Entomopathogenic Fungal Formulations of Mycoinsecticides

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Synthetic chemical insecticides have been the mainstay of insect pest control for more than 50 years. However, the increasing cost of development, pest resistance and resurgence and increased awareness of the environmental effects of excessive dependence on these chemicals have ensured a continuing interest in alternative forms of pest control. Biological control agents such as entomopathogenic fungi have played important roles in the history of microbial control of insects. The first practical attempt was conducted by Metschnikoff in 1879, and then repeated by Krassilstichik in 1888, when *Metarhizium anisopliae* was used against the cockchafer larvae, *Anisoplia austriaca*, and the weevil, *Cleonus punctiventris* (Robert & Humber, 1981).

In Malaysia, the first use of a fungus against insects was reported by Ooi (1979) when *Entomophthora sphaerosperma* was applied against Plutella xylostella. The second publication was only available 14 years later when Ibrahim and Low (1993) reported the successful use of *Beauveria bassiana* and *Paecilomyces fumosoroseus* against *P. xylostella*. Entomopathogenic fungi are gaining increasing attention worldwide as agents for biological control and some have been developed for large-scale commercial production as mycoinsecticides. Uniquely, unlike bacteria or virus, fungi do not have to be ingested; hosts are invaded directly through the integument or natural openings such as spiracles. Therefore, they can infect non-feeding stages such as the eggs and pupae. This manuscript presents a summary of my works on selected isolates of entomopathogenic fungi against various insect pests and mites.

Insects, Mites and Fungal Culture: Cultures of insects and mites were maintained in the laboratory under an ambient environment of 28 ± 2 C, 85 ± 15% RH and 12 h photoperiod. When required distilled water and 5% (w/v) honey were also provided separately as food for the adult insects. All the fungal isolates were maintained on PDA or SDA in the Entomology Laboratory of the Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia. Fungal inocula were prepared from 3-4 week old sporulating cultures suspended in 0.05% aqueous Tween 80. A Neubauer haemocytometer was used to estimate the respective initial conidial concentrations and subsequent appropriate dilutions were prepared thereof.

Dosage-Mortality Bioassay: As a general approach, the test insects were placed either on a "leaf preparation" or on a leaf disc as in the case of spider mites. The dosage-response assays included at least seven inoculum dosages plus a sterile cont rol of 0.05% aqueous Tween 80. Inoculation was done by spraying a minimal amount, 0.05 ml or less of the conidial suspension with a Sigma® hand atomiser that delivered an even droplet spray of 79 µm (VMD). Mortality, including moribund individuals, was recorded daily for 4-12 days depending on the test subjects. The EC_{50}s and LT_{50}s are obtained through a probit programme (S103, Statistical Research Service, Canada DOA, unpublished) based on probit analysis by Finney (1971).

Formulations and Field Trials: Four formulations of microbial control agents have been developed. Dust, wettable powder and oil formulations use conidia as the infective agent, while granular formulation used mycelia as the source of inoculum. Tests for their favourable physical traits and shelf life are still in progress. Field trials have been carried out against *P. xylostella, Crocidolomia binotalis, Phyllotreta striolata* and *Aleurodicus dispersus*. Similar experiments are currently being conducted against *Polyphagotarsonemus latus* and Tetranychus urticae Koch complex, followed by *Epilachna indica* and the aphids in the future. Please refer to Ibrahim & Low (1993), Ibrahim & Yew (1994), Ibrahim & Hashim (1998), Ibrahim & Tan (1999), Ibrahim & Liu (2001), Hashim & Ibrahim (2002), Priyatno & Ibrahim (2002, 2003), Ibrahim & Yeong (2002), Ibrahim (2002) and Ibrahim & Nugroho (2002) for further details.

Dosage-Mortality Bioassay: In general, all the three species of fungi inflicted significant mortality on the insects and mites tested. A concentration of 2×10^7 conidia mL^{-1} was adequate to provide good kill, and significant relationships at $P = 0.05$ were obtained between log concentration and probit mortality values for all the three fungal species. This indicates that infections increased significantly with increasing concentration. The median effective concentrations, EC_{50} values, were very encouraging; generally reached 3 days after treatment (refer to LT_{50}s). This enhances the potential and the desire to minimise the use or replace chemical application by microbial control.

Formulations and Field Trials: Field tests using formulated conidia has been limited to cabbage caterpillars, flea beetles, whiteflies, termites, stored products insects and mites. Conidia *of P. fumosoroseus* in Vesawit® palm oil (rf. no. 2) worked very well against *C. binotalis* but not as encouraging against *P. striolata* and *A. dispersus*. The granular formulations were more successful against the termites (rf. no. 12). Current field tests with the wettable powder formulation have shown encouraging recovery of broad mite infested chilli shoots sprayed with *B. bassiana* and *P. fumosoroseus*, while the rice worm *Corcyra cephalonica* was very susceptible to the dust formulations of *B. bassiana* and *P. fumosoroseus*.

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