THE CHARACTERISTICS OF INDIGENOUS ENTOMOPATHOGENIC FUNGI ISOLATED FROM INSECT PESTS OF OIL PALM

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Keywords: Entomopathogenic-fungi: *Beauveria bassiana*; *Paecilomyces farinosus*; *Aspergillus flavus*; *A. candidus*; *A. ochraceus*; *A. flavicep*; Bagworms: *Metisa plana*; *Mahasena corbetti*; Nettle caterpillars: *Setora nitens*; *Thosea lutea*; Oil palm: *Elaeis guineensis.*

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Original manuscript received: 16 January 1993
Revised manuscript received: 17 August 1993

Various entomogenous fungi imperfecti (Deuteromycotina) were isolated from diseased bagworms collected from several sites. Beauveria bassiana *was very often isolated from* Metisa plana *sampled at Bukit Cloh Estate, Kapar, Selangor. It was identified as one of the most important factors in controlling the worst ever bagworm outbreak occurring in 1991 on the estate.* Paecilomyces farinosus *and* Aspergillus flavus *together were responsible for drastic and complete mortality of* Mahasena corbetti *bagworm reared in PORIM outdoor and indoor insectaries in 1991. On the other hand,* A. candidus *is commonly associated with* M. plana *in indoor insectaries, and can possibly contribute to the moderately high mortality. It has been proved to be a potential agent for the control of the insect pest.* A. ochraceus *and* A. flavicep *were not often found attacking the bagworms, but the study of their pathogenicity should not be neglected. The macroscopic and microscopic characteristics, morphological dimensions and importance of these entomopathogens are described.*

INTRODUCTION

Considerable progress has been achieved in research on the insect mycoses and their application to pest control. However, the study of mycoses in insect pests of oil palm has been somewhat neglected. The major research emphasis has rather been directed towards the study of bacteria (Abdul Halim *et al.*, 1991), parasitoids (Wahid and Kamaruddin,1993) and viruses (Siti Ramlah and Jalani, 1993) whose potentials as microbial control agents were considered to be greater. The activities, prevalence and survival of these biological control agents are very much dependant on environmental conditions. The climate in the tropics is particularly suitable for the propagation and activities of fungi as compared to the other microbes, so it is of obvious interest to investigate fungi as possible microbial control agents for insect pests of oil palm.

The bagworms, *Metisa plana* (Wlk.) and *Mahasena corbetti* (Tams) have been important occasional pests of oil palm, *Elaeis guineensis* Jacq. since 1956 (Wood, 1968). More than 10 thousand hectares of oil palm were seriously attacked by bagworms during 1981 to 1985 (Mohd Basri *et al.*, 1988). Although bagworms are under control, they are still a major threat to oil palm, capable of causing serious outbreaks in Malaysia. A laboratory study on biological control of *M. plana* using one of the most promising fungal pathogens has already been reported (Ramlah Ali *et al.*, 1993).

To-date, very few naturally occurring fungal pathogens have been reported in bagworms, but an entomogenous fungus, *Paecilomyces fumoroseus* has been isolated from *Crematopsyche pendula*, *M. corbetti*, *Cryptothelia cardiophaga* and *Clania tertia* (Sankaran, 1970; Tiong, 1979).

Cordyceps spp. was found effective in the field control of nettle caterpillars, especially *Thosea asigna* (Tiong, 1982). In South-East Asia, *Artona catoxantha* was shown to be attacked by *Beauveria bassiana*, while Chrysalises of Africa were shown to be heavily attacked by *P. farinosus* (Mariau, 1982). The entomopathogenic fungi are distributed within the classes Fungi Imperfecti, Phycomycetes, Ascomycetes and Basidiomycetes (Ferron, 1978).

Before venturing into the detailed study and exploitation of these fungi for field control of oil palm insect pests, some fundamental aspects of these fungi must first be known. First, the morphological features must be available for recognition of these pathogens; second, information on their pathogenicity against various hosts must be reviewed for safe laboratory and field study. The feasibility of developing microbial insecticides depends on their effectiveness, safety, specificity, production, propagation and marketability (Jaques, 1983).

A preliminary study on the pathogenicity of fungal pathogens against bagworms at the Palm Oil Research Institute of Malaysia (PORIM) suggested the possibility of using several fungi for the control of oil palm insect pests. This paper presents the morphological details of several entomogenous fungi isolated from bagworms, and their importance as biological control agents is discussed.

MATERIALS AND METHODS

Infected samples

Infected bagworms were collected individually in sterilized vials. Elimination of saprophytic fungi was ensured by selecting freshly infected samples. Fresh cadavers were collected from indoor and outdoor insectaries as well as from the fields. Regular collection was done during 1990–1992. The oil palm plantations involved in this sampling were PORIM Stations,Tabung Haji Estate and Bukit Lawiang in Johor, Teluk Merbau and Bukit Cloh Estates in Selangor, and Lukut Estate in Negeri Sembilan.

Identification of fungi

Identification of the fungi was based on their colony characters and the morphological details of spore bearing structures. The macroscopic and microscopic structures of several fungi associated with *M. plana* and *M. corbetti* were studied and photographed using a phase-contrast microscope. Determination of species was based on fungal morphology and confirmed by the CAB International Mycological Institute in London.

Macroscopic examination

The infected larvae, adult or pupae, were separately placed on moist sterilized wicking paper and incubated at 28 ± 2°C for a week. Fungal growth was observed daily under a dissecting microscope and the general features of the mycelium and any spore-bearing structures were photomicrographed. Isolates surmised to be pathogens were cultured on to Malt Extract Agar (MEA) in order to arrive at colony descriptions and record the details of microscopic structures.

Microscopic and dimensional morphology

The cellophane tape method was adopted for studying the microscopic characteristics of colonies 1–7 days old. The dimensions of morphological structures, which included, conidia, conidiophores, vesicles, metulae, phialides, rachis and hyphae, were determined using the slide micrometer method.

RESULTS AND DISCUSSION

Most of the parasitic entomogenous fungi of bagworms were in the Deuteromycetes (Fungi Imperfecti), consisting of many species of unrelated genera. The potentials of these entomopathogenic species as control agents for bagworms in oil palm are discussed in order of their importance.

Beauveria bassiana (Balsamo-Criv) Vuill.

When present in an oil palm estate, *B. bassiana* (Balsamo-Criv) Vuill., attacked the adult (*Figure 1-A*), pupae and larvae (*Figure 1-B*) of *M. plana*. This broad-spectrum entomopathogenic fungus was very frequently encountered in Bukit Cloh Estate, Selangor.

Colony structures

B. bassiana is a slow grower and its colonies never exceeded 3 cm in diameter after 14 days at 30°C on MEA. The young colonies were woolly white, later often becoming light yellow and powdery because of abundant conidia.

Microscopic structures

The conidiogenous apparatus formed dense clusters of swollen stalks comprising of subglobose to flask-shaped venters measuring 3–6 × 2.5–3.5 µm (*Figure 1-C*) which extended into zig-zag or denticulate rachides with maximum dimensions of 25 µm long and 1 µm wide (*Figure 1-D*). The smooth-walled globose to subglobose conidia mostly measured 2–3 µm, and were attached on the stalk or rachis. With age the conidiogenous cells became more slender and less densely clustered, as seen in *Figure 1-D*.

Paecilomyces farinosus (Holm ex Gray)

This fungus is a ubiquitous insect pathogen, found regularly in the indoor and outdoor insectaries and estates. It was found to be one of the pathogens responsible for 100% mortality *M. corbetti* cultures in a PORIM outdoor insectary in early 1991.

Colony characteristics

The colonies grew moderately fast, reaching 5–6 cm in diameter in 2 weeks at 30°C on MEA. The powdery culture remained white to luteous throughout (*Figure 2*).

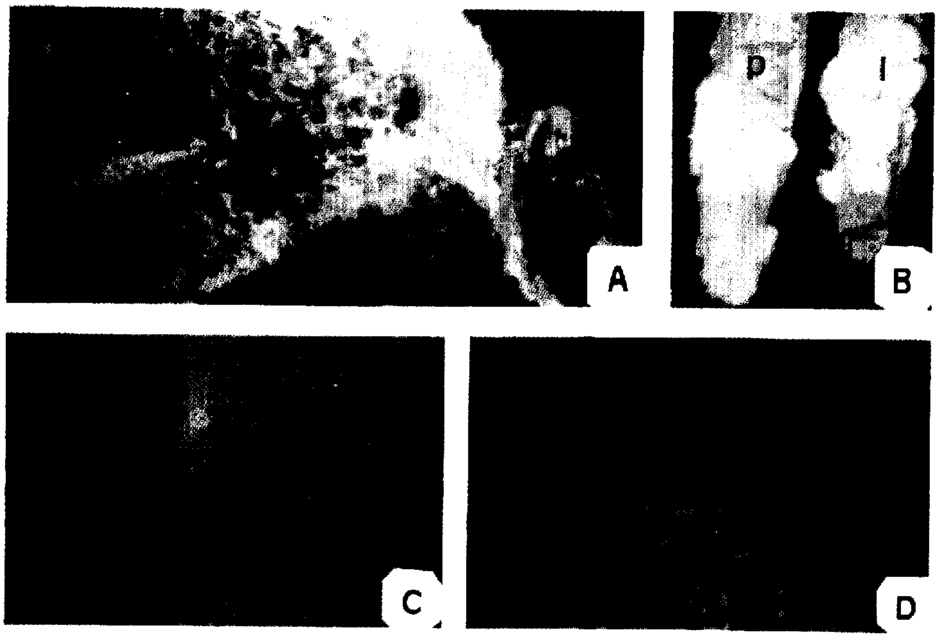

Figure 1. B. bassiana *attacking adult bagworm (A), pupa and larva (B); dense cluster of conidiogenous cells on the left and slender or less clustered on the right (C); flask-shaped venter extending into denticulate rachis (D). Magnification 40x (A and B), 2300x (C) and (4500X) (D). adult (a), pupa (p), larva (l), densely clustered conidiogenous cells (ds), venter(v), rachis (r) and globose to sub-globose conidia (c).*

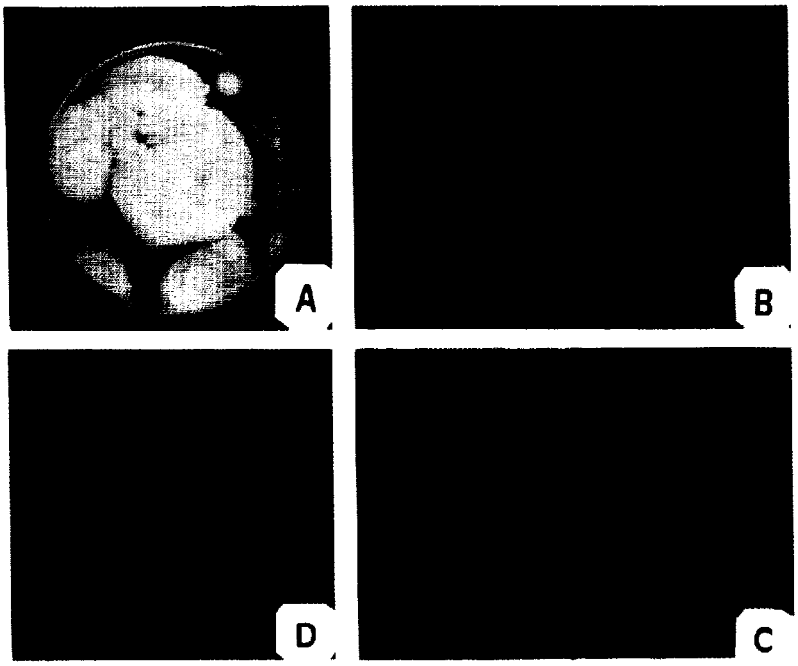

Figure 2. P. farinosus *culture on MEA (A), characteristic structures (B,, flask-shaped phialides (C) and the ellipsoidal conidia (D). Magnification 0.5x (A), 900x (B), 1900X (C) and 3600X (D). vegetative hyphae (h), conidiophore (cp), phialide (p) and conidia (c).*

Microscopic structures

Conidiophores were usually erect, 100–300 µm tall, and bearing several whorls of flask-shaped phialides (*Figure 2-B and 2-C*). The conidia were ellipsoidal to fusiform measured 2–3 x 1–8 µm, and formed in chains of 8 to 16 as seen in *Figure 2-D.*

Aspergillus candidus (Link)

The main distributional range of *A. candidus* is the sub-tropical and tropical regions. Attacks on bagworms were observed quite regularly, especially in rearing cylinders.

Colony characteristics

Colonies were relatively slow-growing, reaching 2.5–3.5 cm in diameter in 2 weeks at 30°C on MEA. The young colonies were normally white, becoming yellowish cream with age. The conidial heads were usually variable in size and shape within the same colony.

Microscopic structures

The conidiophores typically had large globose vesicles bearing inflated club-shaped metulae or primary sterigmata (*Figure 3-A*) and narrow phialides or secondary sterigmata (*Figure 3-B*). The conidia were globose to ellipsoidal and smooth-walled; they measured 2.5–3.5 µm in diameter with the terminal conidia reaching 4 µm diameter and the spore chains sometimes slimming down as seen in *Figure 3-C.*

Aspergillus ochraceus (Wilh)

This fungal species was not often associated with bagworms in the field: it was more often isolated in the indoor insectaries.

Colony characteristics

Colonies were quite fast growing, attaining 4–4.5 cm in diameter in 14 days on MEA at 30°C. The colonies appeared light ochraceus buff to ochraceus in the abundance of conidial heads.

Microscopic structures

The thick-wall conidiophores measured 1–1.5 mm long and were thicked-walled and coarsely roughened (*Figure 3-D*). The vesicles were globose to subglobose, delicately roughened, and mostly 2.5–3 µm in diameter, as in *Figure 3-E.* Sclerotia measured 500–1000 um, and were often present at the later stage, borne singly and pale pink to vinaceous purple when mature.

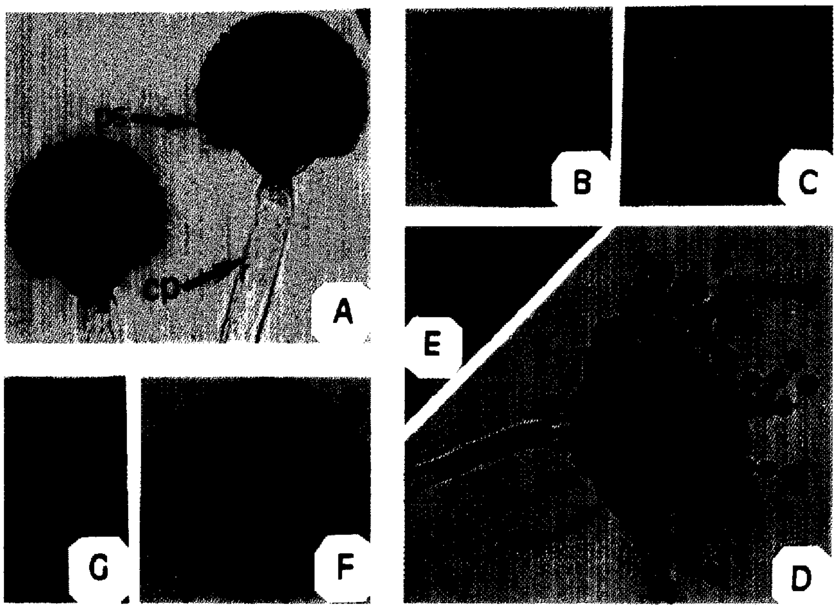

Figure 3. Aspergillus *spp. frequently attacking bagworms.* A. candidus *vesicle with primary sterigmata (A); attached at the extremities are secondary sterigmata (B) and their conidia (C).* A. ochraceus *young vesicle (E) and coarsely roughened mature conidiophore, with biserate sterigmata carrying oval smooth-walled conidia (D).* A. flavus, *showing radiating biserate vesicle (F) and echinulate conidia (G); Magnification 900X (A,B,E and F) and 3600X (C,D and G). conidiophore (cd), primary sterigmata (ps), secondary sterigmata (ss), vesicle (v) and conidia (c).*

Aspergillus flavus (Link)

This fungus is distributed world-wide, but predominates particularly in the tropics and sub-tropics. Its occurrence on bagworms together with *P. farinosus* at PORIM resulted in 100% mortality of *M. corbetti* cultured in insectaries. It was the commonest isolate from all our collections of bagworms. The frequency of occurrence was ascribed to its being ubiquitous and versatile.

Colony characteristics

Colonies were characteristically yellowish green; they were very fast growing, reaching 6–7 cm in diameter in 14 days on MEA at 30°C. The conidial heads were variable in size and shape within the same colony.

Microscopic structures

The rough-walled conidiophores varied from 0.4 mm to 1.0 mm long. The conidial heads were radiating, with short metulae supporting the phialides or biseriate sterigmata (*Figure 3-F*). The conidia were globose to sub-globose, finely roughened to echinulate, and mostly 3.5–4.5 µm in diameter (*Figure 3-G*).

Aspergillus flavicep (Bainier & Sartory)

This is a species of world-wide distribution but its main centres are in the tropical, sub-tropical and Mediterranean zones. Like *A. ochraceus* it was less commonly associated with bagworms.

Colony characteristics

The colonies were rather slow growing, reaching 3.5–4.0 cm in diameter in 14 days at 30°C on MEA. The whitish conidial masses were set among the shiny brownish conidiophores. The reverse colour stained yellowish brown and with time it turned deep reddish brown.

Microscopic structures

The conidial heads appeared radiating when young and became broadly and irregularly columnar at maturity. They were initially white but ultimately became hazel. The vesicles appeared subglobose to elongate, especially when young.

The sterigmata were usually in two series, covering almost the entire head or only the terminal area of the younger head. The conidia were globose to subglobose, smooth and thin-walled, and measured 2.4–3.2 µm in diameter. Present also were tufts of yellow thick walled septate hyphae which simulated the hulle cells.

DISCUSSION

When present in oil palm plantations, *B. bassiana* has appeared to be a better control agent than parasitoids in checking bagworm outbreaks. For instance, the most serious outbreak ever, in April 1991 at Bukit Cloh Estate, was checked by this fungus during a dry season; its ability to withstand the drought (Ferron, 1978) and control the intense outbreak make it a good candidate for biological control. Its performance was even better in the subsequent wet months of 1991. A detailed laboratory study on the pathogenicity of *B. bassiana* had been reported (Ramlah Ali *et al.*, 1993). The exact route of infection has yet to be studied, but preliminary scanning electromicrography of bagworms infected with *B. bassiana* has shown entry through spiracles and mouth-parts.

In the hemipteran insect pest, *Lygus hesperus*, whose feeding habits preclude the ingestion of *B. bassiana* spores, infection still occurred through the body wall, even at a relative humidity as low as 40–50%, which indicates that infection proceeds under conditions that appear too dry to allow the germination of conidia (Campbell *et al.*, 1983). However, almost all other fungi require a humidity above 80% for germination.

As with *B. bassiana*, drought-resistance in *P. farinosus* has been reported (Ferron, 1978). The host range of *P. farinosus* appears unlimited, and it has been used for biological control experiments with various insects (Domsch and Gams, 1980). Its pathogenicity to bees, bumble bees, *Leptinotarsa decemlineata* and *Pyrausta nubilalis* has received particular attention (Ferron, 1978).

Members of the *A. flavus* group are important insect pathogens. However they also have pronounced and obviously undesirable toxic effects on insects and animals. Mycoses of bees, cockchafers, *Melolontha melolontha*, *Tenebrio molitor*, *Laphygma exempta*, *Cimex lectularius*, *Schizoneura lanuginosa* and pine beetles such as *Dendroctonus frontalis* and *Pyrausta nubilalis*, as well as fatal effects on rabbits and guinea pigs have been frequently reported. The members of the *A. flavus* group produce mycotoxins, notably aflatoxins; the toxicology is important and has been much studied. They have been isolated from the alimentary canal of man and the elk and they are particularly common on insects such as, the corn earworm and the corn borer (Domsch and Gams, 1980).

Apergillus spp. are frequently associated with insect diseases (Pierre, 1985; Robert and Yendel 1971). This genus includes *A. candidus*, which has been confirmed to be one of the most virulent entomopathogens. When applied against the spindle bug, *Carvalhoia arecae*, it resulted in 100% mortality in four days from inoculation. The common occurrence of *A. ochraceus* on imagoes of worms has been reported: infection was directly via the hind gut (Raper and Fennell, 1973). The fact that *A. ochraceus* has been isolated from the sputum of a patient with pulmonary disturbance (Almeida, 1939), obviously makes it unattractive as a control agent, for reasons of safety. A culture filtrate of *A. flavicep* has been reported to be toxic to the larvae of the rice moth, *Corcyra cephalonica* (Domsch and Gams, 1980).

The endoparasitic action of fungi when attacking insects requires that they enter the body and establish themselves in the hemolymph or tissues. Several routes of infection have been reported (Ferron, 1978). Infection in *Pyrausta nubilalis* and *Platysamia ceropia* by *A. flavus* was through the integument, while the infection of *B. bassiana* in bagworms was via the spiracles and mouth-parts. Similarly, *A. flavus* and *A. parasiticus* infected certain Orthoptera via tracheal openings, while the germ-tube of *A. flavus* can penetrate the chitinous lining of the hind gut of *Platysamia ceropia* (Raper and Fennell, 1973). Penetration via the integument and chitin layers involved the appropriate enzyme systems in the fungi (Ferron, 1978). Mature spores of *A. ochraceus* infect the gut of bees via the emerging germ-tubes (Raper and Fennell, 1973).

Ingestion of spores and mycelial masses by some insects results in acute disease symptoms or even mortality before the fungus has actually penetrated the gut wall. These effects are thought to be caused by fungal toxins such as beauverin produced by some *Beauveria* spp. and *Paecilomyces* spp., and aflatoxins produced by some of the *Aspergillus* spp. (Ferron, 1978). Death after several days of infection has been attributed to the damaging effect of the mycelial presence, to toxin production, to the histolytic effect or to interference with the tracheole and spiracle system (Raper and Fennell, 1973).

CONCLUSION

Not all insect species are susceptible to the same fungi. Some of the indigenous fungal isolates from bagworms, such as *B. bassiana*, *P. farinosus* and *A. flavus*, have displayed a promising lethal effect on bagworms. Others, like the *A. candidus*, *A. ochraceus* and *A. flavicep*, are known entomopathogens to other insect pests, which were regularly isolated from bagworms. On the basis of their pathogenicity, *B. bassiana*, *P. farinosus* and *A. candidus* are reckoned to be the best three candidates to be examined for the integrated management of bagworms. Although *A. flavus* is a possibility for the control of bagworms, its toxicity to mankind clearly makes it unsuitable for biocontrol.

ACKNOWLEDGEMENTS

The authors would like to thank Dr B C Sutton, Head of the Taxonomic and Identification Service, and Dr Michele Williams of the CAB International Mycological Institute, London, for assistance with the confirmation of fungal species.

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