ROLE OF AIR AND LIGHT IN SCLEROTIAL DEVELOPMENT AND BASIDIOSPORE FORMATION IN *SCLEROTIUM ROLFSII*

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Abstract: *Sclerotium rolfsii* is one of the devastating soil-borne phytopathogens which causes severe loss at the time of seedling development. It also causes leaf spots in several crops and wild plants. Petri plates, containing potato dextrose agar medium, were inoculated with *S. rolfsii*. Two-third area of three, 50% area of three and 100% area of other three plates were sealed with cellophane tape. The other three plates were not sealed. All the plates were incubated at 27±2°C. Two sets of such plates were prepared. One set was incubated in light whereas the other set in the dark. There was no significant difference in mycelial growth and number of sclerotia among them but significant difference was observed when compared to the control, i.e. the plates which were not sealed. Sclerotium and basidiospore formation were directly influenced by air as completely sealed plates failed to produce sclerotia and basidiospores. Basidiospores were produced abundantly in the light and in the dark conditions in unsealed plates only on *Cyperus rotundus* rhizome meal agar medium.

Key words: *Sclerotium rolfsii*, aeration, sclerotia, basidiospores, *Cyperus rotundus* meal agar medium

INTRODUCTION

Sclerotium rolfsii is a soil-borne phytopathogenic fungus found in tropical and subtropical regions of the world and causing serious yield loss in crops of high economic importance. The pathogen is parasitic on a number of cultivated and non-cultivated plants but rarely on cereals (Sarma 2002; Maurya *et al.* 2007). Diseases caused by *S. rolfsii* are initiated either directly from soil-borne sclerotia which germinate to form fine cottony hyphae infecting the collar region of host plants or sclerotia sticking on the lower/upper surfaces of the leaves by rain splashes where they germinate and cause leaf spots (Singh and Pavgi 1965). Soil temperature 25–30°C and soil moisture 90% play significant role in disease development (Gupta *et al*. 2002). Various biotic and abiotic factors which directly or indirectly influence the development of sclerotia were discussed in literature (Punja 1985; Georgiou 1997; Ellil 1999; Sarma 2002).

In vitro basidiospore formation of *S. rolfsii* was reported by several workers (Gato 1930; Mundkur 1934; Mishra and Haque 1960; Kulkarni and Ahmed 1966, Ahmed *et al*. 1967, 1968; Kulkarni and Ahmed, 1967a, b; Punja *et al*. 1982). However, in all these reports, the time required for basidiospore formation ranged from two weeks to a few months. Singh *et al.* (1996) developed a very simple new medium (6% *Cyperus rotundus* rhizome powder, agar agar 20 g and 1 000 cm^3 distilled water) on which basidiospores were formed in 6–8 days. In the present experiment we report the effect of air and light on sclerotial development and basidiospore formation of *S. rolfsii*.

MATERIALS AND METHODS

Isolation and purification of *S. rolfsii*

S. rolfsii was isolated by picking individual sclerotia from the infected chickpea (*Cicer arietinum*) plants collected from the Agricultural Research Farm of Banaras Hindu University. Such sclerotia were surface-sterilized with 0.1% mercuric chloride for a few seconds followed by three washings in sterilized distilled water. The sclerotia were then placed on potato dextrose agar (PDA) medium in Petri dishes and incubated at 27±2°C. Sclerotia formed after

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10 days were picked up with the help of an inoculatation needle and inoculated onto PDA slants for further use.

Experimental design

Fresh rhizomes of *C. rotundus* collected from the field were washed thoroughly in running tap water and then air-dried to remove surface moisture at room temperature (27±2°C). They were powdered in an electric grinder. Two per cent agar powder and 5, 6 and 7% *C. rotundus* rhizome powder were added to one litre of distilled water and then autoclaved at 121°C for 10–15 minutes. Twenty cm^3 of this medium was poured in each Petri dish. After solidification of the medium, mycelial discs (5 mm diam) were cut from 4–6 day-old actively growing culture of *S. rolfsii* and each disc was placed in the centre of Petri plates containing *C. rotundus* rhizome meal agar medium (CRMA) as well as on PDA. Two sets of each of six plates were inoculated. In each set, three plates were wrapped with black paper in order to create dark condition and another set left unwrapped. The plates were incubated at 27±2°C in an incubator. After every 24 h, observations were made for growth, colony characters, mycelial strands, sclerotia initials, colour and induction of basidiospores. Another six plates were inoculated as above but the plates were sealed with lab seal tape to check the aeration and the other three plates were wrapped with black paper and other three left unwrapped.

Another experiment was concurrently conducted to assess the role of air for the development of sclerotia. In this experiment PDA was poured in twelve plates. Each plate was inoculated with a mycelial disc and sealed with the help of lab seal in the following manner, i.e., no sealing (control), half sealed, ⅔ and finally complete sealing. Each set contained 3 plates. After inoculation and sealing, Petri dishes were incubated at 27±2°C in light and the other sealed plates were wrapped with black paper and incubated as above. Visual observations were periodically made for sclerotial development and basidiospore formation.

RESULTS AND DISCUSSION

Dark and light conditions did not affect the fungal growth, size and number of sclerotia. In ⅔ and ½ sealed plates placed in light and darkness affected mycelial growth and number of sclerotia significantly as compared to the control (unwrapped plates). In control plates, sclerotial initials were observed after 4–5 days from inoculation as whitish, tiny, pinhead-like structures and after 5–6 days exudation commenced. In completely sealed plates, the fungal growth was relatively very slow, compact and profusely growing mycelium was observed after 7 to 8 days as compared to the control. Among all completely sealed plates, there was no sclerotium formation even after 15 days from inoculation. In ⅔ and ½ sealed plates, the number of sclerotia was less but they were bigger in size as compared to the control. In control plates, mature sclerotia became brownish at 7th day after inoculation but in ½ and ⅔ sealed plates, such sclerotia were seen only after 9 days. The number and scletorial weight were affected drastically due to improper aeration as average number of sclerotia and weight of 100 sclerotia were more in unsealed plates (control) (Table 1).

In the experiment, it was observed that the growth of the fungus was the same on PDA in either sealed or unsealed plates. Different concentrations of *C. rotundus* rhizome powder induced basidiospores formulation. However, basidiospores were not produced in ½, ⅔ and completly sealed plates, rather mycelial strands formed. Basidiospores were formed on Petri plates were completely covered with mycelial growth i.e. on 5–6th day. Initial basidiospore formation commenced at the periphery and later moved gradually to the center of the Petri plate in CRMA medium. Profuse basidiospores were formed in unsealed plates of CRMA medium (Table 2).

Sclerotia are the asexual structures formed due to the aggregation of fungal mycelium. Biogenesis of scleroia is associated with lipid peroxidation. Georgiou (1997)

Table 1. Effect of air on sclerotial development of *S. rolfsii* on potato dextrose agar medium

Treatment (sealing of plates)	Observation												
	in dark					average No. of sclerotia/ plate	weight [mg]/100 sclerotia	in light					average No. of sclerotia/ plate	weight [mg]/100 sclerotia
	visual observation after [days]							visual observation after [days]						
	5	7	9	11	13			5	7	9	11	13		
No sealing (control)	++	$+++^0$	+++	+++	+++	222	84	+	$+++^0$	+++	+++	+++	240	85
½ sealing	$+^F$	+	++	+++	+++	180	82	$+^F$	+	++	+++	+++	197	79
2/3 sealing	$+^F$	+	++	+++	+++	175	80	$+^F$	+	++	+++	+++	186	80
Complete sealing	–	–	–	–	–	00	00	–	–	–	–	–	00	00

+ sclerotial initial; $+^F$ fewer sclerotial initials; ++ white sclerotia; $+++^0$ optimum size of slightly melanized and exudating sclerotia; +++ dark brown sclerotia; – no sclerotial initials

Table 2. Effect of *C. rotundus* powder on atelial stage development in *S. rolfsii*

Treatment (Sealing of plates)	Concentration [%] of *C. rotundus* powder					
	incubation in dark			incubation in light		
	5	6	7	5	6	7
No sealing (control)	+++	+++	+++	+++	+++	+++
½ sealing	–^{mt}	–^{mt}	–^{mt}	–^{mt}	–^{mt}	–^{mt}
⅔ sealing	–^{mt}	–^{mt}	–^{mt}	–^{mt}	–^{mt}	–^{mt}
Complete sealing	–	–	–	–	–	–

+++ Petri plates full of atelial stage
–^{mt} thick mycelial threads only
– no basidial stage, no mycelial threads

reported direct correlation between the number of sclerotia and lipid peroxidation in the fungal colonies. Several biotic and abiotic factors influence the aggregation of fungal hyphae in the culture medium. Punja and Damini (1996) and Singh *et al*. (2002) reported that sclerotial exudates directly influence the development and maturation of sclerotia. Bhoraniya *et al*. (2002) reported that due to pathogenesis the level of oxalic acid increases in the infected plants and the increase of oxalic acid induces formation of sclerotial initiation at the collar region. It was reported that depletion of exudate inhibits the development of sclerotia of *S. sclerotiorum* (Singh *et al*., unpublished observation). In the present experiment, we found that a proper aeration is essential for the development of sclerotia. It is already known that scletorial development and severity of pathogenesis is maximum in sandy sail which provides more aeration as compared to heavy soil because it has less porosity more compactness, less aeration which hinder the formation of abundant sclerotia.

Regarding sexual stage induction in *S. rolfsii*, the most frequent and early induction of basidiospores was reported by Singh *et al*. (1996) in 6% CRMA medium at 27±2°C in the dark. However, at all the concentrations (5, 6 and 7%) of *C. rotundus* powder, basidiospores were observed both under light and darkness in the presented experiments. The effect of aeration on basidiospore induction and sclerotia formation is reported for the first time for this fungus.

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POLISH SUMMARY

ROLA POWIETRZA I ŚWIATŁA W ROZWOJU SKLEROCJÓW I WYTWARZANIU BAZIDOSPOR PRZEZ *SCLEROTIUM ROLFSII*

Sclerotium rolfsii jest patogenem glebowym, powodującym uszkodzenia siewek, sprawcą plamistości liści w kilku uprawach i na dzikich roślinach. W badaniach wykorzystano płytki Petriego zawierające pożywkę agarową z glukozą, zaszczepioną grzybem *S. rolfsii*. Dwie trzecie powierzchni trzech płytek, 50% powierzchni następnych trzech oraz 100% powierzchni kolejnych trzech zaklejono taśmą celofanową. Trzy płytki nie były zalepione. Wszystkie były inkubowane w 27±2°C. Przygotowano dwa komplety takich płytek. Jeden był inkubowany na świetle, drugi w ciemności. Nie było istotnych różnic między nimi we wzroście grzybni i liczbie sklerocjów, ale istotne różnice zaobserwowano w porównaniu z kontrolą, tj. niezaklejonymi płytkami. Na wytwarzanie sklerocjów i bazidiospor bezpośrednio wpływało powietrze, ponieważ nie stwierdzono istotnych różnic we wzroście grzybni i liczbie sklerocjów w porównaniu z kontrolą, tj. z płytkami, które nie były zaklejone. Tylko na pożywce agarowej z mączką z rozłogów *Cyperus rotundus*, bazydiospory powstawały licznie na świetle i w ciemności w niezaklejonych płytkach.