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Influence of temperature, pH and wavelength of light on cultural characteristics and sporulation of *Alternaria lini*

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Abstract
The effects of environmental variables like temperature, pH and wavelength of light on the development, phenotypic expression, and sporulation of *Alternaria lini* were investigated. To determine the optimal temperature, the petridishes were incubated at 10 °C, 15 °C, 20 °C, 25 °C, 30 °C and 35 °C, Smooth texture with circular growth pattern, gray color of margin with thick mycelium appearance at 25 °C. Pigmentation ranges from black at 20 °C, 25 °C, 30 °C, 35 °C and brown at 10 °C and 15 °C. Sporulation was found excellent at 25 °C and 30 °C, good at 20 °C and 35 °C, moderate at 15 °C and poor at 10 °C. According to the results of the pH study, the optimal conditions for mycelial growth were found to be pH 6 (89.33 mm), followed by pH 7 (75.33). Mycelial growth was observed to be moderate at pH 8 (68.67 mm), then it was observed to be moderate at pH 9 (56.67 mm), and the mycelial growth was observed to be minimal at pH 4 (26.00 mm), then it was observed to be minimal at pH 5 (47.00 mm).Sporulation was found excellent at pH 6 and 7, good at pH 8, moderate at pH 5 and 9, poor at pH 4. Study on wavelength depicted that maximum mycelial growth observed in fluorescent white light (90.00 mm) followed by blue (82.67 mm) and yellow (73.67 mm). Sporulation was found excellent in yellow, blue and fluorescent white, good in red and violet, moderate in green. Pigmentation observed black in all the wavelength of light. Sporulation was found to be moderate in green, but excellent in yellow, blue, and fluorescent white. It was found to be excellent in red and violet, but only good in green. The investigation concluded that the optimal wavelength of light for the growth and sporulation of *Alternaria lini* was fluorescent white light, followed by blue light in second place.

Keywords: *Alternaria lini*, sporulation, pigmentation, mycelial growth

Introduction
The oilseed crop with the second-highest area and production in India is linseed, also known as flax (*Linum usitatissimum* L.), a member of the family Linaceae. It is grown on a total global area of 22.70 lakh ha, yielding 22.39 lakh metric tonnes at productivity of 986 kilogrammes per hectare. It is grown on an area of 3.38 million hectares in India, yielding 1.47 million metric tonnes at productivity of 435 kilogrammes per hectare (FAO, 2013) [2]. *Alternaria alternata*, *Alternaria linicola*, *Aspergillus flavus*, *Aspergillus niger*, *Colletotrichum linicola*, *Curvularia lunata*, *Fusarium moniliforme*, *rhizoctonia bataticola*, *Rhizoctonia solani*. The state of Chhattisgarh relies heavily on rainfed agriculture, with linseed being the most important oilseed crop grown there. A major biotic stress limiting crop yield in hot and humid environment is Alternaria blight, caused by *Alternaria lini* Dey and *A. linicola* Groves and Skolko (Singh *et al.*, 2006; Singh *et al.*, 2009) [13, 14]. The most significant disease threat to linseed farmers is most likely *Alternaria lini*. Seedlings can suffer significant damage from Alternaria blight, which can lead to a significant reduction in plant stand (Singh *et al*., 2017) [15]. Pathogens can cause yield loss and diminished oil quality in their hosts by causing a variety of symptoms, such as spots on the leaves and necrotic lesions on the capsules. Depending on the cultivar and the severity of the disease, Alternaria leaf blight can reduce annual yields by 18-60% Chauhan and Srivastava, (1975) [1], Singh *et al.* (2003) [11], Singh *et al.* (2004) [12]. To that end, the present investigation set out to measure how changes in environmental factors such as temperature, pH, and light wavelength affect the social behaviors of *Alternaria lini*.

Materials and Methods

Implications of temperature variation

This study was carried out to determine the optimal growing conditions for *Alternaria lini*. Each petridish received 20 ml of the medium. A mycelial disc measuring 5 mm in diameter, harvested from a 10-day-old culture of *Alternaria lini* using a sterilised cork borer, was set in the middle of the medium. Incubation of petri dishes were kept at temperatures of 10, 15, 20, 25, 30, and 35 degrees Celsius. For each treatment, we kept three separate replications. Mycelial growth was observed 7 days after inoculation, or when full growth was seen in any of the petridishes. Colony characteristics, including pigmentation, were noted on various treatments. After 10 days of inoculation, sporulation was detected.

Implications of pH variation

Using a digital pH metre and a combination of 0.1N HCL and 0.1N NaOH, we examined the impact of various pH levels on the growth characteristics of *Alternaria lini* before sterilisation of medium. About 20 millilitres of sterilised, pH-specific media were poured into each petri dish. *A* mycelial disc measuring 5 mm in diameter, harvested from a 10-day-old culture of *Alternaria lini* using a sterilised cork borer, was set in the middle of the medium. Three independent replicates of each treatment were kept in an incubator 27±2° C. Mycelial growth was observed after 7 days of inoculation, or when full growth was seen in any of the petridishes. Colony characteristics, including pigmentation, were noted on various treatments. After 10 days of inoculation, sporulation was detected.

Impact of various wavelengths of light

Lights of varying wavelengths were used to observe how they affected the mycelial growth and sporulation of *Alternaria lini* on potato dextrose agar. The colours tested included red, yellow, violet, green, blue, and fluorescent white. Each petridish received 20 ml of the medium. Petridishes were wrapped in various colours of gelatin paper and a 5 mm mycelial disc cut from a 10-day-old culture of *Alternaria lini* was placed in the middle of the medium. Three independent replicates of each treatment were kept in an incubator 27±2° C. Mycelial growth was observed 7 days after inoculation, or when full growth was seen in any of the petri dishes. Colony characteristics, including pigmentation, were noted across treatments. After 10 days of inoculation, sporulation was detected.

Results and Discussion

Influence of temperature on the development, morphology, and sporulation of *Alternaria lini*

The effects of varying temperatures on the mycelial growth, cultural characteristics, and sporulation of *A. lini* have been presented in table 1, along with the data pertaining to those effects. Result depicted that maximum mycelial growth observed at 25 °C (90.00 mm) followed by 30 °C (75.33 mm). Moderate mycelial growth observed at 35 °C (51.67 mm) followed by 20 °C (42.67 mm) and Minimum mycelial growth observed at 10 °C (29.33 mm) followed by 15 °C (37.00 mm). Whereas colony color gray observed at temperature range 25 °C, 30 °C and 35 °C, light gray observed at temperature range 10 °C, 15 °C and 20 °C. Colony character observed cottony texture, circular or irregular growth pattern with thick mycelium appearance at 10 °C, 20 °C and 35 °C. Fluffy texture, irregular growth pattern with thick mycelium appearance at 15 °C. Smooth texture, circular growth pattern with thick mycelium appearance at 25 °C. Velvety texture, irregular growth pattern with thin mycelium appearance. Pigmentation ranges from black at 20 °C, 25 °C, 30 °C, 35 °C and brown at 10 °C and 15 °C. Sporulation was found excellent at 25 °C and 30 °C, good at 20 °C and 35 °C, moderate at 15 °C and poor at 10 °C. The result revealed that 25 °C temperature favored by *A. lini* for suitable growth and sporulation followed by 30 °C. Similar results were reported by Pandey *et al.* (2002) [7], Gupta *et al.* (2013) [3], Sharma (2017) [10], and Shukla *et al.* (2020) [9] regarding the optimal growth and sporulation of *A. lini* at 25 degrees Celsius.

Influence of pH on the development, morphology, and sporulation of *Alternaria lini*

The effects of different pH on the mycelial growth, morphological characteristics, and sporulation of *A. lini* are presented in table 2. Result depicted that maximum mycelial growth observed at pH 6 (89.33 mm) followed by pH 7 (75.33). Moderate mycelial growth observed at pH 8 (68.67 mm) followed by pH 9 (56.67 mm) and minimum mycelial growth observed at pH 4 (26.00 mm) followed by pH 5 (47.00 mm). Whereas colony color observed dark gray at pH 5, pH 6 and pH 7, grayish brown at pH 8 and pH 9, light gray at pH 4. Colony characters observed velvety texture, circular growth pattern with thick or thin mycelium appearance at pH range 5, 6, 7 and 9. Velvety texture, irregular growth pattern with thin mycelium appearance at pH 8. Cottony texture, circular growth pattern with thick mycelium appearance at pH 4. Pigmentation observed black at pH range 5, 6 and 7, dark brown at pH range 8 and 9, olive brown at pH 4. Sporulation was found excellent at pH 6 and 7, good at pH 8, moderate at pH 5 and 9, poor at pH 4. Result revealed that pH 6 favored by *A. lini* for suitable growth and sporulation followed by pH 7. Similar kind of finding on present investigation by Pandey *et al.* (2002) [7], found pH between 6-6.5to be optimum for the growth of the pathogen. Ram *et al.* (2007) [8], Shukla *et al.* (2020) [9] reported pH level 6.5 was observed as most suitable pH for the optimum growth and excellent sporulation of the *A. lini.* Gupta *et al*. (2013) [3] was reported maximum growth and sporulation were observed at 6.5 pH followed by 7.5pH.

Influence of different wavelength of light on the development, morphology, and sporulation of *Alternaria lini*

The data pertaining to the effect of different wavelength of light on mycelial growth, cultural characteristics and sporulation of *A. lini* have been presented in table 3. Result depicted that maximum mycelial growth observed in fluorescent white light (90.00 mm) followed by blue (82.67 mm) and yellow (73.67 mm). Moderate mycelial growth observed in violet (68.67 mm) followed by red (66.00 mm) and minimum mycelial growth observed in green (56.33 mm). Colony color observed gray in all the wavelength of light. Colony characters observed smooth texture, circular growth pattern with thick mycelium appearance in red and violet light. Smooth texture, irregular growth pattern with thick mycelium appearance in yellow and green light. Velvety texture, circular growth pattern with thick mycelium in blue and fluorescent white. Pigmentation observed black in all the wavelength of light. Sporulation was found excellent in

yellow, blue and fluorescent white, good in red and violet, moderate in green. The result revealed that the suitable wavelength of light for growth and sporulation of *Alternaria lini* was found fluorescent white followed by blue light. Kumar *et al.* (1997) [6] reported that maximum mycelial growth in violet light followed by blue. Igbalajobi *et al.* (2019) [5] were observed that different colours of light shows difference in sporulation of *Alternaria alternata*.

Table 1: Influence of temperature on the development, morphology, and sporulation of *Alternaria lini*

Temperature	Radial growth (mm)*	Colony colour	Growth pattern	Colony texture	Thickness of mycelium	Pigmentation	Sporulation **
10 °C	29.33	Light Gray	Irregular	Cottony	Thick	Brown	+
15 °C	37.00	Light Gray	Irregular	Fluffy	Thick	Brown	++
20 °C	42.67	Light Gray	Circular	Cottony	Thick	Black	+++
25 °C	90.00	Gray	Circular	Smooth	Thick	Black	++++
30 °C	75.33	Gray	Irregular	Velvety	Thin	Black	++++
35 °C	51.67	Gray	Irregular	Cottony	Thick	Black	+++
CD at 1%	2.47						
SE(m)±	0.79						
CV	2.53						

* Mean of three replications **Sporulation (+ = poor, ++ = moderate, +++ = good, ++++ = excellent)

Table 2: Influence of pH on the development, morphology, and sporulation of *Alternaria lini*

pH	Radial growth (mm)*	Colony colour	Growth pattern	Colony texture	Thickness of mycelium	Pigmentation	Sporulation **
4	26.00	Light gray	Circular	Cottony	Thick	Olive brown	+
5	47.00	Dark gray	Circular	Velvety	Thick	black	++
6	89.33	Dark gray	Circular	Velvety	Thick	black	++++
7	75.33	Dark gray	Circular	Velvety	Thick	black	++++
8	68.67	Grayish brown	Irregular	Velvety	Thin	Dark brown	+++
9	56.67	Grayish brown	Circular	Velvety	Thin	Dark brown	++
CD at 1%	1.70						
SE(m)±	0.54						
CV	1.56						

*Mean of three replications**Sporulation (+ = poor, ++ = moderate, +++ = good, ++++ = excellent)

Table 3: Influence of different wavelength of light on the development, morphology, and sporulation of *Alternaria lini*

Wavelength of light	Radial growth (mm)*	Colony colour	Growth pattern	Colony texture	Thickness of mycelium	Pigmentation	Sporulation **
Red (620-750)	66.00	Gray	Circular	Smooth	Thick	Black	+++
Yellow (570-590)	73.67	Gray	Irregular	Smooth	Thick	Black	++++
Violet (380-450)	68.67	Gray	Circular	Smooth	Thick	Black	+++
Green (495-570)	56.33	Gray	Irregular	Smooth	Thick	Black	++
Blue (450-495)	82.67	Gray	Circular	Velvety	Thick	Black	++++
Fluorescent white (400-780)	90.00	Gray	Circular	Velvety	Thick	Black	++++
CD at 1%	2.24						
SE(m)±	0.72						
CV	1.71						

*Mean of three replications **Sporulation (+ = poor, ++ = moderate, +++ = good, ++++ = excellent)

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