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Effect of different cultural media on growth of *Sclerotium rolfsii* Sacc. incited bycollar rot of lentil

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Abstract
Sclerotium rolfsii Sacc. is a serious ubiquitous soil borne phytopathogenic fungus, causing Southern blight The fungus is notorious for its ability to induce dark stem rot, during any plant growth stages, followed by drooping and wilting of leaves and gradually wilting of the whole plant. Potato dextrose agar (85.17 mm), followed by Richard's agar (75.50 mm), Beet root dextrose Agar (68.50 mm), Carrot dextrose agar (58.67 mm), Lentil leaf dextrose Agar (54.83 mm), Cornmeal agar (53.00 mm), Czapek'sDox agar (49.00 mm), while minimum growth was recorded in Malt extract Agar (48.33 mm). In respect of sclerotial formation in *S. rolfsii*, the maximum number of sclerotia were formed in Richard's agar (131.00 /plate) followed by Potato dextrose agar (126.67 /plate), Czapek'sDox agar (118.67/ plate), Malt extract Agar (111.33 /plate), Carrot dextrose agar (103.33 /plate), Beetroot dextrose agar (102.00/plate), and Lentil leaf dextrose Agar (98.67/plate), while the minimum number of sclerotial was found in Cornmeal agar (92.67/plate).

Keywords: *Sclerotium rolfsii*, media, mycelial growth, sclerotial development

Introduction
Sclerotium rolfsii Sacc.is a serious ubiquitous soil borne phytopathogenic fungus, causing Southern blight of a wide range of agricultural and horticultural crops (Aycock, 1966 and Anahosur, 2001 [3, 2] and Galdames and Diaz, 2010 [8] and Kwon *et al.*, 2013 and Shen *et al.*, 2014 and Mahadevakumar *et al.*, 2015) [3, 2, 8, 16, 11]. The fungus is notorious for its ability to induce dark stem rot, during any plant growth stages, followed by drooping and wilting of leaves and gradually wilting of the whole plant. Such wilted plants show white cottony fungal thread girdling the basal part of the stem and moving below the stem to roots (Kator*et al.*, 2015 and Sun *et al.*, 2020 and Punja *et al.*, 1985) [10, 15, 14]. This pathogen survives as sclerotia on decayed plant material in the soil which germinate later and attack surrounding host plants (Sachslehner*et al.*, 1997 and Ludwig and Haltrich, 2002) [17, 12]. This fungus is widely distributed and causes heavy economic losses on many crops (Aycock, 1966 and Gurha and Dubey, 1983 and Fery and Dukes, 2002 and Billah, 2017 and Sun *et al.*, 2020) [3, 9, 7, 4, 15]. Under laboratory conditions that culture media have significant effects on these parameters. However, there is little information on pathogen nutritional requirements in semi-synthetic media. Therefore, this study was undertaken to examine the effect of different culture media on mycelial growth and sclerotial formation *S. rolfsii*.

Materials and Method
Collar rot infected diseased plants of lentil were affected from the lentil fields. These diseased plants were examined in the laboratory of Department of Plant Pathology College of Agriculture, Gwalior and isolations were made cultured *S. rolfsii* was isolated on PDA from the plants showing collar rot symptoms of lentil. The pure culture of the fungus was obtained and maintained on PDA for further study. Eight different solid mediums were evaluated for obtaining maximum mycelial growth of the *S. rolfsii*. The experiment was laid out in complete randomized design with replicated thrice times. Eight solid culture media *viz*., Potato Dextrose agar, Czapek'sDox agar, Richard agar, Carrot Dextrose agar, Beetroot Dextrose agar, Cornmeal agar, Malt Extract agar and lentil leaf Dextrose agar were used to compare the growth rate of *S. rolfsii***.** The culture medium was prepared by the standardized method and autoclaved at 121.6 °C, 15 psi pressure for twenty minutes. The required quantities (20 ml) of each medium were poured in 90 mm sterilized Petri dish in aseptic condition. Each Petri plate was inoculated separately with uniform mycelia culture bits (5 or 7 mm disc) cut with the help

of cork borer from young (5 days) vigorously growing culture were placed on the middle of the each pre poured medium and incubated at 25±2 °C (Dela Paz *et al*., 2006). Each treatment was replicated three times. The diameter of the growth of the fungus was measured 3, 5 and 7 days after inoculation and 15th day for number of sclerotia were recorded.

Results

The culture media selection for the growth of *Sclerotium rolfsii* is very essential factor for study the morphological and cultural characteristic. In order to standardized the media for optimum growth of pathogen the various media was under taken during the study**.**

Radial growth of the pathogen

The results of the experiment conducted during 2021 revealed that all the media were found effective against growth and sclerotial production of *Sclerotium rolfsii*. Significant difference in the fungal growth was observed in the tested media at 3, 5 and 7 days after inoculation.In order to study that radial growth of*Sclerotium rolfsii* the same was grown on eight different solid culture media; Potato dextrose Agar, Czapek'sDox Aga, Malt extract Agar, Cornmeal Agar, Richard's Agar, Carrot dextrose Agar and Lentil leaf dextrose Agar and the data are presented in (table-1 and fig-1). The fungus capable of growing on different solid media, but variation in growth and number of sclerotia formed was observed on eight media. The mycelial growth was measured at 3, 5 and 7 day after inoculation. Whereas Sclerotia formation was also recorded at 15 day after inoculation .The minimum mycelium growth (14.00 mm) was recorded in Malt extract Agar medium where as its maximum growth (35.00 mm) was recorded in Potato dextrose agar medium. The growth of Potato dextrose agar medium at 3 day after inoculation was significantly superior over rest of the tested media.

At 5 day after inoculation the maximum growth was recorded in Potato dextrose agar (59.50 mm), followed by Richard's agar (50.83 mm), Lentil leaf dextrose Agar (47.67 mm), Beet root dextrose Agar (44.00 mm), Cornmeal agar (42.33 mm), Carrot dextrose agar (39.67 mm), and Czapek'sDox agar (33.17 mm), while minimum growth was recorded in Malt extract Agar (32.33 mm).

In all the tested medium at 7day after inoculation the maximum fungal growth was recorded in Potato dextrose agar (85.17 mm), followed by Richard's agar (75.50 mm), Beet root dextrose Agar (68.50 mm), Carrot dextrose agar (58.67 mm), Lentil leaf dextrose Agar (54.83 mm), Cornmeal agar (53.00 mm), Czapek'sDox agar (49.00 mm), while minimum growth was recorded in Malt extract Agar (48.33 mm). The remaining media leaf dextrose agar and cornmeal agar were at-par with each other.

In Richard's agar media a special feature was noticed, at first the mycelium aggregate more vigorously at one place. In the same place numerous number of sclerotial initial were formed at 8-9 day after inoculation. The light brown to brown sclerotia were formed at same place after 15 days after inoculation. These sclerotia were associated with the drop of exudate.

Data presented in table-1 depicted that the mean of 3, 5 and 7 days after inoculation of *S. rolfsii* of different media was clearly indicated that the maximum fungal growth was recorded in Potato dextrose agar (59.89 mm), followed by Richard's agar (47.28 mm), Beet root dextrose Agar (43.78 mm), Lentil leaf dextrose Agar (42.50 mm), Cornmeal agar and Carrot dextrose agar (38.78 mm), respectively, Czapek'sDox agar (34.83 mm), whereas minimum fungal growth was recorded in Malt extract Agar (31.67 mm). Potato dextrose agar was found significantly superior over rest of the culture media.

Sclerotia formation per plate

In respect of sclerotial formation in *S.rolfsii*, the maximum number of sclerotia were formed in Richard's agar (131.00 /plate) followed by Potato dextrose agar (126.67 /plate), Czapek'sDox agar (118.67/ plate), Malt extract Agar (111.33 /plate), Carrot dextrose agar (103.33 /plate), Beetroot dextrose agar (102.00/plate), and Lentil leaf dextrose Agar (98.67/plate), while the minimum number of sclerotial was found in Cornmeal agar (92.67/plate). The above sclerotial number clearly indicate that the Richard's agar medium was significantly superior over most of the tested media.

Colour of Sclerotia

Based on sclerotial colour all the tested media were categories in two categories *viz.*, brown and light brown. Light brown colour sclerotia were found four media *viz,*Beet root dextrose agar, Malt extract agar, Carrot dextrose agar and Potato dextrose agar, whereas light brown sclerotial colour were found in media cornmeal agar, Richard's agar, Lentil leaf dextrose Agar and Czapek'sDox agar media.

Shape of sclerotia

Among all the tested media, shapes of sclerotia were categories in two categories *viz.*, round and round to oval. Round shape sclerotia were found in six media *viz*., Beet root dextrose agar, Malt extract agar, Carrot dextrose agar, Lentil leaf dextrose agar, Czapek'sDox agar and Potato dextrose agar, while round to oval shape sclerotia was observed in cornmeal agar and Richard's agar media.

Table-1: *In-vitro* evaluation of culture media for growth of *Sclerotium rolfsii.*

S. No	Culture medium	Radial growth (in mm)				Sclerotia		
		3 DAI	5 DAI	7 DAI	Mean	Number/ Plate	Shape	Colour
1	Beet root dextrose Agar	18.83	44.00	68.50	43.78	102.00	Round	Brown
2	Malt extract Agar	14.00	32.67	48.33	31.67	111.33	Round	Brown
3	Cornmeal Agar	22.00	42.33	52.00	38.78	92.67	Round to oval	Light brown
4	Richard's Agar	15.50	50.83	75.50	47.28	131.00	Round to oval	Light brown
5	Carrot dextrose Agar	17.67	40.00	58.67	38.78	103.33	Round	Brown
6	Lentil leaf dextrose Agar	25.00	47.67	54.83	42.50	98.67	Round	Light brown
7	Czapek'sDox Agar	22.33	33.17	49.00	34.83	118.67	Round	Light brown
8	Potato dextrose Agar	35.00	59.50	85.17	59.89	126.67	Round	Brown
Sem±		0.40	0.58	1.17	-	1.00	-	-
CD at 5%		1.23	1.76	3.56	-	3.02	-	-

*Mean of three replications

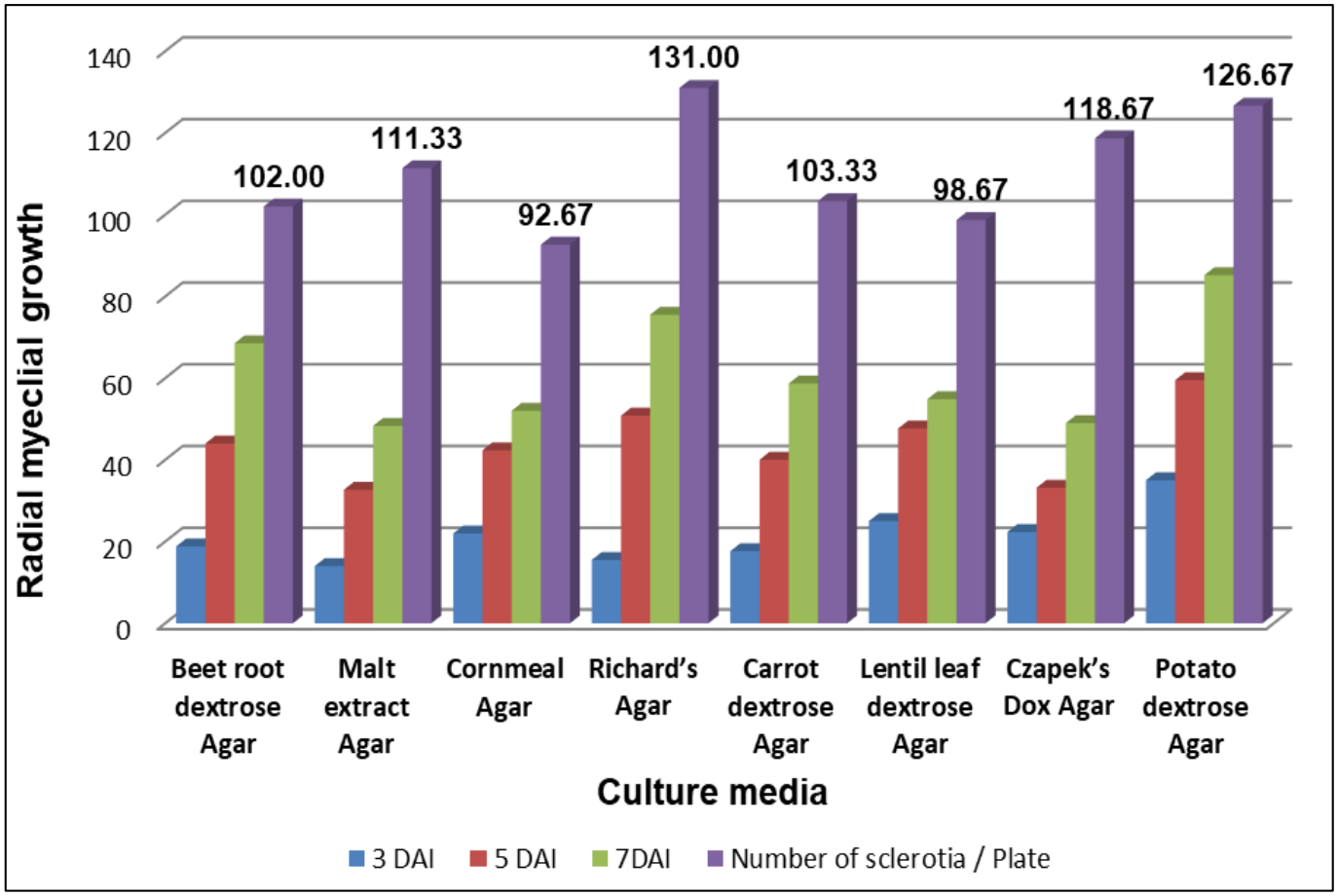

Fig 1: Influence of culture media on mycelial growth and number of sclerotia /plate

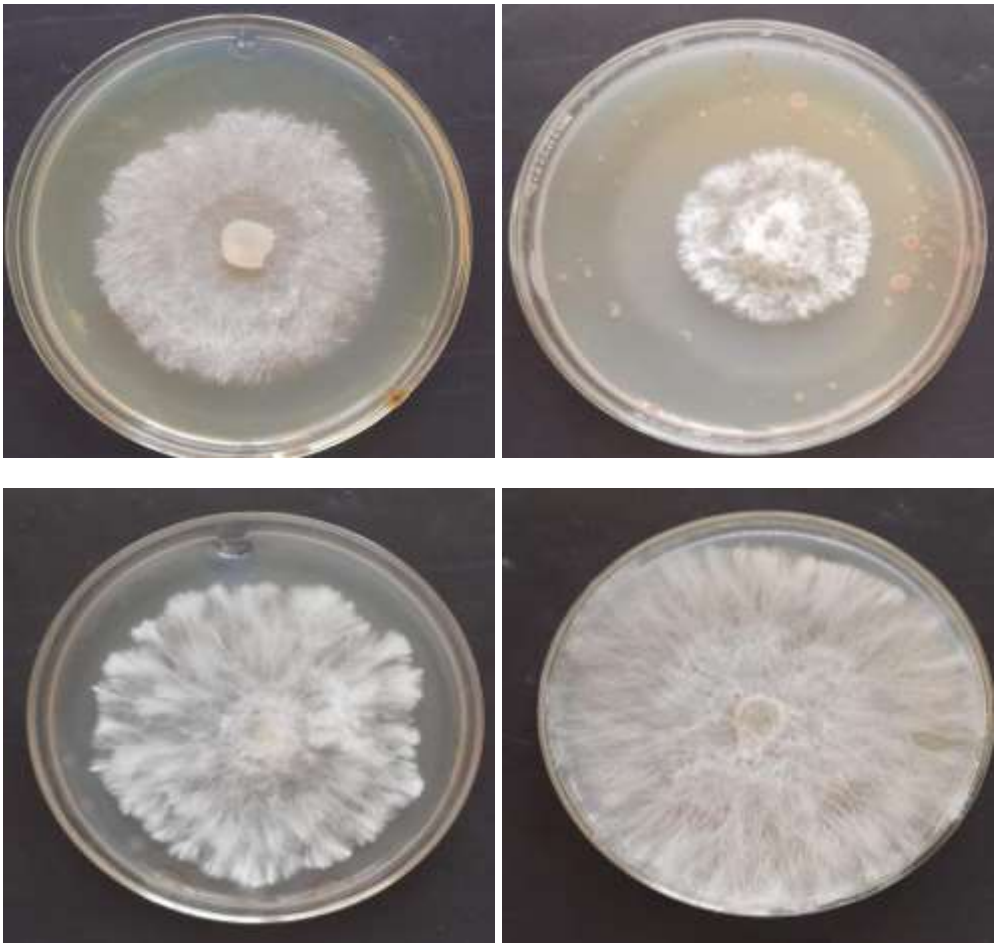

Plate 1: *In-vitro* evaluation of culture media against *S. rolfsii*.

Discussion

In present study eight culture media were evaluated for the growth and sclerotial formation of *S. rolfsii.* At 7 days after inoculation maximum fungal growth was recorded in Potato dextrose agar, followed by Richard's agar, Beet root dextrose Agar, Carrot dextrose agar, Lentil leaf dextrose Agar, whereas minimum fungal growth was recorded in Malt extract agar. Similarly mean of 3, 5 and 7 days after inoculation maximum fungal growth was recorded in Potato dextrose agar, followed by Richard's agar, Beet root dextrose Agar, while minimum fungal growth was recorded in Malt extract Agar. Potato dextrose agar significantly superior over rest of the culture media. Several workers had been reported variation in mycelium growth of fungus in different media because of nutritional status of media. Akram *et al*. (2007) [1] reported that potato dextrose agar was best for the radial growth and sclerotial production of *S. rolfsii*. Bhosale and Verma (2007) [5] showed that at 8 Days after inoculation, mycelial growth was most pronounced (90%) in Richard's medium. The highest number of sclerotia was recorded in linseed meal medium. Chaurasia *et al*., (2013) [6], Zape *et al*., (2013) [18] reported that the most suitable medium for better growth of *Sclerotium rolfsii* was potato dextrose agar (PDA) (90.00 mm).

Conclusion

All of the culture media evaluated greatly increasing the growth of *Sclerotium rolfsii*. Maximum fungal growth was recorded in Potato dextrose agar, whereas minimum fungal growth was recorded in Malt extract agar.

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