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IN VITRO INTERACTION OF pH, TEMPERATURE AND SUGAR CONCENTRATION ON *RHIZOCTONIA SOLANI*

Prajna Samal*, Bireswar Sinha, L. Nongdren Khomba Singh, Shravan M. Haldhar, N. Gopi Mohan Singh, Soumen Pati and Kota Chakrapani

Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal - 795 004, Manipur, India.

*Corresponding author E-mail : prajna.doc98@gmail.com

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ABSTRACT

Rice (*Oryza sativa* L.) is the most principal staple cereal food crop of the world and belongs to the family Poaceae (Gramineae). Rice productivity is affected by numerous pathogens amid which, sheath blight, is one of the most fiscally significant rice diseases worldwide and causes serious grain yield and quality losses. The pathogen associated with sheath blight (ShB) is *Rhizoctonia solani* Kuhn (Teleomorph: *Thanatephorus cucumeris* Frank Donk). Different parameters such as pH, temperature, and sugar concentrations play an important role among different factors affecting fungi growth and spread. In this research, pH, temperature and sugar concentrations at different levels were maintained to study the isolates' mycelial growth variation. The fungal pathogen was maintained as a pure culture on potato dextrose agar (PDA). *In vitro* evaluation was done for ten isolates of *Rhizoctonia solani*. The interaction effects of pH, temperature, and sugar concentration were studied on the radial growth, fresh weight, and dry weight of *R. solani*. The radial growth of isolates increased with increasing temperature (30^0C to 34^0C) and with a pH range of 5 to 6. The maximum fresh and dry weight of the mycelium was found at pH 5 and 30^0C. The radial growth, fresh weight and dry weight of the isolates were found to be maximum at 30^0C and the mycelial weight increased with the increase in sugar concentration. The pH of 5 with an increase in the sugar concentration was found favorable for the growth of *R. solani* isolates. The fresh and dry weight of the mycelium was maximum at pH 5. The increase in sugar concentration did not show much effect on mycelial weight with the changing pH. The present study showed that the pH, temperature, and sugar concentration individually and their combinations significantly affect the growth of *Rhizoctonia solani*.

Key words : Rice, Sheath blight, *Rhizoctonia solani*, pH, Temperature, Sugar concentration, Interaction.

Introduction

One of the principally important food crops in the world is rice (*Oryza sativa* L.). It is a monocotyledonous annual grass on which over half of the world's population depends for their diet. Scientifically known as *Oryza sativa* L. belongs to the genus *Oryza* L., tribe *Oryzeae*, subfamily *Oryzoideae* and the family *Poaceae (Gramineae*) (Lu, 1999). India, Bangaldesh, China, and Pakistan are the four major countries that are chief consumers of rice (FAO, 2002). In 2017, the production of rice was 166.5 million tonnes (FAO, 2017).

Rice is an extensively cultivated crop around the world and country and many fungal pathogens thrust a variety of diseases into the crop. Among this, sheath blight is emerging as an economically significant disease. It is the second most destructive disease after rice blast affecting rice productivity (Ou, 1985). On average, yield loss globally due to ShB ranges from 10–30% (Xie *et al*., 2008) to over 50% during years with severe outbreaks. *Rhizoctonia solani* Kuhn (Teleomorph: *Thanatephorus cucumeris*) is the pathogen associated with sheath blight (Nagarajkumar *et al*., 2004). It is a facultative plant parasite and soil-borne saprotroph (Anees *et al*., 2010).

The fungus has its place in the phylum Basidiomycota, family Ceratobasidiaceae. Symptoms include lesions formed on sheaths of lower rice leaves, which gradually leads to stem softness succeeding stem lodging (Wu *et al*., 2012). The presence of numerous lesions on the sheath usually causes blighting and death of the entire plant. Plants profoundly infected during the early heading and grain filling growth stages produce poorly filled grain.

The average global temperature is increasing due to the changes in the global climate. Elevation of temperature is a key abiotic driver of climate change. And due to the temperature elevation scenarios have projected a rise in the number and severity of epidemics. With ongoing climate change, there is an increasing need to understand the effect of changes in temperature on pathogens. *R. solani* species show diversity in their behavior under different temperatures, sugar concentrations and pH. So, the purpose of this study was to find out the interaction effect of temperatures, pH and sugar concentration on the growth of *R. solani* isolates of rice.

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 (85 mm in diameter) for assessment of growth characters.

Evaluate the effect of pH and temperature on radial growth, dry weight and fresh weight of *R. solani*

The effect of pH and temperature on radial growth, dry weight and fresh weight was evaluated on PDA and Potato Dextrose Broth (PDB) respectively. PDA was prepared in five 250 ml conical flasks, each containing 150 ml media. The pH of the medium was adjusted to 4,5,6,7 and 8 with a help of a digital pH meter using 0.1N Hydrochloric acid and 0.1N Sodium hydroxide. In an autoclave, all conical flasks with media were sterilized at 1.1kg /cm^2 for 20 minutes. For each pH level, 20ml PDA medium was poured into each sterilized petri plate and allowed to solidify. From the actively growing cultures of the different isolates, five mm mycelial discs were placed on the centre of the petri plates. For each treatment, three replications were maintained. It was repeated five times at five different temperatures (26°C, 28°C, 30°C, 32°C and 34°C). Inoculated plates were incubated at different temperatures for 2 days. The growth of all the ten isolates was measured radially after 2 days.

Fresh weight and dry weight of the fungus were studied on Potato Dextrose Broth (PDB) at different pH and temperatures. 30 ml of each broth (4, 5, 6, 7 and 8) was distributed uniformly into 100 ml conical flasks separately and autoclaved at 1.1kg /cm^2 for 20 min and cooled. Five mm mycelial disc pathogen culture was used for inoculating each flask separately. For each treatment, three replications were maintained. It was repeated five times at five different temperatures (26°C, 28°C, 30°C, 32°C and 34°C). Inoculated conical flasks were incubated at different temperatures for five days. Five days after incubation the mycelial mat of the pathogen was filtered through a pre-weighed Whatman No. 1 filter paper separately and dried in hot air oven overnight at 60°C. The mycelial dry weight of the pathogen in each treatment was obtained by subtracting the weight of the filter paper. The dried and cooled filter papers with mycelial mats were then reweighed to estimate the fungal growth on a dry weight basis by using the following formula:

W = W2 - W1

Where,

W = the weight of the mycelial mat,

W1 = the weight of the filter paper

W2 = the total weight of the fungal mycelial mat and filter paper

Evaluate the effect of sugar concentration and temperature on radial growth, dry weight and fresh weight of *R. solani*

The effect of sugar concentration and temperature on radial growth, dry weight and fresh weight was evaluated on PDA and PDB, respectively. The procedure is the same as the above effect but in this case five different concentrations of dextrose i.e., (10,15g, 20g, 25g and 30g) per litre and five different temperatures (26°C, 28°C, 30°C, 32°C and 34°C) was used.

Evaluate the effect of pH and sugar concentration on radial growth, dry weight and fresh weight of *R. solani*

The effect of pH and sugar concentration on radial growth, dry weight and fresh weight was evaluated on PDA and PDB, respectively. The procedure is the same as the above effect but in this case five different concentrations of dextrose *i.e.* (10, 15g, 20g, 25g and 30g) per litre and five different levels of pH (4, 5, 6, 7 and 8) was used.

Statistical analysis

The data recorded in each experiment were subjected to statistical analysis wherever required. ANOVA with factors in combinations. In the present study, the selected

levels of the three factors along with the ten isolates were then evaluated in a three-level factorial design in Randomized Block Design (RBD), where the isolates were common for each combination of physical factors tested. The combinations tested were pH and temperature, temperature and sugar levels and pH and sugar levels. The statistical analysis was done in R software.

Results and Discussion

Effect of pH and temperature on radial growth, dry weight and fresh weight of *R. solani*

The mycelial radial growth of *Rhizoctonia solani* showed a variable trend in response to combinations of changes in pH and temperature. The radial growth of isolates increased with increasing temperature (30^0C to 34^0C) with changes in pH (5 to 6). As per the density graph (Graph 1a), isolate RS-10 had the highest growth and showed less diversity in growth whereas the rest of the other isolates showed wide variation in growth at all pH and temperatures. As per the density graph (Graph 1b), isolate RS-6 has the highest weight and RS-1 showed the least mycelial weight but with wide variation. RS-2 showed less variation in mycelial fresh weight. Rest all of the other isolates shows wide variation in their weight ranging from 1.00 to 2.80g. As per the density graph (Graph 1c), isolate RS-7 has the highest weight an RS-1 shows the least mycelial weight but with wide variation. RS-10 and RS-5 show less variation in mycelial dry weight. The rest of the other isolates show wide variation in their weight ranging from 0.1g to 0.3g.

The results are in partial agreement with the findings reported by Datta *et al*. (2014), who found that pH 6 and temperature 30ºC are most suitable for the growth of *R. solani* fungus. In a previous study by Chaudhary *et al*. (2018) it was found that the maximum hyphal growth of R. *solan*i was measured at 25°C temperature and between pH 4.0 and 9.0, with an optimum of pH 5.6. Pathak *et al*. (2021) studied the effects of temperature and pH on *R. solani* and their findings showed that the highest growth rate of all the isolates was at 30ºC and the highest mean biomass production was observed at pH 7. Nuri and Biswas (2021), studied the growth of *Rhizoctonia solani* Kuhn AG3 on factors like temperature and pH *in-vitro* and concluded that among seven different temperatures, 25ºC has given maximum colony diameter (88.40mm) at 7 days after inoculation and optimum growth was found at 6 pH. As *Rhizoctonia solani* is usually considered the pathogen of tropics and subtropics so it thrives well in most tropical environments. pH of the medium has a deep effect on the rate and

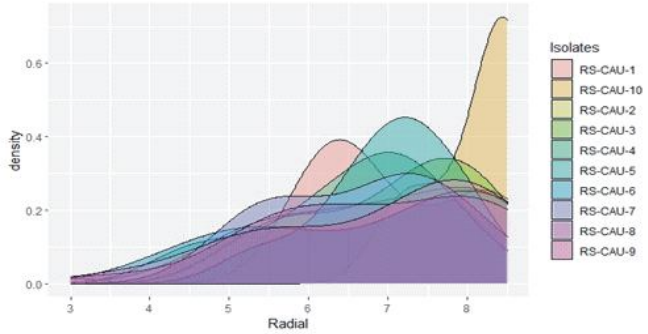

Fig. 1a : Density graph showing radial growth of isolates of *R. solani* at different pH and temperatures.

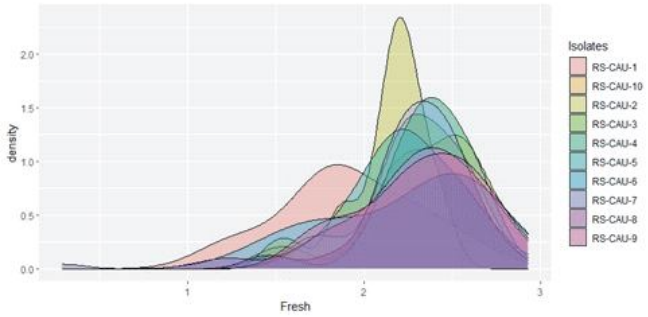

Fig. 1b : Density graph showing fresh weight of isolates of *R. solani* at different pH and temperatures.

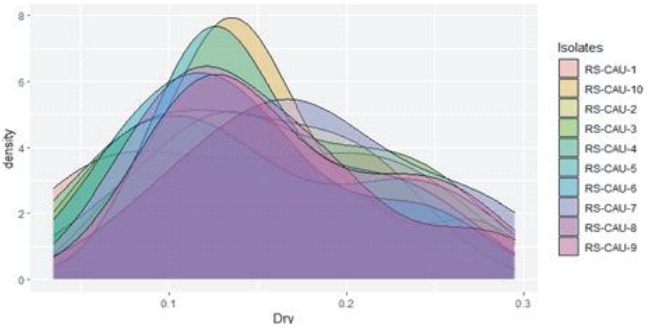

Fig. 1c : Density graph showing dry weight of isolates of *R. solani* at different pH and temperature.

(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)

degree of growth and numerous other life processes of fungi. The fungi commonly use substrates in the form of solution only if the reaction of the solution is favorable to the fungal growth and metabolism. This shows the importance of hydrogen ion concentration for the better growth of fungi.

Effect of temperature and sugar concentration on radial growth, dry weight and fresh weight of *R. solani*

The mycelial radial growth of *Rhizoctonia solani* showed a variable trend in response to combinations of

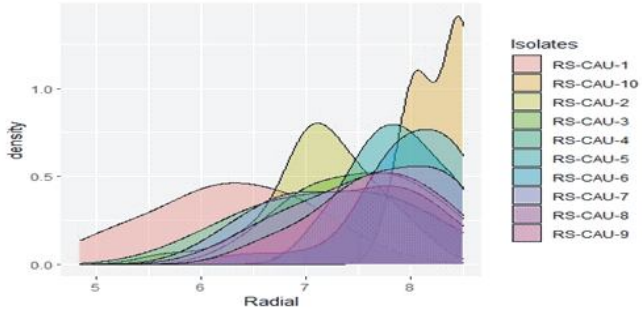

Fig. 2a : Density graph showing radial growth of isolates of *R. solani* at different temperature and sugar concentration.

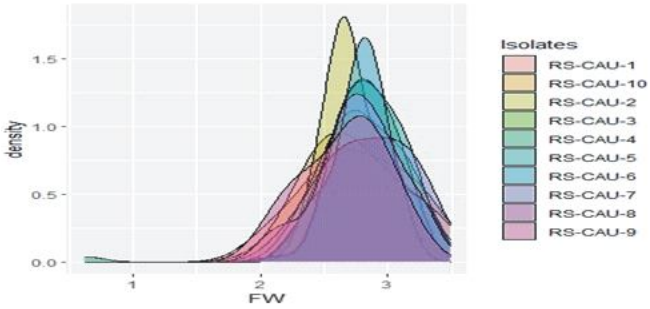

Fig. 2b : Density graph showing on fresh weight of isolates of *R. solani* at different temperature and sugar concentration.

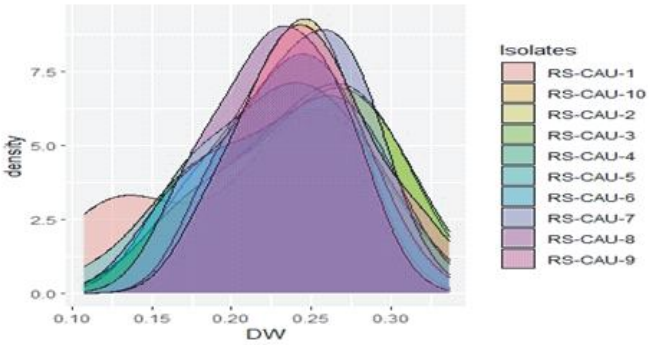

Fig. 2c : Density graph showing dry weight of isolates of *R. solani* at different temperature and sugar concentrations.

(Here,
RS-1 = RS-CAU-1 RS-4 = RS-CAU-4 RS-7=RS-CAU-7
RS-10=RS-CAU-10) RS-2 = RS-CAU-2 RS-5 = RS-CAU-5
RS-8=RS-CAU-8 RS-3 = RS-CAU-3 RS-6 = RS-CAU-6
RS-9=RS-CAU-9

changes in temperature and sugar in PDA medium. The radial growth of isolates increased with the increase in the sugar concentration. As per the density graph (Graph 2a), the isolate RS-10 showed the highest growth and showed less diversity in radial growth. RS-2, RS-5, and RS-6 show less variation in growth for different combinations of temperature and sugar. Isolate RS-1 showed the least growth but with wide variation. The other isolates showed wide variation in growth at all temperatures and sugar concentrations. As per the density graph (Graph 2b), for combinations of temperature and sugar, the isolates showed wide variation. Isolate RS-1 has the highest weight and showed a wide variation in fresh weight. RS-8 showed the least mycelial weight but with wide variation. RS-2 and RS-6 show less variation in mycelial fresh weight. Rest all of the other isolates shows wide variation in their weight ranging from 2.00g to 3.00g. The density of all the isolates is higher between 2.50g to 3.00g. As per the density graph (Graph 3c), for combinations of temperature and sugar, isolates showed a wide variation in mycelial dry weight. Isolate RS-9 has the highest weight and showed a wide variation in dry weight. RS-1 showed the least mycelial weight but with wide variation. The rest of the other isolates show wide variation in their weight ranging from 0.15g to 0.30g. The density of all the isolates is higher between 0.20g to 0.30g of sugar concentration.

The findings are partially similar to the results obtained by Ritchie and McQuilken (2009), who studied the growth of *Rhizoctonia solani* and found that the highest fungal growth is exhibited by when starch as a carbon source was amended into broth medium and optimal growth of *R. solani* was at 30^0C and the metabolic activity was at 25^OC. Chaudhary *et al*. (2018) found that the maximum mycelial growth of *R. solani* was in D-glucose as a carbon source and at 25°C temperature. Nuri and Biswas (2021) studied the growth of *Rhizoctonia solani* Kuhn AG3 *in-vitro* and found that Potato Dextrose Agar supported the best radial growth of the tested fungus and maximum biomass was also harvested from Potato Dextrose Broth and 25ºC has given maximum colony diameter. Temperature variations can modify the thermal performance of a pathogen and thus the host-pathogen interactions in an infectious disease system. The repressive effects of very high sugar concentrations may have been caused by some factors like the number of enzyme molecules accessible and the infusion rate of the sugar restraining the rate of carbohydrate consumption inhibiting normal metabolism.

Effect of pH and sugar concentration on radial growth, dry weight and fresh weight of *R. solani*

The mycelial radial growth of *Rhizoctonia solani* showed a variable trend in response to combinations of changes in pH and sugar in the PDA medium. The radial growth of isolates increased with the increase in the sugar concentration. As per the density graph (Graph 3a), the isolate RS-10 showed highest growth and showed less diversity in radial growth. Isolate RS-7 also shows less variation in growth for different combinations of pH and sugar. Isolate RS-1 showed the least growth but with

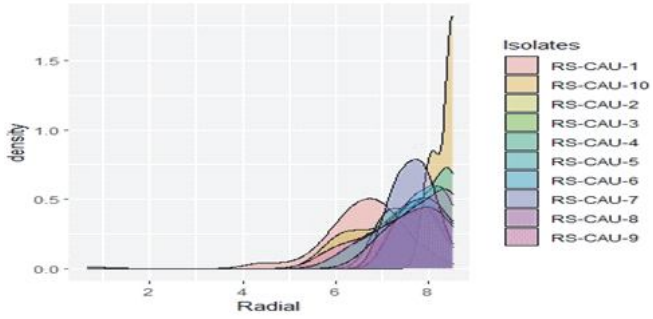

Fig. 3a : Density graph showing the radial growth of isolates of *R. solani* at different pH and sugar levels.

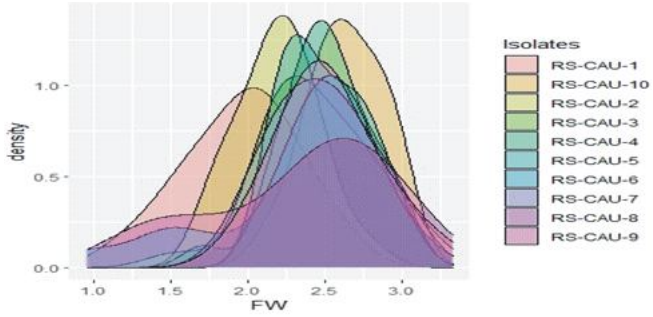

Fig. 3b : Density graph showing the fresh weight of isolates of *R. solani* at different pH and sugar levels.

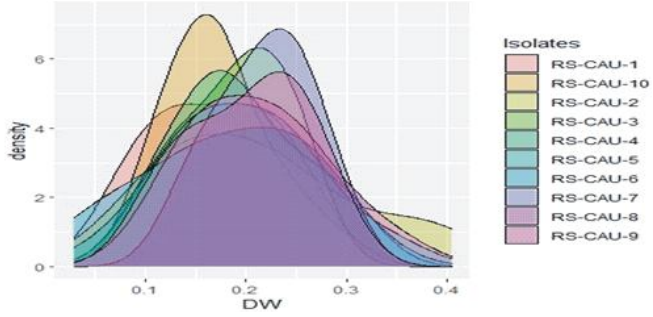

Fig. 3c : Density graph showing the fresh weight of isolates of *R. solani* at different pH and sugar.

(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)

wide variation. And the rest of the other isolates showed wide variation in growth at all pH and sugar concentrations. As per the density graph (Graph 3b), for combinations of pH and sugar, the isolates show wide variation. Isolate RS-10 showed highest weight and less variation in fresh weight. RS-1 showed least mycelial weight but with wide variation. Isolates RS-2, RS-4, and RS-5 showed less variation in mycelial fresh weight. All other isolates showed wide variation in their weight ranging from 1.50g to 3.00g. The density of all the isolates was higher between 2.00g to 3.00g concentration. As per the density graph (Graph 3c), for combinations of pH and sugar concentrations, the isolates showed wide

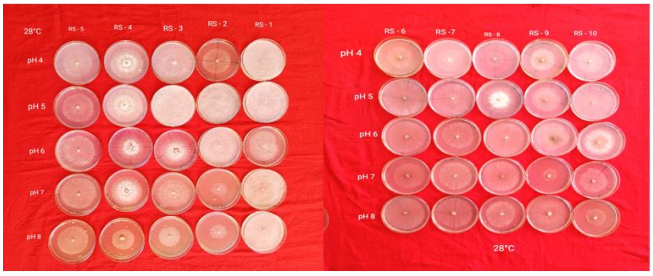

Fig. 4 : Radial growth of isolates of *R. solani* at different pH and 28^{0}C.

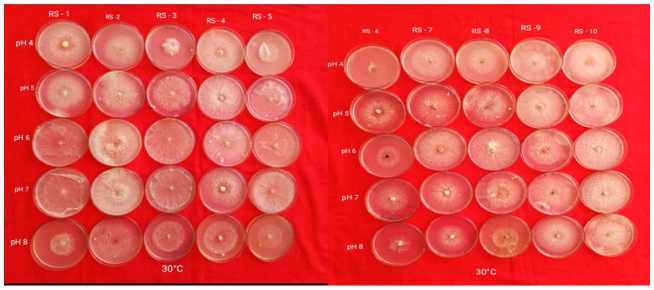

Fig. 5 : Radial growth of isolates of *R. solani* at different pH and 30^{0}C.

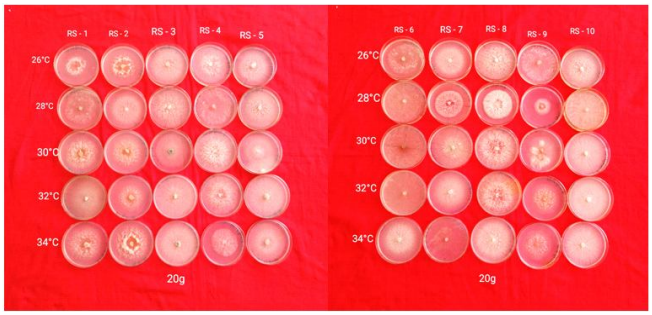

Fig. 6 : Radial growth of isolates of *R. solani* at different temperature and 20g/L sugar concentration.

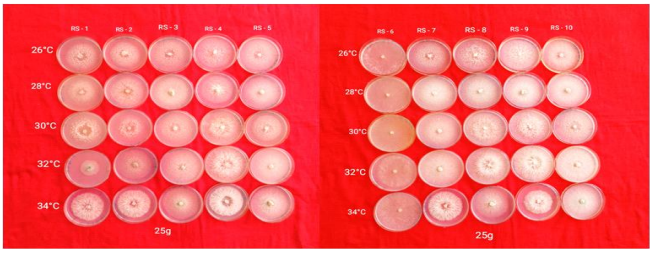

Fig. 7 : Radial growth of isolates of *R. solani* at different temperature and 25g/L sugar concentration.

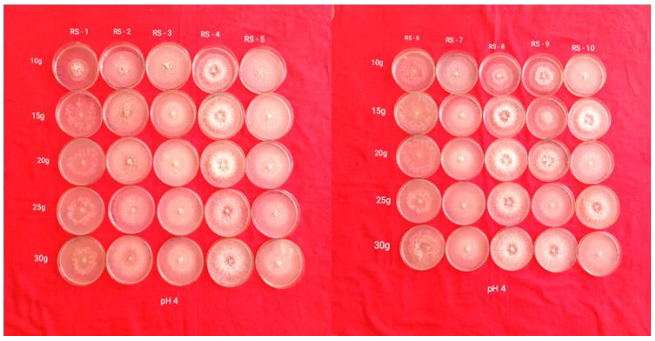

Fig. 8 : Radial growth of isolates of *R. solani* at different sugar concentration and pH 4.

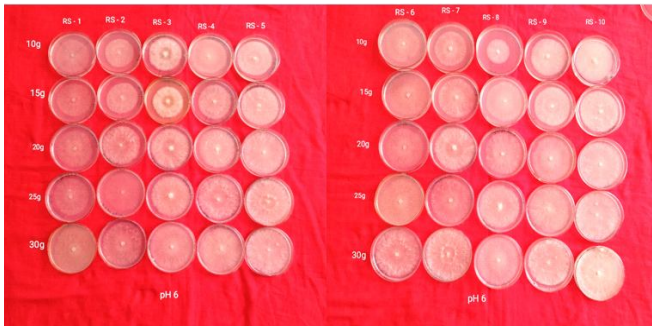

Fig. 9 : Radial growth of isolates of *R. solani* at different sugar concentration and pH 6.

variation. Isolate RS-7 showed highest weight and showed a wide variation in dry weight. RS-1 showed the least mycelial weight but with wide variation. The rest of the other isolates showed wide variation in their weight ranging from 0.10g to 0.35g. The density of all the isolates was higher between 0.15g to 0.30g.

The results are similar to the findings obtained by Nuri and Biswas (2021) who found that maximum biomass was also harvested from Potato Dextrose Broth (26.75mg) and the pH range from 5.5 to 7 is the best for *Rhizoctonia solani* growth, optimum growth was found at 6 pH and Chaudhary *et al*. (2018), who found that the maximum mycelial growth of *Rhizoctonia solani* was observed in D-glucose as a carbon source at pH 5.6. High pH levies stress on the fungal cell which causes problems in the procurement of nutrients which might be the reason for abridged fungal growth even in the presence of high sugar concentration in media.

Conclusion

The changes in the average global temperature are due to climate change. The weather parameter available during 1954–2014 showed an increase in annual maximum temperature (0.1°C per decade) and annual minimum temperature (0.3°C). The host-pathogen interaction is affected by the weather parameters. Current findings showed that *R. solani* grows well under increased trends of temperature. As sheath blight of rice is considered as a minor disease but now there is increase in the incidence of the disease due to rise in temperature. The surge in temperature may offer the pathogen better conditions to thrive and may be one of the factors for pathogen to become more virulent. But current study deals with the temperature, pH and sugar level under in vitro conditions on *R. solani*. More detailed information can be attained if future works would be done on in vivo.

Acknowledgments

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