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Effect of different media, temperature and pH on 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* is a major constraint for low production and productivity. An *in vitro* experiment was conducted to find out effective culture medium, temperature and pH for growth and sporulation of *Fusarium oxysporum* f.sp. *lini*. Study showed that fungus grew best on PDA among eight media tested. The maximum growth of the fungus (87.22 mm) was recorded at 25 °C after seven days of incubation with maximum sporulation and highest growth rate of 12.46 mm per day. Similarly, maximum growth of the fungus (88.16 mm) was recorded at pH 6 with highest growth rate (12.59 mm per day) and maximum sporulation after seven days of incubation.

Keywords: Linseed, *Fusarium oxysporum* f.sp. *lini*, culture media, temperature, pH

Introduction

Linseed (*Linum usitatissimum* L.) is one of the oldest cultivated crops, commonly known as “Ulseec” or “Tisee”. This belongs to family Linaceae and second commercially most important Rabi oilseed crops after rapeseed and mustard in area as well as in production. Linseed 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) [2]. Among the fungal diseases of linseed, wilt caused by *Fusarium oxysporum* f. sp. *lini* is a major constraint responsible for low production and productivity (Kishore *et al.* 2011) [9]. Madhya Pradesh contributes 1.0844 lakh ha areas with 0.328 tones production (Anon. 2020) [1]. *Fusarium* infects linseed at any growth stage of crop growth and may result in cent percent disease incidence in certain cultivars (Kommedahl *et al.*, 1970) [10]. Present work depicts the role of different culture media, temperature and pH levels to understand ecological survival of pathogen, which will be helpful in linseed wilt management strategy under field conditions.

Materials and Methods

Evaluation of various media for the growth of *Fusarium oxysporum* f.sp. *lini*

Eight culture media viz. Reddish root extract dextrose agar, Lentil seed extract dextrose agar, Linseed seed extract dextrose agar, Green gram seed extract dextrose agar, Chickpea seed extract dextrose agar, Carrot extract dextrose agar, Oats seed extract dextrose agar and Potato dextrose agar were evaluated in the laboratory condition to select the most suitable medium for the growth of linseed wilt pathogen *Fusarium oxysporum* f.sp. *lini*.

The media were sterilized at 1.05 kg/cm² and 121.6 °C for 20 minutes in an autoclave. For inoculating different culture media in Petri plates, seven days old culture of target pathogen has grown on potato dextrose agar medium. Five mm disc of inoculum was cut and placed at the center of the plate containing specific medium in an inverted position, so that it came in direct contact with the surface of the medium.

The inoculated Petri plates were incubated at 25±2 °C and radial growth of the pathogen was measured in two directions at right angle with help of a linear scale after 3, 5 and 7 days of incubation.

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Table 1: Media and their composition used during study

S. No.	Name of Media	Ingredients	Quantity
1.	Potato dextrose agar (PDA)	Peeled and sliced potato	200 g
		Dextrose	20 g
		Agar	20 g
		Distilled water	1000 ml
2	Reddish root extract dextrose agar	Reddish root extract	200 g
		Dextrose	20 g
		Agar	20 g
		Distilled water	1000 ml
3	Lentil leaf extract dextrose agar	Lentil leaf extract	200 g
		Dextrose	20 g
		Agar	20 g
		Distilled water	1000 ml
4	Chickpea leaf extract dextrose agar	Chickpea leaf extract	200 g
		Dextrose	20 g
		Agar	20 g
		Distilled water	1000 ml
6	Chickpea seed extract dextrose agar	Chickpea seed extract	200 g
		Dextrose	20 g
		Agar	20 g
		Distilled water	1000 ml
7	Linseed seed extract dextrose agar	Sorghum seed extract	200 g
		Dextrose	20 g
		Agar	20 g
		Distilled water	1000 ml
8	Lentil seed extract dextrose agar	Lentil seed extract	200 g
		Dextrose	20 g
		Agar	20 g
		Distilled water	1000 ml

Effect of different temperature on growth and sporulation of pathogen

The experiment was performed to figure out the most suitable temperature for mycelial growth and sporulation of *F. oxysporum* f.sp. *lini*. Twenty ml of PDA medium was poured in each Petri plate and were inoculated with five mm discs of the actively growing culture of *F. oxysporum* f.sp. *lini*. The inoculated Petri dishes were then; incubated at different temperatures viz., 15, 20, 25, 30 and 35 °C replicated thrice following Completely Randomized Design (CRD) (Sharma *et al.*, 2005) [12]. The data on colony growth were recorded with the help of measuring scale after 3, 5 and 7 days of incubation and the growth rate of colonies mm/day at each temperature was calculated. Sporulation was recorded after seven days with the help of hemocytometer.

Effect of pH on growth and sporulation of pathogen

Different pH levels viz., 2, 4, 6, 8, 10 and 12 were maintained in PDA as described by Sharma *et al.*, 2005 [12]. The pH of the medium was adjusted with the help of HCl and NaOH. After autoclaving, sterilized Petri plates (90 mm) containing equal volume (20 ml) of medium were inoculated centrally with five mm culture disc of actively seven day old growing culture of *F. oxysporum* f.sp. *lini*. The experiment was conducted in Completely Randomized Design (CRD) and each treatment was replicated thrice. Inoculated Petri plates were incubated at 25±2 °C. Observations on the growth of the colonies were taken at three days interval upto seven days and the growth rate mm/day at each pH level was calculated. Sporulation was recorded after seven days with the help of hemocytometer.

Result and Discussion

Effect of different media on the mycelial growth of *Fusarium oxysporum* f.sp. *lini*

Effect of eight different media, viz., Reddish root extract dextrose agar, Lentil seed extract dextrose agar, Linseed seed extract dextrose agar, Green gram seed extract dextrose agar, Chickpea seed extract dextrose agar, Carrot extract dextrose agar, Oats seed extract dextrose agar and Potato dextrose agar on mycelial growth of *Fusarium oxysporum* f.sp. *lini* were studied and observations are presented in table 2.

Out of eight media, maximum mycelial growth was recorded in the Potato dextrose agar (39.50 mm) which was superior over other media. This was followed by Oat seed extract dextrose agar (36.33 mm), Lentil seed extract dextrose agar (32.85 mm) and Green gram seed extract dextrose agar (32.00 mm), Linseed seed extract dextrose agar (26.00 mm), Reddish root extract dextrose agar (25.33), Chickpea seed extract dextrose agar (24.33) and Carrot extract dextrose agar (22.50) exhibited lowest growth of the fungus after 3rd day of inoculation (Table 1).

After five days of inoculation, the mycelial growth was maximum on the Potato dextrose agar (56.50 mm) which was superior over other media. This was followed by Oat seed extract dextrose agar (46.33 mm), Lentil seed extract dextrose agar (45.83 mm), Green gram seed extract dextrose agar (44.66 mm), Linseed seed extract dextrose agar (42.50 mm), Reddish root extract dextrose agar (41.50 mm), chickpea seed extract dextrose agar (37.83 mm) and Carrot extract dextrose agar (35.66 mm) exhibited lowest growth of the fungus after five day of inoculation (Table 1).

After 7 day of inoculation, the mycelial growth was maximum on the Potato dextrose agar (89.16 mm) which was superior over other medium. This was followed by Oat seed extract dextrose agar (73.83 mm), Lentil seed extract dextrose agar (64.33 mm), Green gram seed extract dextrose agar (63.00 mm), Linseed seed extract dextrose agar (58.65 mm), Reddish root extract dextrose agar (56.66 mm), Chickpea seed extract dextrose agar (55.50 mm) and Carrot extract dextrose agar (53.06 mm) exhibited lowest growth of the fungus after 7th day of inoculation (Table 1). Khare *et al.* (1975)^[7] evaluated nine solid media against *F. oxysporum* f.sp. *lentis* and

observed the maximum growth on PDA. Khan *et al.* (2011)^[8] tested *Fusarium oxysporum* f.sp. *ciceri* for variation in growth and cultural characters on five different solid media and found that PDA was best for the growth of different isolates. Similar findings were also recorded by Singh and Kumar (2016)^[13] and Singh *et al.* (2016)^[14] they conclude that Potato dextrose agar and Richard's agar were the best medium for radial growth and sporulation of *Fusarium oxysporum* f.sp. *lentis* as highest colony diameter (76 and 70 mm) and excellent sporulation were observed on these media.

Table 2: Effect of media on mycelial growth of *Fusarium oxysporum* f.sp. *lini* after 3, 5 and 7 days of inoculation (DAI)

Sr. No.	Media	Mycelial growth (mm)		
		3DAI	5DAI	7DAI
1	Green gram seed extract dextrose agar	32.00	44.66	63.00
2	Oats seed extract dextrose agar	36.33	46.33	73.83
3	Chickpea seed extract dextrose agar	24.33	37.83	55.50
4	Linseed seed extract dextrose agar	26.00	42.50	58.65
5	Carrot extract dextrose agar	22.50	35.66	53.16
6	Raddish root extract dextrose agar	25.33	41.50	56.66
7	Lentil seed extract dextrose agar	32.85	45.83	64.33
8	Potato dextrose agar	39.50	56.50	89.16
	C.D.	1.95	2.72	2.22
	SE(m)	0.64	0.89	0.73

Mean of three replications

Effect of temperatures on mycelial growth and sporulation of *Fusarium oxysporum* f.sp. *lini*

The results presented in Table 3 indicate that, the maximum growth of fungus (87.22 mm) was recorded at 25 °C with highest sporulation and growth rate (12.46 mm per day) followed by 69.67 mm radial growth at 30 °C with growth rate of 9.95 mm, 65.22 mm at 20 °C with growth rate of 9.31 mm per day, 45.54 mm at 15 °C with growth rate of 6.50 mm per day. The least growth and sporulation of fungus 24.63 mm was recorded at 35 °C with growth rate of 3.51 mm per day, which exhibits variation in growth at different temperatures

(Plate 3). The present findings are in accordance with earlier observations of Somesh *et al.* (2019)^[15] who stated that optimum temperature range for growth of *Fusarium oxysporum* f.sp. *lini* is 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)^[12] also showed that *Fusarium oxysporum* f.sp. *lini* required optimum temperature of 25±2 °C for growth and sporulation when incubated at five levels of temperature viz., 15, 20, 25, 30 and 35 °C.

Table 3: Effect of different temperatures on colony growth and sporulation of *F. oxysporum* f.sp. *lini*

Temperature (°C)	Average colony diameters (mm) recorded	Mycelial growth rate (mm/day)	Average sporulation per ml
15	45.54	6.50	++
20	65.22	9.31	++++
25	87.22	12.46	+++++
30	69.67	9.95	++++
35	24.63	3.51	+
C.D.	1.79		
SE(m)	0.56		

Mean of three replications

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

The results in Table 4 indicate that maximum sporulation and growth of the fungus (88.16 mm) was observed at pH 6 with highest growth rate of 12.59 mm per day followed by pH 8 where colony diameter was 70.53 mm with 10.07 mm growth rate recorded per day. The least growth and sporulation of fungus (37.81 mm) was observed at pH 2 with growth rate of 5.40 mm per day, which differed significantly from different pH levels.

Data showed that mycelial growth rate decreased from pH 6 to 12 (12.59, 10.07, 9.45, and 8.74 mm/day), and increased from pH 2 to 4 (5.40 and 7.35 mm/day). Studies on *F. oxysporum* f.sp. *lini* during the current study show that the rate

of growth gradually decreased as pH changed from pH 6 to other values (Plate 4). Pal *et al.*, (2019)^[11] confirmed 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.

These results are in conformity with Sharma *et al.* (2005)^[12] who reported optimum pH for growth and sporulation of *F. oxysporum* f. sp. *lini* is 5.5. Gangadhara *et al.* (2004)^[3], Groenewald (2005)^[4], Kishore *et al.* (2011)^[9], Gupta *et al.* (2010)^[5] and Jaruhar and Prasad (2011)^[6] also reported that optimum pH range for growth and sporulation of *Fusarium oxysporum* f.sp. *lentis* was in between 5.0 - 7.0. Khan *et al.* (2011)^[8] reported that optimum pH for growth of *Fusarium oxysporum* f.sp. *ciceri* ranged from 6.5 to 7.0.

Table 4: Effect of different pH levels on mycelial growth and sporulation of *F. oxysporum* f.sp. *lini*

pH Levels	Average colony diameter (mm) recorded	Mycelial growth Rate (mm/day)	Average sporulation per ml
2	37.81	5.40	++
4	51.45	7.35	+++
6	88.16	12.59	+++++
8	70.53	10.07	++++
10	66.20	9.45	++++
12	61.23	8.74	+++
C.D.	1.56		
SE(m)	0.50		

Mean of three replications

Conclusion

In this study, different culture media, temperature and pH strongly influenced the growth and sporulation of *F. oxysporum* f. sp. *lini*. After seven day of inoculation the mycelial growth was maximum on the Potato dextrose agar (89.16 mm) which was significantly superior over other medium. This was followed by oat seed extract dextrose agar (73.83 mm), and Carrot extract dextrose agar (53.06 mm) exhibited lowest growth of the fungus after seven day of inoculation.

It was concluded that after seven days of incubation, the maximum growth of the fungus was at 25 °C with highest growth rate and highest sporulation. In case of pH, maximum growth of the fungus highest growth rate and sporulation after seven days of incubation at 25±2 °C was recorded at pH 6.

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