Research Article

Effect of Different Media, pH and Temperature on Growth and Sclerotia Formation of *Sclerotium rolfsii* Sacc. causing Collar rot of Lentil

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Abstract

Collar rot of lentil caused by *Sclerotium rolfsii* Sacc. is one of the destructive diseases in lentil growing areas of the Madhya Pradesh. *In vitro* studies were conducted on the effect of media, temperature and pH levels on the mycelial growth and sclerotia production of *S. rofsii* Sacc causing collar rot of lentil. The results reveal that the growth of *S. rolfsii* was maximum at 30°C which was reduced significantly below 25°C and 35°C. Of the pH levels tested, pH 6.5 produced maximum mycelial and sclerotia production which was followed by exposing the pathogen to pH 6.0. Among the seven solid and liquid media tested, potato dextrose agar was the best among the solid media and potato dextrose broth was the best among liquid media for the mycelial growth and biomass production of *S. rolfsii*.

Keywords: Media, pH, temperature, *S. rolfsii*, Collar rot, Lentil

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Introduction

Lentil suffers from an attack of a number seed borne diseases such as vascular wilt, collar rot, root rot, stem rot, rust, powdery mildew and downy mildew, which are caused by *Fusarium oxysporum* f.sp. *lentis, Sclerotium rolfsii, Rhizoctonia solani, Uromycis fabae, Erysiphe polygoni and Peronospora lentis,* respectively [1]. Among the diseases, collar rot caused by *S. rolfsii* which is gaining importance. *S. rolfsii* is an economically important pathogen on numerous crops worldwide. It has an extensive host range; at least 500 species in 100 families are susceptible, the most common hosts are legumes, crucifers and cucurbits, and commonly occurs in the tropics, subtropics, and other warm temperate regions [2]. The fast growth of the *S. rolfsii* and its capability of producing excessive sclerotia that may persist in soil for several years [3]. These pathogens exhibit variation in their morphological biological and immunological characteristics and pathogenicity or resistance against harmful environment. Morphogenic and cultural variations are known in many fungal pathogens [4, 5] and as such detailed investigation was carried out on the variations with regards to media, pH and temperature, on the mycelia growth and sclerotia production of *S. rolfsii*.

Material and Methods

Isolate of *S. rolfsii* was recovered from diseased lentil plants from research farm of Jwaharlal Nehru Krishi Vishwa Vidyalaya- Jabalpur. Small pieces of infected tissues 1–2 mm dimension from the advancing margin of the spot, adjacent to healthy portions were cut with blade, washed well in distilled water to remove dust adhered to the infected pieces. Piece were dipped in 0.1 percent mercuric chloride solution for 30 seconds and finally washed well in three changes of sterilized distilled water. The bits were then transferred to PDA slants with the help of inoculating needle under aseptic condition and incubated at 28 ± 1ºC. After 48 hrs, fragments of hyphal growth from the growing tips were transferred to fresh PDA slants. Pure culture was made, following repeated hyphal tip transfer. Pure culture was maintained on PDA slants by sub culturing it at 30 days intervals. For preservation of cultures the plugged end of the culture tubes were dipped in melted wax and stored in a refrigerator at 5 ± 1ºC. Studies of the following physiological aspects of *S. rolfsii* isolates were conducted in laboratory.

Effect of media

Following seven culture media were used to find out the most suitable one for the mycelial growth and formation of sclerotia. Each culture medium was prepared in 1 liter of water and autoclaved at 121.6ºC at 15 psi for 20 min. These were cooled to 45ºC and then poured in 90 mm Petri dishes for solidification. 20 ml of melted medium was poured into each sterilized Petri plates. 5 mm disc of the test fungus were cut with the help of sterilized cork borer from the

margin of seven 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 for each of the pathogen and incubated at 28 ± 1°C. The data on growth were recorded when the fungus reached the rim of the Petri plates (90 mm) in any of the mediums. Variation in the colony diameter, types of fungal growth, pigmentation and/or sporulation and intensity of sclerotial were also recorded after 15 days of incubation.

- Potato Dextrose agar (PDA) medium (Peeled and sliced potato 200g, Dextrose 20g, Agar-agar 20g).
- Richards's agar (RA) medium (Potassium nitrate 10g, Potassium monobasic phosphate 5g, Magnesium sulphate 2.5g, Ferric chloride 0.02g, Sucrose 50g, Agar-agar 20g).
- Czapeks Dox agar (CDA) medium (Sodium nitrate 2g, Di potassium hydrogen phosphate 1g, Magnesium sulphate 0.5g, Potassium chloride 0.5g, Ferrous sulphate 0.01g, Sucrose 30g, Agar-agar 20g).
- Asthana & Hawker's medium (D-Glucose 5g, Potassium nitrate 3.50g, Potassium dihydrogen Phosphate 1.75g, Magnesium sulphate 0.75g, Agar- agar 20g).
- Ashby's agar medium (Mannitol 20g, Di potassium phosphate 0.2g, Magnesium sulphate 0.2g, Sodium chloride 0.2g, Potassium sulphate 0.1g, Calcium carbonate 5g, Agar-agar 15g, final pH (at 25°C) 7.4±0.2).
- Browns agar (BA) medium (Dextrose 2g, Tri basic potassium phosphate 1.25g, Magnesium sulphate 0.75g, Agar-agar 20g).
- Coon's agar (CA) medium (Sucrose 7.2 g, Dextrose 3.60g, Magnesium sulphate 1.23g, Potassium nitrate 2.02g, Potassium di- phosphate2.72g, Agar- agar 15g).

Effect of pH

There were seven different pH level ranging from 5.5 to 8.5 with a difference of 0.5 were prepared by using pH meter and by using either N/10 HCl or NaOH before autoclaving the PDA medium. For each pH value, three replications were maintained. The Petriplates containing sterilized medium was inoculated with 5 mm mycelium disc and incubated at 28 $\pm$ 1ºC. At the interval of 24hrs, the linear growth was measured till 3 days. The number of sclerotia formation per plate was recorded after 15 days.

Effect of temperature

The experiments were conducted to find out, the most suitable temperature for mycelial growth and number of sclerotia formation of *S. rolfsii*. The sterilized poured petriplates with PDA were inoculated with 5 mm disc of the test pathogen of seven days old culture. The petriplates were incubated at 5, 10, 15, 20, 25, 30, 35 and 40°C temperature. Three replications were maintained for each treatment and observation for mycelial growth was recorded after three days. Formation of sclerotia was recorded at fifteen days after incubation.

Results and Discussion

A total of seven media were used for studying the growth of *S. rolfsii*. It is evident from the data presented in **Table 1**, **Figures 1** and **2** that *S. rolfsii* preferred potato dextrose agar (PDA) medium for best growth. Colony diameter was observed significantly superior on potato dextrose agar medium (90.00 mm) followed by Asthana and Hawker's agar medium (58.16 mm) after 3 days of inoculation, whereas, the next best medium was Czapek's Dox agar medium (51.00), Richard's agar (47.50) and Browns agar (39.83). Coon's agar medium (19.66 mm) and Ashby's agar medium (12.50 mm) recorded comparatively less growth of *S. rolfsii.* Potato dextrose agar (PDA) medium was also found best for the sclerotial production of the *S. rolfsii*. Potato dextrose agar, Asthana and Hawker's agar and Browns agar medium showed appressed growth of the fungus while Czapek's dextrose agar, Richard's agar, Coon's agar and Ashby's agar medium showed fluffy growth pattern. The fungus produced dull white to white pigmentation on all the media tested.

Maximum dry mycelial weight (453.33 mg) of *S. rolfsii* was recorded in Potato dextrose broth medium followed by Richard's broth medium, which yielded 231.00 mg dry mycelial weight **Table 2**. Asthana and Hawker's, Ashby's and Coon's broth media did not support the growth, as in all these three media *S. rolfsii* was unable to grow. These results were in confirmation with [6] and [7]. [8] also reported that potato-dextrose medium was most suitable for mycelial growth and sclerotia production of *Sclerotium rolfsii.*

Table 1 Effect of solid media on radial growth and sclerotia formation of *S. rolfsii*

S. No	Name of the medium	Radial growth (mm)		Type of colony	Pigmentation	Degree of Sclerotia Formation (After 15 days)
		After 48 hrs*	After 72 hrs*			
1	Potato dextrose agar	65.00	90.00	Appressed	White	Fair
2	Asthana and Hawker's agar	49.00	58.16	Appressed	Dull white	Poor
3	Czapek's Dox Agar	43.50	51.00	Fluffy	Dull white	Poor
4	Richard's agar	37.16	47.50	Fluffy	Dull white	Poor
5	Browns agar	37.00	39.83	Appressed	White	Fair
6	Coon's agar	15.16	19.66	Fluffy	White	Fair
7	Ashby's agar	10.83	12.50	Fluffy	White	Fair
CD (0.05)		2.433	2.81			

*Average of 3 replications

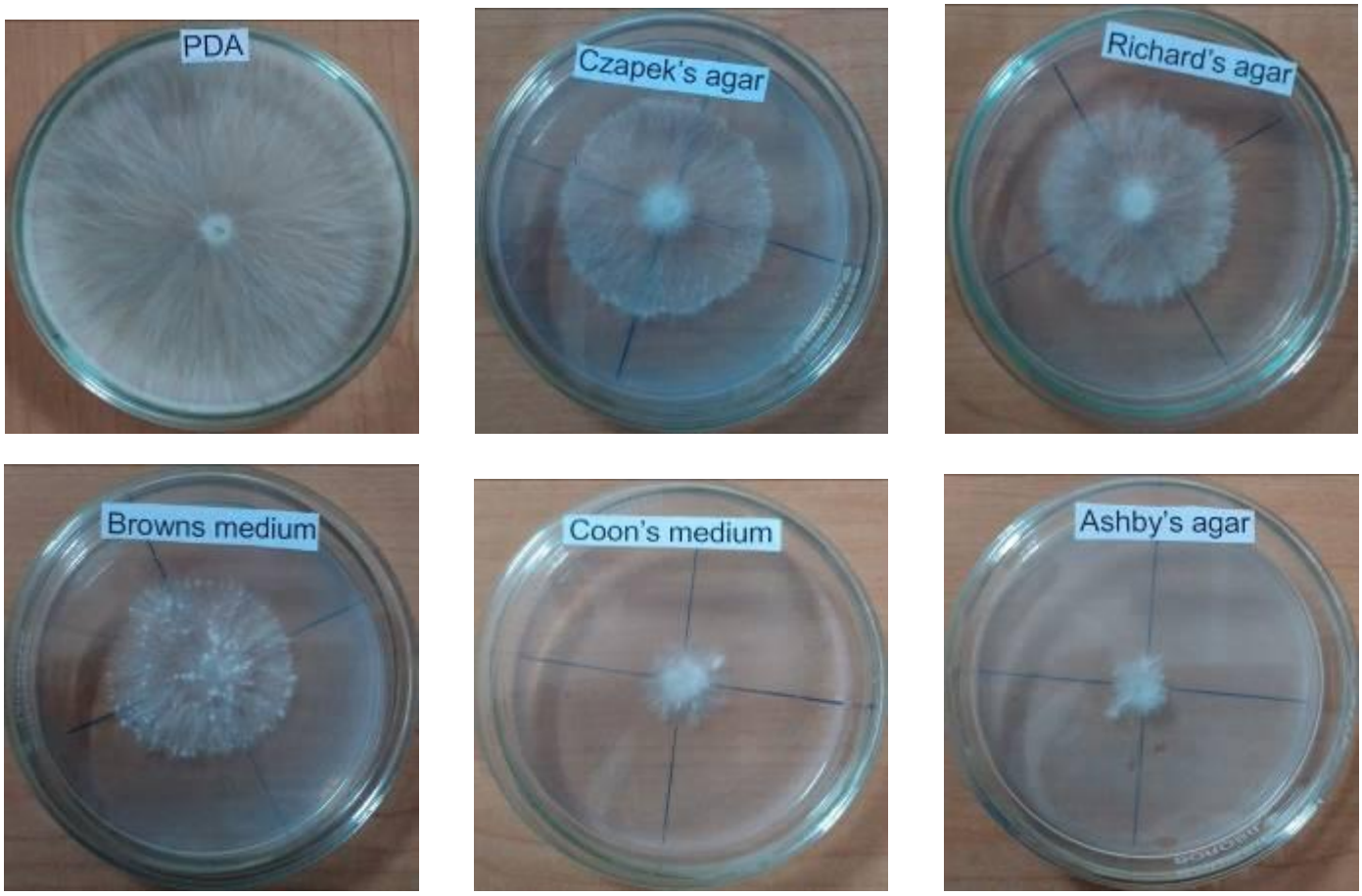

Figure 1 Photographs showing the effect of solid media on radial growth of *S. rolfsii*

Growth of the test fungus was observed at all the pH levels tested but it was maximum at pH 6.5 (90.00 mm) after 3 days of incubation followed by pH 6.0 (86.33 mm) (**Table 3**). Growth of the test fungus decreased by increasing or decreasing the pH level from 6.5 level. Highly acidic and alkaline pH is not suitable for the growth of pathogen. The fungus produced fluffy, appressed and dense compact type of growth pattern at different levels of pH. Excellent sclerotia formation was observed at pH 6.0, 6.5 and 7.0 while fair sclerotia production was recorded at pH 5.5 and 7.5. pH 8.0 and 8.5 supported poor sclerotia formation. The results of the present study are in agreement with those achieved by [9].

The *S. rolfsii* showed highest mycelial growth and sclerotia production at 30°C (90.00 mm), which was significantly superior over the other temperature treatments. No growth and sclerotial formation were recorded at minimum and maximum temperatures of 10 and 40°C. The optimum temperature for the better radial growth of the fungus ranged between 25 to 35°C, whereas, for sclerotial production, it ranged from 20 to 30°C. [10] and [11] observed maximum growth of *S. rolfsii* at 30°C and the excellent sclerotial formation at 25°C in their studies. There results very much support the present studies in which most suitable temperatures level for growth and sclerotia formation of *S. rolfsii* of the test fungus was 30ºC and 25^0C (**Table 4**).

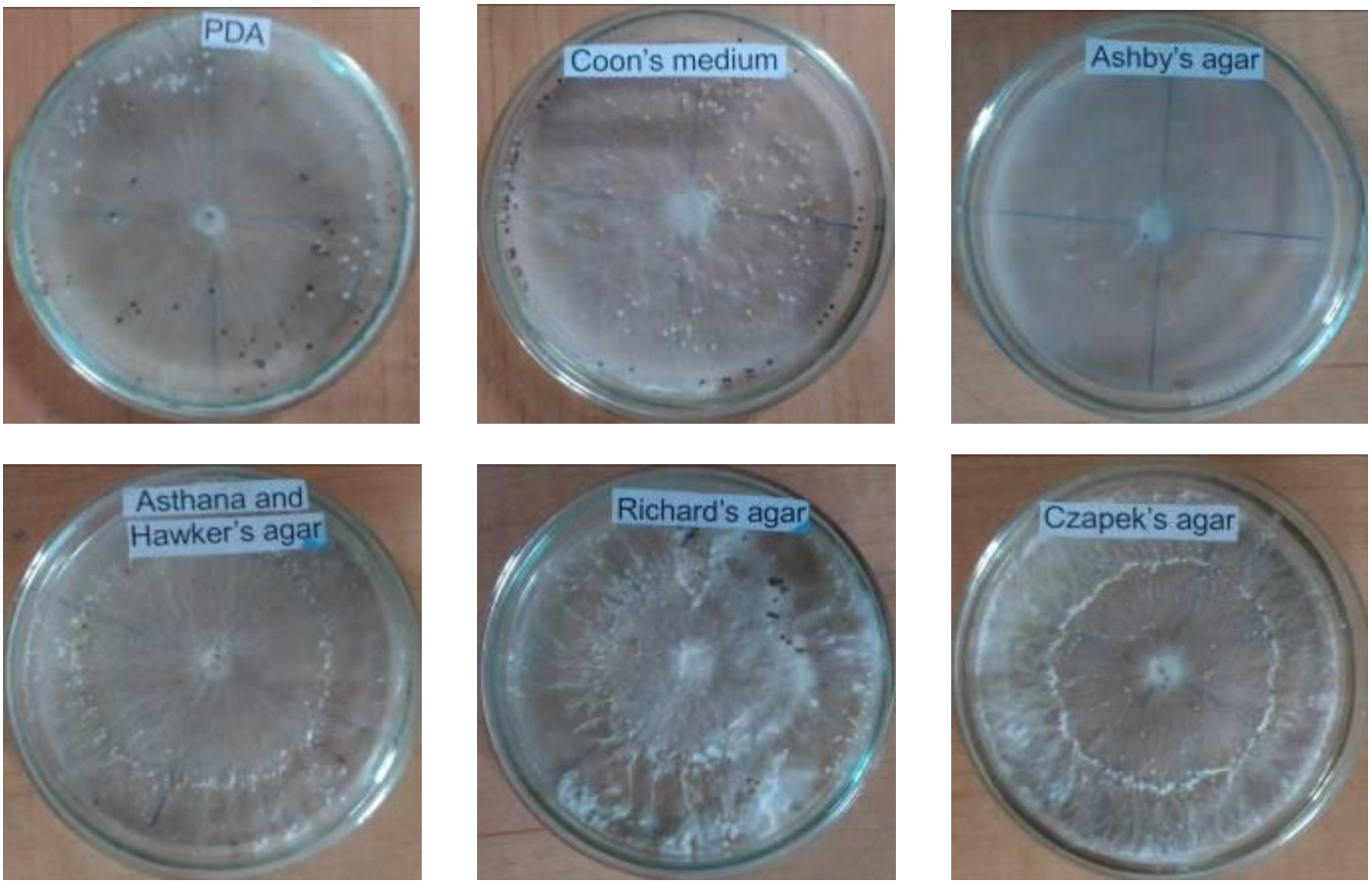

Figure 2 Photographs showing the effect of solid media sclerotia formation of *S. rolfsii*

Table 2 Effect of liquid media on dry mycelial weight and sclerotia formation of S. *rolfsii*

S. No.	Name of the medium	Dry mycelial weight (mg) after 21 days *	Degree of sclerotia formed after 21 days *
1	Potato dextrose broth	453.33	Excellent
2	Richard's broth	231.00	Poor
3	Browns broth	170.00	Poor
4	Czapek's Dox broth	23.66	Poor
5	Asthana and Hawker's broth	0.00	Nil
6	Ashby's broth	0.00	Nil
7	Coon's broth	0.00	Nil
CD (0.05)		2.645	
*Average of 3 replications			

Table 3 Effect of various pH on radial growth and sclerotia formation of S. *rolfsii*

S. No.	pH	Radial growth (mm)		Type of colony	Degree of sclerotia formed after 15 days*
		After 48 hrs*	After 72 hrs*		
1	5.5	41.66	65.00	Fluffy	Fair
2	6.0	58.66	86.33	Fluffy	Excellent
3	6.5	65.33	90.00	Fluffy	Excellent
4	7.0	53.00	78.33	Appressed	Excellent
5	7.5	44.00	66.66	Appressed	Fair
6	8.0	17.33	26.00	Dense compact	Poor
7	8.5	15.00	24.66	Dense compact	Poor
CD (0.05)		2.617	2.939		
*Average of 3 replications					

Table 4 Effect of different temperature on radial growth and sclerotia formation of *S. rolfsii*

S. No.	Temp. °C	Colony diameter (mm) after 168 hrs*	Type of colony	Pigmentation	Degree of sclerotia formed after 15 days*
1	5	0	-	-	Nil
2	10	10.33	Appressed	Dull white	Nil
3	15	25.33	Fluffy	Dull white	Poor
4	20	42.67	Fluffy	White	Fair
5	25	64.33	Appressed	White	Excellent
6	30	90.00	Appressed	Dull white	Excellent
7	35	22.83	Appressed	Dull white	Fair
8	40	0	-	-	Nil
CD (0.05)		2.641			

*Average of 3 replications

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