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Studies on Sclerotium Formation in *Curvularia* Species

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Natural sclerotium formation in two species of *Curvularia* was observed. The sclerotia were spherical to elongated. The scanning electron microscopical observations of sclerotia revealed that the sclerotia were of two distinct layers of cells, outer loosely woven hyphae and inner contact layer of cells. Different lights, viz. red, blue, green, fluorescent or addition of culture filtrate of *Sclerotium rolfsii* in the medium did not affect sclerotium formation.

KEYWORDS: ***Curvularia* species, Electron microscopy, Sclerotium**

Curvularia species occasionally cause leaf spot in several plants. *C. lunata* has been implicated as one of the most important grain molds of rice and millet crops, e.g., ragi (*Eleusine coracana*) and sorghum (*Sorghum bicolor*). The pathogens cause discolouration of the seeds, degenerate endosperm and also infect the embryo resulting in almost 100% loss in viability of the seeds (Rao and William, 1978). Recently, Deshmukh and Raut (1993) confirmed that *Curvularia lunata* can infect all part of the seeds of sorghum.

Sclerotia are resistant multicellular resting structures formed by some filamentous air-borne fungi and soil-borne fungi. In general, three distince stages of sclerotial development are recognised - (i) initiation, (ii) development, and (iii) maturation. Several factors are reported to affect sclerotium formation. In many fungi, light triggers sclerotium formation, for example, *Fusarium oxysporum* (Carlile, 1956); *Aspergillus japonicum* (Heath and Eggins, 1965); *Aspergillus* spp. (Rai *et al.*, 1967; Rudolph, 1962) and Sclerotium rolfsii (Gruelach and Mohr, 1947; Mclellan *et al.*, 1955). Different quality of light differentially affects sclerotium formation in *Verticillium albo-atrum* as blue light inhibits microsclerotial production while yellow, orange and red light promote it (Kaiser, 1964).

Sclerotium formation is affected by several factors. Prithiviraj and Singh (1997) observed an increase in the number of sclerotia in *S. rolfsii* grown on the cultures of *Aspergillus niger* but not on *Penicillium* sp. Various antibiotics and metabolites of soil-borne bacteria, viz., *Bacillus licheniformes* and *Bacillus subtilis* induced sclerotium formation in *S. rolfsii* and *Rhizoctonia solani* while mycelial growth was inhibited (Chet, 1967; Henis and Inbar, 1968). In a recent study, Prithiviraj and Singh (1997) obserbed induction of sexual reproduction in *S. rolfsii* by culture of *B. subtilis*.

Study on sclerotium formation in *Curvularia* species is meager. Perhaps there is only one report on induced sclerotium formation in this fungus (Reddy, 1967). In view of the great significance of sclerotia in fungal survival the present work was taken up to study whether some *Curvularia* species can produce sclerotia by exposing the cultures to light and also by the culture filtrate of *S. rolfsii*. Scanning electron microscopy of the surface morphology of sclerotia has also been done and the results are presented here.

Materials and Methods

The fungi. The tested fungi were isolated on PDA (Peeled potato 250 g, dextrose 20 g, agar 15 g, distilled water 1 *l*) from their respective hosts collected from the Experimental Farm of Banaras Hindu University using standard procedures (Table 1). Pure cultures were made by single spore isolation in the case of *Curvularia* species (Fig. 1) where as in case of *S. rolfsii* pure cultures were obtained from single sclerotia. The cultures were maintained on PDA slants for further use.

Scanning electron microscopy of sclerotia of *Curvularia* species. The sclerotia samples were prepared by fixing them in 2.5% glutaraldehyde prepared in 0.1 M dimethyl arsenic acid at pH 7.2 and stored at 4°C. Just before the electron microscopy work, the specimens were dehydrated in ethylene glycol monoethylether (EGM) with two changes at 12 h intervals. The samples were then kept overnight in 100% water-free acetone. Then, the samples were dried to critical point (Baiters CPD020),

Table 1. The fungi and their hosts used in this study

Fungus	Host
Curvularia lunata	Rice (*Oryza sativa*)
Curvularia species	Dead leaves of moong (*Vigna radiata*)
Sclerotium rolfsii	Chickpea (*Cicer arietinum*)

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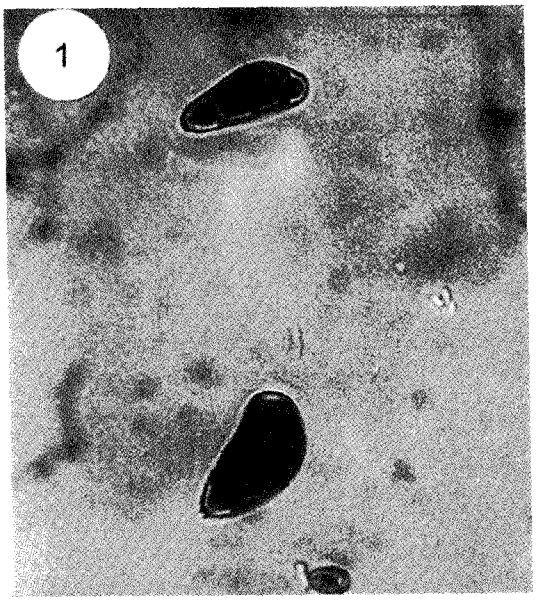


Fig. 1. Conidia of *Curvularia lunata* under high power (×1700).

mounted on aluminum stubs and sputtered with gold-palladium using a Poloron E5000 diode sputtering system. Some of the sclerotia were cut cross-sectionally with a sharp razor blade and the samples were prepared as described above. Scanning electron micrographs were obtained with Hitachi H-800 and Hitachi S430 microscopes.

Effect of culture filtrate of *Sclerotium rolfsii* on sclerotium formation and growth of some *Curvularia* species. Potato dextrose broth was prepared and 100 m*l* of it was poured in each of several conical flasks (500 m*l*). Mycelial bits from young growing cultures of *Sclerotium rolfsii* were inoculated and allowed to grow for 7 days at room temperature. After 7 days the culture broth was filtered through sterilized Whatman filter paper No. 1. The culture filtrate was then added to the melted PDA at the rate of 2, 5, 10, 15 and 20 percent (v/v). The medium containing culture filtrate was poured in Petri dishes. After solidification of the medium, the dishes were inoculated with two species of *Curvularia* separately. The plates were later incubated at 25°C and periodically observed for the formation of sclerotia. The radial growth was measured with the help of a scale holding the plate against the light every 24 h. All the steps were carried out in sterilised condition. PDA without culture filtrate was kept as control. All the experiments were conducted in triplicate.

Effect of different lights on sclerotium formation in *Curvularia* species. Two *Curvularia* species were inoculated separately on Petri dishes containing PDA. The plates were exposed to different light viz., red (128 Lux), blue (124 Lux), green (130 Lux) and white (365 Lux) for 12 h and in darkness for 12 h alternatively till the formation of sclerotia.

Fig. 2. Enlarged view of sclerotia of *Curvularia* species isolated from *Vigna radiata*, formed in pathches which turned whitish on maturation.

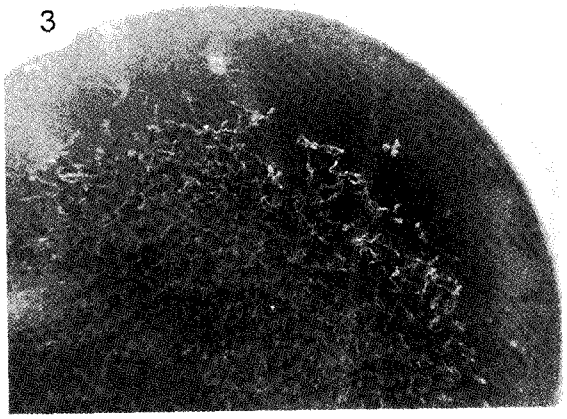
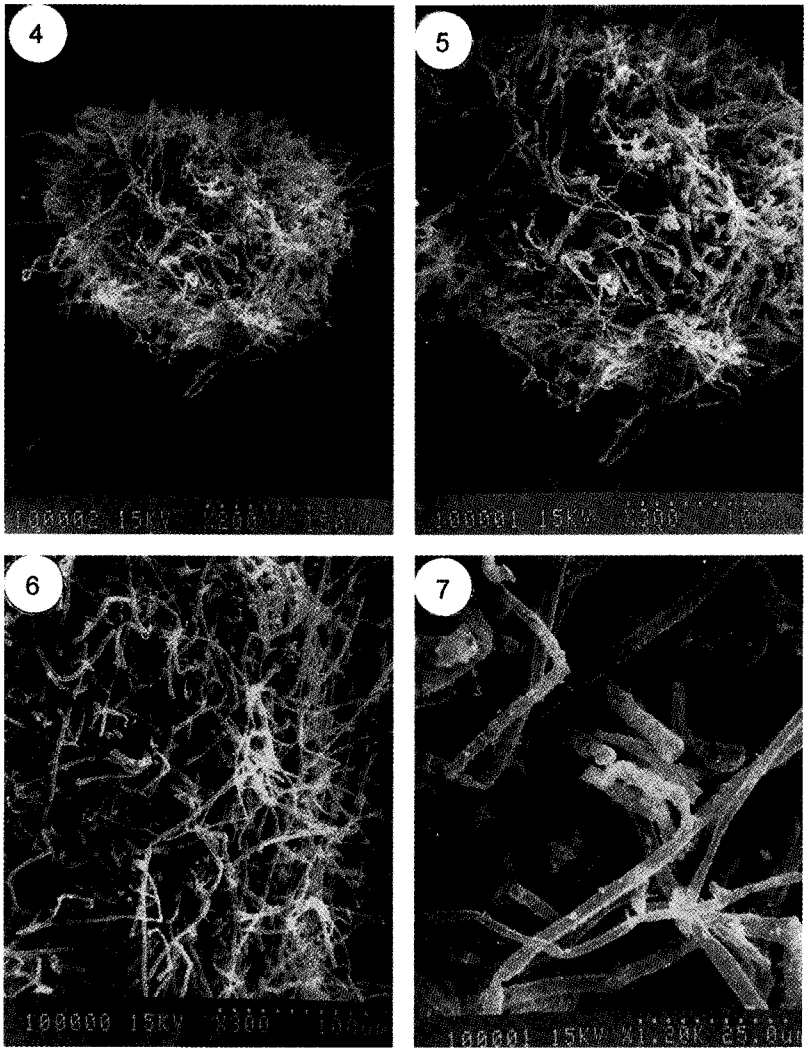


Fig. 3. Enlarged view of culture of *Curvularia lunata* showing elongated sclerotia.

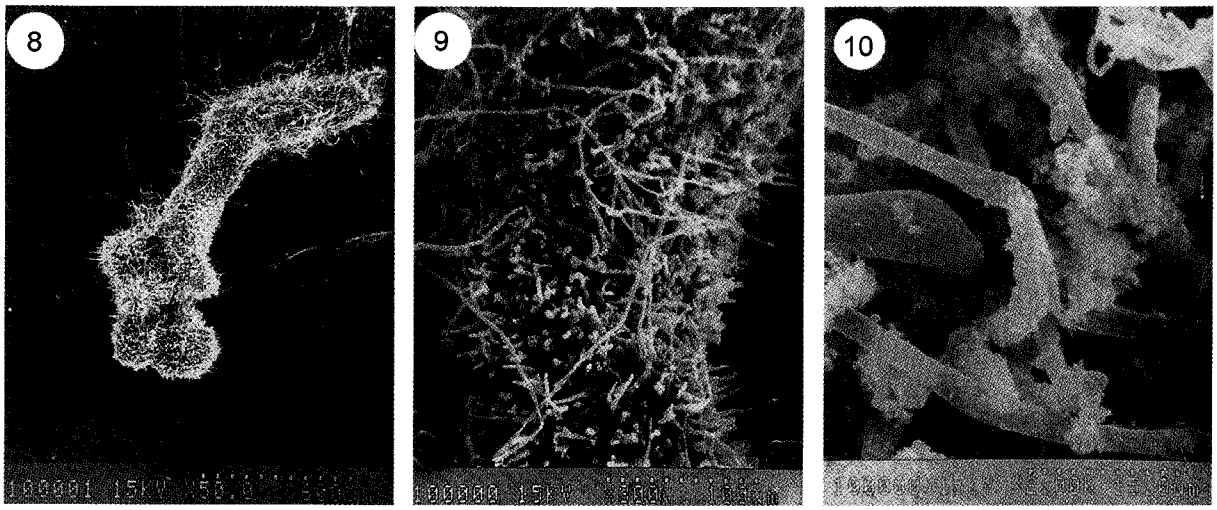
Results and Discussion

Sclerotia of *Curvularia* spp. were formed on cultures grown in Petri plates in both the species isolated from dead leaves of *Vigna radiata* and *Oryza sativa*. The structure of sclerotia and the pattern of sclerotial growth varied in both the species. Sclerotia of *Curvularia* species from *Vigna radiata* were found in patches which turned whitish on maturation on PDA in the Petri plate (Fig. 2), while *C. lunata* produced elongated sclerotia arranged uniformly on PDA (Fig. 3).

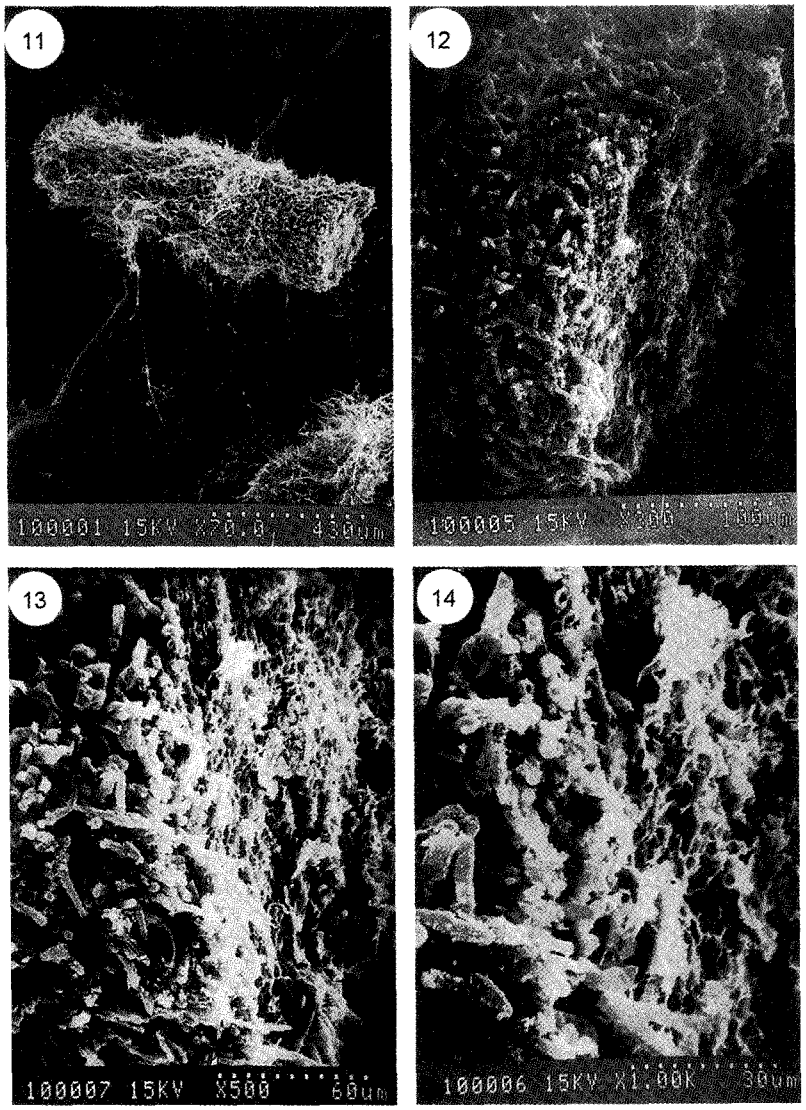
The scanning electron micrographs show spherical to elliptical nature of sclerotia with loosely woven hyphae (Fig. 4). Fig. 5 shows enlarged view of the spherical sclerotium. The outer layer of loosely woven hyphae was distinct (Figs. 6, 7). Sclerotia of some species were elongated with irregularly lobed structures (Fig. 8). Close-up view of the surface of sclerotium revealed loosely interwoven



Figs. 4-7. Scanning electron micrographs (SEM) of spherical sclerotia of *Curvularia* species. **Fig. 4.** SEM of spherical sclerotium showing loosely interwoven hyphae. **Fig. 5.** An enlarged view of Fig. 4. **Fig. 6.** SEM showing a close-up view of the surface of a sclerotium with interwoven hyphae. **Fig. 7.** Enlarged view of Fig. 6.



Figs. 8-10. Scanning electron micrograph (SEM) of elongated sclerotia of *Curvularia lunata*. **Fig. 8.** SEM of elongated sclerotium of *Curvularia lunata* with lobed structures at the end. **Fig. 9.** SEM showing enlarged view of the surface of a sclerotium with interwoven haphae. **Fig. 10.** Enlarged view of part of the sclerotium surface showing hyphal bits.



Figs. 11-14. Scanning electron micrographs (SEM) of cross-section of elongated sclerotium of *Curvularia lunata*. **Fig. 11.** SEM showing elongated sclerotia cut with a razor blade. **Fig. 12.** SEM of the cut portion of sclerotia showing two distinct cell layers: an outer loosely woven hyphal layer and an inner compact arrange ment of cells. **Fig. 13 and 14.** Enlarged view of Fig. 12.

hyphae (Figs. 9, 10). Fig. 11 shows part of the cut sclerotium. Cross-section of the cut sclerotium showed two distinct regions, the outer region of loosely interwoven hyphae and the inner region made of compact tubular hyphal cells (Figs. 12-14).

The effect of different lights, viz., fluorescent, red, blue and green on the growth of two species of *Curvularia* was seen. There was no significant effect of different lights on the growth of *Curvularia* species. Further, the light treatment did not affect sclerotium formation.

Culture filtrate of *S. rolfsii* inhibited the mycelial growth of *Curvularia* spp. and the inhibition of growth was directly proportional to the concentration of culture filtrate in the medium beyond 10% (v/v). But interestingly, culture filtrate less than 10% (v/v) showed stimulatory effect as mycelial growth was better than the control (Tables 2, 3). However, sclerotium formation was not affected by the culture filtrate of *S. rolfsii*. It is already known that the mature sclerotia of *S. rolfsii* consist of four distinct cell layers, (i) a fairly thick skin, (ii) a rind, 2-4 cells thick, made of broad and tangentially flattened cells, (iii) a cortex of thin walled cells with densely stained cytoplasm and (iv) a medulla made of loosely arranged filamentous hyphae with dense contents (Townsen and Willets, 1954). But the structure of sclerotia of *Curvularia* spp. in the present study was relatively simple with only two distinguishable layers, viz., the outer layer of loosely woven hyphae and the inner compact region.

Sclerotium formation in *C. lunata* and *C. pallescens* has been studied earlier by addition of bacterial cellulose,

Table 2. Effect of culture filtrate of *Sclerotium rolfsii* on growth (mm) of *Curvularia lunata*

Days	Culture filtrate					
	0%	2%	5%	10%	15%	20%
1	14±0.81	14.33±0.94	13.66±0.47	12±0.81	4.66±0.94	9.66±0.94
2	25.33±0.47	33±0.87	31±0.81	23.66±1.24	12.66±1.24	10.66±0.94
3	33±0.0	42.66±2.05	44.66±0.47	33.66±1.24	12±0.81	12.33±0.47
4	46±0.81	65.33±1.24	65±0.81	49.33±0.94	13.66±0.47	13±0.81
5	62±0.81	79.33±0.94	82±0.87	63.66±0.94	14±0.81	14.33±1.69
6	68.33±0.94	88±1.63	87.66±0.47	77±1.41	14.66±0.94	13.66±0.47
7	79±0.81	90.66±0.47	92±0.81	89±2.16	16±0.81	13±0.81

Mean±standard deviation.

Table 3. Effect of culture filtrate of *Sclerotium rolfsii* on growth (mm) of *Curvularia* sp. (isolated from *Vigna radiata*)

Days	Cuture filtrate					
	0%	2%	5%	10%	15%	20%
1	11.33±1.24	13.33±0.94	14.33±1.69	12±0.81	9.66±0.47	7.33±0.47
2	23.33±0.47	26±0.81	27±0.81	25±0.81	15.33±0.94	14±0.0
3	30.66±0.94	38.33±1.24	41.33±0.47	34.33±0.47	22.33±0.94	19.33±1.24
4	44±0.81	56±2.16	60.33±0.91	53±0.81	31.66±0.47	28±2.82
5	57.66±0.47	71±0.81	78±0.81	63.66±0.94	39.33±2.05	38±1.63
6	64.66±2.05	82.33±2.05	84.33±0.94	70.33±0.47	46.66±2.49	40.66±5.43
7	73.66±1.24	91±1.63	92±0.94	81.33±1.69	57.66±2.05	55.33±4.49

Mean±standard deviation.

potassium nitrate, ammonium nitrate and ammonium chloride to Richards solution (Reddy, 1967). Further, sclerotia were also produced on organic substrates like rice grain, rice leaves enriched with oats (20%), glucose (0.5%), sucrose (1%) and rice stubbles. However, natural sclerotium formation on common laboratory media like Richard, Czapeks, starch, oat meal or soil extract agar has not been reported earlier. Interestingly, the results presented here show spontaneous formation of sclerotia without addition of any inducing agent(s).

Several workers have obserbed that light affects sclerotium formation in a number of fungi (Carlile, 1956; Heath and Eggins, 1965; Rai *et al.*, 1967). The results of the present study, however, show no distinct effect of light on sclerotium formation in *Curvularia* spp. This may be attributed to differences in the various physiological events leading to sclerotium formation in the present fungus.

Bedi (1958) found that the number of sclerotia formed by *Sclerotinia sclerotiorum* increased significantly when staling products of other fungal culture filtrates or of the same fungus were added to the medium. Similarly, Liu and Wu (1971) observed increased sclerotial number when culture filtrate of *S. rolfsii* was added to the medium and concluded that the presence of some substances in the culture filtrate is responsible for sclerotium formation. However, the results of the present study is in contrary to the above reports as there was no effect of *S. rolfsii* culture filtrate on sclerotium formation. A plausible explanation might be that the mechanisms of sclerotium formation in *S. rolfsii* and *S. sclerotiorum* observed by the above workers could be distinctly different from that of *Curvularia* spp.

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