

## To study the effect of culture media on cultural and morphological characteristics of *sclerotium rolfsii*.

### ABSTRACT

The present study was undertaken to assess the influence of different culture media on the mycelial growth and cultural characteristics of *Sclerotium rolfsii*, an important soil-borne pathogen responsible for collar rot in several crops. Eight culture media, namely Potato Dextrose Agar (PDA), Sabouraud Maltose Agar, Oat Meal Agar, Coon's Agar, Corn Meal Agar, Ashby's Mannitol Agar, Czapek's Dox Agar, and Richard's Agar were evaluated under *in vitro* conditions. Significant variation in radial growth, colony morphology and pigmentation was recorded among the tested media. Maximum mycelial growth was observed on Potato Dextrose Agar (90.00 mm), which was statistically at par with Sabouraud Maltose Agar (89.68 mm) and Oat Meal Agar (89.65 mm). Minimum growth was recorded on Czapek's Dox Agar (67.86 mm). The colony characters ranged from cottony, fluffy, irregular to compact mycelium, while pigmentation varied from pure white to creamy and yellowish white depending on the medium. These results clearly indicate that PDA, Sabouraud Maltose Agar and Oat Meal Agar are the most suitable media for luxuriant growth and sporulation of *S. rolfsii*, and can be recommended for cultural variability studies, pathogenicity assays and further research on this pathogen.

### Introduction

Groundnut (*Arachis hypogaea L.*) is one of the most important oilseed as well as leguminous crops belonging to the family Fabaceae. It is commonly known as peanut, earthnut, monkey nut, and goobers. Groundnut is believed to have originated in South America, from where it spread to Asia and Africa. In India, it is grown both in kharif and rabi seasons under a wide range of agro-climatic conditions, mainly as a rainfed crop in semi-arid regions. It is a short-duration crop with moderate drought tolerance, wider adaptability, and fits well in different cropping systems. Groundnut seeds are rich in oil (40–50%), protein (20–30%), carbohydrates, vitamins, and minerals, making it an important food as well as industrial crop. Being a legume,

it enriches soil fertility through biological nitrogen fixation and also contributes organic matter to the soil. In addition to its edible oil, groundnut haulms serve as nutritious fodder for livestock.

Groundnut is one of the most important legume crops and is a rich source of proteins and edible oil in the human diet. It is considered as an excellent food for both children and adults. Groundnut seeds contain Protein (25–30%), Oil (44–56%), Carbohydrates (18%) and Minerals (4–5%). It is also a good source of essential amino acids such as arginine, lysine and histidine, and provides vitamins like niacin, thiamine, riboflavin and vitamin E. Groundnut oil is rich in monounsaturated (oleic acid) and polyunsaturated fatty acids (linoleic acid), which help in reducing cholesterol levels. Besides, groundnut also contains important bioactive compounds such as resveratrol and phytosterols, which contribute to its antioxidant and cardioprotective properties (Gopalan *et al.*, 1995, Kris-Etherton *et al.*, 1999).

In India, groundnut is mainly grown in the states of Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra, Rajasthan and Madhya Pradesh. The area, production, and productivity of groundnut in the country during the year 2023-24 were about 47.16 lakh ha, 101.80 lakh tonnes and 2,160 kg/ha, respectively. In Maharashtra, the area, production and productivity of groundnut during the year 2023-24 were 1.45 lakh ha, 3.14 lakh tonnes and 2,162 kg/ha, respectively. In the Marathwada region of Maharashtra, the area, production and productivity of groundnut during the year 2023-24 were 0.42 lakh ha, 0.86 lakh tonnes and 2,047 kg/ha, respectively. (As per the Final Advance Estimates 2023-24, Department of Agriculture, Government of Maharashtra).

Groundnut is affected by several fungal, bacterial and viral diseases (Ghewande *et al.*, 2002). The major fungal diseases include stem rot, collar rot, dry root rot, leaf spot (early and late), rust, seedling blight and wilt. The important viral diseases reported are peanut bud necrosis, peanut mottle, peanut clump and rosette. Among these, stem rot or southern blight incited by *Sclerotium rolfsii* is one of the most destructive diseases causing severe damage to groundnut crop and leading to heavy yield losses (Mehan *et al.*, 1994; Mayee and Datar, 1988; Subrahmanyam *et al.*, 1992; Ghewande *et al.*, 2002; Mahato *et al.*, )

In Maharashtra, stem rot of groundnut has been reported as one of the most destructive diseases causing severe economic losses to farmers. *Sclerotium rolfsii* infects the stem near the soil line and spreads rapidly under warm and humid conditions. The fungus produces white mycelial growth on the stem surface, eventually forming small, round, mustard-seed-like sclerotia. The infected stem becomes soft and water-soaked, leading to wilting and death of the plant. The disease also affects pegging and pod formation, resulting in significant reduction in yield (Haari *et al.*, 1991 Mahato *et al.*, 2017).

## Materials and Methods

### The effect of culture media on cultural and morphological characteristics of *Sclerotium rolfsii*

To evaluate the influence on growth and sporulation, eight media were used Potato Dextrose Agar (PDA), Richards' agar, Oat Meal agar, Coon's agar, Corn meal medium, Ashby's mannitol agar, Czapek's dox agar and Sabouraud maltose agar. Except of Potato dextrose agar (PDA), all media were pre-made (make: Hi Media). Sterilize all media in an autoclave at 15 lbs/inch pressure for 20 minutes.

Sterilized Petri plates with a 90 mm diameter were filled with autoclaved and cooled media (20 ml/plate) and allowed to solidify at room temperature. Petri plates of each culture medium (three plates/medium/replication) were infected after the media and solidified by inoculating a 5 mm mycelial disc of a pure culture, that was actively developing seven days after, it was cultured. Three replications of each culture media were made. After that, plates were kept in a BOD incubator at  $27 \pm 2$  °C. After seven days of incubation, observations on mycelial development, colony colour and sporulation were noted. Media giving best radial growth of mycelium were used for further studies.

#### Details of experiment

**Design** : CRD

**Replications** : Three

**Treatments** : Eight (culture media)

#### Treatment details

**M1** : Potato dextrose agar

**M2** : Sabouraud maltose agar

**M3** : Oat meal agar

**M4** : Coon's agar

**M5** : Corn meal medium

**M6** : Ashby's mannitol agar

**M7** : Czapek's dox agar

**M8** : Richard's agar

Visual and microscopic observations were made about the morphological and cultural characteristics of the pathogen, including Sclerotial mycelial development, colony colour, diameter and sporulation.

## Results and discussion

### Effect of culture media

Cultural characteristics *viz.*, colony diameter and mycelial growth of *Sclerotium rolfsii* were studied *in vitro* using eight different culture media and the results obtained are presented in Table I and depicted in Fig. I and PLATE I.

### Mycelial growth

The results (Table -I, Fig I, and Plate I) revealed that, all of the eight culture media tested encouraged better growth of *Sclerotium rolfsii*. The mean colony diameter mycelial growth recorded with all the test media was ranged from 67.86 mm (Czapek's dox agar) to 90.00 mm (Potato dextrose agar). However, significantly highest mean mycelial growth was recorded on Potato dextrose agar (90.00 mm) followed by Sabouraud's dextrose agar (89.68 mm), Oat meal agar (89.65 mm) Coon's agar (88.28 mm), Corn meal agar (86.00 mm), Richard's agar (85.63 mm) and Ashby's mannitol agar (78.33 mm), which were at par with each other. These were followed by the media *viz.*, Richard's agar (85.63 mm), Ashby's mannitol agar (78.33 mm) media which were at par with each other. Comparatively minimum mean mycelial growth of the test pathogen was found on Czapek's dox agar (67.86).

### Colony colour

The data presented in ( Table I ) also revealed that colony colour varied from white, dull white, pale white, creamy white, yellowish white in colour. The colour of the mycelial growth on PDA, Sabouraud maltose Agar, Oat meal Agar, Coon's Agar, Corn meal Agar, Ashby's mannitol Agar Czapek's dox Agar, Richard's Agar was yellowish white, Sabouraud maltose Agar was white to dull white and Czapek's Dox Agar was creamy white.

### Colony character

The data presented in ( Table I ) revealed that there was significant variation in colony character of *Sclerotium rolfsii* depending on the medium . On Potato Dextrose Agar (PDA), the fungus exhibited cottony white mycelium growth. Sabouraud Maltose Agar resulted dull white mycelium, Oat Meal Agar produced white colony of mycelium. Corn Meal Agar exhibited whitish fluffy cottony growth. Ashby's Mannitol Agar leads to white colour mycelium and smooth margin. Czapek's Dox Agar showed colonies with compact creamy white mycelium. On Rechar'd's Agar were initially white but later turned to yellowish colour

The results of the present study on the cultural variability *Sclerotium rolfsii* on different media are in similar line with those reported earlier by several workers *viz.*, Archana *et al.*, (2019) found that *Sclerotium rolfsii* exhibited maximum radial growth (9.0 cm) and profuse

sclerotial production on Potato Dextrose Agar, followed by Host Extract Agar and Oat Meal Agar, while plain agar supported poor growth without sclerotia formation.

**Table .1 Effect of different culture media on mycelial growth of *Sclerotium rolfsii***

<b>Tr No</b>	<b>Media used</b>	<b>Colony diameter (mm)</b>	<b>Colony character</b>	<b>pigmentation</b>
M <sub>1</sub>	Potato dextrose agar (PDA)	90.00	Cottony white mycelium grow	White
M <sub>2</sub>	Sabouraud maltose Agar	89.68	White to dull white mycelium	White to dull
M <sub>3</sub>	Oat meal Agar	89.65	White colony colour of mycelium	White
M <sub>4</sub>	Coon's Agar	88.28	White irregular growth of mycelium	White
M <sub>5</sub>	Corn Meal Agar	86.00	White fluffy cottony growth	Pale White
M <sub>6</sub>	Ashby's Mannitol Agar	78.33	White colour mycelium	White
M <sub>7</sub>	Czapek's Dox Agar	67.86	Aerial with compact creamy white Mycelium	Creamy White
M <sub>8</sub>	Ricahard's Agar	85.63	Initially white and then turn to yellowish colony colour	Yellowish white
	<b>SE(m)</b>	<b>1.93</b>	-	-
	<b>C.D. @ 1%</b>	<b>0.63 %</b>	-	-

\*- Mean of three replications; Figure in parenthesis are arc sine transformed value.

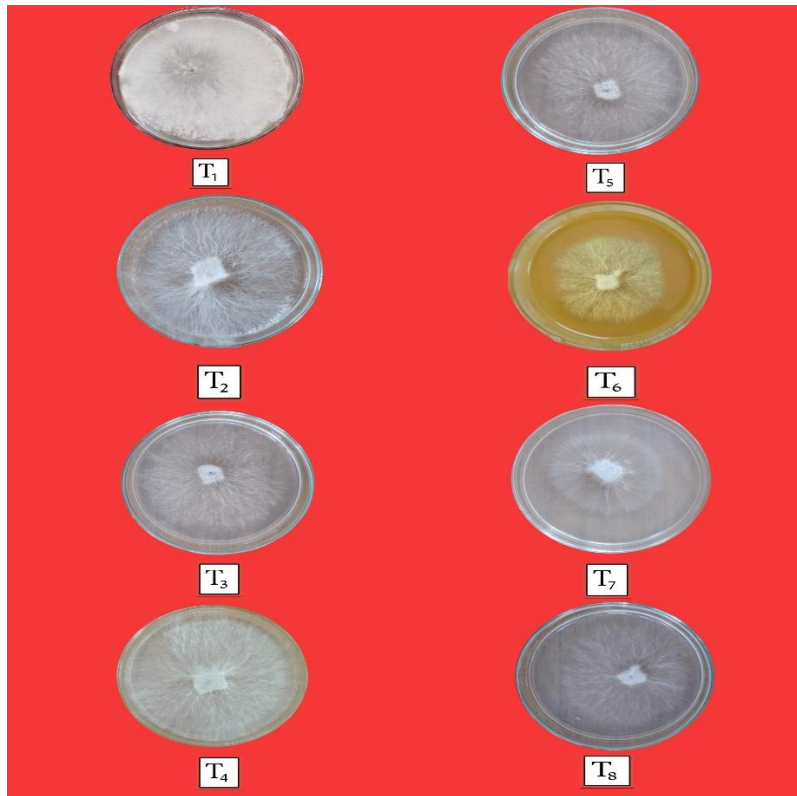
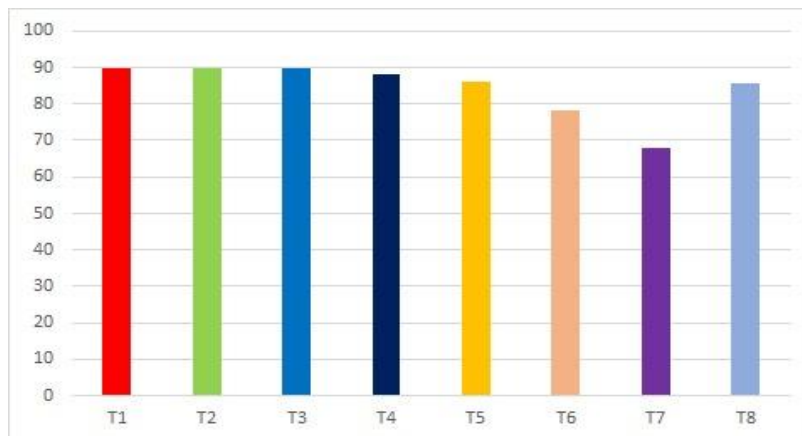


Plate 1. Cultural characteristics viz., colony diameter and mycelial growth of *Sclerotium rolfsii*



T<sub>1</sub> – Potato dextrose agar

T<sub>5</sub> – Corn meal agar

T<sub>2</sub> - Sabouraud maltose agar

T<sub>6</sub> – Ashby’s mannitol agar

T<sub>3</sub> – Oat meal agar

T<sub>7</sub> – Czapek’s dox agar

T<sub>4</sub> – Coon’s agar

T<sub>8</sub> – Rechar’s agar

**Fig.1** *In vitro* effect of different culture media on growth of *Sclerotium rolfsii*

## Conclusion :

Among the different culture media tested, Potato Dextrose Agar (90 mm) and Sabouraud Maltose Agar (89.68 mm) were found to be the most effective in supporting maximum colony diameter with profuse cottony white mycelial growth. These results indicate that PDA and Sabouraud Maltose Agar provide favorable nutrient composition for vigorous mycelial development and can be considered as the most suitable media for maintaining and studying the fungus.

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