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Optimization of pH, temperature, and sugar concentration for the growth of *Rhizoctonia solani* incitant of sheath blight of rice

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Abstract
Rice (*Oryza sativa* L.) is one of the primordial cultivated crops whose productivity is affected by several pathogens, among which, sheath blight, is responsible for yield loss of up to 45%. The pathogen associated with sheath blight (ShB) is *Rhizoctonia solani* Kuhn. pH, temperature, and sugar concentration play an important role among different factors affecting fungi growth and spread. The purpose of the research was to study the effect of pH, temperature, and sugar concentration on the growth of *R. solani* isolates of rice based on the radial mycelial growth on potato dextrose agar. However, *in vitro*, the maximum mycelial growth of the majority of isolates was observed in pH 5 and 7, with temperatures 30 °C and 34 °C, and with higher sugar concentrations. Optimum growth was found at 5 pH, 30 °C temperature, and 20 g/L sugar.

Keywords: Rice, sheath blight, *Rhizoctonia solani*, pH, temperature, sugar concentration

Introduction
Rice (*Oryza sativa*), the staple cereal component, sustains two-thirds of the global population. Rice serves as an important source of energy, fibre, minerals, vitamins, proteins, and antioxidants. Due to the supreme energy and nutritive value, the main living of human beings relies on rice. (Burlando & Cornara, 2014; Goufo & Trindade, 2014; Juliano, 1993) [1, 2, 6].
The largest producer of rice is China followed by India. Several pathogens affect the productivity of rice which has major constraints on production. Among the several pathogens, *Rhizoctonia solani*, which is the causal agent of sheath blight (ShB), is accountable for yield loss up to 45% (Margani and Widadi 2018) [9]. *Rhizoctonia solani* Kunh AG1- IA is a soil-borne saprotroph and facultative parasite. Lesions are caused by the pathogen which in turn affects grain filling and yield in rice (Wu *et al*. 2012) [12]. Crucial symptoms of the disease caused by the pathogen include damping-off and root, crown, and stem rot (Lahlali and Hijri, 2010; Naher *et al*., 2014) [8, 10]. Sundry crops having economic importance globally are often affected by *R. solani* including tomato, beans potato, soybean, tulip, and strawberry (Kotba *et al*., 2018; Lahlali and Hijri, 2010; Ismail & Ismail, 2011; Naher *et al*., 2014) [7, 8, 5, 10].
R. solani species show variability in different temperatures, pH, and sugar levels. There is a diversity of isolates of *R. solani* in behavior and growth under different pH, sugar concentrations, and temperatures. So, the purpose of this study was to find out the effect of temperatures, pH, and sugar concentration on the growth of *R. solani* isolates of rice based on the radial mycelial growth on potato dextrose agar.

Materials and Methods
From the Department of Plant Pathology, College of Agriculture, C.A.U, Imphal ten identified pathogens were collected. On Potato Dextrose Agar (PDA) each of the isolates was maintained as a pure culture. Subculturing of isolates of *Rhizoctonia solani* was done on PDA medium on petri plates (90 mm in diameter) for assessment of growth characters.

To optimize the sugar concentration for different isolates of *R. solani*
150ml of PDA media at different concentrations of sugar (10 g, 15 g, 20 g, 25 g, and 30 g) was prepared in conical flasks (250 ml) per litre. Using the digital pH meter, the pH of the medium was adjusted with 0.1N Sodium hydroxide and 0.1N Hydrochloric acid.

Sterilization of all conical flasks with media was done at 15 lb /inch^2 for 20 minutes in an autoclave. 20 ml PDA medium for each sugar level was poured into each sterilized petri plate and is allowed to solidify. From the actively growing cultures (24 hours old) of different isolates, five mm mycelial discs were placed on the center of the petri plates. For each treatment, three replications were be maintained. Incubation of the inoculated plates was done at 30 ℃ for 2 days. The growth of all the ten isolates was measured radially (in cm) after 2 days of inoculation.

To optimize the pH for different isolates of *R. solani*

To study the mycelial growth variation of isolates five different levels of pH (4, 5, 6, 7, 8) were maintained. The pH was adjusted with HCI or NaOH before the solidification of the PDA media. 150 ml PDA is prepared in five conical flasks (250 ml) and then all conical flasks with media were sterilized at 15 lb /inch^2 for 20 minutes in an autoclave. 20 ml PDA medium was poured into each sterilized petri plate and allowed to solidify for each pH level. From the actively growing cultures (24 hours old) of different isolates, five mm mycelial disc was placed on the centre of the petri plates. For each treatment, three replications were maintained. Then the inoculated plates were incubated at 30 ℃ for 2 days. The radial growth (in cm) of all the ten isolates was measured after 2 days.

To optimize the temperature for different isolates of *R. solani*

Five conical flasks (250 ml) of 150 ml PDA were prepared and the pH of the medium will be adjusted with a help of a digital pH meter using 0.1N Sodium chloride and 0.1N Hydrochloric acid. For 20 minutes in an autoclave, the conical flasks with media were sterilized at 15 lb /inch. Twenty ml of PDA medium was poured into each sterilized petri plate. Then it is allowed to solidify. Five mm mycelial discs were placed on the center of the petri plates from the actively growing cultures (24 hours old) of different isolates. For each treatment, three replications were maintained. Plates that are inoculated were incubated at five different temperatures i.e., 26 ℃ 28 ℃, 30 ℃, 32 ℃, 34 ℃ for 2 days. The growth of all the ten isolates will be measured radially (in cm) after 2 days.

Results and Discussion

To optimize the sugar concentration for different isolates of *R. solani*

Sugar concertation shows a significant effect on the radial growth of different isolates of *R. solani*. The radial growth increases with the increase in sugar concentration but up to a certain concentration (20 g/L). The sugar concentration, 20g/L, shows the maximum growth (8.27 cm) (Table 1) for all the isolates. The maximum radial growth was shown by RS-10 (8.42 cm) and the least growth was shown by RS-9 (6.09 cm) (Table 1). As per the density graph (Fig. 1), RS-10 shows the least diversity in growth whereas RS-2, RS-5, and RS-1 show variation in growth from 7.00cm to 8.20cm. The rest of other isolates show wide variation in growth from 4.00cm to 8.00cm. The results were in agreement with the findings of Ritchie and McQuilken (2009) [11], who confirmed that mycelial growth was hampered at low sugar concentrations, and growth was found to be good at high sugar concentrations.

Table 1: Optimization of radial growth of *R. solani* isolates at different sugar concentrations

Isolates	Radial growth (cm)					Mean
	Sugar concentrations (g/L)					
	10	15	20	25	30	
RS-1	7.72	8.33	8.5	7.37	6.83	7.75
RS-2	7.20	8.50	7.28	7.42	7.18	7.52
RS-3	6.43	7.73	8.50	7.37	5.37	7.08
RS-4	5.73	8.27	8.50	8.50	3.97	6.99
RS-5	8.27	7.72	8.07	8.50	7.63	8.04
RS-6	5.30	6.25	8.28	8.50	6.67	7.00
RS-7	8.50	5.80	8.33	6.33	8.25	7.44
RS-8	6.00	6.95	8.25	8.25	5.18	6.93
RS-9	5.80	4.52	8.5	6.38	5.17	6.07
RS-10	8.5	8.50	8.5	8.50	8.08	8.42
Mean	6.95	7.26	8.27	7.71	6.43	

	S.Em	CD (1%)
Isolate	0.04	0.16
Sugar concentrations	0.03	0.00.12
Isolate*Sugar concentrations	0.10	0.38

To optimize the pH for different isolates of *R. solani*

For all the isolates (Table 2), the maximum radial growth (8.38cm) was found at pH 5. The maximum growth was shown by RS-10, RS-9, and RS-4 (8.50cm), and the least growth was shown by RS-3 (6.42cm). The radial growth for all the isolates of *R. solani* was observed to increase with the increasing pH but up to pH 5. Beyond this pH, the isolates showed wide diversity in radial growth which ranged between 5.00 to 8.50cm (Table 2). As per the density graph (Fig. 2) of the radial growth of isolates at different pH levels, the growth of RS-10, RS-9, and RS-4 does not show much diversity in growth whereas the rest of isolates showed wide diversity in growth from 7.00cm to 8.50cm. The results were similar to the findings of Grosh and Kofoet (2003) [3], who found that the optimum range of pH for fungus was between 5.0-6.0.

Table 2: Optimization of radial growth of *R. solani* isolates at different pH levels

Isolates	Radial growth (cm)					Mean
	pH Levels					
	4	5	6	7	8	
RS-1	8.5	8.5	8.5	8.5	8.02	8.40
RS-2	7.85	7.32	7.82	6.93	5.23	7.03
RS-3	6.92	8.5	5.33	6.12	5.25	6.42
RS-4	8.5	8.5	8.5	8.5	8.5	8.5
RS-5	8.5	8.5	8.5	8.37	8.5	8.47
RS-6	8.08	8.5	7.98	5.97	5.10	7.13
RS-7	8.5	8.5	8.5	8.5	8.12	8.42
RS-8	8.5	8.5	5.47	8.28	5.47	7.24
RS-9	8.5	8.5	8.5	8.5	8.5	8.5
RS-10	8.5	8.5	8.5	8.5	8.5	8.5
Mean	8.24	8.38	7.76	7.82	7.12	

	S.Em	CD (1%)
Isolate	0.03	0.12
pH	0.02	0.08
Isolate*pH	0.07	0.28

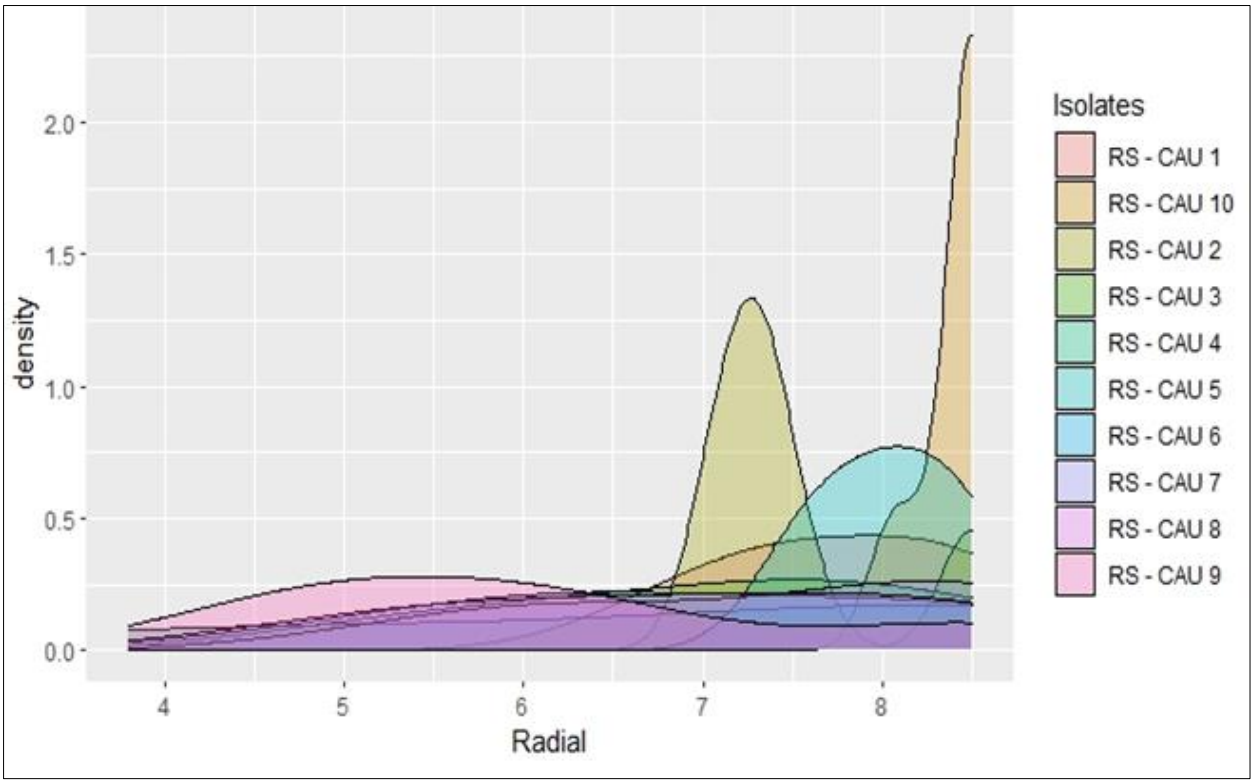
To optimize the temperature for different isolates of *R. solani*: *R. solani* isolates showed growth at a wide range of temperatures. Higher temperatures (30 °C to 34 °C) were found favorable for the radial growth of the fungus. Among

the five different temperatures studied, 30 °C was found to be the most suitable for maximum radial growth (8.5 cm) for all the isolates of *R. solani* (Table 3). The maximum radial growth was found to be in RS-10 (8.49 cm) and the least growth was shown by RS-3 (6.90 cm) (Table 3). The least growth of all the isolates is seen at 28 °C (6.39 cm). As per the density graph (Fig. 3), the isolate RS-10 does not show much diversity in growth whereas RS-7 and RS-8 show diversity in growth which varies between 4.50 cm to 8.40 cm. And the rest of other isolates show wide variation in radial growth ranging from 5.00 cm to 8.00cm. The results were similar to the findings of Hemalatha and Singh (2019) [4], who found that when plates were inoculated with mycelia the growth at 30 °C was maximum which was followed by 25 °C and then 35 °C.

Table 3: Optimization of radial growth of *R. solani* isolates at different temperature levels

Isolates	Radial growth (cm)					Mean
	Temperatures (°C)					
	26	28	30	32	34	
RS-1	7.92	6.60	8.5	8.27	8.5	7.96
RS-2	8.33	5.83	8.5	8.26	8.5	7.89
RS-3	8.5	5.78	8.5	5.60	6.10	6.90
RS-4	6.63	5.12	8.5	8.5	8.50	7.45
RS-5	5.47	7.08	8.5	7.45	7.55	7.21
RS-6	7.9	7.14	8.5	8.5	8.5	8.11
RS-7	5.05	8.46	8.5	8.45	8.48	7.79
RS-8	8.47	4.98	8.49	8.37	8.35	7.73
RS-9	7.07	4.45	8.5	8.48	8.46	7.39
RS-10	8.47	8.49	8.5	8.49	8.5	8.49
Mean	7.38	6.39	8.5	8.04	8.14	

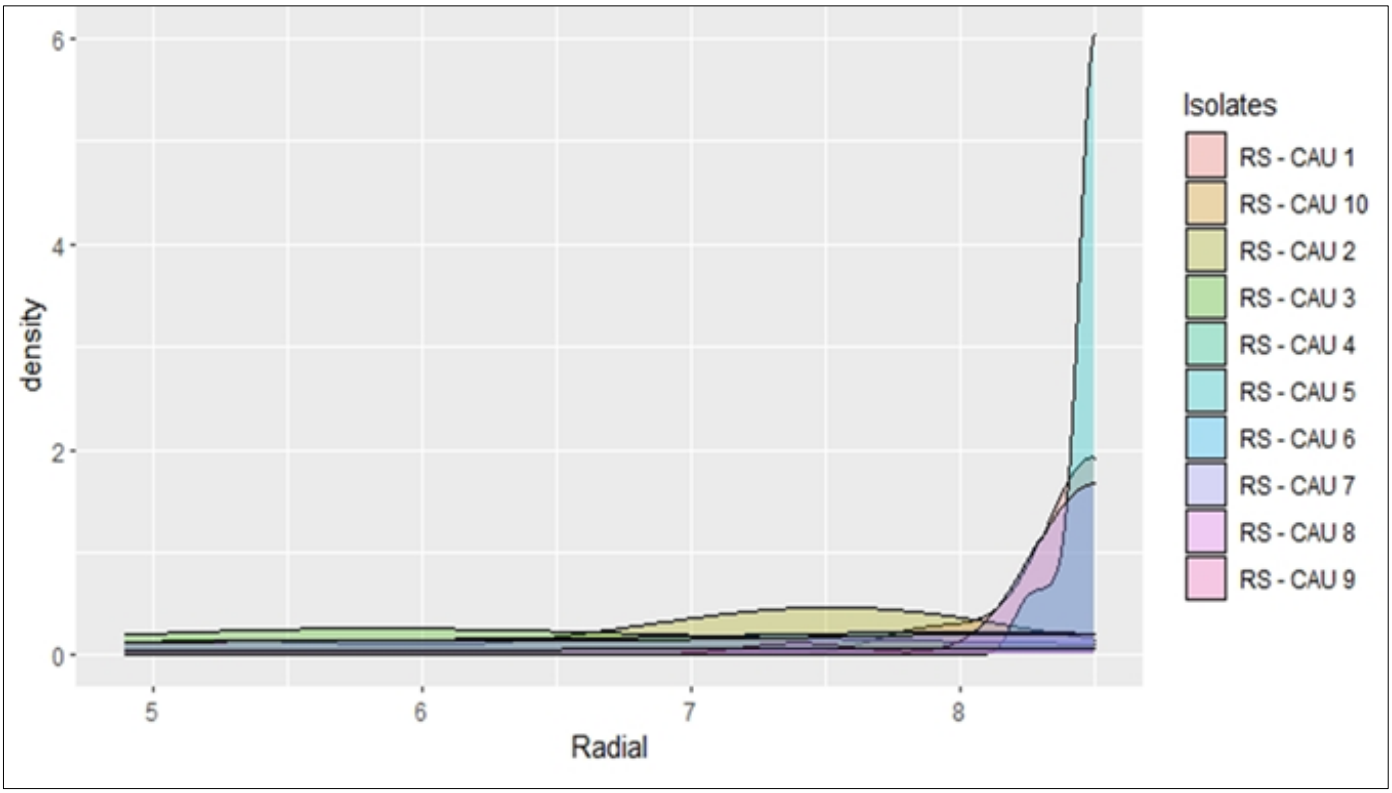
	S.Em	CD (1%)
Isolate	0.04	0.16
Temperature	0.03	0.11
Isolate*Temperature	0.09	0.36



Here,

RS-1 = RS-CAU-1 RS-6 = RS-CAU-6
RS-2 = RS-CAU-2 RS-7 = RS-CAU-7
RS-3 = RS-CAU-3 RS-8 = RS-CAU-8
RS-4 = RS-CAU-4 RS-9 = RS-CAU-9
RS-5 = RS-CAU-5 RS-10 = RS-CAU-10

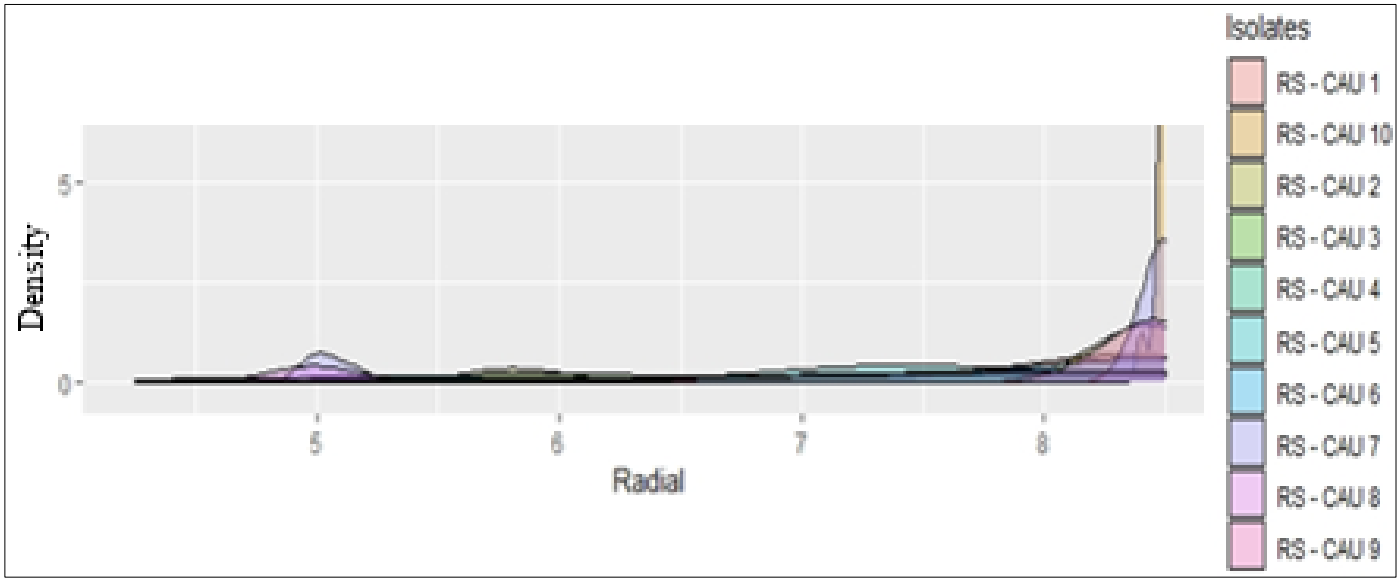
Fig 1: Density graph showing radial growth of isolates of *R. solani* at different sugar concentrations



Here,

RS-1 = RS-CAU-1	RS-6 = RS-CAU-6
RS-2 = RS-CAU-2	RS-7 = RS-CAU-7
RS-3 = RS-CAU-3	RS-8 = RS-CAU-8
RS-4 = RS-CAU-4	RS-9 = RS-CAU-9
RS-5 = RS-CAU-5	RS-10 = RS-CAU-10

Fig 2: Density graph showing radial growth of isolates of *R. solani* at different pH levels



Here,

RS-1 = RS-CAU-1	RS-6 = RS-CAU-6
RS-2 = RS-CAU-2	RS-7 = RS-CAU-7
RS-3 = RS-CAU-3	RS-8 = RS-CAU-8
RS-4 = RS-CAU-4	RS-9 = RS-CAU-9
RS-5 = RS-CAU-5	RS-10 = RS-CAU-10

Fig 3: Density graph showing radial growth of isolates of *R. solani* at different temperature levels

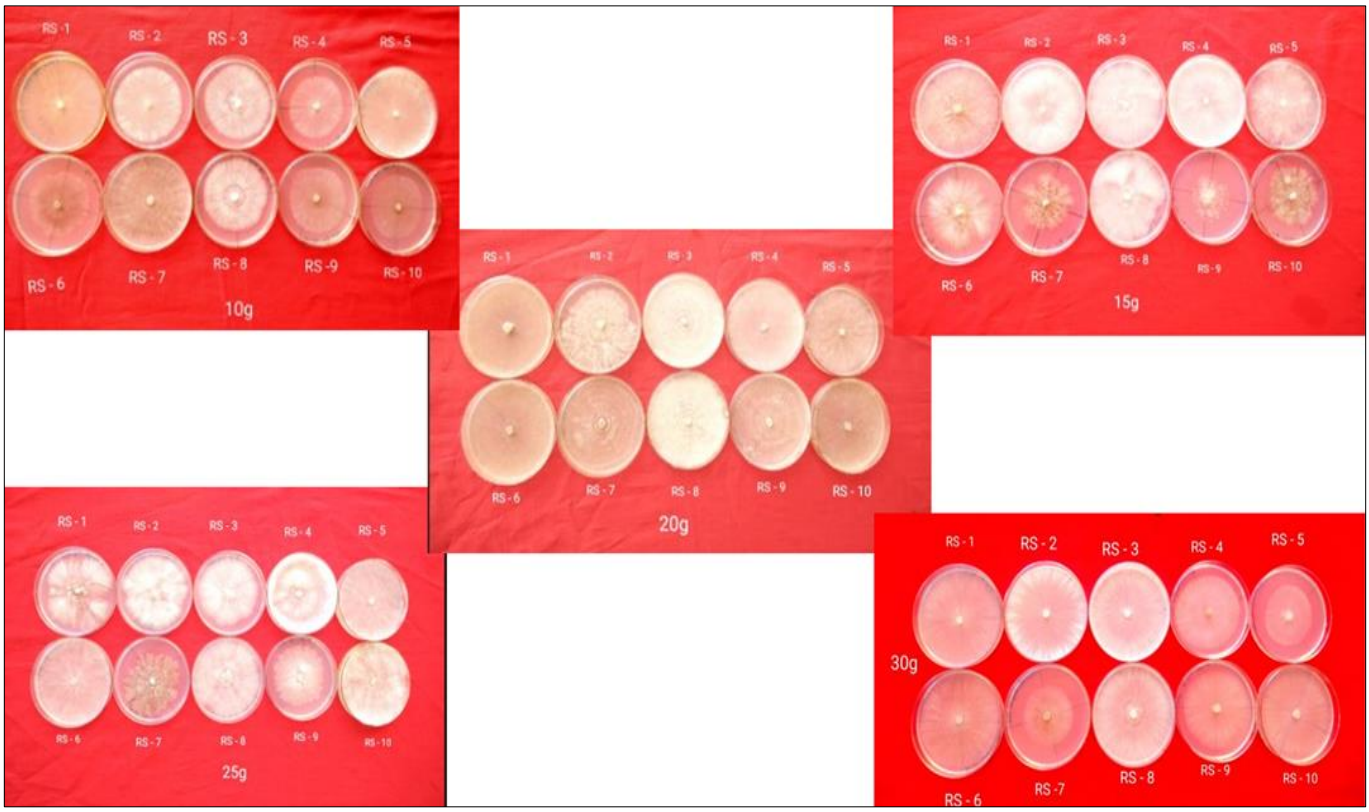


Plate 1: Radial growth *R. solani* isolates at different sugar concentrations

Plate 2: Radial growth *R. solani* isolates at different pH levels

Plate 3: Radial growth *R. solani* isolates at different temperature levels

Conclusion

It was concluded that the isolates of *Rhizoctonia solani* exhibited growth at a wide range of pH, temperatures, and sugar concentrations. The slightly acidic pH (pH 4.0 to 5.0), and higher temperatures (30 °C to 34 °C) were found favorable for the radial growth of the fungus. The isolates showed an increase in growth with increasing sugar concentration but up to a certain point. Optimum growth was found at pH 5, 30 °C temperature, and 20 g/L sugar.

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