Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(2): 1427-1430
Received: 07-01-2019
Accepted: 10-02-2019

Somesh
Department of Plant Pathology, C.S.A. University of Agriculture and Technology, Kanpur, Uttar Pradesh, India

Narendra Singh
Department of Plant Pathology, C.S.A. University of Agriculture and Technology, Kanpur, Uttar Pradesh, India

Lopamudra Behera
Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Rajendra Kumar Bais
Department of Plant Pathology, C.S.A. University of Agriculture and Technology, Kanpur, Uttar Pradesh, India

Ashutosh Tiwari
Department of Plant Pathology, C.S.A. University of Agriculture and Technology, Kanpur, Uttar Pradesh, India

Sumit Kumar
Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Correspondence
Sumit Kumar
Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Effect of temperature and pH on growth and sporulation of *Fusarium oxysporum* f.sp. *lini* (Bolley) Synder and Hensan causing linseed wilt under environmental condition

Somesh, Narendra Singh, Lopamudra Behera, Rajendra Kumar Bais, Ashutosh Tiwari and Sumit Kumar

Abstract
An experiment was conducted on effect of atmospheric temperature and substrate pH on growth, development and sporulation of *Fusarium oxysporum* f.sp. *lini*. The pathogen was collected from linseed growing areas of crop research farm Nawabganj, C.S. Azad University and Technology, Kanpur, UP during *Rabi* 2016-17. The optimum temperature range for growth 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. The pathogen grows over a wide range of pH *i.e.*, from 3.0 to 8.0, but the most suitable pH for its growth was observed to be 7.0 at which the maximum growth of the fungus was recorded which was closely followed by pH 6.5. However, with the increase of acidity or alkalinity, the growth of the fungus was hampered and lowest growth was recorded at pH 3.0.

Keywords: *Fusarium oxysporum* f.sp. *lini*, temperature, pH, sporulation

Introduction
Cultivated Flax (*Linum usitatissimum* L.), commonly known as 'linseed' or 'alsi' is an extensively cultivated oilseed crop. Taxonomically, linseed belongs to order Linales and the family Linaceae. It is one of the oldest cultivated crops, grown either for fibre or oil (Broadfoot WC, 1926) [1]. The origin of Indian type of linseed is traced to be in Ethiopia, through polyphyletic origin of the same is indicated. The cultivation of linseed crop as such goes back to more than 5000 years. The oldest regions of linseed cultivation are reported to be in Asia and on the Mediterranean coast. It is extensively grown in temperate, subtropical and tropical regions of the world. In India it is cultivated mainly at central India, although it's cultivated in patches spread from Himalaya in north to Karnataka in south. Among the oilseed crops grown during *Rabi*, linseed is next in importance to rapeseed and mustard in area as well as in production. The annual area under the cultivation of linseed in the world is 2,764,340 hectares with total production of 2,925,282 tonnes, giving the average yield of 1058.2 kg/ha. Cultivation of linseed in India accounts for about 293,000 hectares with seed production of 125,000 tonnes and national average yield of 426.6 kg/ha (FAOSTAT, 2016). Oil of linseed crop is rich in α-linolenic acid (ALA) and because of this, linseed oil readily polymerizes on exposure to oxygen. Hence it is utilized in the manufacture of paints, oil, cloth, varnish, pad-ink, printed ink, linoleum etc. The energy content of oil is much higher (39.80 MJ/kg) than crude protein (23.88 MJ/kg) or carbohydrate (16.76 MJ/kg). They contain useful carbohydrates, vitamins and provide essential fatty acids. Edible oil cakes are fed to cattle, while non-edible cakes are used as organic manures; it contains about 5 percent nitrogen, 1.4 percent phosphorus and 1.8 percent potash and is one of the oldest commercial oils used for various purposes. Despite considerable increase in productivity and production a wide gap exists between potential yield and the yield realized at farmer's field, which is largely because of a number of biotic and abiotic stresses, to which linseed crop is exposed. Among biotic stresses wilt caused by *Fusarium oxysporum* f. sp. *lini* is the most important disease and known to inflict 80% of theoretical yield losses under conditions favourable for wilt development in linseed (Sattar and Hafiz, 1952) [7]. The crop has poor resistance base against this disease in national gene pool. Extensive studies on occurrence, distribution, seasonal status, symptomatology, cultural and fungicidal management along with integrated management of this seed and soil borne disease have been undertaken. Fungi attack the crop of linseed at different stages of growth including leaf, twigs, Fruits, root and seed inflicting losses in quality and quantity.

Soil temperature, its moisture content and type, affect the incidence of wilt. Tisdale (1917) [10] and Houston and Knowles (1953) [3] reported that wilt pathogen failed to develop below 12 °C and above 38 °C. The incidence of wilt appears to be more at soil pH 5.5 to 7.5 than at higher and lower levels. (Houston and Knowles, 1958; Goel and Swarup, 1964 and Nair, 1957) [4, 2, 8].

Materials and Methods

Naturally affected plants of linseed showing symptoms of wilt disease were collected during *Rabi* 2016-17 from crop Research farm Nawabganj, C.S. Azad University and Technology, Kanpur, UP. Such affected plants were brought to the laboratory and critically examined for the presence of causal organism. The freshly collected diseased materials were used for isolation of the pathogen. The affected roots were first washed in tap water to remove dust particles and then thoroughly washed with sterilized water in order to remove the surface contaminants. The cut pieces were surface sterilized with 0.1 percent mercuric chloride solution under aseptic conditions inside the laminar flow and washed thoroughly three to four times with sterilized water to remove the traces of mercuric chloride. Excess moisture was removed by placing them in the fold of sterilized blotting papers. These pieces were transferred to 2 percent Potato Dextrose Agar (PDA) medium in 90 mm Petri dishes, previously autoclaved at 15 psi. for 20 minutes, with the help of sterilized needles. The petri dishes were then kept at 28±2 °C temperature for 7 days in B.O.D. incubator. These incubated plates were observed for mycelial growth of the causal fungus after 24 hours of inoculation daily once till the growth of the fungus was noted. As soon as the mycelial growth was visible around these pieces, the hyphal tips from the advancing mycelium were cut and transferred into the culture tubes containing Potato-Dextrose Agar medium for further purification, identification and maintenance of culture.

Effect of temperatures on the growth and sporulation of pathogen

The studies were carried out on 2 percent Potato dextrose medium. The fungus was grown at different temperatures viz., 10, 15, 20, 25, 30, 35, 40, 45 and 50 °C. 150ml flat bottom flasks were filled with 50ml of the liquid media and sterilized at 1.1 kg/cm2 for 15 minutes. Three replications were maintained for each temperature and incubated at above mentioned temperatures. Ten days after inoculation, the mycelia mat was filtered on pre- weighed Whatman's filter paper No.42. The fungal mat, which retained on filter paper, was thoroughly washed with sterilized distilled water to remove traces of salt remaining with the mycelium. The filtered mycelia mats were first air dried then in oven along with filter paper at 60 °C for 48 hrs. and subsequently cooled within desiccators. The initial weight of the filter paper was deducted from the total weight to find out the actual weight of the mycelium. Weight of the fungus was recorded for each replication separately.

Effect of pH levels on the growth and sporulation of pathogen

For study of the effect of different pH level on the growth of the fungus, the most suitable Potato dextrose medium was selected. The pH values of the medium were adjusted at 3.0, 4.0, 4.5, 5.0, 5.5, 6.5, 7.0, 7.5 and 8.0 respectively. 50 ml medium was taken in 150 ml conical flasks for study of each pH value in triplicate. The media were sterilized at 1.1 kg/cm2 for 15 minutes. The pH was adjusted by adding N/10 HCl or N/10 NaOH for each pH level.

The flasks containing equal amount of the medium were inoculated with culture disc of equal size of the fungus, cut by the help of sterilized cork borer. The flasks were incubated at 25±2 °C for 10 days and then mycelial mat of the fungus was filtered, thoroughly washed with distilled water and dried in oven at 60 °C for 48 hrs and weighed.

Results and discussion

Effect of different temperatures on the growth and sporulation of the pathogen

It is well known that temperature influences the growth of all microorganisms. Present studies were, therefore, taken up to find out the optimum, minimum and maximum temperature requirements for the growth and sporulation of the test pathogen. In present investigation the fungus was grown on Potato dextrose medium and incubated at nine different temperatures ranging from 10 °C to 50 °C. The average dry mycelium weight was recorded in the Table 1.

The observation recorded in Table1 indicated that, although the pathogen could grow over a wide range of temperature i.e. 10 °C to 45 °C, but the optimum temperature for its growth was found to be 30 °C followed by 25 °C and 35 °C and former two did not differ statistically from each other. The optimum temperature for growth was therefore 25 °C to 30 °C, good growth was recorded at 25 °C and 35 °C However, the minimum growth was recorded at 45 °C and 10 °C. No growth and sporulation were observed at 50 °C temperature. Excellent conidial formation was observed at 25 °C and 30 °C, good at 20 °C and 35 °C, fair at 15 °C, poor at 10 °C, 40 °C and 45oC. Jones and Tisdale (1992) [5] working at Wisconsin Agriculture Experiment Station at US, observed that the optimum temperature, for Linseed wilt pathogen lied between 75 to 82ºF (24-28 ºC) and infection did not occur above 96.4ºF (38 ºC). The present results are in agreement with their findings. Similar results were reported by Mc Rae (1926) [6] from India. However, Souramma and Singh (2004) also found 25 ºC to be the best temperature for growth and sporulation of *Fusarium oxysporum* f. sp. *lini.*

Table 1: Effect of different temperature on growth and sporulation of *Fusarium oxysporum* f.sp. *lini* after 10 days of incubation

S. No.	Temperatures (ºC)	Dry weight of mycelium (mg)	Sporulation
1	10	82.00	Poor
2	15	124.00	Fair
3	20	180.00	Good
4	25	246.00	Excellence
5	30	257.00	Excellence
6	35	214.00	Good
7	40	110.00	Poor
8	45	28.00	Poor
9	50	0.00	-
G.M.	37.89		
Sem±	: 1.27		
CD at 5%	: 3.79		

Effect of different pH levels on the growth and sporulation of the pathogen

The hydrogen ion concentration is also known to have considerable effect on the growth of the fungi. To find the optimum pH level the pathogen was grown on Potato dextrose medium (liquid) adjusted at different pH levels ranging from 3.0 to 8.0. After 10 days of incubation at 28±1 °C, the

mycelial weight was recorded in each treatment as per procedure. The data are presented in Table 2.

It was evident from the data that, although the pathogen grew over a wide range of pH i.e. from 3.0 to 8.0, but the most suitable pH for its growth was observed to be 7.0 at which the maximum growth of the fungus was recorded which was closely followed by 6.5. These two pH levels supported statistically similarly growth of the pathogen. However, with the increase of acidity or alkalinity, the growth of the fungus was hampered and lowest growth was recorded at pH 3.0. The excellent conidia were counted at 6.5 and 7.0 pH, good at 5.5 and 7.5 pH, whereas fair at 5.0 pH level and poor at 3.0, 4.0, 4.5 and 8.0 pH levels. The results are in agreement with finding of Nair (1957) [8] in his studies on factors affecting growth of *Fusarium oxysporum* f. sp. *lini* reported that the incidence of wilt appeared to be more at soil pH 5.5 to 7.5 than at higher and lower level. Souramma and Singh (2004) found that 6.5 to be the most suitable pH level for growth and sporulation of *Fusarium oxysporum* f. sp. *lini.*

Table 2: Effect of different pH levels on growth and sporulation of *Fusarium oxysporum* f.sp. *lini* after 10 days of incubation

S. No.		pH	Dry weight of mycelium(mg)	Sporulation
1		3.0	106.07	Poor
2		4.0	115.00	Poor
3		4.5	138.06	Poor
4		5.0	163.43	Fair
5		5.5	194.00	Good
6		6.5	220.27	Excellence
7		7.0	236.20	Excellence
8		7.5	178.00	Good
9		8.0	116.07	Poor
G.M.	:	163.01		
SEm±	:	1.97		
CD at 5% :		5.85		

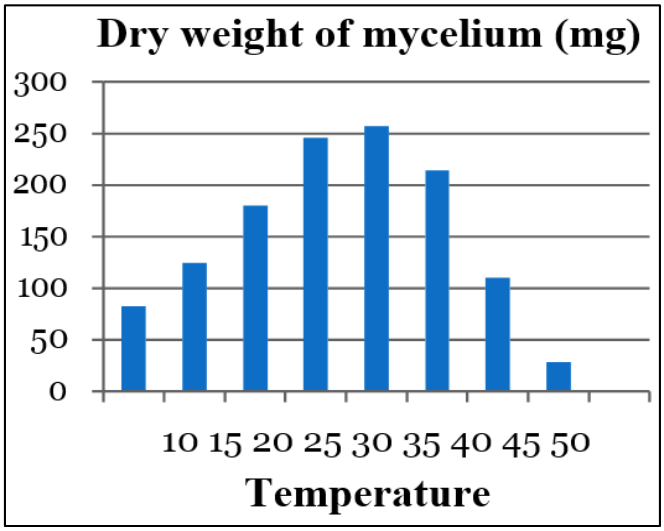

Fig 1: Effect of different temperatures on the growth of *F. oxysporum* f. sp. *lini*

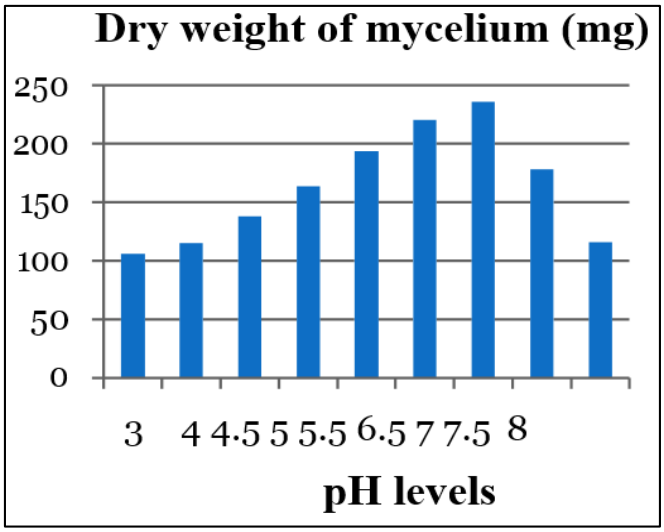

Fig 2: Effect of different pH levels on the growth of *F. oxysporum* f. sp. *Lini*

Conclusion

Wilt disease of linseed caused by *Fusarium oxysporum* f.sp. *lini* was observed on seedlings after third week of sowing crop. Eleven varieties of linseed were evaluated under sick field condition against this disease. The pathogen was isolated and maintained. Effect of different temperature and pH level on growth and sporulation of *Fusarium oxysporium* f.sp. *lini* was evaluated after 10 days of incubation. From this investigation, it was concluded that maximum growth and sporulation were observed at 30 °C temperature followed by 25 °C and 35 °C, where no growth was recorded at 50 °C. The maximum growth and sporulation were observed at 7.0 pH closely followed by 6.5 pH, whereas it was minimum at pH 3.0.

References

1. Broadfoot WC. Studies on the parasitism of *Fusarium lini* (Bolley). Phytopathology. 1926; 16:951-978.
2. Goel LB, Swarup G. Some observations on linseed. Indian Phytopathology. 1964; 17(2):133.
3. Houston BR, Knowles PF. Studies on Fusarium wilt of flax. Phytopathology. 1953; 43(9):491-495.
4. Houston BR, Knowles PF. The determination of pathogenic races of *Fusarium* f.sp. *lini* (Abstr.). Phytopathology. 1958; 48(8):394.
5. Jones LR, Tisdale WB. The influence of soil temperature upon the development of wilt. Phytopathology. 1992; 82:409-13.
6. Mc Rae W. Report of the Imperial Mycologist. Sci Rep. Agric. Res. Inst. Pusa. 1926, 54.
7. Sattar A, Hafiz A. Researches on plant diseases of Punjab. Sci. Mongr. Pak. Assoc. Adv. Sci. 1952; 1(3):158.
8. Nair PN. Factors affecting resistance of flax to *Fusarium lini* Bolley. Dis Abstr. 1957; 17(5):942.
9. Souramma V, Singh J. Effect of temperature and pH on growth and sporulation of wilt causing pathogen, *Fusarium oxysporum* f. sp. *lini* (Bolley) Snyder and

Hansen in linseed (*Linum usitatissimum* L.). Journal of Oilseeds Research. 2004; 21(1):206-207.
10. Tisdale WH. Flax wilt- A study on the nature and inheritance of wilt resistance. Journal of Agricultural Research. 1917; 11:573-605.