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Sunaina Varma
Department of Plant Pathology, College of Agriculture, Swami Keshwanand Rajasthan Agricultural University, Bikaner, Rajasthan, India

Data Ram Kumhar
Head and Professor, College of Agriculture, Swami Keshwanand Rajasthan Agricultural University, Bikaner, Rajasthan, India

Priyanka
Department of Plant Pathology, College of Agriculture, Swami Keshwanand Rajasthan Agricultural University, Bikaner, Rajasthan, India

Mukesh Kumar Sheshma
Department of Plant Pathology, College of Agriculture, Swami Keshwanand Rajasthan Agricultural University, Bikaner, Rajasthan, India

Corresponding Author:
Sunaina Varma
Department of Plant Pathology, College of Agriculture, Swami Keshwanand Rajasthan Agricultural University, Bikaner, Rajasthan, India

In vitro study of different temperature, carbon, nitrogen sources and pH on the sporulation and growth of *Rhizoctonia solani* Kuhn causing root rot of Chilli

Sunaina Varma, Data Ram Kumhar, Priyanka and Mukesh Kumar Sheshma

Abstract
In the present investigation, *in vitro* effect of different temperature, different carbon sources, nitrogen sources and effect of different hydrogen ion concentration were evaluated against *Rizoctonia solani* Kuhn in which mycelial growth was observed and numbers of sclerotia were counted. Among the different temperature *i.e.* 20 ºC, 25 ºC, 30 ºC, 35 ºC and 40 ºC, maximum mycelial growth and sclerotia was observed at 30 ºC. Among the different carbon sources *i.e.* glucose, sucrose, maltose, fructose and lactose maximum mycelial growth and sclerotia were recorded in sucrose source. Similarly different nitrogen sources *i.e.* ammonium chloride, L-alanine, L-agrinine, glutamic acid and ammonium nitrate, most suitable growth media was ammonium nitrate in which maximum mycelial growth and maximum number of sclerotia were recorded and among the different hydrogen ion concentration (pH) levels *i.e.* 6.0, 6.5, 7.0, 7.5 and 8.0 most suitable pH was 7.0 in which maximum mycelial growth and sclerotia were observed.

Keywords: *Rizoctonia solani* Kuhn, carbon sources, nitrogen sources, mycelial growth, sclerotia

Introduction
Chilli (*Capsicum annum* L.) is an important vegetable and spice crop throughout the world. *Rhizoctonia solani* that causes root and stem rot in young transplants, is a major soil borne pathogen of chilli. *Rhizoctonia solani* Kuhn (Teleomorph: *Thanatephorus cucumeris*) is a widespread and destructive fungal pathogen of many plant species. Different types of disease symptoms like damping off, root, crown and stem rot, sheath blight etc. are caused by the pathogen. Narasimhan and Shivakumar, (2016) [9] studied that chilli crop suffers massive yield loss due to root rot caused by *Rhizoctonia solani* and Malhotra *et al.*, (2011) [7] revealed that *Rhizoctonia solani*, which causes damping-off disease of seedlings as well as root and stem rot in young transplants, is a major soil-borne pathogen of chilli (*Capsicum annum* L.). Root rot is very destructive in nature and has been reported from many parts of the world. The disease has registered its presence in almost all the chilli growing areas in India. Grosch *et al.*, (2006) [4] revealed that the widespread soil borne pathogen *R. solani* is responsible for serious damage to many economically important agricultural and horticultural crops as well as trees worldwide.
Root rot incited by *R. solani* is found as an important disease of Rajasthan. It is cosmopolitan and is one of the most successful pathogen which may attack at all stages of plant growth. During warm and dry soil conditions sclerotia directly produce mycelia whereas under favourable conditions ascospores are produced. Mycelium arising from sclerotia causes infection within an area. Sclerotia consist of loosely constructed knots of melanised hyphae with no cellular differentiation. *R. solani* sclerotia only undergo direct myceliogenic germination, whereby vegetative hyphae capable of infecting the host, grow directly out of the sclerotium unlike many other sclerotia forming plant pathogens. The optimal physiological condition for growth and sclerotia production depends upon isolates of *R. solani* and lower and higher optima have been reported for various isolates. Hyakumachi *et al.*, (1988) [5] observed that it is a complex pathogen with great variations among the isolates in terms of myclial colour, zonation, type, and number of sclerotia, size of aerial mycelium, growth rate, saprophytic behaviour, enzyme production and pathogenicity.

Materials and Methods

The pathogen was isolated by infected samples of *Rhizoctonia solani* Kuhn incitant of root rot were collected from chilli diseased fields of different districts of Rajasthan, *viz.* Sri Ganganagar, Hanumangarh, Bikaner (Agricultural Research Station and farmer's field) and Jaipur. The samples collected from diseased plants were used for isolation. The roots were thoroughly washed with tap water to remove soil. Small pieces of about 0.5 cm length were surface sterilized with 1 per cent mercuric chloride solution for 2 minutes, three washings with sterilized distilled water were given, placed on PDA slant in a laminar flow and incubated at 28 ± 1^0C temperature for growth for seven days. To maintain the pure culture of *Rhizoctonia solani*, singal hyphal tip isolation technique was adopted.

The single piece of hypha was demarcated under low power of microscope (10X) and cut with the help of mechanical cutter. Individual piece of hypha was transformed on PDA slants with the help of an inoculating needle. The inoculated slants were kept in Biological Oxygen Device Incubator for growth at 28±1 ºC for 7 days. Thus, the purified cultures of five isolates from diseased chilli field from Rajasthan were maintained by periodical transfers on PDA slants and used for further studies.

Physiological studies

Effect of different temperature

It is a well-known phenomenon that the temperature considerably influences the biochemical activity of pathogens. 20 ml. of PDA was poured in each of sterilized Petri plate. Each Petri plate was inoculated aseptically by placing a 5 mm disc in the centre from actively growing 7 days old culture of pathogen. The inoculated Petri dishes were incubated at 20 ºC, 25 ºC, 30 ºC, 35 ºC and 40 ºC temperatures respectively for 7 days and observations of growth and sporulation were recorded.

Effect of different carbon sources

To find out the effect of various carbon sources *i.e.* glucose, sucrose, maltose, fructose and lactose on mycelial growth of *Rhizoctonia solani*, the sucrose content of basal medium Czapek's dox agar was substituted by adding different sources of carbon on equivalent basis (12.63 g in 30 g of sucrose). Inoculated Petri dishes containing basal medium supplemented by different carbon sources were incubated at 28±1 °C for 7 days and the mycelial growth and sporulation were recorded. Carbon sources used were: glucose, maltose, sucrose, fructose and lactose.

Effect of different nitrogen sources

To find out the effect of various nitrogen sources on mycelial growth of *Rhizoctonia solani*, sodium nitrate of basal medium Czapek's dox agar medium was substituted by adding different sources of nitrogen on equivalent basis (329 mg in 2 g of sodium nitrate) to study the effect of different nitrogen sources on the growth of *Rhizoctonia solani*. The inoculated Petri dishes containing basal medium supplemented with different nitrogen sources were incubated at 28±1 °C for 7 days and observations for mycelial growth and sporulation was recorded. Nitrogen sources studied were: Ammonium chloride, L-alanine, L-agrinine, Glutamic acid and Ammonium nitrate.

Effect of different hydrogen ion concentration (pH)

The study of different pH levels *i.e.* 6.0, 6.5, 7.0, 7.5 and 8.0 were 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 basal medium before autoclaving was adjusted with a difference of 0.5 using N/10 NaOH or N/10 HCl. After autoclaving, the pH was again tested. The inoculated Petri plates were incubated at 28±1 ºC for 7 days and observations of growth and sporulation were recorded.

Result and Discussion

Effect of different temperatures: The effect of temperature on growth of *R. solani* was studied by incubating petri dishes at different temperatures ranging from 20º to 40 ºC. Increasing trend of growth was observed from 20 ºC to 30 ºC. Maximum growth of 47.00 mm was recorded at 30 ºC followed by 36.25 mm at 25 ºC while minimum 15.67 mm at 20 ºC was recorded after 3 days. Maximum growth and number of sclerotia was observed at 30 ºC (90.00 mm and 35 sclerotia) after 5 days. Decreasing trend of growth was found between 35 ºC to 40 ºC and minimum mycelial growth was observed at 40 ºC (35.33 mm) and no sclerotia formation was observed at 40 ºC. On the basis of average (90.00mm) after 5 days, it becomes evident that the maximum mycelial growth of *R. solani* was observed at 30 ºC. There was a significant difference among the different temperatures [Table1].

Table 1: Effect of different temperature on mycelial growth of *R. solani*

Temperature (ºC)	Mycelial growth (mm)*		Mean	No. of Sclerotia
	After 3 days	After 5 days		
20	15.67	22.66	19.17	18
25	36.25	77.66	56.50	23
30	47.00	90.00	68.50	35
35	21.34	41.66	31.50	12
40	20.00	35.33	27.67	-
S.Em (±)	0.08	0.69		
CD (P=0.05)	0.28	2.23		
CV (%)	5.45	2.26		

*****Mean of three replications

Temperature yields considerable effect on growth of fungal organisms. In the present studies increasing trend of mycelial growth of *R. solani* have been observed from 20 ºC to 30 ºC after 3 and 5 days. Maximum growth was observed at 30 ºC. Decreasing trend of growth was found between 35 ºC to 40 ºC and minimum mycelial growth was observed at 20 ºC. Maximum number (35) of sclerotia formation was observed at 30 ºC, while minimum number of sclerotia formation in 35 ºC and scleotia was not formed at 40 ºC. These results show the maximum growth at 30 ºC, which are very much similar and conformity with the results concluded by Goswami *et al.*, 2011 [2] in which they studied the effect of temperature on sclerotia formation and colony growth, 30 °C was the best temperature for the production of sclerotia in *R. solani*. (Grosch and Kofoet, 2003; Ogoshi *et al.*, 1990; Avitia *et al.*, 2013) [3, 10, 1].

Effect of different carbon sources

Table 2 reveals that mycelial growth of *Rhizoctonia solani* was different on Czapek's dox agar basal medium with different carbon sources. Maximum mycelial growth 45.33

mm was recorded in control where sucrose source was used followed by glucose source after 3 days. The growth was gradually increased after 5 days with all the carbon sources. After 5 days maximum mycelial growth 90 mm and number of sclerotia 30 of *R. solani* could be observed on sucrose as compared to glucose, maltose, fructose and lactose. Minimum mycelial growth was in case of lactose, where the mycelial growth was 62 mm and number of sclerotia was 10 after 5 days. No sclerotia were observed before this period.

Table 2: Effect of different carbon sources on mycelial growth of *R. solani*

Carbon sources	Mycelial growth (mm)*		Mean	No. of Sclerotia
	After 3 days	After 5 days		
Sucrose (control)	45.33	90.00	63.17	30
Glucose	42.00	82.33	62.17	23
Maltose	38.67	76.33	57.50	17
Fructose	33.00	68.33	50.67	15
Lactose	31.00	62.00	46.50	10
S.Em (±)	0.86	1.48		
CD (P=0.05)	2.27	4.73		
CV (%)	3.96	3.38		

*****Mean of three replications

Utilization of carbon sources *viz*., glucose, maltose, sucrose, fructose and lactose were tested for their efficacy to support mycelial growth and sclerotial formation of *R. solani* using Czapek's dox agar basal medium. After 72 and 120 hrs, the average maximum radial growth was recorded on sucrose followed by glucose while minimum mycelial growth was recorded on lactose. Maximum number of sclerotia formation was observed on sucrose. This experimental finding supported by (Lakpale *et al.*, 1995; Kumar *et al.*, 2014; Muhsin and Selman, 2013) [11, 6, 8].

Effect of different nitrogen sources

Table 3 reveals the mycelial growth of *Rhizoctonia solani* grown on varied Czapek's dox agar basal medium with different nitrogen sources. Maximum mycelial growth 42.50 mm was recorded where ammonium nitrate (control) was used as nitrogen source followed by glutamic acid and minimum growth 29.57 mm was recorded in ammonium chloride after 3 days. The trend of growth was increasing order after 5 days in all the nitrogen sources. After 5 days, *Rhizoctonia solani* having significantly better growth and more number of sclerotia on nitrogen sources *viz*., Ammonium nitrate (90.00 mm and 37) followed by Glutamic acid (74.66 mm and 32) as compared to Ammonium chloride (59.33 mm and 20).

Table 3: Effect of different nitrogen sources on mycelial growth of *R. solani*

Nitrogen sources	Mycelial growth (mm)*		Mean	No. of Sclerotia
	After 3 days	After 5 days		
Glutamic acid	36.83	74.66	55.75	32
L-alanine	36.00	72.66	54.34	29
L-arginine	32.67	65.66	49.17	24
Ammonium chloride	29.57	59.33	44.45	20
Ammonium nitrate (control)	42.50	90.00	59.92	37
S.Em (±)	0.51	0.86		
CD (P=0.05)	2.64	2.77		
CV (%)	2.71	2.07		

*****Mean of three replications

R. solani having significantly higher growth on nitrogen sources *viz.*, ammonium nitrate, glutamic acid as compared to and ammonium chloride. Maximum number of sclerotia formation was observed on ammonium nitrate, while minimum number of sclerotia formation in ammonium chloride. Among all the tested nitrogen sources ammonium chloride was found least supportive to the mycelial growth and sclerotia formation of *R. solani*. These experimental findings very much similar to findings of Kumar *et al.*, 2014 [6].

Effect of different pH

Hydrogen ion concentration also affected the growth of *Rhizoctonia solani* tested over a wide range of pH. Maximum mycelial growth of 72.86 mm was observed at 7.0 pH followed by 41.66 mm at 7.5 pH while minimum 22.66 mm growth was observed at 6.0 pH after 3 days. The mycelial growth was in increasing trend upto 5 days. Maximum growth and number of sclerotia was observed at 7.0 pH with mycelial growth of 90 mm and 27 sclerotia after 5 days. Minimum mycelial growth 42 mm was observed at pH 6 with 18 sclerotia. Sclerotia was not formed at 8.0 pH. [Table 4].

Table 4: Effect of different pH on mycelial growth of *R. solani*

Ph	Mycelial growth (mm)*		Mean	No. of Sclerotia
	After 3 days	After 5 days		
6.0	22.66	42	32.34	18
6.5	34.33	59.06	46.70	12
7.0	72.86	90	81.44	27
7.5	41.66	70.16	55.92	9
8.0	32.80	61.76	47.29	_
S.Em (±)	1.82	0.92		
CD (P=0.05)	5.81	2.93		
CV (%)	7.72	2.46		

*****Mean of three replications

The mycelial growth of *R. solani* was measured at different pH levels ranging from 6 to 8. Maximum mycelial growth was measured at pH 7.0 followed by pH 7.5 and minimum mycelial growth at pH 6.0 and at pH 8.0. Maximum number of sclerotia formation was observed at pH 7.0 while minimum number of sclerotia formed at pH 7.5 and sclerotia was not formed at pH 8.0. It was thus clear from the finding that the pathogen preferred neutral medium for its growth, which are very much similar and conformity with the results concluded by (Kumar *et al.*, (2014) [6] where they studied that the Maximum mycelial growth was measured 65.1 mm at pH 7.0 and Goswami *et al.*, 2011 [2].

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