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Qualitative and quantitative characteristics of different isolates of *Sclerotium rolfsii* Sacc. on Czapeck Dox Medium

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Ten different isolates of *Sclerotium rolfsii* Sacc. were isolated from different sources and their cultural characteristics like mycelial diameter growth , mean dry mycelial weight, numbers of sclerotia produced, pattern of sclerotial distribution, day's of appearance of sclerotial initials as well as their time of maturity were observed in Czapeck Dox agar/broth. The results of the present study confirmed ample variation among different isolates of *S. rolfsii* Sacc. Isolate number no. 8 and isolate no. 10 was fastest to cover petri plates of nine (9) cm diameter within 72 hrs. of incubation while mean dry mycelial weight of isolate no 9 (867.33 mg) was maximum although it was statistically at par with isolate no. 4 (849.66 mg) and isolate no. 8 (841.66 mg) respectivel. Mean numbers of sclerotia produced was significantly highest in isolate no. 1 (509.3). Likewise, morphological variation between different isolates in respect to mycelium growth, distribution, appearance and maturity of sclerotia were also observed.

Key words: *Sclerotium rolfsii,* mycelial diameter, mean dry mycelial weight, sclerotia

INTRODUCTION

Sclerotium rolfsii Sacc. (teleomorph *Athelia rolfsii* (Curzi) Tu & Kimbrough) is a devastating soil-borne plant pathogenic fungus with a wide host range (Aycock 1966).. Although there are several other sclerotium-producing fungi, the fungi characterized by small tan to dark-brown or black spherical sclerotia with internally differentiated rind, cortex, and medulla were placed in the form genus *Sclerotium*. However, the teleomorphic state was discovered later, confirming that the fungus was a basidiomycete. *Sclerotium rolfsii* usually causes collar rot, but spotted leaf rot with a single tiny sclerotium in the center has also been reported by Singh and Pavgi (1965).

Sclerotium rolfsii Sacc. (Teleomorph: *Athelia rolfsii* (Curzi) Tu and Kimbrough) is a destructive fungal plant pathogens causing diseases in many mono and dicotyledonous plants encompassing more than 500 host species (Punja and Jenkins,1984). In nature ample variation exists with in a same species of this pathogen, studies of variability within the population in a geographical region are important because this reveals the changes occurring in a given population. Thus, the present study was

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carried out to understand *in vitro* variability among different isolates of *S. rolfsii* , on a synthetic medium namely, Czapeck Dox Agar (C.D.A).

MATERIALS AND METHODS

Different isolates of *S. rolfsii* Sacc. were isolated from different sources as listed in Table 1. Samples were collected from different host plants of different area while one isolate was also collected from field soil.

Method of isolation of different isolates of S. rolfsii Sacc. from different crops

Diseased samples collected from different crops were thoroughly washed with sterile distilled water and isolation was made by tissue segment method. The diseased tissue of about 5 to 10 mm segment from the margin of infected part having both diseased and healthy tissues were washed thoroughly in sterile distilled water and was surface sterilized in 0.1% sodium hypochlorite solution for about ½ - 2 minutes (depending upon the nature of tissue) followed by repeated rinsing for 3 – 4 times with sterilized distilled water. The surface sterilized materials were transferred to sterilized Potato Dextrose Agar (PDA) or pathogen specific medium plates aseptically as the case may be. The plates were inverted and incubated at 28 ± 2°C in B.O.D. incubator for 3 – 4 days after the growth of the pathogen. The cultures were purified with single hyphal tip method. Hyphal strands were located and examined under low power of the microscope with the dish inverted and the bit of agar bearing a single hyphal tip is carefully transferred on PDA petri plates.

Further sub culturing was carried out by taking 6 mm discs of the isolates from periphery of 4 day's old culture throughout the experimental period. Cultural and morphological characteristics like rate of mycelial radial growth (mm or cm /day), growth pattern, day's of appearance of sclerotial initials, day's of maturity of sclerotia, size of sclerotia, pattern of sclerotia development, distribution on Petri plates, colour, growth pattern of mycelium and measurement of fungal mycelium were observed by growing the pathogen on C.D.A. medium.

RESULTS AND DISCUSSION

The general morphological features of the isolates grown on CDA medium showed considerable variations which are as given below: -

Isolate 1: Very light cottony growths with curly mycelium, sclerotias were evenly distributed. Appearance of sclerotial initials and their maturity was observed to be 7 and 10 days respectively.

Isolate 2: Dense cottony growth of mycelium, with very few sclerotia at centre of petri plate was observed. Days of appearance and maturity of sclerotia was 8 to 10 days respectively.

Isolate 3: Light cottony growths of sclerotia at centre of petri plate but more at periphery were seen. Days of appearance of sclerotial initials vary from 5 to 6 days, which starts maturing from 7 days onwards.

Isolate 4: Very light cottony growth of mycelium with evenly distributed sclerotia throughout the Petri plate. A sclerotial initial starts appearing within 5 to 6 days and starts maturing from 8 days onwards.

Isolate 5: Light cottony growth of mycelium was seen. Sclerotia were distributed at the peripheral side. Its appearance and maturity varies from 4 to 5 days to 6 to 7 days respectively.

Isolate 6: Dense cottony growth of mycelium. Sclerotia distribution at periphery which starts appearing within 4 to 5 days and starts maturing from 6 days onwards.

Isolate 7: Very light curly, cottony growth of mycelium with sclerotial initials coming at peripheral side within 5 to 6 days followed by its maturity within 8 to 9 days.

Isolate 8: Dense white cottony growth, numerous sclerotia distributed throughout the petri plate were observed. Sclerotial initials start appearing from 3 to 4 day's and got matured within 5 days.

Isolate 9: Light cottony growth of mycelium with numerous sclerotia distributed at peripheral side only. A sclerotial initial starts appearing within 7 to 8 day's while their maturity starts from 9 days onwards.

Isolate 10: Dense cottony growth of mycelium with numerous sclerotia distributed throughout the petriplate followed by appearance of sclerotial ini-

tials from 3 to 4 days after inoculation and their maturity from 5 days onwards was observed.

Variation among different isolates grown on C.D.A medium in respect to different parameters like mean mycelial diameter growth, mean dry mycelial weight, mean numbers of sclerotia produced were also observed (Table 2). As for example Isolate 8 and 10 requires 72 hrs of incubation to cover the Petri plate of 9 cm diameter while Isolate 1, 2, 3,4,5,6 and 7 requires 96 hours of incubation while Isolate 9 requires 120 hrs. to cover the same.

Table 1 : Isolates of *Sclerotim rolfsii* isolated from different sources

Isolate No.	Source
1.	Soil
2.	Chili (*Capsicum annum* L).
3.	Elephant Foot yam (*Amorphophallus* sp.)
4.	Tuberose *(Polyanthus tuberosa)*
5.	Brinjal *(Solanum melongena)*
6.	Tomato *(Lycopersicon esculentum)*
7.	Barley (*Horderum vulgarae* L.)
8.	Cumin (*Cuminum cyminum*)
9.	Potato (*Solanum tuberosum*)
10.	Groundnut (*Arachis hypogaea* L.)

Table 2 : Quantitative characteristics of different Isolates of *S. rolfsii* on C.D.A medium

Isolates	Mean mycelial diameter growth in hours 24 hrs	48 hrs	72 hrs	96 hrs	120hrs	Mean dry mycelial wt (mg)	Mean numbers of sclerotia
1	1.98	4.03	6.0	9.0		483.66	509.3
2	2.75	3.73	7.23	9.0		299.00	62.6
3	2.11	4.11	5.75	9.0		486.33	42.6
4	1.38	4.83	6.8	9.0		849.66	225.6
5	1.1	5.08	6.75	9.0		148.33	30.0
6	2.11	4.06	5.83	9.0		421.33	181.3
7	2.36	4.68	7.0	9.0		164.66	11.6
8	3.08	7.2	9.0			841.66	284.0
9	2.75	3.93	5.41	7.8	9.0	867.33	25.3
10	2.1	6.26	9.0			332.33	294
						SE D =56.3499	SE D =8.8066
						C D (.01) =160.3413	C D (.01) =25.0587

Mean dry mycelial weight of Isolate 9 was maximum (867.33 mg) while minimum mycelial weight was recorded in Isolate 5. Mean numbers of sclerotia produced was maximum in case of Isolate 1 (509.3) and found to be minimum in Isolate 7 (11.6). No variation in respect of hyphal thickness (2.55 to 17.5 micron) was observed among different isolates. Different isolates isolated from different sources differ from each other in few to many characters. No two isolates were exactly similar with each other in all respects. It can be further supported by the evidence of many workers who have also reported variability in this fungus like, Sarma and Singh (2002) who studied variability among 26 isolates of *Sclerotium rolfsii* Sacc. collected from various hosts/soil samples and localities in India. The isolates varied in colony morphology, mycelial growth rate, sclerotium formation, teleomorph production and sclerotial size and color. Further Sarma *et al* (2002) collected 121 isolates of *S.rolfsii* from 15 localities and seven plant species (groundnut, sunflower, soybean, beet, carrot, valeriana, and lupinus) throughout South Africa and compared them. Okabe *et al.* (2000) also observed variants in southern blight fungal isolates in Japan. Ansari and Agnihotri (2000) observed morphological, pathological and physiological variation among the isolates of *S. rolfsii* Sacc. in Soybean.

From the above results it can be concluded that different isolates recorded differs among each other in respect to their cultural and morphological characteristics, mycelial growth pattern, dry mycelial weight, numbers of sclerotia produced, distribution, appearance and maturity of sclerotia, which further confirms variability of the pathogen *S. rolfsii* Sacc.

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