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Effect of temperature and pH levels on the growth and sporulation of *Fusarium oxysporum* f.sp. *lini* causing linseed wilt

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Abstract
Among the fungal diseases of linseed, wilt caused by *Fusarium oxysporum f.sp. lini* (Bolley) is a major constraint responsible for low production and productivity. An *in vitro* experiment was conducted to find out the suitable temperature and pH for the growth and sporulation of *Fusarium oxysporum* f.sp. *lini*. Study showed that after 9 days of incubation, the maximum growth of the fungus was 88.33 mm at 24ºC with highest growth rate of 9.81 mm per day and highest sporulation of 7.9 x 10^6 per ml. In case of pH, maximum growth of the fungus was 86.33 mm at pH 5.5 with highest growth rate of 9.59 mm per day and highest sporulation of 8.2 x 10^6 per ml after 9 days of incubation at 25 ± 2°C

Keywords: linseed, *fusarium oxysporum* f.sp. *lini*, temperature, PH

Introduction
Linum usitatissimum L. commonly known as linseed or flaxseed is an ancient oilseed and fiber crop and extensively grown in the countries of the temperate zone as well as in those of the tropical zone. It is such a valuable crop that every part of the plant has specific economic importance. Flax (*Linum usitatissimum*) is also one of the richest dietary sources of α-linolenic acid (ALA) and is a good source of soluble fiber mucilage in human nutrition (Cunnane *et al.* 1993) [4]. In India, Madhya Pradesh leads in yield and acreage, followed by Uttar Pradesh, Maharashtra, Bihar, Rajasthan, Karnataka and West Bengal. Madhya Pradesh and Uttar Pradesh together contribute to national linseed production to the extent of about 70 per cent (Anonymous 2015) [1]. Cultivation of linseed on marginal and sub marginal soils under input starved conditions and susceptibility of crop to diseases are the root causes of low productivity. Among the fungal diseases of linseed, wilt caused by *Fusarium oxysporum* f. sp. *lini* (Bolley) is a major constraint responsible for low production and productivity along with rust and powdery mildew (Kishore *et al*. 2011) [12]. The crop losses due to wilt in the range of 80-100% have been reported by Sattar and Hafiz (1952) [15]. Present work depicts the role of different temperature and pH levels to understand ecological survival of pathogen, which will be helpful in linseed wilt management strategy in the field.

Material and Methods
Collection and isolation of pathogen
Linseed plants naturally infected and showing typical wilt symptoms were collected from farmer's fields from different locations in district Kangra and Mandi of Himachal Pradesh during 2015- 2016 and brought to the laboratory. The roots and stems of infected plants were washed in tap water to remove adhering soil particles, if any and root bark was removed before isolation to avoid contamination. Discolored vascular tissues from roots of diseased plants were cut as small bits (size 2.5cm) with sterilized sharp blade. These bits were then disinfected with 0.1% solution of mercuric chloride for one or two minutes, then washed thoroughly in sterile distilled water thrice to remove the traces of mercuric chloride, dried in sterile blotter paper and aseptically transferred on Potato Dextrose Agar (PDA) medium in Petri plate, and incubated at 25 ± 2°C for a week. Fungus growth in plate was examined and then sub-cultured on PDA slants. The pure culture of fungus was obtained by adopting single spore techniques as described by Choi (1999) [3]. Identification of the fungal growth was done by mounting in lactophenol and following the standard keys (Booth, 1971) [2].

Effect of different temperature levels on the growth of *Fusarium oxysporum* f.sp. *lini*

In order to study the effect of different temperature levels on the growth of the *F. oxysporum* f.sp. *lini*, 20 ml of PDA medium was poured in each petriplate and was inoculated with 5 mm discs of the actively growing culture of *F. oxysporum* f.sp. *lini*. The inoculated petridishes were then, incubated at different levels of temperature *viz*., 16, 20, 24, 28 and 32ºC, replicated four times in a Completely Randomized Design (CRD) (Sharma 2005) [17]. The data on colony growth were recorded with the help of measuring scale after 3, 6 and 9 days of incubation and the growth rate (rg) of colonies mm/day at each incubation temperature was calculated. Sporulation was recorded after 9 days. The number of spores was counted using known depth of Haemocytometer slide (0.01 cm) under the compound microscope as discribed by Tyagi and Pudel (2014) [19]:-

Number of spores / 100 ml = (V/ N) x 100

Where, N =Average number of spores per square of the four corner square of haemocytometer counted; V= Volume of haemocytometer (0.256 $x10^{-5}$) cc

Effect of different pH levels on the growth of *Fusarium oxysporum* f.sp. *lini*

Eight pH levels *viz.*, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 were maintained on PDA medium as described by Sharma *et al*. (2005) [17]. The pH of the medium was adjusted before autoclaving with the help of HCl (0.1N) and NaOH (0.1N). After autoclaving the medium, sterilized Petriplates (90 mm), containing equal 20 ml volume of medium was inoculated centrally with 5 mm culture disc of the actively growing culture of *F. oxysporum* f.sp. *lini*. The experiment was conducted in Completely Randomized Design (CRD) and each treatment was replicated four times. Inoculated Petriplates were incubated at 25 ± 2°C. Observations on the growth of the colonies were taken at 3 days interval up to 9 days and the growth rate (rg) mm/day at each pH level was calculated. Sporulation was recorded after 9 days by counting the number of macro-conidia and micro-conidia with the help of haemocytometer.

Result and discussion

Effect of different temperature levels on the growth of *Fusarium oxysporum* f.sp. *lini*

It was depicted in Table 1 that, the maximum growth of the fungus was 88.33 mm at 24ºC with highest growth rate of 9.81 mm per day and highest sporulation of 7.9 x 10^6 per ml followed by 70.00 mm at 28ºC with growth rate of 7.78 mm per day and sporulation of 5.3 x 10^6 per ml, 68.00 mm at 20ºC with growth rate of 7.56 mm per day and sporulation of 4.6 x 10^6 per ml and 49.67 mm at 16ºC with growth rate of 5.51 mm per day and sporulation of 2.3 x 10^6. The least growth of fungus was 29.33 mm at 32ºC with growth rate of 3.25 mm per day and least sporulation of 1.2 x 10^6, which differed significantly from the growth at other temperatures (Fig 1). Thus, the optimum temperature range for growth and sporulation of the pathogen was 24-28°C. The present findings are in accordance with earlier observations of Somesh *et al*. (2019) [18] stated that optimum temperature range for growth of *Fusarium oxysporum* f.sp. *lini* was found to be 25 °C to 30 °C. However, the minimum growth was recorded at 45° C and 10°C. No growth and sporulation were observed at 50 °C temperature. Sharma *et al*. (2005) [17] also showed that *Fusarium oxysporum* f.sp. *lini* required optimum temperature of 25 ± 2ºC for the growth and sporulation when incubated at five levels of temperature *viz.*, 15º, 20º, 25º, 30º and 35ºC.

Table 1: Effect of different temperatures on colony growth and sporulation of *Fusarium oxysporum* f.sp. *lini*

Temp. (Cº)	Average colony diameter (mm) 9 days after inoculation	My celial growth rate (mm/day)	Average sporulation per ml
16	49.67	5.51	2.3 x 10^6
20	68.00	7.56	4.6 x 10^6
24	88.33	9.81	7.9 x 10^6
28	70.00	7.78	5.3 x 10^6
32	29.33	3.25	1.2 x 10^6
C.D. (P=0.05)	1.64		
SE(m)+/-	0.52		

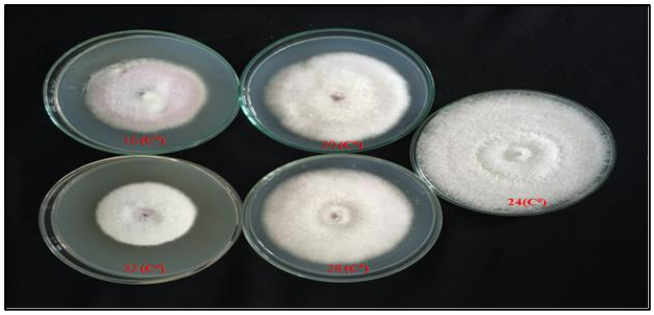

Fig 1: Effect of different temperatures on colony diameter growth of *Fusarium oxysporum* f.sp. *lini*

Landa *et al*. (2001) [13] showed that optimum growth of *F. oxysporum* f. sp. *ciceris* was at 24.5–28.5°C. Similar experiment was conducted by Farooq *et al*. (2005) [5] who observed that temperature of 25ºC and 30ºC were the best for *Fusarium oxysporum* f. sp. *ciceri*, where it has attained maximum growth. Sharma *et al*. (2011) [16] who also reported maximum growth of fungus *Fusarium oxysporum* f. sp. *lycopersici* at 25°C followed by 30°C while minimum average radial growth of fungus was recorded at 15 and 35°C.

Effect of different pH levels on the growth of *Fusarium oxysporum* f.sp. *lini.*

The results in Table 2 indicates that, the maximum growth of the fungus was 86.33 mm at pH 5.5 with highest growth rate of 9.59 mm per day and highest sporulation of 8.2 x 10^6 per ml followed by 84.33 mm at pH 6.0 with growth rate of 9.37 mm per day and sporulation of 8.1 x 10^6 per ml, 83.67 mm at pH 6.5 with growth rate of 9.29 mm per day and sporulation of 7.5 x 10^6 per ml. However, diameter of colony was statistically at par at pH levels of 5.5, 6.0 and 6.5. The least growth of fungus was 24.67 mm at pH 4.5 with growth rate of 2.74 mm per day with least sporulation of 1.1 x 10^7, which differed significantly from the growth at other pH levels. Optimum pH for growth of fungus was recorded at pH 5.5. Data also revealed that growth rate was increasing in descending order (9.59, 9.37, 9.29, 8.89, 8.51 and 7.07 mm/day) from pH 5.5 to 8.0 and growth rate was increasing in ascending order (2.74, 3.14 & 7.19 mm/day) from pH 4.5

to 5.5. The studies conducted on *F. oxysporum* f. sp. *lini* during present investigation indicated that, as the pH decreases or increases from the optimum, the rate of growth gradually decreased (Fig 2).

Table 2: Effect of different pH levels on growth and sporulation of *Fusarium oxysporum* f.sp. *lini*

pH Levels	Average colony diameter (mm) 9 days after inoculation	Mycelial growth rate (mm/day)	Average sporulation per ml
4.5	24.67	2.74	1.1×10^7
5	28.33	3.14	1.5×10^6
5.5	86.33	9.59	8.2×10^6
6	84.33	9.37	8.1×10^6
6.5	83.67	9.29	7.5×10^6
7	80.00	8.89	5.4×10^6
7.5	76.67	8.51	4.7×10^6
8	63.67	7.07	2.1×10^6
C.D (P=0.05)	3.41		
SE(m)+/-	1.12		

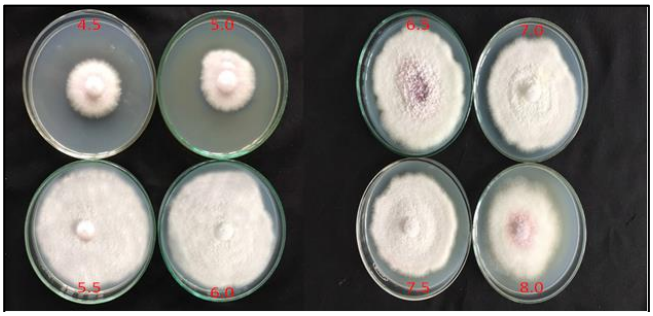

Fig 2:- Effect of different pH levels on colony diameter growth of *Fusarium oxysporum* f.sp. *lini*.

These results are in conformity with Sharma *et al.* (2005) [17] who also reported optimum pH for growth and sporulation of *F. oxysporum* f. sp. *lini* is 5.5. Gangadhara *et al*. (2004) [6], Groenewald (2005) [7], Kishore *et al*. (2009), Gupta *et al*. (2010) [8] and Jaruhar and Prasad (2011) [9] also reported that optimum pH range for growth and sporulation of *Fusarium oxysporum* f.sp. *lentis* were in between 5.0 - 7.0. Khan *et al*. (2011) [10] reported that optimum pH for growth of *Fusarium oxysporum* f.sp. *ciceri* ranged from 6.5 to 7.0. Khilare and Ahmed (2011) [11] reported most suitable pH level for growth of *Fusarium oxysporum* f.sp. *ciceri* was 6.0 and 6.5.

Conclusion

Fungi have the ability to produce a number of secondary metabolites, typically dependent on environmental factors ranging from nutrient concentrations to light, pH and temperature etc. (Pradeep and Pradeep 2013) []. In this study, different levels of temperature and pH strongly influenced the growth and sporulation of *F. oxysporum* f. sp. *lini.* It was concluded that after 9 days of incubation, the maximum growth of the fungus was at 24ºC with highest growth rate and highest sporulation. In case of pH, maximum growth of the fungus was at pH 5.5 with highest growth rate and highest sporulation after 9 days of incubation at 25 ± 2°C.

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