar, and host leaf extract agar produced a scanty colony texture (Table 2, Plate 2).

Similarly, Xiang *et al.* (2014) ^[23] reported that *Colletotrichum truncatum* exhibited significantly faster radial growth on soymilk dextrose agar than on PDA. Kumar *et al.* (2022) ^[16] reported that *C. truncatum* on soybean leaf extract agar recorded the maximum mycelial growth (85.10 mm), followed by corn meal agar (83.13 mm) and PDA (81.33 mm). Joshi *et al.* (2024) ^[11] reported considerable variation in the growth and sporulation of *C. capsici* on 12 different media, with the highest growth recorded on red chilli dextrose agar (90.00 mm), followed by carrot dextrose agar and potato carrot agar; the colonies also differed in colour, texture and margin across the media, and excellent sporulation was observed on PDA, carrot dextrose agar, potato carrot agar and red chilli dextrose agar, indicating that the culture medium strongly influenced both growth and sporulation of the pathogen.

2. Growth characteristics of *Colletotrichum truncatum* on liquid media

The influence of 12 different liquid media on dry mycelial weight and sporulation of *C. truncatum* was evaluated and considerable variation was observed among the media. The maximum dry mycelial weight was recorded in V-8 juice broth (297.43 mg), which was statistically superior to Czapek Dox broth (208.97 mg), followed by potato dextrose broth (198.51 mg) and malt extract broth (183.06 mg), which differed statistically from each other. The minimum dry mycelial weight was observed in water sucrose broth (11.25 mg), which differed statistically from oat meal broth

(27.33 mg), followed by Asthana and Hawker's broth (50.60 mg) (Table 10, Fig. 4, Plate 11). Variation was also noticed in sporulation among the liquid media. Excellent sporulation (++++) was observed in malt extract broth and potato dextrose broth. Oat meal broth recorded good sporulation (+++), whereas water sucrose broth showed moderate sporulation (++). No sporulation was observed in Asthana and Hawker's broth, carrot broth, carrot dextrose broth, Czapek Dox broth, host leaf extract broth, Richard's broth, Sabouraud's broth and V-8 juice broth (Fig. 2, Table 3, Plate 2).

Similar variation was reported by Ekbote *et al.* (1997) ^[8], who observed maximum growth of *Colletotrichum truncatum* after 12 days of incubation in potato dextrose broth, whereas Kulkarni (2009) ^[14] reported a progressive increase in dry mycelial weight from the third day, reaching maximum biomass around the 15th day. Andrews and Nordheim (1987) ^[3] found that the type and nutritional concentration of the culture medium markedly influenced spore production of *C. gloeosporioides*, among 11 media tested, V-8 juice was the most suitable for high sporulation, with an organic nutrient concentration of approximately 6 mg / ml providing a favourable combination of high conidial production and low mycelial biomass, though in the present study V-8 juice broth failed to sporulate. Cascino *et al.* (1990) ^[5] found that increasing the concentration of V-8 juice from 5 to 40 per cent increased *C. gloeosporioides* mycelial production and delayed the initiation of sporulation, with cultures at concentrations above 10 per cent V-8 attaining a maximum spore concentration of approximately 0.80 mg / ml, corresponding to about 10⁷ - 10⁸ spores / ml.

It was observed that V-8 juice broth produced the highest dry mycelial biomass (297.43 mg) but failed to produce sporulation, whereas malt extract broth and potato dextrose broth produced excellent sporulation with comparatively lower dry mycelial weights of 183.06 and 198.51 mg, respectively. Thus, the medium most favourable for vegetative biomass production was not necessarily the most favourable for reproductive development.

Table 1: Mycelial growth and sporulation of *Colletotrichum truncatum* on different solid media

Sl. No.	Media	Mean colony diameter (mm)	Sporulation
1	Asthana and Hawker's agar	60.00*	+++
2	Carrot agar	66.33	+++
3	Carrot dextrose agar	80.00	+++
4	Czapek Dox agar	73.67	++
5	Host leaf extract agar	62.33	+
6	Malt extract agar	80.33	++++
7	Oat meal agar	65.67	++++
8	Potato dextrose agar	90.00	++++
9	Richard's agar	69.33	++++
10	Sabouraud's agar	78.00	+++
11	V-8 juice agar	80.00	-
12	Water sucrose agar	77.00	+++
	S. Em. ±	0.29	
	C. D. at 1 %	1.17	

* Mean of three replications

++++ Excellent sporulation; +++ Good sporulation; ++ Moderate sporulation; + Poor sporulation; - no sporulation

Table 2: Cultural characteristics of *Colletotrichum truncatum* on different solid media

Sl. No.	Name of the media	Colony shape	Growth pattern	Colony colour	Colony margin	Colony texture
1	Asthana and Hawker's agar	Regular	Circular	White	Serrated	Appressed
2	Carrot agar	Irregular	Feathery	White	Serrated	Fluffy
3	Carrot dextrose agar	Regular	Circular	White	Serrated	Appressed
4	Czapek Dox agar	Regular	Circular	White	Serrated	Fluffy
5	Host leaf extract agar	Irregular	Feathery	White	Serrated	Scanty
6	Malt extract agar	Regular	Circular	White	Serrated	Appressed
7	Oat meal agar	Regular	Circular	Dull white	Smooth	Appressed
8	Potato dextrose agar	Regular	Circular	White	Smooth	Fluffy
9	Richard's agar	Irregular	Feathery	Dull white	Serrated	Appressed
10	Sabouraud's agar	Regular	Circular	White	Serrated	Fluffy
11	V-8 juice agar	Regular	Circular	Dull white	Serrated	Appressed
12	Water sucrose agar	Regular	Feathery	White	Serrated	Appressed

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

Sl. No.	Media	Dry mycelial weight (mg)	Sporulation
1	Asthana and Hawkers broth	50.60*	-
2	Carrot broth	124.07	-
3	Carrot dextrose broth	143.89	-
4	Czapek Dox broth	208.97	-
5	Host leaf extract broth	110.78	-
6	Malt extract broth	183.06	++++
7	Oat meal broth	27.33	+++
8	Potato dextrose broth	198.51	++++
9	Richard's broth	88.19	-
10	Sabouraud's broth	152.57	-
11	V-8 juice broth	297.43	-
12	Water sucrose broth	11.25	++
S. Em. $\pm$		0.68	
C. D. at 1 %		2.69	

* Mean of three replications ++++ Excellent sporulation; +++ Good sporulation; ++ Moderate sporulation; + Poor sporulation; - sporulation

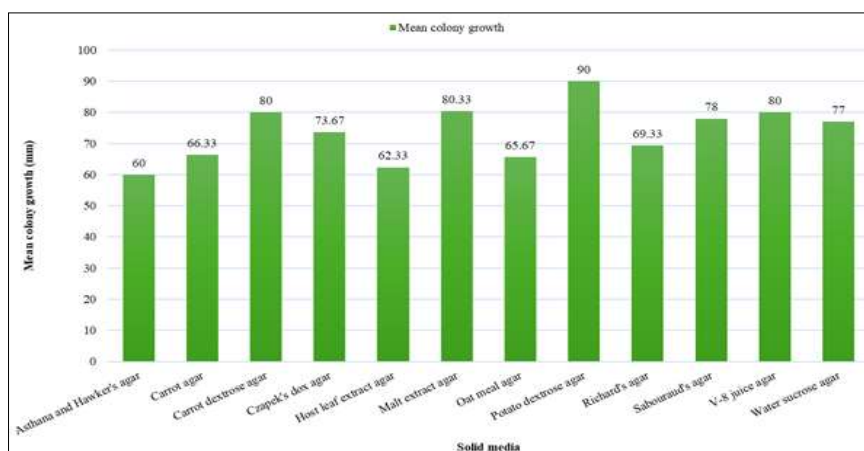
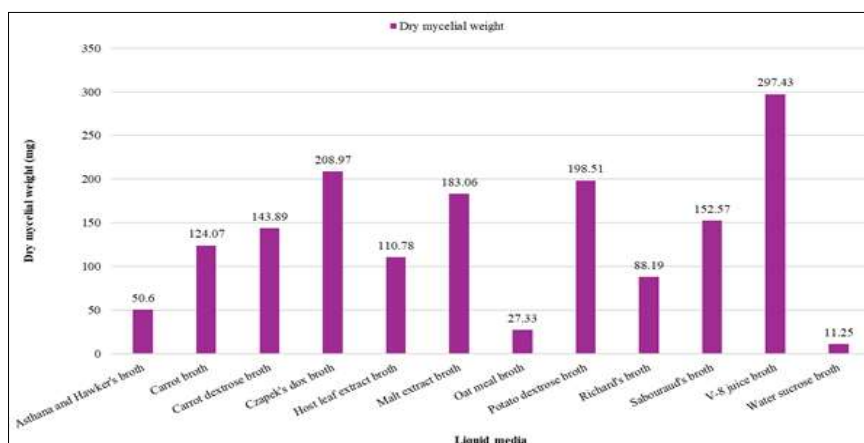
**Fig 1:** Mycelial growth of *Colletotrichum truncatum* on different solid media**Fig 2:** Dry mycelia weight of *Colletotrichum truncatum* on different liquid media



Plate 1: Growth of *Colletotrichum truncatum* on different solid media



Plate 2: Growth of *Colletotrichum truncatum* on different liquid media

Conclusion

Potato dextrose agar was the most suitable for both mycelial growth (90.00 mm) and excellent sporulation of *Colletotrichum truncatum*, while Asthana and Hawker's agar supported the least growth (60.00 mm). In liquid media, V-8 juice broth produced the maximum mycelial biomass (297.43 mg) but failed to sporulate, whereas malt extract broth (183.06 mg) and potato dextrose broth (198.51 mg), despite comparatively lower biomass, favoured excellent sporulation. This confirms that the nutrient composition of the medium differentially governs vegetative growth and reproductive development in the pathogen and that media optimal for biomass accumulation are not necessarily optimal for spore production.

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Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Competing interests

The authors declare that they have no competing interests.

References

1. Agam MN, Raut RA, Jejurkar GB, Sable SB. Evaluation of the fungicides, botanicals and bioagents against *Colletotrichum truncatum* causing anthracnose of soybean in pot culture. *Journal of Pharmacognosy and Phytochemistry*. 2019;8(2):629-634.
2. Andole VC. Soybean. It's cultivation, uses and values in dietetics. 1984;29.
3. Andrews JH, Nordheim EV. Microplate assay for *Colletotrichum* spore production. *Applied and Environmental Microbiology*. 1987;53(4):627-632.
4. Asalkar UA, Hingole DG, Khaire PB, Mete VS. Effect of different solid media on the growth and sporulation of *Colletotrichum gloeosporioides* Penz. and Sacc. causing fruit rot of aonla. *International Journal of Current Microbiology and Applied Sciences*. 2019;8(10):610-616.
5. Cascino JJ, Harris RF, Smith CS, Andrews JH. Spore yield and microcycle conidiation of *Colletotrichum gloeosporioides* in liquid culture. *Applied and Environmental Microbiology*. 1990;56(8):2303-2310.
6. Chandrasekaran A, Rajappan K. Effect of *Colletotrichum truncatum* seed infection on seed

- weight and seedling vigour of soybean. *Plant Disease*. 2002;17(1):72-73.
7. Chauhan GS, Joshi O. Soybean (*Glycine max*) - the 21st century crop. *Indian Journal of Agricultural Sciences*. 2005;75(8):4-6.
 8. Ekbote SD, Padaganur GM, Patil MS, Chattannavar SN. Studies on the cultural and nutritional aspects of *Colletotrichum gloeosporioides*, the causal organism of mango anthracnose. *Journal of Mycology and Plant Pathology*. 1997;27(3):229-230.
 9. Harshitha KN, Palakshappa MG, Yashoda H, Sridevi O, Mallapur CP. Cultural and morphological characters of *Colletotrichum truncatum* on different solid media. *Journal of Farm Sciences*. 2022;35(4):449-451.
 10. Hawksworth DL, Sutton BS, Ainsworth GC. Ainsworth and Bisby's Dictionary of the Fungi. 7th ed. London: Commonwealth Mycological Institute; 1983.
 11. Joshi R, Yenjerappa ST, Aswathanarayana DS, Chavan SS, Kisan B, Lakshmikanth M. Influence of different culture media on growth and sporulation of *Colletotrichum capsici*. *International Journal of Environment and Climate Change*. 2024;14(2):555-561.
 12. Khan M, Sinclair JB. Pathogenicity of sclerotia and non-sclerotia forming isolates of *Colletotrichum truncatum* on soybean plants and roots. *Phytopathology*. 1992;82(3):314-319.
 13. Khare AK, Kumar A, Chouhan A, Sharma S, Ramakrishnan RS, Sharma R, *et al.* *In vitro* efficacy of fungicides against *Colletotrichum truncatum* causing anthracnose of soybean. *Journal of Advanced Biology and Biotechnology*. 2025;28(10):1133-1141.
 14. Kulkarni SA. Epidemiology and integrated management of anthracnose of green gram [dissertation]. Dharwad: University of Agricultural Sciences; 2009.
 15. Kulkarni S, Raja. Studies on the cultural characteristics of *Colletotrichum truncatum*, the causal organism of green gram anthracnose. *Journal of Pharmacognosy and Phytochemistry*. 2019;8(2):2366-2369.
 16. Kumar R, Ravikumar MR, Asthaputre SA, Ganpathi T. Cultural characterization of *Colletotrichum gloeosporioides* causing leaf spot of ginger on different solid media. *The Pharma Innovation Journal*. 2022;11(12):1811-1814.
 17. Mittal RK, Prakash V, Koranne KD. Package of practices for the cultivation of pulses in the hills of Uttar Pradesh. *Indian Farming*. 1993;42(10):1-5.
 18. Roja S, Kulkarni S, Sunkad G, Ajithkumar K, Shobharani M. Cultural and physiological requirements of *Colletotrichum truncatum* causing soybean pod blight. *Biological Forum – An International Journal*. 2022;14(1):85-91.
 19. Shin JH, Vaughn JN, Abdel HH, Chavarro C, Abernathy B, Kim KD, *et al.* Transcriptomic changes due to water deficit define a general soybean response and accession specific pathways for drought avoidance. *BMC Plant Biology*. 2015;15(1):26-32.
 20. Shivakumar KV, Palaiah P, Raja, Mallesh SB. Effectiveness of different cultural media on the growth and sporulation of *Colletotrichum gloeosporioides* causing anthracnose disease of mango (*Mangifera indica* L.). *Advances in Life Sciences*. 2016;5(3):793-796.
 21. Sinclair JB. Discolouration of soybean seeds an indicator of quality. *Plant Disease*. 1992;76(11):1087-1091.
 22. Sinclair JB, Backman PA, editors. *Compendium of soybean diseases*. 3rd ed. St. Paul: APS Press; 1989.
 23. Xiang Y, Herman T, Hartman GL. Utilizing soybean milk to culture soybean pathogens. *Advances in Microbiology*. 2014;4(3):126-132.