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ULTRASTRUCTURE OF *SCLEROTIUM ROLFSII* SACC. AND *SCLEROTIUM DELPHINII* WELCH, AFTER BURIAL IN SOIL

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The external and internal changes of dried and non-dried sclerotia of *Sclerotium rolfsii* Sacc. and *Sclerotium delphinii* Welch, after burial in soil for four weeks, were studied with stereoscan electron and light microscopes respectively. The sclerotia of both species were cultured on malt extract agar and tomato plant tissues. The external surface of dried sclerotia of *S. rolfsii* and *S. delphinii* were seen colonized by soil mycoparasites within two weeks. From third to fourth week most of the dried sclerotia became hollow shells. The internal examination of the sclerotia showed that the reproductive bodies of different mycoparasites embeded within the medullary tissues. The non-dried sclerotia were seen not effected by any soil fungi upto fourth week of burial.

Key words : Ultrastructure, Sclerotia, Mycoparasites.

INTRODUCTION

There are some septate fungi which are capable of forming sclerotia. The sclerotial body consists of firm aggregate of vegetative hyphae. It has an outer protective layer of thickened elements referred to as a "rind". This layer is probably one of the reasons why sclerotia are often able to withstand severe condition of dessication and other adverse conditions (Willetts [1]) and to resist biological degradations. (Butler, Chet and Hanis [2-4]). The role of sclerotia in the life of pathogens of economic plants is well known and by 1887 the structure of several sclerotia had been studied (Willetts [1]).

Townsend and Willetts [5] investigated the development and structure of sclerotia of *S. rolfsii* with light microscope. A mature sclerotium is composed of four distinct cell layers: (1) a thick skin or cuticle (2) a rind, two to four cells consisting of thickened, pigmanted, broad and tangentially flattened cells [3] a cortex of thin-walled cells with densely stained cytoplasm and [4] a medulla, made up of loosely arranged filamentous hyphae, also filled with dense contents.

In recent years the scanning electron microscope has provided a technique by which surface structures and also interaction of host and pathogen can be studied. Using scanning electron microscopy, according to Willetts [1] showed that the outer surface of sclerotium of *S. rolfsii* is an almost continuous layer which is thrown into ridges and troughs to give weavy appearance. The surface of *S. rolfsii* (Willetts [1]) and *S. delphinii* (Javed [6]) were found to be covered with a crust of collapsed material which resulted from drying out of the outer most hyphae.

Our present investigation is an attempt to reveal the external and internal structure of sclerotia of *Sclertium rolfsii* and *Sclerotium-delphinii* after burial in moist soil. These studies were made to observe the effect of microflora of soil on fresh and dried sclerotia which were grown on artificial agar medium and natural tomato plant tissues. These observations were carried out through scanning electron microscope and with light microscope.

MATERIAL AND METHOD

Preparation of sclerotia (a) *Drying of sclerotia.* Sclerotia of *S. rolfsii* and *S. delphinii* from malt extract agar and tomato plant tissues were washed with sterilized water thoroughly and then placed in a well aerated growth chamber for 24 hours at 25°. The relative humidity in the growth chamber remained between 25% and 35%.

(b) *Burial of sclerotia.* The fresh and dried sclerotia of both spices were buried with small quantities of 1mm sieved garden soil in nylon fabric bags. This nylon has a mesh size (60-80 μm) which allows passage of fungal hyphae. The bags were tied with strips and were buried 8-10 cm below the soil surface and moistened them and kept at 15°. These experiments were carried out in pots. The sclerotia were recovered at weekly intervals upto four weeks. These sclerotia were fixed in 5% glutaraldehyde (V/V in 0.1M phosphate buffer).

To study external and internal structure of sclerotia the following procedure was adopted.

(A) For scanning electron microscope studies, sclerotia were air dried for a week and attached to aluminium stubs with a property adhesive. They were coated with carbon and a thin layer of gold under high vacuum (NGN coating unit) and examined directly in a Cambridge 600 Stereoscan microscope at 25KV. Spurr [7].

(B) In order to examine the internal structure of scle-

rotia transvers sections were prepared from fixed sclertia embedded in resin Spurr [7]. Thin sections (1-2 μ) were prepared with a Cambridge (Model-11) ultra microtome using glass knives. The excellent staining for light microscopy has been obtained by the Azur 11-methylene blue method of Richardson *et. al.* [8]. The stained sections were mounted in immersion oil under coverslip and examined with a Vicker's Patholux microscope. The photomicrographs were taken with Kodak Paratomic-X film.

RESULTS AND DISCUSSION

Surface structure of sclerotia. (Electron micrographs) Sclerotia from malt extract agar and tomato plant tissue culture of both fungi *S. rolfsii* and *S. delphinii* had similar appearance. Plate-1 (Fig. 1 and 2) show the enlarged portion of a dried sclerotium before burial in soil. The surface of the sclerotium reveals weavy appearance with conspicuous ridges and troughs have a crack-like appearance but there is no evidence that such cracks actually penetrate the inner cells.

Plate-1. *Surface structure of dried sclerotium before burial in soil.*

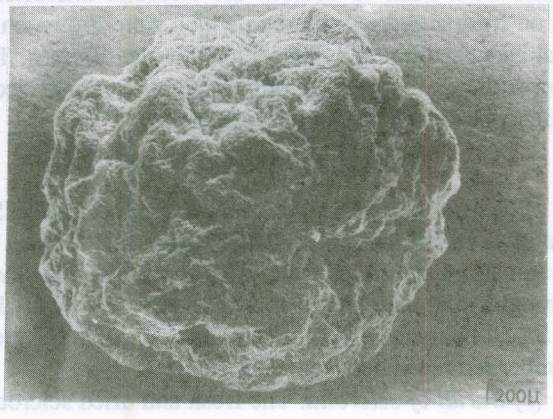

Plate-1. Fig. 1. Scanning electron micrograph of the upper surface of dried sclerotium of *S. delphinii*. Note the conspicuous ridges and troughs giving a wavy appearance.

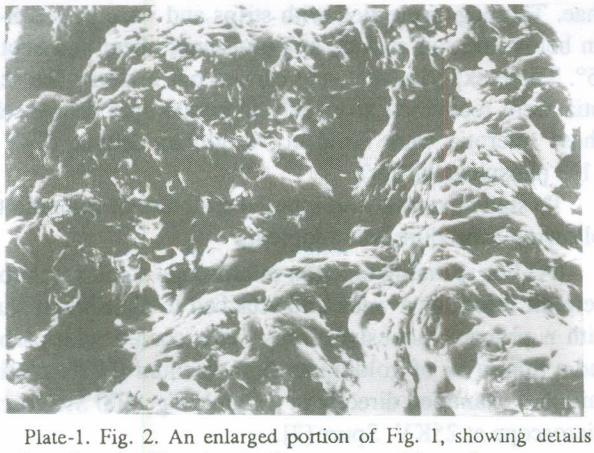

Plate-1. Fig. 2. An enlarged portion of Fig. 1, showing details of troughs and cracks. Note the pitted appearance of the surface.

In the first week, after burial in soil, dried sclerotia from malt extract agar and tomato plant tissue cultures under the scanning electron microscope show sparse covering of fungal mycelium. After two weeks of burial this covering of fungal hyphae was much more conspicuous with sclerotia from malt extract agar and tomato plant tissues, Plate-2 (Fig. 1 and 2). During third and fourth weeks of burial the majority of dried sclerotia of both kind became hollow shells. Spores or fruiting bodies of invading fungi were seen to be associated with these shells. Plate-3 (Fig. 1, 2 and 3).

Fresh (non-dried) sclerotia from malt extract agar and tomato plant tissue culture were also examined for surface changes and invading fungi, after burial in soil. Sclerotia from agar medium showed no surface change or any presence of fungi even after four weeks burial in soil, Plate-4 (Fig. 1 and 2). Some tomato plant tissue sclerotia did bear a few fungal hyphae in the fourth week.

Internal structure of sclerotia. (Light microscope micrographs) Thin sections were made of sclerotia which had been recovered after burial in soil.

Plate-2. *Surface structure of dried sclerotium after two weeks burial in soil.*

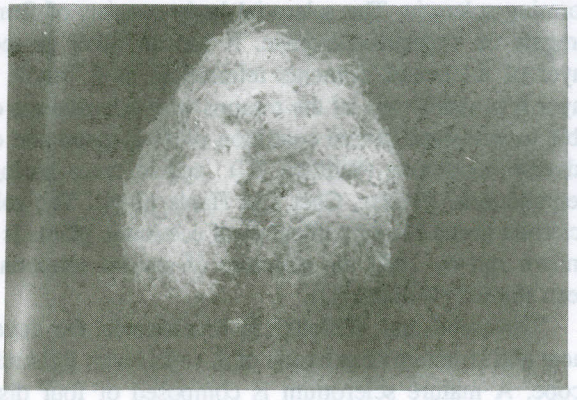

Plate-2. Fig. 1. Scanning electron micrograph of a dried sclerotium of *S. rolfsii* from tomato plant tissue culture covered with mycelium of soil mycoparasites.

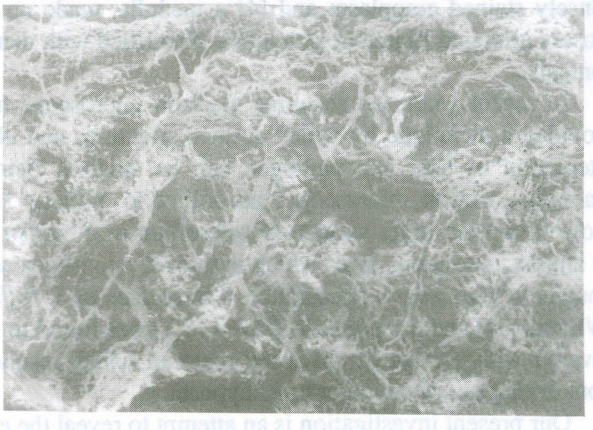

Plate-2. Fig. 2. An enlarged illustration of Fig. 1.

Plate-3. *Decayed sclerotium* of *S. delphinii buried for three to four weeks in soil .*

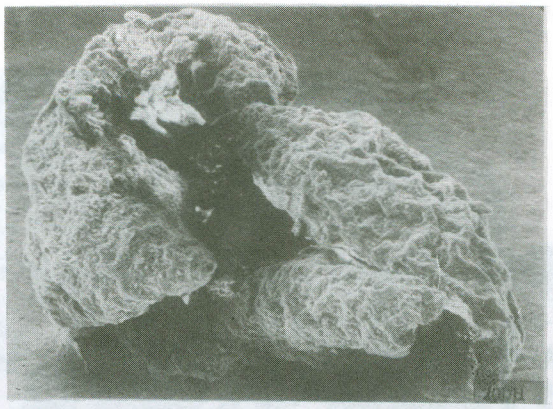

Plate-3. Fig. 1. The decayed sclerotium has become a shell. A large number of spiny bodies-cogonia of *Pythium oligandrum* can be seen within the shell.

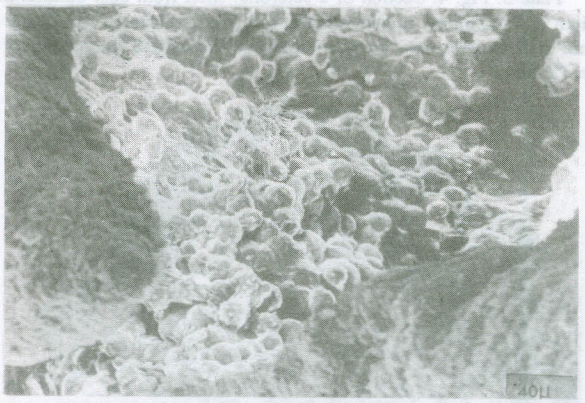

Plate-3. Fig. 2. An enlarged portion of the decayed sclerotium showing central interior part with spiny bodies.

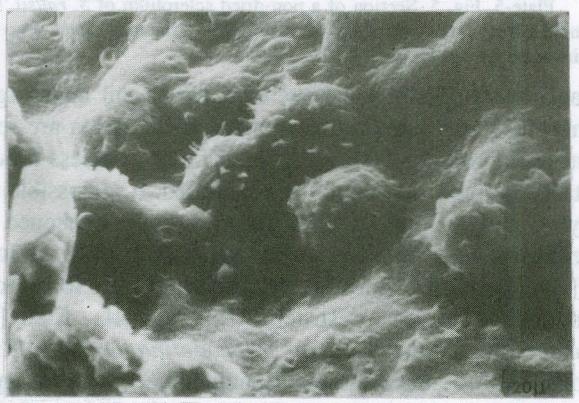

Plate-3. Fig. 3. An enlarged portion of Fig. 2, showing spiny cognia of *P. oligandrum.*

Plate-4. *Surface structure of non dried sclerotium from agar culture buried in soil for four weeks .*

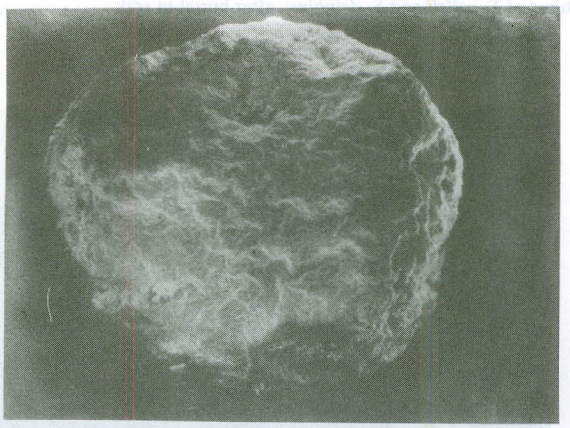

Plate-4. Fig. 1. Non-dried sclerotium of *S. rolfsii.*

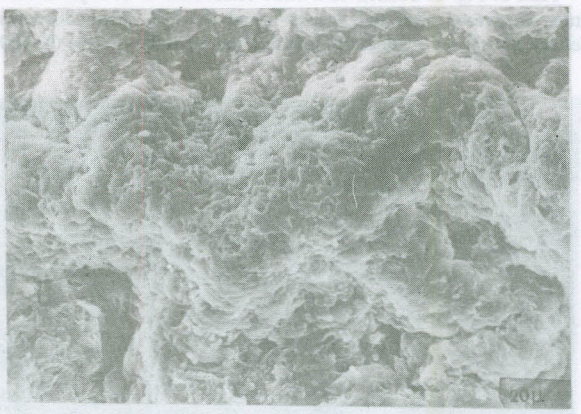

Plate-4. Fig. 2. An enlarged portion of non-dried sclerotium showing clear surface, without any fungal contamination.

From the first week, observations were made to detect invasion by soil fungi in the internal cells of sclerotia. The invasion of fungi was more obvious with sclerotia from tomato plant tissues than with one from malt extract agar. In the second week the majority of dried sclerotia were found to be invaded by different soil fungi. Thin sections of such sclerotia revealed the presence of large number of reproductive structures of these fungal invaders in both malt extract agar and tomato plant tissue cultures. These fungi were isolated and cultured on Czapek's Dox agar and studied under the microscope for identifications.

Amongst the structure observed in the sections of sclerotia, were the characteristic chlamydospores of *Trichoderma* sp. Plate-5 (Fig. 1) spiny cognia of *Pythium oligandrum*, Plate-5 (Fig. 2) and dense mycelium with spores of *Fusarium* sp., Plate-5 (Fig. 3). In the third and fourth weeks of burial the internal tissues of dried sclerotia were severely disorganized and masses of fungal hyphae were present. The spiny bodies of *P. oligandrum* were a prominent feature at this stage, Plate-5 (Fig. 4), section of non-dried sclerotia were also examined but there was no sign of invasion by soil fungi even after four weeks burial in soil, Plate-5 (Fig. 5).

Scanning electron micrographs revealed the irregular surface of dried sclerotia before burial. The dried surface

Plate-5. *Internal structure of sclerotia invaded by soil fungi.*
Sections of sclerotia (1-2 μm) from agar and tomato plant tissue cultures of *S. rolfsii* and *S. delphinii*, after burial in soil.

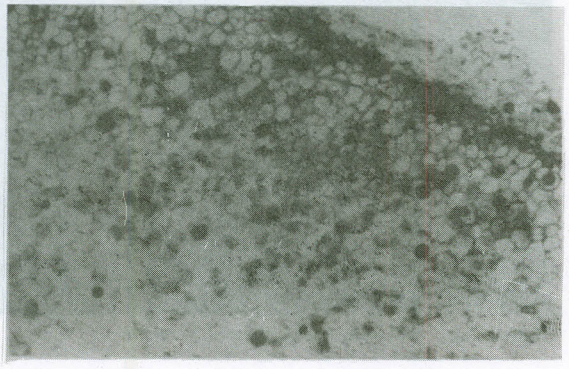

Plate-5. Fig. 1. Section of a dried sclerotium of *S. rolfsii* from agar culture, recovered after two weeks burial in soil. Chlamydospores of *Trichoderma* sp. are scattered throughout the medullary tissue (X-400).

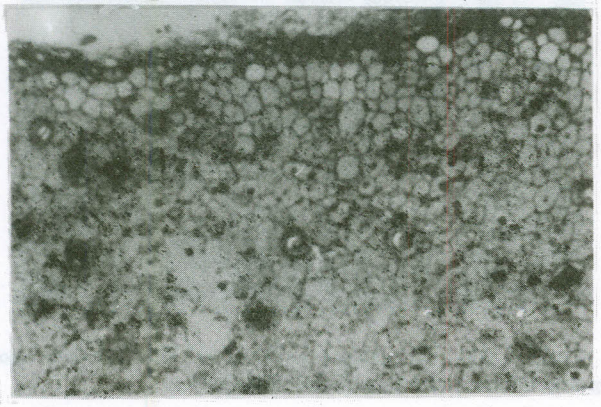

Plate-5. Fig. 2. Section of a dried sclerotium of *S. delphinii* from tomato plant tissues, recovered after two weeks burial in soil. Spiny cogonia of *P. oligandrum* and spores of a *Trichoderma* sp. embeded within the tissue (X-400).

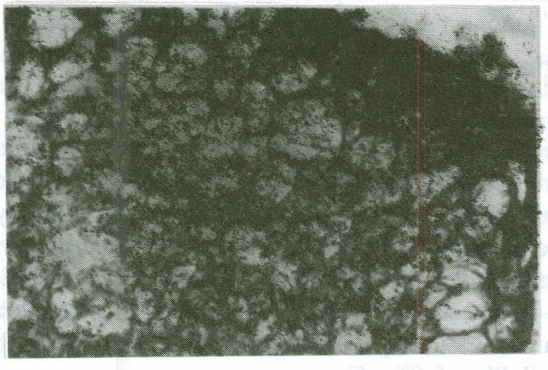

Plate-5. Fig. 3. Section of a tomato plant tissues sclerotium of *S. rolfsii* recovered after two weeks burial in soil. Mycelium with spores of a *Fusarium* sp. is scattered throughout the medullary tissue (X-806).

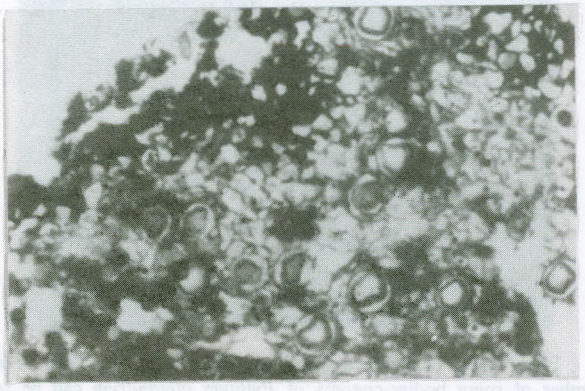

Plate-5. Fig. 4. Section of a sclerotial shell from agar culture. Sclerotium of *S. delphinii* recovered after four weeks burial in soil. Spiny oogonia of *P. oligandrum* are seen scattered throughout the medullary tissues (X-884).

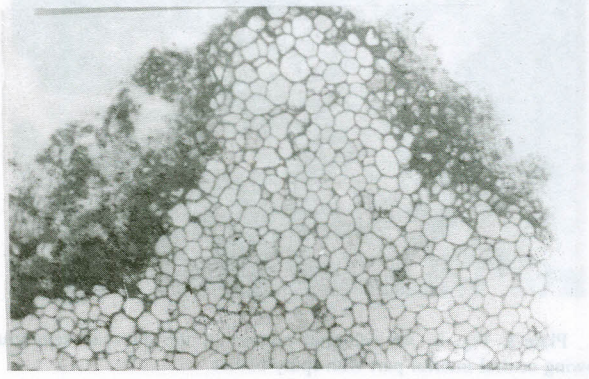

Plate-5. Fig. 5. Section of a non-dried sclerotium of *S. rolfsii* from tomato tissues recovered after four weeks burial in soil. There is no sign of invasion by soil fungi (X-600).

cracks were possibily the site of entry of soil microorganisms. It is well known that melanised structures are resistant to microbial attack and it is difficult to assess how an intact sclerotial rind could be penetrated. It is possible that drying and wetting of sclerotia may have weakened the melanised wall to microbial invasion. According to Jones [9] wall lyzing enzymes of parasitic fungi were found to be responsible for the destruction of sclerotia. Elad [10] pointed out that the ability of *Trichoderma hazianum* Rifai, isolate 203 to attack the plant pathogen *S. rolfsii* is apparently connected with the production by the isolate of chitinase and β-(1-3) glucanase inside the attacked sclerotia during parasitism.

The electron and light microscope micrographs of both *S. rolfsii* and *S. delphinii* showed that the mycoparasites caused degradation of sclerotial cells and the attacked cells lost their cytoplasmic content. It is therefore suggested that the mycoparasites, like *P. oligandrum, Trichoderma hama-*

tum Bain, *T. viride* Pers. ex S.F. Gray and *T. harzianum,* Rifai and *Fusarium* sp. involved in this investigation, utilized cell contents of the host through their enzymes, and thus, enabling them to sporulate intensively on the sclerotial surface and inside the digested cells. Thus these mycoparasites could be used to reduce sclerotial survival and controlling diseases they cause in green houses and fields.

The fresh (non-dried) sclerotia were found uneffected by soil mycoparasites even after four weeks burial in soil. Therefore, it is suggested that drying and wetting of sclerotia cause microbial degradation. (Smith [11]).

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