UNIVERSITI PUTRA MALAYSIA

MICROEMULSION FORMULATIONS OF ROTENONE AND THEIR EFFECTIVENESS AGAINST THE DIAMONDBACK MOTH (LEPIDOPTERA: YPONOMEUTIDAE)

SITI NURULHIDAYAH BINTI AHMAD

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By

SITI NURULHIDAYAH BINTI AHMAD

Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in
Fulfilment of the Requirement for the Degree of Master of Science

April 2009

I dedicated this thesis to;

My beloved Umi & Abah

Siblings of 12

Khairul Khushahiri

Thanks for the endless love

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman : Professor Dzolkhifli Omar, PhD
Faculty : Agriculture

Oil-in-water (O/W) microemulsions were prepared by the titration method through phase diagram study. The mixture consisted of surfactant, oil (as carrier), and water. The surfactants were Agnique PG 8107-U, Agnique PG 9116, and Tween 20 while the oils were Agnique BL 7001, Agnique BL 7002, and xylene. The potential of plant-derived insecticide rotenone (*Derris elliptica*) microemulsion formulations and their effectiveness against crucifer insect pest namely diamondback moth, *Plutella xylostella* were investigated in laboratory. The objectives of this study were, therefore, to formulate rotenone as microemulsion formulations through phase diagram study, to characterize the formulations, and to determine their LC_{50} on diamondback moth by bioassay study.

Twelve phase diagrams of ternary systems were constructed and isotropic region were established. The systems having wider isotropic region were selected and they were Agnique 8107-U/Agnique BL 7001/water, Agnique 9116/Agnique BL 7001/water, Tween 20/Agnique BL 7001/water, Agnique PG 8107-U/Agnique BL 7002/water, Tween 20/Agnique BL 7002/water and Tween 20/Edenor ME/water systems. From these phase diagrams, 13 microemulsion solutions were derived. These microemulsion solutions were further evaluated for miscibility, surface tension, and particle size analysis.

The phase diagram systems containing Tween 20 as the surfactant showed the greater ability to produce a wider isotropic (microemulsion) region compared to others. The miscibility test showed all surfactants mixed readily with water. In interaction with the all oil phases, Tween 20 showed better miscibility compared with Agnique PG 8107-U and Agnique PG 9116 which produce double layers isotropic emulsion in absence of water. The width of isotropic/transparent region in phase diagrams constructed measured in decreasing order were; Tween 20/Agnique BL 7002/ water > Tween 20/Agnique BL 7001/water > Tween 20/Edenor ME/water > Agnique PG 9116/Agnique BL 7001/water > Agnique PG 8107-U/Agnique BL 7001/water > Agnique PG 8107-U/Agnique BL 7002/water > Tween 20/xylene/water > Agnique PG 9116/Agnique BL 7002/water > Agnique PG 9116/xylene/water > Agnique PG 8107-U/xylene/water > Agnique PG 8107-U/Edenor ME/water > Agnique PG 9116/Edenor ME/water.

Three phase diagram systems representing the best microemulsifiable characterization properties and solubility with rotenone were Tween 20/Agnique BL 7001/water, Tween 20/Agnique BL 7002/water and Tween 20/Edenor ME/water systems. Six points in the isotropic regions of the selected phase diagrams were utilized to prepare the microemulsion and coded as M1 to M13. The microemulsions were then subjected to the determination of their surface tensions and particle sizes. The surface tension values of the selected microemulsions were low and their values in decreasing order were; M9 (27.3 mN/m) > M13 (26.9 mN/m) > M2 (26.8 mN/m) > M4 (26.4 mN/m) > M11 (22.8 mN/m) > M7 (22.7 mN/m). The particle size of the microemulsion in decreasing order were; M4 (207.57 nm) > M13 (83.31 nm) > M2 (68.7 nm) > M11 (49.03 nm) > M9 (35.86 nm) > M7 (20.63 nm).

The selected microemusions were used to prepare the rotenone microemulsion formulations. The formulations were then evaluated for their toxicity in comparison with the standard commercial EC formulation (Saphyr®) against the early third instar larvae of the diamondback moth by leaf-dipped bioassay in the laboratory. The mortality of the larvae was recorded at 72 and 96 hours following treatment and data were subjected to the Probit analysis to establish the LC_{50} and LC_{95}. Based on LC_{50} values, the toxicity of formulations for 72 hours after treatment in decreasing order were M11 (204.82 ppm) > M7 (139.71 ppm) > M13 (129.89 ppm) > M4 (122.8 ppm) > M2 (116.94 ppm) > M9 (96.09 ppm) > Saphyr® (96.05 ppm) while for 96 hours after treatment, the toxicity in

decreasing order were M11 (166.63 ppm) > M7 (119.58 ppm) > M13 (105.82 ppm) > M2 (105.22 ppm) > M4 (97.67 ppm) > M9 (87.22 ppm) > Saphyr® (76.86 ppm). The toxicity study indicated that the rotenone microemulsion formulations especially M9 were comparable to commercial rotenone, Saphyr®.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

FORMULASI-FORMULASI MIKROEMULSI ROTENON DAN KEBERKESANANNYA TERHADAP KUPU-KUPU INTAN (LEPIDOPTERA: YPONOMEUTIDAE)

Oleh

SITI NURULHIDAYAH BINTI AHMAD

April 2009

Pengerusi : Professor Dzolkhifli Omar, PhD
Fakulti : Pertanian

Mikroemulsi-mikroemulsi minyak-dalam-air (O/W) telah diperolehi dengan kaedah pentitratan melalui kajian diagram fasa. Campuran mikroemulsi tersebut terdiri daripada surfaktan, minyak (sebagai pembawa), dan air. Surfaktan-surfaktan tersebut ialah Agnique PG 8107-U, Agnique PG 9116, dan Tween 20 manakala minyak-minyak ialah Agnique BL 7001, Agnique BL 7002, dan xylene. Potensi formulasi-formulasi mikroemulsi racun serangga rotenon dari sumber tumbuhan (*Derris elliptica*) dan keberkesanannya terhadap serangga perosak krusifer iaitu kupu-kupu intan, *Plutella xylostella* telah dijalankan di makmal. Objektif-objektif kajian ini termasuklah untuk memformulasikan rotenon sebagai formulasi-formulasi mikroemulsi melalui kajian diagram fasa, untuk mencirikan formulasi-formulasi tersebut, dan untuk menentukan LC_{50} ke atas kupu-kupu intan menerusi kajian bioassai.

Dua belas diagram fasa sistem-sistem ternari telah dihasilkan dan kawasan-kawasan isotropik telah diperoleh. Sistem-sistem yang mempunyai kawasan isotropik yang lebih luas dipilih iaitu sistem-sistem Agnique 8107-U/Agnique BL 7001/air, Agnique 9116/Agnique BL 7001/air, Tween 20/Agnique BL 7001/air, Agnique PG 8107-U/Agnique BL 7002/air, Tween 20/Agnique BL 7002/air dan Tween 20/Edenor ME/air. Daripada diagram-diagram fasa ini, 13 larutan mikroemulsi telah dihasilkan. Kesemua larutan mikroemulsi ini dinilai selanjutnya untuk ujian-ujian keterlarutan, regangan permukaan, dan saiz partikel.

Sistem-sistem diagram fasa yang mengandungi Tween 20 sebagai surfaktan menunjukkan lebih keupayaan untuk menghasilkan kawasan isotropik (mikroemulsi) yang lebih luas berbanding yang sistem-sistem yang lain. Ujian keterlarutan menunjukkan semua surfaktan sedia terlarut dengan air. Dalam interaksi dengan semua fasa minyak, Tween 20 juga menunjukkan keterlarutan yang lebih baik berbanding Agnique PG 8107-U dan Agnique PG 9116 yang menghasilkan dua lapisan emulsi isotropik tanpa kehadiran air. Keluasan kawasan isotropik/ transparensi di dalam diagram-diagram fasa yang dihasilkan diukur dalam turutan menurun iaitu; Tween 20/Agnique BL 7002/air > Tween 20/Agnique BL 7001/air > Tween 20/Edenor ME/air > Agnique PG 9116/Agnique BL 7001/air > Agnique PG 8107-U/Agnique BL 7001/air > Agnique PG 8107-U/Agnique BL 7002/air > Tween 20/xylene/air > Agnique PG 9116/Agnique BL

7002/air > Agnique PG 9116/xylene/air > Agnique PG 8107-U/xylene/air > Agnique PG 8107-U/Edenor ME/air > Agnique PG 9116/Edenor ME/air.

Tiga sistem diagram fasa menunjukkan ciri-ciri karakter pengemulsian dan keterlarutan yang terbaik dengan rotenon ialah sistem-sistem Tween 20/Agnique BL 7001/air, Tween 20/Agnique BL 7002/air, dan Tween 20/Edenor ME/air. Enam titik di dalam kawasan isotropik diagram-diagram fasa terpilih digunakan dalam penyediaan mikroemulsi dan dikodkan sebagai M1 hingga M13. Mikroemulsi-mikroemulsi tersebut telah diuji untuk penentuan regangan-regangan permukaan dan saiz-saiz partikel. Nilai-nilai regangan permukaan bagi mikroemulsi terpilih dalam turutan menurun ialah; M4 (207.57 nm) > M13 (83.31 nm) > M2 (68.7 nm) > M11 (49.03 nm) > M9 (35.86 nm) > M7 (20.63 nm). Saiz partikel bagi mikroemulsi dalam turutan menurun ialah; M9 (27.3 mN/m) > M13 (26.9 mN/m) > M2 (26.8 mN/m) > M4 (26.4 mN/m) > M11 (22.8 mN/m) > M7 (22.7 mN/m).

Mikroemulsi-mikroemulsi terpilih digunakan untuk menyediakan formulasi-formulasi mikroemulsi rotenon. Formulasi-formulasi tersebut kemudiannya dinilai ketoksikannya sebagai perbandingan dengan standard formulasi EC komersil (Saphyr®) terhadap larva instar awal ketiga kupu-kupu intan dengan bioassai celup-daun di makmal. Kematian larva direkodkan pada 72 dan 96 jam selepas rawatan dan data dianalisa dengan analisis Probit untuk mendapatkan LC_{50} dan LC_{95}. Berdasarkan nilai-nilai LC_{50}, ketoksikan formulasi-formulasi yang diperoleh

selepas 72 jam rawatan dalam turutan menurun ialah M11 (204.82 bsj) > M7 (139.71 bsj) > M13 (129.89 bsj) > M4 (122.8 bsj > M2 (116.94 bsj) > M9 (96.09 bsj) > Saphyr® (96.05 bsj) manakala ketoksikan untuk 96 jam selepas rawatan dalam turutan menurun pula ialah (166.63 bsj) > M7 (119.58 bsj) > M13 (105.82 bsj) > M2 (105.22 bsj) > M4 (97.67 bsj) > M9 (87.22 bsj) > Saphyr® (76.86 bsj). Kajian ketoksikan menunjukkan formulasi-formulasi mikroemulsi rotenon terutamanya M9 adalah setanding dengan rotenon komersil, Saphyr®.

ACKNOWLEDGEMENT

I express special gratitude to my main supervisor, Prof. Dr Dzolkhifli Omar as well as my co-supervisor, Prof. Dr Dzulkefly Kuang Abdullah who have taught and mentored me through my study in UPM. I wish to thank my colleagues who have supported me in many ways; Moe, Ivy, Yati, Siti, En Jas, En Zaki and others. Special thanks to Department of Plant Protection (Faculty of Agriculture, UPM), Department of Chemistry (Faculty of Science, UPM) and Mr. Jacky Chan from Cognis Oleochemical Sdn. Bhd for the equipments used and surfactants supplied. To my beloved family and husband; your patience, understanding and endless support is greatly appreciated. You bring me much joy and pride. Thanks are also due to my current employer, Malaysia Palm Oil Board (MPOB) for giving me the opportunity to complete my thesis writing besides my current research work.

I certify that a Thesis Examination Committee has met on 17 April 2009 to conduct the final examination of Siti Nurulhidayah binti Ahmad on her thesis entitled “Microemulsion Formulations of Rotenone and Their Effectiveness Against the Diamondback Moth (Lepidoptera: Yponomeutidae)” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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Date: 9 July 2009

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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SITI NURULHIDAYAH BINTI AHMAD

Date:

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LIST OF ABBREVIATIONS

a. i.	Active ingredient
APG	Alkyl Polyglycoside
BHC	Benzene Hexachloride
bsj	Bahagian per sejuta
Bt	*Bacillus thuringiensis*
CMC	Critical Micelle Concentration
CEPP	Chemical Engineering Pilot Plant
CLCE	Concentrated Liquid Crude Extract
CRD	Complete Randomized Design
DNA	Deoxyribonucleic acid
DDT	Dichloro-diphenyl-trichloroethane
DBM	Diamondback moth
EC	Emulsifiable Concentrate
ED_{50}	Effective Dose at 50%
EPN	Entomopathogenic Nematodes
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GLM	General Linear Module
HLB	Hydrophilic Lipophilic Balance
HPLC	High Performance Liquid Chromatography
IPM	Integrated Pest Management
LC_{50}	Lethal Concentration at 50 %
LD_{50}	Lethal Dose at 50 %
mΩ	Mega ohm
mm	Millimeter
ME	Microemulsion
mN/m	MilliNewtons/meter
nm	Nanometer
NMR	Nuclear magnetic resonance