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LIGHT AND ELECTRON MICROSCOPIC STUDIES IN *PESTALOTIOPSIS ADUSTA* (Ell. & Ev.) Steyaert

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The development of conidiomata in *Pestalotiopsis adusta* was studied using light microscopy and Transmission electron microscopy. Light microscopic studies showed the presence of pycnidial conidiomata in culture whereas the fungus is known to produce only acervular conidiomata on leaves. During the developmental stages, the initially formed temporary conidiogenous cells are sloughed off and released into the pycnidial cavity along with the conidia. Another interesting observation is that the ultrastructure of the wall in the coloured cells as well as in the basal hyaline cell along with the appendage has thrown new light on their biological significance. For the first time a *Pestalotiopsis* sp. has been investigated which has thrown light on the development of the conidiomata with ultrastructural details.

Keywords : Appendage; Conidia; Electron microscopy; *Pestalotiopsis adusta*.

Introduction

Species of *Pestalotiopsis* are usually separated by conidial dimension, pigmentation and appendage characters. Even the morphologically distinctive taxa usually considered to be host specific can be recorded on a wide variety of hosts. Cultural characters are also remarkably homogenous and there is considerable unstable variation in the size, septation and pigmentation of the conidia as well as in appendage characters. The present study reports the development of the conidiomata in *P. adusta* (Ell. & Ev.) Steyaert[1] grown in artificial cultural media.

Materials and Methods

The material used in this study, *P. adusta* was isolated from leaves of *Daphniphyllum neilgherense* collected in 1995 at Kodaikanal (Altitude 2400 metre), Tamil Nadu, India (Herb MUBL No. 3133). The fungus was isolated in pure culture on oat meal agar and potato dextrose agar. The initial stages in the development of conidiomata were studied by slide cultures. To study the formation of conidiomatal cavity and conidia, selected conidiomata with agar were trimmed into 2-3mm square blocks and fixed in 2% glutaraldehyde in 0.1M phosphate buffer (pH-7.2) at 4° C and post fixed in 1% osmium tetraoxide at 4°C. Dehydration in graded series of acetone was followed by two to three changes in fresh araldite and polymerization at 60°C for 24 hours. The semithin sections of about 0.5 µm thickness were cut by ultramicrotome to study the development of the conidiomata using light microscope.

Transmission Electron Microscopy - Ultrathin sections were collected on copper grids and stained with uranyl acetate and lead citrate[2] and viewed using Phillips CM10 Transmission Electron Microscope.

Results and Discussion

Description of the fungus from leaves - Leaf spots were circular to irregular, amphigenous, pale yellow to brown, 2-5mm in diam. Acervuli hypophyllous, circular, scattered. Conidia fusoid, 14.5 - 15.5 x 4.0 - 4.5 µm, median coloured cells slightly constricted at septa , thin walled, lower coloured cell light brown, upper two coloured cells slightly darker than the lower cell, 13.0 µm long; apical cell hyaline, conical with 2-3 divergent setulae, 14-15 µm long; basal cell hyaline with a short pedicel 1-2µm long.

Cultural characters - Colonies on PDA were hyaline with white aerial mycelium, reverse unpigmented, conidiomata initially pycnidial, later becoming acervular, scattered, abundant, black, 100-300 µm in dia., composed of pale brown pseudoparenchymatous cells. Conidiogenous cells lining the cavity of the conidioma, initially undifferentiated from the cells comprising the wall, later formed conidiogenous cells ampulliform to cylindrical, annellidic. Conidia fusiform, 14-15 x 4.0-4.5 µm. Coloured cells lightly constricted at the septa, thin walled, upper two coloured cells slightly darker than the lower cell, apical cell hyaline, with 2-3 divergent setulae of 10-13 µm long; basal cell hyaline, conical with a sharp pedicel 1-2 µm long (Fig. 1).

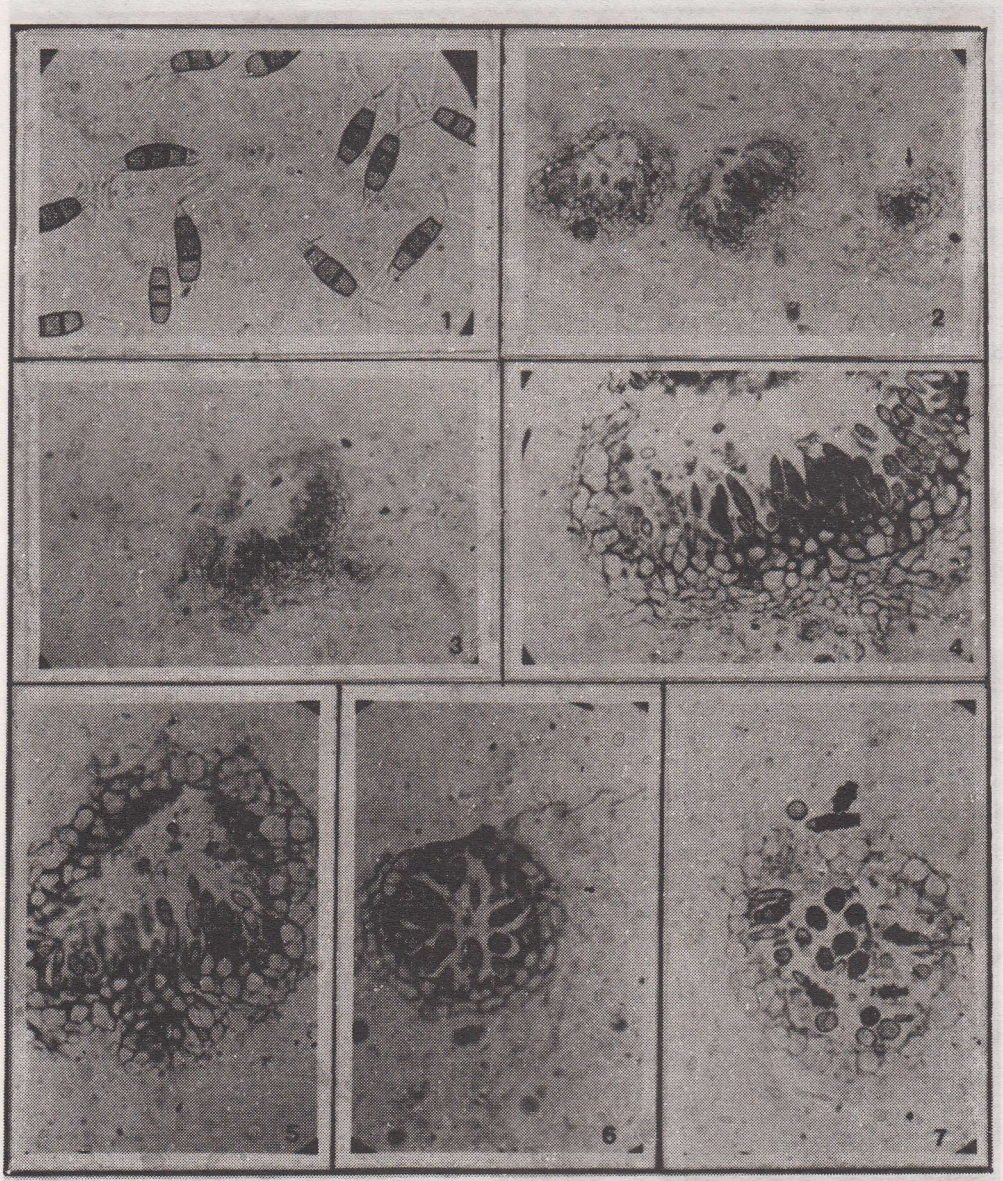
Development of conidiomata - Formation of conidiomatal primordium - The conidiomatal initials were observed as small spherical pseudoparenchymatous structures initially. The cells at the centre of the initial stained deeply when compared with the rest of the cells comprising the pseudoparenchymatous primordium (Fig. 2, arrow). The mode of cavity formation was not very clear from the sections, but once the cavity is formed the cells lining the cavity started producing the conidia (Figs. 6, 7). Concurrently the primordial cells divide to increase the size of the conidioma which became almost spherical to sub-spherical. By the continued growth of the conidioma and the increased production of conidia inside, the shape of the conidioma became flattened during the later stages of the development.

During further development the cells of the wall became thick and pigmented, whereas the conidiogenous cells remained thin walled and hyaline. The temporary conidiogenous cells resemble the cells comprising the wall of the conidioma (Figs. 2,3,9). Later on, the initially formed conidiogenous cells were replaced by permanent conidiogenous cells which were easily differentiated by their shape and size (Figs. 4,5). The later formed conidiogenous cells are cylindrical and elongated. One or two annellations were also visible in some sections (Fig. 5). As the conidioma develop, changes occur in the wall layers also. The wall layers overlying the conidiogenous cells became disorganized and began to disintegrate forming an opening for the release of the conidia (Fig. 3) whereas the wall layers from which the conidiogenous cells arise, became flattened and compressed. Some of the sections showed the presence of stromatic conidiomata where the individual locule produces the conidia inside the cavity and several such locules were seen in the section.

Electron microscopic studies - The electron micrograph of a longitudinal section through a conidium of *P. adusta* shows the presence of an electron dense outer layer and an electron transparent inner layer in all the five cells (Fig. 8). But in the apical and basal hyaline cells due to the drastic reduction in the thickness of the cell wall layers, the apical and basal cells are hyaline when viewed through a light microscope (Figs. 10, 11).

The basal appendage consists of only the inner electron transparent layer but it retains its protoplasmic continuity with the basal cell of the conidium (Fig. 11). The conidium becomes detached by rupturing at the apex of the conidiogenous cell where it is attached to the basal cell of the conidium. In doing so, a small amount of the conidiogenous cell is carried away still attached to the basal cell which is seen as a frill (Fig. 11).

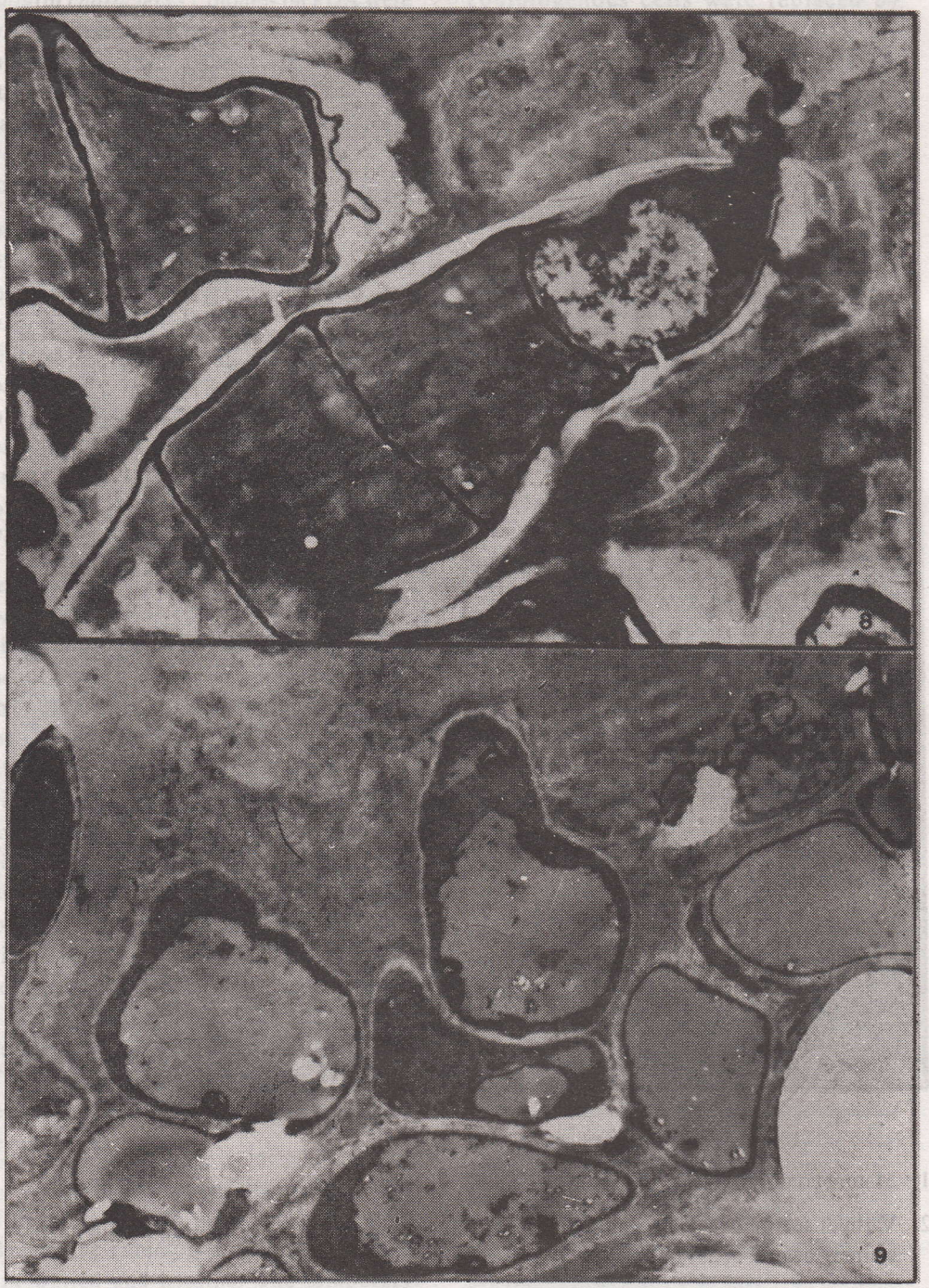
During maturation of the conidium, the cytoplasm of the apical cell undergoes a process of cytolysis. After cytolysis, the inner hyaline layer remained intact in the apical cell but later it is reabsorbed and the apical cell consists of a single layer, particularly the



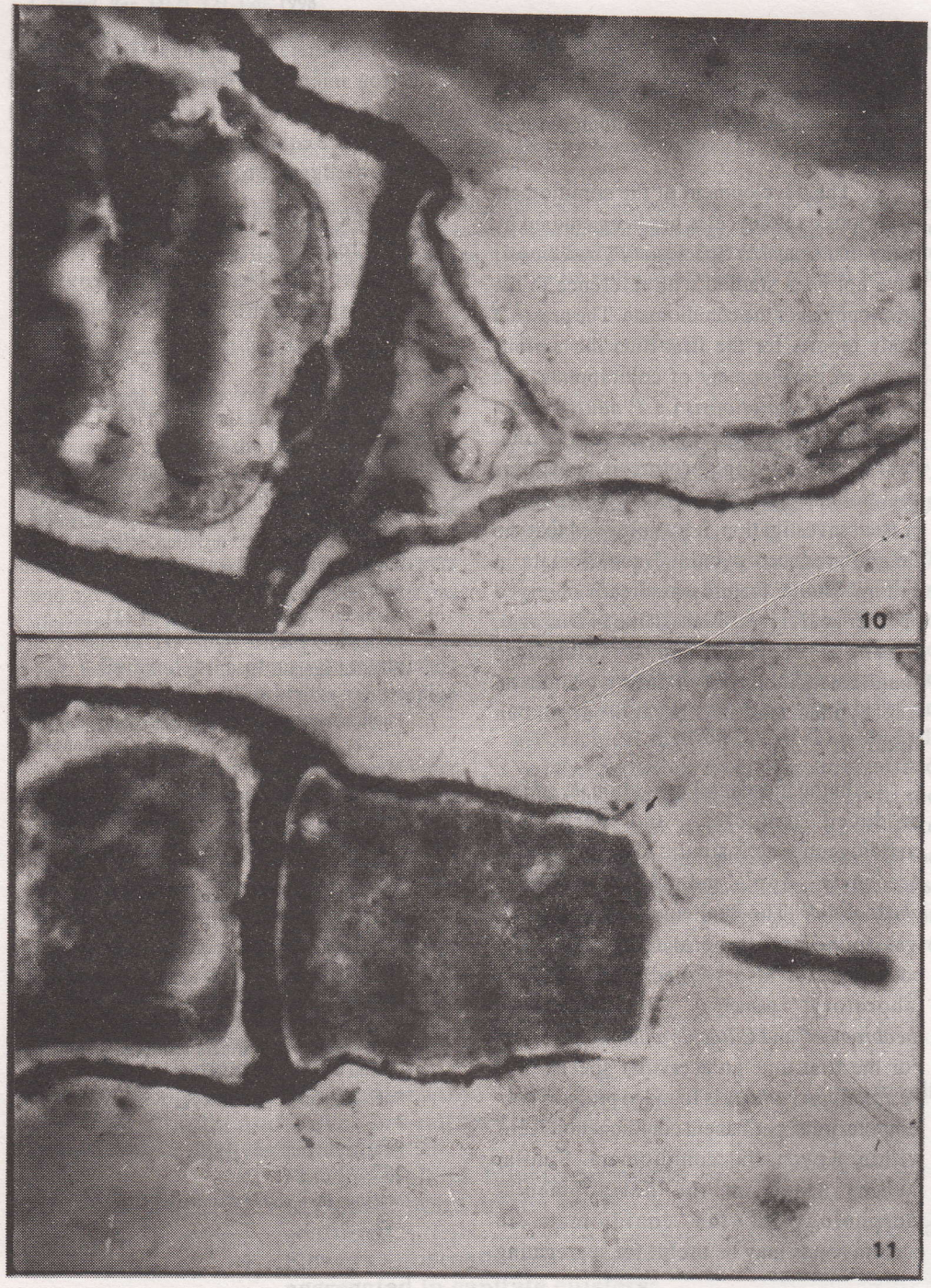
1. Mature conidia X128
2. Vartical sections of young conidiomata X320 (arrow shows the primordium with deeply staining central cells)
3. Vertical section of young conidioma showing the ostiole X320

4,5. Vertical sections of mature conidiomata X678

6,7. Vertical sections of young conidiomata X640



8. TEM Longitudinal section through released conidia X13500
9. TEM section showing the young conidiogenous cells lining the conidiomatal cavity X13500



10. TEM Longitudinal section of a conidium showing the apical cell with the appendage and the uppermost coloured cell X25000

11. TEM Longitudinal section of a conidium showing the basal cell with the appendage and the lowermost coloured cell X25000 (Arrow shows the frill in the basal cell)

melanized outer layer.

The basal cell is hyaline under the light microscope and this is due to much reduced wall layers.

The development of the conidiomata in various coelomycetes has been studied by many in the past[3-13]. Species of *Pestalotiopsis* have not been studied with reference to the development of the conidiomata. The presenct study reports for the first time the various stages of development of conidiomata in a species of *Pestalotiopsis* i.e. *P. adusta* which recorded interesting observations. *Pestalotiopsis* spp. produced acervular conidiomata in natural habitat whereas in the present investigation, it was obseved that the fungus produced pycnidial conidiomata in culture. Similar feature was already observed in other coelomycetes also, like in *Discosia*, a fungus characterised by dimidiate conidiomata in nature producing conidia on free hyphae and also on sporodochia in culture[14].

In the present investigation, *P. adusta* produced temporary and permanent conidiogenous cells similar to those found in *Ascochyta* spp[7]. and *Macrophomina phaseolina*[15]. The dual conidiation process, was found as a general feature in other coelomycetes also studied in the authors laboratory, namely *Botryodiplodia theobromae*[16] and *Ciliochlorella mangiferae*[10]. For the first time an acervular species like *Pestalotiopsis adusta* is found to produce both temporary and permanent conidiogenous cells within a pycnidial conidiomata. Similar studies based on the developmental morphology of the conidiomata in coelomycetes may be useful for segregating them into different groups. Transmission electron microsopic studies have provided useful information on the nature of the wall layers of the conidium and the appendages.

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References

1. Guba E F 1961, *Monograph of Monochaetia and Pestalotia*. Harvard University Press, Cambridge, Mass.
2. Reynolds E S 1963, *J. Cell. Biol.* **17** 208
3. Bary A de 1866, *Morphologie und Physiologie der Pilze Flechten, und myxomyceten, Leipzig*. Eds 2, 1884; English Translation. *Comparative morphology and biology of the fungi; Mycetozoan and Bacteria*, 1887, Oxford.
4. Kempton F E 1919, *Bot. Gaz.* **68** 233
5. Dodge B O 1923, *J. Agric. Res.* **23** 743
6. Punithalingam E 1966, *Trans. Br. Mycol. Soc.* **49** 19
7. Punithalingam E 1979, *Graminicolous Ascochyta species. Mycol. Pap*. No. 142 : Commonwealth Mycological Institute, Kew.
8. Maiello J M and Peterson J L 1976, *Mycologia* **68** 1121
9. Muthumary J and Vanaja R 1986, *Trans. Br. Mycol. Soc.* **87** 109
10. Masilamani S and Muthumary J 1994, *Mycol. Res.* **98** 857
11. Masilamani S and Muthumary J 1995, *Mycol. Res.* **99** 693
12. Muthumary J, Jayachandra J A and Bhagavathy Preetha M 1993, *Mycotaxon* **XLVII** 147
13. Roux C and Van Warmelo K T 1990, *Mycol. Res.* **94** 109
14. Subramanian C V and Reddy KRC 1974, *Kavaka* **2** 57
15. Punithalingam E 1982, *Nova Hedwigia* **36** 249
16. Masilamani S and Muthumary J 1996, *Mycol. Res.* **100** 1383