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Nutritional and physiological studies on the growth and sporulation of *Colletotrichum lindemuthianum* isolated from Dolichos bean

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ABSTRACT

Effect of nine carbon, seven nitrogen sources and various temperature and pH regimes were evaluated *in vitro* on the growth and sporulation of *Colletotrichum lindemuthianum* isolated from diseased dolichos bean. Sucrose and potassium nitrate proved to be the best carbon and nitrogen sources, respectively for the growth and sporulation of the fungus. The temperature range of 25 ^{0}C to 28 ^{0}C and pH range of 6.0 to 7.0 were the optimum for the fungal growth and sporulation Richard's broth.

Key words: Carbon, *Colletotrichum lindemuthianum*, Nitrogen, pH, Sporulation, Temperature

Dolichos bean, *Lablab purpureus* L. (Sweet) is one of the most ancient leguminous crops grown for pulse, vegetable and forage purposes. It is one of the major sources of protein in the diets of South Indians. Anthracnose disease in dolichos bean caused by *Colletotrichum lindemuthianum* is the most devastating disease in many dolichos bean cultivating countries including India (Sharma and Sugha, 1995). The incidence of anthracnose disease has been reported to range from 0.5 to 88.0 per cent in different areas (Padder *et al*., 2010). However, not much systematic research work has been carried out on physiological and nutritional requirements of the pathogen. Hence, the present study was undertaken to determine the most readily utilizable sources of carbon, nitrogen and optimum temperature and pH regimes favourable for the growth and sporulation of the fungus, *Colletotrichum lindemuthianum.*

MATERIALS AND METHODS

***Effect of temperature*:** The fungus *C. lindemuthianum* was isolated from infected dolichos bean leaves and its monosporic culture was maintained on potato dextrose agar slants. The fungus was subjected to different temperature regimes to determine the optimum temperature for its growth and sporulation using Richard's medium. Twenty-five ml of Richard's broth was poured into conical flask under aseptic conditions and was inoculated with 5 mm diameter identical culture discs from an actively growing zone of twelve day old culture of *C. lindemuthianum*. Inoculated flasks were incubated at 5, 10, 15, 20, 25, 28, 30 and 35 ^{0}C. The experiment was replicated thrice. Dry mycelial weight was recorded using electronic weighing balance and the extent of sporulation was recorded using compound microscope twelve days after incubation.

Effect of pH: The desired pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.00 of Richard's broth medium was adjusted using 0.1 N NaOH or HCl. Twenty five ml of media of each pH level was poured into 100ml conical flask under aseptic conditions and each flask was inoculated with 5 mm diameter mycelial disc. Inoculated flasks were incubated at 27±1 ^{0}C. The data were recorded on dry mycelial weight. Sporulation was recorded using compound microscope twelve days after incubation.

***Effect of carbon and nitrogen sources*:** The experiments were conducted to determine the sources of carbon and nitrogen most efficiently utilized by *C. lindemuthianum* for its growth and sporulation. Richard's broth was used as a basal medium for the experimentation. The carbon and nitrogen sources sucrose and potassium nitrate, used in the basal medium were replaced with various carbon and nitrogen compounds.

Nine different carbon sources *viz*., sucrose, maltose, glucose, dextrose, fructose, glycerol, mannitol, lactose and citric acid were incorporated into Richard's broth separately @ 21.053 grams of carbon per litre of medium with potassium nitrate as a constant and uniform source of nitrogen along with the control treatment of no carbon source. Seven nitrogen sources (sodium nitrate, ammonium nitrate, urea, ammonium sulphate, ammonium chloride, L-aspargine and potassium nitrate) were incorporated into Richard's broth @ 1.3855 grams of nitrogen per liter of the medium with control treatment of no nitrogen source and sucrose as the constant and uniform as source of carbon. Each medium (25ml) was poured into 100ml flask and autoclaved. Each of treatment was replicated thrice. All the flasks were aseptically inoculated with12 days old culture of *C. lindemuthianum* and the inoculated flasks were incubated at room temperature (27±1°C) for twelve days. The fungal mycelial mat was filtered through Whatman No. 42 filter paper dried at 60 °C for 24 hours and its weight was recorded. The data thus recorded was statistically analyzed.

RESULTS AND DISCUSSION

The data on effect of temperature and pH are summarized in Table 1.It was observed that maximum fungal growth and

Table 1: Effect of temperature and pH on growth and sporulation of *Colletotrichum lindemuthianum*

Sl No.	Temperature (ºC)	Dry mycelial weight (mg)	Sporulation	pH	Dry mycelial weight (mg)	Sporulation
1	5	0.00	-	3.0	91.67	+
2	10	10.17	+	4.0	132.00	+
3	15	22.47	+	5.0	220.58	+++
4	20	279.23	++	6.0	368.52	++++
5	25	312.8	+++	7.0	319.37	++++
6	28	340.17	+++	8.0	202.25	+
7	30	223.8	++	9.0	169.22	+
8	35	201.8	+	10.0	76.67	+
	SEm±	**1.02**			**1.07**	
	CD at 1%	**4.21**			**4.42**	
	CV	**1.01**			**0.94**	

Sporulation: + + + + = Excellent, + + + = Good, + + = Fair, + = Poor, - = No sporulation

sporulation were recorded at 28^{0}C (340.17 mg dry mycelial weight) followed by 25 ^{0}C (312.8 mg dry mycelial weight) and it was the least at 10 ^{0}C (10.17 mg dry mycelial weight) (Table 1). The present results are in agreement with the earlier workers (Thakur and Khare1991) who observed that growth of *C. lindemuthianum* causing mungbean anthracnose was the highest at 26-29 ^{0}C. Similarly, Hiremath *et al.* (1993) and Kulkarni (2009) also recorded maximum mycelial growth at 25 ^{0}C followed by at 30 ^{o}C. The present study established that the temperature of 28^{0}C supported maximum growth of *C. lindemuthianum* and can therefore be suggested as optimum temperature for its growth and this can be used in future for laboratory studies. The fungus was grown on Richard's broth at different pH levels. The growth of *C. lindemuthianum* at pH 6.0 (368.52 mg dry mycelial weight) was significantly higher compared to other levels and good growth was recorded at a pH range of 6.0 to 7.0 (Table 1). The sporulation was also influenced by pH and excellent sporulation was recorded at pH 6.0 and 7.0 and it was fairly good at pH 5.0. The optimum pH range for the growth of *C. lindemuthianum* was on acidic side and there was sudden decline in growth towards basic pH side indicating that the fungus was acid tolerant. Earlier, it was opined that fungi are relatively more tolerant to acid ions (H+) than basic ions (OH^-) than the bacteria and actinomycetes (Cochrane 1958, Bilgrami and Verma1978). The observations are also in agreement with those of Deshmukh *et al.* (2012) and Kulkarni (2009).

Carbon and nitrogen are required by the fungus as structural and functional constituents. Carbon comprises about 50 per cent of total dry mycelial growth in fungi (Lorena Hernandez Silva *et al.*, 2007). In the present study, among the nine carbon sources tested for *C lindemuthianum*, sucrose supported maximum (390.1mg) growth of the fungus and it was minimum (61.73 mg) in citric acid. (Table 2). The results are in agreement with the observations of Durairaj (1956) and Naik *et al.* (1988) in case *Colletotrichum* sp. causing anthracnose of chilli and betelvine. In the present study, among the various nitrogen sources tested, potassium nitrate

Table 2: Effect of different carbon and nitrogen sources on the dry mycelial weight and sporulation of *Colletotrichum lindemuthianum* in Richard's broth

Sl No.	Carbon sources	Dry mycelial weight (mg)	Nitrogen sources	Dry mycelial weight (mg)
1	Sucrose	390.1	Sodium nitrate	10.83
2	Maltose	220.57	Ammonium nitrate	302.37
3	Glucose	318.5	Urea	178.67
4	Dextrose	357.27	Ammonium sulphate	233.17
5	Fructose	232.1	Ammonium chloride	172.3
6	Glycerol	255.2	L-Aspargine	260.63
7	Mannitol	210.33	Potassium nitrate	318.97
8	Lactose	100.43	Control	8.97
9	Citric acid	61.73		
10	Control	32.33		
	SEm±	**1.58**		**1.51**
	CD at 1%	**6.38**		**6.26**
	CV	**1.26**		**1.41**

(318.97 mg) was found as the best nitrogen source for the growth of *C. lindemuthianum* followed by ammonium nitrate (302.37mg dry mycelial weight) whereas, minimum growth was observed with sodium nitrate (10.83 mg dry mycelial weight) (Table 2). The present studies are in conformity with the results of earlier workers (Naik *et al*. 1988, Sangeetha 2008). Similarly Deshmukh *et al*. (2012) reported that sucrose, glucose (as carbon source) potassium nitrate (as nitrogen source) supported maximum growth of *C. gloeosporioides* on Indian bean. This could be attributed to the fact that sucrose is the simplest form of carbon, which is readily soluble, easily available and utilizable by the fungus. Hence, sucrose and potassium nitrate could be suggested as the best source of carbon and nitrogen, respectively for growth and sporulation of the fungus *C. lindemuthianum*. Moreover, temperature of 25 ^{0}C to 28 ^{0}C and pH of 6.0 to 7.0 are the optimum for the growth and sporulation of the fungus.

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