Plant Archives Vol. 26, Supplement 1, 2026 pp. 2672-2681 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.supplement-1.359

EFFECT OF DIFFERENT CULTURE MEDIA, CARBON SOURCES, AND PH ON MYCELIAL GROWTH AND SCLEROTIA FORMATION OF *RHIZOCTONIA SOLANI* CAUSING SHEATH BLIGHT DISEASE OF RICE

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(Date of Receiving : 25-10-2025; Date of Acceptance : 03-01-2026)

ABSTRACT

Rhizoctonia solani causing sheath blight disease of rice, is one of the major important pathogen causing sever yield losses, reported in all the major rice growing country of the world. Therefore, present investigation carries out to evaluate the effect of different culture media, carbon source and pH on radial mycelial growth and sclerotial distribution of *R. solani*. The pathogen showed the significantly highest radial mycelial growth on Potato dextrose agar (90mm), Malt extract agar (90mm) and Oat meal agar (90mm) medium. In different pH level, significantly maximum mycelial growth of *R. solani* was recorded under pH-7 (90.00 mm) and pH -6 (90.00 mm) on 4th day of inoculation are followed by pH-8 (87.41 mm). Among the different carbon sources, significantly highest radial mycelial growth of *R. solani* was recorded under dextrose (90 mm), sucrose (90 mm), lactose (90.00 mm) on 3rd days of inoculation and mannitol (90 mm) are followed by the galactose (80.91mm) medium.

Keywords **:** Sheath Blight, Rice, *Rhizoctonia solani*, pH, Media, Carbon source.

Introduction

Rice (*Oryza sativa* L.), is the cereal crop and globally most widely consumed, especially important to the rapidly growing populations in South Asian countries (Pareja *et al.,* 2011).

Crops for more than 60% of the world's population. Over 90% of the rice produced in the world is consumed in the Asian countries. India is the number one rice producing country in the southwest Asia with an area of 43.5 million hectares where the annual production is 893.1 million metric tons, and it ranks second to China in production in the world perspective (CMIE Report, 2010). 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

Typical symptoms of sheath blight disease of rice caused by *R. solani* collected from different rice growing districts of Chhattisgarh and adjoining districts of Madhyapradesh, Odisha and Maharshtra states.

Isolation of *Rhizoctonia solani* from infected plant sample

For the isolation of *Rhizoctonia solani*, infected rice plant parts, having typical symptoms along with healthy tissues were cut in to 0.5 cm with the help of sterilized blade. These pieces were washed thoroughly with the tap water and placed into1.0 per cent sodium hypochloride solution for 30 seconds followed by washing thrice with the sterilized water thoroughly. The pieces were then transferred aseptically to petri dishes containing sterilized Potato Dextrose Agar (PDA) and incubated at 28±2ºC under BOD incubator. The petri dishes were examined at regular time intervals for fungal growth radiating from the infected pieces and the 15 isolates were isolated.

Culture media

The following cultural media's used in the study:

1. Potato Dextrose Agar Medium (PDA) Koley and Mahapatra (2015)
2. Malt extract Agar Medium (MEA) Koley and Mahapatra (2015)
3. Oat meal Agar Medium (OMA) Koley and Mahapatra (2015)
4. Czapek's Dox Agar Medium (CDA) Czapek F (1902)
5. Richard's Agar Medium (RA) Koley and Mahapatra (2015)
6. Yeast extract agar
7. Potato dextrose agar

Procedure

Seven culture media were used to find out the most suitable medium for the mycelial growth and sclerotia formation of the *R. solani*., Among them each culture media was prepared in 1 liter of water and for sterilization autoclaved at 121.6ºC at 15 psi for 20 min., These were cooled and then poured in 90 mm petri dishes, 20 ml of melted medium was poured into each sterilized Petri plates and solidified in room temperature, 5 mm disc of the test fungus were cut with the help of sterilized cork borer from six days old culture grown on PDA petri plate.,one disc was placed in the centre of each petri plates. Three replications of each medium were maintained and incubated at 26±1°C. Mycelia growth were recorded after 4 days and sclerotial formation were recorded after 6 days of incubation.

Carbon sources

To study the effect of different carbon sources on mycelia growth and sclerotia formation of *R. solani* isolate RS-1 the carbon source present on potato dextrose agar medium.i.e. sucrose, lactose, galactose, dextrose, sorbitol and mannitol were the carbon sources used in the study. In the sterilised petri dish, the sterilised melting warm medium was poured and allowed to solidify. The petri dish was filled with an actively growing 7-day-old culture of 5 mm discs here three replications were kept. The plates were incubated at 24 ±1ºC. The sclerotial development has been recorded after 5-6 days following inoculation, and observations were made after complete mycelia growth in any one treatment.

Different pH

To determine how pH levels affect the mycelial growth and sclerotial distribution. of *R. solani* isolate RS-1 on potato dextrose agar medium. Tests were carried out at pH values of 4,5, 6, 7,8 and 9. Before sterilisation, pH were adjusted by using pH meter and by using either N/10 HCl or N/10 NaOH before autoclaving the PDA medium. For each pH value, .three replications were maintained. The Petri plates containing sterilized medium was inoculated with 5 mm mycelium disc and incubated at 24 ±1ºC. Mycelia observations were recorded after 4 days and the number of sclerotia formation per plate was recorded after 5 days.

Results and Discussion

Effect of different culture media on mycelial growth of *Rhizoctonia solani*

The investigation different media boosted the radial mycelial growth of *Rhizoctonia solani* showed that Potato dextrose agar (90mm), Malt extract agar (90mm) and Oat meal agar (90mm) were medium significantly showed that the highest mycelial growth in 72 hours of inoculation are followed by Host leaf extract agar (87.08mm), Czapek's dox agar (86.07mm), and Richard'sagar (81.76mm) media, whereas, the minimum radial mycelial growth was recorded in Yeast extract agar (73.97mm) (Table-1, Fig.-1).

Fluffy mycelial texture were observed in Malt extract agar, Richard's agar- agar, Oat meal agar, Potato dextrose agar, Capek's dox agar and Yeast extract agar, and thesparse type texture was observed under Host leaf extract. Potato dextrose agar, Oat meal agar and Richard's agar media had regular type colony of *R. solani* and irregular colony shape was observed in Malt extract agar, Host leaf extract agar, Czapek's dox and Yeast extract agar media whereas the pale white

colony pigmentation was commonly observed in media under the study except the yeast extract media emits the yellowish white pigmentation of *R. solani* colony (Plate-1).

Many workers found that pathogen *R. solani* had great diversity in colony colour, growth pattern and colony diameter (Lal and Kandari, 2009). Potato dextrose agar media supported maximum colony growth of *R. solani* closely followed in Oat meal agar and Malt extract agar (Husain *et al.,* 2016).

Sclerotial distribution

Significantly highest number of sclerotia of *R. solani* were recorded in Potato dextrose agar (75.00) are followed by Oat meal agar (63.33), Richard's agar (62.66), Malt extract agar (31.00), Czapek's dox agar (23.00) and least in Host leaf extract agar (13.00), while no sclerotia formation was recorded with Yeast extract agar medium in the 4 day period.

The influence of different nutrients media *i.e.,* Malt extract agar, Richard's agar- agar, Oat meal agar, Potato dextrose agar, Capek's dox agar, Host leaf extract and Yeast extract agaron sclerotia formation *i.e.,* no colour arrangement of sclerotia of *R. solani* were recorded under the study.

Under the different medium the period of sclerotia formation fully development of sclerotia also differed under the study. The colour of sclerotia of *R. solani* also varied under the media. The brown colour sclerotia was recorded under Malt extract agar, Czapek's dox agar and Host leaf extract agar, while reddish brown colour of sclerotia were seen in Oat meal agar, Potato dextrose agar and Richard's agar media. Peripheral sclerotial arrangement were observed in Oat meal agar, while scattered in Host leaf extract agar and Potato dextrose agar, whereas, Center & scattered sclerotial arrangement was recorded in Czapek's dox agar and Richard's agar.

Similar findings were observed by different workers who observed variation in sclerotial colour, texture and arrangement pattern of *R. solani* in different media (Hoa, 1994; Sinha and Ghufran, 1988; Singh *et al*., 1990). Maximum sclerotial formation was observed in Potato dextrose agar and Oat meal agar (Sharma *et al.,* 2013; Kumar *et al*., 2014; Husain *et al.,* 2016).

Effect of different carbon sources on mycelial growth of *Rhizoctonia solani*

To determine the influence of different carbon sources for supporting the mycelial growth of *Rhizoctonia solani* were tested and grow in solid medium supplemented with six different carbon sources *i.e.,* Mannitol, Galactose. Sorbitol, Lactose, Sucrose, Dextrose on pathogen (*Rhizoctonia solani* khun) mycelial growth, colony characters sclerotia formation. Observation was shown in the (Table-2, Fig.-2).

Among the different carbon sources, significantly highest radial growth of *R. solani*was recorded under dextrose (90 mm), sucrose (90 mm), lactose (90.00 mm) on 3rd days of inoculation and mannitol (90 mm) are followed by the galactose (80.91mm) medium, while minimum growth was recorded under Sorbitol (75.41min) medium on 4th day of inoculation. The sparse colony texture of *R. solani* was recorded in galactose and dextrose carbon sources, while fluffy colony texture growth was recorded in mannitol, sorbitol, lactose and sucrose. The regular colony shape was observed in mannitol, while, irregular colony shape was recorded in sorbitol, sucrose dextrose, galactose and lactose medium (Plate-2). The carbon sources *i.e.,* dextrose, galactose and lactose are effective carbon source for the mycelial growth of *R. solani* (Lakpale *et al.,* 1997).

The no. of sclerotia formation of *R. solani* also varied by different carbon sources. Significantly maximum no. of sclerotia formation was recorded with the dextrose carbon sources (55.33) are followed by mannitol (43.00), lactose (32.00), galactose (32.00)and sucrose (24.00), while, least in sorbitol (23.33) medium. Days taken for sclerotia initiation to maturity also differed on different carbon sources,the sclerotia initiation started at 4 days of incubation in mannitol, lactose and sucrose dextrose, while it was delayed for one days i.e., 5th day of inoculation under galactose and sorbitol.

The dark brown colour sclerotia was observed in mannitol, reddish brown in mannitol, lactose and sorbitol, while pale brown in dextrose and galactose. Peripheral arrangement of selerotia was observed in sorbitol and sucrose, while scattered type were recorded in mannitol, galactose, lactose and dextrose medium.

Kumar *et al*. (2014) also reported *R. solanı* formed maximum no of sclerotia in dextrose followed by glucose.

Effect of different pH on mycelial growth of *Rhizoctonia solani*

Among the different pH level, significantly maximum mycelial growth of *R. solani* was recorded under pH-7 (90.00 mm) and pH -6 (90.00 mm) on 4th day of inoculation are followed by pH-8 (87.41 mm), pH-9 (82.31 mm) and pH-5 (81.58), while, minimum mycelium growth was recorded in pH-4 (63.24 mm) (Table-3, Fig.-3).

The colony texture of *R. solani* also differed under the different pH level. The thread like mycelia growth and pale white colour of *R. solani* were commonly observed under the different pH level whereas, whitish colony colour was recorded in the pH level 4. The colony shape of *R. solani* also slightly varied under the different pH level and the irregular type colony shape of *R. solani* were recorded with the level of pH 5,6,7 and 9 and regular type colony shape were recorded and the level of pH 4 and 8 (Plate-3).

Similar findings were recorded by different workers who recorded pH-6 and pH-7 were effective for the radial growth of the pathogen *R. solani* (Singh and Malhotra, 1994 Goswami *et al*., 2011; Kumar *et al.,* 2014).

The number of sclerotia formation of *R. solani* was greatly varied with influence by pH level and significantly maximum number of sclerotia formation was recorded in pH-7 (62.66), while, least in pH-6 (42.00) followed by pH-5 (22.66), pH-8 (21.33), and pH-9 (17.00). No sclerotia formation was recorded on pH-4 and taken to start of initiation of sclerotia on 4 days and maturity of sclerotia also varied in different pH media. The *R. solani* after 4 days of incubation in pH-7, after 5 days in pH-6 and pH-8, while after 6 days in pH-6.Thebrown colour of sclerotia was recorded in pH-7. pH-8 and pH-9, reddish brown in pH-5 and pH-6. Centre & scattered sclerotia arrangement was found in pH-6 while, scattered in pH-7 and scattered sclerotia arrangement was observed in pH-5, pH-8 and pH-9.

Singh and Malhotra (1994) observed maximum number of sclerotia formation was found in pH-6 in *R. solani*. Goswami *et., al*. (2011) also reported pH-8, pH-4 and pH-7 respectively supported maximum number of sclerotia formation in *R. solani*.

Table 1 : Effect of different media on mycelia growth and sclerotia formation of *Rhizoctonia solani*.

S. No.	Media	Colony Dia. (mm 72 hr)	Mycelial growth	Colony texture	Colony pigmentation	Colony shape	No. of sclerotia per plate	Colour of sclerotia	Arrangement of sclerotia
1.	Malt extract agar	90.00	Thread like	Fluffy	Pale white	Irregular	31.00	Brown	Center
2.	Host leaf extract agar	87.08	Thread like	Sparse	Pale white	Irregular	13.00	Brown	Scattered
3.	Richard's agar	81.76	Thread like	Fluffy	Pale white	Regular	62.66	Reddish brown	Center & Scattered
4.	Czapek's dox agar	86.07	Thread like	Fluffy	Pale white	Irregular	23.00	Brown	Center & Scattered
5.	Oat meal agar	90.00	Thread like	Fluffy	Pale white	Regular	63.33	Reddish brown	Periphery
6.	Yeast extract agar	73.97	Thread like	Fluffy	Yellowish white	Irregular	00.00	-	-
7.	Potato dextrose agar (Control)	90.00	Thread like	Fluffy	Pale white	Regular	75.00	Reddish brown	Scattered
C.D. (P=0.05)		**2.2**					**2.8**		
SE(m)±		**0.79**					**0.94**		

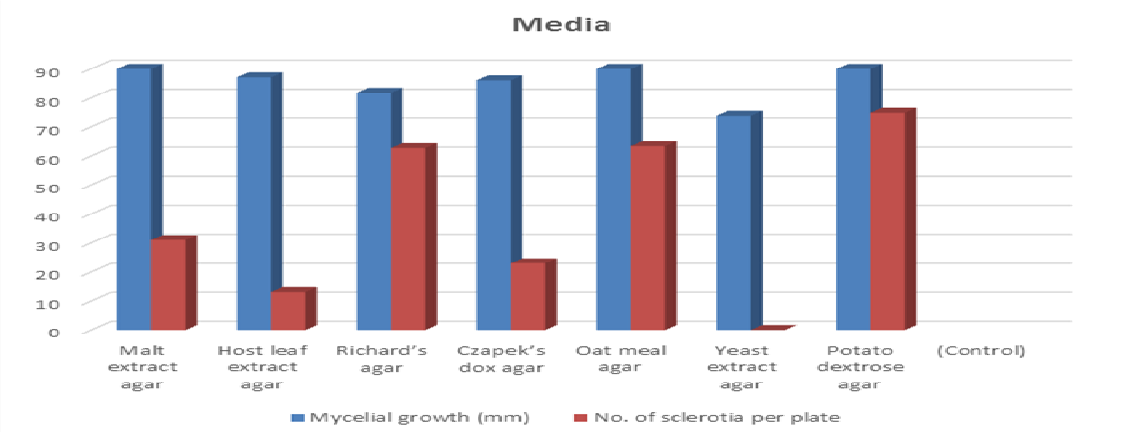

Fig. 1 : Effect of different media on mycelia growth and sclerotia formation of *Rhizoctonia solani*.

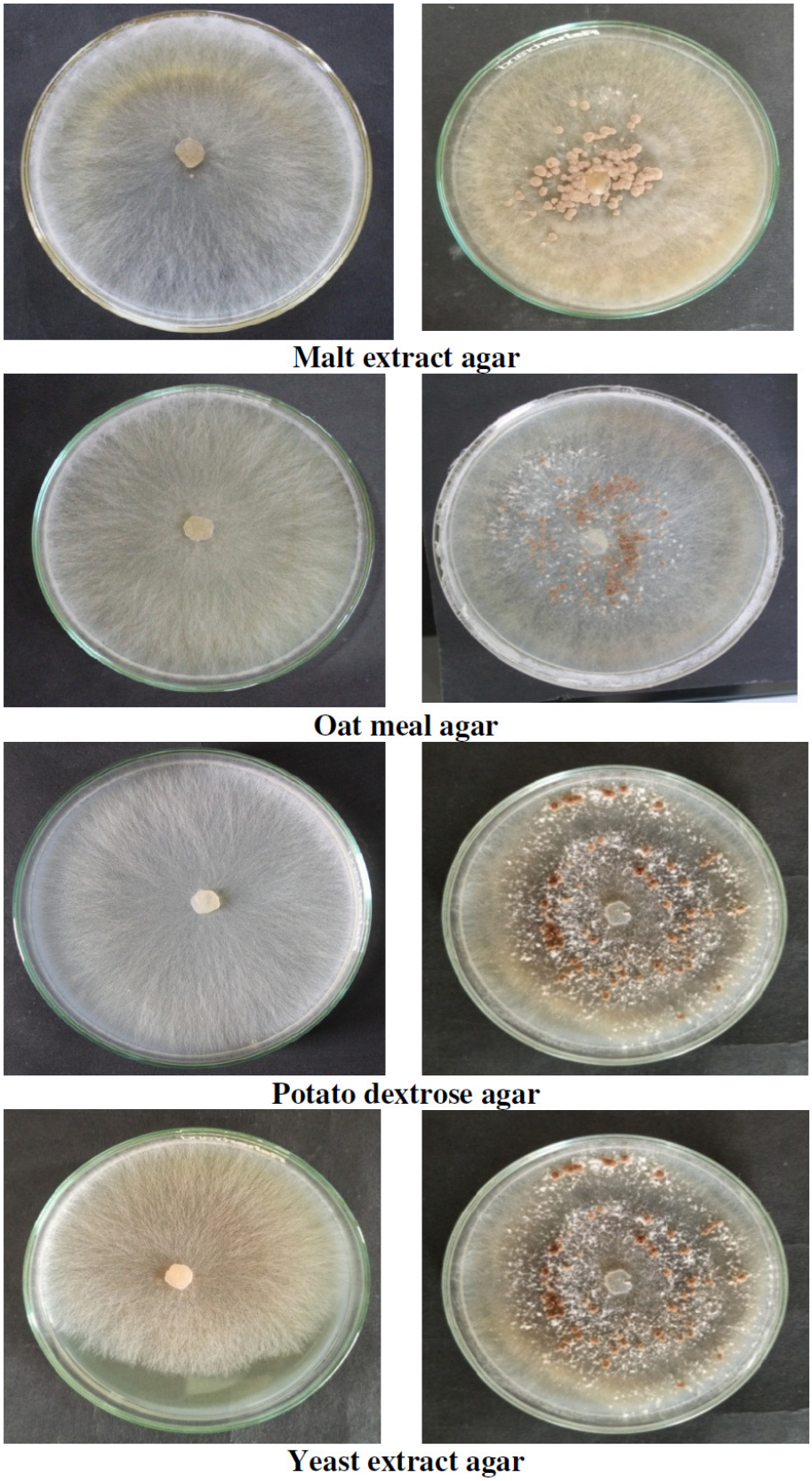

Plate 1 : Effect of different media on mycelia growth and sclerotia formation of *Rhizoctonia solani*.

Table 2 : Effect of different carbon sources on mycelial growth and sclerotia of *Rhizoctonia solani.*

S. no.	Carbon sources	Colony Dia. (mm 72 hr)	Mycelial growth	Colony texture	Colony pigmentation	Colony shape	No. of sclerotia per plate	Colour of sclerotia	Arrangement of sclerotia
1.	Sucrose	90.00	Thread like	Fluffy	Pale white	Irregular	24.00	Brown	Periphery
2.	Galactose	80.91	Thread like	Sparse	Pale white	Irregular	32.00	Pale brown	Scattered
3.	Lactose	90.00	Thread like	Fluffy	Pale white	Irregular	32.66	Dark brown	Scattered
4.	Sorbitol	75.41	Thread like	Fluffy	Pale white	Irregular	25.66	Dark brown	Periphery
5.	Mannitol	90.00	Thread like	Fluffy	Pale white	Regular	43.00	Reddish brown	Scattered
6.	Dextrose (Control)	90.00	Thread like	Sparse	Pale white	Irregular	55.33	Pale brown	Scattered
C.D. (P=0.05)		**2.3**					**3.7**		
SE(m)±		**0.7**					**1.1**		

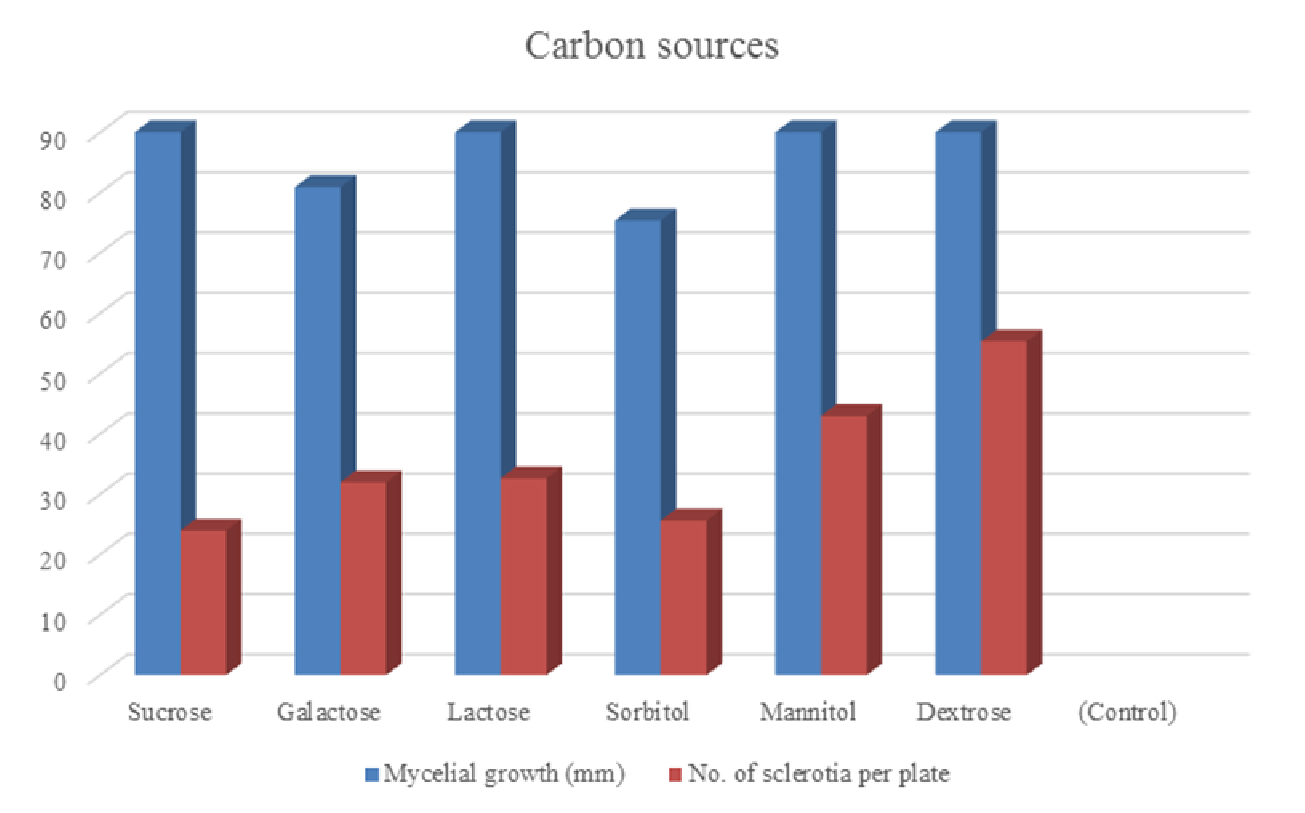

Fig. 2 : Effect of different carbon sources on mycelial growth and sclerotia of *Rhizoctonia solani*

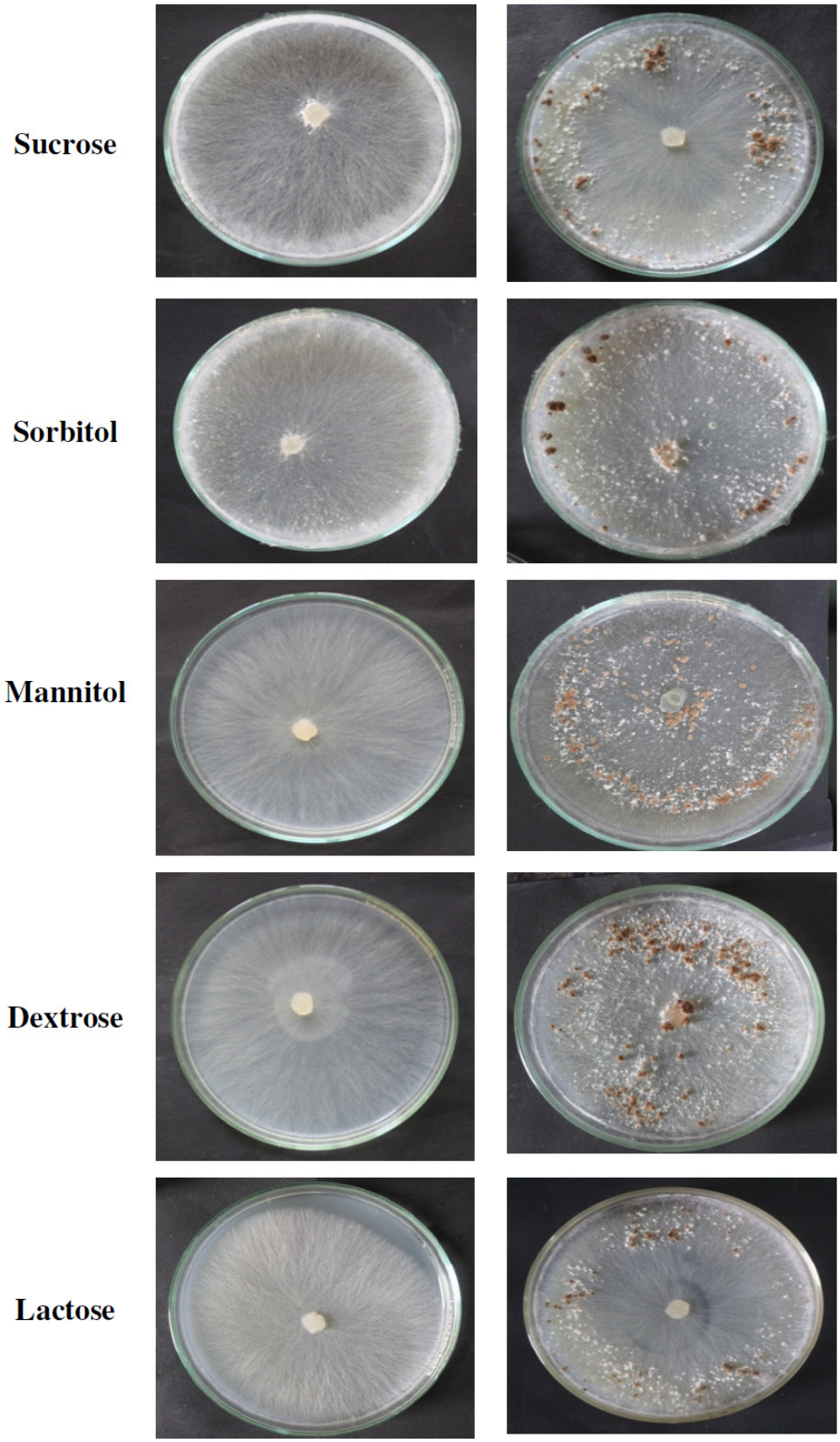

Plate 2 : Effect of different carbon sources on mycelial growth and sclerotia of *Rhizoctonia solani.*

Table 3 : Effect of different pH on mycelial growth and sclerotia formation of*Rhizoctonia solani.*

S. no.	pH	Colony Dia. (mm 72 hr)	Mycelial growth	Colony texture	Colony pigmentation	Colony shape	No. of sclerotia per plate	Colour of sclerotia	Arrangement of sclerotia
1.	4	63.24	Thread like	Fluffy	Whitish	Regular	00.00	-	-
2.	5	81.58	Thread like	Fluffy	Pale white	Irregular	22.66	Reddish brown	Scattered
3.	6	90.00	Thread like	Fluffy	Pale white	Irregular	42.00	Reddish brown	Center & Scattered
4.	7	90.00	Thread like	Sparse	Pale white	Irregular	62.66	Brown	Center & Scattered
5.	8	87.41	Thread like	Sparse	Pale white	Regular	21.33	Brown	Scattered
6.	9	82.31	Thread like	Sparse	Pale white	Irregular	17.00	Brown	Scattered
C.D. (P=0.05)		**2.6**					**3.2**		
SE(m)±		**0.83**					**1.05**		

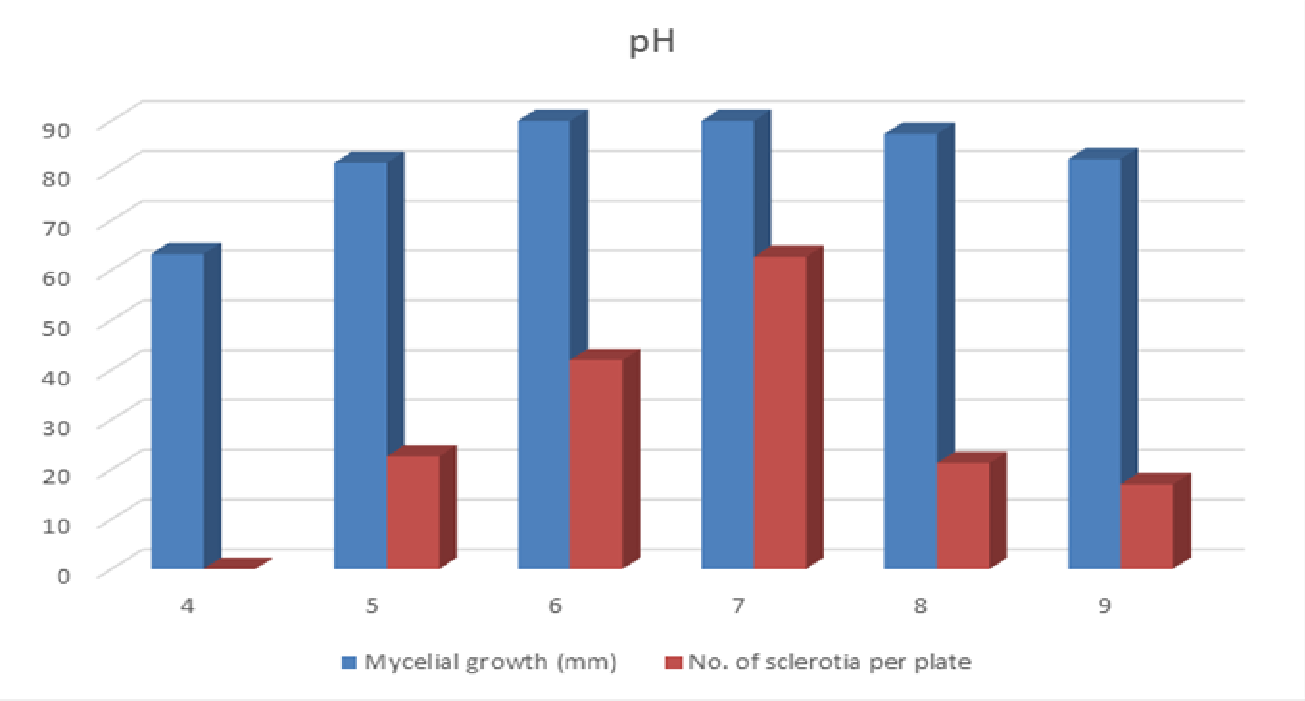

Fig. 3 : Effect of different pH on mycelial growth and sclerotia formation of*Rhizoctonia solani.*

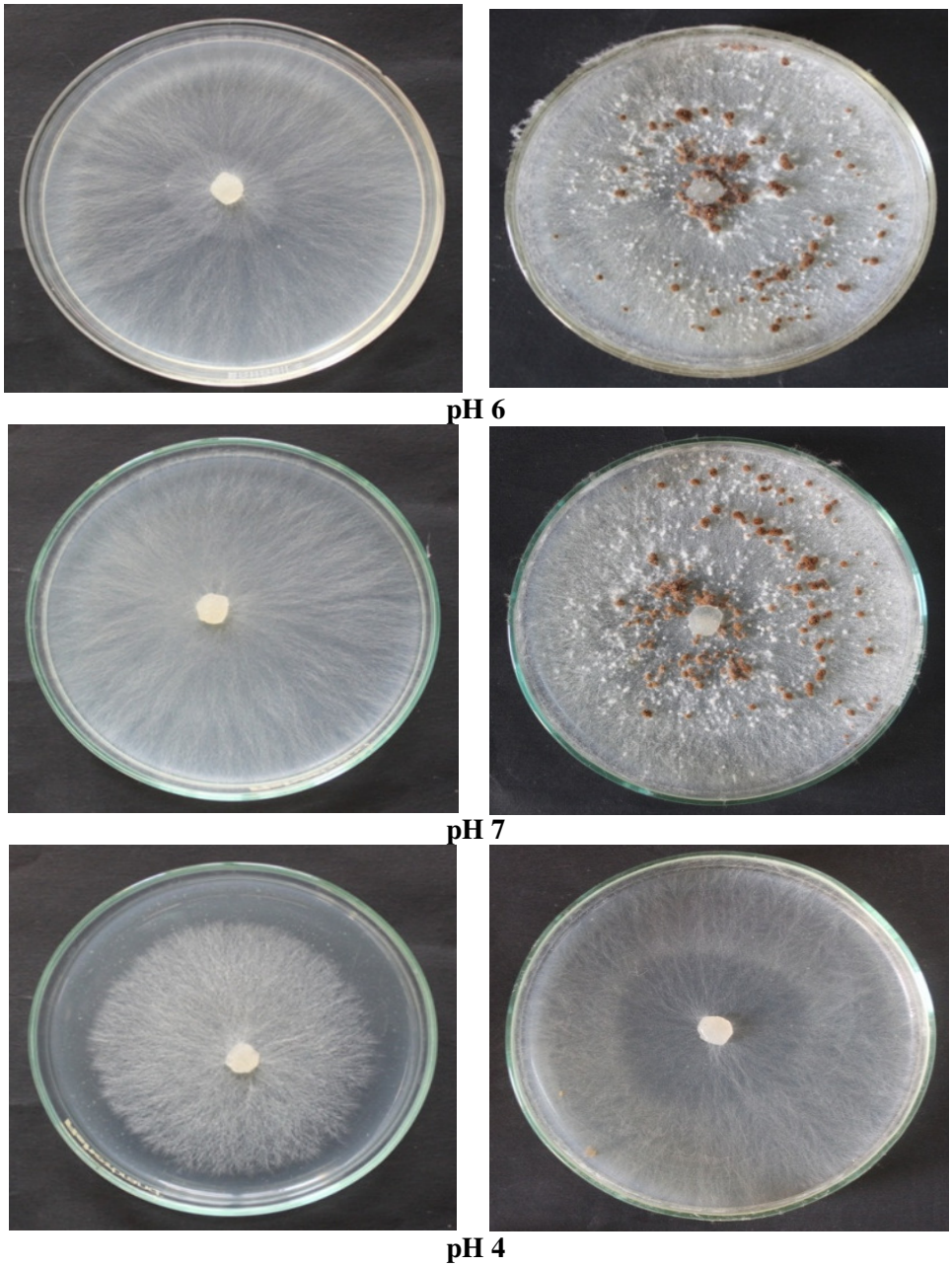

Plate 3 : Effect of different pH on mycelial growth and sclerotia formation of *Rhizoctonia solani.*

Conclusion

Sheath blight of rice caused by *Rhizoctonia solani* is an important disease. The present study was conducted to evaluate the effect of different media, carbon sources, and pH on mycelial growth and sclerotia formation of *Rhizoctonia solani.* The investigation showed different media boosted the radial mycelial growth of *Rhizoctonia solani* were Potato dextrose agar (90mm), Malt extract agar (90mm) and Oat meal agar (90mm) mycelial growth was observed. In the different carbon sources, significantly highest radial growth of *R. solani* was recorded under dextrose (90 mm), sucrose (90 mm), lactose (90.00 mm) on 3rd days of inoculation and mannitol (90 mm). Among the different pH level, significantly maximum mycelial growth of *R. solani* was recorded under pH-7 (90.00 mm) and pH -6 (90.00 mm).

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