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Effect of different physiological parameters on the growth and sporulation of *Rhizoctonia solani* (Kuhn)

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Abstract
Mungbean (*Vigna radiata* L.) is an important pulse crop in Indian continent. Mungbean is infected by several fungal, bacterial and viral diseases but foliar blight disease caused by *Rhizoctonia solaniis* considered as one of the most devastating diseases in the mungbean growing areas of Rajasthan. In the present investigation, effect of different range of temperature, pH, carbon and nitrogen sources with Czapex's dox agar basal medium were evaluated under *in vitro* conditions against *Rhizoctonia solani* Kuhn. Maximum mycelial growth and sclerotia formation of pathogen was observed at 30 ^{0}C. While, minimum mycelial growth and sclerotia formation was at 20 ^{0}C temperature. Similarly, optimum pH for maximum mycelia growth and sclerotia formation of pathogen was 7.0. Maximum mycelial growth (68 mm) and sclerotia formation (32) of *R. solani* was observed on sucrose as compared to other sources. Among all the tested nitrogen sources, glutamic acid supported maximum mycelial growth (57.67 mm) and sclerotia formation (31) as compared to ammonium nitrate 65.50 mm and 36 respectively. Ammonium chloride was found least supportive to the mycelia growth and sclerotia formation 45.42 mm and 20 of the pathogen.

Keywords: *Rhizoctonia solani*, temperature, pH, carbon source, nitrogen sources and Czapex's dox agar

Introduction
Mungbean/green gram *[Vigna radiata* (L.) Wilczek] is one of the most important pulse crop. It is grown in almost all parts of the country. It has proved to be an ideal crop for the spring, summer and *kharif* season. Mungbean belongs to family leguminaceae and sub family papilionaceae. It is the third important pulse crop in the country after chickpea and pigeonpea and covers an area about 29.36 lakh ha with production of 13.90 lakh tonnes (Anonymous 2015) [3]. Mungbean is mainly grown in the states of Rajasthan, Maharashtra, Madhya Pradesh, Orissa, Andhra Pradesh, Tamil Nadu and Uttar Pradesh. In Rajasthan, the total area under cultivation of mungbean was 13.68 lakh ha with the annual production 6.02 lakh tonnes and productivity of 441 kgha^{-1} (Anonymous, 2016) [2]. Mungbean is mainly grown in Bikaner, Jaipur, Bhilwara, Bharatpur, Jodhpur, Kota, Ajmer and Udaipur districts of Rajasthan. It is erect, sub erect deep rooted crop. It requires hot climate and has the capacity to tolerate moisture stress. De-Candolle believes that mungbean has been originated in India. It is grown in these areas since prehistoric period.

This global crop, encounters a number of operational constraints, including pests and diseases that limit its production and yield potentials from seedling to harvest and often provoking grain yield loss of over 35 per cent (Asiwe, 2006) [4]. Brien *et al*. (2008) [5] identified *Rhizoctonia solani* (Kuhn) on the basis of morphological features as causal pathogen on mungbean. *R. solani* is known to affect many crops. It can survive even in the absence of host on dead organic matter or by producing sclerotia or thick walled brown hyphae in plants debris. Sclerotial bodies which are viable over the years. *Rhizoctonia solani* (Kuhn), inciting of foliar blight of mungbean was described by the De Candolle (1815) [6].

In western Rajasthan the incidence of *R. solani* is very severe and hampered the yield production of mungbean crop. Now, it is a well-known soil inhabiting plant pathogen including mungbean, capable of attacking a tremendous range of host plants throughout the world and causing seed decay, damping off, stem cankers, root rots, fruit decay and foliage diseases.

Among the diseases, foliar blight is most severe at seedling and vegetative stage. The disease is characterized by oval or spindle shaped brown–black lesions having length ranging from 0.2-8 cm at soil level near collar region and girdling the basal portion of the stem. Under humid conditions the lesions develop rapidly and coalesces leading to extensive blighting and defoliation (Allen and Lenne, 1998) [1]. The optimal physiological condition for growth and sclerotia production depends upon isolates of *R. solani* and lower and higher optimal have been reported for various isolates (Goswami *et al*. 2011) [8]. Muhsin and Selman (2013) [11] reported the effect of different culture media, temperature, pH, carbon and nitrogen sources for the *R. solani* growth response. Keeping in view the importance of foliar blight disease of mungbean causing by pathogen *R. solani* Kuhn, the present investigations were carried out to unravel the effect of temperature, pH, carbon and nitrogen sources dynamics on mycelial growth and sclerotia production of *R. solani*.

Materials and Methods

Foliar blight affected leaves of mungbean were collected from Crop Research Farm, College of Agriculture, SKARU, Bikaner. The pathogen was isolated from diseased leaves on potato dextrose agar (PDA) and purified through hyphal tip / single sclerotial method (Rangaswami and Mahadevan, 2004) [14]. Pure culture was maintained and stored in refrigerator at 5 ºC for further studies. The different physiological studies were evaluated *in vitro* against *Rhizoctonia solani* Kuhn by following Poisoned Food Technique (Schmitz, 1930) [16].

Physiological Dynamics

Temperature: It is a well-known phenomenon that the different range of temperature considerably influences the biochemical activity of pathogens. The influence of temperature on mycelial growth and sporulation of *R. solani* of mungbean isolate was determined on PDA at 20 ^{0}C, 25 ^{0}C, 30 ^{0}C, 35 ^{0}C and 40 ^{0}C. Mycelial disc from the actively growing three days-old cultures were placed in the centre of Petri dishes and three replications were incubated in incubator maintaining five different temperature levels. The average colony diameter of *R. solani* was assessed at 3 and 5 days and formation of sclerotia was measured after 7 days.

pH: Effect of different hydrogen ion concentration on the growth and sporulation of fungus were studied on growth medium at different pH levels i.e. 6.0, 6.5, 7.0, 7.5 and 8.0. The initial pH of the basal medium before autoclaved 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 30 ± 1 ^{0}C. The average colony diameter of *R. solani* was assessed at 3 and 5 days and formation of sclerotia was measured after 7 days. Three replications were maintained for each pH level.

Biochemical Dynamics

Carbon sources: To find out the effect of various carbon sources on mycelial growth and sporulation of *R. 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). Glucose, maltose, sucrose, fructose and lactose were incorporated separately in the basal medium equivalent to the carbon present in sucrose. Freshly grown 5mm culture disc of *R. solani* was inoculated at the centre of each Petri plates and incubated at 30 ± 1 ^{0}C. Three replications were maintained for each treatment. The average colony diameter of *R. solani* was observed at 3 and 5 days and formation of sclerotia was measured after 7 days.

Nitrogen sources: Effect of various nitrogen sources were studied on mycelial growth and sporulation of *R. 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). The inoculated Petri dishes containing basal medium supplemented with different nitrogen sources i.e. ammonium chloride, L-alanine, L-agrinine, Glutamic acid and ammonium nitrate were incubated at 30 ± 1 ^{0}C. Three replications were maintained for each treatment. The average colony diameter of *R. solani* was recorded at 3 and 5 days and formation of sclerotia was measured after 7 days.

Results and Discussion

Effect of different physiological and biochemical dynamics were evaluated on the mycelial growth and sporulation of sclerotia of *Rhizoctonia solani*. The effects of different range of temperature 20 ºC to 40 ºC were studied on growth and sporulation of *Rhizoctonia solani*. The results depicted that maximum mycelial growth of fungus was recorded 45 mm and 90 mm at 30 ºC after 3 and 5 days incubation. While, minimum growth of the fungus 16 mm and 23 mm at 20 ºC was recorded after 3 and 5 days of incubation. Average mean of mycelial growth of pathogen was 67.5 mm. Similarly, maximum number of sclerotia (37) was recorded at 30 ºC after 5 days of inoculation. Decreasing trend of mycelial growth of test fungus was observed between 35 ºC to 40 ºC temperatures and no sclerotia formation was observed at 40 ºC (Table 1). A number of studies showed that optimal hyphal growth of *R. solani* was observed between 25 ^{0}C to 30 ^{0}C (Grosch and Kofoet, 2003; Tiwari and Khare, 2002) [9, 18]. Goswami *et al.* (2011) [8] 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*, which was followed by 25 °C. This finding corroborated to our studies. Muhsin and Selman (2013) [11], reported that *R. solani* exhibited higher growth and sclerotial production at 30 ^{0}C temperature that was also harmony to our findings. Similarly, Kumar *et al.* (2014) [10] studied the effect of temperature on mycelia growth of *R. solani* AG1-IB urdbean isolate after 24 and 48 hrs of the inoculation. After 48 hrs, the maximum mycelial growth 58.8 mm and 57.2 mm was recorded at 25 °C and 30 °C, respectively which findings result was similar to our studies.

The mycelial growth rate and sporulation of *R. solani* mungbean isolate was measured at pH levels ranging from 6 to 8. The results revealed that maximum fungal mycelial growth was measured 72 mm and 90 mm at pH 7.0 followed by 44 mm and 71 mm at pH 7.5 after 3 and 5 days inoculations and minimum mycelial growth was recorded at pH 6.0 (24 mm and 46 mm) on the same days. Average mean of mycelial growth of pathogen was 81.00 mm. It was thus clearly indicated that pathogen preferred pH 7 medium for their maximum growth and sporulation of sclerotia (Table 2). The results showed that maximum and minimum sclerotial productions (26 and 8) were recorded on medium containing pH 7 and 7.5, respectively. Many fungi are able to grow over a wide pH range, with an optimum between pH 5.5 to 8 (Deacon, 1984) [7]. It has been demonstrated previously that if *R. solani* is able to initiate growth on moderately acid or alkaline media, it will usually modify the pH to one more favourable in order to grow successfully (Sherwood, 1970)

[17]. Muhsin and Selman (2013) [11] reported that *R. solani* exhibited higher growth and metabolite production at pH 6. Mycelial growth of all isolates of *R. solani* occurred between pH 4 and 9, with an optimum of pH 5.6. (Ritchie *et al*., 2009) [15]. This study demonstrated that the pathogen was grown on wide range of pH medium that was partially confirmatory to previous authors' studies. Kumar *et al.,* (2014) [10] studied that the mycelia growth rate of *R. solani* AG1-IB urdbean isolate was measured at pH levels ranging from 5 to 10. Maximum mycelia growth was measured 65.1 mm at pH 7.0 followed by 44.0 mm at pH 6.0 and minimum mycelial growth at pH 5.0 (22.7 mm) and at pH 10.0 (22.9 mm). It was thus clear that the pathogen preferred neutral medium for its growth that corroborated to our findings. Goswami *et al.* (2011) [8] reported the effect of pH on colony growth. All five tested isolates of *R. solani* grew well on PDA medium at all levels of pH within the range of 4-8. The highest colony growth was obtained at pH 7 in case of all the isolates except for isolate DIN-8, where best growth was observed at pH 6. In case of all the tested isolates, significantly lowest growth was observed at pH 4. All the findings revealed that pathogen and its isolates grown at wide range of pH.

The results reveal that mycelial growth of *R. solani* was different on Czapek's Dox Agar basal medium with different carbon sources. Maximum mycelial growth of pathogen was recorded in sucrose containing medium 46 mm and 90 mm followed by glucose 43.33 mm and 81.41 mm after 3 and 5 days inoculation. Similarly, minimum mycelial growth was observed in lactose containing medium 30 mm and 61 mm followed by maltose 37 mm and 75.08 mm after 3 and 5 days inoculation. The growth was gradually increased after 5 days with all the carbon sources. Average mean of mycelial growth of pathogen was 62.37 mm. The highest number of sclerotia (32) was counted in sucrose containing medium followed by sclerotia (22) in glucose containing media after 5 days inoculation. The lactose containing medium supported less production of sclerotia in same conditions (Table 3). Ramteke and Kamble, (2011) [13] reported that among different carbon sources effect, sucrose was the best carbon source followed by lactose and maltose for growth of *Fusarium solani* which is corroborated to our study. Pallavi and Gupta (2013) [12] showed the phosphate solubilisation activity of *Pseudomonas lurida* at different carbon sources and revealed that glucose activated the maximum phosphate solubilisation activity 10th day at 10 ^{0}C followed by maltose at 7th day at 10 ^{0}C. Kumar *et al.,* (2014) [10] reported that utilization of carbon sources viz., dextrose, glucose, maltose, arabinose and cellobiose were tested for their efficacy to support mycelia growth and sclerotial formation of *R. solani* using Asthana and Hawker's medium as the basal medium. After 48 hrs, the average maximum radial growth 43.8 mm was recorded on dextrose followed by maltose (38.9 mm.) and glucose (33.0 mm) which directly supported to our findings.

Rhizoctonia solani was grown on different nitrogen sources containing Czapek's dox agar basal medium. Maximum mycelial growth 38.33 mm and 77 mm was recorded in glutamic acid followed by 37 mm and 73.16 mm in L- alanine as compared to control (ammonium nitrate) 41 mm and 90 mm after 3 and 5 days inoculation. Ammonium chloride containing medium was recorded minimum 31 mm and 59.83 mm mycelial growth of *R.solani* after 3 and 5 days inoculation. Average mean of mycelial growth of pathogen was 57.67 mm. Maximum number of sclerotia (31) was recorded in glutamic acid followed by L-alanine (28) as compared to control (36) (Table 4). Kumar *et al.,* (2014) [10] studied that maximum mycelia growth was observed in nitrogen source asparagine (46.0 mm) followed by potassium nitrate (36.3 mm) after 48 hrs. Maximum number of sclerotia formation was observed on asparagine (58.8) followed by potassium nitrate (48.5) and no sclerotia formation in sodium nitrate. Muhsin and Selman (2013) [11] revealed that $NaNO_3$ was the most suitable for the growth of *R. solani* as compared with the examined nitrogen sources which partially corroborated to our findings. Hence, it can be concluded from the results of the present investigation that *R. solani* grown and sporulated their sclerotia on 30 ^{0}C temperature, pH 7.0; grow best on sucrose and glucose carbon sources and glutamic acid nitrogen source.

Table 1: Effect of different temperature on mycelial growth and sclerotia formation of *Rhizoctonia solani*

Temperature (^{0}C)	Mycelial growth (mm)			No. of Sclerotia
	3 days	5 days	Mean	
20	16.00	23.00	19.5	17
25	38.00	79.00	58.5	24
30	45.00	90.00	67.5	37
35	20.00	43.00	31.5	13
40	19.66	37.16	28.41	-
S Em ±	1.15	1.03	1.09	
CD (P = 0.05)	3.68	3.29	3.48	
CV (%)	7.21	3.28	5.24	

Mean of three replications

Table 2: Effect of different pH on mycelial growth and sclerotia formation of *Rhizoctonia solani*

pH	Mycelial growth (mm)			No. of Sclerotia
	3 days	5 days	Mean	
6.0	24.00	46.00	35.00	18
6.5	35.66	60.00	47.83	13
7.0	72.00	90.00	81.00	26
7.5	44.00	71.00	57.50	8
8.0	35.00	64.00	49.50	-
S Em ±	1.41	1.31	1.36	
CD P = 0.05	4.51	4.20	4.36	
CV (%)	5.81	3.44	4.63	

Mean of three replications

Table 3: Effect of different carbon sources on mycelial growth and sclerotia formation of *Rhizoctonia solani*

Carbon sources	Mycelial growth (mm)			No. of Sclerotia
	3 days	5 days	Mean	
Lactose	30.00	61.00	45.50	11
Glucose	43.33	81.41	62.37	22
Maltose	37.00	75.08	56.04	18
Fructose	35.00	66.83	50.91	16
Sucrose	46.00	90.00	68.00	32
S Em ±	1.06	1.18	1.12	
CD (P = 0.05)	3.39	3.77	3.58	
CV (%)	4.81	2.73	3.77	

Mean of three replications

Table 4: Effect of different nitrogen sources on mycelial growth and sclerotia formation of *Rhizoctonia solani*

Nitrogen Sources	Mycelial growth (mm)			No. of Sclerotia
	3 days	5 days	Mean	
Glutamic acid	38.33	77.00	57.67	31
L-alanine	37.00	73.16	55.00	28
L-arginine	34.00	64.00	49.00	24
Ammonium chloride	31.00	59.83	45.42	20
Ammonium nitrate (Control)	41.00	90.00	65.50	36
S Em ±	1.29	1.18	1.24	
CD (P = 0.05)	4.12	3.77	3.95	
CV (%)	6.16	2.81	4.49	

Mean of three replications

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