

Original Research Article

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Studies of Physiological Parameters on the Growth of Sclerotio Formation of *Rhizoctonia solani* with Okra (*Abelmoschus esculentus* L. Moench)

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Rhizoctonia root rot of okra caused by *Rhizoctonia solani* is an important disease which is known to cause heavy loss in Rajasthan as well as in India. Among physiological parameters effect of temperature, effect of pH and effect of relative humidity were study in vitro. The fungus grew at all the temperature level ranged from 15°C to 35°C. Maximum mycelial growth of the fungus (90mm) was observed at 30°C. In different pH levels that maximum dry mycelium weight (630gm) of *Rhizoctonia solani* was observed at 6.5 pH level. In different levels of relative humidity the maximum mycelial growth (90mm) of *Rhizoctonia solani* was observed at 100 and 90 per cent relative humidity.

Introduction

Okra [*Abelmoschus esculentus* (L.) Moench] is a member of the family Malvaceae. Earlier, its botanical name was *Hibiscus esculents* (L.) Moench under the section *Abelmoschus* of *Hibiscus*, established by Linnaeus in 1737. It is an important summer/rainy season vegetable crop, extensively used globally for its nutritional and health benefits. Okra seeds are good sources of quality edible oil and proteins (Berry *et al.*, 1988). The okra plants are used for controlling diseases like stone in kidney, leucorrhoea, backache and goutre in

human beings. Mucilage extract of stem and roots of okra is used for clarifying sugarcane juice for making jaggery (Gur). The fruits of okra contain carbohydrate (6.4%), protein (1.9%), fat (0.2%), fibre (1.2%), minerals (0.7%) and moisture (89.6%). (Anonymous, 2013). Okra is cultivated throughout the country for its immature tender fruits, occupying an area over 532.66 thousand hectares with an annual production of 6346.37 thousand metric tonnes. Major okra growing states are Andhra Pradesh, West Bengal, Bihar, Gujarat, Odisha, Uttar Pradesh, Haryana and Rajasthan. In Rajasthan, it is

grown in an area of 3.95 thousand hectares with an annual production of 12.27 thousand metric tonnes (Anonymous, 2014). This crop suffers severely from the vagary of diseases caused by fungi, bacteria, viruses and nematodes in the field. Okra is attacked by several fungal pathogens, which not only reduces the potency of seed, but also degrades the health beneficial and nutritional quality components. Root rot (*Rhizoctonia solani*) is major destructive fungal diseases (Anonymous, 2003). The genus *Rhizoctonia* was described by De Candolle (1815), now, it is a well-known saprophyte, notorious soil inhabiting plant pathogen, capable of attacking a tremendous range of host plants throughout the world, causing seed decay, damping-off, stem cankers, root rots, fruit decay and foliage diseases. Young culture of *R. Solani* shows profuse mycelia growth and dirty white sclerotia while older ones are abundantly branched with constriction at the point of origin and dark brown sclerotia with variable shape and size (Verma *et al.*, 2006). Crop losses by root rot of okra (*Rhizoctonia solani*) is ranged from negligible to 50 per cent depending on the extent of severity and different stages of crop (Safiuddin *et al.*, 2014). Sunder *et al.*, (1996) harvested inoculum of *R. Solani* from incubation temperature of 25-30°C and pH 6.5-7.5 that produced maximum disease on cotton whereas inoculum grown at 20°C and 35°C resulted in lower seedling mortality. Tiwari (1997) found maximum mycelial growth and sclerotia formation at 25°C, followed by 30°C. Mycelial growth and sclerotial formation were optimum at pH 6.0-7.0, and there was no growth at pH values 3.0 and 9.0. Haq, I., Khan, S. M., and Ahmad, R. (1999) conducted a study with six isolates of *R. Solani* from rotted roots of cotton. They finalized that temperature levels of 30°C and 35°C were optimum for mycelial growth and sclerotial formation. Ali *et al.*, (1998) also reported that relative humidity values of 93

per cent and above gave optimum growth of *R. Solani* isolates from carrot.

Materials and Methods

Effect of physical parameters on the growth and sclerotia formation of the pathogen

All the glasswares were thoroughly cleaned and rinsed with distilled water. Chemicals of analar grade were used. Five different synthetic and semi-synthetic media were prepared by weighing the different constituents of each medium and autoclaved at 1.045 kg/cm² for 20 minutes. In all three physiological experiments, inoculation was done with 5 mm diameter bit taken from 7 days old fungal culture and incubated at 30±1°C (except for temperature study) for 7 days. The each experiment, under physiological study was arranged in completely randomized design (CRD) with three replications.

Effect of temperature

Effect of temperature on mycelial growth of *Rhizoctonia solani* was studied *in vitro*. Twenty ml of sterilized PDA was poured in each sterilized Petriplate. Inoculation was made with 5 mm disc of 7 days old culture of *Rhizoctonia solani* taken with the help of sterilized cork borer and incubated at different levels of temperature viz. 15, 20, 25, 30 and 35°C for 7 days. Observations on mycelial growth and sclerotia formation were recorded at 7th day of incubation.

Effect of pH

To study the effect of different pH levels on mycelial growth and sclerotia formation, the pH of medium (broth) was adjusted from at 6.0, 6.5, 7.0, 7.5 and 8.0 using citrate phosphate buffer before sterilization with the

help of pH meter. Flask having liquid medium of each pH level were inoculated with 5 mm disc of seven days old fungus culture. Observations on mycelial growth and sclerotial formation of the fungus were recorded at 7th day of incubation.

Effect of relative humidity

To study the effect of relative humidity on mycelial growth and sclerotia formation of *Rhizoctonia solani*, 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 proportion in glass desiccators according to the method suggested by Buxton and Mellanby (1934). The composition of the acid solution used was as follows.

RH (%)	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
100	0.00	Only distilled water

Petriplates containing PDA medium were inoculated with 5 mm disc of 7 days old culture of *Rhizoctonia solani*, taken 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 7th days. Observations on mycelial growth and sclerotia formation were recorded at 7th day of incubation.

Results and Discussion

In vitro evaluation of physiological parameters

Effect of temperature

The entire microorganisms grow under certain

range of temperature within which a minimum, optimum and maximum temperature could be located. It is evident from the data (Table 1) that the fungus grew at all the temperature levels ranged from 15°C to 35°C. Maximum mycelial growth of the fungus (90 mm) was observed at 30°C at 7th day of incubation followed by 25°C (85.00 mm) and 35 °C (84.03 mm) and found at par with each other. A gradual decrease in mycelial growth was observed at 20°C (76.66 mm) and lower temperature minimum mycelial growth (50.76 mm) of the fungus was observed at 15°C. Maximum sclerotia formation was observed at 30°C, sufficient at 25°C and few at 20°C, no sclerotia formation was observed at 15°C and 35°C. Present results are in accordance with the results of Inam-ul-Haq *et al.*, (1999), Singh *et al.*, (1999), Grosch and Kofoet (2003) and Ray and Kumar (2009) who observed maximum mycelial growth and sclerotia formation of the fungus was observed at 30°C after 7 days of incubation. Optimum temperature i.e. 25°C to 35°C for mycelial growth and sclerotia formation of *Rhizoctonia solani*

Effect of pH

To evaluate the effect of pH on mycelium growth and sclerotia formation, the fungus was exposed directly to different levels of pH i.e. 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 2) showed that maximum dry mycelial weight (630 mg) of *Rhizoctonia solani* was observed at 6.5 pH level. A significantly decrease in dry mycelial weight was observed at pH 7 (546 mg), 6.0 (422 mg) and 7.5 (380 mg). Minimum dry mycelial weight (320 mg) was observed at 8.0 of pH level and maximum sclerotia formation at pH 6.5, sufficient at pH 7.0 and few at 6.0 pH level, no sclerotia

formation observed at pH 7.5 and 8.0. Similar results have also been reported by Singh *et al.*, (1999), Singh *et al.*, (1974) and Grosch and Kofoet (2003) who evaluate the effect of pH on mycelium growth and sclerotia formation the fungus was exposed directly to different levels of pH. Maximum dry mycelial weight and sclerotia formation by *Rhizoctonia*

solani were observed at 6.5 pH level. A significantly decrease in dry mycelial weight and sclerotia production were observed at pH 7, 6.0 and 7.5. Maximum sclerotia formation was noticed at pH 6.5 followed by pH 7.0 while no sclerotia formation was observed at pH 7.5 and 8.0.

Table.1 Effect of temperature on mycelial growth and sclerotia formation of *Rhizoctonia solani* after 7 days

Temperature (°C)	Mycelial growth (mm)*	Sclerotia formation
15	50.76	-
20	73.66	+
25	85.00	++
30	90.00	+++
35	84.03	-
SEm±	1.01	
CD (p = 0.05)	3.29	

* Average of three replications

- = Absent, + = Few, ++ = Sufficient, +++ = Abundant

Table.2 Effect of pH on mycelial growth and sclerotia formation of *R. solani* after 7 days at 30 ± 1°C

pH level	Dry weight of mycelial growth (mg)*	Sclerotia formation
6.0	422	+
6.5	630	+++
7.0	546	++
7.5	380	-
8.0	320	-
SEm±	8.67	-
CD (p = 0.05)	28.28	-

* Average of three replications

- = Absent, + = Few, ++ = Sufficient, +++ = Abundant

Table.3 Effect of relative humidity on mycelial growth and sclerotia formation of *Rhizoctonia solani* after 7 days at $30 \pm 1^\circ\text{C}$

Relative humidity (%)	Mycelial growth (mm)*	Sclerotia formation
60	45.25	-
70	66.00	+
80	85.66	+
90	90.00	++
100	90.00	+++
SEm _±	1.20	
CD (p = 0.05)	3.93	

* Average of three replications

- = Absent, + = Few, ++ = Sufficient, +++ = Abundant

Fig.1 Effect of temperature on mycelial growth of *R. Solani* after 7 days

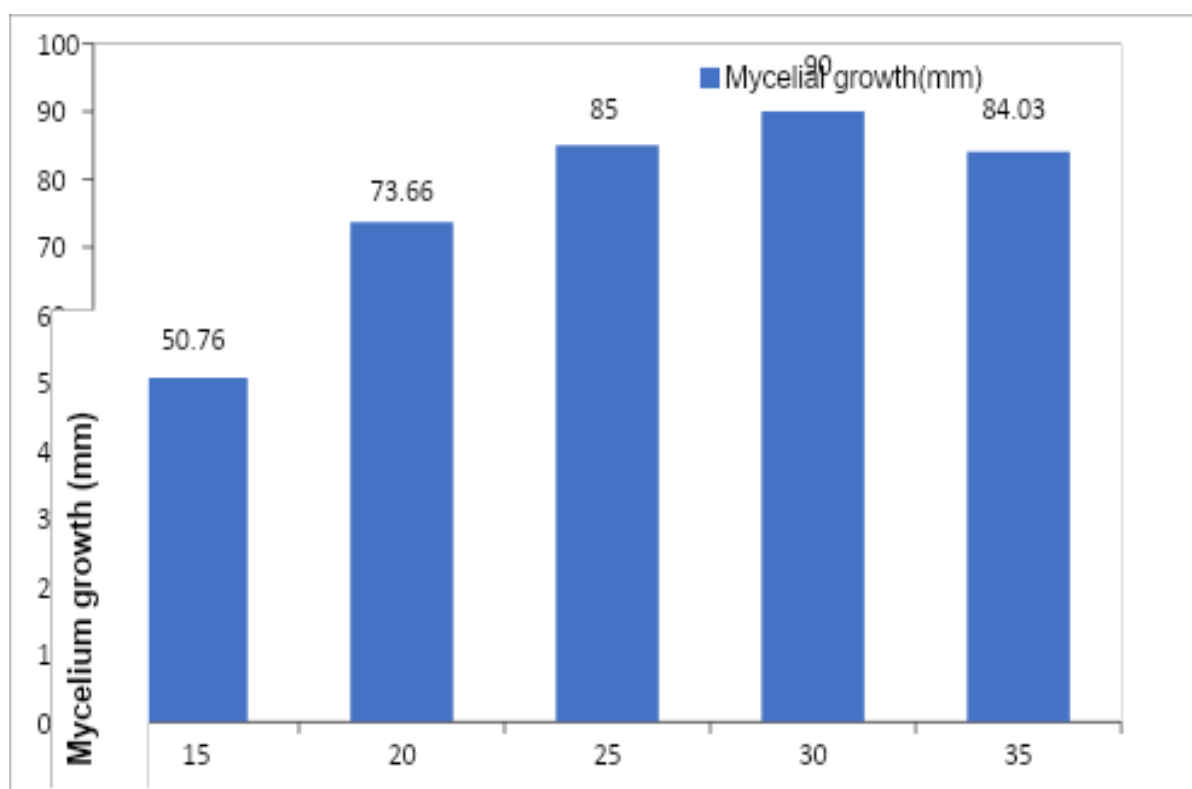


Fig.2 Effect of pH on mycelial growth of *R. solani* after 7 days at $30 \pm 1^{\circ}\text{C}$

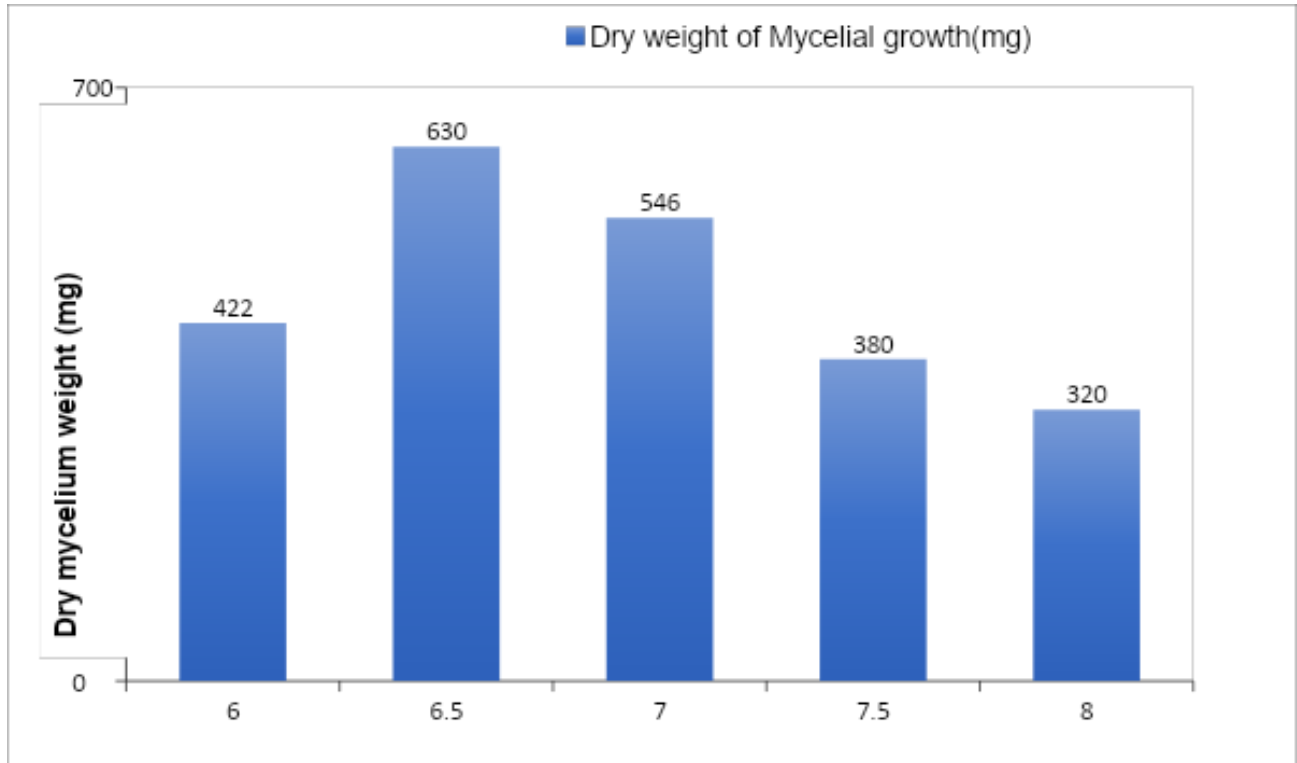
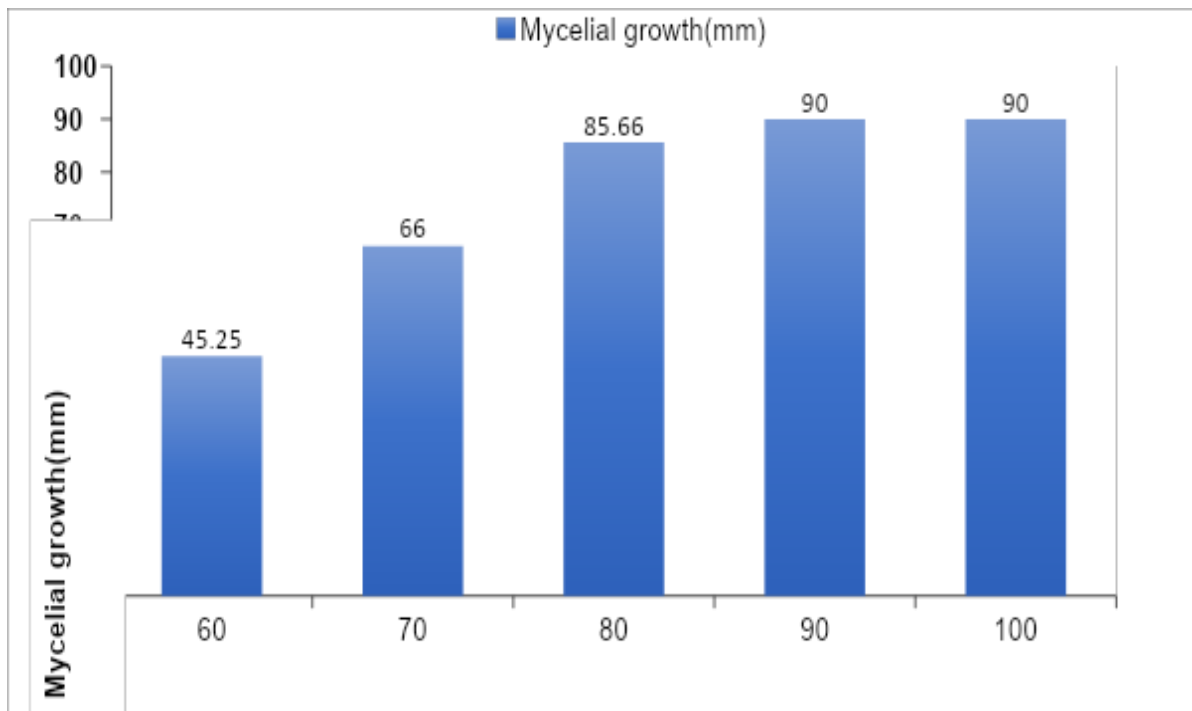


Fig.3 Effect of relative humidity on mycelial growth of *R. solani* after 7 days at $30 \pm 1^{\circ}\text{C}$



Effect of relative humidity

To evaluate the effect of atmospheric moisture, the fungus was exposed directly to different levels of relative humidity viz. 60, 70, 80, 90, 100 per cent and incubated at $30\pm 1^{\circ}\text{C}$ for 7 days. It was observed that all the five humidity levels include the growth of *Rhizoctonia solani*. Perusal of data (Table 3) showed that maximum mycelial growth (90 mm) of *Rhizoctonia solani* was observed at 100 and 90 per cent relative humidity. A significantly decrease in mycelial growth was observed at 80 per cent (85.66 mm) and 70 per cent (66.00 mm) relative humidity. Minimum mycelial growth (45.25 mm) was observed at 60 per cent relative humidity and maximum sclerotia formation at 100 per cent relative humidity, sufficient at 90 per cent and few at 70, 80 relative humidity, no sclerotia formation observed at 60 per cent relative humidity. In the present investigation, it was observed that pathogen (*Rhizoctonia solani*) grew efficiently and sclerotia produced enormously at 80 to 100 per cent relative humidity, whereas, decline was observed at lower humidity levels. Maximum growth and sclerotia formation by *Rhizoctonia solani* has also been observed best at 90 to 100 per cent relative humidity by earlier workers (Ali et al., 1998 and Marcelo and Vega, 1988).

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