Indon. J. Plant. Prot. ISSN 0853-7376 Vol. I. No. 1 : 19 -27 , 1995

VARIOUS STAGES OF PINK FUNGUS (*UPASIA SALMONICOLOR*) IN JAVA

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ABSTRACT

Pink fungus in Java is classified as *Upasia salmonicolor* (Basidiomycetes; Corticiaceae) and its anamorph is *Necator decretus*. This fungus is a serious pathogen which attacks many woody plants. The pink fungus in Java exhibits five developmental stages on the surface of the host bark : I. An initial **cobweb stage**, as thin, white, cobweb-like hyphal layer, which creeps over the surface of the bark, during which penetration of the host occurs; II. **Pseudonodular stage**, as conical white pustules occurring only on lenticels or cracks, and only on shady side of branches; III. **Teleomorph**, occurs as **pink incrustation and pink pustules** on shady side of branches; IV. **Nodular stage**, as globose white pustules occurring chiefly on intact bark, but also on the lenticels or cracks, on exposed side of branches; V. **Anamorph**, as small orange-red sporodochium, on exposed side of branches.

Key words: Pink fungus, Corticiaceae, Basidiomycetes, *Necator*

INTISARI

Jamur upas di Jawa diklasifikasikan sebagai *Upasia salmonicolor* (Basidiomycetes; Corticiaceae) dan anamorfnya adalah *Necator decretus*. Jamur ini adalah patogen yang penting, yang menyerang banyak tumbuhan berkayu . Jamur upas di Jawa membentuk lima stadium pada permukaan kulit kayu : I. **Stadium rumah laba-laba**, berupa lapisan miselium tipis seperti sarang laba-laba di permukaan kulit kayu, sebagai stadium permulaan penetrasinya ke dalam jaringan inang; II. **Stadium bongkol semu**, berupa pustul putih berbentuk kerucut hanya terdapat di permukaan lentisel atau epidermis yang pecah, dan hanya di sisi cabang yang terlindung; III. **Teleomorf**, dapat berupa **kerak merah jambu** dan **pustul merah jambu**, terdapat pada sisi cabang yang terlindung; IV. **Stadium bongkol**, berupa pustul putih membulat terutama terdapat pada permukaan yang utuh, tetapi juga di permukaan lentisel atau celah epidermis, di sisi cabang yang terbuka; V. **Anamorf**, berupa sporodokium kecil berwarna merah jingga, di sisi cabang yang terbuka.

Kata kunci : Jamur upas, Corticiaceae, Basidiomycetes, *Necator*

INTRODUCTION

In most phytopathological literature the causal agent of tropical pink disease which attacks many woody plants in Java and other tropical countries has been classified as *Corticium* (Petch, 1911; Rant, 1912; Brooks and Sharples, 1914; Bally, 1929; Hilton, 1958; Mundkur, 1959; Cunningham, 1963; Verma and Munjal, 1979/1980); *Pellicularia* (Dastur, 1946); *Botryobasidium* (Venkatarayan, 1950); and *Phanerochaete* (Julich, 1975). Recently Burdsall (1985) excluded this species from *Phanerochaete* because of its large basidiospores and short-celled subhymenial elements, and made the combination *Erythricium salmonicolor* (Berk. & Br.) Burds. Most recently Tjokrosoedarmo (1992) excluded this species of pink fungus in Java from *Corticium* and proposed a new genus *Upasia* (Tjokr. & Rifai) because of its multinucleate hyphal cells and its inamyloid basidiospores, and made the combination *Upasia salmonicolor* (Berk. & Br.) Tjokr. with the characteristics of its five developmental stages which composed into sexual and asexual cycles, and its monilioid-chains of short-rectangular cells of subhymenial elements.

Most previous authors have concentrated only on the teleomorph, especially the morphology of its basidia, basidiospores, and hyphal system. This study reports an extended study of morphology and anatomy of all stages included the teleomorph and anamorph, using fresh specimens on living hosts, and not using specimens from herbarium.

MATERIALS AND METHODS

Materials studied were specimens of pink fungus on 23 species of living host plants in Java, Indonesia. They were *Acacia auriculiformis*

A. Cunn. ex Benth.; *Acalypha godseffiana* Mast.; *Acalypha wilkesiana* Muell. Arg.; *Artocarpus integra* Merr. (jackfruit); *Calliandra sancti-pauli* Hassk.; *Camellia sinensis* (L.) O.K. (tea); *Cinchona ledgeriana* (Howard) Moens (cinchona); *Cinchona succirubra* Pav. et Klotzsch (cinchona); *Citrus aurantifolia* (Christm. et Panz.) Swingle (lime); *Coffea arabica* L. (coffee); *Crotalaria striata* DC.; *Euphoria longan* (Lour.) Steud. (longan); *Gardenia augusta* Merr.; *Gnetum gnemon* L.; *Hakea saligna* Knight.; *Hevea brasiliensis* Muell. Arg. (rubber); *Lantana camara* L. (lantana); *Mangifera indica* L. (mango); *Nerium oleander* L.; *Pyrus malus* L. (apple); *Tecomaria capensis* (Thumb.) Spach; *Tephrosia candida* (Roxb.) DC.; *Theobroma cacao* L. (cocoa).

Specimens on four of these hosts, -*Pyrus malus*, *Cinchona ledgeriana*, *Coffea arabica*, and *Tephrosia candida* - were studied in more detail to elucidate the morphology and anatomy of all stages of pink fungus which in its life cycle develops all stages completely.

The specimens were collected from apple orchards in Batu (Malang) and Pagilaran (Pekalongan), cinchona plantations in Pagilaran (Pekalongan) and Bungamelur (Sukabumi), longan plantation in Pringsurat (Temanggung), and some gardens and fields in Yogyakarta, Indonesia.

The morphology of various stages were studied macroscopically on living hosts, photographed in the laboratory, and microscopically by sectioning specimens previously fixed in FAA II (formalin alcohol acetic acid II) and mounting sections in cotton blue lactophenol.

The formation of basidiospores were studied by examining pieces of fresh and actively growing teleomorph incrustation collected and fixed in FAA II directly each hour over a period of 24 hours. Freehand sections were prepared, mounted in cotton blue lactophenol and observed microscopically. Good slide preparations were drawn using camera lucida, and photographed.

RESULTS AND DISCUSSION

This study found five developmental stages of pink fungus in Java, which will be numbered for convenience : I. **Cobweb stage**; II.**Pseudonodular** stage; III. **Teleomorph** (basidiocarp, basidioma, *Corticium* stage); IV. **Nodular stage**; and V. **Anamorph** (conidioma, *Necator* stage) (Fig 1). Not only macroscopic, but also microscopic morphology and anatomy of the stages (Fig 2) are shown in this paper.

The previous authors found only four stages : I. Cobweb stage; II. Nodular stage; III. Corticium stage; IV. Necator stage (Brooks and Sharples, 1914; Butler, 1918; Mundkur, 1959; Semangun, 1988; Widjanarko and Bakri, 1969). They considered pseudonodular stage as nodular stage. This study found four differences between pseudonodular and nodular stages, those are :
1. occurrence; 2. development; 3. tissue cells; and 4. maturation (Table 1).

Table 1. The differences between pseudonodular stage and nodular stage

	Pseudonodular stage	Nodular stage
1. Occurrence	- On the surface of lenticels or cracks, on shaded bark (in sexual cycle)	- Chiefly on intact bark but also on lenticels or cracks, on exposed bark (in asexual cycle)
2. Development	- Develops from symphogenous aggregation of cobweb stage mycelia, is not affected by environmental factors	- Develops from compound meristogenous aggregation of cobweb stage mycelia, is affected by environmental factors (sunshine and rain water)
3. Tissue cells	- Rounded or irregular cells	- Flattened cells, as the results of cell fissions
4. Maturation	- At maturation develops into pink pustules (teleomorph pustules)	- At maturation develops into small orange-red sporodochial anamorph

I. **Cobweb stage** is a thin, white, cobweb-like or net-like hyphal layer, which creeps over the surface of the bark, both on shady and exposed side of a branch, it consists of 2-3 layers of hyphae, which anastomose freely, and develop perpendicular branches.

The cobweb stage is the early stage in disease development, since the penetration of the fungus into the living host tissue takes place at this stage. In the life cycle of pink fungus, cobweb stage initiates the other four stages. That is why cobweb stage is the most important stage for the disease development and the life cycle of this fungus.

II. **Pseudonodular stage** is recognized by conical or hemisphaerical white pustules, occurring only on lenticels or cracked epidermis, never on intact bark, on the lower or shady side of a branch.

The pustule tissue consists of irregular rounded cells, 8-20 x 6-15 µm. The surface cells are slightly flattened, and will later function as basal layer of the teleomorph pustules. At this time the pseudonodular pustules develop a pink colouration, and this stage develops into teleomorph pustules on the surface of those pseudonodular pustules.

The previous authors considered these pseudonodular pustules as nodular pustules. This study found the differences between both kind of pustules (Table 1).

III. **Teleomorph** (*Corticium* stage) is the basidiocarp (basidioma) of the fungus , occurs in two morphological variants, those are **pink incrustation** (teleomorph incrustation), an extensive resupinate colony of the fungus on the surface of the bark, and **pink pustules** (teleomorph pustules), occurring on the surface of pseudonodular pustules. Both variants occurring only on shady/lower side of branches or encircling a shaded stem.

The anatomy of the teleomorph showed that the hyphal system is monomitic, hyphae lacking clamp-connection, cystidia or gloeocystidia lacking. The teleomorph is composed of four layers: 1) **Basal layer**, a loose net-like or cobweb-like hyphal layer, actually it is mature cobweb stage mycelia which branching horizontally and vertically; 2) **Intermediate layer**, composed of loosely arranged perpendicular branches of basal layer, each giving rise to dichotomous or irregular branches : 5-24 x 3-6 µm; 3) **Subhymenial layer (subhymenium)**, composed of moniliform chains of short rectangular sterile cells, arising as the continuation branches of the intermediate layer with very short branches, the length of these branches is about the same as the width; each branch in this layer has capability of branching, so that this layer consists of abundant chains, compactly arranged, devergent below but with their apices always perpendicular to the basal layer; 4) **Hymenial layer (hymenium)**, composed of holobasidia without cystidia or gloeocystidia; the *holobasidia* are hyaline, subclavate to cylindric, 12-24 x 4,5-9 µm, and bear 2-4 hyaline sterigmata each bearing one basidiospore; the *sterigmata* are slender and conical, straight or slightly curved inward, 4,5-9 µm long; the *basidiospores* are hyaline, globose to ovoid, thin-walled, smooth, inamyloid, 6-7,5 x 4,6-6 µm.

Basidiospores are formed by actively growing teleomorph incrustation/pustules on living hosts more abundantly at night, with maximum basidiospore formation of pink fungus on cinchona, tephrosia, and apple, was recorded from about midnight to 05.00 (Table 2).

Table 2. Basidiospore formation on cinchona, tephrosia, and apple

Time	Basidiospore formation on		
	Cinchona	Tephrosia	Apple
01.00	+	+	+
02.00	+	+	+
03.00	+	+	+
04.00	++	++	++
05.00	+++	++	+++
06.00	++	++	+
07.00	-	-	-
08.00	--	-	-
09.00	---	--	-
10.00	--	--	-
11.00	--	---	---
12.00	-	-	--
13.00	-	-	-
14.00	--	-	-
15.00	---	-	-
16.00	--	--	--
17.00	-	-	-
18.00	-	-	+
19.00	-	-	-
20.00	+	+	-
21.00	-	-	+
22.00	-	-	+
23.00	+	+	+
24.00	++	++	++

Note : +++ many basidiospores
++ quite a few of basidiospores
+ few basidiospores
- basidia bearing sterigmata, without basiospore
-- basidia without sterigma and basidiospore
--- sterile cells only

The previous authors had different opinions about this stage. Zimmermann's figure about this stage, showed long-celled subhymenial layer (Rant, 1912), while Brooks and Sharples' figure showed the short-celled subhymenial layer (Brooks and Sharples, 1914). Burdsall (1985) found also the short-celled subhymenial element. Unfortunately there is no further information in those three references, such as what are the short or long cells in the subhymenial layer.

This study found that subhymenial layer composed of abundant moniliform chains of short-rectangular cells, as the continuation branches of the intermediate layer, compactly arranged, divergent below but their apices almost perpendicular to the basal layer, as described above (**Fig. 2C**).

Brooks and Sharples (1914) found difficulty to find basidiospores. This study recorded the basidiospore formation at night, with the maximum basidiospore formation at about 05.00. Basidiospore formation happens only at a particular time (Table 2).

IV. **Nodular stage** appears as globose white pustules on the exposed or upper side of a branch, occurring chiefly on intact bark, but also on lenticels or cracks. This stage is composed of compact flattened cells as the result of cell fission, young pustules are white and covered by a mantle of interwoven hyphae. Later this mantle breaks open since it is pushed by basipetal chains of holothallic conidia produced by conidiogenous cells -the surface cells of mature nodular pustule- and the colour changes to orange red after developing into the anamorph.

V. **Anamorph** (*Necator decretus*): This stage composed of small orange red sporodochial conidiomata (sporodochium) , occurring on the exposed side of a branch, chiefly on intact bark, but also on lenticels or cracks. The sporodochium composed of a **stroma** (the nodular tissue), **conidiogenous cells**, and **basipetal chains of holothallic conidia** (Fig. 2E). The conidia are individually hyaline, but orange red in mass, ovoid, rectangular or irregular in shape, relatively thick-walled, 9-26 x 8-12 µm.

The previous author, Zimmermann, who studied pink fungus in Java found anamorph composed of pycnidia which globular in shape, and later breaks opened after maturing.This study also found globular form of this stage (Fig 3A), but this is a horizontal section of sporodochium, not a pycnidium. This form was found from vertical section of sporodochium in horizontal position (Fig 3Bb). The vertical section showed a sporodochium (Fig 2E,4A). The scanning electron micrograph also showed a sporodochium (Fig 4B).

The result of this study showed that pink fungus in Java exhibits five developmental stage : I. Cobweb stage, as thin hyphal layer on the surface of the host bark; II. Pseudonodular stage, as white pustules on lenticels or cracked epidermis; III. Teleomorph, as pink incrustation and pink pustules; IV. Nodular stage, as white pustules chiefly on intact bark; and V. Anamorph, as small orang-red sporodochia.

It is concluded that cobweb stage (I) is the weakest form of those five stages, and it is supposed as the easiest stage to be destroyed. That is why for controlling of the pink disease, one must concern with the cobweb stage.

I wish to thank Prof. Bryce Kendrick, and Prof. Dr. Ir. Haryono Semangun, for their comments and reviewing the manuscript.

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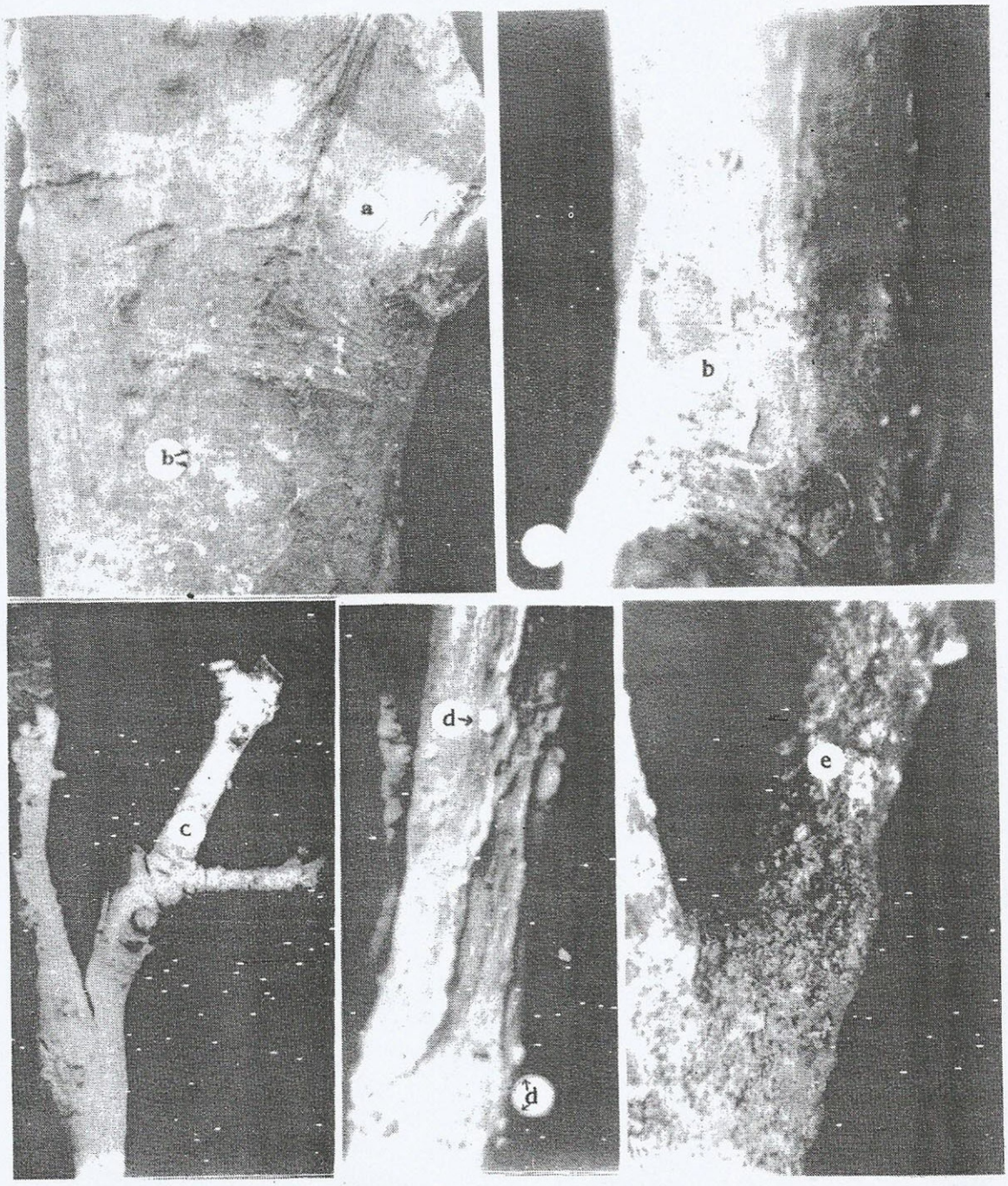

Figure 1. The five tages of Upasia salmonicolor on apple. a. Cobweb stage; b. Pseudonodular stage; c. Telemorph; d. Nodular stage; e. Anamorph (sporodochia).

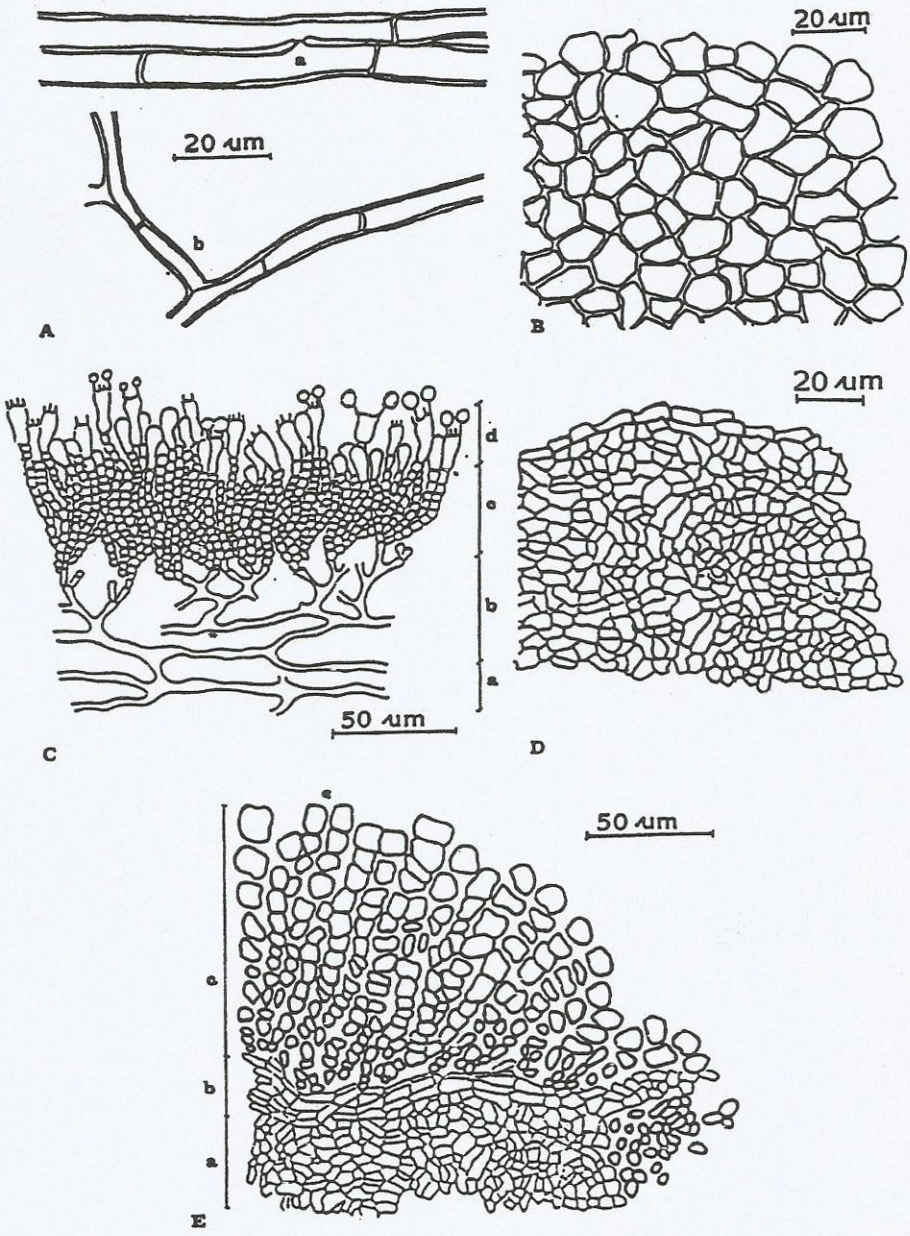

Figure 2. Microscopic morphology and anatomy of *Upasia salmonicolor* stages. A. **Cobweb stage** mycelia: anastomosis (a), perpendicular branch (b); B.**Pseudonodular stage** tissue cells; C. **Teleomorph** anatomy: basal layer (a), intermediate layer (b), subhymenial layer (c), and hymenial layer (d); D. **Nodular stage** tissue cells; E. **Anamorph** anatomy: stroma (a), conidiogenous cells (b), and basipetal chains of conidia (c)

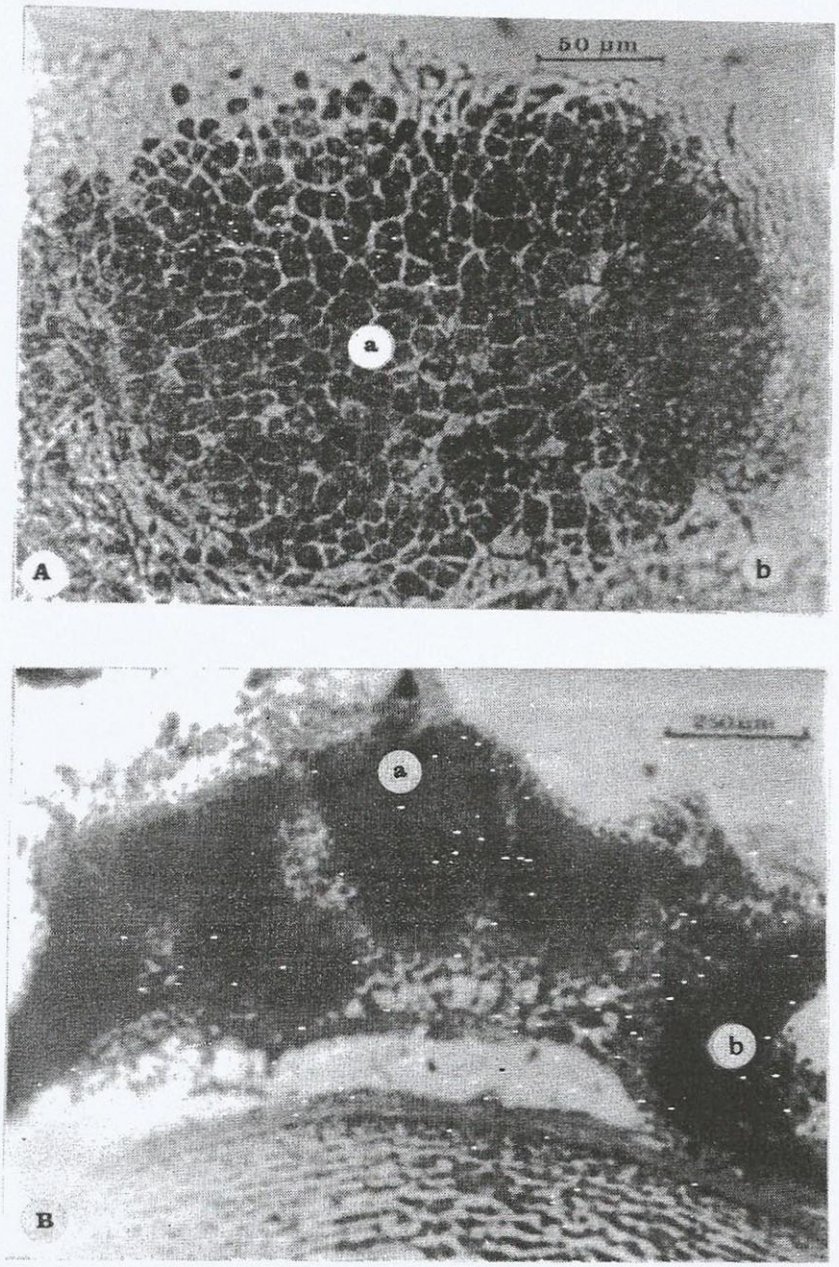

Figure 3. Sporodochial anamorph A. Horizontal section, globose in shape: a. conidia; b. stroma
B. Compound sporodochium: a. Vertical position; b. Horizontal position

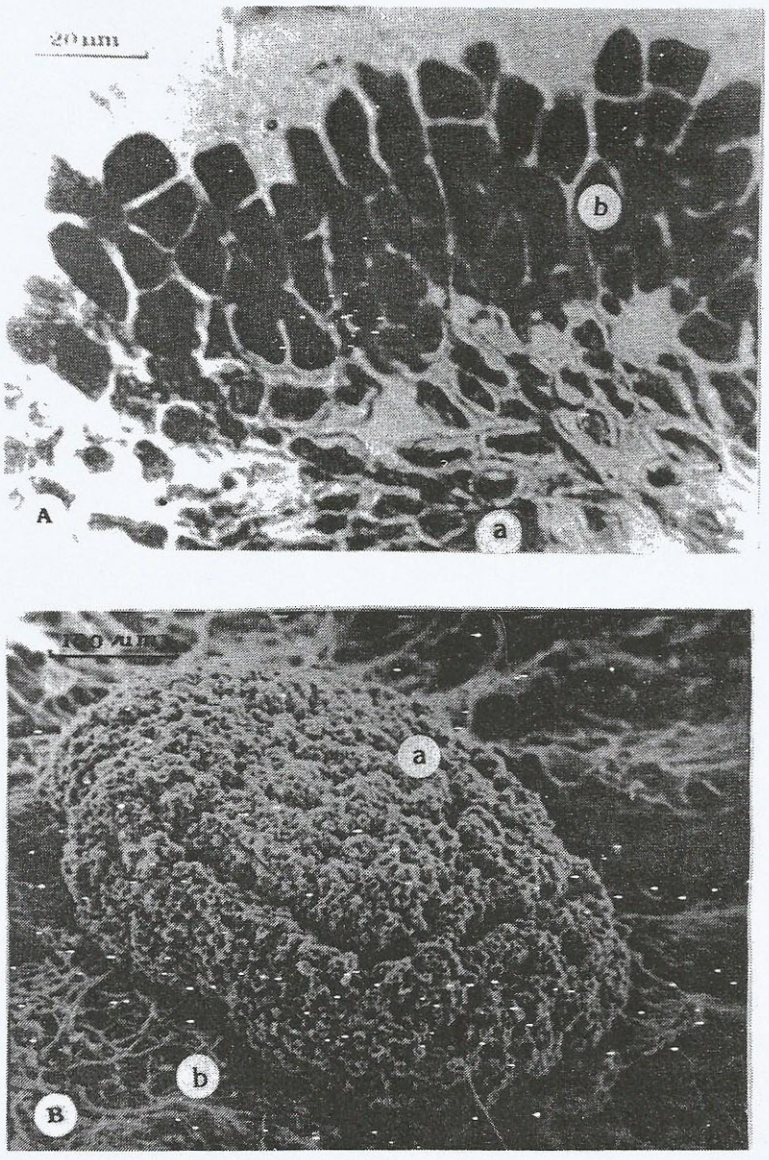

Figure 4. Sporodochial anamorph A. Vertical section of sporodochium: a. Stroma; b. Konidium
B. Scanning electron micrograph of sporodochium: a. Cobweb stage mycelia:
b. Mass of conidial chains.