OIL NANO-EMULSION FORMULATIONS OF AZADIRACHTIN
FOR CONTROL OF Bemisia tabaci GENNADIUS

NOORHAZWANI BINTI KAMARUDIN

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NOORHAZWANI BINTI KAMARUDIN

MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA
2013
OIL NANO-EMULSION FORMULATIONS OF AZADIRACHTIN FOR
CONTROL OF *Bemisia tabaci* Gennadius

By

NOORHAZWANI BINTI KAMARUDIN

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

July 2013
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DEDICATION

Dedicated to:

My mother (Bahiah Bt Abd Aziz) and My Father (Kamarudin Harun)

For their true love, support and inspiration
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

OIL NANO-EMULSION FORMULATIONS OF AZADIRACHTIN FOR CONTROL OF Bemisia tabaci GENNADIUS

By

NOORHAZWANI BT KAMARUDIN

July 2013

Chairman : Dzolkhifli Omar, PhD.
Faculty : Agriculture

Current water emulsion insecticides only provide limited control of Bemisia tabaci. Oil droplets were found to be more effective as they spread much better on leaf surfaces compared to either water alone or water that contained adjuvant. Thus oil nano-emulsion formulation derived from azadirachtin was developed as an effort to control the population of whiteflies, B. tabaci. Oil nano-emulsion system was developed for insecticide formulations by constructing ternary phase diagrams with 70% (w/w) emulsion system constituted of non-ionic surfactant(s), carrier, water, and 30% (w/w) neem oil as an active ingredient. The non-ionic surfactant was alkylpolyglucosides while carrier or oil phase was dimethylamide. Ternary phase diagrams of the mixed surfactant systems MBL510H: MBL530B at mixed surfactant
ratios (MSRs) of 5:5, 6:4, 7:3, 8:2, 9:1 exhibited larger isotropic (I) phase than the single surfactants of either MBL510H or MBL530B.

The points were selected from the ‘I’ phase and homogenous region for pre-formulation. Most of the points selected were from regions with high proportion of oil, low proportion of water and adequate proportion of surfactant to mix with active ingredient and to form water-in-oil (W/O) emulsion. Sixteen formulations miscible with neem oil were selected. In the stability study, all the selected formulations were stable under centrifugation and storage at room temperature (25°C). However, at 54°C after 14 days storage, F3, F7, F9, F10, and F12 showed phase separation, transformed to two opaque phases. The mean particle size of nano-emulsions ranged between 150.00 and 450.00nm except for F9 with mean particle size of 640.44nm. All sixteen formulations showed surface tension lower than water (72.00mN/m). The formulation F14 (29.90mN/m), F15 (29.93mN/m) and F16 (29.86mN/m) showed lower surface tension compared to other formulations. The zeta potential values of F14 (39.60mV), F15 (39.20mV) and F16 (38.80mV) were higher compared to the other formulations. The value is related to the stability of colloidal dispersions and high zeta potential value will confer stability.

In the biological activity study, the adult B. tabaci were used to test the toxicity of the oil nano-emulsion formulation. The result showed the mortality of the adults was higher with the increase of time exposure. The mortality rate of B. tabaci showed that the oil nano-emulsion formulations gave excellent efficacy with LC_{50} value of 3.70ppm at 96 h after treatment. In the measurement of spread area study, three
different levels of formulation toxicities were used to determine the spreading coefficient and evaluate the mode of action of the formulation on the early nymphal instar’s *B. tabaci*. The studies have proved the interaction between spread area and mortality rate. The larger the spread area of the droplet result in increased of mortality. In this study, F15 formulation with low mean lethal concentration gave the larger spread area on the leaves surfaces. As a result, the formulation also gave highest mortality rate on early nymphal instar of whiteflies due to the spreading ability of this formulation. This finding has proved the mode of action of oil nano-emulsion formulation in killing the early nymphal instars of *B. tabaci* by giving wider coverage of active material on leaves surface and brings larger areas of cuticle into contact with the insecticides, resulting in better retention and enhanced the biological effect.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
Sebagai memenuhi keperluan untuk Ijazah Master Sains

NANO-EMULSI MINYAK DARI AZADIRACHTIN UNTUK PENGAWALAN

*Bemisia tabaci* GENNADIUS

Oleh

NOORHAZWANI BT KAMARUDIN

Julai 2013

Pengerusi : Dzolkhifli Omar, PhD
Fakulti : Pertanian

Racun serangga emulsi air yang sedia ada hanya memberikan kawalan terhad kepada
*Bemisia tabaci*. Titisan minyak didapati lebih berkesan kerana ia merebak lebih baik pada permukaan daun berbanding air sama ada bersendirian atau air yang mengandungi adjuvan. Oleh itu, formulasi minyak nano-emulsi yang bersumberkan dari azadirachtin telah dihasilkan sebagai satu usaha untuk mengawal populasi lalat putih, *B. tabaci*. Sistem minyak nano-emulsi telah dihasilkan untuk formulasi racun serangga dengan membina diagram fasa ‘terner’ pada sistem emulsi 70% (b/b) yang mengandungi surfaktan nonionik, pembawa, air, dan minyak mambu 30% (b/b) sebagai bahan aktif. Surfaktan bukan ionik yang digunakan adalah akilpoliglukosida manakala pembawa atau minyak adalah dimetiamid. Diagram fasa terner bagi sistem
surfaktan campuran MBL510H: MBL530B pada nisbah campuran (MSRₜ) 5:5, 6:4, 7:3, 8:2, 9:1 mempamerkan fasa isotropic (I) yang lebih besar berbanding surfaktan tunggal MBL510H atau MBL530B.

Kawasan fasa I dan fasa homogenus adalah kawasan di mana pra-formulasi dipilih. Kebanyakan titik yang dipilih adalah dari kawasan yang mempunyai kadar minyak yang tinggi, kadar air yang rendah dan kadar surfaktan yang mencukupi untuk bercampur dengan bahan aktif serta untuk membentuk emulsi air dalam minyak (W/O). Enam belas formulasi terlarut campur dengan minyak mambu telah dipilih. Dalam ujian kestabilan, semua formulasi yang dipilih stabil pada proses emparan dan simpanan pada suhu bilik (25°C). Walau bagaimanapun, pada 54 °C selepas 14 hari penyimpanan, F3, F7, F9, F10 dan F12 menunjukkan peminisah fasa, berubah kepada dua fasa legap. Min saiz zarah bagi nano emulsi ialah di antara 150.00 dan 450.00nm kecuali untuk formulasi F9 dengan min saiz zarahnya 640.44nm. Keseluruhan 16 formulasi menunjukkan ketegangan permukaan lebih rendah daripada air (72.00mN/m). Formulasi F14 (29.90mN/m), F15 (29.93mN/m) dan F16 (29.86mN/m) menunjukkan ketegangan permukaan yang lebih rendah berbanding dengan formulasi yang lain. Nilai potensi zeta bagi formulasi F14 (29.90mN / m), F15 (29.93mN / m) dan F16 (29.86mN / m) adalah lebih tinggi berbanding dengan formulasi lain. Nilai yang diperolehi mempunyai kaitan dengan kestabilan penyebaran koloid dan nilai potensi zeta yang tinggi akan memberikan kestabilan.

Dalam kajian aktiviti biologi, B. tabaci dewasa telah digunakan untuk menguji ketoksikan formulasi minyak nano emulsi. Kematian lalat putih dewasa meningkat
seiring dengan peningkatan masa pendedahan. Kadar kematian *B. tabaci* menunjukkan bahawa formulasi minyak nano-emulsi memberi keberkesanan yang sangat baik dengan nilai LC$_{50}$ sebanyak 3.70ppm pada 96 jam selepas rawatan. Dalam kajian penentuan kawasan penyebaran, tiga formulasi dengan aras toksik yang berbeza telah digunakan untuk menentukan pekali penyebaran dan menilai ketoksikan formulasi pada pada nimfa lalat putih peringkat awal. Kajian telah membuktikan terdapat interaksi antara luas kawasan penyebaran dan kadar kematian. Semakin besar kawasan penyebaran titisan, semakin meningkat kadar kematian. Dalam kajian ini, formulasi F15 yang mempunyai kepekatan LC$_{50}$ paling rendah telah memberikan penyebaran kawasan yang lebih besar pada permukaan daun. Hasilnya, formulasi juga turut memberikan kadar kematian tertinggi kepada peringkat awal nimfa lalat putih disebabkan keupayaan penyebaran formulasi ini. Hasil penemuan ini telah membuktikan kesan tindakan formulasi minyak nano-emulsi dalam membunuh nimfa lalat putih peringkat awal iaitu dengan memberi liputan bahan aktif yang lebih meluas di atas permukaan daun dan memberi kawasan yang lebih besar bagi kutikel bersentuhan dengan racun serangga, lantas menyebabkan pengekalan yang lebih baik dan meningkatkan kesan biologi.
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In the Name of Allah, the Most Gracious, the Most Merciful...

First of all, I thank God Almighty for giving me the strength and patient along my way to complete my master’s project with much faith and determination. Besides that, I would like to thank my family especially my father and my mother for giving the support and advice during my journey in completing my thesis.

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APPROVAL

I certify that a Thesis Examination Committee has met on 29 July 2013 to conduct the final examination of Noorhazwani binti Kamarudin on her thesis entitled “Oil Nano-Emulsion Formulations Of Azadirachtin for Control of Bemisia tabaci Gennadius” 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.

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Declaration by graduate student

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<tr>
<td>g</td>
<td>gram(s)</td>
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<tr>
<td>kg</td>
<td>kilogram(s)</td>
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<td>mL</td>
<td>mililitre(s)</td>
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<td>cm</td>
<td>centimetre(s)</td>
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<td>d</td>
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<td>°</td>
<td>degree</td>
</tr>
<tr>
<td>°C</td>
<td>degree(s) Celsius</td>
</tr>
<tr>
<td>APG</td>
<td>Alkylpolyglucosides</td>
</tr>
<tr>
<td>CRD</td>
<td>Complete Randomized Design</td>
</tr>
<tr>
<td>DAT</td>
<td>Day after treatment</td>
</tr>
<tr>
<td>PM</td>
<td>post meridem, after noon</td>
</tr>
<tr>
<td>RM</td>
<td>Ringgit Malaysia</td>
</tr>
<tr>
<td>S.E</td>
<td>Standard Error</td>
</tr>
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<td>UPM</td>
<td>Universiti Putra Malaysia</td>
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<tr>
<td>a.i</td>
<td>active ingredient</td>
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<tr>
<td>w/w</td>
<td>weight over weight</td>
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<td>w/v</td>
<td>weight over volume</td>
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<td>ppm</td>
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<td>&amp;</td>
<td>and</td>
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<td>viz.</td>
<td>videlicet, that is, namely</td>
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xxii
i.e. exempli gratia, for example

et al. et alii, and others
CHAPTER 1

INTRODUCTION

Whiteflies are among the major key pest of many fruits, vegetables and ornamental crops. There are highly polyphagous, and damage a broad range of food and non-food crops by direct feeding, impairing product quality through the excretion of honeydew, and transmission of over 100 plant viruses (Jones, 2003). Some of these viruses such as tomato yellow leaf curl virus (TYLCV) are high economic importance and causes high economic losses on tomato in the Mediterranean basin (Morione & Luis-Arteaga, 1999). Although there are approximately 1,200 species of whiteflies worldwide, only a few of their species cause the highest damage on agricultural crops. Among the species, *Bemisia tabaci* is the most important species in agriculture.

*Bemisia tabaci* is often difficult to control using insecticides as all stages are normally located on the underside of the leaf (S. Chu et al., 1998). Furthermore, *B. tabaci* has developed high levels of resistance against several chemical classes of insecticides. Pesticide resistance usually arises from the overuse and misuse of pesticides, which is often due to lack of available alternatives (Denholm, 1988). The use of insecticides also has negative impact on environment, non-target organism and human health. These have encouraged the development of alternative methods of control. Thus, biopesticides are being developed to control *B. tabaci* around the world.
Biopesticides are pesticide in which the active ingredient (a.i) is derived from virus, fungus, bacteria or natural product from plant sources. The use of biopesticide in crop protection is a practical and sustainable alternative to the synthetic organic-based insecticides. They could maintain biological diversity of predators (Grange & Ahmed, 1988), reduce environmental contamination and human health hazards. Plant sources commonly used as biopesticide include *Azadirachta indica*, *Derris sp.*, and *Cymbopogon nardus*. Azadirachtin extracted from *Azadirachta indica* has a broad mode of action. Thus, it is difficult for the insects to build resistance to this compound. Besides, the use of agro-based carrier materials in the pesticide formulation has become more important as they are relatively biodegradable, low in toxicity and from renewable resources than those from mineral oil derived commodities (Chow et al., 1992).

Water-based formulation cannot fully control the whiteflies due to morphological and ecological characteristics of the leaf such as a waxy cuticle, and the whiteflies tendency to colonize the underside of leaves making it difficult for active ingredient (a.i) to reach the target (Osborne & Landa, 1992). Oil-based formulations droplets were found to spread much better on leaf surfaces than either water alone or water that contained adjuvant (McWhorter & Barrentine, 1988). The wider spread enables the active ingredient (a.i) to reach the target pest especially sessile insects such as whiteflies.

Aside from having good spreading ability, the formulations should also have good penetration of the active ingredient (a.i) towards the target pest. This can be achieved
by having a nano droplet size formulation. Nano-emulsion is a non-equilibrium colloidal system comprising of oil phase, surfactants and water, offers better absorption having extremely a small size droplets (100-600nm) (Shafiq et al., 2007; Solans et al., 2003) and thus could be uniformly distributed (Gutierrez et al., 2008). Oil-phase in nano-emulsion increase bioavailability of active ingredient (a.i) which allows better penetration into the waxy layers and cuticle of the leaf. However, there is limited information on the development of nano-emulsion system for oil-based biopesticide.

Thus, the objectives of this study were to:

1. Prepare oil nano-emulsion formulation of azadirachtin and determine the physiochemical properties of the formulations;

2. Evaluate the toxicity of oil nano-emulsion formulations against *Bemisia tabaci* and,

3. Verify the mode of action on oil nano-emulsion formulation in killing *Bemisia tabaci*. 
BIBLIOGRAPHY


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