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FORMULATION OF MYCOINSECTICIDES AS WATER-DISPERSIBLE GRANULES FOR VEGETABLE INSECT PEST CONTROL

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FORMULATION OF MYCOINSECTICIDES AS WATER-DISPERSIBLE GRANULES FOR VEGETABLE INSECT PEST CONTROL

By

IVY CHAI CHING HSIA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements of the Degree of Doctor of Philosophy

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Dedicated to my beloved parents.
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

FORMULATION OF MYCOINSECTICIDES AS WATER-DISPERSIBLE GRANULES FOR VEGETABLE INSECT PESTS CONTROL

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February 2011

Chairman: Professor Dzolkhifli Omar, PhD

Faculty: Agriculture

Microbial pesticides have been widely accepted as a biological alternative to chemical insecticides in controlling insect pests. Where disease-causing (entomopathogenic) fungi are being manipulated and used inundatively for insect pest control, such microbial pesticides are known as mycoinsecticides. The infective propagules known as conidia produced by the entomopathogenic fungi was mass-produced using semi-solid fermentation to be used as an active ingredient in a mycoinsecticidal product. The aims of this study was to formulate the conidia as water-dispersible granules (WG), to delve into WG formulation methods, and to evaluate the factors that influence the biological and physical performances of the WG-conidia formulations in both laboratory and glasshouse conditions.
The study initiated with the screening for the best entomopathogenic fungal isolate to be used as active ingredient. Five fungal isolates *i.e.* *Metarhizium anisopliae* (MPs, MaBg and MaCc1a), *Beauveria bassiana* (BbGc) and *Paecilomyces fumosoroseus* (PfPx) were screened by exposing each isolate’s conidia to wet-heat and oven-heat stress through a series of temperatures. Isolate MPs showed the best tolerance to the heat stresses and was selected as the active ingredient in all WG-conidia formulations. Inert ingredients and/or additives were then selected based on the ability of the conidia of MPs to survive more than 80% germination upon contact with each ingredient at different temperatures and exposure duration. Following the selection of inert ingredients and/or additives, ternary phase diagrams were constructed to obtain five ‘Surfactant systems’, in which a total of 22 different WG-conidia formulations were prepared. All 22 formulations were tested and rated for their physical and biological performances using four parameters *i.e.* conidial fresh viability, viability after 7 days in storage, suspensibility, and dispersibility. Five formulations were rated as ‘Good’, 12 formulations as ‘Satisfactory’ and five was ‘Uns satisfactory’. Six WG-conidia formulations containing additives were selected and tested for their storage stability at different temperatures and exposure period. Results showed that shelf life of WG-conidia formulations were greatly reduced when storage temperature and exposure period increased. Shelf life improved when additive(s) was added to the formulations. Formulations containing sodium alginate and sodium acetate showed better conidial germination (80%), 15 days after formulation (DAF) at 15°C, than formulations without additives. While the formulation containing sodium alginate showed 27% conidial germination at 30DAF, 15°C, most WG-conidia formulations did not store well beyond this period and temperature.
The effectiveness of the WG-conidia formulation was evaluated through a time-mortality response bioassay against the insect pests of economic importance in Malaysia, *Plutella xylostella* and *Epilachna indica*. The WG-conidia formulation, 3B5gK, was comparatively satisfactory to unformulated conidia in causing death to both insect pests with median lethal time ($LT_{50}$) of 6.5 days and 5 days for *P. xylostella* and *E. indica*, respectively. Relative potency of unformulated conidia was 1.3-1.6 times than that of 3B5gK. In a pre-field trial conducted in the glasshouse to investigate the effects of different volume application rates of WG-conidia formulations on the larvae of *P. xylostella*, the formulation containing sodium alginate, 7A5gBAl caused the quickest mortality of larvae ($LT_{50}=72$ hours) at high volume application rate compared to three other WG-conidia formulations and unformulated conidia. Most WG-conidia formulations showed potential to be further developed as mycoinsecticidal products due to their good biological potency and physical performances in both laboratory conditions and pre-field application. However, further studies should be done to improve shelf lives of the product and their applicability in reduced cost, which are imperative factors for any microbial products to be acceptable by end users.
FORMULASI KULAT SEBAGAI RACUN ‘WATER-DISPERSIBLE GRANULES’ UNTUK KAWALAN SERANGGA PEROSAK TANAMAN

Oleh

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Februari 2011

Pengerusi: Profesor Dzolkhifli Omar, PhD
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Percubaan pertama adalah pemilihan kulat yang terbaik antara lima isolat kulat yang tersedia, iaitu MPs, MaBg, MaCc1a daripada kulat *Metarhizium anisopliae*, BbGc (*Beauveria bassiana*) dan PfPx (*Paecilomyces fumosoroseus*) untuk dijadikan bahan aktif racun. Isolat-isolat didedahkan kepada suhu tinggi dalam bentuk wap basah dan wap kering (oven). Isolat kulat MPs menunjukkan toleransi tertinggi kepada suhu dan seterusnya konidinya dipilih sebagai bahan aktif untuk kesemua fomulasi WG-konidia. Kemudiannya, bahan-bahan lengai dan aditif-aditif untuk diformulasikan bersama konidia dipilih berdasarkan keupayaan konidia MPs untuk hidup >80% apabila terdedah kepada bahan lengai dan aditif tersebut untuk sesuatu masa dan suhu tertentu. Selepas bahan-bahan lengai dan aditif-aditif telah dipilih, diagram fasa-tiga darjat diolah supaya lima ‘Sistem Surfaktan’ didirikan dan 22 formulasi WG-konidia disediakan. Setiap formulasi WG-konidia itu diuji dan dipangkatkan berdasarkan keupayaan biologi dan fisikal mereka, iaitu percambahan segar konidia, percambahan konidia selepas 7 hari distorkan, keupayaan untuk mengampai, dan keupayaan untuk melerai apabila dimasukkan ke dalam air. Lima formulasi telah dikategorikan sebagai ‘Baik’, 12 formulasi dikategorikan sebagai ‘Memuaskan’, manakala 5 lagi ‘Tidak Memuaskan’. Enam formulasi terpilih kemudiannya diuji untuk kestabilan-dalam-simpanan dengan mendedahkan formulasi-formulasi kepada suhu dan tempoh yang berbeza. Ujian menunjukkan bahawa kestabilan kesemua WG-konidia menurun dengan mendadak apabila suhu dan tempoh dedahan meningkat. Jika dibandingkan, kestabilan WG-konidia yang mengandungi aditif adalah lebih tinggi daripada formulasi yang tiday dirumus dengan aditif. Formulasi yang mengandungi kombinasi aditif - sodium alginat dan sodium asetat merekodkan percambabahn konidia sebanyak 80% selepas 15 hari terdedah kepada suhu 15°C.
Sementara itu, formulasi yang mengandungi sodium alginat merekodkan 27% germinasi selepas 30 hari terdedah kepada suhu 15°C, walaupun pada suhu tersebut, kebanyakan formulasi WG-konidia telahpun menjadi tidak stabil melebihi 30 hari dalam simpanan.

Keberkesanan formulasi-formulasi WG-konidia ditentukan melalui ujikaji masa-respon terhadap serangga perosak yang penting di Malaysia iaitu *Plutella xylostella* dan *Epilachna indica*. Keupayaaan membunuh median (LT\(_{50}\)) bagi formulasi WG-konidia, 3B5gK, didapati memuaskan dengan LT\(_{50}\) = 6.5 hari dan LT\(_{50}\) = 5 hari untuk *P. xylostella* dan *E. indica*, masing-masing. Potensi relatif konidia segar adalah 1.3-1.6 kali daripada 3B5gK. Dalam ujikaji pra-lapangan di rumah kaca untuk mengetahui kesan isipadu aplikasi seburan berbeza (VAR) kepada larva *P. xylostella*, boleh dirumuskan bahawa formulasi WG-konidia 7A5gBAI menyebabkan kematian yang paling pantas iaitu 72 jam menggunakan VAR tinggi, berbanding 3 formulasi WG-konidia lain dan konidia segar. Formulasi-formulasi WG-konidia telah menunjukkan potensi untuk dikembangkan sebagai produk mikoinsecticid kerana menunjukkan prestasi biologi dan fisikal yang memuaskan di makmal dan lapangan. Namun, kajian yang lebih harus dilakukan untuk memperbaiki kestabilan dalam simpanan dan pengurangan kos pembuatan dan penggunaan, iaitu dua faktor terpenting dalam penerimaan-guna pakai produk mikrobial oleh para pengguna.
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I certify that an Examination Committee met on **23 February 2011** to conduct the final examination of **Ivy Chai Ching Hsia** on her Doctor of Philosophy thesis entitled “**Formulation of Mycoinsecticides as Water-Dispersible Granules for Vegetable Insect Pests Control**” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (High Degree) Regulations 1981. The Committee recommends that the candidate be awarded the Doctor of Philosophy.

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Date:
I declare that the thesis is my original work except for quotations and citations which has been duly acknowledged. I also declare that it has not been previously or is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institution.

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IVY CHAI CHING HSIA

Date:
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