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J. Mycopathol, Res, 54(1) : 45-48, 2016;
ISSN 0971-3719

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Physiological and nutritional studies on the growth and sporulation of *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams. Scri causing Anthracnose of Field bean *Lablab purpureus*

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Received : 20.03.2015 RMS Accepted : 17.07.2015 Published : 25.04.2016

Nine carbon, seven nitrogen sources and various temperature and pH regimes were tested for their effect on growth and sporulation of *Colletotrichum lindemuthianum* causing anthracnose of field bean. The optimum range of temperature required for the fungus was found to be 25^{0}C to 28^{0}C. Studies on pH revealed that fungus produced the maximum dry mycelial weight and sporulation at pH 6.0 and pH 7.0 in liquid media respectively. Out of nine carbon sources tested, sucrose proved to be the best for the growth and sporulation of the fungus. Among the different nitrogenous sources tested, potassium nitrate showed good growth and sporulation

Key words: Carbon, *Colletotrichum lindemuthianum*, nitrogen, pH, temperature, sporulation

INTRODUCTION

Field bean, *Lablab purpureus* L. Sweet (2n = 22) which belongs to the family Fabaceae, is one of the most ancient crops among cultivated plants. It is a multipurpose crop grown for pulse, vegetable and forage purposes. The crop is grown for its green pods while dry seeds are used in various food preparations. It is one of the major sources of protein in the diets of South Indians.

Field bean anthracnose is the most devastating disease in many field bean cultivating countries including India (Sharma and Sugha, 1995). The fungus *Colletotrichum lindemuthianum* which causes anthracnose of field bean was first observed in 1875 by Lindemuth at Popplesdori in Germany. Saccardo and Magnus (1878) first described it as *Gloeosporium lindemuthianum*. Lakshmi Ramakrishnan (1964) surveyed the seed borne infection intensity of *C. lindemuthianum* on Field bean and reported it to the extent of 13.40 per cent. However, not much systematic research work has been carried out on physiological and nutritional characteristics of the pathogen. To determine the most readily utilizable source of carbon, nitrogen and favorable temperature and pH by the fungus *Colletotrichum lindemuthianum* the present study was undertaken.

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MATERIALS AND METHODS

Physiological studies

Effect of temperature

The pathogen was subjected to different temperature conditions to study the best-suited

temperature level for the growth and sporulation of the fungus. Richard's medium was used in the experiment to study the growth and sporulation. Twenty-five milliliters of Richard's medium was poured into each petriplate under aseptic condition and inoculated with 5 mm diameter identical culture discs from an actively growing zone of twelve day old culture. The experiment was replicated thrice. Inoculated conical flasks containing Richard's medium were incubated at 5, 10, 15, 20, 25, 28, 30 and 35^{0}C. Dry mycelial weight and the extent of sporulation, were recorded in the liquid cultures twelve days after the incubation.

Effect of pH

The effect of pH of medium on the growth and sporulation of *C. lindemuthianum* was studied with selected pH range of 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.00 grown on Richard's broth. Twenty five millilitre of liquid medium was poured into a 100ml conical flask under aseptic conditions. The reaction of the medium was adjusted to the desired pH by adding 0.1N NaOH or 0.1N HCl (Naik *et al,* 1988). The medium was buffered with Disodium hydrogen phosphate citric acid buffer according to the schedule of Vogel (Vogel, 1951). Flasks were sterilized at 121^{0}C at 15 psi for 20 minutes. Each flask was inoculated with 5 mm diameter mycelial disc in sterile conditions.Inoculated flasks were incubated at 27±1^{0}C for twelve days and the dry mycelial weight and extent of sporulation were obtained.

Nutritional Studies
Effect of carbon and nitrogen sources

The experiment was conducted to find out the source of carbon and nitrogen which can be most efficiently utilized by the fungus for its growth and sporulation. Monosporic culture of *C. lindemuthianum* from the infected field bean leaves was obtained in pure culture and maintained on potato dextrose agar slants. Richard's medium (Magnesium sulphate-2.50 g, Potassium di hydrogen phosphate -5.00 g, Potassium nitrate-10.00 g, Sucrose - 50.00 g, Distilled water 1000.00ml) was taken as the basal medium. The carbon and nitrogen nutrition was studied by replacing the sucrose and potassium nitrate in the basal medium 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 liquid basal medium. Potassium nitrate was added as a source of nitrogen in all the treatments. Carbon sources were added to the basal medium @ 21.053 g of carbon per liter of medium. Sodium nitrate, ammonium nitrate, urea, ammonium sulphate, ammonium chloride, L-aspargine and potassium nitrate were used as different nitrogen sources and incorporated into Richard's agar medium @ 1.3855 g of nitrogen per liter of the medium. In control, no nitrogen source was added. Sucrose was used as source of carbon in all the treatments. Twenty-five ml. of each medium was poured into 100ml flasks, plugged with non-absorbent cotton and autoclaved at 121°C (at 15psi pressure) for 20 minutes. Each of the treatments was replicated thrice. All the flasks were aseptically inoculated with 5mm fungal discs from an actively growing zone of twelve day old culture. 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 and the dry mycelial weight was recorded after drying it in hot air oven maintained at 60°C for 24 hours. The data thus recorded was statistically analyzed.

RESULTS AND DISCUSSION

Temperature is one of the important factors governing distribution, growth, reproduction and survival of the fungus. It also plays an important role in infection and disease development. An effort was made to know the optimum temperature for the growth and sporulation. In the present study, it was observed that maximum fungal growth and sporulation was recorded at 28^{0}C (340.17 mg) followed by 25^{0}C (312.8) and it was least at 10^{0}C

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

Temperature (^{o}c)	Dry mycelial weight (mg)	Sporulation
5	0.00	-
10	10.17	+
15	22.47	+
20	279.23	++
25	312.8	+++
28	340.17	+++
30	223.8	++
35	201.8	+
SEm±	1.02	
CD at 1%	4.21	
CV	1.01	

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

(10.17mg). The results are presented in table 1.The present results are in agreement with the results obtained by Thakur and Khare (1991) they

Table 2 : Effect of pH on dry mycelial weight and sporulation of *Colletotrichum lindemuthianum*

pH level	Mean dry mycelial weight (mg)	Sporulation
3.0	91.67	+
4.0	132.00	+
5.0	220.58	+++
6.0	368.52	++++
7.0	319.37	++++
8.0	202.25	+
9.0	169.22	+
10.0	76.67	+
SEm±	1.07	
CD at 1%	4.42	
CV	0.94	

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

observed that growth of *C. lindemuthianum* was highest at 26-29^{O}C. Hiremath *et al*, (1993) also recorded maximum mycelial growth at 25^{0}C followed by 30^{o}C. The present study established

Table 3 : Effect of different carbon sources on the dry mycelial weight and sporulation of *Colletotrichum lindemuthianum* in Richard's broth

Carbon sources	Dry mycelial weight (mg)
Sucrose	390.1
Maltose	220.57
Glucose	318.5
Dextrose	357.27
Fructose	232.1
Glycerol	255.2
Mannitol	210.33
Lactose	100.43
Citric acid	61.73
Control	32.33
SEm±	1.58
CD at 1%	6.38
CV	1.26

that temperature of 28^{0}C supported maximum growth which could be suggested as optimum temperature for growth of *C. lindemuthianum*. This optimum temperature can be used in future for laboratory studies. Similar observation were also reported by Sunil Kulkarni (2009),Wasantha Kumara and Rawal (2008)

A living organism requires a particular medium for the growth and development. A wide range of pH supported the growth of *C. lindemuthianum*. The fungus was grown on Richard's broth at different pH levels. The growth of *C. lindemuthianum* at pH 6.0 (368.52 mg) was significantly high compared to the other levels. Good growth was found at a range of 6.0 to 7.0 pH. The results are presented

Table 4 : Effect of Nitrogen sources on the dry mycelial weight and sporulation of *Colletotrichum lindemuthianum* in Richard's broth

Nitrogen sources	Dry mycelial weight (mg)
Sodium nitrate	10.83
Ammonium nitrate	302.37
Urea	178.67
Ammonium sulphate	233.17
Ammonium chloride	172.3
L-Aspargine	260.63
Potassium nitrate	318.97
Control	8.97
SEm±	1.51
CD at 1%	6.26
CV	1.41

in Table 2. The sporulation was also influenced by the pH and is known to play a crucial role. In present investigation, the excellent sporulation was found in 6.0 and 7.0 pH and good sporulation was noticed at 5.0 pH. The optimum pH range was obtained towards acidic pH side and sudden decline were observed towards basic pH side which indicated that fungus was acid tolerant. Cochrane (1958) and Bilgrami and Verma (1978) also opined that in contrast to bacteria and actinomycetes fungi are relatively more tolerant to acid ions (H+) than basic ions (OH-). The observations are in agreement with those of Deshmukh *et al*, (2012) and Sunil Kulkarni (2009).

Carbon occupies a unique position among the essential elements required by living organisms. As a component of both structural and functional constituents, carbon comprises of about 50 per cent of total dry mycelia growth in fungus (Lorena Hernandez Silva *et al,* (2007). Among the nine carbon compounds tested in the present study for *C lindemuthianum*, sucrose supported maximum (390.1mg) growth of the fungus whereas minimum growth was observed in citric acid (61.73 mg) excluding control or without any carbon source in the respective medium. The results are presented in table 3. The result was on par with the results obtained by Durairaj (1956) and Naik *et al*, (1988). Similarly Deshmukh *et al*, (2012) reported that sucrose and glucose supported maximum growth of *C. gloeosporioides* on Indian bean. This could be attributed to the fact that sucrose is the simplest of carbon, which is readily soluble and easily available to the fungus. In the present investigation the

fungus *C. lindemuthianum* preferred sucrose followed by dextrose for its good growth.

Similar to carbon, nitrogen is required by the fungus as structural and functional constituent. In the present study among the various nitrogen sources potassium nitrate (318.97 mg) was recorded to be the best nitrogen source for growth of anthracnose fungus which was significantly followed by ammonium nitrate (302.37mg). Among the nitrogen sources minimum growth was observed on sodium nitrate (10.83 mg). The results are presented in table 4. The present studies are also in conformity with the results of earlier worker (Naik *et al*, 1988) and Sangeetha (2008). Similarly Deshmukh *et al*, (2012) reported potassium nitrate supported maximum growth of *C. gloeosporioides* on Indian bean. Hence potassium nitrate could be suggested as a best source of nitrogen for its growth.

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