
UNIVERSITI PUTRA MALAYSIA

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

IVY CHAI CHING HSIA

FP 2011 44

# 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**

**February 2011**

*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**

By

**IVY CHAI CHING HSIA**

**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 'Unsatisfactory'. 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.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**FORMULASI KULAT SEBAGAI RACUN 'WATER-DISPERSIBLE GRANULES' UNTUK KAWALAN SERANGGA PEROSAK TANAMAN**

Oleh

**IVY CHAI CHING HSIA**

**Februari 2011**

**Pengerusi: Profesor Dzolkhifli Omar, PhD**

**Fakulti: Pertanian**

Penggunaan racun perosak dalam bentuk mikroorganisma telah lama diterima-guna pakai sebagai alternatif kepada racun kimia dalam mengawal serangga perosak. Kulat yang boleh menyebabkan penyakit kepada serangga perosak, atau lebih dikenali sebagai kulat entomopatogen boleh dimanipulasikan untuk dijadikan racun serangga atau mikoinsectisid. Bahan jangkitan/sebaran atau konidia yang dikeluarkan oleh kulat entomopatogen boleh dimanipulasikan dan hasilkan secara besar-besaran melalui fermentasi separa-pepejal untuk dijadikan bahan aktif racun serangga. Objektif kajian ini adalah untuk merumus konidia ini sebagai bahan aktif racun serangga dalam bentuk granul tersebar-air (WG), iaitu bentuk formulasi butiran kering yang terbaru dalam pasaran. Kajian ini juga akan menyelami cara dan proses konidia diformulasikan sebagai WG-konidia, serta penilaian kepada faktor-faktor yang mempengaruhi prestasi biologi dan fisikal formulasi-formulasi di makmal dan rumah kaca.

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 konidianya 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 prestasi 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 dibuat bandingan, 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 percamabahn 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 7A5gBAl 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.

## ACKNOWLEDGEMENTS

I would like to express my deepest gratitude and appreciation to my supervisor Prof. Dzolkhifli bin Omar, that throughout my Ph.D, candidacy had provided me not only guidance but important feedbacks in order to complete this dissertation and research. He was very supportive of my opinions and ideas and there was definitely no one as patient as him in assessing my work. My mum and dad should be put in the highest honour for firstly, allowing me to pursue a subject so unusual yet endearing to my heart. I dedicate this doctorate to them for they have not only endured but respected my pursue of this degree. This journey would not have come to a closure without the generous financial assistance of the Kuok Foundation Berhad who was very supportive of my research throughout my 3-year tenure. Not forgetting the members of the Faculty of Agriculture, UPM, especially the officers in the Department of Plant Protection who have handled all my vital administrative matters. Not forgetting the lab mates and lab assistant from Toxicology laboratory (year 2005-2010) - Arshia Hematpoor, Norhayati Ngah, Mahbub Morshed, Mogeret Sidi, Dahlia Shahbuddin, Ahmad Termizi, Aizat, Zaki, En. Jagasi, and lab assistants from Microbiology Lab who have strongly supported me technically and given me the priceless joy I felt while working in the lab. Deepest appreciation also to members of the faculty that have advised and motivated me directly - Dr. Tan Yee How, Prof. Yusof Ibrahim, Dr. Rita Muhammad, and Dr. Kamaruzaman Sijam.

I am thankful for the time and delight that was shown throughout my journey in UPM. Without these people and also others that I've not mentioned, I would not have been what I am today.

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.

Members of the Examination Committee were as follows:

**LAU WEI HONG, Ph.D.**
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

**RITA MUHAMAD, PhD**
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

**AHMAD SAID SAJAP, PhD**
Professor
Faculty of Forestry
Universiti Putra Malaysia
(Internal Examiner)

**ROY PETER BATEMAN, PhD**
International Pesticide Application Research Consortium (IPARC)
Imperial College London, Silwood Park Campus, Ascot
United Kingdom
(External Examiner)

_____
**Shamsuddin Sulaiman, Ph.D.**
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia
Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

**DZOLKHIFLI B OMAR, PhD**
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

**YUSOF B IBRAHIM, PhD**
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

**TAN YEE HOW, PhD**
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

_____ _____
**HASANAH MOHD GHAZALI, PhD**
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

I declare that the thesis is my original work except for quotations and citations with 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.

_____

**IVY CHAI CHING HSIA**

Date:

**TABLE OF CONTENTS**