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Physiological Studies of *Sclerotium rolfsii* Sacc. Causing Stem Rot of Groundnut (*Arachis hypogaea* L.)

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A B S T R A C T

Different media, carbon, nitrogen, temperature and pH were used on the mycelial growth and biomass production of *Sclerotium rolfsii* Sacc (*in vitro*) causing stem rot of groundnut. Total ten culture media used in the culturing the *Sclerotium*. Out of ten media PDA (90mm; 763.3mg) showed maximum growth of *S. rolfsii* followed by Nutrient Agar 71.3mm; 233.3mg). In carbon sources significant growth was observed in sucrose (90mm; 730mg) and followed by starch, dextrose and Maltose, while in lactose significantly reduced the growth on both solid and broth. Potassium nitrate was the best among the ten nitrogen sources. Maximum growth and biomass showed at temperature 30°C which was reduced significantly below 20°C and above 35°C. Mycelial growth was observed at all pH tested, maximum growth was recorded at pH 6 (87.3 mm) and dry weight 270mg.

***Key words*:** Culture media, Carbon, Nitrogen, pH, Temperature, Peanut, *Sclerotium rolfsii*

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop in India, belongs to family Leguminosae. Groundnut pods are consumed directly as raw, roasted as well as boiled. It is important for its high oil content ranges from 44 to 56 percent as well as total protein 16.2 to 36 percent [1]. It is also a source of fibre, iron, magnesium, phosphorus, niacin, and vitamin E as well as phytoestrogen, flavones and other antioxidant compounds. The major groundnut producing countries in the world namely India, China, Nigeria, Senegal, Sudan, Burma and the United States of America. India is the second largest groundnut producer in the world under 6.7 million cultivations. The major groundnut growing states are Gujarat, Andhra Pradesh, Karnataka, Tamil Nadu and Maharashtra which together account for about 80% of area and 81% of production in India [2]. Although in various states of India groundnut is cultivated in one or more (kharif, rabi and summer) seasons, nearly 80% of acreage and production comes from kharif crop (June-October). In Maharashtra, it is mainly cultivated in Dhule, Kolhapur, Satara, Pune, Ahemadnagar, Nashik and Sangali. In 2019, over 187500 hectares of land in the state was used for groundnut production [3]. Many factors are responsible for decreasing production of groundnut in recent years among which the soil-borne fungal diseases like root rot, collar rot, stem rot and pod rot are very important. Stem rot is caused by *Sclerotium rolfsii* Sacc. has become a severe disease in groundnut growing regions. This disease causes severe damage and yield losses over 25% [4]. The present investigation was conducted on the variations like media, carbon and nitrogen sources, temperature and pH on the mycelial growth and mycelial dry weight of *Sclerotium rolfsii*.

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MATERIALS AND METHODS

Collection and isolation of the pathogen

Infected plants of groundnut were collected from field under survey and brought back in Research Laboratory, K.V. Pendharkar. Small pieces of infected tissue of about 0.5 to 1 cm from infected region were cut with sterile scalpel. The pieces were surface sterilized in 0.5% sodium hypochlorite solution for 5 min and rinsed in three changes of sterile distilled water. Then dried in between sheets of sterile filter paper and transferred onto fresh PDA medium impregnated with streptomycin, and incubated for seven days at 28 ± 2°C. Plates were observed periodically for growth of the pathogen. The isolates were further purified by using bit of mycelium from each colony on potato dextrose agar (PDA). The culture thus obtained was stored in refrigerator at 5°C for further studies and was sub cultured periodically. The pathogen was identified as *Sclerotium rolfsii* based on its morphological and colony characters [5].

Effect of different media

Mycelial growth of S. rolfsii on solid medium

The mycelial growth of *S. rolfsii* was cultured on ten different media: namely Potato Dextrose Agar (PDA), Czapek Dox Agar (CZA), Nutrient Agar (NA), Waksman's medium,

Fungi Kimming Agar (FKA), Yeast Dextrose Agar (YDA), Malt Extract Agar (MEA), Richard's solution, Corn Meal Agar (CMA) and Water Agar (WA). All these media were prepared autoclaved at 121°C for 30 min and poured into Petri plates. Plates were inoculated with a 5 mm mycelial disc cut from a 7-day old culture of *S. rolfsii* and incubated at 30°C. Three replicates for each individual treatment were maintained.

Growth on liquid medium

Broths were prepared, sterilized and poured 20 ml of broth in Erlenmeyer flasks (100 ml). After that, the medium was inoculated with 5 mm mycelial disc of *S. rolfsii* obtained from 7 days old culture grown on PDA. The inoculated plates were incubated for 7 days at room temperature (27 ± 2°C). At the end of the incubation period, the mycelial mats were filtered through filter paper (Whatman No. 41) and dried in hot air oven at 105°C for 48 h and the mycelial dry weight was calculated. Three replications were maintained for each treatment.

Effect of different carbon and nitrogen

Mycelial growth of S. rolfsii on solid medium

The *in vitro* growth of the fungus was tested with ten different carbon sources and nitrogen sources. Potato Dextrose Agar medium was taken as the basal medium for the study, dextrose replaced by various carbons on equivalent weight basis. For nitrogen, prepared medium for each nitrogen source an amount 1% and autoclaved at 15 lb pressure for 20 min then poured in sterile Petri plate at 20 ml quantities. After cooling, they were inoculated with 5 mm mycelial disc of the pathogen obtained from 7 days old culture and incubated for 7 days. Control was set without any carbon and nitrogen source. The linear growth of the pathogen was measured in mm at the end of the incubation period. Three replications were maintained for each treatment.

Growth on liquid medium

Potato dextrose broth was taken as the basal medium for the study amended with different carbon and 1% nitrogen sources respectively on equivalent weight basis. Broth were prepared, sterilized and inoculated as described previously. At the end of the incubation period the mycelial dry weight was calculated prescribed as early. In both the methods, three replications were maintained for each treatment.

Effect of different temperatures

Solid medium: Petri dishes were poured and inoculated as described previously and incubated in BOD at different temperature (5, 10, 15, 20, 25, 30 and 35°C) for 7 days. The mycelial growth of the pathogen was measured in mm at the end of incubation period.

Liquid medium: Erlenmeyer flasks (100 ml) containing 20 ml of potato dextrose broth were sterilized, inoculated and incubated at different temperatures namely: 5, 10, 15, 20, 25, 30 and 35°C for 7 days in BOD incubator. At the end of the incubation period the mycelial dry weight was calculated prescribed as early. In both the methods, three replications were maintained for each treatment.

Effect of different pH

Solid medium: Potato dextrose agar of different pH levels (3.0, 4.0, 5.0, 6.0, 7.0 and 8.0) prepared by using 0.1N HCl or 0.1N NaOH and autoclaved. Petri dishes were poured and inoculated as described previously and incubated for 7 days at room temperature (28 ± 2°C). Observation recorded after incubation.

Liquid medium: Potato dextrose broths of different pH levels (3.0, 4.0, 5.0, 6.0, 7.0 and 8.0) were prepared, sterilized and inoculated. At the end of the incubation period the mycelial dry weight was calculated prescribed as early. In both the methods, three replications were maintained for each treatment.

RESULTS AND DISCUSSION

Effect of different media

S. rolfsii isolates were able to grow on all tested culture media, out of all the maximum growth as well dry weight observed on Potato Dextrose Agar 90 mm, 763.3mg respectively (Table 1). Indicated that, Nutrient Agar was moderate the growth as compared to PDA (71.3mm; 233.3mg). However, in Richard's Solution Agar and broth growth was minimum (16.3mm; 60mg). Previous studies also showed a similar pattern, in that *S. rolfsii* preferred PDA medium for best growth *S rolfsii* [6-8].

Table 1 Effect of different media on the mycelial growth and dry weight of *S. rolfsii*

Name of media	Mycelial growth of in mm*	Dry weight in mg*
Potato dextrose agar	90	763
Czapek dox agar	50	00
Malt extract agar	64.6	207.6
Nutrient agar	71.3	233.3
Waksman's medium	69	140
Fungi Kimming agar	57.6	210
Hansen's medium	66.6	220
Richard's solution agar	16.3	60
Dextrose yeast extract peptone agar	52.6	97
Water agar	72.6	17

*Means of three replications

Effect of different carbon sources

Ten carbon sources tested, sucrose recorded the maximum mycelial growth of 90 mm and dry weight of 730 mg (Table 2). In this table observed that the growth of *S. rolfsii* was higher the growth and followed by starch (88mm; 570mg) and dextrose (88mm; 480mg).

Table 2 Effect of different carbon sources on the mycelial growth and dry weight of *S. rolfsii*

Name of carbon source	Mycelial growth of in mm*	Dry weight in mg*
Dextrose	88	480
Cellulose	86	320
D- Fructose	72.3	530
Galactose	73	500
D+ Glucose	80.3	640
Lactose	42	320
D+ Maltose	87	570
Starch	88	570
Sucrose	90	730
Xylose	74.7	580
Control	36	230

*Means of three replications

Effect of different nitrogen sources

Maximum growth of *S. rolfsii* was observed in Ammonium chloride, Ammonium nitrate and Potassium nitrate 90 mm and mycelial dry weight was highest in Potassium nitrate (263mg) followed by ammonium chloride(226mg) and ammonium nitrate (223mg) (Table 3). Minimum growth on

media containing urea (50mm: 23mg). No growth observed in plate containing Cobalt nitrate, Lead nitrate and silver nitrate on the both solid as well broth [13].

Table 3 Effect of different Nitrogen sources on the mycelial growth and dry weight of *S. rolfsii*

Name of Nitrogen	Mycelial growth of in mm*	Dry weight in mg*
Ammonium chloride	90	226
Ammonium nitrate	90	223
Ammonium sulphate	72.3	196
Calcium nitrate	83	146
Cobalt nitrate	00	00
Lead nitrate	00	00
Potassium nitrate	90	263
Silver nitrate	00	00
Sodium nitrate	88	196
Urea	50	023
Control	56	080

*Means of three replications

Table 4 Effect of different temperatures on the mycelial growth and dry weight of *S. rolfsii*

Temperature ℃	Mycelial growth of in mm*	Dry weight in mg*
5	00	00
10	17.6	13
15	43.3	51.6
20	62.6	153.3
25	87.3	246.6
30	90	346.6
35	79.3	293.3

*Means of three replications

Effect of different temperatures

As shown in (Table 4), *Sclerotium rolfsii* was able to grow over a range of temperatures varying from 10°C to 35°C whereas no growth was observed at 5°C. Among the temperature levels tested, 30°C was found to be more favorable for the mycelial growth of *S. rolfsii* (90 mm) and also the highest mycelial dry weight of 346.66 mg, which was followed by 25° (87.3mm; 246.6mg), 35(79.3mm; 293.3mg) (Table 4). Previous published reports also observed that the optimum temperature for the *in vitro* growth of *S. rolfsii* is in the range of 25 to 30°C [11-16]. Recent study on the *S. rolfsii* showed rapid mycelial growth at 30°C [7].

Table 5 Effect of different pH levels on the mycelial growth of *S. rolfsii*

pH of media	Mycelial growth of in mm*	Dry weight in mg*
3.0	11.6	63.3
4.0	30.3	103.3
5.0	64	160
6.0	87.3	270
7.0	75	186.6
8.0	32.3	120

*Means of three replications

Effect of different pH

Sclerotium rolfsii was able to grow with wide range of pH level that is, 3.0 to 8.0 but it was maximum at pH 6.0 (87.3 mm) and dry weight 270mg followed by pH 7.0 (75mm; 186.6mg), pH 5.0 (64mm; 160mg). Mycelial growth and dry weight of *Sclerotium rolfsii* was reduced at pH 5 and pH 8. This result was compared with the earlier reports [16-18].

CONCLUSION

Effect of different nutritional sources and factors were observed the growth and dry weight of *Sclerotium rolfsii.* It is s concluded that Potato Dextrose Agar is more suitable for the growth of *Sclerotium rolfsii*, Sucrose and also Potassium nitrate are best among the nutritional factors and the optimum temperature is 30° C and optimum pH is 6.

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