

Effects of pH and media composition and temperature on growth and sporulation of *Alternaria lini*

Rakesh Babu Sharma

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Abstract

An experiment on effect of various substrate composition, atmospheric temperature and substrate pH on growth, development and sporulation of *Alternaria lini*. The pathogen was collected from linseed growing areas of Hamirpur district of Uttar Pradesh during rabi 2013-14. Four growth media of various compositions were prepared as suggested by various workers. For the study of the effect of different pH levels, Potato dextrose agar medium was used and adjusted at different pH levels viz. 3.5, 4.5, 5.5, 6.5, 7.5, 8.5 and 9.0 with the help of Phillips pH meter by using N/10 hydrochloric acid and sodium hydroxide solution for the maintenance of pH. Inoculated Petri plates were kept in incubators set at the temperatures (10, 15, 20, 25, 30 & 35⁰ C) for 7-10 days. Three replications were used for each treatment. The cultures were examined after 7 - 10 days of incubation for minimal growth, normal growth and maximal growth. The observation were recorded and tabulated as per standard procedures. The results showed that the highest radial growth of 33mm was recorded from potato dextrose agar (PDA) media. The further superiority sequence of the media was as Oat Meal (Richard's agar), Corn Meal agar (Czapeck (Dox) agar, Malt Extract agar respectively with the radial growth of pathogen colony in the order 30 mm>27 mm>24 mm>21 mm>18 mm respectively. The results also revealed that Malt extract agar was poorest among the six culture media undertaken for present study. The maximum growth of the pathogen was observed at 25⁰ C with maximum radial growth of 36 mm followed by 30⁰ C with 26 mm radial growth. The growth of the pathogen was significantly decreased to 10- 22mm at 20⁰ C, 15⁰ C and 10⁰ C. At higher than optimum temperature 35⁰ C, growth decreased rapidly and was just 11 mm in diameter. The pathogen grow over a wide range of pH ranging from 3.5 to 9.0 but the maximum growth in the tune of 35mm with excellent sporulation was recorded at pH 6.5 followed by 7.5 with radial growth of 31.5 mm. The growth was progressively decreased at 5.5 (21mm), 8.5(20.5mm), 4.5 (14mm) respectively. The media with maintaining pH 9.0 and 3.5 showed the minimum radial growth of the pathogen 8mm and 8.5mm respectively indicating the adverse effect of pH on radial growth of colony and behavior of sporulation.

Key words: *Alternaria lini*, growth medium, temperature, pH, linseed blight, sporulation.

1. **Rakesh Babu Sharma**, Research Scholar, M.G. C.G. Univ. Chitrakoot, Satna (M.P.) India
E-mail: rakeshsharma1780@gmail.com.
Cell: +918423972365

Introduction

Among the oilseed crops raised during *rabi*, linseed is next in importance to rapeseed-mustard in area as well as in production. In technical oil production, it ranks first in the country.

Each and every part of the linseed plant is utilized commercially, either directly or after processing. Seed contains 33 to 47% of oil. On a very small scale, the seed is directly used for edible purposes. About 20% of the total oil produced is used at farmer's level, and the rest 80% oil goes to industries in various forms, such as boiled oil, borated oil, eposidized oil, aluminated oil, urethane oil, isomerized oil etc. The oil is rich (> 66%) in linolenic acid, and is a perfect drying oil. Hence it is utilized in the manufacture of paints, oil cloth, varnish, pad-ink, printed ink, linoleum etc.

The oil cake is a good feed for milch cattle and poultries and hence priced 50% higher than rapeseed-mustard cake. It is good in taste and contains 36% protein, 85% of which is digestible. It is also used as organic manure. It contains about 5% N, 1.4% P_2O_5 and 1.8% K_2O .

Linseed is globally cultivated for its fibres and is called flax. Fibres are used for the manufacture of linen. The stem yields fibre of good quality having high strength and durability. The weather resistant fibre is lustrous and blends very well with wool, silk, cotton etc. Strong twines, canvas, suitings, shirtings and various indispensable products for defence purposes are manufactured from it. Woody matter and short fibres may be used as raw pulp for making paper of quality comparable with that of currency notes. The rough and strong linseed fibre can effectively be used for low-cost roofing tiles based on convertible polymers and for fibre-reinforced plastic (FRP). 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 *Alternaria* blight caused by *Alternaria lini* and *A. linicola* is the most important disease in eastern India and known to inflict 40–60 % of theoretical yield losses in linseed. The crop has poor resistance base against this disease in national gene pool. Extensive studies on occurrence, distribution, seasonal status, symptomology, 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 stage of growth including leaf, twigs. Fruits, root and seed inflicting losses in quality and quantity. The common diseases of linseed are blight, cankers, damping off, leaf spots, root rot, rust, wilt and powdery mildew etc. of these disease *Alternaria* blight. present survey conducted during Rabi season of 2013-14 in different linseed growing areas of Bundelkhand especially Rath area of Hamirpur districts which showed wide occurrence of leaf spot and black bud disease of linseed caused by *Alternaria lini*.

Keeping in view of the above facts the present study has been carried out with following objectives:

1. To study the effect of physical parameters like, temperature, pH on growth and sporulation of the pathogen.
2. Growth competition the pathogen in different culture media and pathogenicity test.

Material and Methods:

Preparation of Media:

The media were prepared by mixing the ingredients as per amount given against each media. Conical flasks of 150 ml. Capacity was filled up with 50 ml of prepared medium. Three replications of each medium were taken. Flasks containing medium in the specific amount were taken and sterilized at 15 lb/inch² pressure for 20 minutes in an autoclave.

After sterilization 10 ml of each medium was aseptically poured in sterilized Petri plates, inoculated with pure spore suspension of *Alternaria lini* in laminar air flow and were incubated at $25 \pm 2^{\circ}$ C for 7 - 10 days. The radial growth and sporulation were recorded and tabulated.

For the study of the effect of different pH levels on the growth and sporulation of the pathogen, Potato dextrose agar medium was used and adjusted at different pH levels viz. 3.5, 4.5, 5.5, 6.5, 7.5, 8.5 and 9.0 with the help of Phillips pH meter by using N/10 hydrochloric acid and sodium hydroxide solution for the maintenance of pH.

Flasks of 150 ml volume filled with 50 ml. culture media of different pH which were sterilized at 15 lb/in² pressure for 20 minutes in an autoclave.

Seven sterilized Petri plates were filled with each range of (pH) of culture media and maintained at $25 \pm 2^{\circ}$ C temperature in incubator for 7 - 10 days. Three replications were used for each treatment. The data were recorded and analyzed as per standard procedures. After preparation of various media the spores of *Alternaria lini* were inoculated and kept for growth and development of the pathogen in each medium separately.

Effect of temperature on the growth and sporulation of *Alternaria lini* on PDA:

Effect of temperature on the growth of the pathogen was studied at seven different temperatures ranges viz. 10⁰ C, 15⁰ C, 20⁰ C, 25⁰ C, 30⁰ C and 35⁰ C in BOD incubator.

The experiment was performed as; flasks of 150 ml volume were filled with 50 ml of culture media and sterilized in autoclave at 15 lb/in² pressure for 20 minutes. The sterilized culture media was poured in seven Petri plates and labeled properly. These Petri plates were inoculated with equal amount of mycelial disc from actively growing culture of the fungus (*Alternaria lini*) cut with the help of sterilized cork borer in a laminar airflow.

Inoculated Petri plates were kept in incubators set at the temperatures (10, 15, 20, 25, 30 & 35⁰ C) for 7-10 days. Three replications were used for each treatment. The cultures were examined after 7 - 10 days of incubation for minimal growth, normal growth and maximal growth. The observation were recorded and tabulated as per standard procedures.

Results and discussions

Table1: Effect of media pH on growth and sporulation of *Alternaria lini*.

S. No.	pH level	Radial growth of colony in mm	Sporulation
1	3.5	8	Poor
2	4.5	14	Good
3	5.5	21	Good
4	6.5	35	Excellent
5	7.5	31.5	Excellent
6	8.5	20.5	Good
7	9.0	8.5	Poor
		Av. = 19.78	

Effect of pH level of culture media on growth and sporulation of *Alternaria lini*:

The pH of the medium affects the rate of growth and sporulation of the pathogen is well established. In present study, seven pH levels, viz. 3.5, 4.5, 5.5, 6.5, 7.5, 8.5 and 9.0 were maintained in growth medium to study the effect of pH on radial development and growth behavior of colonies to find out the most suitable and most adverse pH for survivability of pathogen studied (Table 1). It is clear from the table that the pathogen grow over a wide range of pH ranging from 3.5 to 9.0 but the maximum growth in the tune of 35mm with excellent sporulation was recorded at pH 6.5 followed by 7.5 with radial growth of 31.5 mm. The growth was progressively decreased at 5.5 (21mm), 8.5(20.5mm), 4.5 (14mm) respectively. The media with maintaining pH 9.0 and 3.5 showed the minimum radial growth of the pathogen 8mm and 8.5mm respectively indicating the adverse effect of pH on radial growth of colony and behavior of sporulation. Thus it can be concluded that the range of pH from 6.5 to 7.5 was most suitable for the maximum growth and sporulation of the pathogen while lowering down the pH showed adverse effect for the same (Fig 1).

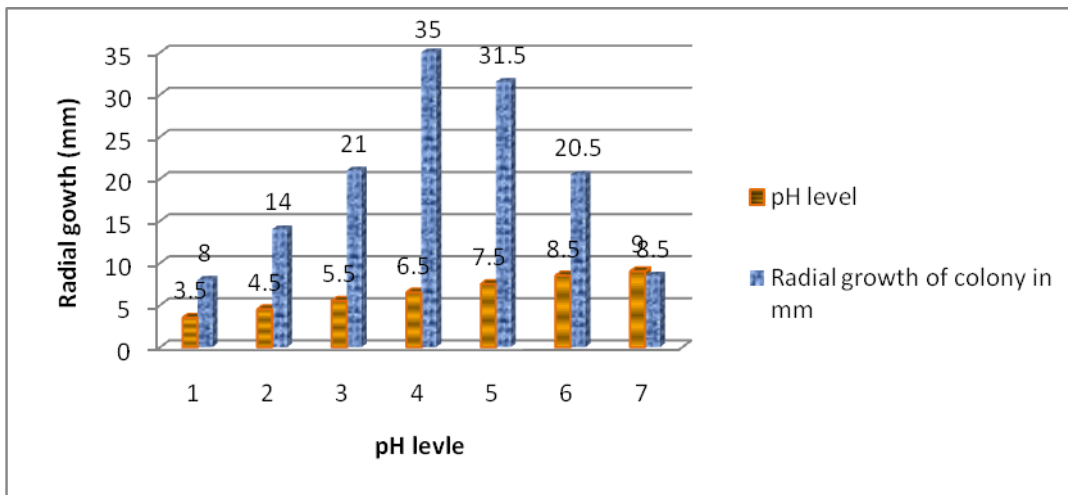


Plate 1: Effect of different pH on growth and sporulation of *Alternaria lini*.

Effect of growth media on Growth competition of *Alternaria lini*:

The effect of various growth media on development of average diameter of fungal colonies was studied and the results are presented in table-2 and the extent of sporulation in the various media was recorded. The results indicated that the highest radial growth of 33mm was recorded from potato dextrose agar (PDA) media. The further superiority sequence of the media was as Oat Meal (Richard's agar), Corn Meal agar (Czapeck (Dox) agar, Malt Extract agar respectively with the radial growth of pathogen colony in the order 30 mm>27 mm>24 mm>21 mm>18 mm respectively. The results also revealed that Malt extract agar was poorest among the six culture media undertaken for present study.

Table 2: Effect of different culture media on growth and sporulation of *Alternaria lini*.

S.No.	Name of the culture media	Radial growth of colony in mm	Sporulation	Colour of mycelium	Colony shape
1	Potato dextrose Agar	33	Excellent	White	Circular
2	Oat meal agar	30	Good	Light olive	Circular
3	Richard's agar	27	Good	Olive greenish	Irregular
4	Corn meal agar	24	Good	Light olive	Circular
5	Czapeck (Dox) agar	21	Poor	Pale olive gray	Irregular
6	Malt extract agar	18	Poor	Grayish olive	Circular
		AV. = 25.5			

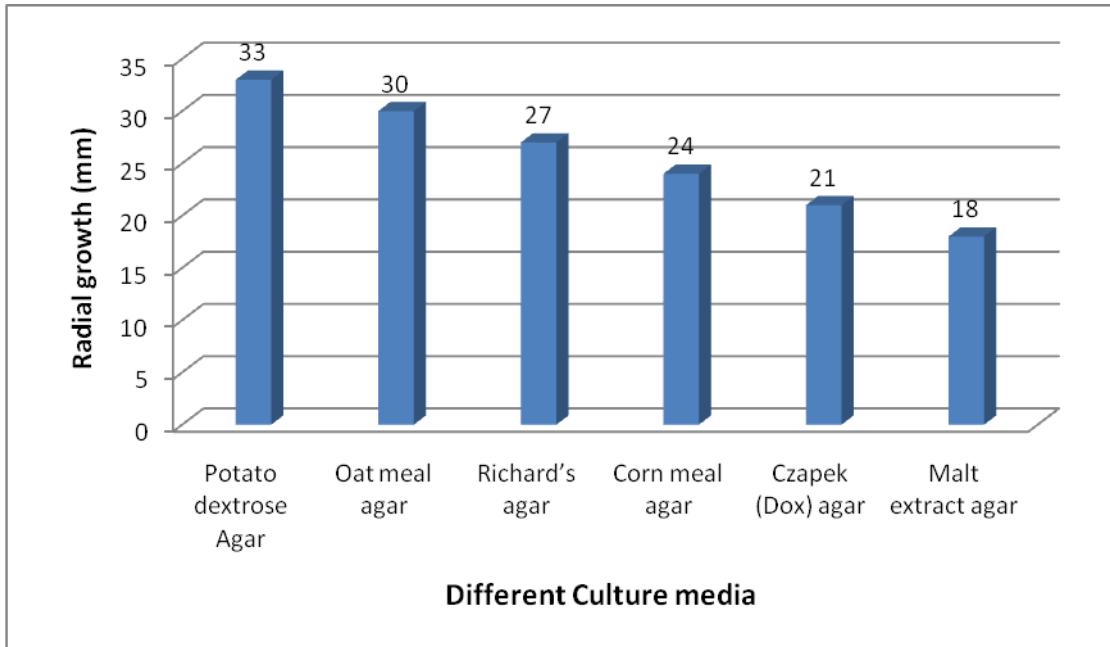


Fig. no. 2: Effect of different culture media on growth and sporulation of *Alternaria lini*.

Growth and sporulation of *Alternaria lini* on selected media:

Pure culture of *Alternaria lini* on maintained PDA was used for studying the growth and sporulation on different culture media. The selected media and their composition are as follows:

Composition of Potato Dextrose Agar Media:

Peeled potato	:	200 gm
Dextrose	:	20 gm
Agar-agar	:	20 g
Distilled water	:	1000 ml

Composition of Richard's solution:

Potassium nitrate	:	10.0 gm
Magnesium sulphate	:	2.5 mg
Ferric chloride	:	0.02 g
Sucrose	:	2.0 gm
Agar-agar	:	50.0 gm
Distilled water	:	500.0 ml

Composition of Oat meal media:

Oat meal	:	30.0 gm
Agar-agar	:	20.0 gm
Distilled Water	:	1000 ml

Composition of Corn meal agar media:

Maize	:	30.0 gm
Agar-agar	:	20.0 gm
Glucose	:	30.0 gm
Distilled Water	:	1000 ml

Composition of Czapek's Dox media:

Sodium nitrate	:	2.0 gm
Potassium phosphate	:	0.5 gm
Potassium chloride	:	0.05 gm
Magnesium sulphate	:	0.01 gm
Ferrous sulphate Sucrose	:	30.0 gm
Agar-agar	:	15.0 ml
Distilled water	:	1000 ml

Composition of Malt extracts agar Media:

Malt extract	:	15 gm
Di-potassium hydrogen phosphate	:	1.0 gm
Ammonium chloride	:	1.0 gm
Citric acid N/1	:	15 gm
Agar-agar	:	20 gm
Distilled water	:	1000ml

Effect of temperature on growth and sporulation of *Alternaria lini*:

Among the environmental factors, temperature is the most important factors which influence the growth and sporulation of the pathogen. In our findings the optimum suitable temperature for the growth and sporulation of *Alternaria lini* was 25⁰ C on PDA the same

finding was given by Roten, (1994). According to Hatzipapus *et al.*, (2002), the fungus (*Alternaria.lini*) grew at a wide temperature range of 15⁰ C – 35⁰ C with on optimum at 25⁰ C.

The temperature is the most important factors which affects the metabolic activity of pathogen. In the present study, six different temperatures *viz.*, 10⁰ C, 15⁰ C, 20⁰ C, 25⁰ C, 30⁰ C and 35⁰ C were taken for study of their influence on the growth and sporulation of the pathogen and to explore the most favorable temperature for maximum mycelia growth and sporulation of the *Alternaria lini*. Result (Fig. no.3) indicated that the maximum growth of the pathogen was observed at 25⁰ C with maximum radial growth of 36 mm followed by 30⁰ C with 26 mm radial growth. The growth of the pathogen was significantly decreased to 10- 22mm at 20⁰ C, 15⁰ C and 10⁰ C. At higher than optimum temperature 35⁰ C, growth decreased rapidly and was just 11 mm in diameter (Fig no.3).

Our result indicates that 25⁰ C ± 2⁰ C was the optimum temperature for the maximum growth and sporulation of the pathogen followed by 30⁰ C and 20⁰ C respectively (Table no. 3 Fig. no.3).

The influence of pH concentration on the growth and sporulation, of the pathogen was studied. It was found that the pathogen could grow at a wide range of pH 3.5 to 9.0. in the present study, maximum growth and sporulation was observed at pH of 6.5. This finding was supported with the observation of Ram *et al.*, (2007) who reported that maximum growth and sporulation was favored by 6.5 pH media of PDA.

In present investigation out the best media for the growth and sporulation of *Alternaria lini* was explored. Potato dextrose agar media was found to be superior most followed by Oat meal and Richard's media. Pandey *et al.*, (2006), and Ashour and Elkhadi, (1959) also found similar results Ram *et al.*, (2007) stated P.D.A. is the best media for growth and sporulation of *Alternaria*.

Table 3 : Effects of Temperature on growth and sporulation of *Alternaria lini*.

S. No.	Temperature (°C)	Radial growth of colony in mm	Sporulation
1	10	10	Poor
2	15	10.5	Poor
3	20	22	Good
4	25	36	Excellent
5	30	26	Good
6	35	11.00	Poor
		<i>Av. = 19.25</i>	

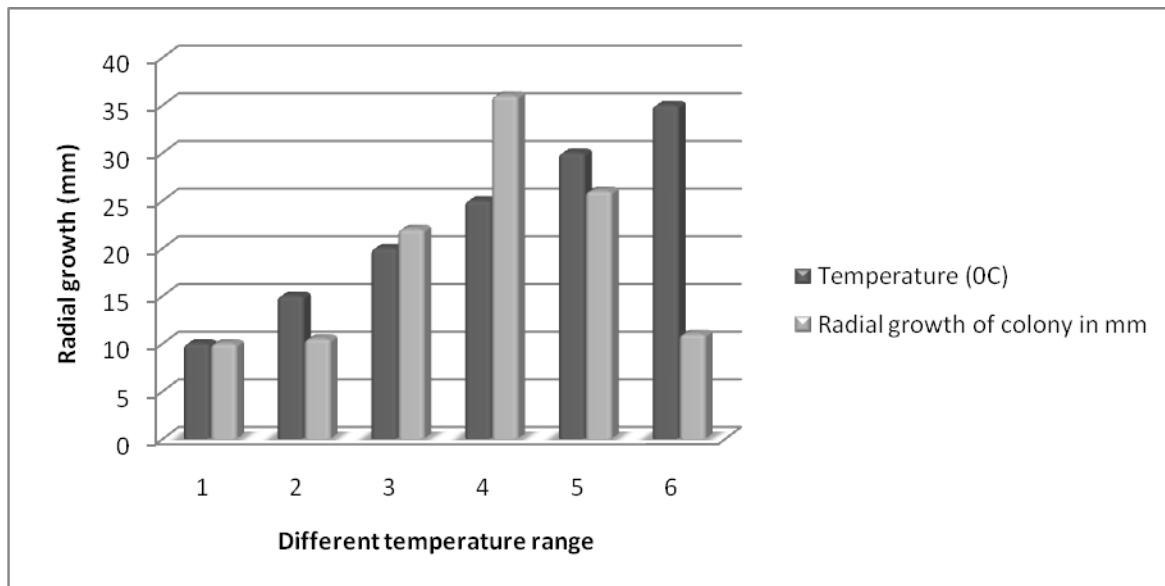


Plate3: Effect of different temperature on growth and sporulation of *Alternaria lini*.

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