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EFFECT OF CULTURAL MEDIA AND TEMPERATURE ON GROWTH AND SPORULATION OF COLLETOTRICHUM TRUNCATUM OF SOYBEAN IN VITRO

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Abstract

Among the fungal diseases infecting soybean crop, anthracnose/pod blight caused by Colletotrichum truncatum is one of the most important and destructive disease. In the present investigation ,the rate of growth of Colletotrichum truncatum has been compared in various culture media and temperature. The diversity in cultural characteristics viz., colony diameter, mycelial growth and sporulation of C. truncatum was studied in vitro using ten culture media. Of the culture media tested significantly highest (90.00 mm) growth was recorded on PDA, followed by Oat Meal Agar (82.20 mm) and Czapek's dox Agar (80.23 mm).The maximum [excellent (++++)] sporulation was found in Potato Dextrose Agar, Oat Meal Agar, Richard's Agar; whereas, media viz., Czapek's dox Agar, Modified Czapek's dox Agar, Sabouroud's Agar and V8 Juice Agar exhibited fair (++) sporulation. The test fungus exhibited differential response to various temperature regimes and pH levels tested. The studies indicated that the test fungus could grow in a wide temperature range of 10-40^{o}C; however, it could proliferate better with significantly highest mycelial growth of 82.40 and 77.80 mm at temperatures of 25 and 30^{o}C, respectively which also exhibited good (+++) to excellent (++++) sporulation. Lowest mycelial growth recorded at temperature of 10^{o}C (21.50 mm) and 40^{o}C (16.20 mm).This is important for further study of disease management.

Key words- Soybean, temperature regimes ,Colletotrichum.,sporulation

I. INTRODUCTION

Soybean is the world's foremost provider of protein and oil. Soybean growing major states in the country are Madhya Pradesh, Maharashtra, Karnataka and Andhra Pradesh. Soybean plant health is a critical component of profitable soybean production. *Colletotrichum truncatum*, is the most common species recorded on soybean[5] and the crop soybean is susceptible to *C. truncatum* at all stages of development particularly from bloom to pod fill.

II. MATERIAL AND METHODS

A roving survey was conducted in soybean growing areas during *Kharif* 2011-12 and *Kharif* 2012-13 in the eight districts of Marathwada region to assess anthracnose intensity (PDI) and pod infection. Diseased leaves (anthracnose) and pods (blighted) of soybean collected from various fields were brought to the laboratory and washed thoroughly in running tap water. These diseased specimens (leaves, pods) were blot dried and cut with sharp sterilized blade into small bits (5mm) keeping half

healthy and half diseased portion intact. These pieces were surfaces sterilized with 0.1% aqueous solution of mercuric chloride ($HgCl_2$) for two minutes and then washed by giving three changes with sterile distilled water to remove traces of mercuric chloride and blot dried. The surface sterilized diseased pieces were the inoculated on the solidified and cooled PDA (Potato dextrose Agar) medium in petri plates under aseptic conditions of Laminar-air-flow cabinet, (Make : ACS, Bangalore). Inoculated plates were then incubated in BOD incubator (Make : MAC, Delhi) at 27 $\pm$ 2°C temperature. After a week of incubation, the well-developed mycelial growth free from any contaminant was obtained. Following single hyphal-tip technique, the fungus was transformed/ sub-cultured aseptically on the PDA slant in test tubes. Through frequent sub-culturing, the fungus was purified and pure culture was maintained on Agar slants in test tubes stored in refrigerator for further studies. Based on morphological and cultural characteristics of the fungus and microscope observations on spores and mycelium of the fungus (Miller and Roy, 1982; Lenne, 1992; Sinclair and Backman, 1989), the fungus has been identified and confirmed as *Colletotrichum truncatum.* (Schw).

Effect of culture media

The cultural characters of *C. truncatum* were studied on 10 different culture media *viz.,* Ashby's Mannitol Agar, Beijerinckia Agar, Conn's Agar, Czapek's Dox Agar, Modified Czapek's Dox Agar, Oat Meal Agar, Potato Dextrose Agar, Richard's Agar, Sabouraud's. Maltose Agar, V_8 Juice Agar. Except Potato Dextrose Agar (PDA) Richards Agar, all the media used were ready made (Make : Hi media). All the media were sterilized in Autoclave at 15 lbs / $inch^2$ pressure for 20 min.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 $\pm$ 1^0C. 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, and sporulation were also recorded at 7 and 15 days of incubation, respectively. The sporulation was graded as follows. Sporulation grade

Sl. 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	-

Experimental details

Design : CRD
Replications : Three
Treatments : Ten (Culture media)

T_1 : Ashby's Mannitol Agar T_2 : Beijerinckia Agar
T_3 : Czapek's dox Agar T_4 : Conn's Agar
T_5 : Modified Czapek's dox Agar T_6 : Oat meal Agar
T_7 : Potato Dextrose Agar T_8 : Richard's Agar
T_9 : Sabouraud's Maltose Agar T_{10} : V_8 Juice Agar

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 (20 ml/plate) was poured into 90 mm diameter sterile glass petriplates (90 mm dia.). After solidification,

5 mm disc from actively growing 7 days old culture of *C. truncatum* were cut and inoculated to solidified petriplates and incubated for 15 days in the BOD incubator (Make : MAC, Delhi) adjusted to required temperature levels. Each treatment was replicated thrice. After incubation period, colony diameter and sporulation from solid media were recorded.

Experimental Details

Design : CRD
Replications : Three
Treatments : Seven

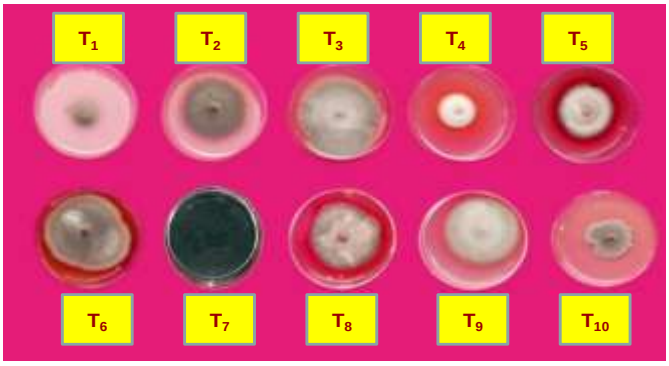
T_1 : 10^0C T_2 : 15^0C
T_3 : 20^0C T_4 : 25^0C
T_5 : 30^0C T_6 :35^0C
T_7 : 40^0C

III. RESULTS AND DISCUSSION

The effect of different culture media on growth and sporulation of *C. truncatum*.

The diversity in cultural characteristics *viz*., colony diameter, mycelial growth and sporulation of *C. truncatum* studied *in vitro* using ten culture media and the results obtained are presented in Table 1 and depicted PLATE I.

PLATE I



Effect of various culture media on growth and sporulation of *C.truncatum*

T_1 – Ashby's Mannitol agar
T_2 ~ Beijerinkia Agar
T_3 ~ Czapek's dox Agar
T_4 ~ Conn's Agar
T_5 ~ Modified Czapek's dox Agar
T_6 ~ Oat meal Agar
T_7 ~ Potato Dextrose Agar
T_8 ~ Richards Agar
T_9 ~ Sabourad maltose Agar
T_{10} ~ V8 Juice Agar

Table 1 : Effect of culture media on growth and sporulation of *C. truncatum*

Treatments	Colony Diameter (mm)*	Cultural characteristics	Sporulation
Ashbys Mannitol Agar	35.20	Grayish black dense mycelial mat	-
Beijerinickia	72.20	Dark grayish dense mycelial mat	+
Czapek's dox Agar	80.23	Light gray coloured mycelia with rough margin	++
Conn's Agar	45.10	Dense mycelial mat	-
Modified Czapek dox Agar	58.90	Creamish white mycelial mat	++
Oat meal Agar	82.20	Dense mycelial mat	++++
PDA	90.00	Light gray coloured mycelia with smooth margin	++++
Richard's Agar	65.40	Grayish black dense mycelial mat	++++
Sabouraud's maltose Agar	78.00	Dull white colour mycelia	++
V8 Juice Agar	46.20	Loose to dense mycelial mat	++
S.E. +	**1.15**		
C.D. (P=0.05)	**3.39**		

*-Mean of three replications

++++ : Excellent; +++ : Good; ++ : Fair; + : Poor Excellent sporulation was found on PDA, Richard's Agar, and Oat Meal Agar. Poor sporulation was found on Ashbys Mannitol Agar and Conn's Agar

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 mean colony diameter recorded with all the test media was ranged from 35.20 mm (Ashbys Mannitol Agar) to 90 mm (PDA). However, significantly highest mean mycelial growth (90.00 mm) was recorded on PDA. The second and third best media were Oat meal Agar (82.20 mm) and Czapek's Dox Agar (80.23 mm). This was followed by Sabouraud's maltose Agar (78.00 mm), Beijerinickia (72.20 mm), Richard's Agar (65.40 mm) Modified Czapek's dox Agar (58.90 mm), V8 Juice Agar (46.20 mm) Conn's Agar (45.10 mm) and Ashby's Mannitol Agar was found least suitable which recorded minimum mean mycelial growth (35.20 mm) of the test pathogen .

All the culture media exhibited a wide range of colony morphology and colour. The colony growth produced on all the culture media was irregular, profuse, wooly with smooth to irregular margins. Colour of colonies varied from grayish black to dark grayish, creamish white to dull white.

The maximum [excellent (++++)] sporulation was found in Potato Dextrose Agar, Oat meal Agar and Richard's Agar. Media *viz*., Czapek's dox Agar, Modified Czapek's dox Agar, Sabouraud's maltose Agar and V8 Juice Agar exhibited Fair (++) sporulation. Whereas, poor (+) sporulation was recorded on Beijerinkia Agar while on Conn's Agar and Ashby's Mannitol Agar, there was no any sporulation induced by the test pathogen.The present results are in confirmation with the findings of Chacko *et al*. (1978) who tested seven different media for growth and sporulation of *Colletotrichum* species and maximum growth and sporulation of fungus was observed on Richard's Agar, Potato Dextrose Agar and Czapek's Agar. The growth and sporulation was observed in Potato Dextrose Agar and Oat Meal Agar because of both the agars supplied maximum amount of carbon, nitrogen and mineral elements to the respective organism. Sinclair and Backman (1989) also found that Potato

Dextrose Agar and Oat Meal Agar were good or suitable medium for *C. truncatum* growth and sporulation.

These results are in consonance with earlier findings *viz*., Wong *et al*. (1983), Singh and Shukla (1986), Sinclair (1988), Shirshikar (1995), Varaprasad (2000), Mandeep and Munshi (2003) and Laxman (2006).

IV. EFFECT OF TEMPERATURE REGIMES

The fungus *C. truncatum* was grown on Potato Dextrose Agar medium at different temperatures *viz*., 10, 15, 20, 25, 30 35 and 40^{o}C to know the suitable temperature requirement for their maximum radial growth and sporulation.

Table 2 : Effect of temperature regimes on growth and sporulation of *C. truncatum*

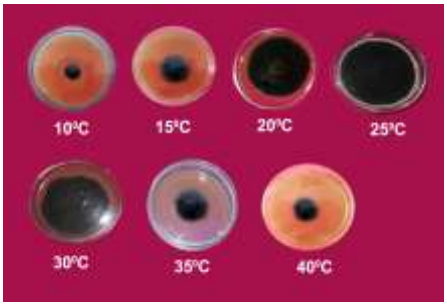
Temp. levels (^{0}C)	Colony diameter* (mm)	Sporulation
10	21.50	-
15	32.40	+
20	62.00	+++
25	82.40	++++
30	77.80	+++
35	40.20	++
40	16.20	-
S.E.	**1.33**	
C.D.	**4.04**	

*-Average of three replications

++++ : Excellent; +++ : Good; ++ : Fair; + : Poor; - : No

Results (Table 2 and PLATE II) revealed that, maximum mean colony diameter of fungus at temperatures of 25^{o}C (82.40 mm) and 30^{o}C (77.80 mm)was significantly superior over all other temperatures. Lowest mean colony diameter was obtained at temperatures of 10^{o}C (21.50 mm) and 40^{o}C (16.20 mm).

PLATE II



Effect of various temperature regimes on growth and sporulation of *C.truncatum*

All the temperature regimes exhibited a wide range of sporulation from no (-) to excellent (++++). However excellent (++++) sporulation was recorded at temperature 25^{o}C Good (+++)

sporulation was recorded at temperature 30°C and 20°C it was fair (++) at 35°C; whereas poor (+) sporulation was recorded at 15°C. At the temperatures of 10°C and 40°C there was no sporulation of the test pathogen.The present results are in agreement with the results obtained by Mishra and Gupta (1994), Shirshikar (1995), Murthy (1997), Varaprasad (2000), Singh and Singh (2001) and Laxman (2006), who reported that optimum temperature for growth and sporulation of *C. truncatum* was 25°C to 30°C.

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

V. CONCLUSION

The pathogen, *C. truncatum* was successfully isolated on PDA from naturally disease foliage/pod specimens of soybean collected during survey and maintained for further studies. The pathogen, *C. truncatum* grow better and sporulated good to excellent on a wide range of synthetic and non synthetic culture media tested; however, Potato Dextrose Agar was found most suitable.

The temperature in the range of 25-30°C, pH in the range of 5.5 to 7 and alternate dark and light (each for 12 hrs duration) were found most favourable for better growth and sporulation of *C. truncatum*.

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