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Assessing the effects of different Media, Temperature and pH on the Growth and Sclerotial Formation of *Rhizoctonia solani* Kuhn causing web Blight of Urdbean

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ABSTRACT: *Rhizoctonia solani* Kuhn, a primary urdbean pathogen, produces web blight disease, which causes significant crop losses in all of Madhya Pradesh's key urdbean-growing regions. A range of abiotic elements influence this soil-borne disease. The current study looked at medium, pH, and temperature as the three key factors that influence *Rhizoctonia solani* development and sclerotia formation. Each of the three, including the media, Temperature and pH have a significant impact on the rate of growth of fungal mycelial and sclerotial production. Fungal growth on PDA was most active at pH 7.0 and 30°C. There was no growth observed below 15°C.

Keywords: Media, Ph. *Rhizoctonia solani* Temperature, Web blight.

INTRODUCTION

Vigna mungo (L.) Hepper's Urdbean is an important pulse crop of Madhya Pradesh It is a nutritious, short duration crop with a good source of digestive protein (25-28%), carbohydrate (62-65%), fiber (3.5-4.5), ash (4.5-5.5) on dry basis. it also contains a lot of phosphoric acid (Singh, 1982). In India during *kharif* 2020-21, area covered under black gram is 30.48 lakh ha with the production of 19.48 lakh tones and 441 kg/ha productivity. Madhya Pradesh is the major producer of black gram with 14.38 lakh ha area and 8.29 lakh tons production (Annonymous, 2020).

The fundamental cause for urd bean's low production is the crop's vulnerability to a variety of biotic and abiotic limitations. Among the biotic constraints, urdbean Web blight caused by *Rhizoctonia solani* Kuhn [Teleomorph stage: *Thanatephorous cucumeris* (Frank) Donk] is regarded as one of the most serious. Depending on the circumstances, yield losses from this illness range from 20 to 30%. Water soaking areas on the leaves are followed by browning or necrotic circular lesions. As a result, infected leaves die and fall off, and pod symptoms include irregular brown lesions that may merge to form big areas, as well as seed damage on affected pods (Dwivedi and Saxena 1974) which causes significant loss to the crop and economic loss to the farmers. On collar region, reddish brown lesion occurs causing collar rot and resulting in stem canker symptoms (Kaiser, 1970).

The best physiological conditions for growth and sclerotia generation vary depending on the isolate of *R. solani*, with lower and higher optimal values observed for different isolates (Goswami *et al.*, 2011; Muhsin and Selman 2013). There is currently insufficient information on the survival of *R. solani* isolated from urdbean in Madhya Pradesh. With the importance of web blight disease of urdbean caused by pathogen *R. solani* Kuhn in mind, the current study was carried out to determine the influence of different culture media, temperature, and pH on *R. solani* growth and sclerotia generation.

MATERIAL METHOD

Isolation of pathogen. The leaves of urdbean, which had the typical indications of web blight disease, were gathered from the research farm of Jawaharlal Nehru Krishi Vishwa Vidyalaya in Jabalpur, Madhya Pradesh. Pathogen isolation was performed on lesions that presented the early and prominent clinical signs. To eliminate residual soil and control surface contamination, infected leaves were rinsed with sterile water. Young diseased leaves were cut into 2 to 3 mm pieces with a sterilized blade (containing 2/3 healthy and 1/3 sick portions). These leaf fragments have been surface sterilized. After sterilization, leaf pieces were rinsed three times with distilled water in a solution of 1% sodium hypochlorite. 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 BOD incubator at a temperature of 30±°C for 48 hrs.

Identification of the isolate of *R. solani.* The pathogen was identified on the basis of cultural and morphological traits. Slide was prepared with lactophenol stain and for the morphological characteristics inspected under a compound microscope.

Cultural characteristics and Radial growth. *Rhizoconia solani* cultural features were investigated using seven different culture media, including Potato Dextrose Agar (PDA), Czapek's Dox Agar (CZDA), Asthana and Hawkers agar, Coons agar, Richard's medium, Browns agar, and Ashby's agar. Each culture medium was prepared in one litre of water and autoclaved for 20 minutes at 121.6°C and 15 pressure. After cooling to 45°C, they were placed on 90 mm Petri plates for solidification.

Effect of different temperature level on radial growth and sclerotia formation of *R. solani.* To investigate the influence of temperature on the radial growth of *R. solani* isolated from urdbean, five different temperatures were chosen: 10, 15, 25, 30, and 35°C. Each therapy was repeated three times. Each Petri plate received 20 ml of sterilized PDA medium. When the media had set, equal mycelial discs (5 mm in diameter for fungal growth) were cut with a sterilized cork borer and inserted (inoculated) in the center of each Petri plate. For radial development and sclerotia production, the inoculated Petri plates were incubated at various temperatures. The radial growth of the fungus was observed after 48 and 96 hours.

Effect of various pH levels on radial growth and sclerotia formation of *R. solani.* The set of different pH levels of 5, 5.5, 6, 6.5, 7, 7.5, and 8 were created, and pH was altered in the PDA medium by adding suitable amounts of HCL and NaOH. There were three replications for each pH value. PDA was used as the basal medium. The media was pipetted into a 100 ml Erlenmeyer flask, and the pH of the medium was adjusted to get the appropriate level by adding 0.1 N HCL or 0.1 NaOH. With the use of a sterilized cork borer, the flasks containing sterilized medium were infected with a 5 mm mycelial disc cut from the margin of a 3 day old culture of *R. solani.* To quantify mycelial growth, inoculated Petri plates were incubated at 301°C for 72 hours.

RESULT AND DISCUSSION

Effect of solid different solid and liquid media culture media. All the tested media supported the growth of *R. solani* varied significantly on all media tested. PDA supported maximum mycelial growth (90 mm) and also support sclerotia production followed by Czapek's dox Agar (88.3 mm), Richard's agar and coon's agar (72.1 mm) (Table 1). The Brown`s agar, Asthana and Hawker's agar and supported mycelial growth to the tune of 65.3, 60.3 and 23.3 mm respectively. No sclerotia production was found Asthana and Hawker's agar and Ashby's agar medium. Minimum mycelial growth was recorded on Ashby's agar (23.3 mm). Growth pattern was observed as abundant in Potato dextrose agar Czapek's Dox agar and Richard's agar medium and moderate in Asthana and Hawker's agar, Coon's agar and Brown's agar whereas slight were found in Ashby's agar. Ritchie *et al.* (2009) concluded from their study that in all the tested isolates of *R. solani* number of sclerotia produced were more when they were grown on PDA. Sharma *et al.* (2013); Kumar *et al.* (2014) also reported that PDA medium supported maximum mycelial growth which support the result of present study.

Among the various liquid media tested, significantly higher dry mycelial weight and microsclerotia formation was yielded in Potato dextrose broth (776.0 mg) as compared to the rest of the liquid media. The next best medium in order of merit was Richard's broth (602.0 mg). Jha and Dubey (2000); Tandel *et al*. (2012) also found maximum mycelial and microsclerotia production of *R. solani* on Richard's medium followed by potato dextrose broth. Tandel *et al*. (2012) also found maximum mycelial and microsclerotia production of *R. solani* on potato dextrose broth. The present finding tallies with present results.

Effect of various temperature levels on radial growth and sclerotia formation of *R. solani.* The maximum radial growth (88.2 mm) and sclerotia production was recorded at 30°C followed by 25°C (79.2 mm), 20°C (72.2 mm), 35°C (65.5 mm), 40°C (29.2 mm) after 96 hrs. The minimum radial growth (18.3 mm) was recorded at 10 °C after 96 hrs (Table 2). Growth pattern was observed as abundant in 25 and 30°C and moderate at 20 and 35°C and slight at 10, 15 and 40°C. Hemalatha and Singh (2019) reported that 30°C temperature found significantly superior in mycelia radial growth which supports our findings. Singh *et al.* (1974) found that fungal growth of *R. solani* was maximum at 30°C. Tiwari and Khare (2002) reported optimum hyphal growth at 25°C while sclerotia production was observed at 30-35°C in various media.

Effect of various pH on radial growth and sclerotia formation of *R. solani.* The optimal pH for the radial growth and sclerotia formation of *R. solani* was 7 followed by 6.5 and 7.5. A pH of 8 and 5 was the least favorable for the pathogen (Table 3). Singh *et al.* (1974) reported similar results and observed that pH 7.0 was good for mycelia growth as well as sclerotial production of *R. solani*. Datta *et al.* (2014) reported that best growth and sclerotia formation of *R. solani* occurred best at pH 6.0.

Table 1: Effect of solid and liquid media on radial growth and sclerotia of *R. solsni.*

Sr. No.	Name of the medium	Solid Media		Liquid Media	
		Colony diameter (mm) after 96 hrs*	Sclerotia production	Dry mycelial weight (mg) after 21 day*	Sclerotia Production
1.	Potato dextrose agar	90.0	++++	776.0	++++
2.	Czapek's Dox agar	88.3	++++	590.0	++++
3.	Richard's agar	82.4	+++	602.0	++++
4.	Coon's medium	72.1	++	383.0	+++
5.	Browns medium	65.3	++	147.3	++
6.	Asthana and Hawker's agar	60.3	+	263.0	++
7.	Ashby's agar	23.3	-	102.3	-
	CD (0.05)	2.46		0.039	

*****Average of 3 replications

Table 2: Effect of different temperature level on radial growth and sclerotia formation of *R. solani.*

Sr. No.	Temp. ^{o}C	Colony diameter (mm) after 96 hrs*	Sclerotia production
1.	10	18.3	-
2.	15	56.3	-
3.	20	72.2	+
4.	25	79.2	++++
5.	30	88.2	++++
6.	35	65.5	+
7.	40	29.2	-
	CD (0.05)	2.22	

*Average of 3 replications

Table 3: Effect of different Ph level on radial growth and sclerotia formation of *R. solani.*

Sr. No.	pH	Colony diameter (mm) after 96 hrs*	Sclerotia production
1.	5.0	17.1	-
2.	5.5	52.0	-
3.	6.0	70.3	++
4.	6.5	77.2	++++
5.	7.0	89.3	++++
6.	7.5	74.2	++
7.	8.0	23.6	-
CD (0.05)			

*Average of 3 replications

CONCLUSION

Rhizoctonia solani Kuhn, a primary urdbean pathogen, produces web blight disease, which causes significant crop losses in all of Madhya Pradesh's key urdbean-growing regions. The current study looked at medium, pH, and temperature as the three key factors that influence *Rhizoctonia solani* development and sclerotia formation. The test pathogen growth on PDA was most active at pH 7.0 and 30°C.

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Conflict of Interest. None.

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