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TO STUDY THE ROLL OF pH ON GROWTH AND SPORULATION OF *ALTERNARIA LINI* IN THE CROP LINSEED, BUNDELKHAND REGION, INDIA

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Abstract

In this study, seven categories of pH as 3.5, 4.5, 5.5, 6.5, 7.5, 8.5, and 9.0 was taking under consideration and observations were recorded for each category of pH respectively, on growth and sporulation of *Alternaria Lini* in the crop linseed, Bundelkhand Region, India. The most favorable condition of pH for the radial growth and sporulation of the *Alternaria lini* on PDA were found 6.5 out of seven categories of pH. Highest mycelial radial growth was obtained 35 mm with pH 6.5 followed by 31.5 mm with pH 7.5, which is statistical significant (CD=4.67)at 5% level of significance. Whereas, lowest mycelial growth was found 8 mm with pH 3.5 followed by 8.5 mm with pH 9.0 Which is found at par at 5% level of significance. The results revealed that the mycelial radial growth and sporulation of *Alternaria lini* is increased from 8 to 35 mm with respect to increase of pH from 3.5 to 6.5. Further, the mycelial radial growth and sporulation of *Alternaria lini* was decreased from 31.5 mm to 8.5 with pH 7.5 to 9.0, respectively.

Keyword: *Alternaria Lini*, Effect pH, Linseed, Mycelial Radial Growth and Sporulation, *RBD*

Introduction

Study was carried out to fulfill the object at research farm of the BNPG College Rath, Hamirpur and Samples are collected randomly from the field of farmers of the Bundelkhand Region during *rabi* seasons in the years 2013-14and 2014-15. Linseed is an annual herb, belong to family Linaceace and genus linum(Sharma R. B., 2015). The plant is an erect annual herb which attains a height of 1- 1.2 metres, leaves are small, alternate, simple, linear or lanceolate, with smooth surface, margins entire (Sharma and Singh, 2022). It is one of the important oil seed and fibre yielding crops of India (Sharma, 2015). India is the third largest producer in the world. However, the average national productivity (403 kg/ha) is for below the world average (851 kg / ha) (Radhamanij *et al*., 2006). Oil flax seed contains about 5.1-11.7 per cent carbohydrates, cotyledons contain on an average 25-45% fat, and up to 30% of protein (Stramkala *et al*., 2003). Most *Alternaria* species are saprophytes that are commonly found in soil or on decaying plant tissues. Some species are pathogenic that collectively cause a range of disease with economic impact on large variety of important agronomic host plant (Thomma, 2003).

Fruit rot of beer caused by Alternaria *alternata* (Fr) Kessler maximum growth and sporulation were favored by 6.5 pH. He noticed that the best suitable temperature for the growth and sporulation was 25 ± 2^0 C to 30 ± 2^0 C. (Ram *et al*., (2007).The scope of regular use of fungicides is limited due to the cost and adverse environmental hazards besides development of resistance in pathogens.

Geographically Rath, Hamirpur district lies in the Sub-tropical zone at a latitude and longitudinal range of 79.7^0 East and 25.5^0 North. It is located to an elevation of 526 feet's from the sea levels. The annual rainfall ranges between 900-1000 mm received mostly from last week of June to Last September with occasional showers in winter. The study area covered by boundaries in east

Mahoba District near 65km from head quarter west Jalaun (Orai) near 55 km, nourth hamirpur 85km and in south Harpalpur 45km from Study side (AICRP, Mauranipur, 2014).

Mainly, four types of soils are found in the district namely Mar, Kabar, Padua and Ranker. Mar is loamy black soil, retentive price in comparison to other soils, Kabar like Mar (loamy), but fighter in colors and fertility. It is sometimes mixed with Padua is a lighter type of soil lacking in organic matter. It is yellow in color and smooth in texture. Ranker is reddish-colored very light consisting of gravel and least fertile of the lot (Sharma R. B., 2016).

To see the effect of pH on growth and sporulation of *Alternaria Lini* in the crop linseed, Bundelkhand Region, India, This study is carried out.

Methods and Materials

Total number of 250 samples was collected follow standard methods. These samples were analysed and recorded readings with different levels of pH, in the laboratory of BNPG College Rath, Hamirpur, and Uttar Pradesh, India. Especially, leaves and buds were collected, and samples were procured in polythene bags. The samples were sterilized thoroughly with the help of cotton dipped in alcohol and brought to laboratory for examination and isolation of the pathogen. All the work was done in sterilized and aseptic conditions to prevent the other contaminations. In order to make the experiment free from unwanted microbes, sterilization is prerequisite. For the sterilization of glassware, first the Petri plates and other glassware were thoroughly washed with detergent, water and then sun dried. After the washing, Petri plates were sterilized in the oven at 160 -180°C for 4 - 6 hours. Many small instruments like forceps, scalpels, needles, bores etc. were ordinarily sterilized by dipping them in 95 % alcohol followed by flaming. These instruments are repeatedly sterilized during the operation to avoid contamination. The mouths of culture vessels were also flamed before pouring or inoculation. Before inoculation and pouring of the media, hands are repeatedly sterilized with 75 % alcohol to avoid contamination. Inoculation of fungus into the Petri plates was done under laminar air flow. Before each experiment, ultra violet radiations were given on for 15 minutes to kill the microbes. After switching of U.V. radiations, the inoculation was done. Then PDA media was poured into 5 conical flasks and these were plugged with cotton plugs. Conical flasks with media were autoclaved for sterilized at 121°C at 151b/in^2 pressure for 20 minutes then flask containing media were allowed to cool until the flask can be handled by hand. Media was poured into already sterilized Petri plates under aseptic conditions. Collected samples showing typical diseased symptoms were used for isolation of the pathogen. The infected leaves and buds were first thoroughly washed with distilled water. Cross section of lesion was cut of 5 to 10 mm square, containing both the diseased and healthy looking tissues. Surfaces of the cut portions, were sterilized by dipping in 0.1 % Mercuric chloride (Hg Cl_2) as surface sterilant solution for 30 seconds. The treated pieces were washed in three washes with sterile water and then dried on clean, sterile paper towels to remove the sterilant. Aseptically transferred the pieces onto sterilized petriplates containing PDA media usually one piece per plate are inoculated. The inoculated plates were incubated in an inverted position at 25 ± 2° C for 3-5 days (Aneja, 1996). The whitish mycelial growth appeared around the pieces placed in the Petri plates. Further the hyphal tips of mycelium were transferred aseptically in PDA culture tubes the culture obtained from different diseased pieces was subjected to preliminary microscopic examination, which revealed the presence of pathogen responsible for disease development. Finally the culture was purified by single spore technique to keep the fungus viable, active and fresh. Culture of the pathogens was multiplied by regular sub-culturing on PDA both in Petri plates and culture tubes, and was kept in a refrigerator. After the incubation for 4 days, a temporary mount slide was prepared in cotton blue and lectophenol from various isolates collected from different location and examined under microscope for their shape and size of the pathogen. 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 were sterilized at 15 Ib/in^2 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± 2^0 C temperature in incubator for 7 - 10 days. Three replications were used for each treatment. Result were recorded and analyzed.

Results and discussions

Identification of the isolated pathogen is done on the basis of morphological variation in character. The characters were same as reported by Dey 1933. The hyphae were septate, branched, hyaline, colorless turned pale than olive gray. The mycelial width was 3-5.3 µm. Conidiophores are septate erect, branching, or non-branching geniculte, olive buff to dark olive buff, 21.5-100.5µm wide. Effect of pH on growth and sporulation of *Alternaria lini* is shown in Table 1 and statistically analyzed, shown in Table 2. 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) and (Fig 1 &2).

Table1 Effect of media pH on growth and sporulation of *Alternaria lini (mean of mean values)*

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	

It is clear from the table that the pathogen grows over a wide range of pH ranging from 3.5 to 9.0 and found statistically significant difference at 5% level of significance except pH 3.5 and 9.0, but the maximum growth in the tune of 35 mm 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 8 mm and 8.5 mm, respectively indicating the adverse effect of pH on radial growth of colony and behavior of sporulation which is found statistically at par at 5% level of significance. 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.

Table 2 ANOVA

Sources of variance	**DF**	**SS**	**MSS**	**Fc**	**Ft**
replication	2	2.860952	1.430476		
Treatments	6	1997.723	332.9538	621.7901	2.99612
Error	12	6.425714	0.535476		
Total	20				
CD	4.67				

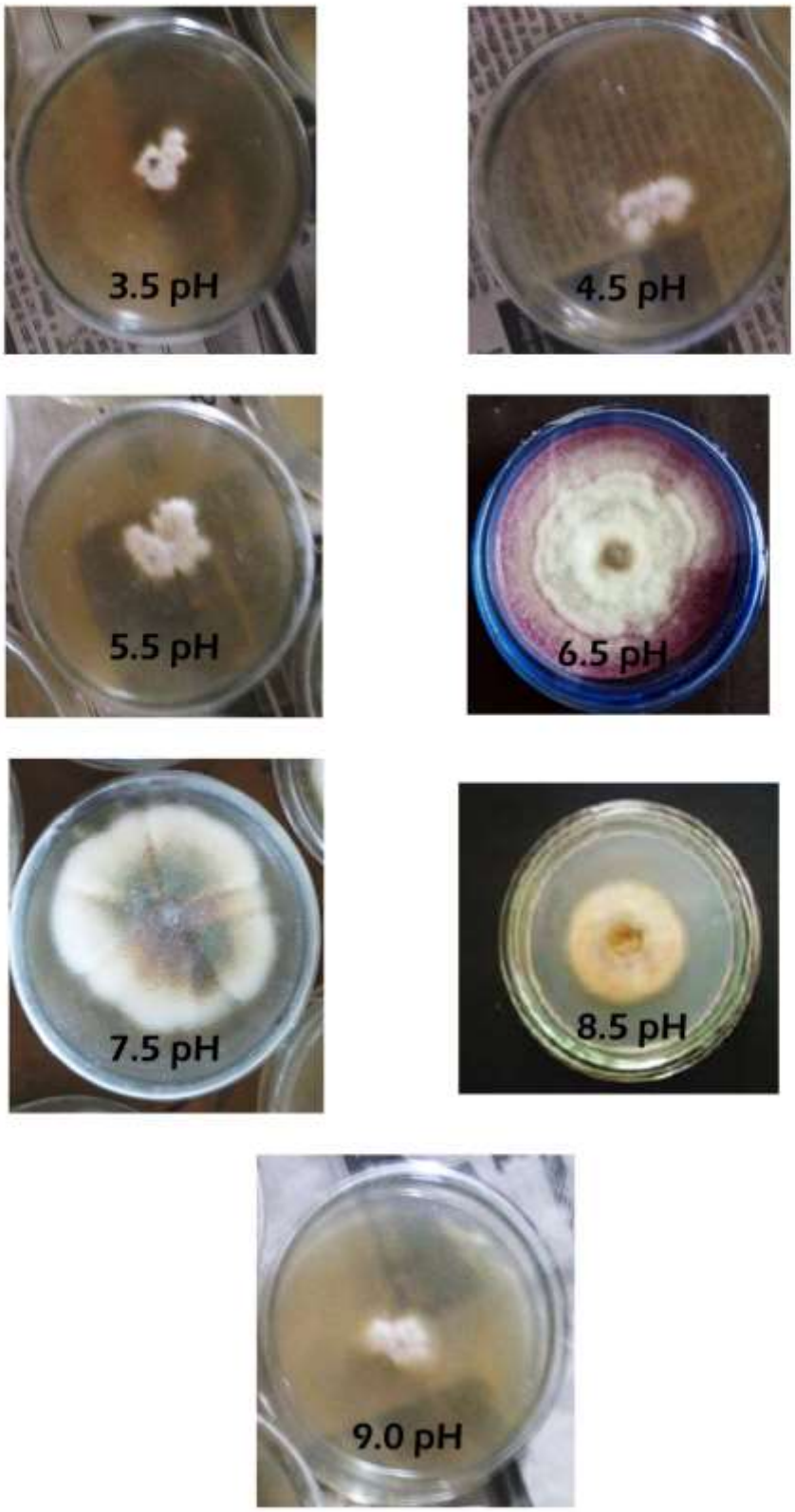

Figure 1 Effect of media pH on grwth and sporulation of *Alternaria Lini*

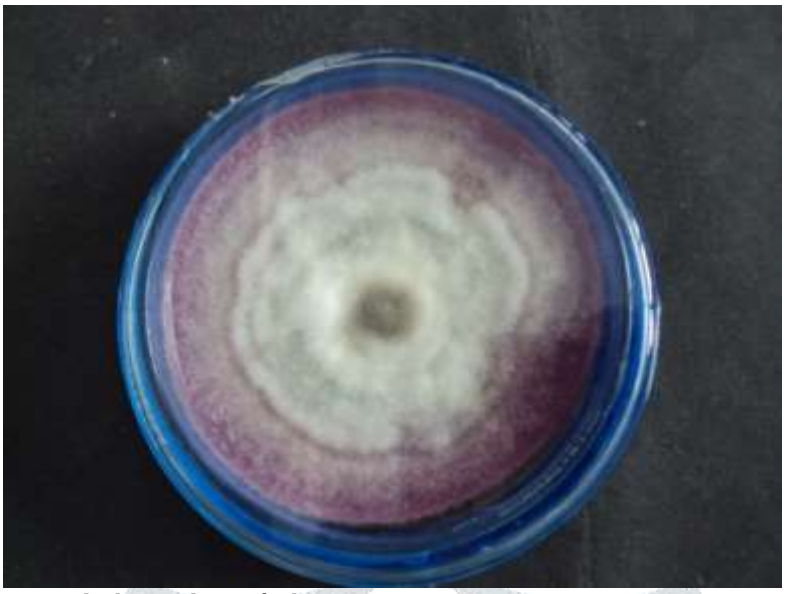

Fig. 2 The best colony of *Alternaria Lini* at pH 6.5

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