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### Evaluation of environmental factors for optimum growth of *Rhizoctonia solani* causing root rot of cluster bean (*Cyamopsis tetragonoloba*)

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**Abstract**

Clusterbean [*Cyamopsis tetragonoloba* L.) Taub.] is popularly known as “Guar” or “Guwar” and belongs to family *Fabaceae* of kingdom *Plantae*. It is an important legume crop and mainly grown under rainfed conditions of arid and semi arid regions of tropical India during *Kharif* and *Zaid* seasons. It is tolerant to drought, deep rooted and can be grown for different purposes viz., vegetable, green fodder, green manuring, production of seed and for endospermic gum (30-35 percent). Root rot caused by *Rhizoctonia solani* is an important and becoming severe problem of clusterbean in Rajasthan as well as India under the changing scenario of climate. This study was undertaken to find the most favorable and critical point of temperature, relative humidity and pH factors for optimum growth of the *Rhizoctonia solani* that may help in survival, spread and cause infection in healthy hosts. The most favorable temperature for flourishing and mycotic growth of *Rhizoctonia solani* was 30 °C (90.00 mm) followed by 25 °C (80.25 mm). Among various level of relative humidity, 90 per cent relative humidity was most favorable for maximizing growth (90.00 mm) while 6.5 pH was highly suited for maximum growth of the pathogen (632 mg dry mycelial weight). Conclusively, this pathogen requires higher temperature for fast multiplication that is prevalent in arid region of Rajasthan and due to these favorable factors this disease may be gaining importance day by day.

**Keywords:** Clusterbean, *Cyamopsis tetragonoloba*, *Rhizoctonia solani*, optimum growth, temperature, P<sup>H</sup>, relative humidity etc.

**Introduction**

Clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.] is also known by various names like “Guar” or “Guwar” and it belongs to family *Fabaceae* of kingdom *Plantae*. It is an important legume crop and mainly grown under rainfed conditions of arid and semi arid regions of tropical India during *Kharif* and *Zaid* seasons. It is considered as one of the most drought tolerant grain legumes and very valuable within crop rotation cycle as it lives in symbiotic association with nitrogen fixing bacteria. It is tolerant to drought, deep rooted and can be grown for different purposes viz., vegetable, green fodder, green manuring, production of seed and for endospermic gum (30-35 percent). Green pods of clusterbean are nutritionally rich in energy (16 kcal), moisture (81g), protein (3.2 g), fat (1.4 g) carbohydrate (10.8 g), vitamin-A (65.31 IU), vitamin-C (49 mg), calcium (57 mg) and iron (4.5 mg) per 100 g of edible portion (Kumar and Singh, 2002) [1]. It is well adapted to conditions prevailing in Rajasthan like hot desert areas (Jaisalmer, Barmer and Jodhpur) and is being grown in areas receiving annual rainfall from 350 to 750 mm. Cultivation for vegetable purposes, it favors long days for growth and short days for producing flowers. In Rajasthan, clusterbean as a vegetable crop is cultivated throughout the state for its green pods (immature pods) occupying an area 694 hectares with production of 976 metric tonnes (Anonymous, 2016) [2]. For seed production, it is grown in arid and semi-arid regions mainly during rainy season while, for vegetable purpose during *Zaid* and rainy seasons. As vegetable crop, it produces green pods continuously for a long time, thus it needs regular feeding along with much care from pests especially from diseases. Through, it is a hardy crop, but some important diseases like root rot, Alternaria blight, bacterial blight, powdery mildew etc. severely damage the crop. Among these, root rot caused by *Rhizoctonia solani* is one of the major diseases occurs in Rajasthan and elsewhere, wherever this crop is grown. Lodha *et al.* (1986) [3] observed 31.0 per cent root rot incidence of

clusterbean with 32.11 per cent yield loss in arid regions of Rajasthan. *Fusarium solani* also recorded to cause root rot of clusterbean (Mathur and Shekhawat, 1987) [11]. The crop suffers from a number of phytopathogenic fungal and other diseases, but root rot or charcoal rot caused by *Rhizoctonia* sp. is a serious disease of clusterbean (Prasad, 1944; Dhingra and Sinclair 1978 and Lodha, 1993) [12, 4, 8]. Sharma and Tripathi (2001) [14] observed that web blight (*Rhizoctonia solani*) of urdbean is favoured by high relative humidity (80%). Singh and Malhotra (1994) [17] recorded optimum temperature (28 °C) for the growth and sclerotia formation of *Rhizoctonia solani*. Sukanya *et al.* (2016) [18] studied *Macrophomina phaseolina* at various pH levels (3, 4, 5, 6, 7, and 8). They recorded optimum dry mycelia weight at pH 6.0. Therefore, this study was undertaken to find out the most favorable and optimum temperature, relative humidity and pH factors for fast growth and multiplication of *Rhizoctonia solani* under the changing climate of Rajasthan.

### Material and Methods

The diseased sample was collected and experiment was conducted in laboratory during 2018 at Department of Plant Pathology, S.K.N. College of Agriculture, Jobner, Jaipur (Rajasthan). All the glassware were cleaned with potassium dichromate sulphuric acid solution, washed with sterilized water, sterilized in hot air oven at 160 °C for two hours. Potato dextrose agar medium was sterilized by autoclaving at 1.045 kg cm<sup>2</sup> pressure for 20 minutes. Roots of diseased plants of clusterbean were first washed under the tap water and then cut into small pieces along with healthy portion. These pieces were surface sterilized by dipping in 1 per cent sodium hypochlorite solution for 1 minute. After three consecutive washings with sterilized distilled water, the pieces were transferred to autoclaved potato dextrose agar medium in Petriplates and incubated at 30±1 °C in BOD incubator for 7 days. The fungal colonies emanating from bits were examined on 7 days of incubation. Pure culture of the fungus was obtained by hyphal tip cut method on plain agar medium. For this, hyphal tips were obtained from culture slants after 96 hours of incubation and were suspended in sterilized distilled water. The dilution of suspension was adjusted such that in one loopful, 5-10 hyphal tips could be counted under the low power objective of the microscope. One ml of above suspension was spread in Petriplates containing 20 ml sterilized plain agar medium. After 12-24 hours of inoculation, the germinating hypha were located under the microscope and marked with the help of dummy objective and then transferred to PDA slants and kept in BOD for further growth. The culture was maintained by periodical transfer on PDA slants for further studies. The isolated fungus was identified on the basis of morphological characters. The culture was also got confirmed from ITCC, Division of Plant Pathology, IARI, New Delhi and identified as *Rhizoctonia solani* with ID No. 10.659.17.

**Influence of relative humidity:** For ascertaining the impact of relative humidity on mycelial growth of *Rhizoctonia solani*, six different levels of relative humidity i.e. 50, 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 according to the

method suggested by Buxton and Mellanby (1934) [3]. The composition of the acid solution used was as follows.

RH (%)	Stock solution (ml)	Distilled water (ml)
50	405.0	278.0
60	374.0	396.0
70	348.0	510.3
80	294.0	640.0
90	161.0	712.0
100	0.00	Only distilled water

Petriplates containing sterilized PDA medium were inoculated with 5 mm disc of 7 days old culture of *Rhizoctonia solani*, 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 30±1°C for 7 days with four replications. Observations on mycelial growth was recorded when either one of plates full with mycelial growth.

**Effect of temperature:** It is a well known phenomenon that the temperature exerts considerable influence on the biochemical activities of pathogens that play an important role in fast multiplication of the cellular organisms. Twenty ml of sterilized PDA was poured in each of sterilized Petri dish. Each Petri dish was inoculated aseptically by placing in the centre a 5 mm disc from actively growing 7 days old culture *Rhizoctonia solani* on PDA. The inoculated Petri dishes were incubated at 20, 25, 30, 35 and 40 °C temperature with four replications. Observations on mycelial growth was recorded when either one of plates full with mycelial growth.

**Effect of hydrogen ion concentration (P<sup>H</sup>):** The study of different pH levels was undertaken with a view to ascertain the effect of different hydrogen ion concentrations of the medium on growth of the fungus. The initial pH of the potato dextrose broth, before autoclaving was adjusted from 6.0 to 8.0 with a difference of 0.5 using N/10 NaOH or N/10 HCl. After autoclaving the pH was again tested and potato dextrose broth was filled in flasks (250 ml capacity). The flasks were inoculated with 5 mm disc from actively growing 7 days old culture of *Rhizoctonia solani* and incubated at 30±1°C for 7 days with four replications. Observations on mycelia growth was recorded at 7<sup>th</sup> day of incubation.

### Result and Discussion

**Influence of relative humidity:** The effect of relative humidity on the mycelial growth was studied at different levels viz., 50, 60, 70, 80, 90 and 100 per cent by incubating at 30±1°C for 7 days. It was observed that all the six humidity levels include the growth of *Rhizoctonia solani*. Perusal of data (Table 1) showed that maximum mycelial growth (90 mm) of *Rhizoctonia solani* was observed at 90 and 100 per cent relative humidity. A significant decrease in mycelia growth was observed at 80 per cent (83.55mm), 70 percent (67.81mm) and 60 per cent (41.16) relative humidity. Minimum mycelial growth (38.50 mm) was observed at 50 per cent relative humidity. Our findings are in the agreement with the results of earlier workers (Ali *et al.*, 1998 and Marcelo and Vega, 1988) [1, 10]. They observed maximum growth of *Rhizoctonia solani* at 80 to 90 per cent relative humidity while studying with the pathogen of other crops.

**Table 1:** Effect of relative humidity on mycelial growth of *Rhizoctonia solani*

Relative humidity (%)	Mycelial growth (mm*)
50	38.50
60	41.16
70	67.81
80	83.55
90	90.00
100	90.00
SEm+	1.52
CD (p=0.05)	4.67

\*Average of four replications

**Effect of temperature:** All the microorganisms grow under the certain range of temperature within which a minimum, optimum and maximum temperature could be located. It is cleared from the data (Table 2) that the fungus grow at all the temperature levels ranged from 20 °C to 40 °C but flourished and maximum mycelial growth (90 mm) of the fungus was observed at 30 °C at 5th day of incubation followed by 25 °C (80.25 mm) and 35 °C (78.65 mm) and found at par with each other. A gradual decrease in mycelial growth was observed at 20°C (63.66 mm) and 40 °C (21.15 mm) with lowest mycelial growth. Temperature is one of the important factors for the growth of an organism which also influences the occurrence and development of disease and most of the organisms grow between 0 to 42°C (Wolf and Wolf, 1947) [19]. Favorable temperature in the range of 25°C to 30°C for higher mycelial growth of *Rhizoctonia solani* has also been observed by Haq *et al.* (1999), Singh *et al.* (1999), Grosch and Kofoet (2003) and Ray and Kumar (2009) [6, 15, 5, 13].

**Table 2:** Effect of temperature on mycelial growth of *Rhizoctonia solani*

Temperature (°C)	Mycelial growth (mm)*
20	63.66
25	80.25
30	90.00
35	78.65
40	21.15
SEm+	1.09
CD (p=0.05)	3.35

\*Average of four replications

**Effect of pH:** To know the effect of pH on growth of the fungus, it was exposed directly to different levels of pH viz. 6.0, 6.5, 7.0, 7.5 and 8.0 and incubated at 30±1 °C for 7 days. It was observed that all the five pH levels include the growth of *Rhizoctonia solani*. Perusal of data (Table 3) showed that maximum dry mycelial weight (632 mg) of *Rhizoctonia solani* was observed at 6.5 pH. A significant decrease in dry mycelial weight was observed at pH 7 (548 mg), 6.0 (423 mg) and 7.5 (380 mg). Minimum dry mycelial weight (315 mg) was observed at 8.0 of pH level. Our results are in corroboration with the findings of Singh *et al.* (1999), Singh *et al.* (1974) and Grosch and Kofoet (2003) [15, 16, 5].

**Table 3:** Effect of pH on mycelial growth of *R. solani* after 7 days of incubation

pH level	Dry weight of mycelium (mg)*
6.0	423.00
6.5	632.00
7.0	548.00
7.5	380.00
8.0	315.00
SEm+	8.16
CD (p=0.05)	25.13

\*Average of four replications

## Conclusion

The most favorable temperature for flourishing and mycotic growth of *Rhizoctonia solani* was 30 °C. Among various level of relative humidity, 90 per cent relative humidity and 6.5 pH were most favorable for maximizing growth of the pathogen. Therefore, it can be concluded that pathogen requires higher temperature for fast multiplication that is prevalent in arid region of Rajasthan and due to these favorable factors this disease may be gaining importance day by day.

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