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EFFECT OF DIFFERENT pH LEVELS ON THE GROWTH AND SPORULATION OF *FUSARIUM OXYSPORUM* SCHLECHT. F. SP. *LENTIS* (VASUDEVA AND SRINIVASAN) THE CAUSAL ORGANISM OF WILT DISEASE OF LENTIL

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ABSTRACT
Effect of pH on the growth and sporulation of *Fusarium oxysporum* Schlecht . f . sp. *lentis* (Vasudeva and Srinivansan) after incubation of two weeks *in vitro* culture in sucrose nitrate medium was studied. pH level 6.0 was found optimum for the growth as well as sporulation of the fungus. Sporulation of chlamydospore was however found best in the pH level 4.0. Further increases in the pH level show retarding effect on growth and sporulation. Size of the spores increases with increase in the pH range.

INTRODUCTION

Fusarium oxysporum Schlecht f sp. *lentis* (Vasudeva and Srinivasan) causes a serious wilt disease in lentil (*Lens esculenta* Moench.) which is an important pulse crop of Palamu commissionary (23º52'North latitude and 84º17'East longitude). Due to this disease there is huge loss of the yield in lentil. Vasudeva and Srinivasan (1952) have reported that Wilt disease of lentil caused by *Fusarium* spp. is one of the serious disease and it causes huge loss of the standing crop throughout the world. The extent of the damage to the crop due to the disease ranges from 20-24% annually, (Saxena *and* Johansen, 1990 and Ali, 2007). There were various reports that indicate *Fusarium* spp grow at different pH levels for growth and sporulation (Wilson, 1946; Srobar, 1978; Prasad *et al*., 1992; Souramma and Singh, 2004; Groenewald, 2005). The pathogen is soil born and infects the host during seedling stage through root and blocks the vascular system (Vasudeva and Srinivasan, 1952). For the successful cultivation of the lentil it is necessary to investigate the physiology of the fungus. Therefore, effect of different pH level on the growth and sporulation of *Fusarium oxysporum* Schlecht. f. sp. *lentis* was undertaken.

MATERIALS AND METHODS

The fungus was isolated from the infected plant of lentil (*Lens esculenta* Moench). Monospore culture as described by Prasad and Chaudhary (1966, 1967) was employed in the present study. From the culture of the isolates, pure culture was obtained using 10^{-5} decimal level dilution plate technique. Sucrose nitrate medium consisting of sucrose (50.0g), KNO_3 (10.0g), KH_2PO_4 (5.0g), $MgSO_4$. $7H_2O$ (2.5g) and 1000 mL double distilled water used as basal medium. There were 10 different pH level ranging from 2.0 to 6.5 with a difference of 0.5 were prepared by using pocket size pH meter HANNA Instrument Co. Mouritius by using either N/10 HCl or NaOH before autoclaving. 100 mL of the medium was taken in 250 mL conical flask and five replicate sets were used in each case. The solution was autoclaved at 15 psi for 15 minutes. The inoculation was done with 3 mm discs of the fungus culture cut with a sterilized cork borer from a margin of 10 days old colony growing in Potato Dextrose Agar (PDA) medium. Flasks' were then inoculated at 26ºC $\pm$ 2ºC for two weeks. The mean dry weight of the mycelium was determined as described by Prasad and Chaudhary (1966). The number of spores was counted using known depth of Haemocytometer slide (0.01 cm) using the formula:
Where,

Number of spores / 100 mL = V/NX100

N = Average number of spores per square of the four corner square of haemocytometer counted.

Table 1: Effect of different pH levels of mycelia growth, spore population and size of spore change of *Fusarium oxysporum Schlecht. F.* sp. *Lentis* (Vasudeva and Srinivasan). Incubation period: 2 Weeks

S. No.	pH range	Dry wt. in mg.	Spores in millions /100mL medium*			Size of spores in μ*		
			Macro conidia	Micro conidia	Chlamydo spores	Macro conidia	Micro conidia	Chlamydo spores
1	2.0	37.00	0.67	2.62	0.39	26.20	7.20	4.90
2	2.5	53.00	0.78	3.63	3.51	27.70	7.30	4.90
3	3.0	73.00	1.17	4.06	3.64	28.20	8.10	6.10
4	3.5	93.00	1.55	7.42	5.20	28.90	8.90	6.30
5	4.0	124.00	1.69	9.37	4.03	29.20	9.60	7.30
6	4.5	169.00	1.95	13.68	3.71	30.70	9.80	7.50
7	5.0	198.00	2.19	19.68	3.71	31.20	10.70	7.50
8	5.5	276.00	2.62	69.14	2.73	33.30	12.30	8.20
9	6.0	476.00	5.86	154.03	2.19	34.20	12.90	8.30
10	6.5	210.00	3.69	132.42	3.51	31.10	9.20	8.50
SE±		15.24	1.50	5.78	1.74	0.05	0.18	0.24
C.D. at p= (5%)		30.97	3.14	11.75	3.54	0.11	0.36	0.48

*mean of five replicates

V = Volume of haemocytometer (0.256×10^{-5}) cc
Length of the spores was measured by calibrated ocular micrometer under compound microscope (10 x 45 x of magnification).

RESULTS AND DISCUSSION

The mean dry weight of mycelium and sporulation of three spore forms of the fungus on different pH levels is recorded in Table 1. The perusal of the results showed that *Fusarium oxysporum* Schlecht. f. sp. l*entis* grew maximum in pH 6.0. Very high acidic range of pH showed very poor growth of mycelium. Mycelial mat accumulation increased with increase in pH but declined after pH 6.0. Sporulation of the macroconidia and microconidia was observed to be maximum at pH 6.0. Least sporulation occurred in pH 2.0. Chlamydospores produced were noted to be the maximum at pH 4.0. Length of macroconidia and microconidia were the maximum at pH 6.0 and thereafter it started to decline. Diameter of chlamydospores increased with increase in pH level.

The growth and sporulation of many fungi have been studied under the influence of various pH levels. Mix (1933) found that pH range from 4 to 8 showed good growth for *Phyllosticta solitaria*. Wilson (1946) observed acid soil (pH 4.2) support growth of *Fusarium* spp. where as a pH near neutrality prevents growth. Chaudhary (1971) and Prasad *et al*. (1992) reported 6.0 pH level as the best for the growth and sporulation of *Fusarium moniliform*e v *subglutinans*e Wr. and Rg. Munjal and Gautam (1977) observed that maximum fungal growth and sporulation occurred at pH 6.0 and 5.5 respectively for *Septoria humuli*. Srobar (1978) found pH 6 to be the most suitable for the growth of all species while a highly acidic medium was unsuitable for sporulation of all species causing fusarioses disease in wheat. Jat and Goyal (1978) found that growth and sporulation of *Claviceps microcephala* to be optimum at pH 7.5 and 6.0 respectively. Nair(1957); Souramma and Singh (2004); Groenewald (2005); Kishore *et al*. (2009) also found pH 5.5 to 7.0 to be the best for growth and sporulation of *Fusarium oxysporum f.* sp. *lini* (Belley). Such reports are in agreement with present findings for this pathogen. This indicates that unusual acidity badly hampered the growth and sporulation of the fungus. On the other hand sporulation of chlamydospores at more acidic range indicates that this spore is characteristically different from other two spores and more tolerable to stressed condition than the macro and micro conidia.

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