UNIVERSITI PUTRA MALAYSIA

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

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By

MARZIYEH CHOUPANIAN

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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Chairman : Professor Dzolkhifli Omar, PhD
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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®) 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_{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_{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

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Pengerusi : Profesor Dzolkhifli Omar, PhD
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Sitophilus oryzae dan *Tribolium castaneum* lebih dikenali sebagai perosak kosmopolitan di mana ianya boleh menyebabkan kerosakan yang teruk terhadap produk simpanan pada setiap tahun. Penggunaan racun perosak secara berlebihan telah mengakibatkan pelbagai masalah serius seperti pencemaran udara, sisa kimia pada bahan makanan dan perkembangan strain yang resisten terhadap racun. Produk berasaskan tumbuhan boleh menjadi sumber alternatif untuk mengawal perosak berlandaskan beberapa sebab seperti lebih bersifat mesra alam, bebas sisa kimia, senang terurai, dan kos efektif. Minyak neem merupakan salah satu racun berasakan tumbuhan yang mengandungi azadirachtin sebagai kandungan aktif. Ia telah digunakan secara meluas terhadap pelbagai spesis perosak. Akan tetapi, masalah minyak neem adalah kestabilan molekul azadirachtin yang rendah oleh penguraian foto. Ketika ini, formulasi berasaskan emulsi telah menarik minat yang tinggi kerana lebih bersifat mesra alam, mempunyai potensi dan kegunaan yang lebih luas. Justeru itu, kajian ini di jalankan bertujuan untuk menghasilkan formulasi nano-emulsi berasaskan minyak neem untuk meningkatkan kestabilan dan ketoksikan terhadap *S. oryzae* dan *T. castaneum*. Formulasi nano-emulsi ini dihasilkan dengan membina diagram fasa 'terner' dan empat formulasi dikodkan sebagai NF1, NF2, NF3 dan NF4 telah dipilih dari kawasan isotropik. Semua formulasi yang dipilih boleh bercampur dengan minyak neem dan mengandungi sama ada surfaktan polisorbat bukan ionik (Tween 80) atau alkilpoliglukosida (MBL 510H). NF1 dan NF2 mengandungi MBL510H, manakala NF3 dan NF4 mengandungi Tween 80. Semua formulasi yang dipilih adalah stabil selepas proses emparan dan penyimpanan pada suhu ambien iaitu 25°C. Walau bagaimanapun, NF2 menunjukkan lapisan pemisahan fasa pada suhu 54°C selepas 14 hari penyimpanan dan berubah kepada dua lapisan keruh. Keputusan min saiz partikel bagi formulasi terpilih yang baru disediakan menunjukkan bacaan diantara 200-600 nm, yang mana ianya boleh di kategorikan sebagai formulasi nano-emulsi. Saiz partikel yang terkecil dimiliki oleh NF3 dan NF4 dengan Tween80 sebagai surfaktan. Keputusan potensi zeta bagi formulasi juga adalah di dalam linkungan 31 hingga 39 mV. Keputusan juga menunjukkan kelikatan dan pH formulasi nano-emulsi adalah diantara 65 to 88 Pa.s dan 3-5, masing-masing. Ketoksikan formulasi nano-emulsi terhadap *S. oryzae* dan *T. castaneum* dewasa dinilai menggunakan kaedah celupan kertas turas dan juga kaedah rawatan makanan. Minyak neem dan formulasi EC azadirachtin yang komersil (Neemix®) digunakan sebagai kawalan positif. Terdapat interaksi di antara formulasi dan kepekatan. Kepekatan formulasi yang tinggi meningkatkan kadar kematian serangga. Ketoksikan formulasi selepas 24 jam didedahkan bagi kaedah-kaedah yang digunakan terhadap kedua-dua serangga dengan urutan menaik adalah

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 $LT_{50,}$ formulasi NF3 pada kepekatan 1% mempunyai kadar kematian *S. oryzae* dan *T. castaneum* yang 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_{50} 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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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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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:

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LIST OF ABBREVIATIONS

%	Percent
°	Degree
℃	degree(s) Celsius
g	gram
d	day (s)
h	hour (s)
APG	Alkylpolyglucoside
LT_{50}	Lethal Time 50
LT_{90}	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

Abbott, W. (1925). A method of computing the effectiveness of an insecticide *Journal of Economic Entomology,* 18: 265-267.

Allotey, J., & Azalekor, W. (2000). Some aspects of the biology and control using botanicals of the rice moth, *Corcyra cephalonica* (Stainton), on some pulses. *Journal of Stored Products Research,* 36(3): 235-243.

Anjali, C. H., Sharma, Y., Mukherjee, A., & Chandrasekaran, N. (2012). Neem oil (*Azadirachta indica*) nanoemulsion- a potent larvicidal agent against *Culex quinquefasciatus*. *Pest Management Science,* 68(2): 158-163.

Anton, N., Benoit, J. P., & Saulnier, P. (2008). Design and production of nanoparticles formulated from nano-emulsion templates- a review. *Journal of Controlled Release,* 128: 185-199.

Arabi, F., Moharramipour, S., & Sefidkon, F. (2008). Chemical composition and insecticidal activity of essential oil from *Perovskia abrotanoides* (Lamiaceae) against *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). *International Journal of Tropical Insect Science,* 28(03): 144-150.

Azeem, A., Rizwan, M., Ahmad, F. J., Iqbal, Z., Khar, R. K., Aqil, M. and Talegaonkar, S., (2009). Nanoemulsion components screening and selection: a technical note. *American Association of Pharmaceutical Scientists*, 10: 69-76.

Barakat, A., El-Mahy, S., Moustafa, O., & El-Hadek, M. (2006). Joint action between some vegetable oils and their methylated form against the 4th larval instar of *Spodoptera littoralis* Boisd. *Journal of Pest Control & Environmental Science,* 14(2): 91-105.

Baseeth , S. S., & Sebree, B. R. (2010). Renewable surfactants in spray adjuvants. *Lipid Technology,* 22: 79-82.

Bell, C. H., & Wilson, S. M. (1995). Phosphine tolerance and resistance in *Trogoderma granarium* Everts (Coleoptera: Dermestidae). *Journal of Stored Products Research,* 31(3): 199-205.

Bittner, M. L., Casanueva, M. E., Arbert, C. C., Aguilera, M. A., Hernandez, V. J., & Becerra, J. V. (2008). Effects of essential oils from five plant species against the granary weevils *Sitophilus zeamais* and *Achanthoscelides obtectus* (Coleoptera). *Journal of the Chilean Chemical Society,* 53(1): 1444-1448.

Bowers, B. W., Ohta, T., Cleere, J. S., & Marsell, P. A. (1976). Discovery of insect anti-juvenile hormones in plants. *Science,* 193: 542-547.

Butterworth, J. H., & Morgan, E. D. (1968). Isolation of a substance that suppresses feeding in locusts. *Journal of Chemical Society Chemical Communications,* 35: (1) 23-24.

Calderilla-Fajardo, S. B., Cazares-Delgadillo, J., Villalobos-Garcia, R., Quintanar-Guerrero, D., & Ganem-Quintanar, A. (2006). Influence of sucrose esters on the in vivo percutaneous penetration of octyl methoxycinnamate formulated in nanocapsules, nano-emulsion and emulsion. *Drug Development and Industrial Pharmacy*, 32: 107-113.

Campbell, J. (2002). Influence of seed size on exploitation by the rice weevil, *Sitophilus oryzae*. *Journal of insect behaviour*, 15(3): 429-445.

Champ, B.R. and Dyte, C.E. (1976). Report of the FAO global survey of pesticide susceptibility of stored grain pests. FAO Plant Protection Series No. 5, FAO Rome.

Chaudhry, M. (1997). A Review of the Mechanisms Involved in the Action of Phosphine as an Insecticide and Phosphine Resistance in Stored Product Insects. *Pesticide Science*, 49(3): 213-228.

Chen, F., Wang, Y., Zheng, F., & Liang, W. (2000). Studies on cloud point of agrochemical microemulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 175: 257-262.

Chen, G. and Tao, D., (2005). An experimental study of stability of oil-water emulsion. *Journal of Fuel Processing Technology*, 86: 499-508.

Coelho, M. B., Marangoni, S., & Macedo, M. L. (2007). Insecticidal action of *Annona coriacea* lectin against the flour moth *Anagasta Kuehniella* and the rice moth *Corcyra cephalonica* (Lepidoptera: Pyralidae). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 146(3): 406-414.

Cotton, R. T. (1950). *Insect Pests of Stored Grain and Grain Products, Identification, Habits and Methods of Control*. New Dehli: Biotech Books Press.

Dukhin, A., & Bridge, G. (10 Sep, 2002). United States Patent No. US 6,449,563 B1.

Dureja, P.; Johnson, S., (2000). Photodegradation of azadirachtin – A: a neem based pesticide. *Current Science*, Bangalore, 79: 1700-1703.

El-Shafei, G. M., El-Said, M. M., Attia, H. A., & Mohammed, T. G. (2010). Environmentally friendly pesticides: Essential oil-based w/o/w multiple emulsions for anti fungal formulations. *Industrial Crops and Products*, 31: 99-106.

Flanangan, J., Kortegaard, K., Pinder, D. N., Rades, T., & Singh, H. (2006). Solubilisation of soybean oil in microemulsions using various surfactants. *Food Hydrocolloids*, 20: 253-260.

Finney, D., (1971). Probit analysis (3rd ed.): Cambridge Universiti Press.

Gamble, J. S. (1902). *A Manual of Indian Timbers*. Dehradun, Indian.

Garry, V. F., Griffith, J., Danzl, T. J., Nelson, R. L., Whorton, E. B., Krueger, L. A., & Crevenka, J. (1989). Human genotoxicity: pesticide applications and phosphine. *Science*, 246 (13): 251-255.

Gi, H. J., Chen, S. N., Hwang, J. S., Tien, C., & Kuo, M. T. (1992). Studies of Formation and Interface of Oil-Water Microemulsion. *Chinese Journal of Physics,* 30: 665-678.

Gill, J. S., & Lewis, T. (1971). *Systemic action of an insect feeding deterrent*. Nature, London, 232: 402-403.

Green, J. M., & Beestman, G. B. (2007). Recently patented and commercialized formulation and adjuvant technology. *Crop Protection,* 26: 320-327.

Gupta, S., Jafar, S., Jaiwal, V., Raman, S. P., & Maithani, M. (2011). Review on titrimetric analysis. *International Journal of Comprehensive Pharmacy,* 2(5): 1-6.

Gutierrez, J. M., Gonzalez, C., Maestro, A., Sole, I., Pey, C. M., & Nolla, J. (2008). Nano-emulsions: New applications and optimization of their preparation. *Current Opinion in Colloids and Interface Science,* 13: 245-251.

Hameed A., Freed S., Hussain A., Iqbal M., Hussain M., Naeem M., Sajjad A., Hussnain H., Sadiq M. A., and Tipu A. L. (2012). Toxicological effects of neem (*Azadirachta indica*), Kanair (*Nerium oleander*) and spinosad (Tracer 240 SC) on the red flour beetle (*Tribolium castaneum*) (Herbst.). *African Journal of Agricultural Research*, 7(4): 555-560

Hammad, A. F., Zournajian, H., & Talhouk, S. (2001). Efficacy of extracts of *Melia azedarach* L. callus, leaves and fruits against adults of the sweet potato whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae). *Journal of Application Entomology,* 125: 483-488.

Hanaor, D., Michelazzi, M., Leonelli, C., Charles, C., Sorrell, C.C., (2012). The effects of carboxylic acids on the aqueous dispersion and electrophoretic deposition of ZrO_2. *Journal of the European Ceramic Society*, 32: 235-244.

Haque, M. A., Nakakita, H., Ikenaga, H., & Sota, N. (2000). Development-inhibiting activity of some tropical plants against *Sitophilus Zeamais* Motschulsky (Coleoptera: Curculionidae). *Journal of Stored Products Research*, 36(3): 281-287.

Hassan M., Sadique M. A., Shafique M., Sagheer M., and Aleem M. (2005). Comparative efficiency of ethanol leaf extract of *A. Virides* and *Salsola barysona* (Shultes) and cypermethrin against *Trogoderma granarium*. *Pakistan Journal of Agricultural Science*, pp. 4-42.

Hemayet, U. M., Carlos, R., Kenichi, W., Arturo, L. Q., Tadashi, K., Haruhiko, F., Kunieda, H. (2001). Phase behavior and formation of reverse cubic phase based emulsion in water/poly (oxyethylene) poly (dimethylsiloxane) surfactant/silicone oil systems. *Langmuir*, 17(17): 5169-5175.

Hill, D. S. (2002). *Pests of Stored Foodstuffs and their Control*. The Netherlands: Kluwer Academic Publishers, pp. 496.

Hoeller, S., Sperger, A., & Valenta, C. (2009). Lechithin based nanoemulsions: A comparative study of the influence of non-ionic surfactants and the cationic phytosphingosine on physicochemical behavior and skin permeation. *International Journal of Pharmaceutics,* 370: 181-186.

Ignacimuthu, S., & Jayaraj, S. (2005). *Green Pesticides for Insect Pest Management*. New Dehli: Narosa Publishing House, pp. 181-187.

Izquiredo, P., Feng, J., Esquena, J., Tadros, T. F., Dederen, J. C., & Garcia, M. J. (2005). The influence of surfactant mixing ratio on nano-emulsion formation and stability. *Journal of Colloid Interface Science,* 285: 388-394.

Jafari, S. M., Assadpoor, E., & Bhandari, B. (2008). Re-coalescence of emulsion droplets during high-energy emulsification. *Food Hydrocolloids*, 22: 1191-1202.

Jbilou, R., Ennabili, A., & Sayah, F. (2006). Insecticidal activity of four medicinal plant extracts against *Tribolium castaneum*(Herbst)(Coleoptera: Tenebrionidae). *African Journal of Biotechnology,* 5(10): 936-940.

Jeyasankar, A., Raja, N., & Ignacimuthu, S. S. (2005). *Green Pesticides for Insect Pest Management: Botanical pesticides for insect control*. New Dehli, India: Narosa Publishing House Pvt. Ltd.

Kabir, V.G., (2002). Insecticides for theprotection of bagged grain products. *Zashchita i Karantin Rastenii,* (2): 36.

Kareem, A. (1999). *Biopesticides and Insect Pest Management*. New Dehli: Phonix publishing house.

Kartsev, V.N., Shtykov, S.N., Bogomolova, I.V. and Ryzhov, I.P., (2009). Thermodynamic stability of microemulsion based on sodium dodecyl sulfate. *Journal of Molecular Liquid,* 145: 173-176.

Ketkar, C. M. (1976). *Final Technical Report-Utilisation of Neem (Azadirachta indica* A. Juss*) and its By Products.* Pune, India: Sadhna Press.

Khalequzzaman, M., & Jesmun, N. (2001). Toxicity of Nine Insecticides to Adult *Tribolium castaneum* (Herbst). *Journal of Biological Science,* 1(11): 1043-1045.

Kim, S. I., Roh, J. Y., Kim, D. H., Lee, H. S., & Ahn, Y. J. (2003). Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis*. *Journal of Stored Products Research,* 39(3): 293-303.

Knowles, A. (2008). Recent developments of safer formulations of agrochemicals. *Environmentalist,* 28: 35-44.

Koehler, P. G. (2008). Rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae): EDIS.

Kogteva, E.F., Zakladnoi, G.A., (2001). Mixtures of methoprene with insecticides for protectionof stored grain. *Zashchita i Karantin Rastenii*: 7, 17-18.

Koul, O. (1996). *Mode of Azadirachtin action, in Neem (eds)*. New Dehli: New Age International Publishers Ltd.

Koul, O. (1999). Insect growth regulation and antifeedant effects of neem extracts and azadirachtin on two aphid species of ornamental plants. *Bioscience,* 24: 85-90.

Koul, O., & Wahab, S. (2004). *Neem: Today and in the New Millennium*. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Kumar, M., Misra, A., Babbar, A. K., Mishar, A. K., Mishar, P., & Pathak, K. (2008). Intranasal nanoemulsion based brain targeting drug delivery system of risperidone. *International Journal of Pharmaceutics,* 358: 285-291.

Leatemia , J. A., & Isman, M. B. (2004). Insecticidal Activity of Crude Seed Extracts of Annona spp., *Lansium domesticum* and *Sandoricum koetjape* Against Lepidopteran Larvae. *Entomology,* 32(1): 30-37.

Lee, G. W., & Tadros, T. F. (1982). Formation and stability of emulsions produced by dilution of emulsifiable concentrated. *Colloids and Surfaces,* 5: 105-115.

Lin, J. J., & Lin, S. F. (2003). Phase inversion of self-aggregation Mannich amines with poly (oxyethylene) segments. *Journal of Colloid and Interface Science,* 258(159): 310-321.

Liu, X., Guan, Y., Ma, Z., & Liu, H. (2004). Surface modification and characterization of magnetic polymer nanospheres prepared by miniemulsion polymerization. *Langmuir,* 20(23): 10278-10282.

Lu, J.-H., & He, Y.-Q. (2010). Fumigant toxicity of *Ailanthus altissima* Swingle, *Atractylodes lancea* (Thunb.) DC. and *Elsholtzia atauntonii* Benth extracts on three major stored-grain insects. *Industrial Crops and Products,* 32(3): 681-683.

Lyklema J. (1995). Fundamentals of Microfluids. Academic Press, New York.

Maclagan, S. D., & Edward, D. (1935). The Experimental Analysis of the Growth of an Insect Population, pp.126-139.

Malouki, M. A., Cavani, L., Halle, A., Ciavatta, C., & Richard , C. (2009). Photosensitizing properties of formulation adjuvants. *Journal of Photochemistry and Photobiology A: Chemistry*, 203: 186-191.

Mason, T. G., Wilking, J. N., Meleson, K., Chang, C. B., & Graves, S. M. (2006). Nanoemulsions: formation, structure, and physical properties. *Journal of Physics: Condensed Matter*, 18: 635-666.

Matthews, G. A. (2006). *Pesticides: Health, Safety and the Environment*: Wiley-Blackwell.

Mohan, S., & Fields, P. G. (2002). A simple technique to assess compounds that are repellent or attractive to stored-product insects. *Journal of Stored Products Research,* 38(1): 23-31.

Morales, D., Gutie, J. M., Garci, M. J., & Solans, Y. C. (2003). A study of the Relation between Bicontinuous Microemulsions and Oil/Water Nano-emulsion Formation. 17: 7196-7200.

Mordue, A. J., & Blackwell, A. (1993). Azadirachtin: an update. *Journal of Insect Physiology,* 39: 903-924.

Moreira, M. D., Picanco, M. C., Barbosa, L. C., Guedes, R. N., Campos, M. R., Silva, G. A., & Martins, J. C. (2007). Plant compounds insecticide activity against Coleoptera pests of stored products. *Pesquisa Agropecuria Brasileira,* 42(7): 909-915.

Moretti, M. D., Passino, S. G., Demontis, S., & Bazzoni, E. (2002). Essential Oil Formulations Useful as a New Tol for Insect Pest Control. *AAPS PharmSciTech,* 3(2): 64-74.

Mulqueen, P. (2003). Recent advances in agrochemical formulation. *Advances in Colloid and Interface Science, 106*: 83-107.

Nair, K. S. S. (2007). *Fumigation for Insect Control*. New Delhi, India: Gene-Tech Books Press.

Narang, A. S., Delmarre, D., & Gao, D. (2007). Stable drug encapsulation in micelles and microemulsions. *International Journal of Pharmaceutics,* 345: 9-25.

Negahban, M., Moharramipour, S., & Sefidkon, F. (2006). Chemical Composition and Insecticidal Activity of *Artemisia scoparia* Essential Oil Against Three Coleopteran Stored-Product Insects. *Journal of Asia-Pacific Entomology,* 9(4): 381-388.

Negahban, M., Moharrampour, S., & Sefidkon, F. (2007). Fumigant toxicity of essential oil from *Artemisia sieberi* Besser against three stored-product insects. *Journal of Stored Products Research,* 43(2): 123-128.

Nilsson, T., Fricke, C., & Arnqvist, G. (2002). Patterns of divergence in the effect of mating on female reproductive preformance in flour beetles. *International Journal of Organic Evolution,* 56(1): 111-120.

Ntoukam, G., Kitch, L. W., Shade, R. E., & Murdock, L. L. (1997). A novel method for conserving cowpea germplasm and breeding stocks using solar disinfestation. *Journal of Stored Products Research,* 33(2): 175-179.

Ogendo, J. O., Kostyukovsky, M., Ravid, U., Matasyoh, J. C., Deng, A. L., Omolo, E. O., Shaaya, E. (2008). Bioactivity of *Ocimum gratissimum* L. oil and two of its constituents aganst five insect pests attaching stored food products. *Journal of Stored Products Research,* 44(4): 328-334.

Padin, S., Dal Bello, G., & Fabrizio, M. (2002). Grain loss caused by *Tribolium castaneum, Sitophilus oryzae* and *Acanthoscelides obtectus* in stored durum

wheat and beans treated with Beauveria bassiana. *Journal of Stored Products Research,* 38(1): 69-74.

Pai, A., Feil, S., & Yan, G. (2007). Variation in polyandry and its fitness consequence among populations of the red flour beetle, *Tribolium castaneum. Proceedings of the Royal Society B,* 276: 145-151.

Pathak , C., & Tiwari, S. (2010). Toxicological effects of neem *Azadirachta indica* A. Juss leaf powder against the ontogeny of *Corcyra cephalonica* (Staint.) (Lepidoptera: Pyralidae). *Journal of Biopestides,* 3(3): 617-621.

Prasantha , B. D. (2002, September 3-5). Efficacy of burnt plant material smoke for protection of stored paddy against infestation of *Sitophilus oryzae* (L.), 25: 171.

Puri, H. S. (1999). *Neem, the Divine Tree Azadirachta indica*. Amsterdam: Harwood Academic Publishers.

Rahman, A., & Talukder, F. (2006). Bioefficacy of some plant derivatives that protect grain against the pulse beetle, *Callosobruchus maculatus*. *Journal of Insect Science*, 3: 1-10.

Rajendran, S., & Sriranjini, V. (2008). Plant products as fumigants for stored product insect control. *Journal of Stored Product Insect,* 44(2): 126-135.

Rees, D., & Rangsi, V. (2004). *Insects of Stored Products*: CSIRO.

Ribeiro, B. M., Guedes, R. N., Oliveira, E. E., & Santos, J. P. (2003). Insecticide resistance and synergism in Brazilian populations of *Sitophilus zeamais* (Coleoptera: Curculionidae). *Journal of Stored Products Research,* 39(1): 21-31.

Rosano, H. L., Weiss, A., & Gerbacia, W. E. (1967, September). In Proceedings of the 12th Internation Congress on Surface Active Substances, pp. 1,453.

Ruscoe, C. N. (1972). Growth disruption effects of an insect antifeedant. *Nature New Biology,* 236: 159-160.

Rybinski, W., Guckenbeihl, B. and Tesmann, H., (1998). Influence of co-surfactants on microemulsions with alkylpolyglucosides. *Colloids and Surface A: Physicochemical and Engineering Aspects*, 142: 333-342.

Sadurni, N., Solans, C., Azemar, N., & Garcia-Celma, M. J. (2005). Studies on the formation of O/W nano-emulsions, by low-energy emulsification methods, suitable for pharmaceutical application. *European Journal of Pharmaceutical Science,* 26: 438-445.

Santos, F. K., Neto, E. L., Moura, M. C., Dantas, T. N., & Neto, A. A. (2009). Molecular behavior of ionic and nonionic surfactants in saline medium. *Colloids and Surfaces A: Physicochemical Engineering Aspects,* 333: 156-162.

Saxena, R. C., Liquido, N. J., & Justo, H. D. (1981). Neem seed oil, a potential antifeedant for the control of the rice brown planthopper, Nilaparvata lugens, in Natural Pesticides from the Neem Tree *(Azadirachta indica A. Juss.)*. Germany.

Schmutterer, H. (1995). *The Neem Tree, Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and other Purposes.* Weinheim, Germany: VCH Publishers.

Schmutterer, H. (1990). *Properties and Potential of Natural Pesticides from the Neem Tree, Azadirachta indica*. Annual Review of Entomology, Stanford, 35: 271-297.

Schoonhoven, L. M. (1982). Biological aspects of antifeedants. *Entomology*, 31: 57-69.

Shaaya, E., Kostijukovski, M., Eilberg, J., & Sukprakarn, C. (1997). Plant oils as fumigants and contact insecticides for the control of stored-product insects. *Journal of Stored Products Research*, 33(1): 7-15.

Shafiq, S., Faiyaz, S., Sushma, T., Farhan, J. A., Khar, A. K., & Ali, M. (2007). Design and development of ramipril nanoemulsion formulation. *Journal of Biomedical Nanotechnology*, 3: 28-44.

Shazali, M. E., & Smith, R. H. (1986). Life history studies of externally feeding pests of stored sorghum: *Corcyra cephalonica* (Staint) and *Tribolium castaneum* (Hbst). *Journal of Stored Products Research*, 22(2): 55-61.

Singh, S. N. (1999). *Pest Management an Ecofriendly Approach*: The Hindu Survey of Indian Agriculture.

Slama, K., & Williams, C. M. (1966). *'Paper factor' as an inhibitor of the embryonic development of the European bug, Pyrrhocoris apterous*. Nature, 61: 210-219.

Sokoloff, A. (1974). The biology of *Tribolium castaneum* with special emphasis on genetic aspects: Oxford University Press.

Solans, C., Esquena, J., Forgiarini, A., Uson, N., Morales, D., & Izquierdo, P. (2003). *Absorption and Aggregation of Surfactants in Solution, in Nano-emulsions: Formation, Properties and Applications.* New York Marcel Dekker.

Somasundaran, P., Somil, C. M., & Purohit, P. (2006). Silicone emulsions. *Advances in Colloid and Interface Science*, 135: 121-135.

Sonneville-Aubrun, O., Simmonet, J. T., & L'Alloret, F. (2004). Nanoemulsions: A new vehicle for skincare products. *Advances in Colloid and Interface Science*, 145-149.

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.

Swarnalatha, S., Selvi, P. K., Kumar, A. G., & Sekaran, G. (2008). Nanoemulsion drug delivery by ketene based polyester synthesized using electron rich carbon/silica composite surface. *Colloids and Surfaces B: Biointerfaces*, 65: 292-299.

Tadros , T. F., Izquierdo, P., Esquena, J., & Solans, C. (2004). Formation and stability of nano-emulsions. *Advances in Colloid Interface Science*, 108: 303-318.

Tapondjou, A. L., Adler, C., Fontem, D. A., Bouda, H., & Reichmuth, C. (2005). Bioactivities of cymol and essential oils of Cupressus sempervirens and Eucalyptus saligna against *Sitophilus zeamais* Motschulsky and *Tribolium canfusum* du Val. *Journal of Stored Products Research,* 41(1): 91-102.

Taylor, P. (2003). Ostwald ripening in emulsions: estimation of solution thermodynamics of the disperses phase. *Advanced Colloid Interface,* 106: 261-285.

Tewary, D. K., Bhardwaj, A., & Shanker, A. (2005). Pesticidal activities in five medicinal plants collected from mid hills of western Himalayas. *Industrial Crops and Products,* 22(3): 241-247.

Thakur, A.K., (1999). Laboratory evaluation of some organophosphatic insecticides in stored rice as grain protectants against *Sitophilus oryzae* L. *Himachal Journal of Agricultural Research* 24: 74-78.

Tripathi, A. K., Prajapati, V., Aggarwal, K. K., & Kumar, S. (2001). Toxicity, Feeding Deterrence, and Effect of Activity of 1, 8-Cineole from *Artemisia annaua* on Progeny Production of *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Journal of Economic Entomology,* 94(4): 979-983.

Varma, J., & Dubey, N. K. (2001). Efficacy of essential oils of *Caesulia axillaris* and *Mentha arvensis* against some storage pests causing biodeterioration of food commodities. *International Journal of Food Microbiology,* 68(3): 207-210.

Wang, C. J., & Liu, Z. Q. (2007). Foliar uptake of pesticides- present status and future challenge. *Pesticide Biochemistry and Physiology,* 87: 1-8.

Weaver , M. A., Jin, X., Hoagland, R. E., & Boyette, C. D. (2009). Improved bioherbicidal efficacy by *Myrothecium verrucaria* via spray adjuvants or herbicide mixtures. *Biological Control,* 50: 150-156.

Wheeler, G. S., Slansky, F. J., & Yu, S. J. (2001). Food consumption, utilization and detoxification enzyme activity of larvae of three polyphagous noctuid moth species when fed the botanical insecticide rotenone. *Entomologia Experimentalis et Applicata,* 98: 225-239.

Williams, C. M. (1956). *The juvenile hormone of insects*. Nature, pp. 178-212.

Yoon, C., Kang, S.-H., Jang, S.-A., Kim, Y.-J., & Kim, G.-H. (2007). Repellent Efficacy of Caraway Oils for *Sitophilus oryzae* (Coleoptera: Curculionidae). *Journal of Asia-Pacific Entomology,* 10(3): 263-267.

Zakladoni, G. A., & Ratanova, V. F. (1987). *Stored-Grain Pests And Their Control*. New Dehli: Oxonian Press.

Zettler, J., Halliday, W., & Arthur, F. (1989). Phosphine resistance in insects infesting stored peanuts in the southeastern United States. *Journal of Economic Entomology,* 82(6): 1508-1511.