JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2012. Vol. **6**(2), p. 881-887

Morphological and Physiological Characterization of *Sclerotium rolfsii* Pathogenic to Groundnut (*Arachis hypogaea* L.)

M. Saraswathi and M.N. Reddy

Department of Applied Microbiology, Sri Padmavati Mahila Visva Vidyalayam, Tirupati - 517 502, India.

(Received: 18 February 2012; accepted: 21 April 2012)

***Sclerotium rolfsii* is one of the devastating soil-borne phytopathogens which cause severe loss at the time of seedling development. The requirements of carbon, nitrogen, sulphur and phosphorus for growth of *Sclerotium rolfsii* were studied in vitro. The best source of carbon for growth appeared to be sucrose. Ammonium phosphate appeared to be the best source of phosphorus sources. Magnesium sulphate supported best growth among sulphur sources and proline and potassium nitrate supported best growth for organic and inorganic nitrogen sources.**

Key Words: *Slerotium rolfsii*, Stem rot, Nutritional requirements, Groundnut, Morphology.

Sclerotium rolfsii is a ubiquitous soil-borne fungal pathogen known to cause disease on worldwide range of agricultural and horticultural crops (Kaveriappa, 1979). It infects more than 500 plant species in 100 families throughout the world (Punja, 1985; Sarma *et al*., 2002; Adandonon, 2005; Ganesan *et al*., 2007). Most *S.rolfsii* diseases have been reported on dicotyledonous hosts, but with several monocotyledonous species also being infected (Aycock, 1966). In 1983, Patil and Rane reported that all the 35 hosts, including important cultivated crop plants, were susceptible to the pathogen, indicating the wide host range of parasitism of *S.rolfsii*. Secondary hosts are numerous, among them food crops and ornamentals, most of them being economically important. There are also a variety of weed hosts.

* To whom all correspondence should be addressed.
E-mail: saraswathiphd@gmail.com

Sclerotium rolfsii causes severe damage during any stage of crop growth (Ganesan *et al*., 2007) and attacks all parts of the plant but stem infection is the most common and serious. Serving as a protective structure, sclerotia contain viable hyphae and serve as primary inoculum for disease development (Aycock, 1966; Nalim *et al*., 1995; Cilliers *et al*., 2000) as well as its principle means of dispersal and sole organs by which the fungus survives adverse environmental condition, awaiting germination and infection of susceptible hosts when favorable conditions return (Okereke and Wokocha., 2007). Keeping in view the losses caused by this fungus, the aim of the present investigation therefore was to evaluate the different morphological aspects and nutritional requirements of *S.rolfsii*.

MATERIALS AND METHODS

Isolation of *S.rolfsii*

During the survey of groundnut fields around Srikalahasti and Tirupati areas of Chittoor District, and also other Rayalaseema Districts of

A.P. *Sclerotium rolfsii* was found to be associated with the infected hypocotyls region of groundnut at early stages of growth and development. The plants showing southern blight symptoms were brought to the laboratory for making isolation according to the tissue segment method on PDA, pure culture was obtained by transferring the sclerotia to PDA plates. The stock culture was maintained on PDA slants in a refrigerator at 10ºC and sub cultured for every two months.

Morphological characterization of *S.rolfsii*

Effect on growth

Six culture media viz., Potato dextrose agar, Czapek-Dox agar, Rose Bengal agar, Richard's agar, Coon's agar and Saboraud's agar were used for studying the effect of culture media on mycrelial growth and sclerotial production. Sterilized and cooled medium was poured into sterilized petri plates (90 mm) @ 20 ml and allowed to solidify. A 3 mm mycelial disc cut from the actively growing 3 day old culture by means of a sterilized cork borer was inoculated at the centre of the plate. The plates were incubated at room temperature 29±2^{0}C for 10 days. Three replicates were maintained for each culture media. The radial growth of the mycelium was measured on alternate days to estimate the rate of growth.

Effect on sclerotial germination

All the six media as mentioned above were inoculated with three sclerotia for each media and incubated at room temperature (29±2^{0}C) and radial growth was recorded for every 24 hr. Triplicates were maintained for each media.

Effect on enhanced sclerotial production (Narain and Mishra,1979)

The media were sterilized in an autoclave at a pressure of 15 lbs psi for 20 min, each medium was poured into 10 cm petri plates and triplicates were maintained for each of the media. When the medium was solidified 3 mm mycelial disc cut from the 3 day old culture of *S.rolfsii* was inoculated at the centre of the plate and incubated at room temperature for 5 days. After incubation, when the mycelium had uniformly covered the entire surface of the medium in Petri plate, 3 mm agar discs were cut out. These operations were aseptically done with the help of cork borer, six such agar discs, approximately equidistant from one another, were randomly removed. Observations on sclerotial development were made 8 days after cutting the agar discs.

Nutritional characterization of *S.rolfsii*

A number of carbon, phosphoros, sulphur and nitrogen (organic and inorganic) compounds were tested for their effect on growth of the fungus. Different carbon sources were incorporated separately in the basal medium devoid of any carbon source, at the same carbon level as present in the medium with 3% sucrose. Similarly different sources of nitrogen (organic and inorganic), sulphur and phosphorus were added separately at the same levels of nitrogen (as a $NaNO_3$), sulphur (as in $MgSO_4$) as phosphorus (as in K_2HPO_4) present in the basal (Czapek-Dox) medium. Starch and cellulose, whose molecular weight is uncertain, were supplemented in amounts equal to sucrose by weight (3%) in the medium.

In all the nutritional studies 25 ml of the medium were taken in conical flasks, P^H adjusted to 6.0 and sterilized. A 3 mm disc of actively growing mycelium cut from the periphery of a 3 day old culture with the help of a sterilized cork borer was used to inoculate into each of the flasks. The flasks were incubated for 10 days at 29±2^{0}C at stationary culture.

At the end of the incubation period, growth of the fungus was estimated by harvesting the mycelial mat produced in liquid media by filtering through a pre-weighed Whatman No.1 filter paper disc washing several times with water and drying. Growth is expressed as mycelial dry weight in mg/flask.

RESULTS

Effect on growth

The fungus was grown on different solid media and observed for various morphological characteristics— colony diameter, growth rate, colour and texture of the mycelium, sclerotial production, size and shape, colour, average number and distribution on the culture plate. Thick, white, cottony and fast growth with average colony diameter was observed on Saboraud's agar (Table 1) followed by Potato dextrose agar and Rose Bengal agar. Thin, pale, white, cottony and slow growth with average colony diameter was observed on Czapek-Dox agar, Coon's agar and Richard's agar medium (Table 1).

Table 1. Effect of different solid media on growth of *S.rolfsii*.

Type of media	Colony diameter (mm)			Growth rate	Color of the mycelium	Texture of the mycelium
	1 day	3 days	5 days			
Potato Dextrose agar	16.5	66.5	99	Rapid	White, cottony	Thick
Rose Bengal agar	15.5	71	95	Rapid	White, cottony	Thick
Czapek-Dox agar	19	49.5	64	Slow	Pale white, cottony	Thin
Coon's agar	17	49	60	Slow	Pale white, cottony	Thin
Saboraud's agar	11	34	52	Rapid	White, cottony	Thick
Richard's agar	18	76	101	Slow	Pale white, cottony	Thin

* Each value is an average of 3 replicate samples

Table 2. Effect of different solid media on sclerotial production of *S.rolfsii*

Type of media	Initiation of sclerotia (days)	Maturation (days)	Size of (mm)	Shape of sclerotia	Color of sclerotia	Average number
Potato Dextrose agar	Throughout the plate after 8 days	13	1.4	Round	Brown	++
Rose Bengal agar	Centre of the plate after 6 days	9	1.2	Round	Light brown	+
Czapek-Dox agar	-	-	-	-	-	-
Coon's agr	-	-	-	-	-	-
Saboraud's agar	Throughout the plate after 5 days	11	2	Irregular/oval	Brown	+++
Richard's agar	-	-	-	-	-	-

* Each value is an average of 3 replicate samples
- = No sclerotial production; + = Low level (20-30 sclerotia)
++ = Medium level (30-40 sclerotia); +++ = High level (more than 40 sclerotia

Of the six media tested, small, fuzzy and white sclerotia were first initiated in Saboraud's agar followed by Rose Bengal agar and potato dextrose agar. Rose Bengal agar significantly reduced the time for maturation of sclerotia (9 days) as against the highest 11 days in PDA. No sclerotial production was observed on CZDA, Coon's agar and Richard's agar medium (Table 1).

Effect on sclerotial germination

Of the six media tested for the germination of sclerotia, growth was first initiated in Saboraud's agar followed by RBA and PDA within 24 hr after inoculation. In the other media growth was initiated after 48 hr of inoculation. Maximum growth was recorded in Saboraud's agar followed by PDA, RBA, Czapek-Dox agar, Coon's agar and Richard's agar (Table 2 and Fig 1).

Nutritional characterization of *S.rolfsii*

Effect of different sources

The fungus failed to grow in the absence of all the nutrients viz., carbon, phosphorous, magnesium or nitrogen sources in the basal medium (Czapek-Dox medium). Of the different carbon sources tested, sucrose was proved to be the best source of carbon (Fig 1), ammonium phosphate for phosphorus sources (Fig 2). Among the sulphur sources, magnesium sulphate and ammonium sulphate yielded maximum growth of the fungus (Fig 3) and for nitrogen sources tested, tryptophan, proline and potassium nitrate supported good growth of *S.rolfsii* (Fig 4).

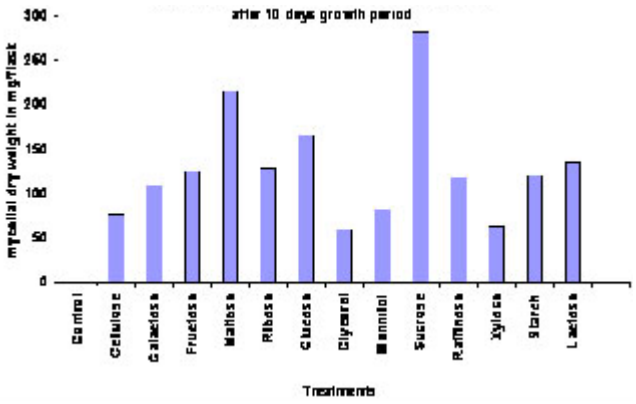

Fig. 1. Effect of various carbon sourceso n the growth of *S. rolfsii.* Results are expressed as mg dry weight of the mycelium/flask, after 10 days growth period

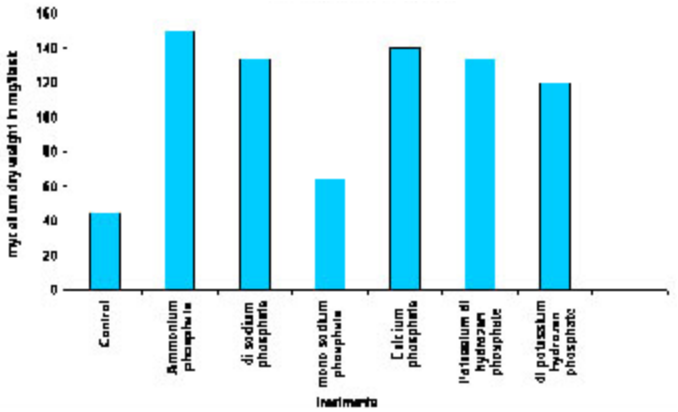

Fig. 2. Effect of various phorphorus sources on the growth of *S.rolfsii*. Results are expressed as mg dry of the myselium/flask, after 10 days growth period

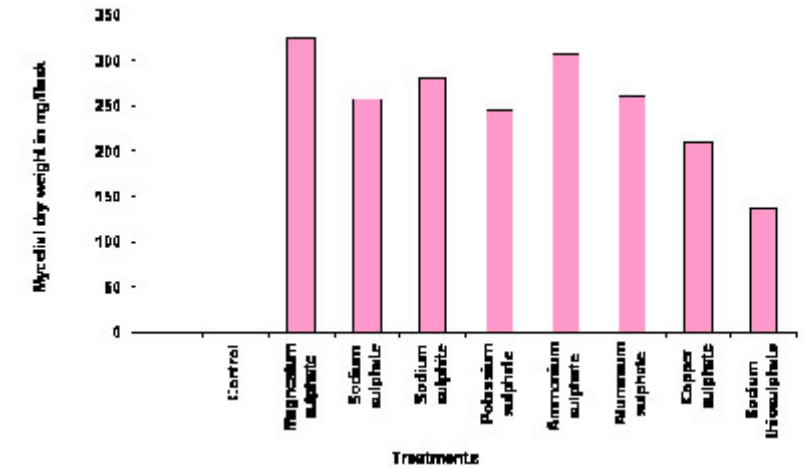

Fig. 3. Effect of sulphur sources on the growth of *S.rolfsii*. Results are expressed as mg dry of the myselium/flask, after 10 days growth period

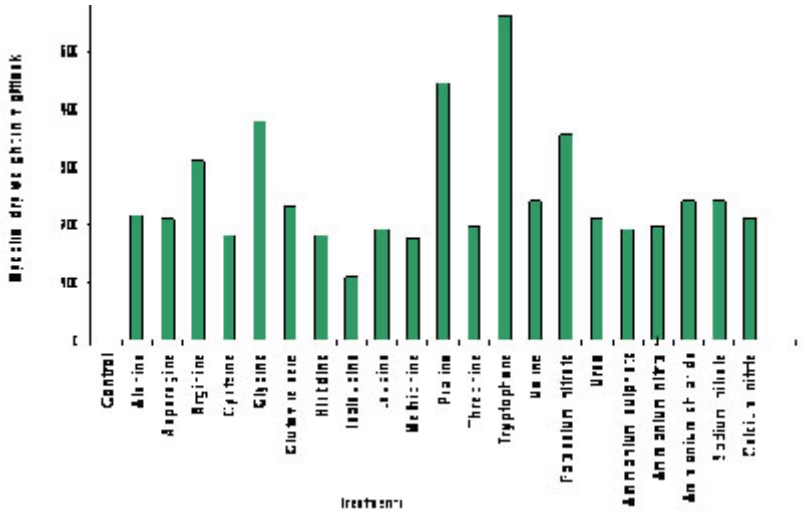

Fig. 4. Effect of varios nitrogen(organic and inorganic) sources on the growth of *S.rolfsii.* Results are expressed as mg dry of the myselium/flask, after 10 days growth period

DISCUSSION

In the present study S.Rolfsii exibited wide variation in the growth rate, colour nad texture of the mycelium, sclerotial production in various pathogens isolated from different hosts (Gosh and Sen, 1973; Suriachandraselven and Seetharaman, 2003; Okereke and Wokocha., 2007).Several earlier workers (rawn., 1991; Singh and Kaiser, 1994; Sarma *et al*., 2002; Sudarshan *et al*., 2010) also reported that PDA supported the highest growth of various pathogens and they concluded that glucose proved the best carbon source for the linear growth of the *S.rolfsii.* PDA containing glucose probably supported the best growth of the pathogen. However, other media viz., Czapek-Dox agar, Richard's agar also supported good growth of *S.rolfsii*

Fungi derive food and energy from the substrate upon which they grow in nature. In order to culture the fungi in the laboratory, there is no universal substrate or artificial medium upon which all the fungi can grow and reproduce (Sandhya Rani and Murthy, 2004).Pathogenic fungi usually exhibit certain degree of specificity in utilizing various nutritional substances for their growth and sporulation (Shreemali, 1973).

Carbohydrates, being the chief metabolic products of the photosynthetic plants, have special importance in the nutrition of the plant pathogens. In the plants they exist in both simple, easily utilizable monosaccharides and disaccharides and also as complex polysaccharides. The utilization of the former entails no strain on the organism, whereas the latter requires adoptation of certain special mechanisms, mostly enzymatic.

Among the carbohydrates tested, sucrose was found to be the best, for the mycelial growth and followed by dextrose, fructose and starch as also observed by other workers (Prasad *et al*., 1986). Other carbohydrates tested supported moderate to poor growth. Poor utilization or non-utilization of a carbon source may be due to atleast in part to the low absorption rate by the fungal cells or in some degree to the structural differences between sugars.

Among various phosphorus compounds maximum growth of the fungus was observed in ammonium phosphate followed by calcium phosphate. Little growth was observed in mono sodium phosphate, may be due to increase in pH. Among sulphur compounds tested magnesium sulphate supported maximum growth of the fungus whereas copper sulphate showed little growth. This may be due to the increase in the final pH value.

Among the inorganic nitrogen sources,

potassium nitrate supported better growth than that of ammonium salts (Subramanian, 1967). Among the organic nitrogen sources tryptophan and proline supported good growth.

Although cultural studies may not yield the most desirable information with respect to the elucidation of pathogenic mechanisms, they do provide valuable data on the potential of the pathogen to utilize various substances known to be present within host tissues.

The behavior of the pathogen during the process of infection indicates that both morphological and physiological barriers govern the process. Susceptibility of host, conducive environment coupled with innate parasitic potentialities to overcome the host barriers and ability to proliferate in the host tissues are the pre-requisites for an organism to become pathogenic. Consequently, pathogenicity is an expression of interaction of two compound systems, the host and the parasite.

ACKNOWLEDGEMENTS

The authors are thankful to Sri Padmavati Mahila VisvaVidyalayam, Tirupati for providing facilities.

REFERENCES

1. Adandonon, A., Theresa, A.S.A., Nicolaas A. and Gina Sanders. Genetic variation among *Sclerotium* isolates from Benin and South Africa, determined using mycelial compatibility and ITS rDNA sequence data. *Australian plant pathology.* 2005; **34**: 19-25.
2. Aycock, R. Stem rot and other diseases caused by *Sclerotium rolfsii* or the status of Rolf's fungus after 70 years. *N. C. Agri. Exp. Stn. Tech. Bull*. 1966; 174.
3. Carroll D. Rawn., Induction of sclerotia in Sclerotium rolfsii by short low-temperature treatment. Journal of General Microbiology. 1991; **137**: 1063-1066.
4. Cilliers, A.J., Herselman, L. and Pretorius, Z.A. Genetic viability within and among mycelial compatibility groups of *Sclerotium rolfsii* in South Africa. *Phytopathology* 2000; **90** (9): 1026-1031.
5. Ganesan, S., Ganesh, K.R. and Sekar, R. Integrated management of stem rot disease (*Sclerotium rolfsii*) of groundnut (*Arachis hypogaea* L.*)* using *Rhizobium* and *Trichoderma harzianum* (ITCC-4572). *Turk. J. Agric. For* 2007; **31**: 103-108.
6. Gosh, S. K. and Sen, C. Comparative physiological studies on four isolates of *Macrophomina phaseolina*. *Indian Phytopathology.* 1973; **XXVI**: 615-621.
7. Kaveriappa, K.M. Mutual aversion in brinjal isolates of *Sclerotium rolfsii*. *Indian Phytopathology.* 1979; **32**: 475-477.
8. Nalim, F.A., Starr, J.L., Woodard, K.E., Segner, S. and Keller, N.P. Mycelial compatibility groups in Texas peanut field populations of *Sclerotium rolfsii*. *Phytopathology.* 1995; **85**: 1507-1512.
9. Narain, A. and Mishra, S.K. Characteristics of an isolate of *Sclerotium rolfsii* on ragi. *Indian Journal of Mycology and Plant Pathology.* 1979; **9**(1): 1-4.
10. Okereke, V.C. and Wokocha R.C. *In vitro* growth of four isolates of *Sclerotium rolfsii* Sacc in the humid tropics. African Journal of Biotechnology. 2007; **6** (16): 1879-1881.
11. Patil, M.B. and Rane, M.S. Studies on host range effect of plantage on susceptibility and varietal reaction of groundnut to *Sclerotium rolfsii*. *Indian Journal of Mycology and Plant Pathology.* 1983; **13**(2):183-186.
12. Prasad, B.K., Sinha, T.S.P. and Prasad, A. 1986a. Influence of nutritional factor pH and temperature on growth of *Sclerotium rolfsii* Sacc. isolated from tomato fruit. *Indian Journal of Mycology and Plant Pathology.* 1986; **16**(2):209-212.
13. Punja, Z.K. The biology, ecology, and control of *Sclerotium rolfsii*. *Annual Review of Phytopathology.* 1985; **23**:97-127.
14. Sandhya Rani, G. and Murthy, K.V.M.K. Cultural and nutritional characteristics of *Colletotrichum gloeosporioides*, the causal organism in cashew anthracnose. *Journal of Mycology and Plant Pathology.* 2004; **34**(2): 317-318.
15. Sarma, B.K., Singh, U.P. and Singh, K.P. Variability in Indian isolates of *Sclerotium rolfsii*. *Mycologia.* 2002; **94** (6): 1051-1058.
16. Shreemali, J.L. The effect of carbon and nitrogen sources on the growth and sporulation of six different isolates of *Botryodiplodia theobromae*. *Indian Phytopathology*. 1973; **XXVI**: 220-224.
17. Singh, R.D.N. and Kaiser, S.A.K.M. Effect of different culture media and pH levels on growth and cultural characteristics of charcoal rot pathogen (*Macrophomina phaseolina*) infecting maize. Crop Research. 1994; **107**: 282-287.

18. Subramanian, C.V. Nitrogen utilization of some plant pathogenic fungi. *Symposium on Physiology of fungi.* 1967; **35**: 11-22.
19. Sudarshan Maurya, Udai Pratap Singh, Rashmi Singh, Amitabh Singh and Harikesh Bahadur Singh. Role of air and light in sclerotial development and basidiospore formation in *Sclerotium rolfsii*. *Journal of Plant Protection and Research.* 2010; **50** (2):206-209.
20. Suriachandraselvan, M. and Sitaraman, K. Effect of culture media on growth and sclerotial production of different isolates of *Macrophomina phaseolina* infecting sunflower. *Indian Journal of Mycology and Plant Pathology.* 2003; **33**(3): 226-229.