Research Article

Effect of Physical Factors on Growth of *Alternaria Alternata* Inciting Leaf Blight of Isabgol

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Abstract

The experiments were conducted at Department of Plant Pathology, S.K.N. College of Agriculture, Jobner (Rajasthan). *Alternaria alternata* was isolated from leaves of isabgol and observed to be pathogenic under artificial conditions.Maximum mycelial growth for *Alternaria alternata* was recorded at 25 ^{0}C. Temperature and maximum nycelial growth of Alternaria alternata 6.5 pH and 100 per cent relative humidity on PDA medium in vitro.

Keywords: *Alternaria alternata*, fungicide, leaf blight and *plantago ovate*, isabgol

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Introduction

Blond psyllium (*Plantago ovata* Forsk.) commonly known as *isabgol*, is an annual herb with narrow linear rosette like leaves belonging to the family Plantaginaceae. *Isabgol* is an important cash crop cultivated for its export and being of important medicinal value is reported to have larger demands and is traded in major medicinal markets of the world. *Isabgol* has pharmaceutical importance to treat dysentery, chronic constipation and chronic diarrhoea and as laxative demulcents, emollients and diuretics. India commands nearly monopoly in the production and export of the seed and husk to the world market. India is earning about Rs. 1600 million as foreign exchange from the export of blond psyllium products to countries like USA, Germany, France, England, Spain and Belgium [1].

In India, the *isabgol* crop is mainly grown as commercial crop in Gujarat, Rajasthan and Madhya Pradesh. However, the crop is spreading to other non-traditional parts of the country such as Haryana, Uttar Pradesh and Karnataka. In Rajasthan, it is being cultivated in 190081 hectares area with a total production of 99950 tonnes of seeds with an average productivity of 525 kg/ha [2]. In Rajasthan, lsabgol mainly cultivated in Barmer, Jalore, Nagaur, Jodhpur and Jaisalmer districts. Presently, Rajasthan is on the top in productivity in India.

[3] reported a number of pathogens viz., Fusarium wilt (*Fusarium oxysporum),* damping off (*Pythium ultimum* trow), leaf blight (*Alternaria alternata* (Fr.) Keissler), downy mildews (*Peronospora plantaginis*) and powdery mildew (*Erysiphe cichoracearum* D.C.) affecting this crop. Alternaria blight has become a serious problem in recent years. It has been found that downy mildew affected crop is more prone to be attacked by *A. alternata*. It causes considerable damage every year and sometimes become very severe which results in total loss of yield [4]. Hence, present investigations were carried out to test the efficacy of plant extracts and fungicides against leaf blight of isabgol incited by *A. alternata*.

Material and Methods

All the glasswares were thoroughly cleaned and rinsed with distilled water. Chemicals of analar grade were used. Different synthetic and semi-synthetic media were prepared by weighing the different constituents of each medium and then adding the distilled water to make up the volume 1000 ml and autoclaved at 1.045 kg/cm^2 for 20 minutes. Inoculation was done with 5 mm disc of mycelial mat taken from 7 days old fungal culture and incubated at 25 $\pm$ 1^{0}C (except temperature study) for 10 days. The each experiment under physio-pathological studies was arranged in completely randomized design (CRD) with three replications.

Effect of temperature on mycelial growth

To study the effect of temperature on mycelial growth of *Alternaria alternata* five levels of temperature were maintained in BODs. Twenty ml of sterilized PDA medium was poured in each sterilized Petriplates. Inoculation was

made with 5 mm disc of 7 days old culture of *Alternaria alternata* with the help of sterilized cork borer and incubated at 5 different levels of temperature *viz*., 15, 20, 25, 30 and 35 ^{0}C for 10 days. Observations on mycelial growth were recorded at 4th and 7th day after inocubation. Each treatment was replicated three times.

Effect of relative humidity on mycelial growth

To study the effect of relative humidity on mycelial growth of *Alternaria alternata*, five different levels of relative humidity *i.e*. 60, 70, 80, 90 and 100 per cent were maintained by using the concentrate sulphuric acid and sterilized distilled water in different proportions in glass desiccators by the method suggested by [5]. The composition of the acid solution was used as followed.

Relative humidity (%)	Stock solution (ml)*	Distilled water (ml)
60	374.0	396.0
70	348.0	510.3
80	294.0	640.0
90	161.0	712.0
* 50% v/v solution of concentrate sulphuric acid.		

Petriplates containing PDA medium were inoculated with 5 mm disc of 7 days old culture of *Alternaria alternata* with the help of sterilized cork borer. Inoculated Petriplates were immediately accommodated in glass desiccators containing mixture of sulphuric acid and distilled water in required proportion and incubated at 25 $\pm$ 1^{0}C for 10 days. Observations on mycelial growth were recorded after 10 days of incubation.

Effect of pH

To study the effect of different levels of pH on mycelial growth, the pH of medium (broth) was adjusted at 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 using citrate phosphate buffer before sterilization with the help of pH meter. Flasks having liquid medium of each pH level were inoculated with 5 mm disc of seven days old fungus culture. Flasks were incubated at 25 $\pm$ 1°C and the observation on mycelial growth (on dry weight basis) was recorded after 7th day of inoculation.

Effect of different solid media

To study the effect of different solid media on mycelial growth observation and treatments were similar to the previous experiments.

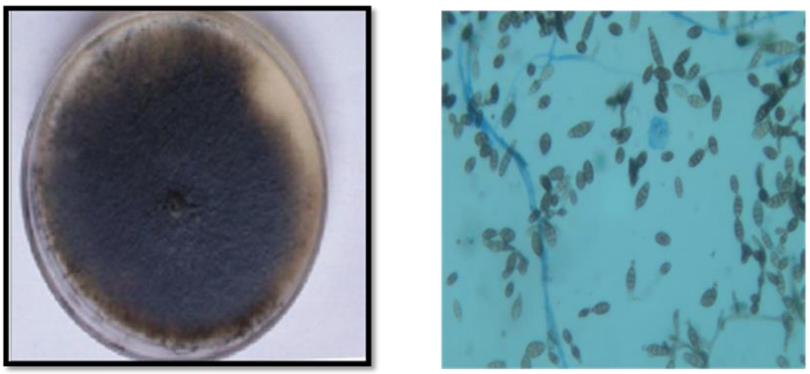


Plate: 1 Pathogenicity test and culture of *Alternaria alternata*

Result and Discussion

Effect of temperature on mycelial growth of Alternaria alternata

The effect of different temperature levels *viz.*, 15, 20, 25, 30 and 35 ^{0}C was studied on PDA as basal medium with essentially pH 6.5. This was aimed to know the optimum temperature for growth of *Alternaria alternata*. Results depicted in **Table 1**, **Figure 1 and Plate 1** revealed that fungus could grow at all the temperature levels *viz.*, 15, 20, 25, 30 and 35 ºC. Maximum mycelial growth (88.00 mm) was observed at 25 ºC. However, the temperature of 20 ºC, 30 ºC and 35 ^{0}C resulted in 75.00 mm, 71.00 mm and 65.00 mm mycelial growth of *Alternaria alternata*, respectively, but differ significantly from the growth at 25ºC.

Temperature is most important physical environmental factor for regulating the growth and reproduction of fungi. Ultra low or high temperature adversely affects the germination and growth. The results obtained in the present investigation showed that optimum temperature for growth is 25 ºC. Similar results were recorded by [6-9] recorded optimum growth of *A. alternata* at 27 ^{0}C.

Table 1 Effect of different levels of temperature on mycelial growth of *Alternaria alternata* at 7th day of incubation *in vitro*

S. No.	Temperature (^{0}C)	Mycelial growth (mm)*
1	15	61.00
2	20	75.00
3	25	88.00
4	30	71.00
5	35	65.00
SEm±		1.082
CD (p=0.05)		3.052
*Average of three replications		

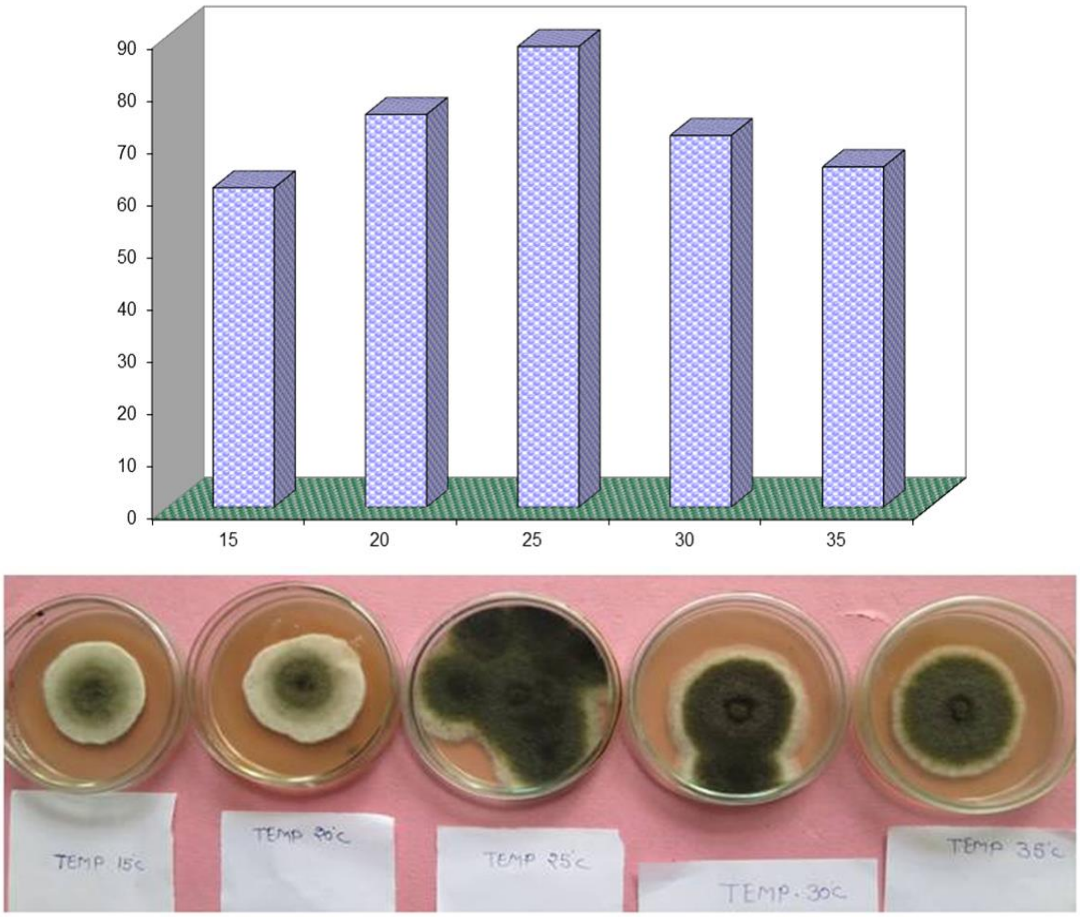


Figure 1 Effect of different levels of temperature on mycelial growth of *Alternaria alternata* at 7th day of incubation *in vitro*

Effect of relative humidity on mycelial growth of A. altermata

To evaluate the effect of atmospheric moisture, the fungus was exposed directly to different levels of relative humidity *viz.*, 50, 60, 70, 80, 90 and 100 per cent and incubated at 25 ± 1 ^{0}C for 7 days. It was observed (**Table 2**, **Figure 2**) that all the six humidity levels induced the growth of *Alternaria alternata*. Maximum mycelial growth (87.50 mm) was recorded at 100 per cent relative humidity which was at par with 90 per cent (85.00 mm) relative humidity. A significantly decrease in mycelial growth was observed at 80, 70 and 60 per cent humidity. Minimum mycelial growth (40.00 mm) was observed at 50 per cent relative humidity.

Table 2 Effect of relative humidity on the mycelial growth of *Alternaria alternata* at 10 days of incubation at 25 ± 1^0C *in vitro*

S. No.	Relative humidity (%)	Mycelial growth (mm)*
1	50	40.00
2	60	47.25
3	70	65.50
4	80	79.00
5	90	85.00
6	100	87.50
SEm+		1.21
CD (p=0.05)		3.97
*Average of three replications		

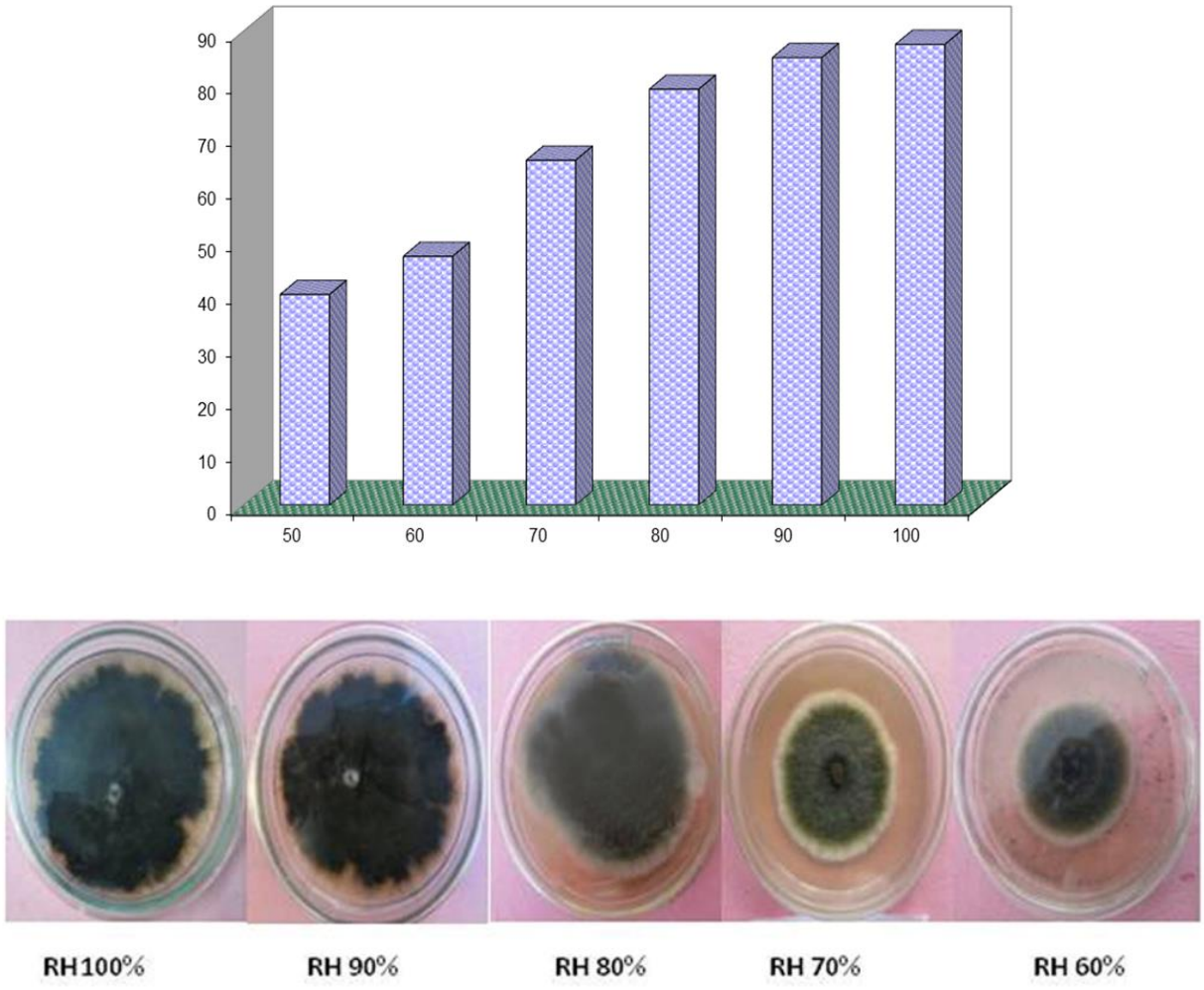


Figure 2 Effect of relative humidity on the mycelial growth of *Alternaria alternata* at 10 days of incubation at 25 ± 1^0C *in vitro*

In vitro studies on different levels of relative humidity revealed that 100 per cent relative humidity supported maximum mycelial growth of *Alternaria alternata* while minimum mycelial growth at 50 per cent relative humidity. The results are in close conformity with the observations of [10] who observed maximum growth of *Alternaria alternata* at 90-100 per cent relative humidity and [9] also reported that 90 to 100 per cent relative humidity was most suitable for mycelial growth of *Alternaria alternata*.

Effect of different pH levels on growth of Alternaria alternata

In general, fungi are capable of growing within a wide range of hydrogen ion concentrations of the medium while, most of them grow best in neutral or slightly acidic medium. The pH preference of most of the foliar pathogens

ranges between 5.0 and 6.5 which obviously favour the establishment of pathogen in their host. Hydrogen ion concentration governs several physiological and metabolic processes of microorganisms. The relationship of pH to the mycelial growth of *Alternaria alternata* was determined at different pH levels *viz*. 4.0 to 8.0 at 25 ± 1 ºC for 7 days.

Of all the eight pH levels, pH 6.5 was found to be ideal and produced the maximum dry mycelial weight of 845 mg followed by pH 6.0 (820 mg) which was at par to each other. The dry mycelial weight was lowest at pH 9 which recorded 290 mg (**Table 3 Figure 3**). The pH below six and above 6.5 was noticed to be inhibitory to the growth. Similar results were also recorded by [7]

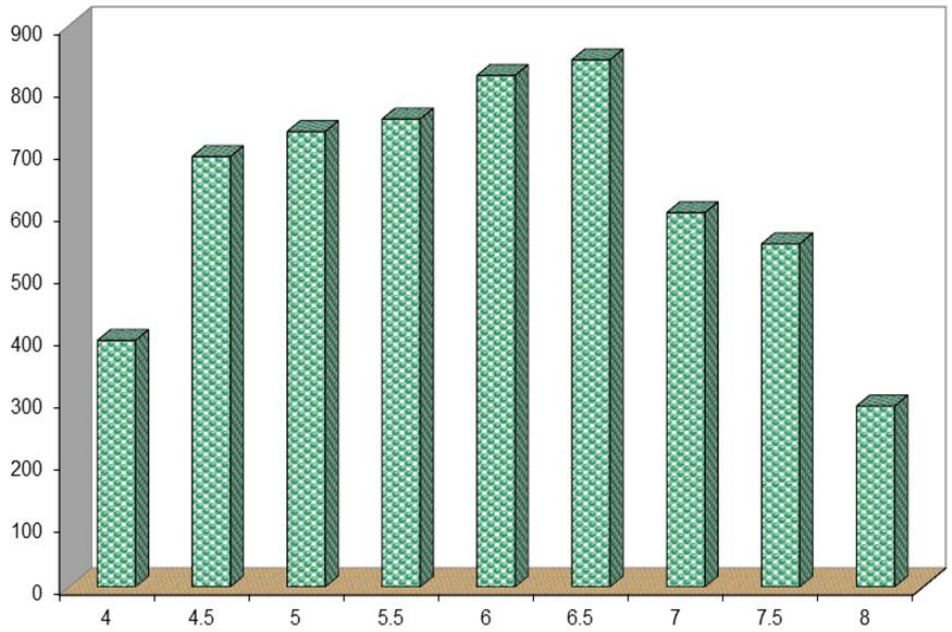
Table 3 Effect of pH on the mycelial growth of *Alternaria alternata* on PD broth at 10 days of incubation at 25 ± 1^0C *in vitro*

S. No.	pH	Dry Mycelial weight (mg)*
1	4.5	690
2	5.0	730
3	5.5	600
4	6.0	820
5	6.5	845
6	7.0	750
7	7.5	550
8	8.0	290
SEm+		12.046
CD (p=0.05)		36.108
*Average of three replications		

Fungi generally utilize substrates in the form of solution only if the reaction of solution conducive to fungal growth and metabolism [11]. This brings importance of hydrogen ion concentration for better fungal growth. In this set of experiment taking different pH, the most suitable with maximum dry mycelial weight was 6.5. This shows that fungus prefers near neutral medium. The results obtained in the present study are in accordance with the results of [12-14].

Effect of solid media on mycelial growth of Alternaria alternata

To find out a suitable medium for the mycelial growth of *Alternaria alternata*, five different synthetic and semi synthetic media were tested *in vitro*.



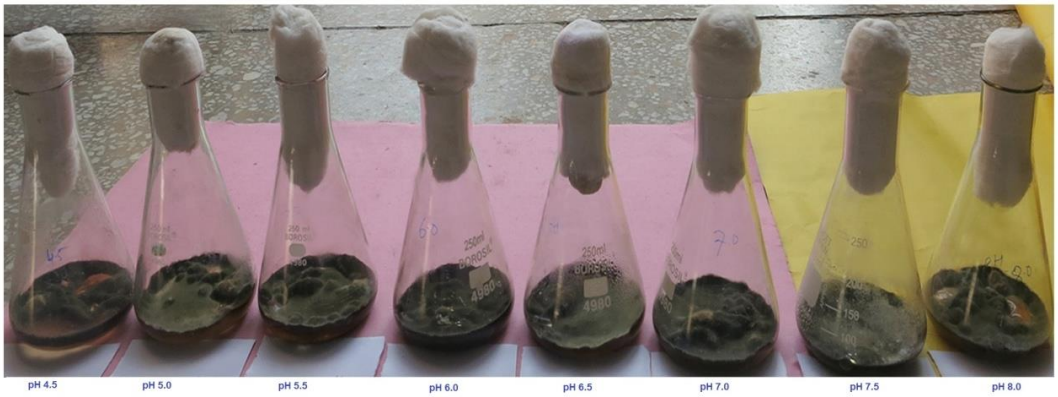


Figure 3 Effect of pH on the mycelial growth of *Alternaria alternata* on PD broth at 10 days of incubation at 25 ± 1^0C *in vitro*

Table 4 Effect of solid media on mycelial growth of *Alternaria alternata* at 25±1°C

S. No.	Medium	Mycelial growth (mm)* After 7 days
1.	Czapeck's medium	55.55
2.	Martin's medium	37.77
3.	Oat meal medium	63.33
4.	Potato dextrose agar medium	85.55
5.	Richard's medium	76.70
	SEm±	2.80
	CD (p=0.05)	8.34
*Average of three replication		

On perusal of data (**Table 4**) revealed that among the five different solid media understudy, the potato dextrose agar was significantly superior in supporting maximum mycelial growth (85.55 mm) at 7th day of incubation followed by Richard's media (76.70 mm) and oat meal agar (63.33 mm). Minimum growth of the fungus was observed on Martin's medium (37.77 mm). The potato dextrose agar medium was best supporter of growth of the fungus. The PDA medium was selected for further studies.

Physiological studies for this pathogen were carried out extensively covering media, temperature, pH and relative humidity. Out of the five media tested, PDA proved to be the best in terms of growth. Similar results were obtained by [12, 15-17], [8] and [13] reported that *Alternaria alternata* fungus grew well on potato dextrose agar medium.

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