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Physiological Study of *Sclerotium rolfsii* Sacc.

Azhar Hussain, [1]Sh. Muhammad Iqbal, Najma Ayub and [1]Abdul Majeed Haqqani
Quaid-i-Azam University, Islamabad, Pakistan
[1]National Agricultural Research Centre, Park Road, Islamabad, Pakistan

Abstract: ***In vitro*** studies were conducted on the effect of temperature, pH levels, culture media, carbon and nitrogen sources on mycelial growth of ***Sclerotium rolfsii*** Sacc. Growth of ***S. rolfsii*** was maximum at 25^{0}C after 7 days of inoculation, which was reduced significantly below 20^{0}C and above 35^{0}C. All the tested pH levels (5 to 8) were found equally suitable for growth of fungus. This fungus grew best on cornmeal agar medium among the culture media that were tried. All the carbon sources were found to be the best while peptone was the best among the nitrogen sources.

Key words: ***Sclerotium rolfsii***, culture media, pH, carbon, nitrogen, mycelial growth

Introduction

Sclerotium rolfsii Sacc. is an important soil-borne pathogen causes severe damage to many economically important crops and plants (Mirza and Qureshi, 1982). The fungus affects nearly 500 plant species comprising Composite and Leguminosae. Graminaceous species are less susceptible (Mahen ***et al.***, 1995). The pathogen is also known to cause collar rot disease in chickpea (Bashir ***et al.***, 1986). Seedling mortality 54.7 to 95.0% in chickpea due to infestation of ***S. rolfsii*** has been reported (Mathur and Sinha, 1968; 1970; Kotasthane ***et al.***, 1976). The present investigation was conducted to study the effect of physiological factors on the growth and sclerotial production of the fungus.

Materials and Methods

Studies of the following physiological aspects of ***S rolfsii*** were conducted ***in vitro.***

Effect of culture media

Five culture media viz; Chickpea seed meal extract agar (CSMA) medium (chickpea seed meal extract 20 g, dextrose 20 g and agar 20 g), Potato dextrose agar (PDA) medium (potato starch 20 g, dextrose 20 g and agar 20 g), cornmeal agar medium (cornmeal 20 g, dextrose 20 g and agar 20 g), Czepkdox agar medium (sodium nitrate 2 g, potassium nitrate 1 g, magnesium sulphate 0.5 g, potassium chloride 0.5 g, ferrous sulphate 3 g, sucrose 30 g and agar 20 g) and Sabouroud's agar medium (dextrose 40 g, peptone 10 g and agar 20 g) were used to find out the most suitable one for the mycelial growth of the fungus. Each culture medium was prepared in 1 lit of water and autoclaved at 120^{0}C at 15 psi for 20 min. These were cooled to 45^{0}C and then poured in 9 cm petri dishes for solidification.

Effect of different Carbon and Nitrogen Sources

CSMA medium (in one liter of water) was used as the medium for studying the effect of carbon and nitrogen sources.

Nitrogen Sources

Three nitrogen compounds viz; Potassium nitrates 10 g, Sodium nitrate 8.5 g and Peptone 2.5 g were amended in cornmeal agar medium.

Carbon Sources

Three carbon compounds viz; glucose 13.5 g, sucrose 12.5 g and starch 12.5 g were tried individually as a constitute of carbon source in cornmeal agar medium.

Effect of Temperature

The fungus *S. rolfsii* was inoculated in CSMA medium using five petri dishes for each temperature, which was applied at 10, 15, 20, 25, 30 and 35^{0}C.

All these experiments were conducted in five replicates. Plates were inoculated by placing one sclerotium per plate as an amount in the centre of the petri dishes. Plates were incubated at 25^{0}C (except for the study of temperatures) when observations on linear growth were recorded after 7 days of inoculation.

Effect of different pH levels

The test fungus was inoculated on cornmeal agar medium whose pH was adjusted to 5.0, 6.0, 7.0, 8.0 and 9.0.

Results and Discussion

Effect of culture media

The results of the experiment revealed that the Cornmeal agar and CSMA media were the best for the radial growth of *S. rolfsii* as this fungus gave maximum growth of 8.5 and 8.0 cm respectively, after 7 days of inoculation followed by Waksman agar medium which showed growth of 7.0 cm (Fig. 1). Borromeo (1967) tested some culture media for the growth of *Ganoderma lucidum* and found that malt extract agar medium was the best. The study indicated that more media could be tested to identify the most suitable medium for *S. rolfsii*. CSMA medium has already been proved effective for the growth of *Ascochyta rabiei* (Iqbal *et al.*, 2002). Backman and Rodriguez-Kabana (1976) developed a selective medium for the isolation of *S. rolfsii* associated with groundnut from the soil.

Effect of different carbon and nitrogen sources

The results of this experiment indicated that all starch was the best carbon source for growth of the fungus whereas the other C-sources did not showed good results (Fig. 2). The fungus may utilize certain simple form of complex carbon compounds into simple form, which may be readily metabolized (Bais *et al.*, 1970).

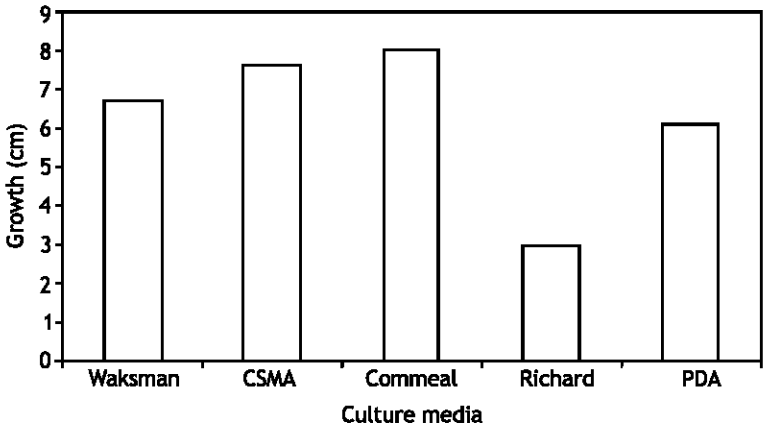

Fig. 1: Effect of different culture media on the growth of *Sclerotium rolfsii*

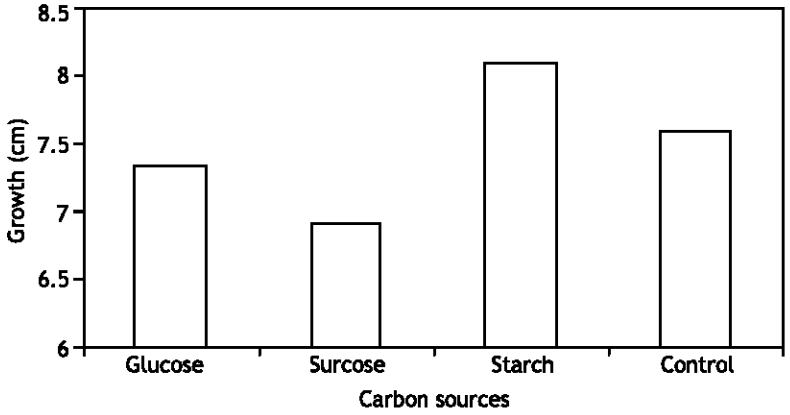

Fig. 2: Effect of different carbon sources on the growth of *Sclerotium rolfsii*

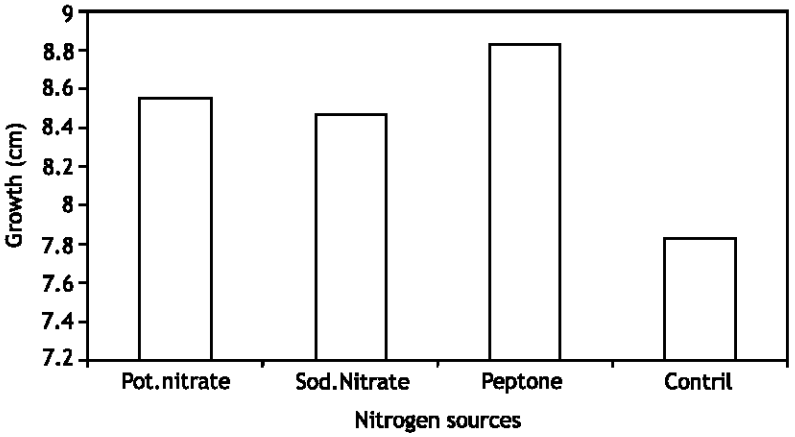

Fig. 3: Effect of different nitrogen sources on the growth of *Sclerotium rolfsii*

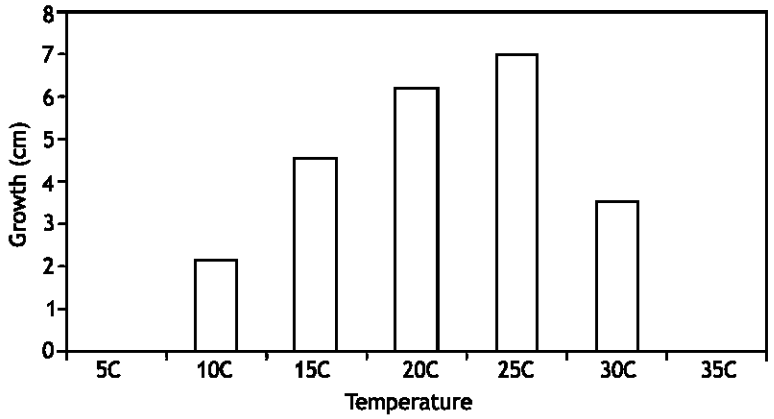

Fig. 4: Effect of different range of temperature on the growth of ***Sclerotium rolfsii***

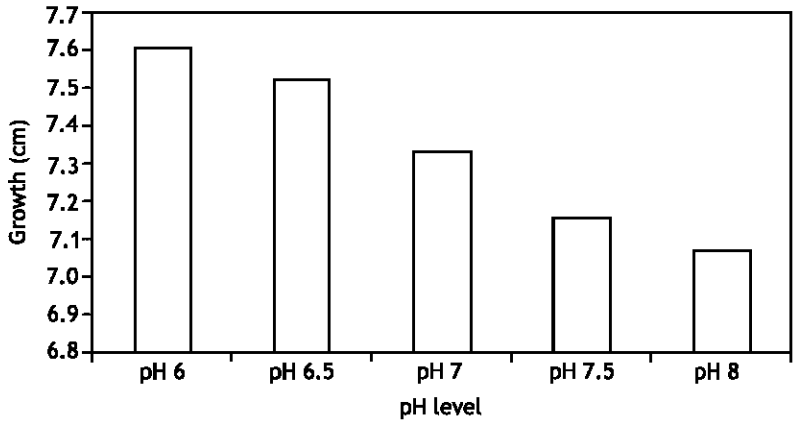

Fig. 5: Effect of different pH levels on the growth of ***Sclerotium rolfsii***

As is evident from Fig. 3, Peptone (9.0 cm) was found to be best source of nitrogen for *S. **rolfsii***. It was followed by Potassium nitrate. On potassium nitrate (KNO_3), the growth of fungus was 8.2 cm after 5 days of inoculation. Similar observations were made by Brook (1951) for the mycelial study of ***Morchella esculenta***.

Results of our study indicated that the role of C: N ratio is very important. The fungus readily colonizes organic substances in the soil. Increased inoculum potential and disease severity are positively correlated with the food base of organic substances. Crop debris that serves as a food base can also serve as an infection bridge. The fungus becomes active primarily at the soil surface and a mat of hyphea is found over the basal portion of plants (Mahen ***et al.***, 1995).

Effect of Temperature

As evident from Fig. 4, the fungus grew at the temperature range of 10 -30^{0}C. However, growth of the fungus was drastically reduced below 15^{0}C and started to decline above 30^{0}C, as

these temperatures did not favour much growth of the fungus. It was observed that at 25°C, the fungus attained the maximum growth (9.0 cm) while at 20° C, it was 6.0 cm after 7 days of inoculation. No growth was observed at 5°C as well as at 35°C. Mahen ***et al.*** (1995) also reported that the suitable temperature for the growth of this fungus is 27-30 °C, although the temperature range was 8-40°C. Mahen ***et al.*** (1995) reported the observations of ***S. rolfsii*** associated with the stem and pod rots of groundnut. The variation in the optimum temperature of the pathogen of groundnut and that of the collar rot of chickpea in the present study indicated the pathogenic variability of ***S. rolfsii***.

Effect of different pH levels

Growth of the fungus was obtained at all the pH levels tested but it was maximum at pH 6.0 (Fig. 5). Growth of the fungus decreased by increasing the pH level. This study has been supported by Mahen ***et al.*** (1995) who published that the growth occurs over a wide range of pH (1.4 - 8.8), the optimum being pH 3.5.

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