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Pycnidia and sclerotia of *Macrophomina phaseolina* (Tassi) Goid on jute and identity of the sclerotial isolates

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Morphology and development of sclerotia and pycnidia of *Macrophomina phaseolina* (Tassi) Goid described and application of the names *Rhizoctonia bataticola* (Taub) Butler and *Sclerotium bataticola* Taub. to different sclerotia-forming strains discussed. One strain is linked with an ascomycete.

Key words : Sclerotia, Pycnidia, Clamp connection, *Rhizoctonia*, Sclerotium, *Macrophomina*, Identity.

Jute both *Corchorus capsularies* L. and *C. olitorius* L. suffer from seedling blight, stem rot and root rot (Sawada. 1916 ; Shaw 1924 ; Varadarajan and Patel 1943). Ghosh (1961) stated that different isolates from infected jute did not always produce pycnidia (on jute) but universally produced sclerotia or sclerotia like agglomeration of cells.

MATERIALS AND METHODS

Small bits of root, stem or tuber of infected jute, roselle, cotton and potato were surface sterilized with 0.01 per cent mercuric chloride, washed with distilled water and were placed on sterilized PDA (Potato dextrose agar) and incubated at 37°C

Hirsute or downy hyphal growth started in all plates within 24 hrs. and sclerotia appeared within 72 hrs.

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The typical cultural characters are described below as observed with the isolate obtained from pycnospores developed on *C. olitorius* L.

Cultural Characters

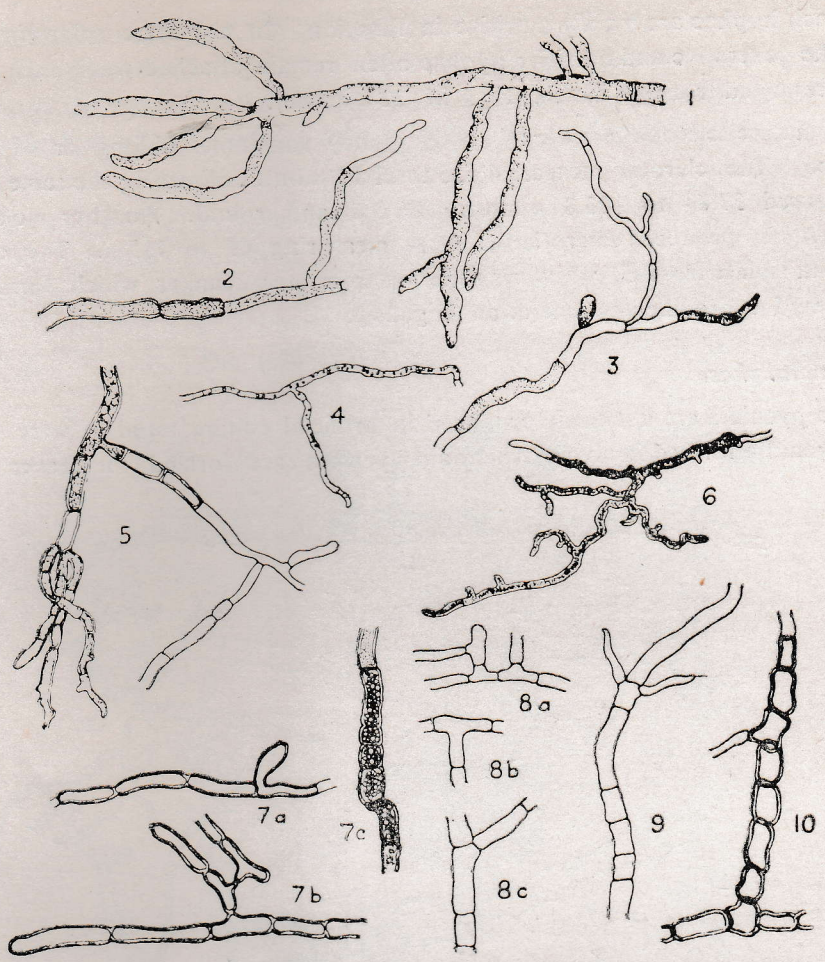
After 36 to 48 hrs. pale pinkish grey or greyish white hyphae grow on the bits of the host tissue and on the medium ; most of the dishes (7.5 cm diam.) are covered within 96 hrs. and rarely after 120 hrs. The colour of mycelia changes, after covering the plate to mostly mouse grey, some greenish grey and rarely black. In all cases abundant small black sclerotia develop on the surface and immediately below the surface of the medium. In some, mycelia roll over the glass. Generally, the hyphal growth from dead tissues of a jute root on PDA starts as greyish white or pinkish grey cottony mass, later spreading radially and filling the plate. The colour of the mycelia is influenced by the sclerotia which are greenish grey to start with but are finally black.

Mycelia in PDA consist of hyphae of different dimensions, thickness of wall, colour and cell content which are correlated with function.

Functionally the hyphae may be classified as nutritive, vegetative and generative types. The nutritive hyphae are mostly submerged, narrow 1.8 to 3.2 μ, thin walled and hyaline. They arise as branches from vegetative hyphae, which creep along the surface of the medium and send both submerged and aerial branches.

The young advancing vegetative hyphae are full of granular contents towards the base and somewhat hyaline towards the apex full of protoplasm (Fig. 1). The branching at the growing region is different from the typical description for the genus *Rhizoctonia* ; branches, more than one, may arise from the advancing hyphae almost at the tip (Figs. 1-5). Before the first septum is formed 8 or more branches and 3 to 4 secondary branches may arise from the advancing main hypha : the first septum is often formed at 1 to 1.6 mm away from the tip. The tip of the growing hypha and its branches are rounded and never acute, but the branch initials may arise as small conical outgrowth (buds) which subsequently have rounded tips (Figs. 1,3).

The older vegetative hypha is characteristic in having branches at right angles with a slight constriction at the base, with a septum near the point of branching (Figs. 7b, 8a-c & 9). Mature hyphae 3.4 to 4.8 μ broad, are greenish grey to brownish grey. Old hyphae turn deep brown to bright amber brown with snuff brown walls sometimes with a pale violet tinge (Fig 6). Cell contents are often in form of globules or are absent. A tendency to form chlamydospores is seen (Figs. 24 a to h).



Figs. 1-10. Characteristics of vegetative hyphae of *M. phaseolina* (for explanation see text)

Formation of Sclerotia

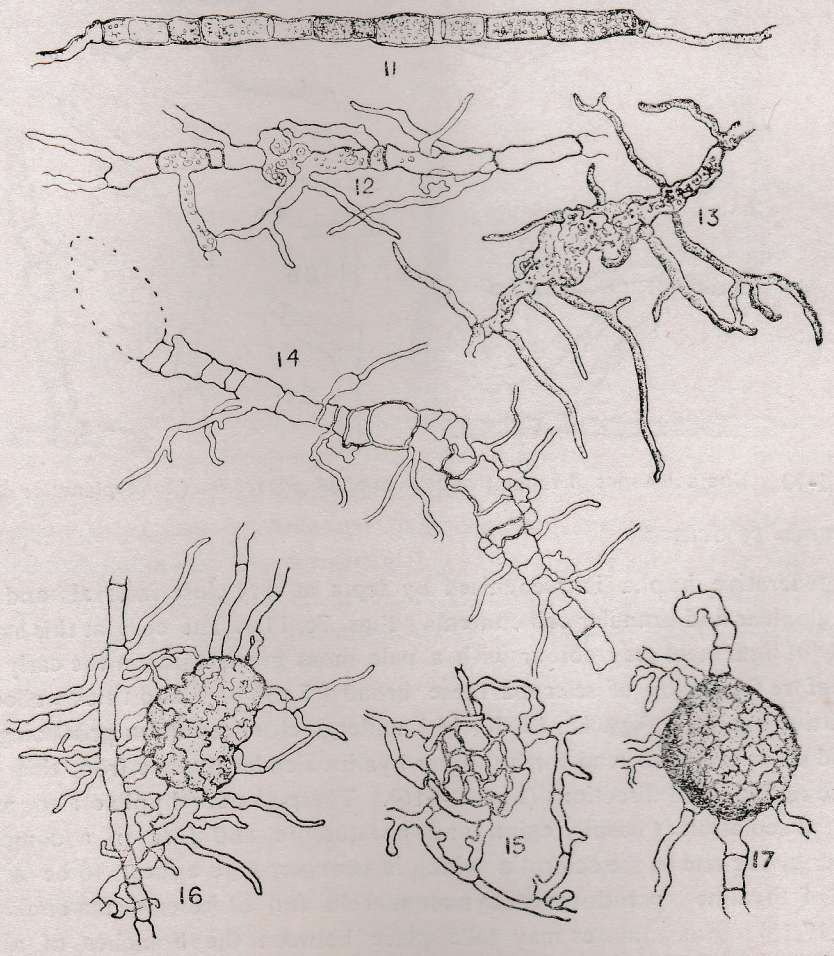
The generative hypha is recognised by septa at very close intervals and by the large globular and granular cell contents (Figs. 7c, 11). The cells at this stage are usually of light steel grey colour with a pale moss green shine in the early stages. Just before formation of sclerotia these broad (7 to 9 μ.) and thick walled stout generative hyphae (Figs. 11, 12, 13, 14) undergo close septation producing barrel shaped short cells and send forth thinner hyaline slender hyphae from this closely septate region in all directions (Figs. 14-16). These slender hyphae also undergo repeated septation at basal region and anastomose and fuse in a complicated manner giving rise to the sclerotia which in younger stage appear to be a round mass of hyaline pseudoparenchymatous cells full of reserve materials (Figs. 15,16,17,18). Anastomoses may take place between the branches of a single generative hypha (Fig. 16) or between two separate hyphae (Fig. 20). The slender

branch hyphae are mainly nutritive in function. In a mature sclorotia portions of the generative and nutritive hyphae often remain attached when such sclerotia are removed from the matrix (Figs. 21, 22 a and b).

The mature sclerotia are dark (mostly round) sometimes oblong or of irregular shape. The sclerotia measure 46 35 to 81.13 μ on *C. olitorius* ; the same on PDA measured 37.84 to 123.8 μ mostly 86 0 μ and round. Another isolate from *C. olitorius* produced sclerotia on PDA measuring 69 to 115 μ. The exception was an isolate from *C. capsularis* suffering from stem canker which formed only chlamydospore like agglomeration (Fig. 24j).

Pycnidial phase

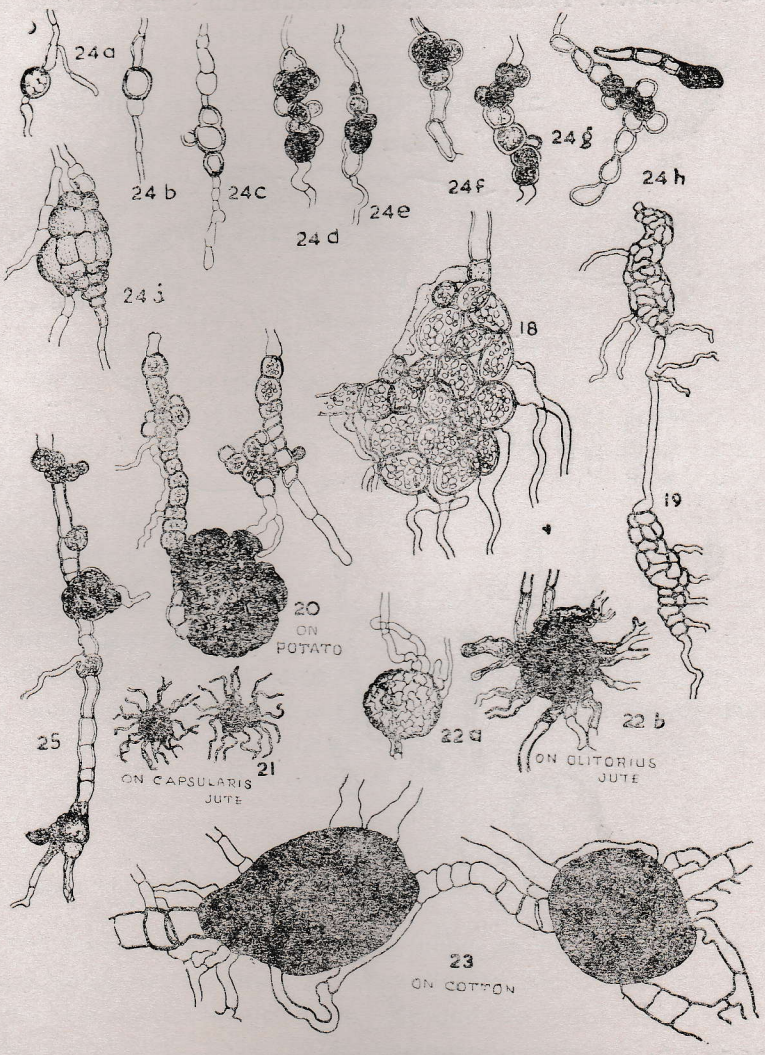
Since pycnidia are not easily produced in artificial culture, studies were confined to pycnidia formed in vivo. Abundant pycnidia are formed on leaves and on



Figs. 11-17. Different stages in formation of sclerotium of *M. phaseolina* (for explanation see text)

stems of both *capsularis* and *olitorius* plants infected with stem rot or root rot. Bits of leaf-lamina bearing pycnidia, after surface sterilization, on transfer to PDA yielded the typical sclerotial phase as described earlier, Pycnospores in sterile distilled water on transfer to articial media also produced the same sclerotial phase.

The pycnidia from *C. olitorius* in transmitted light, are spherical, light brown to snuff brown, ostiolate - the ostiole having a dark rim ; the wall of the pycnidium is pseudoparenchymatous without any stroma (Fig. 26). Pycnidia range from 98.5×98 5 μ to 151.5×121.2 μ in leaf aud 151.5×151.1 μ to 181.8×166 6 μ on stems of *C. olitorius*. The wall is 10-16 μ thick. The wall of the pycnidium



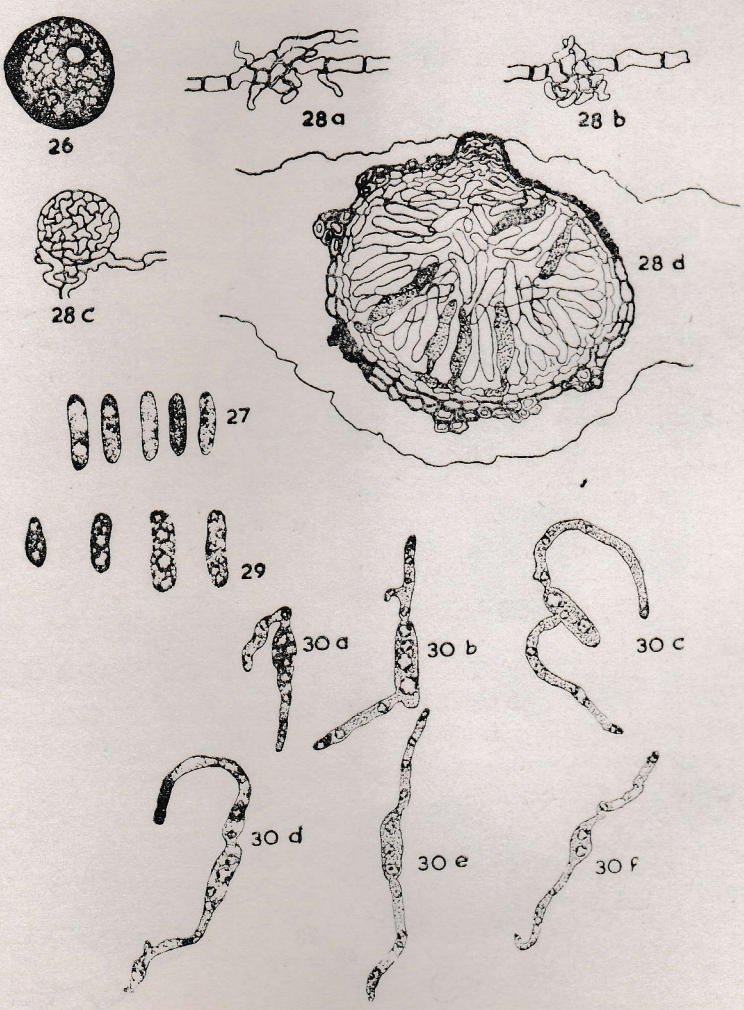
Figs. 18-25. Dif erent stages in formation of sclerotium and chlamydospore of *M. phaseolina* (for explanation see text)

consists of one layer of dark pseudoparenchymatous cells, and one layer of hyaline cells. Next to these layers is the sporogenous layer (Fig. 28d).

The pycnospores are hyaline, cylindro-elliptical, thin walled, 13 μ to 29 μ $\times$5.0 to 9.5 μ (Fig. 27).

Development of Pycnidia

Abundant pycnidia are produced on leaves of jute particularly of *C. capsularis* var. JRC 412, from May to August on 22°N latitude Diseased leaves with pycnidia in various stages of development were fixed in Bouin's fluid ; at the time of fixation 0.2 g of urea along with 0.15 g chromic acid was used ; the fixation time was 50 minutes. Paraffin blocks were made from some of these and stained



Figs. 26-30. Stages of development of pycnidium and germination of pycnospore of *M. phaseolina* (for explanation see text)

sections were prepared. Some fixed leaves were kept immersed in lacto-phenol ; bits of this leaf tissue were teased in laetophenol cotton blue. It was found that in an infected leaf, hyphae ramify in all directions. These hyphae are hyaline and full of protoplasm with many shiny spherical bodies of various sizes. A hypha undergoes repeated septation resulting in almost isodiametric cells recalling those during formation of sclerotia (Figs. 28 a-b). Later the cells send out branches which twist on the central cells ultimately producing a small sphere of pseudoparenchymatous cells. Till this period the entire sphere remains sunken in the lysigenous cavity of the leaf tissues.

Two or more contiguous hyphae may take part in formation of the sphere This type of development has been termed compound meristogenous type by Kempton (1919). It is not clear, if simple meristogenous development i.e. formation of pycnidium from a single hyphal strand is the usual mode of development of pycnidia of *M. phaseolina.* When the sphere attains about two thirds of the normal size all traces of stroma is lost. At this phase there is no differentiation between outer layer of cells. Although it is very difficult to say anything definitely about the formation of the ostiole, it probably is formed much later by breakdown of two or three cells in each of the outer layers A section through an immature pycnidium of half of the normal size show that the innermost layer of cells are slightly more elongated than the outer cells. These elongated cells later develop into very short conidiophore. In a mature pycnidium the outer layers of cells become dark brown to almost black and angular (Fig. 28d). The inner wall consists of usually 2 layers of hyaline cells. Next to this is the fertile layer of short conidiphores and the conidia. Ostiole formation starts probably after the first spores are cut off.

The fertile layer takes violet blue stain with haematoxylin and the nuclei turn violet. Conidiophore abstricts spores singly. A pycnospore may contain 2-4 nuclei of different sizes. These nuclei are of disperse type. During abstriction of spores the conidiophore shows 2-6 nuclei (Fig. 29). On germination these nuclei divide and half of them pass into the germ tube leaving in the spore case the rest of the nuclei which degenerate in course of 24 hrs (Figs. 30 a-d).

DISCUSSION

Sawada (1916) described a pycnidia-bearing fungus on jute in Formosa which he called *Macrophoma corchori* Saw. He showed that the fungus had a sclerotial phase similar to what was isolated from jute and described by Shaw (1912) as *Rhizoctonia solani* Kuhn. Shaw (1924) subsequently compared the fungus from jute in India with that isolated from jute by Sawada and confirmed that the two were the same.

Ashby (1927) had shown that *Macrophoma corchori* Saw, *Macrophomina phillippines* Petrak and *Macrophoma phaseoli Maubl.* were different names for the same fungus. He justified the separation by Petrak (1923) of the new genus *Macrophomina* from *Macrophoma* 'to include pycnidial forms devoid of stroma with long narrow thin walled elliptical spores which remain hyaline and continuous'. Since Ashby's publication (1927) the pycnidial stege has been accepted as *Macrophomina phaseoli* (Maubl) Ashby and the sclerotial phase as *Rhizoctonia bataticola* (Taub) Butler. Goidanich (1947) changed the name to *Macrophomina phaseolina* (Tassi) Goid.

Henson and Valleau (1927) opposed the use of the name *R. bataticila* (Taub) Butler on the ground that the genus *Rhizoctonia* has usually been connected with a basidiomycete ; but *R. bataticola* which never shows any real clamp connection and has a pycnidial phase, cannot be considered as such. Hence they are of opinion that *R. bataticola* (Taub) Butler should be known by its former name *Sclerotium bataticola* Taub. This view is supported by Reichert and Hellinger (1947) and needs serious consideration. They suggest that strains of *R. bataticola* (Taub) Butler which never produced pycnidia should not be described as *Macrophomina phaseoli* (Maubl) Ashby i.e. *M. phaseolina* (Tassi) Goid, but should be known by the name *Sclerotium bataticola* Taub.

Ghosh (1961) found some isolates of *R. bataticola* (Taub) Butler from potato, *Hibiscus sabdariffa* var *altissima* and cotton which failed to produce pycnidia on *Corchorus olitorius* L. but could infect jute causing various degrees of damage.

Sclerotial character	Pycnidia formed on jute or not	Pycnospore character	Identify
45 to 125 μ, black hard, contour smooth, with or without hyphal projection	Yes	hyaline, ellipsoid to ellipso-cylindrical 13-29 μ x 5.0-9.5 μ	*Macrophomina phaseolina* (Tassi) Goid.
45 to 125 μ, black smooth, with hyphal projection or only chlamydospore like agglomeration on a stout hyphal axis.	No	None	*Rhizoctonia bataticola* (Taub) Butler.
126 μ and above brownish black to black, irregular contour, not very hard, with stout hyphal projections.	No	None	*Sclerotium bataticola* Taub.

Ghosh *et al* (1964) described an ascigerous fungus *Orbilia obscura* sp. nov which was collected from the base of the stem of *C. capsularis* plants raised in the gamma garden with 20 Curie source at the Jute Agricultural Research Institute, Barrackpur. Monosporus culture raised from ascospores of the fungus developed a greenish grey mycelia on PDA with large brownish black sclerotia which are not very compact. They measured 160 to 275 μ; this sclerotial phase goes near *R. bataticola* (Taub) Butl.

To *Rhizoctonia batatcola* (Taub) Butler has been ascribed many fungi which may not be allied but produce carbonaceous sclerotia, greenish grey or dark grey mycelia and hyphae without clamp connection. It is an artificial assemblage. Therefore the following scheme of nomenclature somewhat on Haigh's (1930) line who recognised three groups A, B & C on the basis of differences in size of sclerotia, may prove convenient.

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