International Journal of Research in Engineering, Science and Management
Volume-3, Issue-1, January-2020
www.ijresm.com | ISSN (Online): 2581-5792

Effect of Temperature and pH on Growth of *Alternaria Lini* Causes Blight Disease in Linseed

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***Abstract*: The study was carried out to evaluate the in vitro application of temperature and pH in the control of *Alternaria lini (A. lini)*, contributing agent of blight disease in Linseed. *A. lini* was inoculated in Potato Dextrose Agar (PDA) media at variable temperature (10°C-35°C) and pH (3.5-9.0). Highest mycelial growth was obtained at 25°C temperature and pH 6.5. Whereas lowest mycelial growth was achieved at 35°C and pH 3.5, results revealed that certain acidic medium and a certain temperature can be applicable to inhibit the mycelial growth and sporulation of *A. lini* to save Linseed crop from infection.**

***Keywords*: *Alternaria lini*, Blight disease, Growth, pH, Sporulation, Temperature.**

1. Introduction

Linseed or flaxseed (*Linum usitatissimum* L., 2n=30), is an annual herb, belongs to family Linaceace and genus *Linum* [1]. It is a *Rabi* crop and an annual dicotylednous plant [2]. It is known as beginning crop which is being evaluated as a crop platform for the economic importance as manufacturing of bio-industrial and nutritious food products. It is also the sixth largest oilseed crop in the world [3]. Linseed is one of the richest source of α-linolenic acid (ω-3 fatty acid) and soluble mucilage [4]. Linseed oil contains ω-3 (57%), ω-6 (16%), monosaturated fatty acid (18%) and saturated fatty acid (9%) [5] Linseed is grown as either oil crop or a fiber crop. Canada is the world's largest producer of flax (38% of total production) [2].

Although Linseed has important contribution in Indian economy because of its wide industrial applications yet the average production of Linseed is relatively low as compare to other countries. In India, linseed is grown mostly under rainfed (63%), utera (25%), irrigated (17%) and in input starved environment. Major Linseed producing states i.e. Madhya Pradesh, Chhattisgarh, Maharashtra, Jharkhand, Uttar Pradesh and Odisha [6]. It acts as a host to various diseases and usually raised as a mixed crop with other crops in rainfed marginal lands which is the main cause of its lower production in India. Alternaria blight is one of the most dangerous diseases of Linseed crop. It causes by a specific pathogen named *Alternaria lini*, it affects all the aerial parts of plant and destroys chlorophyll of much larger area of leaf lamina, due to its necrotrophic behaviour. This infection causes a drastic fall in available photosynthetic area of plant, which ultimately affecting the significant yield of crop [7]. Thus Alternaria blight disease decreases the up to 28-60% of total productivity of Linseed [8]. This disease was firstly reported by Dey in 1933 [9]. Later Siddiqui (1963), reported the occurrence of Alternaria blight on linseed cultures at IARI, New Delhi and other parts of the country [10]. Sangwan (2005), studied the fungal disease of linseed in India *viz.*, rust, powdery meldew, Alternaria blight, wilt and seed rot [11].

This is the most common Linseed crop disease but very little information and literature is available for understanding the behaviour and control of this disease. The chemical fungicides can be used to control this pathogen, but this is the crop usually grown by marginal farmers who are generally unable to use costly chemical fungicides to save their crops from *A. lini*, moreover their use has some hazardous environmental impacts. In this view, researchers are focusing to find out some cost effective as well as ecofriendly approaches to control this disease causing pathogen (*A. lini*) for better and improved productivity of Linseed. Previous attempts as use of biocontrol agents [12], slight change in sowing dates [13], ethanomedicinal control approaches [1] were made to inhibit this pathogen. Manipulation of planting conditions such as soil pH, environmental temperature etc. could be the useful tactic for disease control. *A. lini* pathogens are able to infect only under certain favorable environmental conditions. So a slight change in planting conditions may lead to inhibit their development and growth on Linseed. A very little literature reported on the pH and temperature management for blight free linseed farming. Hence an investigation was undertaken to evaluate different pH and environmental temperature of sowing of Linseed to observe most suitable condition to control this disease.

2. Material and methods

A. Collection of fungi

Leaves and buds of linseed showing the characteristics symptoms were collected from agriculture fields of Jhansi. These collected plant parts were sterilized thoroughly with alcohol and were brought to laboratory for isolation of disease causal pathogen (*A. lini*).

B. Isolation of the pathogen

Infected leaves and buds of linseed were firstly washed with triple distilled water. Cross section of lesion containing infected tissues was cut of up to 5 to 10 mm square. Surface sterilization procedure was followed by sterilization of the cut portions with 0.1% mercuric chloride ($MgCl_2$) solution for 30 seconds. Treated infected tissues were further washed with sterile water and then air dried. Finally, the infected tissues were transferred into petri plates containing PDA media and placed in biological oxygen demand (BOD) incubator for inoculation. After 2-3 days, whitish mycelia growth was appeared around the infected tissue. The hyphal tips of mycelium were transferred in PDA culture plates. After microscopic examination of obtained culture, presence of pathogen (*A. lini*), responsible for blight disease development (in collected plant parts) was observed.

C. Effect of temperature on inoculation of A. lini

Conical flasks of 150 ml volume were filled with 50 ml of PDA culture media and sterilized in autoclave at 15 Ib/in^2 pressure for 20 minutes. The sterilized culture media was poured in six petri plates. These petri plates were further inoculated with equal amount of mycelial disk. Inoculated petri plates were kept at six different temperatures ranges viz, 10°C, 15°C, 20°C, 25°C, 30°C and 35°C for 7 days. Three replications were used for each treatment.

D. Effect of pH on inoculation of A. lini

For this study seven conical flasks of 150 ml containing 50 ml PDA culture of different pH (pH adjusted) such as 3.5, 4.5, 5.5, 6.5, 7.5, 8.5 and 9.0 have been taken. The pH was adjusted by using 0.1 N hydrocloric acid and sodium hydroxide solutions. Resulting culture media were sterilized in autoclave at 15 Ib/in^2 pressure for 20 minutes. All sterilized culture media were poured in seven different petri plates (sterilized) which were further inoculated with equal amount of mycelial disk and maintained at 25±2°C temperature in BOD incubator for 7 days. Three replicates of the same experiments were performed.

3. Statistical analysis

Statistical analysis was performed using the standard procedure described by Gomez and Gomez (1986) [14].

4. Results

Identification of isolated pathogen was carried out on the basis of its morphological characteristics. This study revealed that the morphological characteristics of isolated pathogen were same as reported by Dey (1933) [9] for *Alternaria lini*.

Table 1 and graph 1, depicts the results of variable temperature studies. Six different temperatures as 10°C, 15°C, 20°C, 25°C, 30°C and 35°C were taken for this study to find out most suitable temperature for the optimum growth and excellent sporulation of *A. lini* pathogen. Results indicated that the highest mycelial growth of *A. lini* was obtained at 25°C with maximum radial growth of 48 mm (Figure 1:a) followed by 20°C (24 mm), 30°C (23 mm), 15°C (22 mm) and 10°C (13 mm). Lowest mycelial growth was obtained at 35°C, a rapid decrement in mycelial (11 mm) was observed at this temperature. Thus, 25°C temperature was observed as most suitable temperature for the optimum growth and excellent sporulation of the *A. lini.*

Table 2 and graph 2, showed results of effect of pH studies. For this study seven different pH levels, viz. 3.5, 4.5, 5.5, 6.5, 7.5, 8.5 and 9.0 were taken to observe most suitable pH for the optimal growth and excellent sporulation of *A. lini*. Maximum growth (37 mm) and excellent sporulation was observed at pH 6.5 (Figure 1:b) followed by 7.5 (35 mm), 5.5 (21 mm), 8.5 (20 mm), 4.5(19 mm) and 9.0 (14 mm). Minimum radial growth of *A. lini* was observed at pH 3.5 (12 mm). Thus, pH level 6.5 was observed as most suitable pH for the optimum growth and excellent sporulation of the *A. lini.*

5. Discussion

The morphological characters of isolated and identified pathogen were closely resembled with the description given by Dey, (1933) [9]. Moreover it also showed similar cultural character which was described by Siddiqui, (1963) [10] and Simmons, (1967) [15] which suggested that the isolated and identified pathogen was *A. lini*. PDA used as a growth medium for isolate the pathogen (*A. lini*) form infected portion of samples. Among the environmental factors, temperature is the most important factor which directly influences the growth and sporulation of the pathogen (*A. lini*). In present study the optimum suitable temperature for the growth and sporulation of *A. lini* was 25°C on PDA, finding was reported by Roten, (1994) [16]. According to Hatzipapus et al. (2002) [17], the fungus *A. alternata* can grow over a wide range of temperature from 10°C to 35°C with an optimum growth at 25°C on PDA.

The mycelial growth of *A. lini* was also measured over a wide range of pH (from 3.5 to 9.0).

Results revealed that hydrogen ion concentration also have a considerable effect on growth of *A. lini*. Maximum growth and the best sporulation was observed at pH of 6.5, whereas minimum mycelial growth was obtained at pH 3.5. Results also suggested that the pH below six and above seven leads to the mycelial growth inhibition. These results showed much similarity with the results reported by Gawai el al., (2018) [18], that maximum growth and sporulation of *A. alternata* was favoured at pH 6.5. Gupta *et al.*, (2013) [19] also observed that the pH 6.5 was the best, and pH 4.5 was poor, followed by 3.5 and 9.0 for mycelial growth of *A. lini*. Hubbali et al (2010) [20]

reported that the best growth of *A. alternata* was at pH range of 6.0- 6.5 and temperature range of 25 - 30°C.

Pathogens showed genetic variability with changing climatic conditions. So, it is essential to understand the morphological and physical dynamics that helpful to control several devastating diseases. Therefore, it can be concluded from the present investigation results, that *A. lini* can grow best at 25-30°C temperature and pH 6.5 on media of PDA under continuous light condition.

Table 1
Effect of different temperature on the growth and sporulation of *A. lini.*

S. No.	Temperature (°C)	Radial growth of colony (mm)	Sporulation
1	10	13	Poor
2	15	22	Good
3	20	24	Good
4	25	48	Excellent
5	30	23	Good
6	35	11	Poor
Average		23.5	

Table 2
Effect of different pH on growth and sporulation of *A. lini*

S. No.	pH	Radial growth of colony (mm)	Sporulation
1	3.5	12	Poor
2	4.5	19	Good
3	5.5	21	Good
4	6.5	39	Excellent
5	7.5	35	Excellent
6	8.5	20	Good
7	9.0	14	Poor
Average		22.85	

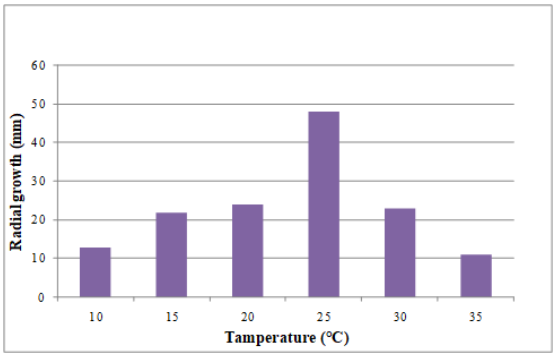

Fig. 1. Effect of temperature on growth of *A. lini*

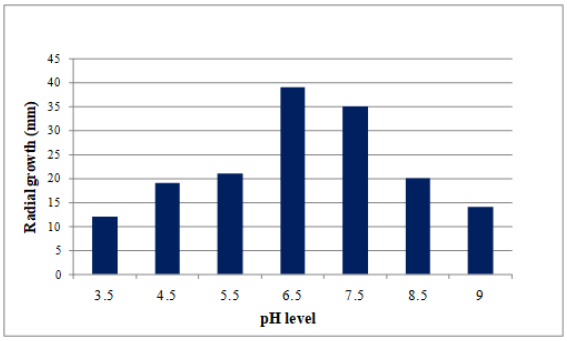

Fig. 2. Effect of pH on growth of *A. lini*

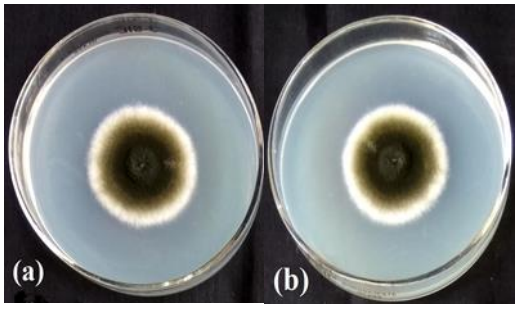

Fig. 3. Maximum growth and sporulation of *A. lini* in suitable a) temperature (25°C) and b) pH level (6.5)

6. Conclusion

This paper presented an overview on effect of temperature and ph on growth of *alternaria lini* causes blight disease in linseed.

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