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Physiological studies of *Alternaria cyamopsidis* causing Alternaria blight of cluster bean

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Abstract
Physiological studies were conducted at SKNAU, Jobner on *Alternaria cyamopsidis* Rang and Rao inciting Alternaria blight of clusterbean. Results revealed that different levels of relative humidity and temperature play a significant role in mycelial growth of *Alternaria cyamopsidis. In vitro* studies of different levels of relative humidity reveals that, 90 to 100 per cent relative humidity produce maximum mycelial growth 88.90 mm and 85.67 mm, respectively. In temperature studies maximum mycelium growth (88.92mm) was observed at 25 ºC followed by 30 and 20 ºC.

Keywords: *Alternaria cyamopsidis*, mycelial growth, RH, temperature

Introduction
Clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.] Commonly called as "Guar" is an important arid legume crop. India enjoys unique status in the cultivation of guar in the world because of congenial climatic condition for the crop growth. Rajasthan has largest area under cultivation of guar (82.1%) followed by Haryana (8.5%), Gujarat (8.3%) and Punjab (1%) which in turn producing 64, 22.12 and 2 per cent of guar seeds (Bagenia and Chaturvedi, 2018) [2]. Clusterbean suffers from various fungal, bacterial and viral diseases. The production of clusterbean in terms of grain and fodder is limited mostly due to the Alternaria blight disease caused by *Alternaria cyamopsidis* in Northern India and Rajasthan. The maximum severity of the blight takes place between bloom and pod setting. Higher yield losses (43-78%) were recorded when leaves were infected at seedling stage than at old stage (Sharma, 1981 and Anonymous, 2013-14) [8, 1].
Generally, all the fungal species prefer a temperature range of 20-30℃ with neutral pH (7.0). Optimum temperature is one of the important factors for the growth and sporulation of fungi which also influences the occurrence and development of disease. Thus the present investigations have been made to determine the optimal conditions for the growth of *Alternaria cyamopsidis* including temperature and relative humidity.

Material and Methods
Collection and isolation of pathogen
Alternaria blight infected plant samples of clusterbean were collected from major clusterbean growing areas of Rajasthan. Isolations were made from the infected plants showing typical symptoms of Alternaria blight. Small pieces of the leaves of cluster bean plant were cut from the diseased portion along with some healthy tissues, surface sterilized for 1-2 minutes in 1.0 per cent Sodium hypochlorite solution followed by three washings with sterilized distilled water. These bits were transferred aseptically to Potato Dextrose Agar in Petri plates separately. Incubation was done at 25 ± 1 °C for 7 days. Sub-culturing from uncontaminated peripheral growth was made on PDA slants. Pathogen was purified by using single spore technique.

Effect of relative humidity
To study the effect of relative humidity on mycelial growth of *Alternaria cyamopsidis*, six different levels of relative humidity i.e. 50, 60, 70, 80, 90 and 100 per cent was maintained in desiccators by using the concentrate sulphuric acid and sterilized distilled water in different proportions. The different relative humidity levels were maintained by the method suggested by Buxton and Mellanby (1934) [3].

Table 1: Composition of the acid solution used were as follows

S. No	Relative humidity (%)	Stock solution (ml)*	Distilled water (ml)
1.	50	514.0	420.0
2.	60	374.0	396.0
3.	70	348.0	510.3
4.	80	294.0	640.0
5.	90	161.0	712.0
6.	100	0.00	Only distilled water

*50% v/v solution of concentrate sulphuric acid

Petri plates having PDA medium inoculated with 5mm disc of seven days old fungus culture was kept in desiccators having solution of different level relative humidity and then incubated at 25±1 °C. Observations on radial growth was recorded after 7 days of incubation

Effect of temperature

Effect of temperature on growth of *Alternaria cyamopsidis* was studied *in vitro*. 20 ml of sterilized potato dextrose agar medium was poured in each sterilized Petri plate. Inoculation was made with 5 mm disc from 7 days old fungal culture and incubated at 7 different temperatures *viz.*, 15, 20, 25, 30, 35 and 40 °C. Observation on radial growth was recorded after 7 days of inoculation.

Result and Discussion

Isolation and pathogenicity test of pathogen

Isolation of the pathogen from diseased plants of cluster bean was done on potato dextrose agar (PDA) medium. After seven days of incubation at 25±1 °C, growth of fungus was developed. Pure culture of the pathogen, obtained by single sporing on PDA, yielded *Alternaria cyamopsidis*.

Effect of relative humidity

To evaluate the effect of atmospheric moisture, the fungus was exposed directly to different level of relative humidity. It was observed (Table 2 and Plate- 1) that all the six humidity levels (50 to 100 per cent) induced the growth of *Alternaria cyamopsidis*. Significantly best mycelial growth (88.90 mm) was recorded at 90 per cent relative humidity followed by growth at 100 per cent (85. 67 mm) relative humidity level. A significant decrease in mycelium growth was observed at 80, 70 and 60 per cent humidity level. Minimum mycelium growth was observed at 50 per cent relative humidity level. It can be concluded that high humidity favoured the growth of *A. cyamopsidis*.

In general optimum relative humidity enhances the fungal growth and disease development in plants. In present study relative humidity near saturation *i.e.* 90 to 100% was found to increase mycelial growth of *A. cyamopsidis*. *In vitro* studies of different levels of relative humidity reveals that, 90 to 100 per cent relative humidity produce maximum mycelial growth of *A. cyamopsidis*. Similarly results were reported by Prasad and Ahir (2013) [7]. They reported maximum mycelium growth of *A. alternata* infecting brinjal, at 100% relative humidity. While Yenjerappa and Padaganur (1993) [10] found 95% RH best for maximum spore germination of *A. cyamopsidis*. Chen *et al.* (2000) [4] reported the conidial germination of *Alternaria tenuissima* was maximum at 90 to 100 per cent relative humidity.

Effect of temperature

The temperature ranges for the growth vary for all microorganisms as well as for host pathogen interactions. It is evident from the data presented in table 3 and Plate- 2 that the fungus grew in a range of 15 to 35 °C temperatures, under study. Maximum mycelial growth (88.92 mm) was observed at 25 °C. A sudden fall in mycelial growth was observed at 40 °C. No growth observed at 40 °C temperature. However, 30 °C, 20 °C and 35 °C favoured good growth of *A. cyamopsidis* but differ significantly for growth at 25 °C. It can be concluded that 25 °C is the optimum temperature for mycelial growth of *A. cyamopsidis*.

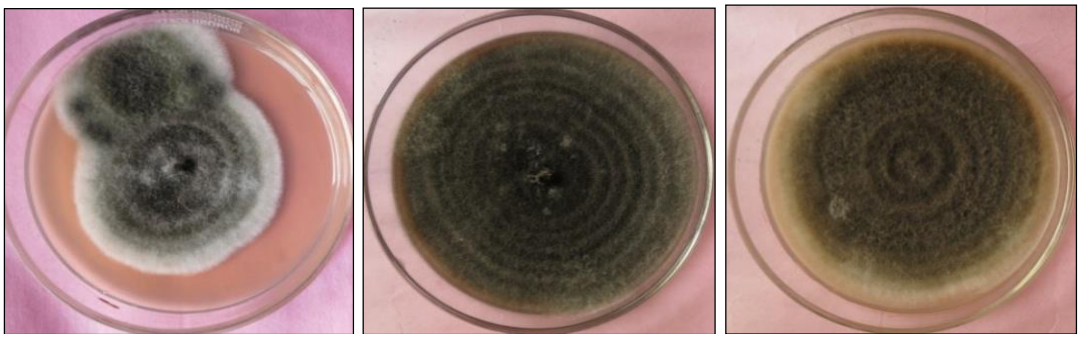
Table 2: Effect of relative humidity on mycelial growth of *A. cyamopsidis* incubated for 7 days at 25 +1 ºC

S. No	Relative humidity (%)	Mycelial growth* (mm)
1	50	52.11
		(46.21)
2	60	63.88
		(53.06)
3	70	69.83
		(56.68)
4	80	81.00
		(64.16)
5	90	88.90
		(70.54)
6	100	85.67
		(67.76)
	S.Em+	0.56
	CD (p=0.05)	1.69

*Average of three replications

**Values in parenthesis are angular transformed

Relative humidity 50% **Relative humidity 60%** **Relative humidity 70%**



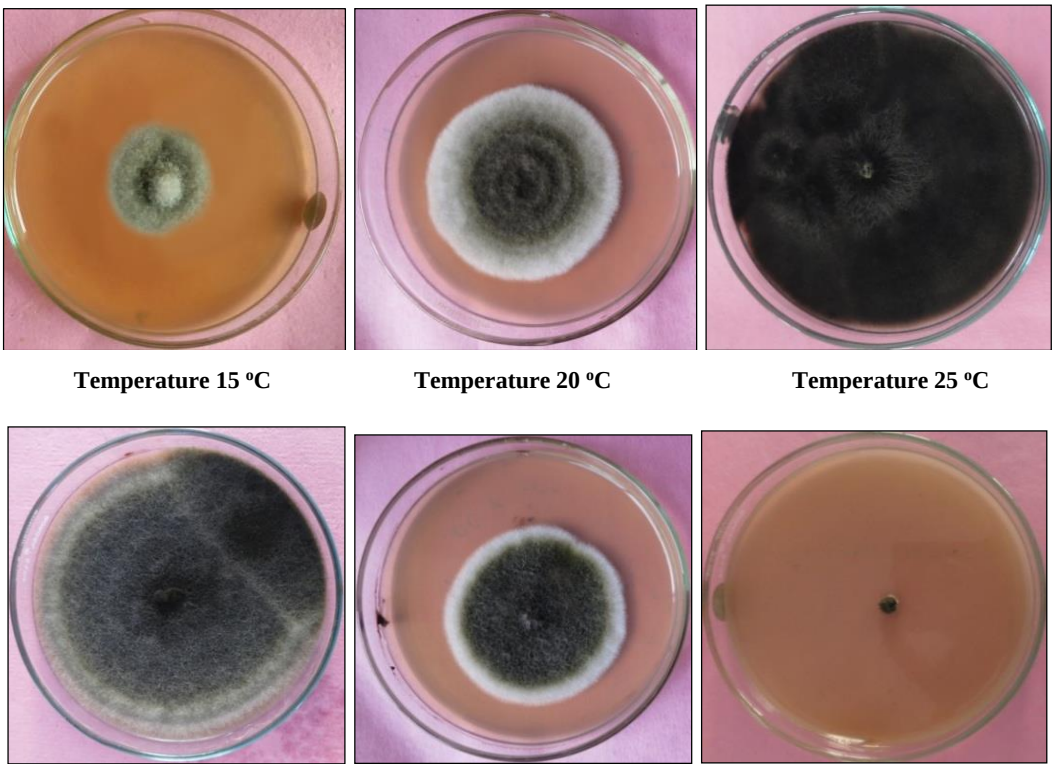
Relative humidity 80% **Relative humidity 90%** **Relative humidity 100%**

Plate 1: Effect of relative humidity on mycelial growth of *Alternaria cyamopsidis*

Table 3: Effect of different temperature levels on mycelial growth of *A. cyamopsidis*

S. No	Temperature (ºC)	Mycelial growth* (mm)
1	15	25.13
		(30.09)
2	20	69.45
		(56.45)
3	25	88.92
		(70.56)
4	30	87.22
		(69.05)
5	35	54.33
		(47.48)
6	40	0.00
		(0.00)
	S.Em+	0.59
	CD (p=0.05)	1.79

*Average of four replications
**Values in parenthesis are angular transformed



Temperature 15 ºC **Temperature 20 ºC** **Temperature 25 ºC**

Temperature 30 ºC **Temperature 35 ºC** **Temperature 40 ºC**

Plate 2: Effect of different temperature levels on mycelial growth of *A. cyamopsidis*

In general optimum temperature enhances the fungal growth and disease development in plants. In present study temperature around 25-30 ºC was found to increase mycelial growth of *A. cyamopsidis*. Neergard (1945) [6] reported that good growth and sporulation of various species of *Alternaria* was observed in temperature range of 23-28 ºC. Similarly,

Yenjerappa and Padaganur (1993) [10] found maximum spore germination of *A. cyamopsidis* at 30 °C temperature followed by 25 °C. While Wang and Dong (1991) [9] and Hubballi *et al.* (2010) [5] reported that favourable temperature for growth of *A. alternata* was 20-25ºC and 25-30°C, respectively.

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