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Effect of Different Carbon and Nitrogen Sources on Mycelial Growth and Sclerotial Formation of *Sclerotium rolfsii Sacc*. causing Stem Rot of Wheat

***Vikram*[1*], *Praful Kumar*[2] *and Ashwarya L. Tandon*[3]**
[1]*Department of Plant Pathology, College of Agriculture,*
Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh), India.
[2]*Department of Plant Pathology, College of Agriculture and Research Station,*
Indira Gandhi Krishi Vishwavidyalaya, Raipur, Bemetara (Chhattisgarh), India.
[3]*Department of Plant Pathology, College of Agriculture,*
Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh), India.

(Corresponding author: Vikram)*
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ABSTRACT: Nutritional requirements in semi-synthetic media is very essential for growth and survival of *S. rolfsii.* However, there is little information on the nutritional requirements of *S. rolfsii*. Therefore, *In vitro* experiments were conducted at the Department of Plant Pathology, College of Agriculture (Indira Gandhi Krishi Vishwavidyalaya) Raipur, Chhattisgarh, to evaluate the effect of different carbon and nitrogen sources on mycelial growth and sclerotial formation of *Sclerotium rolfsii Sacc.* causing stem rot of wheat. The treatments comprised of the six-carbon sources *viz.*, Glucose, Lactose, Maltose, Mannitol, Starch, and Sucrose, and six nitrogen sources *viz.*, Glycine, Barium nitrate, Magnesium nitrate, Potassium nitrite, Sodium nitrate, and Urea with separate control, for both carbon and nitrogen, were maintained by using Dextrose as carbon source and Sodium nitrate as nitrogen source. Radial growth, number of sclerotia, mycelial density, colony shape, mycelia texture, sclerotia color, and arrangement of sclerotia were observed. Among six carbon sources maximum mycelial growth (90.00mm) was recorded in sucrose, starch, glucose, mannitol, maltose, and control and minimum (28.67 mm) was in lactose. The highest sclerotia (280.33 per plate) was formed in control and no sclerotia in lactose. Glucose, Maltose, and control produced abundant mycelial density. All the carbon sources along with control produced regular colony shape as well as fluffy dense at the margin. All the carbon sources produced dark brown and spherical sclerotia except Starch and Maltose which produced dull brown and irregular sclerotia. The sclerotia were peripheral patterns in all the carbon sources except starch. Similarly among six nitrogen sources maximum mycelial growth (90.00mm) was recorded in control and no radial growth was found in urea. Barium nitrate, Glycine, and control produced abundant mycelial density. All the nitrogen sources along with control produced regular colony shape as well as fluffy semi-cottony dense at the margin. Barium nitrate, Glycine, and control produced dark brown and spherical sclerotia. The sclerotia were peripheral patterns in all the nitrogen sources except magnesium nitrate.

Keywords: *Sclerotium rolfsii*, Stem rot, Wheat, Carbon source, Nitrogen source.

INTRODUCTION

Wheat is an important cereal crop and staple food of the vast majority of the human population. Presently in the world, wheat is grown over an area of 240.4 m ha with a production of 757.92 mt and productivity of 3,438 kg/ha. India stands fourth among wheat-producing countries both in respect of area and production. In India, it is grown over an area of 30.71 m ha with a production of 101.20 mt and productivity of 3,295 kg/ha (Anonymous, 2019). Karnataka is unique in wheat cultivation wherein all three cultivated species, viz., *Triticum aestivum* L., (Bread wheat), *T. durum* (Marconi wheat), and *T. dicoccum* (Khapli, Sadaka, or Emmer wheat) are grown in tropical climates characterized by the prevalence of high temperature during the crop growth.

Sclerotium rolfsii Sacc. is a serious ubiquitous soil borne fungus, causing Southern blight of agricultural and horticultural crops (Anahosur, 2001 and Mahadevakumar *et al.*, 2015). The fungus is notorious for its ability to induce dark stem rot, gradually wilting the whole plant. White cottony fungal thread girdles the

basal part of the stem and moves below the stem to roots (Kator *et al.,* 2015, Sun *et al.,* 2020). This pathogen survives as sclerotia on decayed plant material in the soil which germinate and attack surrounding host plants (Ludwig and Haltrich, 2002). Pectinolytic enzymes and oxalic acid are secreted by S. *rolfsii* during its pathogenesis (Ansari and Agnihotri, 2000). These compounds are essential for degradation cell walls, hydrolyzing pectin, and altering host defensive responses (Ferrar and Walker 1993). It infects economically important crops such as grains, pulses, oilseed crops, and others, and is known to grow in dry, arid climates. The pathogen has become very important in Madhya Pradesh., where most of the farming is rainfed.

To determine the optimal environmental and nutritional conditions for *S. rolfsii* growth and survival, under laboratory conditions temperatures, pH, aeration, and culture media have significant effects (Ayed *et al.*, 2018a and Ayed *et al.,* 2018b). However, there is little information on the nutritional requirements of *S. rolfsii* in semi-synthetic media. Therefore, this study was undertaken to examine the effect of different nitrogen and carbon sources on the mycelial growth, and sclerotial formation of *S. rolfsii*.

MATERIALS AND METHODS

A. Effect of carbon sources on mycelial growth and sclerotial formation of S. rolfsii

To evaluate the effects of carbon sources Potato Dextrose Agar media was used as the basal medium. In Potato Dextrose Agar media, Dextrose was replaced with six carbon sources viz., Glucose, Lactose, Maltose, Mannitol, Starch, and Sucrose, on an equivalent weight basis and autoclaved at 15 lb. pressure for 30 min. Plates containing each of the six tested carbon sources were inoculated with 5 mm mycelial disc cut from 5 days old *S. rolfsii* culture plate previously grown on Potato Dextrose Agar and incubated at 25±1ºC. Observations were taken after full growth in any one treatment and the sclerotial development was recorded on 15 days after inoculation. Colony characters and sclerotial characters were also measured at the time of observation.

B. Effect of nitrogen sources on mycelial growth and sclerotial formation of S. rolfsii

The mycelial growth of *S. rolfsii*, as well as their Sclerotial development, were evaluated on six nitrogen Sources *viz*., Glycine, Barium nitrate, Magnesium nitrate, Potassium nitrite, Sodium nitrate, and Urea. Czapek's dox solid media was taken as the basal medium for the study. Sodium nitrate was substituted with various nitrogen sources on an equivalent weight basis in Czapek's dox solid media, which was autoclaved at 15 lb. pressure for 30 minutes. Plates containing each of the six nitrogen's examined were inoculated with a 5 mm mycelial disc cut from a 5 days old *S. rolfsii* culture previously grown on PDA at 25±1°C. There were three replicate plates for each individual treatment. Observations were taken after full growth in any one treatment. Colony characters were also recorded at the time of observation.

RESULT AND DISCUSSION

A. Effect of carbon sources on mycelial growth and sclerotial formation of S. rolfsii

In the present investigation, six carbon sources, viz., sucrose, starch, glucose, lactose, mannitol, and maltose on radial growth and sclerotia formation of *S. rolfsii* were studied, and the result presented in Table 1 and illustrated in Plate 1. Significantly maximum mycelial growth (90.00 mm) was recorded in sucrose, starch, glucose, mannitol, maltose, and control and minimum mycelial growth (28.67 mm) was found in lactose. Sclerotia formation was observed at 15 days after inoculation significantly 280.33 sclerotia per plate produced in control which was highest among all the carbon sources under-tested and the no sclerotia produced in lactose. From the result, it was evident that there was a difference between colony characteristics on different carbon sources. The abundant mycelial density was observed in Glucose, Maltose, and control followed by moderate to abundant growth was recorded in Sucrose and Starch and moderate mycelial density was observed in mannitol. All the carbon sources produced regular colony shape of *S. rolfsii*, as well as fluffy dense at margin mycelial texture, was observed. Sclerotial formation followed mycelial aggregation with 10 to 15 days. Sclerotia were dark brown to dull brown in color. All the carbon sources it was observed dark brown and spherical sclerotia except Starch, Mannitol, and maltose which produced dull brown and irregular sclerotia. The sclerotia were peripheral pattern in singly or joined together in all the carbon sources glucose produced central & peripheral, mannitol produced less & peripheral and starch produced sclerotia in Scattered all over the plate.

In Table 1 it is clearly indicated that carbon source sucrose, starch, glucose, mannitol, maltose, and control was also supported the growth of *S. rolfsii* and the highest sclerotia of *S. rolfsii* was produced on control.

Optimal radial growth was supported by using Sucrose, Starch, Glucose, Mannitol, and Maltose whereas less mycelial growth was noted using lactose. These findings are in accordance with the findings of Survase *et al.* (2006) who reported significant mycelial growth of *S. rolfsii* using maltose and glucose. Bhagat (2013) reported that maltose and glucose support good growth of *S. rolfsii.* Divya and Narayanba (2017) recorded the best growth of the fungus when glucose was used as the main carbon source. The preference of glucose over other carbon compounds may be due to its fast metabolization by fungi (Garraway and Evans 1984). This was similar to the findings of Xiao *et al*. (2012).

The utilization of various carbon compounds may depend on the activity of the fungus to utilize simpler forms or on its ability to convert the complex carbon compounds into simpler forms, which may be easily utilized (Muthukumar and Venkatesh, 2013).

Table 1: Effect of carbon sources on mycelial growth and sclerotial formation of *S. rolfsii*.

Sr. No.	Carbon Sources	Radial Growth (mm)*	Number of Sclerotia (Number per plate)	Colony Characteristics			Sclerotial Characteristics		
				Mycelial Density	Colony Shape	Mycelial Texture	Colour	Shape	Arrangement of Sclerotia
1.	Sucrose	90.00	215.00	Moderate to abundant	Regular	Fluffy dense at margin	Dark brown	Spherical	Peripheral
2.	Starch	90.00	193.00	Moderate to abundant	Regular	Fluffy dense at margin	Dull brown	Irregular	Scattered all over plate
3.	Glucose	90.00	178.33	Abundant	Regular	Fluffy dense at margin	Dark brown	Spherical	Centre & Peripheral
4.	Lactose	28.67	0.00	no	no	no	no	no	no
5.	Mannitol	90.00	71.00	Moderate	Regular	Fluffy dense at margin	Dull brown	Spherical	Less & Peripheral
6.	Maltose	90.00	165.00	Abundant	Regular	Fluffy dense at margin	Dull brown	Irregular	Peripheral
7.	Control	90.00	280.33	Abundant	Regular	Fluffy dense at margin	Dark brown	Spherical	Peripheral
	SEm±	0.025	1.78						
	C.D. (P= 0.05)	0.077	5.45						
	C.V.	0.537	1.95						

*Average of 3 replication

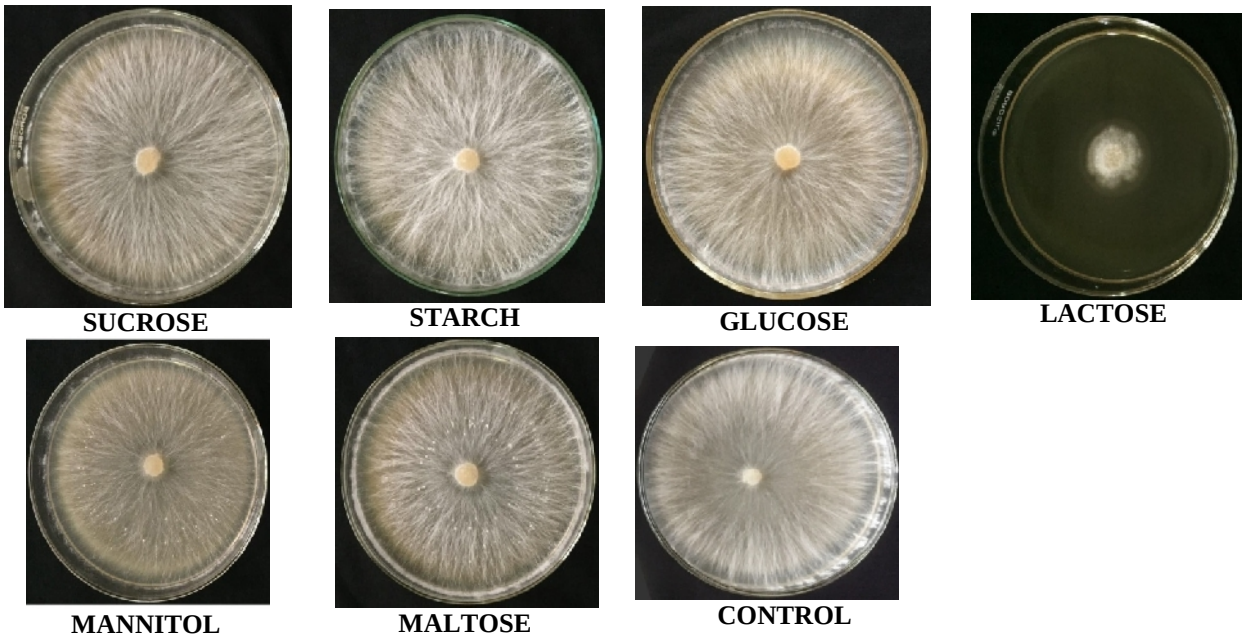

Plate 1: Effect of carbon sources on mycelial growth of *S. rolfsii*.

B. Effect of nitrogen sources on mycelial growth and sclerotial formation of S. rolfsii

The study was conducted to know the effect of different nitrogen sources on the growth of *S. rolfsii*. In this study six nitrogen sources *viz*., urea, potassium nitrate, magnesium nitrate, barium nitrate, sodium nitrite, and glycine, were investigated, on radial growth and sclerotia formation in *S. rolfsii*, and results were reported in Table 2 and illustrated in Plate 2. Significantly maximum mycelial growth was recorded in control followed by potassium nitrate (76.67 mm), barium nitrate and glycine (76.00 mm), magnesium nitrate (73.00 mm), sodium nitrate (57.00), and no radial growth found in urea. From the result, it was evident that difference between nitrogen sources with reference to colony characteristics. The abundant mycelial density was observed in Barium nitrate, Glycine, and control followed by moderate to abundant mycelial density was observed in potassium nitrate and magnesium nitrate, and poor mycelial density was observed in sodium nitrate. All the nitrogen sources showed regular colony shape, as well as fluffy semi-

cottony dense at margin mycelial texture, was observed in Potassium nitrate, Barium nitrate, Sodium nitrate, and Glycine followed by fluffy and cottony dense at margin type mycelial texture were observed in magnesium nitrate and control.

In table 1 it is clearly indicated that all the nitrogen sources except urea were also supported the growth of *S. rolfsii* and the highest sclerotia of *S. rolfsii* was produced on control.

Potassium nitrate, Magnesium nitrate, Barium nitrate, Sodium nitrate, and Glycine were found to be the most suitable N sources for *S. rolfsii* sclerotial formation. However, complete inhibition of any sclerotial development was recorded using urea. These results are in agreement with, Hussain *et al.* (2003) and Basamma (2008) found that potassium nitrate had the ability to enhance *S. rolfsii* mycelial growth. Muthukumar *et al.* (2013) also said that peptone had the highest mycelial growth (89.66 mm) and dry weight (790.33 mg) among the nine nitrogen sources studied (ammonium chloride, ammonium nitrate, ammonium sulfate, calcium nitrate, peptone, potassium nitrate, sodium nitrite, sodium nitrate, and urea). *S. rolfsii* was reported to lowest mycelial growth and dry weight when grown in calcium nitrate and ammonium chloride.

Table 2: Effect of nitrogen sources on mycelial growth and sclerotial formation of *S. rolfsii.*

Sr. No.	Nitrogen Sources	Radial Growth (mm)*	Colony Characteristics			Sclerotial Characteristics		
			Mycelial Density	Colony Shape	Mycelial Density	Colour	Shape	Arrangement of Sclerotia
1.	Urea	0.000	No	No	No	No	No	No
2.	Potassium nitrate	76.67	Moderate to abundant	Regular	Semi-cottony	Dull Brown	Spherical	Less and Peripheral
3.	Magnesium nitrate	73.00	Moderate to abundant	Regular	Cottony	Dull Brown	Irregular	Scatterd all over plate
4.	Barium nitrate	76.00	Abundant	Regular	Flat to semi-cottony	Dark Brown	Spherical	Center and Peripheral
5.	Sodium nitrate	57.00	poor	Irregular	Flat	Dull Brown	Irregular	Less and Peipheral
6.	Glycine	76.00	Abundant	Regular	Flat to cottony	Dark Brown	Spherical	Center and Peripheral
7.	Control	90.00	Abundant	Regular	Flat to cottony	Dark Brown	Spherical	Peripheral
	SEm±	0.045						
	C.D.(P=0.05)	0.139						
	C.V.	1.228						

*Average of 3 replications.

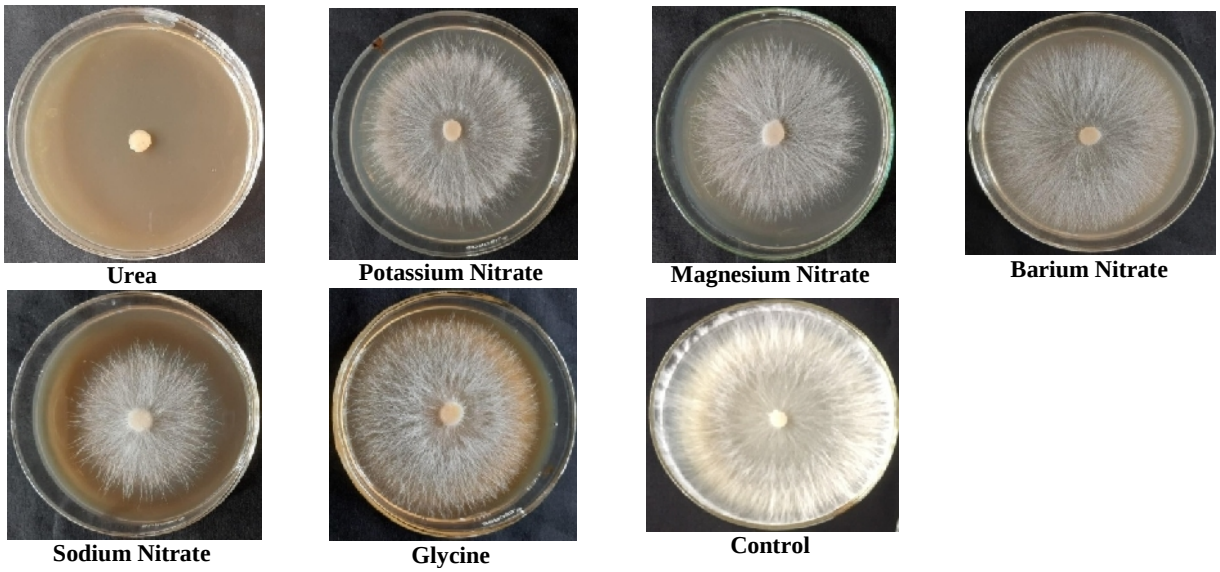

Plate 2: Effect of nitrogen sources on mycelial growth of *S. rolfsii*.

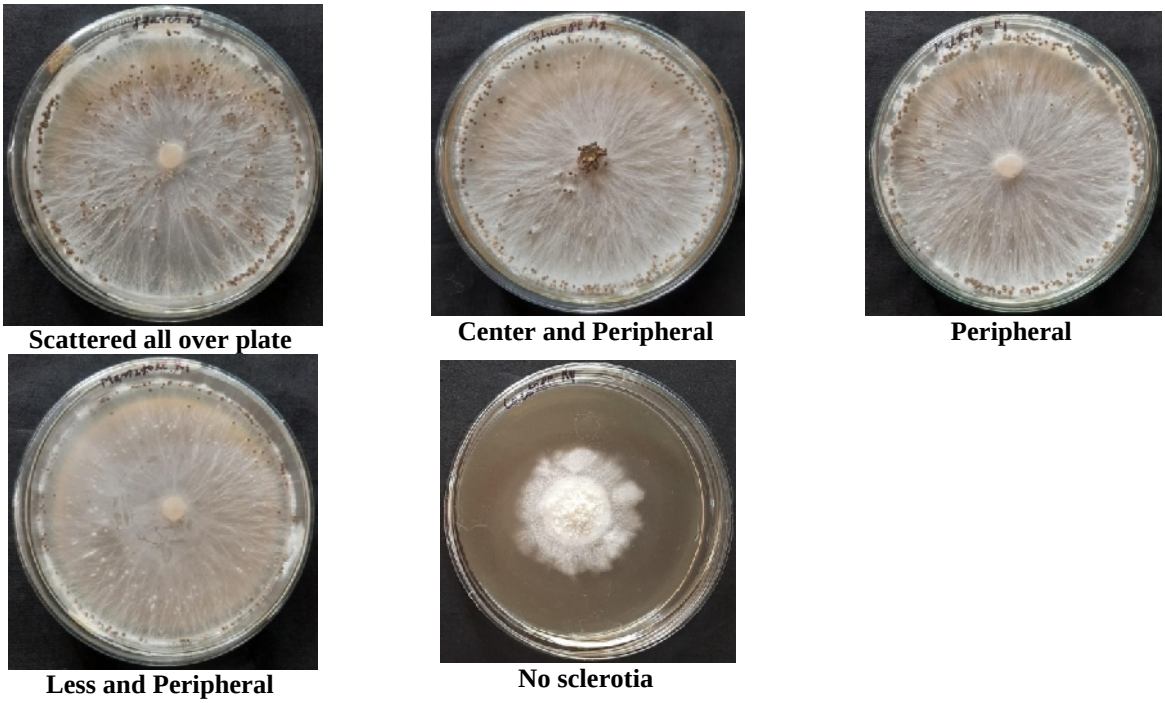

Plate 3: Arrangement of Sclerotia of *S. rolfsii*.

FUTURE SCOPE

In this investigation, considerable variations in mycelial growth, and sclerotial development were observed using different N and C nutritional sources for *S. rolfsii* culture under laboratory conditions. However, to provide a greater understanding of the biology of *S. rolfsii*, further investigations into the effect on pathogen development under field conditions are needed. Such investigations would improve our understanding of the pathogen's population dynamics in soil and help to implement effective disease control methods.

Conflict of Interest: The authors declare that there is no conflict of interest.

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