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Physiological Studies on Major Fungi of Leaf Rot Disease of Coconut

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Abstract

Influence of culture media, pH, temperature and sources of carbon, and nitrogen on growth of major fungi of coconut leaf rot - *Colletotrichum gloeosporioides, Exserohilum rostratum* and *Fusarium solani* - was investigated *in vitro*. Potato dextrose agar was found to be best for the growth of all the three fungi. Czapek dox agar, glucose nitrate agar and Sabouraud dextrose agar were other favourable media for *C. gloeosporioides* and *E. rostratum*; Czapek dox agar to *F. solani*. Carrot agar and oat meal agar were less favourable for the growth of the fungi. In liquid media, high mycelia yield of *C. gloeosporioides* and *E. rostratum* occurred in potato dextrose broth, oat meal broth and Czapek dox broth and *F. solani* in oat meat broth. Carrot broth was least favourable for all the three fungi. Higher growth of *C. gloeosporioides* and *F. solani* occurred in the pH range of 6.0-6.5 while *E. rostratum* preferred a slightly more acidic pH. Higher growth of all the fungi occurred in the temperature range of 25°C-30°C. Mannitol provided good growth of *C. gloeosporioides* in solid medium; fructose, glycerol, sorbitol and glucose were the other, next in order of preferred carbon sources for the fungus. Mannitol and sorbitol were favourable for the growth of *E. rostratum*. Glycerol was good to *F. solani*, besides other carbon sources such as sorbitol, glucose and fructose in influencing the fungal growth. In liquid medium also sorbitol and glucose were the preferred carbon sources, besides mannitol, by all the three fungi. All the three fungi preferred to grow best in sodium nitrate as the source of nitrogen both in solid and liquid media, followed by potassium nitrate, as compared to other sources of nitrogen tested. These findings would be helpful in understanding the physico-cultural behaviour of the fungi and also in context of their growth requirements.

Key words : *Colletotrichum gloeosporioides, Exserohilum rostratum, Fusarium solani*, culture media, pH, temperature, carbon, nitrogen, growth, physiology, coconut, leaf rot.

Introduction

Leaf rot of coconut is a part of root (wilt) disease complex, prevalent endemically in southern districts of Kerala besides its incidence in northern districts of the state and also certain districts of Tamil Nadu. Leaf rot is due to a complex of fungi where in *Colletotrichum gloeosporioides*, *Exserohilum rostratum* and *Fusarium solani* are the major pathogens (Srinivasan, 2002). *In vitro* studies of fungi are undertaken to understand their responses to various conditions and for characterization, cultural characters etc. Cultural characters of fungi depend on media used and fungal growth and their sporulation are reportedly varied in different media (Bilgrami, 1964; Bainade *et al*, 2002a, b; Netam *et al*, 2002; Chidananda

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Swamy and Srikant Kulkarni, 2003). Different species of fungi and isolates of a fungus are shown to differ in their nutritional requirements (Mohammed and Mahmood, 1976). Environmental factors affect the metabolic activities related to growth and sporulation of fungi (Chandi Ray and Purkayastha, 1977). The optimal pH for their growth is variable. Difference in temperature optima may also exist among species of fungi. Certain fungal pathogens tend to cause severe plant disease at their optimal temperatures of growth, as for example *Fusarium* wilt of tomato was reported more serious in a relatively warm climate than in cooler climate (Walker, 1971). Carbon plays an important role in the nutrition of fungi. About half of the mycelial dry weight of fungi is due to carbon. Carbon compounds are the chief sources of energy, which is produced by their oxidation. The carbon source utilizing capacity of a fungus is dependant upon the species involved. For example, good mycelial yield of *C. gloeosporioides* was obtained by Lal and Tandon (1972) with glucose, sucrose, fructose, mannitol and glycerol. Sucrose and lactose were found to be good for *Helminthosporium*, but the fungus grew poorly in starch. While sucrose and glucose significantly enhanced the growth of *Lasiodiplodia theobromae*, only poor growth of the fungus was noticed in maltose, lactose and mannitol; also, potassium nitrate favoured the fungus to attain higher growth over other nitrogen sources tried (Shelar *et al*, 1998). Nitrogen is one of the essential elements for the growth and sporulation of various fungi. Diverse reports on utilization of nitrogen compounds by fungi have been reported by other workers also (Fergus, 1952; Converse, 1953; Hachskaylo *et al*, 1956). Brijesh Kamal *et al* (2002) observed maximum growth of *Botrytis cinerea* with sodium nitrate as the source of nitrogen than other nitrogen sources.

Therefore, cultural studies on fungi have received wider attention. Although attempt on growth requirements of certain fungi of the leaf rot disease was made earlier (Menon and Nair, 1951), more knowledge on physico-cultural characters of the fungi was felt necessary, taking into account current understanding on the fungi involved in the disease (Srinivasan and Gunasekaran, 1996, 2000). Hence, experiments were conducted and the results accrued from the studies on effect of culture media, pH, temperature regimes and carbon, and nitrogen sources on growth of major fungi of the disease are presented in this article.

Materials and Methods

Cultures of fungi : Fresh cultures of *Colletotrichum gloeosporioides*, *Exserohilum rostratum* and *Fusarium solani* were made out from leaf rot affected palms through conventional isolation and purification process, and utilized for various studies.

Culture media : Growth of the fungi in different solid culture media was studied in two experiments (table 1) and the media were prepared as per standard procedure. Sterile medium was poured into each of 90 mm petri dishes (15-20 ml) and agar disc (5 mm diameter) of 5 day old culture (earlier grown in potato dextrose agar) of each fungus was inoculated onto centre of each petri dish. The petri dishes were incubated at 30±2^{0}C. Three replications were maintained for each fungus per medium. The colony diameter was measured at different intervals. Cumulative mean colony diameter was computed. Growth of the fungi was also studied in different liquid culture media (table 2). The agar discs of the fungi were inoculated individually into each sterile liquid medium in 250 ml conical flasks (25 ml) and incubated. After 7 days of incubation the mycelial mats from flasks were harvested onto previously weighed Whatman No. 1 filter papers, washed in distilled water and dried in an oven at 60° C for 72 hours. Thereafter, they were cooled (passed through desiccators) and mycelial weight determined.

Medium pH : The effect of pH on growth of the fungi was studied in potato dextrose liquid medium (25 ml of sterile medium per flask of 100 ml capacity). The pH of the medium was adjusted to various levels, from 4.0 to 9.0 (table 3). Five mm mycelial disc of each fungus was inoculated individually into flasks, incubated at 30±2°C. After 7 days of incubation the mycelial mat was harvested using pre-weighed Whatman No. 1 filter paper and dry weight of mycelial mat determined as explained earlier.

Temperature : The effect of temperature on growth of the fungi was studied in potato dextrose agar medium. The petri dishes poured with the sterile medium was inoculated with the representative fungi as detailed earlier and incubated at different temperature regimes, from 05 to 50°C (table 4). The colony diameters of the fungi were measured for three successive days. Cumulative mean colony diameter of the fungi was computed.

Carbon sources: The carbon utilizing capacity of the fungi in solid medium was studied in two experiments (table 5). In the experiment I, fructose, glucose, glycerol, mannitol, sorbitol, starch and sucrose were used as sources of carbon in corresponding sugar based agar medium. In experiment II, fructose, glucose mannitol, sorbitol and sucrose were used as carbon sources in Czapek dox agar medium where in sucrose was qualitatively replaced with other corresponding sugar for carbon source, keeping the other constituents of the medium same. Sterile medium was poured into 90 mm petri dishes (15-20 ml). Mycelial discs (5 mm diameter) from the actively growing cultures of fungi (previously grown in potato dextrose agar) was individually inoculated onto centre of each petri dish and incubated at 30±2°C. Colony diameter was measured at different day's interval. Three replications were maintained for each fungus per carbon source in each experiment. Cumulative mean colony diameters of the fungi were computed. The effect of different carbon sources on growth of the fungi was studied in liquid medium also (table 6), using Czapek dox broth (sucrose qualitatively replaced with other corresponding sugar for carbon source, keeping other constituents of the medium same). The medium containing each carbon source (25 ml/ conical flask of 100 ml capacity) was inoculated with the fungi individually (5 mm diameter mycelial disc). The inoculated flasks were incubated at 30±2°C and three replications maintained. After seven days of incubation mycelial mat of each fungus was harvested in pre-weighed Whatman No. 1 filter paper. The mycelial mat was gently washed with sterile distilled water and dried in an oven at 60°C for 72 hours. Thereafter, they were cooled (passed through desiccators) and mycelial weight determined.

Nitrogen sources : To study the effect of different sources of nitrogen on the growth of the fungi both solid and liquid media were used. Two experiments were conducted for solid media. In experiment

I, ammonium acetate, ammonium oxalate, ammonium nitrate, peptone and sodium nitrate were used as sources of nitrogen. In experiment II, the nitrogen sources employed were ammonium oxalate, sodium nitrate, potassium nitrate, ammonium chloride and ammonium sulphate (table 7). For this in experiment II, the composition of the medium is as follows: Nirogen source - 2 g, Sucrose - 30 g, KCl - 0.5 g, KH_2PO_4 - 1g, $MgSO_4.7H_2O$ - 0.5 g, $FeSO_4.7H_2O$ - 0.1 g, Agar - 20 g, D. Water - 1000 ml, pH: 5.5-6.5. The fungi were inoculated on to solid media, incubated and colony diameters measured and cumulative mean colony diameter computed as described earlier. The liquid medium with different nitrogen sources (table 6) was also inoculated and incubated as detailed under method for evaluation of carbon sources. The medium constituents for the study in liquid medium are as above, but without agar. The dry weight of the fungal mycelial mat was determined appropriately.

Results and Discussion

The growth rate and cultural characters of leaf rot fungi varied with the culture media. In experiment I, *C. gloeosporioides* grew to the highest extent in potato dextrose agar (42.4 mm) and least growth of the fungus occurred in carrot agar (28.9 mm). Colour of the fungus did not vary much among media. However, the fungus produced abundant aerial mycelium in potato dextrose agar medium. *E. rostratum* also grew at higher rate in potato dextrose agar (51.8 mm) followed by Czapek dox agar and glucose nitrate agar media. Carrot agar was least effective to the fungus (28.2 mm). A difference in growth of *F. solani* was also found among media. The fungus was favoured by potato dextrose agar, glucose nitrate agar and Czapek dox agar, even as its general growth rate was slower than other fungi. In experiment II also, *C. gloeosporioides* preferred to grow fast in potato dextrose agar, besides its growth in Czapek dox agar and sabouraud dextrose agar media. Oat meal agar and carrot agar were less preferred by the fungus. A similar trend of media effect was seen in the growth of *E. rostratum* also. In the case of *F. solani*, higher growth occurred in potato dextrose agar, Czapek dox agar and oat meal agar than in sabouraud dextrose agar and carrot agar media. Difference on effect of solid media on the growth of leaf rot fungi is thus evidenced (table 1).

Variation in growth potential of the fungi in different liquid media was also seen. Among the fungi *F. solani* showed a dispersed growth in all the liquid media tested as compared to a compact mycelial mat developed by *C. gloeosporioides* and *E. rostratum*. In oat meal broth, Czapek dox broth and potato dextrose broth higher mycelial yield of *C. gloeosporioides* (140-300 mg) and *E. rostratum* (220-360 mg) was observed over Sabouraud dextrose broth and carrot broth (90-120 mg/100-200 mg). *F. solani* (130 mg) and *C. gloeosporioides* (300 mg) preferred oat meal broth for developing higher level of mycelium as compared to other liquid media (table 2).

Variation was found in mycelial dry weight of all the fungi with change of medium pH. Growth of *C. gloeosporioides* was found to be high (260 mg) in the pH range of 6.0 to 6.5. Gradual decrease in growth of the fungus occurred in above or below this level of pH range. *E. rostratum* preferred a slightly acidic pH (4.0-6.0) than *C. gloeosporioides* and that fungus relatively tolerated the low pH. However, with increase in pH level the

Table 1 : Effect of different solid culture media on growth of major fungi of leaf rot (Mean of 3 replications).

S. No.	Culture medium	Cumulative mean colony diameter (mm) in Expt. I*				Cumulative mean colony diameter (mm) in Expt. II**			
		Colletotrichum gloeosporioides	*Exserohilum rostratum*	*Fusarium solani*	Mean	*Collectotrichum gloeosporioides*	*Exserohilum rostratum*	*Fusarium solani*	Mean
1.	Potato dextrose agar	42.4	51.8	27.3	40.5	35.6	41.0	31.4	35.9
2.	Sabouraud dextrose agar	42.3	25.6	25.1	30.9	37.2	33.1	27.2	32.5
3.	Czapek dox agar	31.4	43.4	26.6	33.8	35.6	39.5	33.4	36.2
4.	Carrot agar	28.9	28.2	19.9	25.7	30.7	33.6	29.4	31.2
5.	Glucose nitrate agar	37.4	43.1	27.7	36.0	-	-	-	-
6.	Oat meal agar	-	-	-	-	22.3	28.6	31.2	27.4
	Mean	36.5	38.4	25.3		32.3	35.2	30.5	

*Mean of 1, 2, 3 and 4 days of incubation.
**Mean of 3, 4, 5 and 6 days of incubation.

mycelial production of *E. rostratum* declined. The optimum pH for *F. solani* was in the range of 6.0-6.5 (100-110 mg), similar to *C. gloeosporioides*; with an increase or decrease in the level of pH the growth of this fungus also declined (table 3).

Higher growth of all the three fungi took place in the temperature range of 25°C-30°C. *C. gloeosporioides*, *E. rostratum* and *F. solani* yielded cumulative mean colony diameters of 36.8 mm, 46.0 mm and 27.9 mm, respectively at 30°C after 3 days of incubation. The fungi failed to grow at the higher temperature of 40°C. In lower temperature levels also (5°C-10°C) these fungi failed to establish growth. At 15°C also *E. rostratum* failed to establish while other fungi responded with a scanty growth. The mean response of all the fungi to temperature regimes for different days of incubation generally remained similar. The mycelial growth rate of *F. solani* was slower than that of other fungi. However, the mean optimal temperature for all the fungi is shown to be in similar order (table 4).

The difference in growth rate of leaf rot fungi in differential media has been revealed in the study that is in conformity with certain other reports (Bilgrami, 1964). Abiotic factors like pH and temperature does have effect on the growth of the fungi. Menon and Nair (1951) reported a better growth of *Helminthosporium halodes* in oat meal agar and *Gloeosporium* sp. in Brown's agar medium. In the present study, utility of potato dextrose agar for all the three fungi and also other media such as Sabouraud dextrose agar and Czapek dox agar have been noted. Preference of oat meal broth by *C. gloeosporioids* and *F. solani* and in addition to the medium potato dextrose broth and

Table 2 : Effect of different liquid culture media on growth of major fungi of leaf rot (Mean of 3 replications).

S. No.	Culture medium	Mycelial dry weight of fungi on 7th day of incubation (mg)			
		Colletotrichum gloeosporioides	***Exserohilum rostratum***	***Fusarium solani***	**Mean**
1.	Potato dextrose broth	140	220	040	133
2.	Oat meal broth	300	250	130	227
3.	Czapek dox broth	230	360	060	217
4.	Sabouraud dextrose broth	120	200	070	130
5.	Carrot broth	090	100	040	077
	Mean	180	230	070	
	C. D. (P = 0.05)	020	010	010	

Table 3 : Effect of different pH on the growth of major fungi of leaf rot in potato dextrose liquid medium (Mean of 3 replications).

S. No.	Level of pH in the medium	Mycelial dry weight of fungi on 7th day of incubation (mg)			
		Colletotrichum gloeosporioides	***Exserohilum rostratum***	***Fusarium solani***	**Mean**
1.	4.0	130	250	060	147
2.	4.5	170	290	060	173
3.	5.0	210	260	070	180
4.	5.5	250	230	090	190
5.	6.0	260	220	110	197
6.	6.5	260	160	100	173
7.	7.0	230	150	090	157
8.	7.5	230	130	080	147
9.	8.0	220	100	080	133
10.	8.5	180	090	070	113
11.	9.0	190	070	060	107
	Mean	210	180	110	
	C.D. (P = 0.05)	030	010	010	

Czapek dox broth by *C. gloeosporioides* and *E. rostratum* are of importance.

Bainade *et al* (2002 a) observed higher growth of *Macrophomina phaseolina* in potato dextrose agar, besides several other media *in vitro*. The pathogen could grow in the temperature range of 20-30°C, maximally at 30°C and it could grow in the pH range of 6-8 (Bainade *et al*, 2002 b). Netam *et al* (2002) found excellent growth and sporulation of *F. oxysporum* f. sp. *solani* in potato dextrose agar. This fungus also attained significantly higher growth at 30°C and the growth was minimal at pH 4.0 than at pH 7.0. Brijesh Kamal *et al* (2002) found

Table 4 : Effect of temperature on growth of major fungi of leaf rot in potato dextrose agar medium (Mean of 3 replications).

S. No.	Temperature	Cumulative mean colony diameter (mm)*			
		Colletotrichum gloeosporioides	*Exserohilum rostratum*	*Fusarium solani*	Mean {C.D. (P=0.01) : 11.4}
1.	05	0	0	0	0
2.	10	0	0	0	0
3.	15	07.0	0	05.8	04.3
4.	20	26.1	26.6	21.8	24.8
5.	25	29.2	41.1	26.0	32.1
6.	30	36.8	46.0	27.9	36.9
7.	35	22.0	26.3	11.5	20.8
8.	40	0	0	0	0
Mean		15.1	17.8	11.6	

*Mean of 1, 2 and 3 days of incubation.

best growth of *Botrytis cinerea* in potato dextrose agar; the fungus preferred temperature regime of 20-25°C (maximum growth at 25°C) and pH range of 4.5-6.0 (maximum growth at pH 6.0). Chidananda Swamy and Srikant Kulkarni (2003) reported maximum growth of *C. capsici* in potato dextrose broth medium; maximal growth at pH 6.5 and temperature at 30°C. Sandeep Raheja and Thakore (2002) found favourable effect of pH 5.5 on *C. gloeosporioides*.

Although the leaf rot fungi can generally grow in the temperature regime of 15°C-30°C, higher growth of the fungi took place in the range of 25°C-30°C and maximal growth at 30°C that may account for abundance of the fungi in nature (Srinivasan and Gunasekaran, 1996, 2000). Such a result is corroborated with the report of Shelar *et al* (1998). The optimal pH for *C. gloeosporioides* and *F. solani* is in the range of 6.0-6.5 and tolerance of *E. rostratum* to lower pH (4.0-6.0) is other points of interest. The preference of plant pathogenic fungi for an acidic pH for their growth are confirmed (Montgomery, 1936). Depressing effect of higher acidity or alkalinity on growth of the fungi is in conformity with the report of Menon and Nair (1951).

The source of carbon significantly influenced the growth of *C. gloeosporioides*, *E. rostratum* and *F. solani*. In experiment I, mannitol provided good growth of *C. gloeosporioides* (39.7 mm) followed by fructose, glycerol, sorbitol and glucose. Starch and sucrose supported the fungal growth to the lesser extent only (16.4-24.8 mm). Mannitol and sorbitol favoured *E. rostratum* to a good extent (37.4-39.1 mm). Other sugars seem to have comparatively less influenced the fungus. Glycerol was found to be good for the growth of *F. solani* (30 mm). That was followed by sorbitol, glucose and fructose (21.0-26.3 mm). In general, starch and sucrose were found less favourable for all the three fungi. Influence of carbon sources on the fungi was observed in experiment II also. Effect of sorbitol, glucose

Table 5 : Effect of different carbon sources in solid media on growth of major fungi of leaf rot (Mean of 3 replications).

S. No.	Carbon source	Cumulative mean colony diameter (mm) in Expt. I*				Cumulative mean colony diameter (mm) in Expt. II**			
		Colletotrichum gloeosporioides	*Exserohilum rostratum*	*Fusarium solani*	Mean	*Colletotrichum gloeosporioides*	*Exserohilum rostratum*	*Fusarium solani*	Mean
1.	Fructose	31.4	29.2	21.0	27.2	32.6	48.4	26.7	35.9
2.	Glucose	28.8	34.6	22.3	28.6	36.7	56.7	38.3	43.7
3.	Glycerol	31.6	34.8	30.0	32.1	-	-	-	-
4.	Mannitol	39.7	37.4	18.8	31.9	35.8	66.4	36.6	46.3
5.	Sorbitol	28.9	39.1	26.3	31.4	38.9	55.4	38.5	44.2
6.	Starch	24.8	30.4	20.9	25.4	-	-	-	-
7.	Sucrose	16.4	20.0	15.3	17.2	32.7	52.5	34.1	39.8
	Mean	28.8	32.2	22.1		35.3	55.7	34.8	
							C.D. (P=0.01): 09.8		

*Culture medium used: Fructose nitrate agar, glucose nitrate agar, glycerol nitrate agar, mannitol nitrate agar, sorbitol nitrate agar, starch nitrate agar and sucrose nitrate agar, respectively for the corresponding carbon sources (Mean of 1, 2 and 3 days of incubation).

**Czapek dox agar medium used with changes in carbon source, replacing sucrose with appropriate sugar (Mean of 3, 4 and 5 days of incubation).

and mannitol were on par and favourable to the growth of *C. gloeosporioides* (36.7-38.9 mm). *E. rostratum* was favoured more by mannitol, glucose and sorbitol (55.4-66.4 mm). Sorbitol, glucose and mannitol were favourable (36.6-38.5 mm) for *F. solani* (table 5). The variable effect of carbon sources on fungi has been corroborated in liquid media also (table 6). Sorbitol, glucose and mannitol yielded more dry weight of mycelia of *C. gloeosporioides* (160-170 mg) and *F. solani* (120-140 mg). The mycelial yield of *E. rostratum* was higher in mannitol and sorbitol (240-290 mg) than other sugars. Therefore, differential effects of carbon sources on leaf rot fungi are evidenced.

Difference in the utilization of nitrogen sources by the fungi has been found. In experiment I, all the three fungi were found to grow best in sodium nitrate as the source of nitrogen - *C. gloeosporioides* (28.8 mm). *E. rostratum* (34.6 mm) and *F. solani* (25.0 mm) - as compared to other sources of nitrogen. For *C. gloeosporioides*, peptone was next in the order of merit (26.5 mm), whereas for *E. rostratum* ammonium nitrate and ammonium oxalate (20.9-21.0 mm). Moderate effect of ammonium acetate and peptone on *F. solani* (16.3-18.0 mm) was noticed. Ammonium nitrate served least to the growth of both *C. gloeosporioides* and *F. solani*, while *E. rostratum* grew least in peptone as nitrogen source.

Table 6 : Effect of different carbon and nitrogen sources in liquid media on growth of major fungi of leaf rot (Mean of 3 replications).

S. No.	Dry weight of mycelium on 7[th] day of incubation (mg)									
	Carbon source*					Nitrogen source**				
	Source of carobn	*Colletotrichum gloeosporioides*	*Exserohilum rostratum*	*Fusarium solani*	Mean	Source of nitrogen	*Colletotrichum gloeosporioides*	*Exserohilum rostratum*	*Fusarium solani*	Mean
1.	Sucrose	110	200	100	137	Sodium nitrate	220	280	240	247
2.	Glucose	170	230	140	180	Potassium nitrate	160	190	140	163
3.	Fructose	130	210	090	143	Ammonium chloride	060	050	030	047
4.	Sorbitol	170	240	140	183	Ammonium sulphate	130	120	140	130
5.	Mannitol	160	290	120	190	Ammonium oxalate	180	140	100	140
	Mean	150	230	120		Mean	150	160	130	
	C.D. (P = 0.05)	010	010	010		C.D. (P = 0.05)	010	030	010	

*Czapek dox broth medium used with changes in carbon source, replacing sucrose with appropriate sugar, ** See text for medium details.

Table 7 : Effect of different nitrogen sources in solid media on growth of major fungi of leaf rot (Mean of 3 replications).

S. No.	Nitrogen source	Cumulative mean colony diameter (mm) in Expt. I*				Cumulative mean colony diameter (mm) in Expt. II**			
		Colletotrichum gloeosporioides	*Exserohilum rostratum*	*Fusarium solani*	Mean	*Colletotrichum gloeosporioides*	*Exserohilum rostratum*	*Fusarium solani*	Mean (C.D. (P=0.01)=09.5)
1.	Ammonium acetate	23.0	19.1	18.0	20.0	-	-	-	-
2.	Ammonium oxalate	24.4	20.9	16.1	20.5	36.8	25.7	24.0	28.8
3.	Ammonium nitrate	16.4	21.0	13.1	16.8	-	-	-	-
4.	Peptone	26.5	17.6	16.3	20.1	-	-	-	-
5.	Sodium nitrate	28.8	34.6	25.0	29.5	43.3	59.4	43.4	48.7
6.	Potassium nitrate	-	-	-	-	36.0	40.7	34.2	36.9
7.	Ammonium chloride	-	-	-	-	11.6	19.6	08.5	13.2
8.	Ammonium sulphate	-	-	-	-	31.4	17.6	27.8	25.6
	Mean	23.8	22.6	17.7		31.8	32.6	27.6	

*Culture medium used: Glucose ammonium acetate agar, glucose ammonium oxalate agar, glucose ammonium nitrate agar, glucose peptone agar, glucose sodium nitrate agar, respectively for the corresponding nitrogen sources (Mean of 1, 2 and 3 days of incubation).

**See text for medium details (Mean of 3, 4 and 5 days of incubation).

In experiment II also all the three fungi preferred sodium nitrate as the best source of nitrogen. Potassium nitrate was next in order of preference for the growth of fungi. *C. gloeosporioides* and *F. solani* least preferred ammonium chloride, while *E. rostratum* least favoured by ammonium sulphate (table 7). In liquid culture medium also (table 6) all the three fungi preferred sodium nitrate as best nitrogen source, as the mycelia yield stood higher, followed by potassium nitrate. Ammonium chloride had least supported the growth of the fungi in liquid media. Therefore, variation in effect of sources of nitrogen on leaf rot fungi is evident.

The utilization of carbon sources is critical for fungi in synthesis of cellular constituents and to chiefly derive energy by their oxidation. The preferential utilization of mannitol, besides other carbon sources - sorbitol, glucose, fructose and glycerol - by *C. gloeosprioides* in the study is generally in agreement with the observations of Lal and Tandon (1972). Chandi Ray and Purkayastha (1972) noticed sorbitol as a most favourable source of carbon for *C. corchorum*. Mannitol and sorbitol as being most preferably utilized by *E. rostratum* in the current study assumes importance of such carbon source in the fungal growth requirement. The utilization of glycerol by *F. solani* in sold medium and certain other carbon sources (such as sorbitol, glucose and mannitol) being effectively utilized by the fungus in liquid medium pointed out the nutritional preferential status of the fungus. Nitrogen source also plays an important role in the growth of fungi and the fungal growth rate depends upon the source of nitrogen. Brijesh Kamal *et al* (2002) recorded maximum growth of *B. cinerea* in sodium nitrate amended culture medium followed by potassium nitrate. The results of the present study revealing sodium nitrate as the most preferred source of nitrogen for all the three fungi is strongly supported by such a report. The results accrued from these studies are helpful in understanding the general physico-cultural behaviour of leaf rot associated fungi *in vitro* that are strategically useful and also in their nutritional preferences.

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