A STUDY ON A CAPROPHILOUS FUNGUS BELONGING TO THE GENUS *Isaria* FROM TAIWAN[1]

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Abstract: *Isaria* sp., a caprophilous fungus, has been intensively studied and found to have produced at least 4 kinds of conidiospores during the course of its development. Macroconidiospores are thick-walled, and often found in a binucleate stage; these are oval and borne in a terminal cluster on short curved conidiophores, covering almost the entire surface of the synnema. The thin-walled microconidiospores are of three kinds, the spherical, and the rod shaped, which are usually irregularly arranged in loose clusters, and the oidia, the free hyphal cells breaking up from hyphal tips.

Both macro- and microconidiospores may multiply by mitosis—this is more frequently noted in the macroconidiospores—or by budding.

Isaria (*Spicaria*) species are all entomogenous, parasitic on insects but the present fungus is saprophytic on caprophilous habitat.

The main characteristics of the present fungus consists of its white synnema; and hyaline, one-celled ovoid spores, which cover most of the surface of the synnema. These characteristics closely resemble *Isaria cretacea* (Barnett, 1960), but it is parasitic on insects rather than being saprophytic on dung. This species has not been previously reported in Taiwan.

INTRODUCTION

This uncommon fungus was isolated from some old dog-dung in 1966. It consisted of a branched synnemata of various lengths, growing erect on the substratum (Pl. I, 4). In the spring of 1969, another collection was found on fresh horse dung, arising after a nice crop of *Pilobolus* which was then being studied. It differed from the 1966 collection by having single columns instead of branched ones (Pl. I, 1). They revived exceedingly well when transplanted into petri-dishes on moistened filter paper and sterilized rabbit dung pellets. Thus the culture could be maintained in a good living condition, and was available for research for an indefinite period of time. However, there were always some contaminants such as, *Mucor* or *Thamnidium* associated with the organism (Pl. II. 8). It was not until 1970 that pure cultures of this fungus were successfully developed on synthetic media in vitro. Intensive studies revealed that the asexual reproduction of this fungus consisted of some 4 types of conidiospores, each of which developed into septate hyphae. Whether or not all these hyphae gathered to form synnema, is not yet clear.

Pure cultures of this fungus were then sent to several prominent mycologists for examination. Grateful thanks are due to Dr. G. W. Martin who has kindly studied my culture and determined the systematic position of this fungus as belonging to the genus *Isaria* (Martin, 1971). Since then my investigation has centered around all available information concerning *Isaria* to learn what previous studies by many

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experienced mycologists have yielded (Petch, 1924) but their final comment ends with the words: "it is still in a confused state" (Barron & Smith, 1959).

The present report consists of the results of my observations on *Isaria* which may be useful to others who are interested in this fungus.

MATERIAL AND METHOD

1. Materials used for this study were mainly based on the cultures isolated from the horse dung collected in 1969. Occasionally, our stock culture grown on rabbit pellet extract was used. In addition, fresh materials from repeated experiments were used for comparisons.
2. In order to learn the mode of growth and the methods of reproduction of the present fungus, the following techniques were applied: (a) spore germination in distilled water, (b) spore suspension poured over weak agar medium for germination, (c) slide cultures, (d) single spore isolation, and (e) the use of hanging drop cultures.
3. In order to maintain living material for detailed study, a portion of the collection was transplanted into sterile petri-dishes on moistened filter paper to which one or two sterilized rabbit pellets were added. New cultures on rabbit dung pellet were readily obtained in a couple of days.
4. The medium most frequently used for cultivation was weak agar medium (maltose 3 gr., peptone 1 gr., agar 20 gr., and distilled water, 1000 mls.)
5. All the illustrations were made by PM-7 with an Olympus microscope FHF-TR-III

OBSERVATION AND RESULTS

1. Asexual reproduction.

(1) Conidiospores. *Isaria* sp. reproduces asexually by means of conidiospores. Several types of conidiospores were observed. The macroconidiospores came from whorls and clusters in the primary mycelia (Pl. III, 4, 5). The microconidiospores were observed of three forms, the rod shaped, the spherical, and the oidia. The rods (Pl. II, 3) and sphericals (Pl. II, 2) are usually irregularly arranged in loose clusters, and the oidia (Pl. III, 1) are the free hyphal cells breaking up from tips of somatic hyphae. These are shown in Pl. III, 3, 5; Pl. VI, 2; and Pl. IV, 4.

The conidiophores bearing the oval macroconidiospores were short and curved, each produced 3–4 thick-walled macroconidiospores (Pl. III, 4, 5). These spores were obtained by suspending the synnema in distilled water and then the suspension was poured into weak agar plates. An examination of this thin white coating accumulated on the plate about 20 hrs. later, revealed numerous macroconidiospores (Pl. III, 4, 5). Some were still attached to the tips of conidiophores. These spores sometimes increased in number by mitosis or by budding as seen in Pl. I, 6; Pl. II, 1, 4.

(2) Germination. Upon germination of the above spores, profuse mycelia were produced. They soon became prostrate and constituted the thicker white coating on the agar surface (Pl. IV, 6).

(3) The initiation of synnemata. At first, minute white knots of pin-point sizes gradually appeared on the white coating (Pl. IV, 6). These extended upwardly to form the synnemata.

In the second stage, mycelia gathered closer and became erect about 12 days after the sowing of the spores (Pl. V, 5). About 16 days after sowing there was the initiation of a central part, the medulla (Pl. II, 6). In the meantime, there developed profuse short hyphae all around the upright columns and covered the most of the surface of the synnema.

In the third stage, short and somewhat reflected conidiophores, developed, each gave rise to 4 or more oval thick-walled macroconidiospores (Pl. III, 4, 5). This I believe, is the final and mature stage in the development of a synnema. This is also the most important step showing the type of conidiophores and the mode of their growth in relation to the conidiospores, thus exhibiting the distinguishing characteristic of the genus (Barron, 1968). It is also a very difficult process and requires a painstaking technique to obtain the conidiospores and conidiophores properly mounted on a slide without disturbing the intact spores. In this final and mature stage of development of the synnema the hyphal layer of the conidiospores extend as far as its apex. The conidiospores were borne most abundantly on the middle portion of the columm with the apex remaining bare (Pl. II, 8) and the basal part also becoming a bare stalk (Pl. II, 7). It took about 10 days at a suitable temperature of 17–20 C, to complete the cycle.

(4) The marked effect of light on the growth and development of synnemata of *Isaria* sp. was also observed in this study (Pl. IV, 1–6). The early spore germination and their development into mycelia was not effected by light, and it was found that there was no difference between the cultures grown in dark and light, but the growth of synnemata seems to have been strongly influenced by light.

DISCUSSION AND CONCLUSION

1. The present fungus, a species of *Isaria* (Martin, 1971), has been studied by many experienced mycologists and considered as a difficult fungus to assign to a right taxonomic position (Barron, 1968).
2. The present study has revealed that the asexual reproduction of *Isaria* sp. found in Taiwan involves not only the formation of macroconidiospores, but also microconidiospores in three different forms, i. e. rod shaped, spherical, and oidia. The main attributes of the genus *Isaria* consist of white synnema and hyaline, one-celled spores that cover most of the surface of the synnema.
3. Most of the species of *Isaria* are entomogenous but the present specimen was found as caprophilous. Species growing on plant tissues have been reported but no record of them being caprophilous is known. This species grows well on both rabbit pellets and horse dung.
4. In comparing the figures of *I. cretacea* given by Barnett (1960) with the present species, it is evident that ours resembles *I. cretacea*, especially its short curved conidiophores with their terminal conidiospores. Also that the synnema in culture is found turning towards the light if receiving one-sided illumination, but it differs in that *I. cretacea* is entomogenous and ours is caprophilous.

   Because of the two evidences given above, namely, the type of conidiophores and conidiospores and the response of synnema to the effect of light, it is apparent that the present species may well be *Isaria cretacea*, and if so, this is the first record of *I. cretacea* from Taiwan.
5. About 10 days are required to complete its life cycle with the production of

conidiospores. Sexual reproduction is not known to occur in this fungus.

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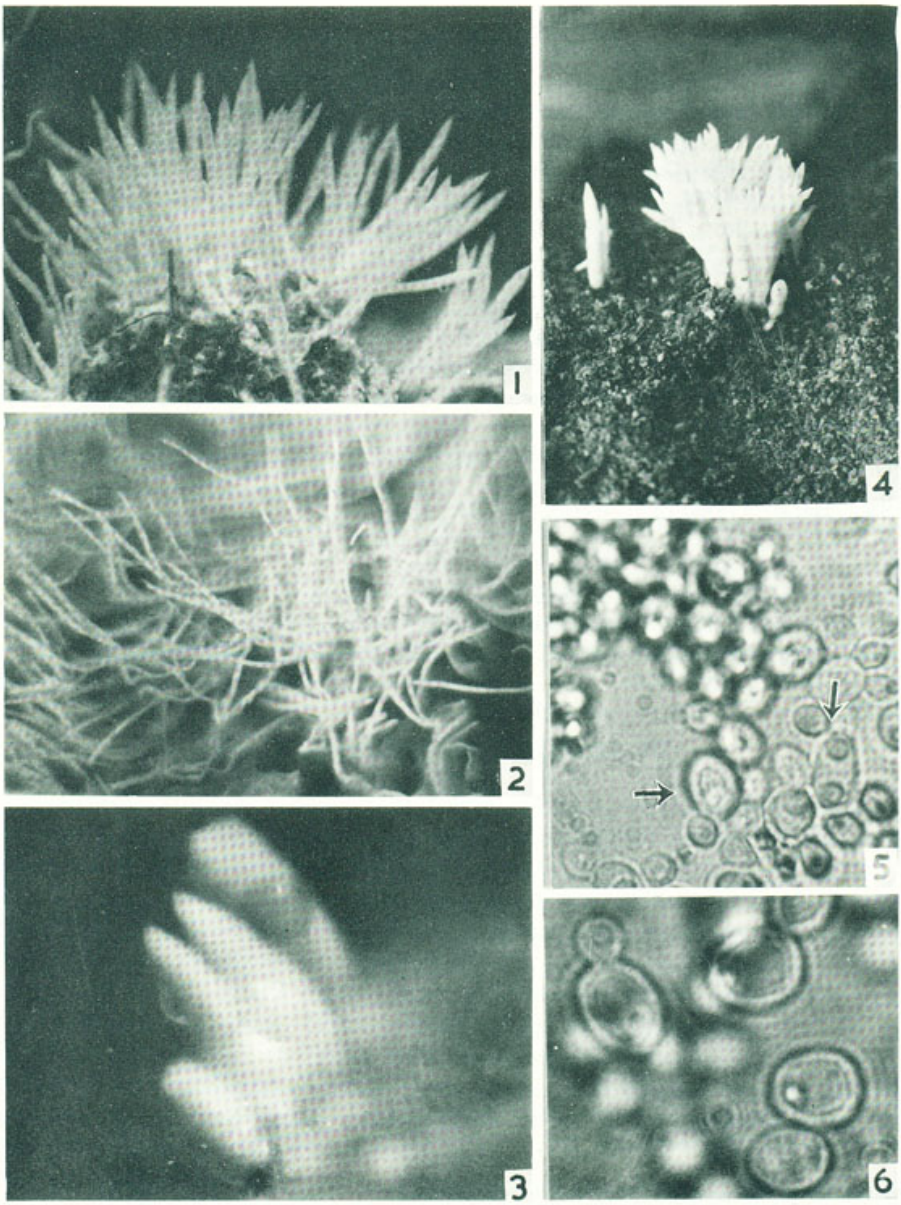
Plate II Explanations, see page 187.

1. Macroconidiospores and germination in suspension. × 600
2. Spherical conidiospores and mycelium 22 hrs. on slide culture. × 400
3. Rod-shaped conidiospores and mycelium 22 hrs. on slide culture. × 400
4. Germination of spherical conidiospores. × 600
5. Showing initiation of erect synnema, 12 days after spore suspension poured on weak agar medium. × 100
6. Showing apical portion of young synnema and the formation of central medulla. × 40
7. Culture grown in vitro showing the bare basal portion of synnemata and spores all borne on the upper portion of the synnemata. × 4
8. Synnemata with profuse short hyphae all around, medulla in center, and bare apices; Mucor and Thamnidium are shown in the background as associated organisms. × 6.3

Plate I



1. Habit on dog-dung, taken a few days after collection in laboratory. (1969) × 1
2. Portion of a culture transplanted to a glass jar growing on moistened filter paper with rabbit dung pellets. × 2/3
3. Culture in vitro on weak agar and rabbit pellets, note the excreted drops which are common in cultures. × 2
4. Habit in nature (Collected 1966) showing branched synnemata. × 1
5. Spore suspension (macroconidiospores) seeded on agar plate showing binucleate cells. × 1000
6. Budding by macroconidiospores. × 1500

Plate II

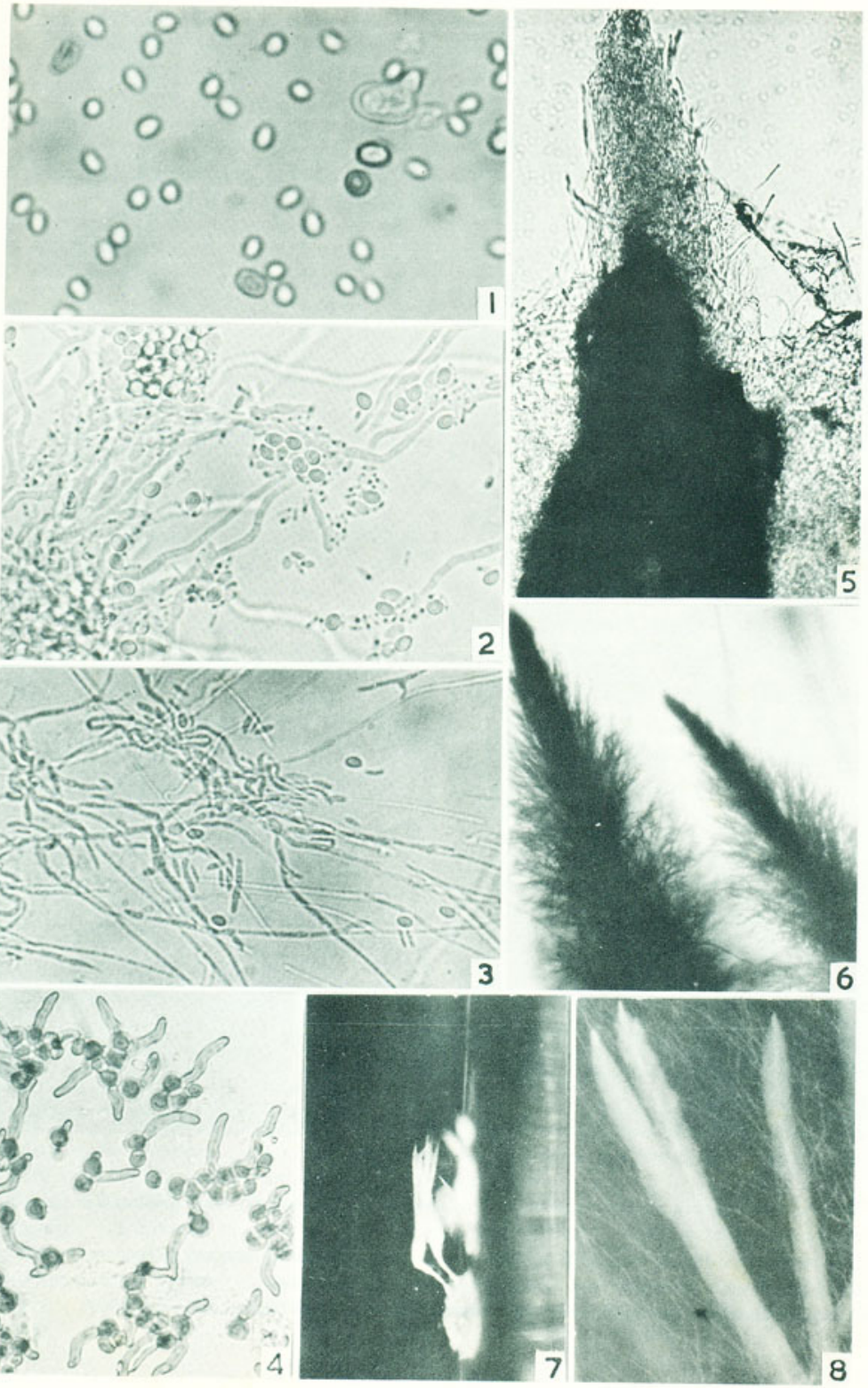
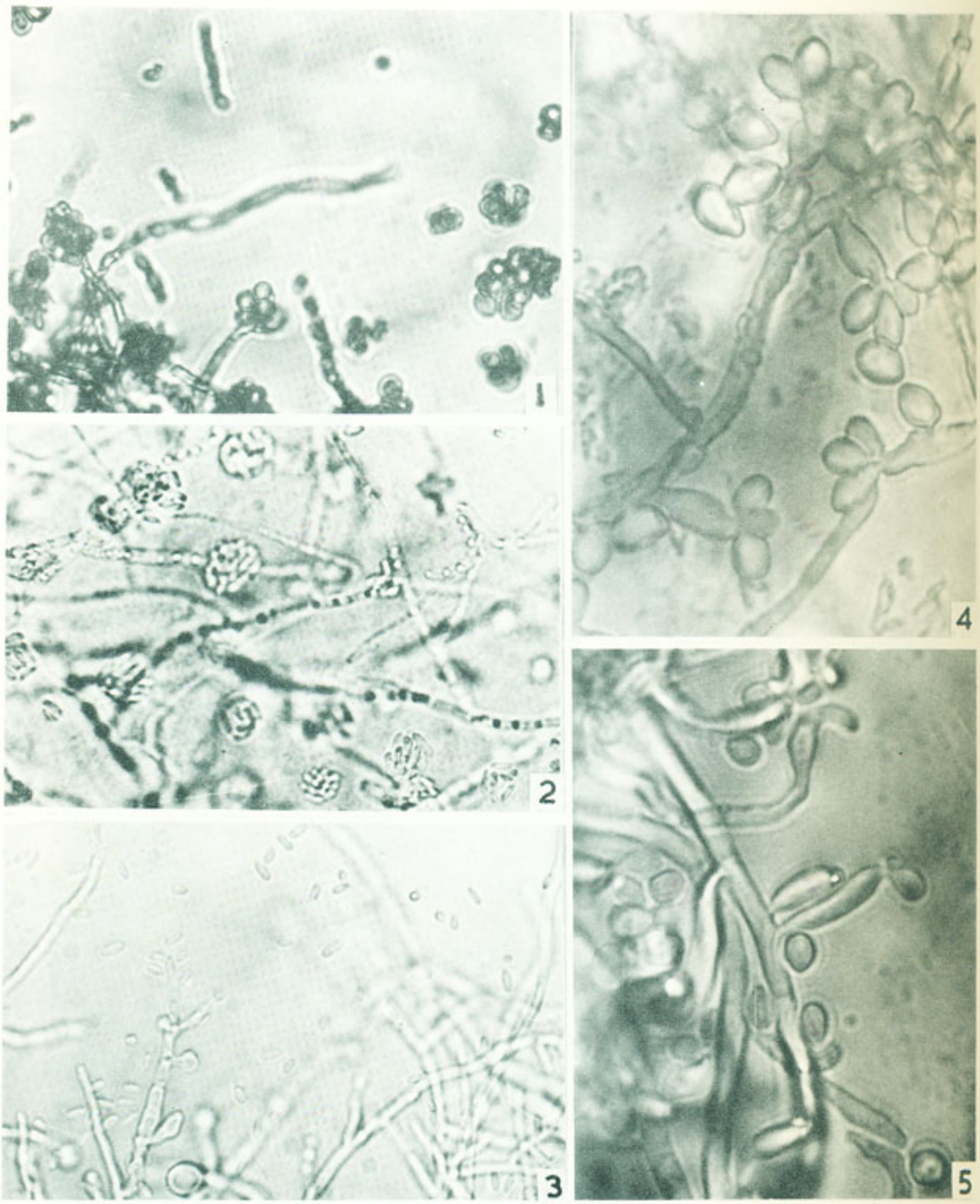
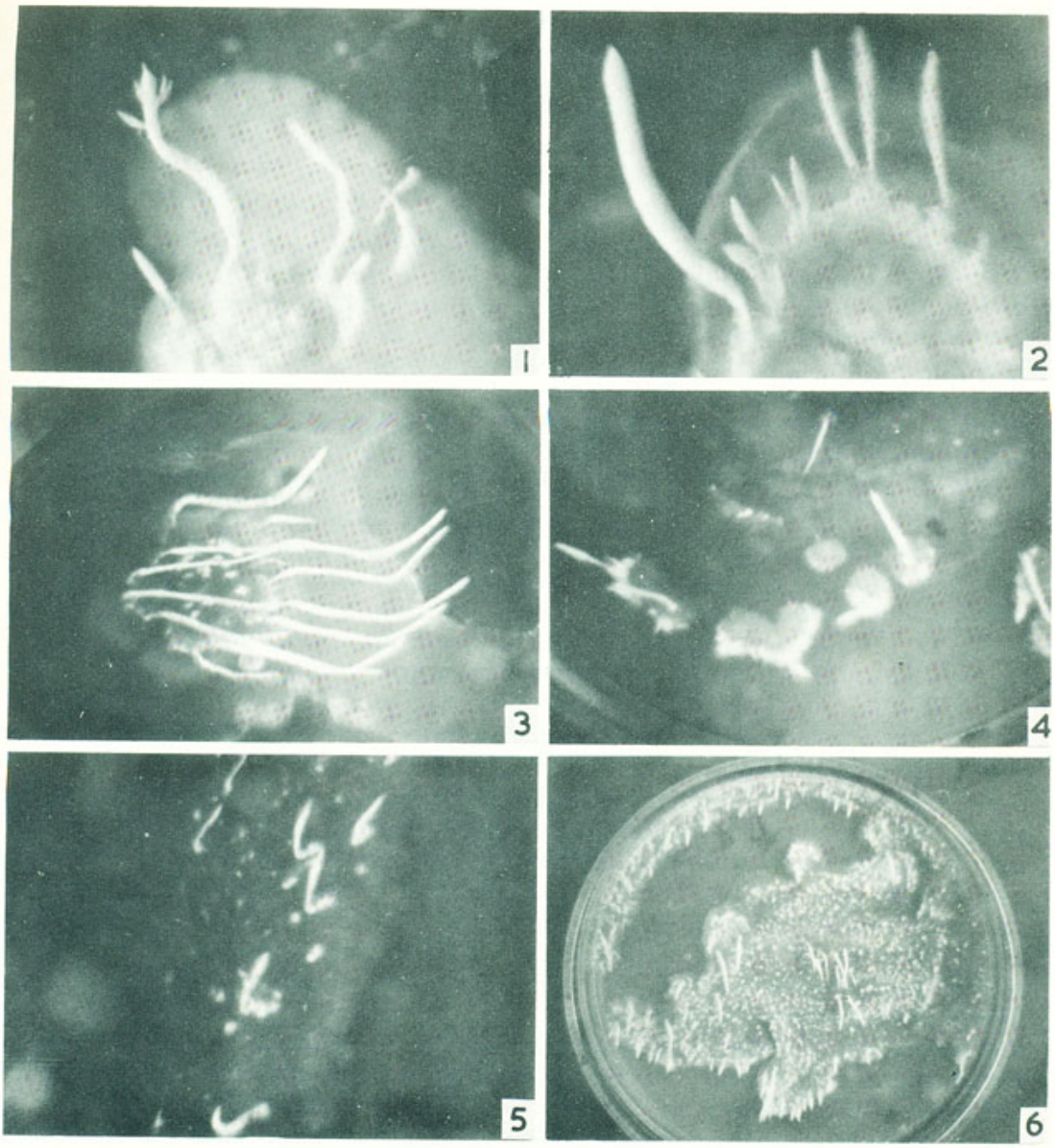


Plate III



1. Microconidiospores, spherical-shaped in loose apical cluster, and oidia-like free hyphal cells in chains. × 600
2. Clusters of conidiospores may be formed on any portion of the mycelium. × 600
3. Conidiospores showing direct connection with the hyphae. × 600
4, 5. Macroconidiospores in whorls and swollen curved conidiophores. × 1500

Plate IV



1. Branched tip of an older synnema, and various lengths of others arising from the white coating of the medium surface. 23 days after spore suspension sprayed on agar medium. (Photo taken with Asahi, Pentax with no. 1 & 2 rings)
2. Synnemata growing toward the source of light. (Taken with no. 1, 2 & 3 rings).
3, 5. Showing the reverse of phototropism, when the direction of light source changed.
4. Culture growing in dark showing synnemata at random direction.
6. Showing culture grown under one-sided light source as Figs. 1 and 2, but in younger stage. Note the minute white dots from which synnemata arise (Photo taken with rings no. 1, 2 & 3).