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Effect of Different Culture Media, Temperature and pH for Growth of *Rhizoctonia solani* Causing Sheath Blight Disease in Rice Mat Nursery

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ABSTRACT

Over the last decade Sheath Blight of Rice as a major constant in rice mat nursery production has been identified globally and integrated disease resistance strategy for Sheath Blight of Rice has been primary focus in south east Asia particularly in the *kharif* and *rabi* rice growing areas like Bangladesh, Eastern India, Terai region of Nepal and Pakistan. *Rhizoctonia solani* (Kuhn.) is the causal agent of Sheath Blight Disease in Rice Mat Nursery. It is a soil inhabiting fungal pathogen which favors hot and humid temperature. Keeping in view the consequentiality of the disease due to transmute in climatic conditions, studies were conducted on the cultural and morphological diversity of the pathogen in different media, pH and temperature conditions. This research was conducted at Department of Plant Pathology in Uttar Banga Krishi Viswavidyalaya. The fungus *Rhizoctonia solani* gives highest mycelial growth on PDA (Potato dextrose Agar) media which is 60.78 mm and it is followed by Glucose peptone Agar media (55.33 mm) and V-8 juice Agar media (51.11 mm) whereas the lowest growth was observed in Czapek Dox Agar media (19.66 mm) followed by Oat meal Agar media (26.66 mm). In different pH, maximum mycelial growth was observed at pH 7 i.e., at neutral pH (62.33 mm) while they showed less growth in too acidic pH i.e., at 4 and at too high pH i.e., at pH 10. In different temperature, the highest mycelial growth was observed at 26ºC (62.91 mm) to 28ºC (62.05 mm) i.e., they prefer normal temperature for their growth.

Keywords Sheath Blight, Rice, *Rhizoctonia solani,* PDA, pH, Temperature.

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INTRODUCTION

Rice (*Oryza sativa* L.), the world's most widely consumed cereal crop, especially important to the rapidly growing populations in South Asian countries (Pareja *et al.* 2011). Rice is a very important crop of India as well as West Bengal. Now a days rice transplanting is done mechanically by machines. This technology

is becoming very popular in Northern parts of West Bengal. It reduces labor cost. For machine transplanting rice seedlings are raised in mat nursery. Farmers are facing the problem in mat nursery of the crop which is affected by number of diseases. Out of those Sheath Blight Disease caused by *Rhizoctonia solani* is responsible for yield loss upto 45% (Margani and Widadi 2018). The ShB pathogen, *R. solani* Kuhn., survives in the soil and water as sclerotia that remain viable for up to 3 years and form mycelia when coming into contact with plants (Kumar *et al.* 2009). The early disease symptoms are the formation of lesions on the sheath leading to softness and lodging of the sheath and inhibition of grain filling (Wu *et al.* 2012). The fungus spreads rapidly via contact between plant parts such as tillers and leaves, and also via sclerotia (densely packed hyphal masses) present in surface water (Tsiboe *et al.* 2017).

MATERIALS AND METHODS

Collection of samples

In the mat nursery Sheath Blight Disease affected rice leaves or sheaths were collected from different farmers field of North Bengal under the supervision of Uttar Banga Krishi Viswavidyalaya. In the nursery irregular patches and bird nest like symptoms were found (Plate 1). The pathogen isolated from those diseased leaves or sheath showing typical symptoms were taken for observation.

Isolation of *Rhizoctonia solani* from sample

These disease samples were cut in small pieces and washed in water to remove the soil particles and other dirt materials. During isolation these bits were surface sterilized by 70% ethyl alcohol solution followed by washing twice in sterile distilled water, dried these bits by placing it on sterile blotter paper and then those bits were transferred to Potato dextrose Agar (PDA) slants which was made previously by using forceps and those inoculated PDA slants were placed in BOD incubator for incubation at 28 ± 2^{0}C (Shukla and Ratan 2019). Pure culture was maintained and stored in refrigerator at 5ºC for further studies.

Media

Different media used in the study are listed below:

a) Potato dextrose Agar
b) Glucose peptone Agar
c) Richard's Agar
d) Czapek (Dox)'s Agar
e) Host extract Agar
f) Cooks Agar
g) Corn meal Agar
h) Oat meal Agar
i) V-8 juice Agar
j) Rose Bengal Agar

All these media were prepared by adding their respective ingredients to distilled water in flasks, sealed with cotton plugs and sterilized in autoclave at 121ºC (15 psi) for 20 minutes. The media were then poured separately in sterile petri dishes and allowed to cool. The isolated pathogen was transferred to different above media through hyphal tip / single sclerotial method (Rangaswami and Mahadevan

Plate 1. Rice Sheath Blight Disease symptoms in mat nursery.

2004) and then those are kept in BOD incubator at a temperature of 28±1ºC. The radial mycelial growth was measured day by day after incubation. The colony diameter, colony growth pattern, colony growth rate was recorded by visual observation at 24, 48 and 72 hrs after inoculation. The number, size, texture (smooth or rough) and pattern of production (central, peripheral and scattered) of sclerotia was noted at 10 days after inoculation.

pH and temperature

The mycelial growth of the pathogen was measured at seven different pH levels ranging from 4 to 10 on PDA. The pH range was adjusted by adding NaOH or HCl with continuous stirring before solidifying the PDA media. Mycelial tip from the actively growing three days-old cultures were placed in the center of petri dishes for each pH levels and inoculated plate were kept at 28±1ºC. The average colony diameter was assessed at 24, 48 and 72 hrs after inoculation.

The influence of temperature on mycelial growth of the pathogen was determined on PDA at 20, 22, 24, 26, 28 and 30ºC. Mycelial tip from the actively growing three days-old cultures were placed in the center of petri dishes (90.00 mm) and incubated in incubator maintaining six different temperature levels. The average colony diameter was measured at 24, 48 and 72 hrs after inoculation.

RESULTS AND DISCUSSION

Mycelial structures and other characters like colony growth rate, sclerotial color, texture of *R. solani* from rice mat nursery were studied in different media. The growth was found highest in PDA (Potato dextrose Agar media) with is 60.78 mm growth and it is followed by Glucose peptone Agar media (55.33 mm) and V-8 juice Agar media (51.11 mm) whereas the lowest growth was observed in Czapek Dox Agar media (19.66 mm) followed by Oat meal Agar media (26.66 mm) while moderate growth of mycelia was observed in Rose Bengal Agar media (35.22 mm), Richard's Agar media (40.33 mm), Cooks' Agar media (38.78 mm), Corn meal Agar (45.11 mm) and Host Agar media (49.66 mm). On the contrary, Corn meal Agar did not support rapid growth of the fungus as they were moderate in these essential nutrients (Khan *et al.* 2016). In conclusion, maximum growth in PDA may be due to presence of some additional nutrients in this media (Devi and Singh 1998). Color of maximum colonies are greyish to dark gray in color but some colonies in Glucose peptone Agar and Oat meal Agar are whitish in nature (Table 1).

The location of the sclerotia in the petri plates are mainly aerial but some grows in surface also like in Cooks Agar media, V-8, juice Agar media and in Czapek Dox Agar media while some sclerotia formed are in peripheral, central or scattered form. Color of sclerotia mainly varies from light brown to yellow brown, but in PDA and V-8 juice Agar media they have whitish sclerotia color (Aboshosha *et al*. 2007, Fernandez *et al.* 2006). Texture is mainly rough but some are also smooth like in Richards Agar media, Glucose peptone Agar media, Corn meal Agar and

Table 1. Effect of different culture media on mycelial growth of *R. solani.*

Media	Mycelial growth (mm)			Mean	Colony color
	24 hrs	48 hrs	72 hrs	(mm)	
Potato Dextrose Agar	23.0	69.33	90.0	60.78	Greyish Black
Richard's Agar	8.33	34.66	78.0	40.33	Dark Gray
Czapek (Dox)'s Agar	5.66	14.33	39.0	19.66	Greyish
Glucose peptone Agar	17.33	58.66	90.0	55.33	Whitish Black
Host extract Agar	14.66	47.33	87.0	49.66	Greyish Black
Cooks Agar	10.0	33.33	73.0	38.78	Greyish
Oat meal Agar	7.66	19.33	53.0	26.66	Whitish Gray
Corn meal Agar	12.33	43	80.0	45.11	Dark Greyish Black
Rose Bengal Agar	9.0	28.66	68.0	35.22	Dark Gray
V-8 juice Agar	12.0	51.33	90.0	51.11	Dark Greyish
CD (p=0.05)	1.58	3.67	8.45	2.11	-
CV (%)	2.32	5.34	7.45	2.92	-

Table 2. Effect of different culture media on sclerotia formation of *R. solani.*

Media	Formation of sclerotia	Location of sclerotia	Texture of sclerotia	Sclerotia color
Potato dextrose Agar	Peripheral	Aerial	Rough	Whitish Brown
Richard's Agar	Central	Aerial and surface	Smooth	Light Brown
Czapek (Dox)'s Agar	Scattered	Surface	Rough	Yellow Brown
Glucose peptone Agar	Peripheral	Aerial	Smooth	Yellow Brown
Host extract Agar	Scattered	Aerial	Rough	Light Brown
Cooks Agar	Scattered	Surface	Rough	Whitish Brown
Oat meal Ngar	Peripheral	Aerial and surface	Rough	Yellow Brown
Corn meal Agar	Peripheral	Aerial and surface	Smooth	Light Brown
Rose Bengal Agar	Central	Aerial	Smooth	Light Brown
V-8 juice Agar	Scattered	surface	Rough	Whitish Brown
CD (p=0.05)	-	-	-	-
CV (%)	-	-	-	-

Table 3. Effect of different pH on the mycelial growth of *R. solani.*

pH	Mycelial growth (mm)			Mean (mm)
	24 hrs	48 hrs	72 hrs	
4	8.40	24.66	52.33	28.46
5	9.66	37.66	70.33	39.22
6	12.66	51.66	83.00	49.11
7	21.00	76.00	90.00	62.33
8	17.33	58.66	90.00	55.33
9	11.66	49.66	87.00	49.44
10	8.66	37.33	71.00	39.00
CD (p=0.05)	0.45	2.26	2.17	1.23
CV (%)	1.22	2.89	6.39	1.62

Rose Bengal Agar media (Table 2).

The mycelial growth rate of *Rhizoctonia solani* was measured in pH ranging from 4 to 10. Maximum mycelial growth was observed at pH 7 i.e., at neutral pH (62.33 mm) while they were showing less growth in too acidic pH i.e., at 4 and at too high pH i.e., at pH 10 (Table 3).

Table 4. Effect of different temperature on the mycelial growth of *R. solani.*

Temperature (^{0}C)	Mycelial growth (mm)			Mean (mm)
	24 hrs	48 hrs	72 hrs	
20	12.6	43.00	65.00	40.20
22	16.5	51.66	73.00	47.05
24	17.6	56.66	84.00	52.75
26	20.4	78.33	90.00	62.91
28	22.5	73.66	90.00	62.05
30	18.2	67.00	81.00	55.40
CD (p=0.05)	1.03	3.56	8.58	2.50
CV (%)	2.31	3.84	7.34	2.99

Mycelial growth of the pathogen was observed at different temperature ranging from 20ºC to 30ºC. The growth was found to be maximum at 26ºC (62.91 mm) to 28ºC (62.05 mm) i.e. they prefer normal temperature for their growth. Lowest growth was observed at 20ºC (40.2 mm) (Table 4). *R. solani* exhibited higher growth and metabolite production at 30^{0}C (Muhsin and Selman 2013).

CONCLUSION

Fungal growth in different media, pH and temperature characteristics were assessed over time. The fungus produced highest growth in PDA (Potato dextrose Agar) media and also produced better growth in the temperature of 26°C to 28°C and at neutral pH of 7. Under this condition, the number of fungal colony and sclerotia of *Rhizoctonia solani* was produced highest.

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REFERENCES

Aboshosha SS, Atta Alla SI, El-Korany AE, El-Argawy E (2007) Characterization of *Macrophomina phaseolina* isolates affecting sunflower growth in El-Behera governorate, Egypt. *Int J Agric Biol* 9(6): 807-815. https://api.semanticscholar.org/CorpusID:90545602

Devi TP, Singh RH (1998) Cultural variation of *Macrophomina phaseolina* isolates collected from *Vigna mungo*. *Ind Phytopathol* 51(3): 292- 293.

Fernandez RB, Santiago AS, Delgado SH, Mayek-Perez N (2006) Characterization of Mexican and Non-Mexican isolates of *Macrophomina phaseolina* based on morphological characteristics, pathogenicity on bean seeds and endoglucanase gene. *J Pl Pathol* 88 (1): 53-60. https://www.researchgate.net/publication/242754115

Khan I, Hussain H, Shah B, Ullah W, Naeem A, Ali W, Khan N, Adnan M, Junaid K, Shah SRA, Ahmed N, Iqbal M (2016) Evaluation of phytobiocides and different culture media for growth, isolation and control of *Rhizoctonia solani in vitro. J Entomol Zool Stud* 4(2): 417-420. https://www.researchgate.net/publication/301530265

Kumar KVK, Reddy MS, Kloepper JW, Lawrence KS, Groth DE, Miller ME (2009) Sheath blight disease of rice (*Oryza sativa* L.)—An overview. *Biosci Biotechnol Res Asia* 6(2): 465–480. https://www.biotech-asia.org/?p=8629

Margani R, Widadi S (2018) Utilizing Bacillus to inhibit the growth and infection by sheath blight pathogen, *Rhizoctonia solani* in rice. IOP conference series: Earth and environmental science, pp 142. 012070. https://doi.org/10.1088/1755-315/142/1/012070

Muhsin TM, Selman MS (2013) *In vitro*, optimization of growth and bioactivity of antibacterial metabolite produced by *Rhizoctonia solani* Kuhn. *J Basrah Res* 39 (1A): 101-111. https://www.iasj.net/iasj/download/c5be3609b1bdd444

Pareja L, Fernández-Alba AR, Cesio V, Heinzen H (2011) Analytical methods for pesticide residues in rice. *Trac Trend Anal Chem* 30(2):270–291. https://doi.org/10.1016/j.trac.2010.12.001

Rangaswami G, Mahadevan A (2004) Disease of crop plants in India. Prentice-Hall of India Private Limited Publisher, New Delhi, India, pp 507.

Shukla A, Ratan V (2019) Management of early blight of potato by using different bio-agents as tuber dressing and its effect on germination and growth. *Int J Curr Microbiol Appl Sci* 8 (6): 1965-1970. doi: https://doi.org/10.20546/ijcmas.2019.806.233.

Tsiboe F, Nalley LL, Durand A, Thoma G, Shew A (2017) The economic and environmental benefits of sheath blight resistance in rice. *J Agricult Res Econ* 42(2): 215–235. https://doi.org/10.22004/ag.econ.257999

Wu W, Huang J, Cui K, Nie L, Wang Q, Yang F, Shah F, Yao F, Peng S (2012) Sheath blight reduces stem breaking resistance and increases lodging susceptibility of rice plants. *Field Crops Res.* 128: 101–108. https://doi.org/10.1016/j.fcr.2012.01.002