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Influence of Culture Media and Environmental Factors on Growth and Sporulation of *Colletotrichum gloeosporioides* (Penz.) Sacc. Causing Anthracnose of Brinjal (*Solanum melongena* L.)

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Abstract

Colletotrichum gloeosporioides, isolated from affected brinjal plants was studied for their morphological characteristics, mycelial growth and sporulation, spore germination and appressoria formation in different culture media and environmental conditions. Leaf extract agar (LEA) and potato dextrose agar (PDA) supported optimum growth. Sporulation was best in oatmeal agar (OMA) and yeast extract mannitol agar (YEMA). PDB supported best growth among the liquid media tested. Highest mycelial dry weight, spore germination and appressoria formation were recorded at 28 C and pH 6.0. Mannitol showed optimum growth among the tested carbon sources while peptone showed best growth among different nitrogen sources tested. The present study will help to maintain the fungus in the laboratory condition for preparation of inocula for different studies concerning control of the pathogen.
Key words : *Colletotrichum gloeosporioides*, Brinjal, Anthracnose, Growth, Sporulation.

Brinjal is a major commercial vegetable crop and is grown all over India except at high altitude. It is extensively cultivated in sub-Himalayan West Bengal. Anthracnose is one of the important diseases of brinjal that causes severe damage of the plants and subsequent crop loss. The disease is caused by *Colletotrichum gloeosporioides* (Penz.) Sacc. (1, 2). Fungi derive food and energy for their normal growth and physiology from the host or from the substrate of their growth. It is necessary to culture the fungus in artificial media to produce inocula in the form of spore or mycelia. Importance of spores as inoculum has been recognized. Studies have been conducted on the effects of various media-components and physiological parameters that lead to maximum sporulation (3—5). The present study was undertaken to assess the morphological characteristics of the fungi, the effects of different culture media, incubation periods, carbon sources, nitrogen sources temperature and pH on the mycelial growth and sporulation of fungus *C. gloeosporioides.*

Methods

Fungal Culture

The fungus *C. gloeosporioides* was isolated from infected leaves and fruits of brinjal plants from the Barobisha region of Jalpaiguri District in West Bengal. Following verification of Koch's postulates the pathogen was identified in the laboratory and was further confirmed by the Division of Plant Pathology, IARI, New Delhi (Identification No. 5446.02).

Observation of Morphology

The pathogen was cultured in oatmeal agar (OMA) and potato dextrose agar (PDA) for ten days. A bit of fungal mycelia was taken from PDA slants, placed on a clean grease free slide and stained with lactophenol and cotton blue. For study of spores, pink masses of spores (acervuli), distinctly produced on the surface of the OMA slants were carefully taken

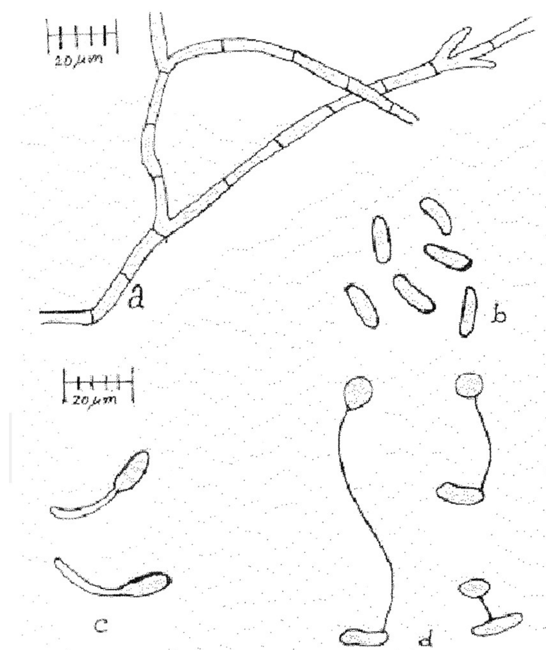

Figure 1. (a) Hypha of *C. gloeosporioides* with septa and branching. (b) Spores of *C. gloeosporioides.* (c) Germinating spores with germ tubes. (d) Germinated spores with appressoria formed.

out, placed on a slide and stained with lacto phenol and cotton blue. Length and breadth of spores, breadth of mycelia were measured by ocular micrometer standardized by stage micrometer under light micros-cope. The details of the morphology of the fungus were noted.

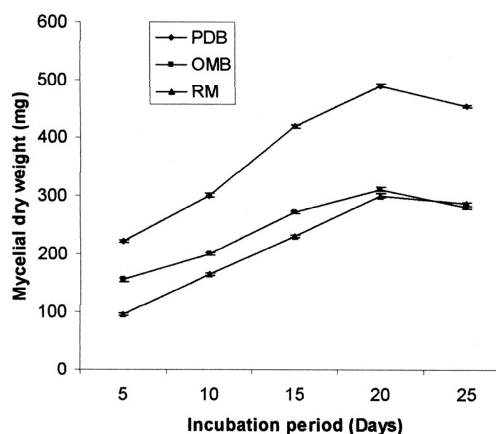

Figure 2. Growth of *Colletotrichum gloeosporioides* after different incubation periods in three different liquid media. PDB = Potato dextrose broth ; OMB = Oat meal broth ; RM = Richard's medium.

Influence of Culture Media

To assess the vegetative growth of *C. gloeosporioides* in solid media, nine different media viz. potato dextrose agar (PDA), oat meal agar (OMA), leaf extract agar (LEA), Czapek Dox agar (CDA), Richards's agar (RA), yeast extract mannitol agar (YEMA), malt extract agar (MEA), potato carrot agar (PCA) and nutrient agar (NA) were used. Mycelial blocks (4—6 mm) were cut from the advancing zone of hypha and placed in sterile petriplates (70 mm di-

Table 1. Mycelial growth and sporulation of *Colletotrichum gloeosporioides* in different solid media. *Mean of three replications. Data after ± represent standard error values. Spn = Sporulation, – = Nil, + = Poor, ++ = Fair, +++ = Good, ++++ = Excellent.

Medium of growth	Radial growth (mm)* and sporulation (days) 1		2		3		4		5	
	Growth	Spn	Growth	Spn	Growth	Spn	Growth	Spn	Growth	Spn
PDA	14.17± 1.09	–	31.17±0.93	–	45.83 ± 1.42	–	56.50± 1.04	–	70.00 ± 0.00	–
OMA	10.83± 0.93	–	22.83±0.83	+	35.83 ± 1.09	++	44.83± 1.36	+++	54.50 ± 1.04	++++
LEA	15.00± 0.76	–	33.17±1.17	–	50.50 ± 1.04	–	60.83± 0.93	–	70.00 ± 0.00	–
CDA	10.33± 0.73	–	23.17±0.60	–	36.83 ± 0.60	–	48.67± 0.88	–	59.67 ± 1.59	+++
RA	10.00± 0.44	–	21.83±0.60	–	38.67 ± 0.88	–	55.50± 0.86	–	70.00 ± 0.00	–
YEMA	9.83 ± 0.67	–	22.00±0.76	+	34.17 ± 1.09	++	46.00± 1.26	+++	51.00 ± 1.00	++++
MEA	10.17± 0.67	–	22.33±0.93	–	32.83 ± 1.17	–	47.17± 1.36	–	58.50 ± 1.04	–
PCA	11.17± 0.93	–	26.00±1.26	–	36.17 ± 1.09	–	46.50± 0.76	–	56.17 ± 1.17	–
NA	8.17 ± 0.60	–	19.00±1.00	–	26.83 ± 0.83	–	34.17± 0.93	+	41.33 ± 1.01	++
CD at 5%	0.83		1.59		1.87		1.95		1.60	

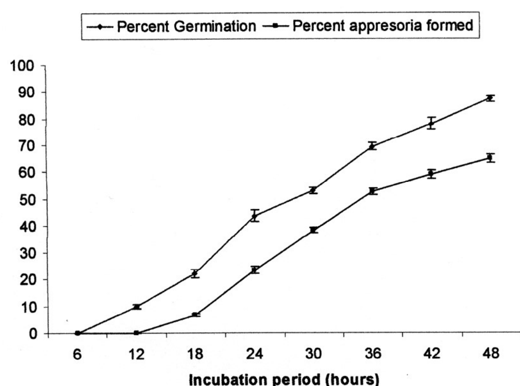

Figure 3. Effect of different incubation periods on spore germination and appressoria formation of *Colletotrichum gloeosporioides.*

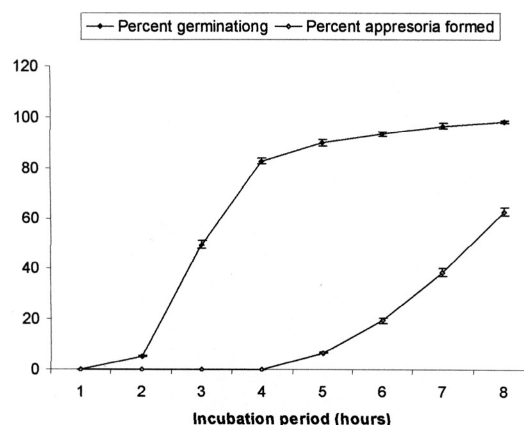

Figure 4. Effect of different incubation periods on spore germination and appressoria formation of *Colletotrichum gloeosporioides* adding brinjal leaf decoction.

ameter) containing 10 ml of different sterile medium and were incubated for maximum 5 days at 28±1 C. Radial growth of mycelia was measured at regular intervals to assess the mycelial growth in different solid media. To assess the mycelial growth in liquid media, mycelial agar discs (4—6 mm diameter) obtained similarly as mentioned above were transferred to conical flasks of 250 ml, each containing 40 ml of different sterilized liquid medium e.g. potato dextrose broth (PDB), oatmeal broth (OMB) and Richard's medium (RM) and incubated at 28±1 C. The growing fungal mycelia were strained through double-layered cheese cloth after 5, 10, 15, 20 and 25 days of incubation, then blotted on a blotting paper and dried in the oven at 60 C. They were cooled and the final weight was noted.

Assessment of Spore Germination

The fungus was initially cultured in OMA for 10 days at 28±1 C for adequate sporulation. To this, 4—6 ml sterile distilled water was added asceptically and the surface of the fungal culture was gently scrapped using a sterile inoculation needle. The tubes were gently shaken for mixing of the spores with distilled water. The resultant mixture was strained through a muslin cloth and the filtrate was used as spore suspension. Sterile distilled water was added to the suspension to adjust the concentration of the spores following hemocytometer count. Spore suspension (30 µl) of the fungus was placed on slides and the slides were incubated in a humid chamber (in petriplates) at 28±1 C. The slides were stained with cotton blue-lacto phenol after the desired period of incubation and observed under light microscope. Finally, the percent spore germination [no. of germinated spores / no. of spores counted) × 100], average germ tube length and percent appressoria formed

Table 2. Effect of different pH on growth of *Colletotrichum gloeosporioides* in PDB. *Mean of three replications. Data after ± represent standard error values. Dry weight of inoculating mycelial block was 10 mg.

	Mycelial dry weight (mg)* after incubation period (days)				
pH	3	6	9	12	15
4.0	35.00 ± 1.73	135.00 ± 1.53	180.00± 1.53	165.00 ± 1.53	150.00 ± 1.15
5.0	27.00 ± 1.15	71.00 ± 1.15	152.00± 1.53	170.00 ± 1.15	148.00 ± 1.53
5.5	38.00 ± 1.15	150.00 ± 1.17	190.00± 1.73	225.00 ± 2.08	210.00 ± 1.73
6.0	40.00 ± 1.00	155.00 ± 2.00	200.00± 2.52	231.00 ± 1.54	215.00 ± 1.00
6.5	32.00 ± 1.15	142.00 ± 1.73	188.00± 2.08	215.00 ± 1.53	200.00 ± 1.53
7.0	34.00 ± 1.00	142.00 ± 1.53	185.00± 2.08	175.00 ± 1.53	160.00 ± 2.08
8.0	10.00 ± 1.00	50.00 ± 1.53	75.00 ± 1.53	105.00 ± 2.08	115.00 ± 1.15
CD at 5%	2.30	3.44	4.00	3.02	3.09

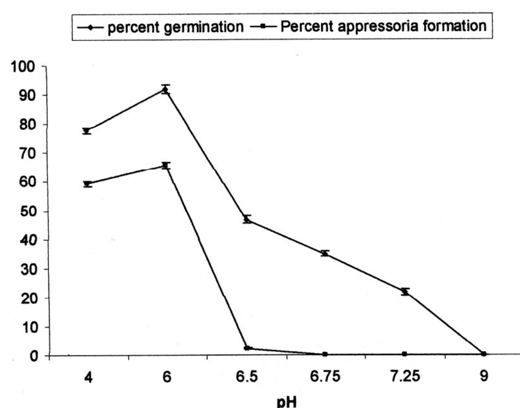

Figure 5. Effect of different pH on spore germination and appressoria formation of *Colletotrichum gloeosporioides.*

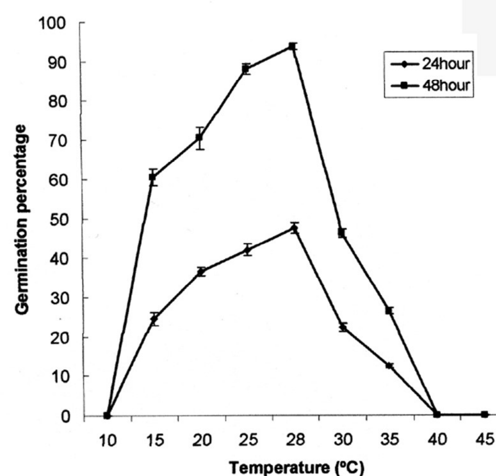

Figure 6. Effect of different temperatures on spore germination of *Colletotrichum gloeosporioides.*

in each case were calculated for each slide.

Spore germination, germ tube elongation and appressoria formation of *C. gloeosporioides* was studied after different periods of incubation. Thirty microliter of spore suspension (1×10^6/ml) drops were placed on slides in triplicates and allowed to incubate for 6, 12, 18, 24, 30, 36, 42 and 48 hours at 28±1 C in a humid chamber. In another set, slides were allowed to incubate for 1, 2, 3, 4, 5, 6, 7 and 8 hours at 28±1 C in a humid chamber.

Influence of Different pH

Sterilized PDB medium (25 ml in 100 ml Erlenmeyer flask) was adjusted to pH 4.0, 5.0, 5.5, 6.0, 6.5, 7.0 and 8.0 separately. The pH of the medium was adjusted by adding 1 (N) NaOH or 1 (N) HCl drop-wise into the medium before sterilization. Mycelial mat of *C. gloeosporioides* (4 mm diameter) was cut from the advancing zone of a petriplate and was placed separately in the media of different pH and incubated at 28±1 C. Mycelial dry weights were noted after 3, 6, 9, 12 and 15 d of incubation.

To study the effect of different pH on spore germination, solutions of different pH values of 4.0, 6.0, 6.50, 6.75, 7.25 and 9.0 were prepared by mixing 0.01 M K_2HPO_4 and 0.01 KH_2PO_4 and sterilized. The spores of the test fungus were suspended in the sterile solutions of different pH (1×10^6/ml) and allowed to germinate on glass slides in triplicates for 48 hours at 28±1 C.

Influence of Different Temperature

To assess the growth of *C. gloeosporioides* at

Table 3. Effect of different temperatures on mycelial growth of *Colletotrichum gloeosporioides* in PDB. *Mean of three replications. Data after ± represent standard error values. Dry weight of inoculating mycelial block was 10.0 mg.

Temperatures (C)	Mycelial dry weight (mg)* after incubation period (days)				
	3	6	9	12	15
8	10.00 ± 1.04	10.00 ± 0.50	10.00 ± 1.00	10.00 ± 0.50	10.00 ± 0.58
13	13.00 ± 1.00	18.00 ± 1.00	55.00 ± 1.00	60.00 ± 1.00	60.00 ± 3.51
18	22.00 ± 1.26	58.00 ± 2.08	125.00± 1.73	158.00 ± 1.53	145.00 ± 1.53
23	24.00 ± 0.76	135.00 ± 2.00	182.00± 3.05	208.00 ± 2.00	195.00 ± 1.73
28	25.00 ± 1.53	145.00 ± 2.08	198.00± 2.52	225.00 ± 3.21	210.00 ± 1.53
33	23.00 ± 1.26	125.00 ± 0.58	176.00± 2.08	195.00 ± 1.00	181.00 ± 2.65
38	15.00 ± 1.26	31.00 ± 1.15	46.00 ± 2.00	55.00 ± 1.53	50.00 ± 2.08
CD at 5%	2.37	3.08	4.30	3.24	4.20

Table 4. Effect of different carbon sources on the growth and sporulation of *Colletotrichum gloeosporioides.* Mean of three replications. Data after ± represent standard error values. Mwt (mg) = Mycelial dry weight in mg, Spn = Sporulation, – = Nil, + =Poor, ++ = Fair, +++ = Good, ++++ = Excellent. *Basal medium without any carbon source.

	Incubation period (days)									
	3		6		9		12		15	
Carbon source	Mwt (mg)	Spn	Mwt (mg)	Spn	Mwt (mg)	Spn	Mwt (mg)	Spn	Mwt (mg)	Spn
Glucose	10.00± 0.50	–	21.00± 1.53	++	71.00 ± 2.52	+++	86.00±2.00	+++	80.00 ± 1.00	++++
Sorbitol	11.00± 0.50	–	24.00± 0.50	+++	82.00 ± 1.15	+++	100.00±2.52	++++	90.00 ± 2.02	++++
Sucrose	18.00± 1.00	–	28.00± 1.53	++	50.00 ± 1.32	++	56.00±0.76	++	55.00 ± 0.58	+++
Lactose	10.00± 1.00	–	19.00± 1.15	–	30.00 ± 1.50	+	45.00±0.58	++	40.00 ± 1.80	++
Mannitol	12.00± 1.00	–	23.00± 0.76	++	86.00 ± 1.04	+++	105.00±1.53	++++	96.00 ± 1.50	++++
Inositol	10.00± 0.57	–	21.00± 1.00	+	69.00 ± 1.44	++	92.00± 2.52	+++	81.00 ± 2.00	++++
Control*	4.00 ± 0.50	–	8.00 ± 0.76	–	14.00 ± 0.76	–	18.00± 1.53	–	20.00 ± 0.76	–
CD at 5%	1.56		2.28		3.04		3.41		3.00	

different temperatures, the test fungus was inoculated in sterile PDB (25 ml in 100 ml Erlenmeyer flask). The inoculated flasks were incubated at different temperatures viz. 8, 13, 18, 23, 28, 33 and 38 C. After 3, 6, 9, 12 and 15 d of incubation, the fungal mycelia were harvested and strained through muslin cloth, blotted and dried at 60 C. Finally the dried mycelia were cooled and mycelial dry weights were noted.

To study the influence of different temperature on spore germination, spore suspension of *C. gloeosporioides* was prepared. Sterile distilled water was added to attain optimum concentration (1×10^6/ml) of the spores. Spore suspension drops (30µl) were placed in different slides in triplicates and incubated at different temperatures (10, 15, 20, 25, 28, 30, 35, 40 and 45 C).

Effect of Different Carbon Sources

The nutritional requirements of a pathogen are of considerable interest as it may help in understanding the physiology of disease development. A basal medium (glucose 1% ; asparagine 0.2% ; KH_2PO_4 0.1% ; $MgSO_4$, $7H_2O$ 0.05% ; Zn^{++}, Mn^{++} and Fe^{+++} 2 µg/ml) was used for the purpose. The different carbon sources tested were glucose, sorbitol, sucrose, lactose, mannitol and inositol. The equivalent amount of carbon present in 1% glucose was used as standard and added separately to the basal medium. The medium (25 ml) was taken in 100 ml Erlenmeyer flasks and sterilized by autoclaving at 15 lb. p.s.i. for 15 minutes. After cooling, the pathogen was inoculated using 4 mm mycelial discs in PDA and incubation was allowed for 3, 6, 9, 12 and 15 days. Erlenmeyer flasks of 100 ml capacity were used where each flask contained 25 ml of sterilized media. In control sets, no carbon sources were used in the basal medium. After incubation for the specified time periods, the mycelia were harvested, dried at 60 C and weighed.

Effect of Different Nitrogen Sources

To assess mycelial growth and sporulation of *C. gloeosporioides* on different nitrogen sources (both inorganic and organic) modified Asthana and Hawker's medium A (glucose 10 g ; KNO_3 3.5 g ; KH_2PO_4 1.75 g ; $MgSO_4$, $7H_2O$ 0.75 g ; agar 20 g and distilled water 1 liter) without agar was used as basal medium. The quantity of various nitrogen sources was so adjusted as to give the same amount of nitrogen as furnished by 3.5 g of KNO_3 in the basal medium. The medium with required nitrogen sources was prepared and 25 ml of media was dispensed in each 100 ml Erlenmeyer flask taking 3 flasks for each nitrogen source. All the media were sterilized by autoclaving at 15 lb. p.s.i. for 15 minutes and after cooling, the test fungus was inoculated and incubated for 3, 6, 9, 12 and 15 days at 28±1 C temperature. In control set, no nitrogen source was used in the basal medium. After specified incubation periods, the mycelia were harvested, dried at 60 C and

Table 5. Effect of different nitrogen sources on the growth and sporulation of *Colletotrichum gloeosporioides.* Mean of three replications. Data after ± represent standard error values, Mwt (mg) = Mycelial dry weight in mg. Spn = Sporulation, – =Nil, + = Poor, ++=Fair, +++ = Good, ++++ = Excellent. * Basal medium without any carbon source.

Nitrogen sources	Incubation period (days) 3 Mwt (mg)	3 Spn	6 Mwt (mg)	6 Spn	9 Mwt (mg)	9 Spn	12 Mwt (mg)	12 Spn	15 Mwt (mg)	15 Spn
Organic										
Peptone	17.50 ±0.29	–	44.50 ± 0.86	+	70.67 ± 1.20	++	82.00 ± 2.65	+++	70.17 ± 1.96	++++
Yeast extract	36.33 ± 1.76	–	52.23 ± 1.13	+	60.00 ± 1.00	+	60.33 ± 2.52	+++	60.17 ± 1.48	++++
Beef extract	30.17 ± 1.09	–	46.17 ± 1.09	+	57.00 ± 1.15	+	63.00 ± 2.65	++	55.67 ± 1.45	+++
Glycine	11.67 ±0.67	–	15.17 ± 0.83	–	16.83 ± 1.09	+	15.17 ± 1.04	++	14.83 ± 0.60	++
Trypton	29.50 ±1.26	–	44.00 ± 1.53	–	51.17 ± 1.92	+	56.17 ± 1.89	++	51.33 ± 1.20	++
Inorganic										
Ammonium sulfate	5.67 ±0.17	–	20.00 ± 0.50	–	46.17 ± 0.60	–	57.17 ± 1.61	–	50.00 ± 1.04	–
Ammonium phosphate	5.33 ±0.17	–	14.17 ± 0.44	–	32.00 ± 1.00	–	42.00 ± 1.73	–	42.00 ± 1.53	–
Ammonium nitrate	5.17 ±0.60	–	13.33 ± 0.67	–	25.33 ± 0.88	–	35.33 ± 1.53	–	30.17 ± 0.93	–
Sodium nitrate	4.67 ±0.44	–	5.83 ± 0.33	–	10.17 ± 0.60	–	15.83 ± 1.04	–	15.67 ± 0.67	–
Potassium nitrate	4.17 ±0.33	–	5.17 ± 0.60	–	10.33 ± 0.33	–	15.00 ± 1.73	–	12.00 ± 0.50	–
Control*	4.33 ±0.44	–	5.17 ± 0.33	–	6.17 ± 0.33	–	7.83 ± 0.76	–	8.67 ± 0.33	–
CD at 5%	1.30		1.30		1.64		1.70		1.88	

weighed. The results are tabulated. After each of incubation periods, the extent of sporulation was also recorded in five different grades based on visual observations.

Statistical Analysis

Statistical analysis was done with the help of Statistical Package for the Social Sciences (SPSS), version 11.0, SPSS Inc., Chicago, Illinois.

Results and Discussion

Microscopic study of the fungus revealed that mycelia and conidia were hyaline. The length and breadth of conidia ranged between 13—16 µm and 4—6 µm respectively. The mature conidia were light, one-celled and hyphae were septate. The diameter of the mature hyphae was 3—5 µm (Fig. 1). Similar observations regarding conidial size and shape have been reported by several authors (6, 7).

Table 1 shows that *C. gloeosporioides* grew in all the media tested but LEA, PDA and RA supported best growth. In case of spore formation, huge masses of pinkish acervuli were found in oat meal agar and yeast extract mannitol agar. Bean meal agar, potato dextrose agar and Richard's agar supported good growth of *C. gloeosporioides* causing leaf blight of *Chrysanthemum* (8). Composition of the culture media and the pH appear to influence the growth, sporulation and morphology of the conidia of *S. schenckii* (9).

Maximum growth of *C. gloeosporioides* was recorded in PDB at 20 days of incubation (Fig. 2).

After 20 days, mycelial dry weight declined due to autolysis and depletion of the media. OMB and RM also showed similar growth pattern. Study on the growth of *C. gloeosporioides* isolated from cashew anthracnose in different solid and liquid media revealed that Richard's agar and potato dextrose agar supported good growth and sporulation (10).

Studies on the mycelial growth at different pH showed that the mycelial dry weight of *C. gloeosporioides* was maximum at pH 6.0 and lowest at pH 8.0 (Table 2). Table 3 shows that mycelial dry weight was maximum at 28 C. At 8 C no mycelial growth was recorded. Our results were similar to the results obtained by previous workers (8, 11—14). Thakare and Patil (8) observed that the optimum pH for growth of *C. gloeosporioides* causing leaf blight in chrysanthemum ranged from 4.1 to 6.8. Kang et al. (15) reported the optimum pH for growth of this fungus to be around 6.0.

During the present study, germination of spores began after 10—12 hours of incubation in sterile distilled water. Percent germination of spores, germ tube length and percent appressoria formed were found to increase with increasing time interval. After 48 h of spore germination, germ tube length and appressoria formation were recorded as 87.17%, 97.33 µm and 64.67% respectively (Fig. 3). When brinjal leaf extract was added in spore suspension, germination of spores started after 2 hours of incubation and nearly all the spores were germinated within 8 h of incubation (Fig. 4). The percent germination of spores, germ tube length and percent appressoria formed were 98.3%, 142.5 µm and 62.5% respectively after 8 h of incubation. Similar results were observed by Kuo (6) who used a two-step method to study the conidial germination and appressorium formation in the mango anthracnose fungus *C. gloeosporioides* during a 9 hour period. Spore germination of *Bipolaris carbonum* was also observed to begin within 2—4 hour *in vitro* (16).

Among the different pH tested, pH 6.0 showed highest germination (92.00%). Figure 5 shows that percent of germination sharply declined at pH 7.25 and no germination was found at pH 9.00. Highest percentage of appressoria formation (65.67%) was observed at pH 6.0. Germ tube length (142.33 µm) was highest at pH 6.75 where no appressoria were formed and percent germination was only 34.77. In a similar study, optimum pH for growth and sporulation of *Alternaria zinniae* was found in the range of pH 6.0—6.5 (4). Effect of temperature on spore germination was studied and results are presented in Figure 6. Results revealed that spore germination was optimum at 28 C (93.53% after 48 h) but no germination occurred at 10 C or below and 40 C or above.

Fungi show differential affinity to various carbon and nitrogen sources for their growth. Some common carbon sources have been used to select best carbon source for *in vitro* growth of the fungi. Table 4 shows that mannitol (105.00 mg) was the best carbon source for optimum growth and sporulation of *C. gloeosporioides.* Sorbitol produced mycelial growth and sporulation next to mannitol. Similarly, some nitrogen sources were also tested to get the best nitrogen source for the growth of *C. gloeosporioides.* Highest growth and sporulation were observed in peptone (82.00 mg) among the organic nitrogen sources tested. Beef extract and yeast extract also showed satisfactory growth and sporulation after 12 d of incubation. Among the inorganic nitrogen sources tested, ammonium sulfate (57.17 mg) and ammonium phosphate (42.00 mg) showed medium growth without any sporulation (Table 5). In a similar study on mycelial growth and sporulation of *C. gloeosporioides* highest growth and sporulation were recorded when mannitol was used as carbon source and peptone was used as nitrogen source (17). Several workers (4, 18, 19) studied the influence of various carbon and nitrogen sources on fungal metabolism.

The result of the present study is crucial for further studies on the fungus as a pathogen of brinjal and also during other host-pathogen interaction studies.

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