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Effect of different media, light conditions and temperature on growth of *Sclerotium rolfsii in vitro*

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Abstract
Sclerotium rolfsii is a well-known soilborne fungus causes root rot, stem rot, collar rot, wilt and foot rot in several plants. The pathogen was exposed to various media, light conditions and temperatures in order to ascertain mycelial growth and the number of sclerotia of *S. rolfsii.* The results demonstrated that potato dextrose agar was the best solid media for *S. rolfsii* mycelial growth and sclerotia formation out of the six solid media evaluated. In the light experiment, alternate cycles of 13 hours light and 11 hours darkness resulted in the better mycelium growth as compared to the constant light and dark conditions. Among the different temperature six levels tested, the growth of *S. rolfsii* was maximum at 28 °C.

Keywords: *Sclerotium rolfsii*, Media, Light conditions, Temperature, etc

Introduction
Sclerotium rolfsii is polyphagous, ubiquitous, facultative and most destructive soil borne fungus. It is an anamorph of *Athelia rolfsii*. Pier Andrea Saccardo, initially identified the species in 1911, based on specimens brought to him by Peter Henry Rolfs, who believed the unnamed fungus was the cause of tomato blight in Florida. He named the species *Sclerotium rolfsii* and placed it in the old *Sclerotium* genus.
According to Aycock (1996) [3], the host range of *S. rolfsii* is very broad, encompassing several horticultural and agronomical crops all over the world. It attacks over 500 species of plants from over 100 families. The pathogen has a significant economic impact on a variety of crops.
Root rot, stem rot, collar rot, wilt and foot rot are all diseases caused by *S. rolfsii*, a soil-borne plant pathogen. It primarily targets host stems, though it can infect any portion of a plant in the right conditions including roots, fruits, petioles, leaves and flowers.
Many fungal pathogens have morphogenic and cultural variations, hence a comprehensive study of the effects of media, light and temperature on mycelial growth and sclerotia formation in *S. rolfsii* was carried out.

Material and Method
Isolation, identification and maintenance of pathogen
Typical collar rot symptoms showing samples were collected from the gram crop in field of College of Agriculture Pune, Maharashtra. The infected samples were brought to the laboratory and subjected to tissue isolation. The infected portion of the plant was cut into small bits in such a way that each bit consisted of infected as well as healthy tissues. The bits were surface sterilized with 0.1% mercuric chloride solution for one minute followed by serial washing in three changes of sterilized water to remove the traces of mercuric chloride. Later on, these bits were aseptically transferred on sterilized Petri plates containing 20 ml previously sterilized PDA medium. These petri plates were incubated at room temperature (27 ± 2 °C). The pure culture thus obtained was microscopically examined for identification and maintained on PDA slants at 27 ± 2 °C for further investigations.

Effect of different media on growth of *S. rolfsii*
With a view to find superior media for the growth of *S. rolfsii*, six culture media *viz.,* Potato dextrose agar, Oat meal agar, Malt extract agar, Yeast dextrose agar, Richard's agar and Soybean casein agar were used. The media were sterilized in an autoclave at 1.054 kg/cm² pressure for 30 min. and then cooled media were poured (@20 ml/plate) in sterilized glass petri plates and allowed to solidify at room temperature and inoculated by placing sclerotia of *S. rolfsii* in the centre of plates. Plates were incubated at room temperature (27± 2 °C).

Effect of different light conditions on growth of *Sclerotium Rolfsii*

The study of different light conditions was undertaken with a view to ascertain the effect of different light conditions on growth of fungus. Petri plates inoculated with *S. rolfsii* were placed under four different light conditions *viz*., continuous dark, continuous light, alternate day and light and Ultra Violet radiation. Daily observations were recorded.

Effect of different temperature levels on growth of *Sclerotium rolfsii*

Effect of different temperature levels on mycelial growth of *S. rolfsii* was studied *in vitro*. Twenty ml of sterilized PDA medium was poured in each sterilized petri plates. Inoculation was made with sclerotia and incubated at 6 different levels of temperature *viz*., 0 °C, 4 °C, 10 °C, 20 °C, 28 °C and 45 °C. Observations on growth were recorded.

Result and Discussion

Growth of *Sclerotium rolfsii* on different media

The results presented in (Table1 and Plate 1) revealed that all the culture media tested showed variation in growth and amount of sclerotia formation of *Sclerotium rolfsii*. The radial growth of *Sclerotium rolfsii* was maximum on potato dextrose agar (73.00 mm) as compared to rest of media. After PDA, mycelial growth on oat meal agar and malt extract agar were as 68.00 mm and 65.00 mm, respectively. The growth was 56.50 mm on Richard's agar and 42.00 mm on soybean casein agar. The minimum growth was observed in yeast dextrose agar medium (16.50 mm).

Potato dextrose agar showed abundant, upraised, white cottony growth of mycelium with profuse amount of sclerotia formation. In yeast dextrose agar, growth of mycelium was poor, irregular and dull white cottony without formation of sclerotia.

Results of present study on the effect of different culture media on growth of *Sclerotium rolfsii* are in conformity with those reported by earlier workers Ayed (2018) [4], Shiva *et al.* (2019) [11], Sravani and Ramchandra (2020) [12] who reported maximum growth and sclerotia formation of *S. rolfsii* on potato dextrose agar media.

Table 1: Effect of different culture media on growth of *S. rolfsii*

Tr. no.	Treatment	Colony diameter (5 DAI in mm)	Colony growth characters	Sclerotia formation (15 DAI)
T1	Potato dextrose agar	73.00	Abundant, upraised, white cottony growth	Profuse
T2	Oat meal agar	68.00	Regular, upraised, white cottony growth	Fair
T3	Malt extract agar	65.00	Regular, flat, white cottony growth	Fair
T4	Yeast dextrose agar	16.50	Poor, irregular, dull white cottony growth	Nil
T5	Richard's agar	56.50	Moderate, fluppy, dull white cottony growth	Moderate
T6	Soybean casein agar	42.00	Poor, regular, dull white cottony growth	Poor
SE (m)±		0.37		
CD (0.01)		1.51		

*DAI- Days after inoculation

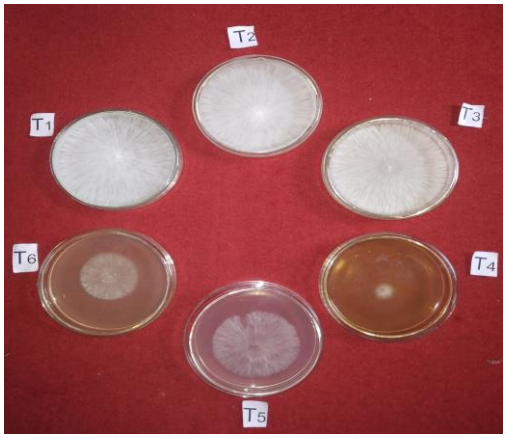

Plate 1: Growth of *S. rolfsii* on different media

T1- Potato dextrose agar
T2- Oat meal agar
T3- Malt extract agar
T4- Yeast dextrose agar
T5- Richard's agar
T6- Soybean casein agar

Growth of *Sclerotium rolfsii* under different light conditions

The results revealed (Table 2) that the exposure of pathogen to alternative cycles of 13 hrs light and 11 hrs darkness produced the maximum growth of mycelium (86.40 mm) of *S. rolfsii* which was significantly superior over other tested treatments. The mycelial growth of pathogen exposed to continuous dark resulted in minimum growth as 59.20 mm and continuous light resulted in moderate mycelial growth as 72.80 mm of *S. rolfsii*. There was no mycelial growth of pathogen which exposed to UV radiation.

Abundant, upraised, white cottony growth of mycelium occurred in alternate light and dark conditions with profuse amount of sclerotia formation in plate. Regular, white cottony growth in continuous light with fair amount of sclerotia were formed and under continuous dark condition poor amount of sclerotia were formed in plate.

These results were in confirmation with Muthukumar and Venkatesh (2013) [10], Tushar and Patel (2019) [14] who reported that *S. rolfsii* was exposed to alternate light and dark condition recorded maximum growth with a greater number of sclerotia formation.

Plate 2: Growth of *S. rolfsii* under different light conditions

T1 – Continuous dark
T2 – Alternate day and light
T3 – Continuous light
T4 – U. V. radiation

Table 2: Effect of light and darkness on growth of *S. rolfsii*

Tr. no.	Treatment	Colony diameter (5 DAI in mm)	Colony growth characters	Sclerotia formation (15 DAI)
T1	Continuous darkness	59.20	Regular, white cottony growth	Poor
T2	Alternate light and darkness	86.40	Abundant, upraised, white cottony growth	Profuse
T3	Continuous light	72.80	Regular, upraised, white cottony growth	Fair
T4	U.V. radiation	0.00	No growth	Nil
SE (m)±±		0.29		
CD (0.01)		1.18		

*DAI- Days after inoculation

4.3.3 Growth of *S. rolfsii* at different temperature levels

S. rolfsii was incubated at six different temperature levels from the results presented in Table 3 that the significantly higher mycelial growth (68.00 mm) of *S. rolfsii* was noticed at incubation of 28 ˚C as compared to other attempted treatments. No growth was observed at temperatures 0 ˚C, 4 ˚C and 45 ˚C.

At 28 ˚C temperature, there was abundant, upraised, white colony mycelial growth with profuse sclerotia structure formation. There were no sclerotia formation at 0 ˚C, 4 ˚C and 45 ˚C.

The findings of this experiment are found to be matched with reports of Maiti and Sen (1988), Ayed (2018) [4], Shiva *et al.* (2019) [11], Sravani and Ramchandra (2020) [12]. They concluded that maximum growth of the pathogen occurred at 28 ˚C.

Table 3: Effect of different temperature levels on growth of *S. rolfsii*

Tr. no.	Treatment	Colony diameter (5 DAI in mm)	Colony growth characters	Sclerotia formation (15 DAI)
T1	0 ˚C	0.00	No growth	Nil
T2	4 ˚C	0.00	No growth	Nil
T3	10 ˚C	38.00	Poor, irregular, white cottony growth	Nil
T4	20 ˚C	66.00	Regular, white cottony growth	Fair
T5	28 ˚C	68.00	Abundant, white cottony growth	Profuse
T6	45 ˚C	0.00	No growth	Nil
SE (m)±±		0.15		
CD (0.01)		0.60		

*DAI- Days after inoculation

Plate 3: Growth of *S. rolfsii* under different temperature levels
T1- 0 ˚C
T2- 4 ˚C
T3- 10 ˚C
T4- 20 ˚C
T5- 28 ˚C
T6- 45 ˚C

Conclusion

Potato dextrose agar found the most suitable medium for mycelial growth of *S. rolfsii*. It produced maximum growth in alternate light and dark condition and at temperature 28 °C.

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