Growth and Sporulation of *Curvularia eragrostidis* as Affected by Media and Nitrogen Sources

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Abstract

Out of eight synthetic and semi-synthetic solid and liquid culture media tested, host leaf extract agar and potato dextrose agar were the best semi-synthetic media for the growth of the *Curvularia eragrostidis*. Among liquid media, oat meal broth, potato dextrose broth and Richards' broth medium were best for the growth and sporulation of *C. eragrostidis*, while nitrogenous sources tried asparagine, L-glutamic acid, ammonium nitrate, potassium nitrate, ammonium sulphate and calcium nitrate proved to be the best for the growth and sporulation of the pathogen. Sodium nitrate and urea showed poor growth and sporulation of the *C. eragrostidis*.

Keywords: *Curvularia eragrostidis*, *Hymenocallis littoralis* Media, Nitrogen Sources

1. Introduction

Studies on nutrition and growth of the pathogen would be useful in future investigations on the physiology of the fungus and on the possible production of toxins or enzymes involved in pathogenesis. Also knowledge of cultural methods and conditions to promote spore production would be advantageous in obtaining adequate amounts of inoculums. This study was conducted to determine the appropriate substrate, method of cultivation and types of nitrogen sources for growth and sporulation of *Curvularia eragrostidis.* Colony of *C. eragrostidis* rapidly grew on PDA medium and covered the whole 90 mm diameter petriplate within 8 days at room temperature (27±2 °C). The fungus produced initially profuse brownish black mycelial growth, which gradually turned into dark black in colour on PDA and produced profuse septate, dark brown mycelial growth. Conidia measured 18.62-21.28×10.64-13.30 µm (Average 20.61×12.10 µm^2). Conidia are ellipsoidal, straight, mostly median portion thick with three transverse septa and very dark brown central cells. *C. eragrostidis* was found constantly associated with Spiderlilly causing leaf blight and the scientific information on this disease is very scanty. Hence, the investigation was undertaken on this problem for generating more scientific information.

2. Materials and Methods

Curvularia eragrostidis was isolated from spider lilly leavescontaining tip blight disease on the farmer's fields of village Itarva, Navsari, Gujarat, India. Pathogenicity was confirmed by tests on healthy spider lilly plants in the Net house. Stock cultures were maintained on potato dextrose agar (PDA). The eight different media of semi-synthetic (Potato Dextrose Agar (PDA), Potato Carrot Sucrose Agar (PCSA), Oat Meal Agar (OMA), Spider lilly leaf extract agar medium) and synthetic types (Czapek's (Dox) Agar (CzDA), Richards' Agar (RA), Asthana and Hawker's Agar (A & HA), Elliot's Agar (EA)) in solid and liquid states were tested for growth and sporulation of *C. eragrostidis.*

2.1. Solid media

These agar agar based sterilized media were poured aseptically into 90 mm diameter pre-sterilized (oven temperature 180 °C for 20 minutes) Petri plates @ 20 ml plate^{-1}. After solidification, 5 mm diameter culture block of 8 days old pure culture of *C. eragrostidis* was placed in centre of the petri plates with the help of sterilized cork borer. Three repetitions were kept for recording observation on colony diameter and characters of the fungus. The Petri plates were incubated at 27±2˚C temperature. The radial growth was daily measured till the plates were covered with the fungal mycelium. The data thus, obtained were statistically analysed.

2.2. Broth media or liquid media

All the solid media were used as broth media with the same ingredients except addition of agar agar. Fifty ml of these broths were poured into 150 ml volume of conical flasks and were plugged with non-absorbent cotton and autoclaved at 1.2 kg cm^{-2} pressure for 20 minutes. The flasks were inoculated

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aseptically by placing 5 mm diameter culture block of *C. eragrostidis* cut aseptically with the help of cork borer from 8 days old pure culture. The flasks were incubated at 27±2 ˚C temperature and after 10 days of incubation mycelial mats were harvested on previously weighed; oven dried Whatman's filter paper no. 42 giving sufficient washing with warm (80 ˚C) distilled water. The filter papers with mycelial mats were dried in an oven at 60 ˚C till constant weight was obtained. The observations were recorded to compare the dry mycelial weight. The spore count was recorded from the fourth repetition. At the end of incubation period, the whole mycelial mat with substrate was homogenized in 150 ml sterile distilled water with the help of grinder. The homogenate was filtered through muslin cloth. A drop of suspension was examined under microscope. The numbers of conidia per microscopic field under low power magnification (10 X) were recorded from four randomly selected microscopic fields in each case. The data, thus obtained were graded as +=poor (below 5), ++=moderate (6- 15), +++=good (16-30) and ++++=excellent (above 30).

2.3. Nitrogen sources

For the testing of nitrogen sources for growth and sporulation of fungus, Hundred ml of sterilized liquid Richard's medium was poured in to 250 ml conical flasks. Potassium nitrate (KNO_3) in the basal medium was replaced by nitrogen sources *viz.,* urea (46% N), potassium nitrate (13% N), sodium nitrate (16% N), calcium nitrate (14% N), ammonium nitrate (33.5% N), ammonium sulphate (20.6% N), asparagine (20% N), and L-glutamic acid (18% N) (Table 1). Nitrogen sources were added singly to furnish 1.38 g of nitrogen l^{-1} of basal medium. The basal medium without nitrogen source served as control. Each treatment was replicated four times. Then pH of the medium was adjusted to 6.0 by adding 0.1 N CH_3COOH or 0.1 N NaOH with the help of pH Tester 30. After sterilizing at 1.2 kg cm^{-2} pressure for 20 minutes in the autoclave, the flasks were inoculated under aseptic condition with 5 mm diameter culture block cut from 8 days old actively growing pure culture of *C. eragrostidis* and transferred to each flask were incubated at room (27±2 °C). Mycelial mats were collected from three repetitions in each case after 15 days on previously weighed Whatman's filter paper no. 42 and dried in oven at 60°C for 3 consecutive days. The average dry weight of the mats was statistically analysed. The sporulation of the fungus was recorded from fourth replication as described earlier. The data were subjected to statistically analysis.

3. Results and Discussion

3.1. Influence of medium on sporulation

The results on the growth and sporulation are presented in (Table 2). The colony and cultural characters of the fungus recorded in different solid media are presented in (Table 3).The results revealed that among all solid media tested; the mycelial growth of *C. eragrostidis* was significantly higher on host leaf extract agar (87.67 mm) as compared to the rest. The next best in order of merit was PDA (85.11 mm) which was statistically at par with OMA (84.77 mm) which in turn was at par with RA (83.89 mm) followed by A & HA (81.89 mm) and PCSA (76.11 mm). The least mycelial colony growth was observed in CzDA (40.24 mm) and EA (35.89 mm). In the liquid media, oat meal broth supported significantly superior growth (1037.00 mg) over the rest of the media tested. The next best in order of merit was Richards' broth (714.67 mg) which was statistically at par with potato carrot sucrose broth (681.67 mg) and potato dextrose broth (657.67 mg). The next best in order of merit was host leaf extract broth (438.33 mg) which was at par with Czapek's (Dox) broth (388.00 mg). While Asthana and Hawker's broth (151.67 mg) and Elliot's broth (114.67 mg) yielded in poor mycelial dry weight. Regarding sporulation, the fungus produced excellent sporulation on potato dextrose broth and host leaf extract broth while good sporulation was observed in Richards' broth and potato carrot sucrose broth, whereas Asthana and Hawker's broth and Czapek's (Dox) broth medium produced moderate sporulation while the fungus *C. eragrostidis* produced poor sporulation on Elliot's broth and oat meal broth medium. Considering the over all performance of different solid media host leaf

Table 1: Various nitrogen sources used *in vitro*

Sr. No.	Group	Source of nitrogen	Chemical formula	Molecular weight	Nitrogen content (%)
I	Inorganic sources	Ammonium sulphate	$(NH_4)_2SO_4$	132.14	20.6
		Ammonium nitrate	$NH_4\ NO_3$	80.04	33.5
		Sodium nitrate	$NaNO_3$	85	16
		Calcium nitrate	$Ca(NO_3)24H_2O$	263.13	14
II	Organic sources	Urea	NH_2CONH_2	60.06	46
		L- Glutamic acid	$C_5H_9NO_4$	147.13	18
		Asparagine	$C_4H_8N_2O_3$	132.2	20
III	Normal Richards'Agar media (Control)	Potassium nitrate	KNO_3	101.11	13
IV	Richards' broth without nitrogen sources	-	-	-	-

Table 2: *In vitro* evaluation of different solid and liquid media on growth and sporulation of *Curvularia eragrostidis*

Sr. No.	Name of medium	Solid media colony diameter after 8 days (mm)	Liquid media	
			Dry mycelial weight(mg)	Sporulation
1.	Potato dextrose	85.11	(2.82)* 657.67**	++++
2.	Potato carrot sucrose	76.11	(2.83) 681.67	+++
3.	Host leaf extract	87.67	(2.64) 438.33	++++
4.	Richards'	83.89	(2.85) 714.67	+++
5.	Czapek's (Dox)	40.24	(2.59) 388.00	++
6.	Asthana and Hawker's	81.89	(2.18) 151.67	++
7.	Oat meal	84.77	(3.02) 1037.00	+
8.	Elliot's	35.89	(2.06) 114.67	+
	SEm±	0.669	0.022	
	CD (*p*=0.05)	1.988	0.065	
	C.V. %	1.812	1.43	

*: Figures indicate logarithmic transformed values; **: Figures indicate original values; Sporulation (no. of conidia); +: Poor (below 5); ++: Moderate (6-15); +++: Good (16-30); ++++: Excellent (above 30)

extract agar and potato dextrose agar were found useful while in liquid media oat meal broth, Richards' broth and potato dextrose broth were found useful. These media were found better for the growth and sporulation of *Curvularia* sp. by earlier workers (Kilpatrik, 1958; Aulakh, 1970; Weststeijn and Okafor, 1971; Singh, 1971; Somal, 1975; Kore and Bhide, 1981 and Olufolaji, 1984).

3.2. Growth on different nitrogen sources.

The results presented in Table 4 revealed that among the eight nitrogenous sources tested, asparagine was statistically found significantly best, giving maximum growth of the fungus (1229.67mg). Next best in order of merit was L-glutamic acid (1115.00mg), followed by ammonium nitrate (958.67mg) which was statistically at par with potassium nitrate (920.00mg) followed by ammonium sulphate (824.33mg) which was at par with calcium nitrate (823.67 mg). These all nitrogenous sources produced excellent mycelial growth while

Table 4: Effect of various nitrogen sources on growth and sporulation of *Curvularia eragrostidis in vitro*

Sr. No.	Name of nitrogen source	Average dry mycelial weight (mg)	Sporulation
1.	Urea	392.33	+
2.	Potassium nitrate	920.00	+++
3.	Sodium nitrate	468.00	+
4.	Calcium nitrate	823.67	+++
5.	Ammonium nitrate	958.67	++++
6.	Ammonium sulphate	824.33	+++
7.	Asparagine	1229.67	++++
8.	L-Glutamic acid	1115.00	++++
9.	Control	205.00	++
	SEm±	13.33	
	CD (*p*=0.05)	39.61	
	C.V. %	3.00	

Sporulation (no. of conidia); +: Poor (below 5);++: Moderate (6-15); +++: Good (16-30); ++++: Excellent (above 30)

Table 3: Colony/Culture characteristics of *C. eragrostidis* on different solid media

Sr. No.	Media	Cultural characters
1.	Potato dextrose agar	Fast growing circular, brownish black profuse mycelial growth, which gradually turned into dark black colour, Excellent sporulation.
2.	Potato carrot sucrose agar	Colony smooth, dirty black with good sporulation.
3.	Host leaf extract agar	Fast profusely growing circular colony, initially dull brownish black, which gradually turned into dark black in colour, Excellent sporulation.
4.	Richards' agar	Whitish black mycelial colony with undulating topographic growth and good sporulation.
5.	Czapek's (Dox) agar	Dull black fluffy mycelial colony with moderate sporulation.
6.	Asthana and Hawker's agar	Whitish black mycelial fluffy colony with good sporulation.
7.	Oat meal agar	Milky fluffy white mycelial colony with poor sporulation.
8.	Elliot's agar	Blackish white flat mycelial colony with poor sporulation.

remaining nitrogenous sources, viz., sodium nitrate and urea showed poor growth of the fungus.

Regarding sporulation, the fungus produced excellent sporulation on asparagine, L-glutamic acid and ammonium nitrate while good sporulation was observed in ammonium sulphate, calcium nitrate and potassium nitrate. Sporulation was found very poor in urea, sodium nitrate and control i.e., Richards' agar medium (without nitrogen). Thus asparagine, L-glutamic acid and ammonium nitrate were found to be the best source for sporulation of the *C. eragrostidis*.Therefore, it is very clear that urea and sodium nitrate did not support the growth, while the rest of the nitrogenous sources stimulated the growth and sporulation of *Curvularia eragrostidis.* These nitrogen sources were found better for the growth and sporulation of *Curvularia* sp. by earlier workers (Olufolaji, 1984; Hangug, 1990; Aulakh 1970 and Hasija 1971).

4. Conclusion

Out of eight synthetic and semi-synthetic solid and liquid media tested, host leaf extract agar and potato dextrose agar were the best semi-synthetic media for the growth of the fungus. In liquid media, oat meal broth, potato dextrose broth and Richards' broth medium were best for the growth and sporulation of *C. eragrostidis*. However,nitrogenous sources viz., asparagines, L-glutamic acid, ammonium nitrate, potassium nitrate, ammonium sulphate and calcium nitrate produced excellent mycelial growth and sporulation while remaining nitrogenous sources viz. sodium nitrate and urea showed poor growth of the *Curvularia eragrostidis*.

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