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TOXICITY OF NANO-EMULSION FORMULATIONS OF AZADIRACHTIN AGAINST Tribolium castaneum (Herbst) & Sitophilus oryzae (L.)

MARZIYEH CHOUPANIAN

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By

MARZIYEH CHOU Panian

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

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DEDICATIONS

To my amazing father
for all his love and support and providing me the best education possible

To my beloved mother
for all her inspirations, patience and prays

&

To my wonderful siblings Ali & Marjan
for their unending encouragements and love

I wouldn’t have gotten to this level of education if it wasn’t for you
Loving you all...
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree Master of Science

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MARZIYEH CHOUPANIAN

April 2016

Chairman : Professor Dzolkhifli Omar, PhD
Faculty : Agriculture

Sitophilus oryzae and Tribolium castaneum are known as the most destructive and cosmopolitan pests which can cause a huge damage on most of common stored products annually. Massive use of synthetic pesticides has created serious problems such as hazards to the environment, residues in foodstuffs, and development of resistant strains. Plant-derived products could be the alternative to control the pests for many reasons such as being eco-friendly, residue-free, biodegradable and cost-effective. Neem oil is one of the plant-based pesticides comprises of azadirachtin as active ingredient. It has been used extensively against an array of pest species. Nonetheless, the problem with neem oil is the low stability of azadirachtin molecule due mainly to photo-degradation. Recently, emulsion-based formulations have gained immense interest due to their green characteristics, wide range of potential and utilization. Thus, this study was conducted to develop nano-emulsion formulations of neem oil with improved stability and toxicity against S. oryzae and T. castaneum. The nano-emulsion formulations were developed by constructing ternary phase diagrams, and four formulations coded as NF1, NF2, NF3 and NF4 were selected from the isotropic regions. All the selected formulations were miscible with neem oil and comprised of either nonionic polysorbate (Tween 80) or alkylpolyglucoside (MBL 510H) surfactant. NF1 and NF2 contained MBL510H, while NF3 and NF4 contained Tween80. All the selected formulations were stable under centrifugation and storage at ambient temperature of 25°C. However, NF2 showed phase separation at 54°C after 14 days storage and transformed to two opaque phases. The results of the mean particle size of the selected formulations showed the droplet size ranging between 200-600 nm, which can be considered as nano-emulsion formulation. The smallest droplet sizes were obtained from the NF3 and NF4 with Tween80 as surfactant. The zeta potential and surface tension of the formulations ranged between 31 to 39 mV and 30.52 to 33.33 mNm\(^{-1}\). The results also indicated viscosity and pH of the nano-emulsion formulations ranging 65 to 88 Pa.s, and 3 to 5, respectively. The toxicity of the nano-emulsion formulations against the adults of S. oryzae and T. castaneum were evaluated using filter paper impregnation method and food impregnation method. Neem oil and commercial EC formulation of azadirachtin (Neemix\textsuperscript{®}) were used as positive control. The results showed the interaction between formulations and concentrations of azadirachtin. The higher the concentration of a formulation results in increased of the mortality. The toxicity of the formulations against both insects for both methods in increasing order was NF3≥ NF4> NF1≥ NF2> Neemix> Neem oil at 24 h after exposure. The NF3 was found to be the most toxic formulation with 58.25 and 43.5% mortality against S. oryzae and T. castaneum, respectively, via food impregnation method and
after 24 h of exposure. However, lower mortality of 25.25% and 21% against *S. oryzae* and *T. castaneum*, respectively, of the filter paper impregnation method were obtained. This result indicated that the food impregnation method is more effective to control both insects. The rate of mortality as indicated by the LT\textsubscript{50} value of 9.61 h and 11.27 h against *S. oryzae* and *T. castaneum*, respectively, at 1% concentration of the NF3 for the food impregnation method was the fastest among the formulations tested. Similar trend for the NF3 was obtained for the filter paper impregnation method with LT\textsubscript{50} values of 17.01 h and 19.26 h against *S. oryzae* and *T. castaneum*, respectively. This could be due to the smaller particle size of the formulation. Polysorbate surfactant is more efficient in comparison with alkylpolyglucoside surfactant due to the higher mortality of the NF3 and NF4 on both insects. The results revealed significant increase of mortality from the nano-emulsion formulations. There is therefore immense potential for development of neem oil from these nano-emulsion formulations.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

KETOKSIKAN FORMULASI NANO-EMULSI AZADIRACHTIN TERHADAP Tribolium castaneum (Herbst) & Sitophilus oryzae (L.)

Oleh

MARZIYEH CHOUPANIAN

April 2016

Pengerusi : Profesor Dzolkhifli Omar, PhD
Fakulti : Pertanian

NF3 > NF4 > NF1 > NF2 > Neemix > minyak neem. Bagi kaedah rawatan makanan dan selepas 24 jam, NF3 merupakan formulasi yang tinggi ketoksikan dengan kadar kematian *S. oryzae* dan *T. castaneum* pada 58.25 dan 43.5%, masing-masing. Walau bagaimanapun, kadar kematian *S. oryzae* dan *T. castaneum* bagi kaedah celupan kertas turas adalah rendah iaitu pada 25.25% dan 21%, masing-masing. Keputusan ini menunjukkan bahawa kaedah rawatan makanan adalah lebih efektif untuk mengawal kedua-dua serangga. Berdasarkan nilai LT<sub>50</sub>, formulasi NF3 pada kepekatan 1% mempunyai kadar kematian *S. oryzae* dan *T. castaneum* yang paling paling Pantas berbanding formulasi lain iaitu pada 9.61 jam dan 11.27 jam, masing-masing bagi kaedah rawatan makanan. Keputusan yang sama telah perolehi pada kaedah celupan kertas turas terhadap *S. oryzae* dan *T. castaneum* bagi NF3 dengan nilai LT<sub>50</sub> iaitu 17.01 jam dan 19.26 jam, masing-masing. Tween 80 merupakan surfaktan yang lebih baik berbanding MBL510H berdasarkan jumlah kadar kematian yang diperolehi pada NF3 dan NF4 terhadap kedua-dua serangga. Keputusan juga mendedahkan peningkatan kadar kematian yang signifikan pada formulasi nano-emulsi. Maka, penghasilan minyak neem dalam formulasi nano-emulsi ini mempunyai potensi yang sangat besar.
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Finally, I would like to pay my deepest appreciation to my beloved parents and siblings for their unconditional love and encouragements throughout my life and supporting me during the entire of my study.
I certify that a Thesis Examination Committee has met on 25 April 2016 to conduct the final examination of Marziyeh Choupanian on her thesis entitled "Toxicity of Nano-Emulsion Formulations of Azadirachtin Against Tribolium castaneum (Herbst) & Sitophilus oryzae (L.)" 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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LIST OF ABBREVIATIONS

% Percent
° Degree
°C degree(s) Celsius
g gram
d day (s)
h hour (s)
APG Alkylpolyglucoside
LT<sub>50</sub> Lethal Time 50
LT<sub>90</sub> Lethal Time 90
min minute
ml milliliter
cm centimeter
mm milimeter
nm nanometer
O/W oil in water
rpm revolution per minute
S.E Standard Error
w/w weight/weight
no. number
et al. et alii, and others
CHAPTER 1

INTRODUCTION

There are several factors resulting to 10-25% annual losses in post-harvest products such as insect losses, microbial decomposition and other causes (Mohan & Fields, 2002; Jbilou et al., 2006). This losses could reach to 40% in some of the developing countries with lack of modern storage technologies (Moreira et al., 2007). Stored agricultural products are in danger of qualitative and quantitative infestation by more than 600 species of beetle pests, 70 species of moths and about 355 species of mites worldwide (Rajendran & Sriranjini, 2008).

Insects are known as the most destructive and serious pests of stored products, particularly in tropical and semi-tropical areas (Tripathi et al., 2001). Among the 600 species of the reported beetle pests the rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) and the red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae) are the most destructive and important pests of stored products especially in South-East Asia (Hill, 2002; Yoon et al., 2007; Stefanazii et al., 2011).

Integrated pest management (IPM) is one of the most common strategies to control stored cereal pests, but the employment of synthetic pesticides against pests caused many problems to the environment and human health which leads to restriction of methyl bromide usage in Europe since 2005 due to its consuming properties on the stratospheric ozone layer (Haque et al., 2000; Barakat et al., 2006).

The side effects of synthetic pesticides are not limited to human health and environmental pollution, also it is considered as a threat to non-target species. Additionally, contamination of the stored grain products due to the residue of synthetic insecticides is another negative results of the synthetic pesticides (Garry et al., 1989). Synthetic insecticide resistance impact, human health concerns and toxic residues on stored foodstuffs, and increased cost of application are the most important concerns of using synthetic insecticides. Phosphine is one example of synthetic insecticide that has caused resistant pests in more than 45 countries. Nair (2007) has reported methyl bromide and phosphine as the two major fumigant insecticides which cause the most number of resistance pests. Therefore, the global agriculture is urgently in need of developing biodegradable pesticides with more safety to non-target organisms as an alternative to synthetic insecticides (Pathak & Tiwari, 2010).

Biopesticides employed from natural based products such as powders, extracts and essential oils have been proved to be non-hazardous to human and the environment against various insect pests (Barakat et al., 2006; Bittner et al., 2008). Various pesticidal properties such as 384 antifeedants, 297 repellents, 27 attractants and 31 with growth inhibiting properties, have been recorded from more than 2000 botanical plants over the world (Jeyasankar et al., 2005; Tewary et al., 2005). The usage of botanical plants has been recorded before the fabrication of synthetic pesticides such as DDT (Jeyasankar et al., 2005). Among botanical plants, one of the most effective alternatives against insect
pests is neem. Neem, *Azadirachta indica* A. Juss, a versatile tree of family Meliaceae, is known as a multi-purpose tree which is used in different areas such as agriculture, forestry, medicine and household for more than three decades (Koul & Wahab, 2004). Neem based pesticides is being used mostly in IPM due to their effectiveness not only on mortality, but also for the different effects on physiological and behavioral effect on the target insects. Additionally, neem based pesticides showed wide range of repellent and antifeedant impacts against insect pests (Ketkar, 1976). Neem consisted of various active ingredients which the most effective one against insect pests is azadirachtin. Azadirachtin is a tetranortriterpenoid and poses classical insect growth regulatory (IGR) effects on its mechanism of action (Mordue & Blackwell, 1993; Koul, 1996b). Although, neem and other botanical insecticides have exhibit wide range of promising properties such as toxicity and biological activity against insect pests the problems associated with botanical insecticides volatility, poor water solubility, and oxidation tendency must be resolved while they are prepared to be used in pest control system (Moretti et al., 2002).

Nano-emulsion formulation system is a promising method to produce valuable source of botanical insecticides with purpose of not only preventing of degradation and destruction of pesticide by evaporation, but also obtaining a controlled release of pesticides and promoting their handling, as well. Nano-emulsion is a non-equilibrium colloidal system comprising of oil phase, surfactants and water. This system exhibits extremely small droplet size (100-600 nm) and uniformly distributed which cause better adsorption (Solans et al., 2003; Shafiq et al., 2007). Besides, nano-emulsion formulation system shown stronger impact than botanical crude extract (Anjali et al., 2012). On the other hand, the pest control system could enjoy lower toxicity of nano-pesticides towards non-target organisms by nano-emulsion formulation system compared with botanical crude extracts (Solans et al., 2003).

In this study, emulsion formulations contained neem oil were developed from the pseudoternary phase diagrams. The selected formulations were then exposed to low-energy centrifugation to obtain nano-emulsion formulation. The toxicity of the nano-emulsion formulations was then evaluated against the adults of *S. oryzae* and *T. castaneum*.

1.1 **Research Objectives**

1. To prepare and characterize oil nano-emulsion formulations of azadirachtin.

2. To evaluate toxicity of the nano-emulsion formulations against adults of *S. oryzae* and *T. castaneum*. 

REFERENCES


Stefanazzi, N., Stadler, T., & Ferrero, A. (2011). Composition and toxic repellent and feeding deterrent activity of essential oils against the stored grain pests *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae). *Pest Management Science,* 67: 639-646.


