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Effect of physiological parameter on the radial growth and sclerotial formation of *Rhizoctonia solani* Kuhn causing web blight of mungbean

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Abstract
Rhizoctonia solani Kuhn, is one of the most important pathogens of mungbean, causing aerial blight, which accounts for heavy losses in mungbean growing areas of MP. Three most common factors associated with the growth and sclerotia formation of pathogen is media, pH and temperature which has been studied in the present study. All three factors significantly affect the growth of the fungal mycelia and sclerotial production. In this investigation, The growth of *R. solani* was investigated using a total of seven solid mediums from which PDA media produced maximum mycelium growth (90.00 mm) and from seven different liquid media Potato dextrose broth (776.33 mg) produced the highest dry mycelial weight. The mycelium growth was tested at different temperatures *viz*., 10, 15, 20, 25, 30, 35 and 40°C in *in vitro* conditions, among which maximum mycelium growth was observed at 30°C (88.33 mm) and among different pH *viz*., 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 the highest mycelium growth was recorded at pH 7.0 (89.10 mm).

Keywords: *Rhizoctonia solani*, media, pH, temperature, web blight

Introduction
Mungbean (*Vigna radiata* (L.) Wilczek) is an important pulse crop In Madhya Pradesh. It belongs to family the Fabaceae or Leguminous. The origin of mungbean is the Indian subcontinent. It is also known as golden gram, mung and green gram. For India's vegetarian population, the mungbean is a significant source of nutritional proteins, vitamins and minerals. Mungbean seeds contain 22.9 gm protein, 61.8 gm carbohydrate, 1.2 gm fat, 4.4 gm crude fibre and 3.5 gm ash per 100 gm of the sample (Adsule *et al*., 1986) [1]. Sprouting, there is an increase in thiamine, niacin, and ascorbic acid concentration. Mungbean has been demonstrated to have anti-hypertensive, anti-inflammatory, anti-diabetic and anti-tumour properties. (Matousek 2009) [14]. The poor productivity of the mungbean has likely been caused by a number of biotic and abiotic factors. Among the various biotic factors, Web blight disease of the mungbean caused by *Rhizoctonia solani* Kühn [Teleomorph: *Thanatephorus cucumeris* (Fr.) Donk] is recognized as one of the most serious limitations. In MP, it affects mungbean productivity by up to 20–30%. Grain production and seed quality are seriously affected by this disease. The only sclerotia of *R. solani* undergo direct myceliogenic germination, through which vegetative hyphae capable of infecting the host, grow directly out of the sclerotium unlike many other sclerotia forming plant pathogens (Coley-Smith JR and Cooke RC.1971) [3]. At present, very little information is available on the survival of *R. solani* isolated from mungbean. Therefore it was important to study the effect of pH, temperature and media on mycelia growth and sclerotia formation of the pathogen.

Materials and Methods
Isolation of pathogen
The leaves of mungbean, which showed the characteristic symptoms of web blight disease was collected from research farm of Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur M.P. For pathogen isolation, the lesions that displayed the first and most noticeable symptoms were selected. Using sterile water, infected leave was washed to remove any remaining soil. and control the surface contamination. With a disinfected blade, young diseased leaves were divided into 2 to 3 mm pieces (containing 2/3 healthy and 1/3 diseased sections).

These leaf pieces were surface sterilized using a solution of 1% sodium hypochlorite and after sterilization, leaf pieces were washed with distilled water thrice. Then, under aseptic circumstances in the inoculation chamber, they were put on pre-sterilized blotter paper to make them free of any excessive moisture. The Pieces of the diseased leaves were transferred directly to the PDA (Potato Dextrose Agar) medium. Petri plates were kept in B.O.D. (Biological Oxygen Demand) Incubator at a temperature of 30± °C for 48 hrs.

Identification of the isolate of *R. solani*

Pathogens were identified based on cultural and morphological traits. Slide was prepared with lactophenol stain and for the morphological characteristics inspected under a compound microscope.

Effect of culture media

The seven cultures medium listed below were tested to see which was the best for sclerotia formation and mycelial growth.

1. Potato Dextrose agar medium (PDA) (peeled and sliced potato 200g, Agar-agar 20g, Dextrose 20g)
2. Richard's agar (RA) medium (Potassium monobasic phosphate 5g, Potassium nitrate 10g, Magnesium sulphate 2.5g, Ferric chloride 0.02g, Sucrose 50g, Agar-agar 20g)
3. Czapek's Dox agar (CDA) medium (Sodium nitrate 2g, Magnesium sulphate 0.5g, Di potassium phosphate 1g, Ferrous sulphate 0.01g, Potassium chloride 0.5g, Sucrose 30g, Agar-agar 20g)
4. Asthana and Hawker's medium (D-Glucose 5g, potassium dihydrogen phosphate 1.75g, Potassium nitrate 3.50g, Magnesium sulphate 0.75g, Agar-agar 20g)
5. Ashby's agar medium (Mannitol 20g, Magnesium sulphate 0.2g, Di potassium phosphate 0.2g, Potassium sulphate 0.1g, Sodium chloride 0.2g, Calcium carbonate 5g, Agar-agar 15g, final pH (at 25 ℃) 7.4±0.2).
6. Brown agar (BA) medium (Dextrose 2g, Magnesium sulphate 0.75g, Tri basic potassium phosphate 1.25g, Agar-agar 20g)
7. Coon's agar (CA) medium (Sucrose 7.2g, Magnesium sulphate 1.23g, Potassium diphosphate2.72g, Potassium nitrate 2.02g, Dextrose 3.60g, Agar- agar 15g).

Effect of pH

Using a calibrated pH metre, potato dextrose agar's pH was adjusted to eight different values, including 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0. Using 0.1 N NaOH or 0.1 N HCl solutions before the PDA medium was autoclaved. Three replications were kept for each pH value. 5mm mycelium discs were used to inoculate on Petri plates containing sanitized PDA media, which were then incubated at a temperature of 30±1 °C. The mycelium growth was measured at48 hours and 96 hours. Sclerotia formation test ranges on various pH levels were reported.

Effect of temperature

The purpose of the studies was to determine the ideal temperature for mycelial growth and sclerotia formation of *R. solani*. The 5 mm disc of the test pathogen was inoculated on sterilized PDA medium, poured in petri discs. Three replications were kept for each different temperature. The petri dishes were incubated at seven different temperature 10 ℃, 15 ℃, 20 ℃, 25 ℃, 30 ℃, 35 ℃ and 40 ℃. The mycelium growth was recorded at 48 hours and 96 hours and sclerotia formation was also recorded.

Results and Discussion

Solid medium

The growth of *Rhizoctonia solani* was investigated using a total of seven solid mediums (Table 1). Maximum mycelium growth was found on PDA medium. Czapek's agar media came in second place with a colony diameter of 79.33 mm, followed by Richard's agar medium (56.83 mm). Minimum colony growth diameter was recorded in Ashby's agar medium. After 15 days of inoculation the formation of sclerotia was observed in which excellent sclerotia formation was seen in Potato dextrose agar medium and Czapek's Dox agar medium. Good sclerotia was seen in Richard's agar medium, fair in Brown's agar and Coon's agar medium. Asthana and Hawker's agar and Ashby's agar medium exhibited poor sclerotia formation. The present finding has been supported by other workers. According to Huang-Shi Chen and U-XiYing (2004) [6], PDA was the best growing medium for *Rhizoctonia solani*. and Ritchie *et al.*, (2009) [17] also observed from research and conclude that in all the tested media for *R. solani* the number of sclerotia produced was more when they were grown on PDA.

Liquid medium

Seven different liquid mediums were used to study the growth of *Rhizoctonia solani.* Potato dextrose broth (776.33 mg) produced considerably more dry mycelial weight in comparison to the other liquid media. Richard's broth (601.67 mg) was the next best medium in terms of merit. Dry weight of mycelium was good in Czapek's Dox broth (589.33 mg) and Coon's broth (382.67 mg) but very poor in Brown's broth, and Ashby's broth (Table 1). The development of sclerotia was seen after 15 days of inoculation. On Potato dextrose broth, Czapek's Dox and Richard's broth, *R. solani* produced excellent sclerotia. On Asthana and Hawker's broth, Coon's and Brown's broth it was found to be fair. Although on Ashby's broth, it was poor. These findings resemble with the observations of Khan *et al.*, (2016) [9] also found that the *R. solani* biomass produced on potato dextrose broth was the highest.

Temperature

The growth of *R. solani* was studied at seven different temperature levels viz. 10, 15, 20, 25, 30, 35 and 40 °C. the maximum radial growth was recorded at 30 °C (88.2mm), followed by 25 °C (79.2mm), 20 °C (72.06 mm), 35 °C (65.50 mm), and 40 °C (29.40 mm).and minimum radial growth was recorded at 10 ℃ (18.3 mm) (Table 2, Figure-1). After 15 days the formation of sclerotia was observed. Abundant sclerotia production was found at 30 °C and 25 °C. Sclerotia did not develop at 10 °C, 15 °C, or 40 °C. The present findings have been supported by other researchers Meena and Chattopadhyay (2002) [15] also found highest growth of *R. solani* found at 30 °C temperature. Grosch and Kofoet (2003) [7] also found that 20°-30 °C temperature was best for growth of *R. solani.*

pH

The effect of seven pH levels, namely 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0, on sclerotia formation and radial growth of *R.*

solani was investigated (Table 3; Figure -2). The maximum colony diameter was found at pH 7.0 (89.10 mm). The next maximum growth was recorded at 6.5 pH (77.06 mm) followed by 7.5 pH (74.43 mm). Pathogen growth was reportedly inhibited by pH values of 5.0 and 8.0 and the growth was 17.06 mm and 23.40 mm, respectively. These results give conformance to the finding of Kumar *et al.*, (2014) [10] found that the optimum pH for mycelium growth and sclerotia production was pH 7.0 reported that best growth and slcerotia formation of *R. solani* occurred best at pH 6.0.

Table 1: Effect of solid and liquid media on radial growth and sclerotia formation *Rhizoctonia solani*

Name of the medium	Solid media		Liquid media	
	Colony diameter (mm) after 96 hrs*	Sclerotia formation	Dry mycelium weight (mg) after 21 days	Sclerotia formation
Potato dextrose agar	90.00	++++	776.33	++++
Czapek's agar	88.17	++++	589.33	++++
Richard's agar	87.20	++++	601.67	+++
Coon's agar	72.27	+++	382.67	++
Brown's agar	65.40	++	147.10	+
Asthana and Hawker's agar	60.43	++	263.00	++
Ashby's agar	23.40	+	102.10	+
SE(m) ±	1.163		1.298	
CD(0.05)	3.562		2.342	

*****mean of three replications

++++ excellent Sclerotia formation, +++ good Sclerotia formation, ++ Fair Sclerotia formation, + poor Sclerotia formation

Table 2: Effect of different temperature on radial growth and sclerotia formation *Rhizoctonia solani*

S. No.	Temp.℃	Colony diameter (mm) after 96 hrs*	Sclerotia formation
1	10	18.33	+
2	15	56.50	+
3	20	72.06	++
4	25	79.06	++++
5	30	88.33	++++
6	35	65.50	+
7	40	29.40	+
SE(m) ±		0.525	
CD(0.05)		1.607	

*****mean of three replications

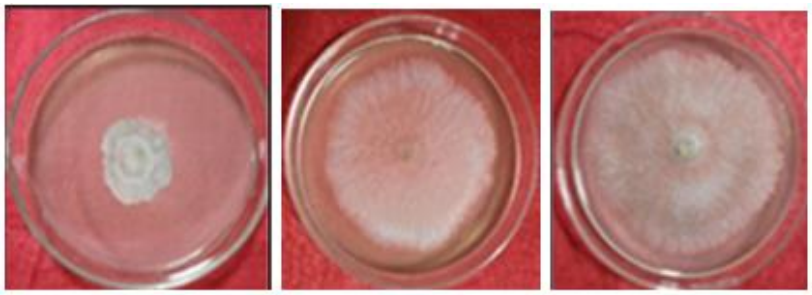
++++ excellent Sclerotia formation, +++ good Sclerotia formation, ++ Fair Sclerotia formation, + poor Sclerotia formation

Table 3: Effect of various pH levels on radial growth and sclerotia formation *Rhizoctonia solani*

S. No.	pH	Colony diameter (mm) after 96 hrs*	Sclerotia formation after 15 days
1	5.0	17.06	+
2	5.5	52.03	+
3	6.0	70.10	+++
4	6.5	77.06	++++
5	7.0	89.10	++++
6	7.5	74.43	+++
7	8.0	23.40	+
SE(m) ±		0.719	
CD(0.05)		2.202	

*****mean of three replications

++++ excellent Sclerotia formation, +++ good Sclerotia formation, ++ Fair Sclerotia formation, + poor Sclerotia formation



10 °C. 15 °C 25 °C

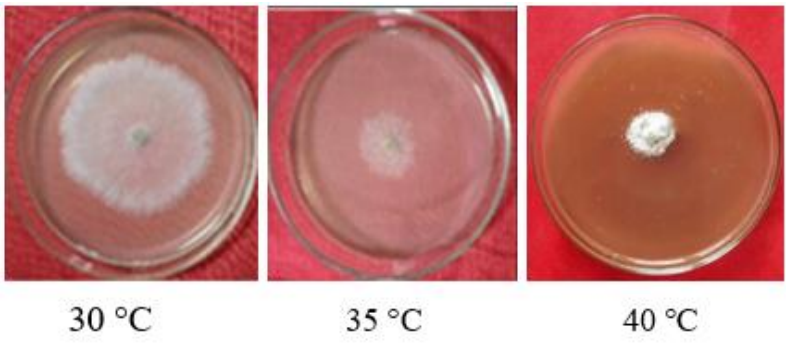


Fig 1: Effect of different temperatures on mycelium growth of *R.solani*

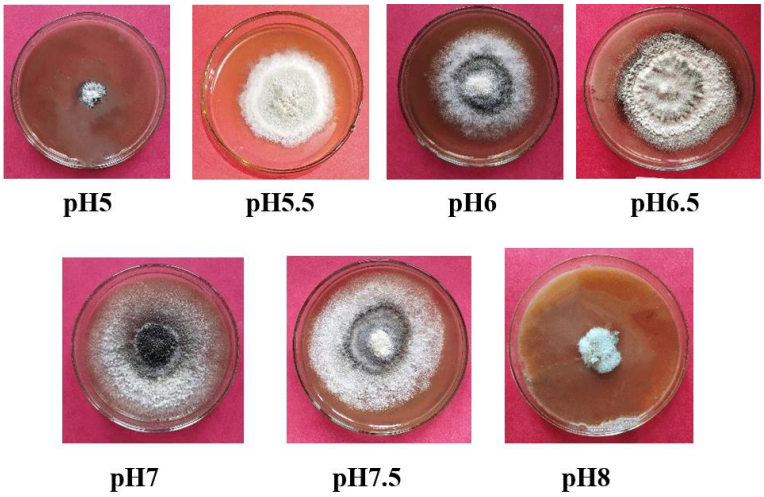


Fig 2: Effect of different pH on mycelium growth of *R. Solani*

Conclusion

Rhizoctonia solani Kuhn causes aerial blight, which contributes for significant losses in MP mungbean growing areas. The three most common parameters linked with pathogen growth and sclerotia formation addressed in this study are medium, pH, and temperature. All three of these parameters have a substantial impact on the proliferation of fungal mycelia and sclerotial formation. PDA media yielded the most mycelium growth (90.00 mm), whereas Potato dextrose broth (776.33 mg) yielded the greatest dry mycelial weight of the test pathogen. Mycelium growth was greatest at 30 °C (88.33 mm) and pH 7.0 (89.10 mm).

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