In vitro effect of various nitrogen, carbon sources and pH regimes on the growth and sporulation of *Colletotrichum gloeosporioides* Penz. and Sacc causing anthracnose of Indian bean

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ABSTRACT

Eight carbon, ten nitrogenous sources and various pH regimes were tested for their effect on growth and sporulation of *C. gloeosporioides*. Out of eight carbon sources tested, starch and xyllose proved to be the best for the growth and sporulation followed by glucose and sucrose. Out of ten different nitrogenous sources tested, potassium nitrate proved to be the best for the growth and sporulation of the pathogen. Studies on pH revealed that fungus produced the maximum dry mycelial weight and sporulation at pH 5.5 and pH 6.5 in liquid media respectively. Thus the use of undegraded organic manures, potassium nitrate bases fertilizers and slightly acidic soil favors the disease.

Key words:*Colletotrichum gloeosporioides,* pH regimes, growth, sporulation

INTRODUCTION

Indian bean (*Lablab purpureus* L.) is an important pulse crop of Gujarat. There are two cultivated types of Indian bean *viz., typicus* and *lignosus* (Shivashankar *et al*., 1971). Typicus is a garden type and is cultivated for its soft and edible pods. Lignosus is known as field bean and mainly cultivated for dry seed as pulse and is more popularly recognized as 'Wal', 'Wal–papdi' or 'Valor' in Gujarat state. The green pods are used for vegetable purpose whereas ripe and dried seeds are consumed as split pulse. The seeds can be sometimes soaked in water overnight and when germination initiates, they can be sun-dried and stored for future use. The fodder has good palatability and the cattles are nourished well. It can also be used as nitrogen fixing pulse crop. The fresh/immature pods contain 4.5 per cent proteins and 10 per cent carbohydrates (Kay, 1973).Occurrence of anthracnose in popular variety NPS1 of Walpapdi or Indian bean was observed seriously in south Gujarat in *rabi* 2007 and isolated and identified as *C. gloeosporioides*. To determine the most readily utilizable source of carbon, nitrogen and favorable pH by the fungus *C. gloeosporioides* the present study was undertaken.

MATERIALS AND METHODS

The best growth and sporulation of the fungus was obtained on PDA and Richards' medium and so Richards' synthetic medium was used as standard medium for all the remaining physiological studies.

Nitrogen sources

A 20 mL of sterilized Richards' agar medium was poured into sterilized Petriplates (90 mm diameter). Potassium nitrate in the basal medium was replaced by various inorganic and organic sources of nitrogen *viz*., ammonium carbonate (29% N), ammonium oxalate (22.5% N), ammonium phosphate (13% N), ammonium sulphate (20.5% N), sodium nitrate (16.5% N), calcium nitrate (17% N), peptone (14.2-15.5 % N), urea (46% N) and potassium nitrate (14% N). Nitrogen sources were added singly to furnish 1.38 g nitrogen per litre of basal medium, without nitrogen source served as control. Each treatment was replicated four times. These Petriplates were inoculated aseptically with 5 mm diameter culture block of mycelium obtained from the 10 day old actively growing pure culture with the help of sterilized cork borer. Inoculated Petriplates were incubated at room temperature (27 $\pm$ 2°C). The mycelial colony diameter was recorded every day until the complete coverage with fungal growth in the plate which was kept as a control at ambient temperature and sporulation was also counted from the plate of fourth replication in each treatment (Verma and Prasad, 1975; Sonai Rajan and Muthukrishnan, 2010).

Carbon sources

To determine the most readily utilizable source of carbon by the fungus *C. gloeosporioides,* carbon sources were inoculated in the Richards' medium which was taken as a basal medium and prepared by dissolving the ingredients except

carbon source (sucrose). The quantity of various sources of carbon such as sucrose (45% C), lactose (42% C), starch (44% C), glucose (40% C), mannitol (40% C) and galactose (40% C) were calculated to furnish 21.053 g carbon /litre medium. The quantity of starch (complex polysaccharides) taken was similar to that of sucrose. The different carbon sources dissolved separately in the medium. 20 mL of sterilized Richards' agar was poured into sterilized Petriplates. Plates without carbon source served as absolute control. Each treatment was replicated four times. The rest of the procedure adopted was similar to experiment on nitrogen sources (Verma and Prasad, 1975).

pH

The pH of the media was adjusted between 4.5 to 9.0 using 0.1 N NaOH and 0.1 N HCl with the help of Backman's pH meter. The effect of different pH regimes on growth and sporulation of the fungus in liquid media were studied. In case of liquid medium, 50 mL of the medium was poured in 150 mL conical flask.

The flask containing liquid medium were inoculated with 10-day old culture discs (5 mm diameter) of *C. gloeosporioides* with the help of sterile cork- borer under aseptic condition. Four replications were maintained for each treatment and incubated at room temperature (27 ± 2^{o}C). Dry mycelial weight, sporulation and drift in pH after incubation period were recorded from liquid medium.

Sporulation was graded according to the number of conidia as : - = no sporulation, + = poor sporulation (0-50 conidia), ++ = fair sporulation (51-100 conidia), +++ = good sporulation (101-150 conidia) and ++++ = excellent sporulation (>150 conidia) per microscopic field.

RESULTS AND DISCUSSION

Nitrogen sources

Ten different nitrogenous sources were tested in solid Richards' agar medium to know their effect on the growth and sporulation of the fungus.

It is observed from the results that maximum growth of *C. gloeosporioides* was recorded in potassium nitrate. The next best in order of merit was ammonium sulphate, sodium nitrate and by peptone while calcium nitrate showed moderate growth of the pathogen. Rest of the nitrogenous sources showed poor growth as compared to control (Table 1).

Sporulation of *C. gloeosporioides* was excellent in potassium nitrate, ammonium sulphate and sodium nitrate while good sporulation recorded in peptone and calcium nitrate. Ammonium carbonate, ammonium oxalate and Richards' agar medium (without nitrogen) supported poor sporulation of the fungus.

Table 1. Effect of various nitrogen sources on the growth and sporulation of *C. gloeosporioides in vitro.*

Name of nitrogen source	Average diameter of mycelial colony, mm	Sporulation
Richards' agar without nitrogen (Control)	14.33	+
Ammonium carbonate	24.66	+
Ammonium oxalate	42.66	+
Ammonium phosphate	45.66	++
Urea	52.66	++
Calcium nitrate	60.66	+++
Peptone	64.33	+++
Sodium nitrate	84.00	++++
Ammonium sulphate	84.66	++++
Potassium nitrate	88.00	++++
S.Em. $\pm$	0.59	
C.D.at 5%	1.67	
C.V.%	1.8	

Purkayastha and Sengupta (1975) found peptone; casamino acid and potassium nitrate were favourable for both sporulation and mycelial growth of *C. gloeosporioides* Penz. and Sacc., incitant of jute anthracnose. The present results are also tallied with above results.

From the foregoing study, it can be concluded that potassium nitrate, ammonium sulphate and sodium nitrate were good sources of nitrogen for excellent growth and sporulation of *C. gloeosporioides*.

Carbon sources

Eight different carbon sources were tested in solid Richards' agar medium to know their effect on the growth and sporulation of the fungus. The results indicated that significantly maximum mycelial growth was recorded in starch which was at par with xylose. The next best in order of merit were glucose, sucrose, mannitol , galactose and lactose while very poor growth was recorded in Richards' agar medium without carbon (control) compared to other carbon containing sources (Table 2).

The fungus produced excellent sporulation in starch, xylose and sucrose while good sporulation was observed in glucose. Mannitol produced moderate sporulation while poor sporulation was recorded in galactose and lactose.

The maximum growth and sporulation was recorded in starch added medium. Yang *et al*. (2000) reported glucose was the best carbon source for mycelial growth and sucrose was the best for sporulation of *C. musae* from banana fruit. The present results are also akin to the results shown by different workers.

Table 2. Effect of various carbon sources on growth and sporulation of *C. gloeosporioides in vitro.*

Name of Carbon Source	Average diameter of mycelial colony, mm	Sporulation
Richards' agar without carbon (Control)	23.00	-
Lactose	43.00	+
Galactose	51.33	+
Mannitol	69.00	++
Sucrose	72.66	++++
Glucose	81.00	+++
Xyllose	86.33	++++
Starch	88.00	++++
S.Em. ±	0.61	
C.D at 5%	1.83	
C.V.%	1.7	

PH

Richards' medium with and without agar was found superior and so it was used as basal medium for the remaining physiological studies. The dry mycelial weight, sporulation and drift in pH after incubation period were recorded in liquid medium (Table 3).

The results clearly indicated that the fungus could grow and sporulate in wide range of pH *i.e.* from 4.0 to 8.0, in liquid medium. The dry mycelial weight was significantly higher in pH 5.5 which was statistically at par with pH 6.0.The next best in order of merit was pH 5.0. pH 6.5 followed by pH 4 and pH 7.0. The poor growth of the fungus was recorded at pH 7.5 and pH 8.0.

The sporulation of *C. gloeosporioides* was found the highest at pH 5.0, pH 5.5 and pH 6.0. It was good at pH 6.5, fair at pH 4.0 and pH 7.0 while it was found poor at pH 7.5 and pH 8.0. Excellent growth and sporulation of *C. gloeosporioides* was recorded at pH 5.5 to 6.0 which was in line with that of Thakare and Patil (1995); Patel (2000); Prashanti and Kulkarni (2003) and Patel (2004) for *C. gloeosporioides* from chrysanthemum, turmeric and chilli.

Out of eight carbon, ten nitrogen sources and various pH regimes tested, the growth and sporulation of *C. gloeosporioides* of carbon sources starch and xyllose proved to be the best followed by glucose and sucrose. Out of nitrogenous sources tested, potassium nitrate proved as the best for the growth and sporulation of the pathogen. Where as studies on pH revealed that fungus produced the maximum dry mycelial weight and sporulation at pH 5.5 and pH 6.5 in liquid media, respectively. So the disease prevailed in the potassium nitrate based fertilizers and slightly acidic soil and undegraded or partially degraded organic manures in the soil.

Table3. Effect of different pH regimes on dry mycelial weight, sporulation and final pH of *C. gloeosporioides*.

pH	Liquid medium (after 12 days)		
	Av. dry mycelial weight, mg	Sporulation	Filtrate pH
4.0	1.968 * (90.66) **	++	5.5
5.0	2.315 (206.00)	++++	6.0
5.5	2.464 (290.33)	++++	6.6
6.0	2.453 (282.66)	++++	7.2
6.5	2.187 (153.00)	+++	7.3
7.0	1.959 (90.00)	++	7.4
7.5	1.731 (53.00)	+	7.5
8.0	1.709 (50.33)	+	7.8
S. Em. ±	0.080	-	
C.D. at 5%	0.030		
C.V. %	0.7		

* Figures indicate logarithmic transformed values and ** Figures indicate original values

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