



UNIVERSITI PUTRA MALAYSIA

**ANALYSIS OF LETHALITY OF AZADIRACHTIN, GARLIC OIL AND
ROTENONE AGAINST *Chrysoperla nipponensis*
(NEUROPTERA: CHRYSOPIDAE)**

NURFATIN RUSLAN

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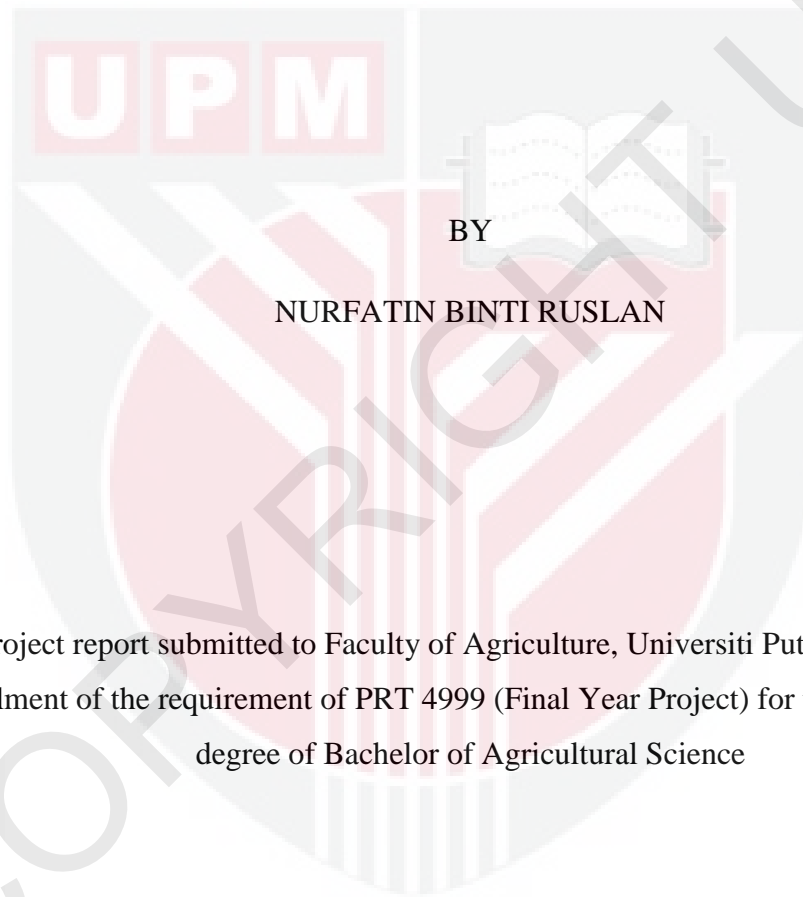
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BY

NURFATIN BINTI RUSLAN

A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science

Faculty of Agriculture
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2015/ 2016

CERTIFICATION

This project report entitled **Analysis of Lethality of Azadirachtin, Garlic Oil and Rotenone against *Chrysoperla nipponensis* (Neuroptera: Chrysopidae)** is prepared by **Nurfatin Binti Ruslan** and submitted to Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agriculture Science.

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ABSTRACT

Botanical insecticides are widely used nowadays to control insect pest population due to their lower toxicity to human and safer to the environment. However, the use of these insecticides in the field can also affect the population of the beneficial insects. *Chrysoperla nipponensis* is one of the important beneficial insects and its larvae act as predators. The information on the effects of botanical insecticides to the larvae of *C. nipponensis* is scarce. The efficacy of three botanical insecticides, azadirachtin, garlic oil and rotenone was tested on the 3rd instar larvae of *C. nipponensis* based on exposure to insecticide residues in laboratory. The larvae were exposed individually to the insecticide residues for 2 hours and then monitored daily for survivorship over a period of 5 days. The percentage of moribund and dead larvae was recorded to determine the efficacy of insecticides. Garlic oil and rotenone showed an increasing efficacy, in which the percentage of dead and moribund larvae increased by > 10% after 5-d, while azadirachtin showed stable efficacy with variability of toxicity of < 10% after similar period. Lethality index for the botanical insecticides was developed based on quantification of immediate and delayed effects of insecticides exposure on the larvae of *C. nipponensis*. Garlic oil showed the highest lethality index (23.06%) on the larvae of *C. nipponensis*, followed by rotenone (20.00%) and azadirachtin (15.00%). The lower index values indicate the three botanical insecticides do not give detrimental effect on the 3rd instar larvae of *C. nipponensis*.

ABSTRAK

*Racun serangga botani digunakan secara meluas pada masa kini bagi mengawal populasi serangga perosak kerana kurang beracun kepada manusia dan persekitaran. Walau bagaimanapun, penggunaan racun serangga dalam bidang ini juga boleh memberi kesan kepada populasi serangga berfaedah. *Chrysoperla nipponensis* merupakan salah satu daripada serangga berfaedah yang penting dan larvanya bertindak sebagai pemangsa. Keberkesanan tiga racun serangga botani, azadiraktin, minyak bawang putih dan rotenone telah diuji ke atas larva instar ke-3 *C. nipponensis* berdasarkan pendedahan kepada sisa-sisa racun serangga di dalam makmal. Larva telah diujisecara individu terhadap sisa-sisa racun serangga selama 2 jam dan dipantau setiap hari kemampuannya untuk hidup dalam tempoh 5 hari. Peratusan larva hampir menemui ajal dan mati direkodkan untuk menentukan keberkesanan racun serangga. Minyak bawang putih dan rotenone menunjukkan keberkesanan yang semakin meningkat, di mana peratusan larva mati dan hampir menemui ajal meningkat sebanyak > 10% selepas 5 hari, manakala azadiraktin menunjukkan keberkesanan yang stabil dengan kepelbagaian ketoksikan <10% selepas tempoh yang sama. Indeks kematian bagi racun serangga botani telah dibina berdasarkan kuantifikasi kesan serta-merta dan kesan jangka masa panjang selepas pendedahan racun ke atas larva *C. nipponensis*. Minyak bawang putih menunjukkan indeks kematian tertinggi (23.06%) ke atas larva *C. nipponensis*, diikuti oleh rotenone (20.00%) dan azadirachtin (15.00%). Nilai indeks yang rendah menunjukkan ketiga-tiga racun serangga botani tidak memberi kesan yang memudaratkan kepada larva instar ke-3 *C. nipponensis*.*

CHAPTER 1

INTRODUCTION

Botanical insecticides are referred to the products extracted or chemical isolated from the plant parts. They undergo certain processes and are used in the field to control and manage the insect pest population. Botanical insecticides are less toxic materials to human and the surroundings compared to the synthetic insecticides. Botanical insecticides have been chosen to be used as one of the alternatives to control the pest population. History has shown the early botanical insecticide, nicotine was introduced in the XVII Century. Nicotine obtained from tobacco leaves can kill plum beetles.

Besides, it does not show favourable effectiveness response when being tested by the scientific methods. The use of botanical insecticides was replaced following the introduction of synthetic insecticides after the Second World War. Synthetic insecticides are the popular chemical control and widely used nowadays by the farmers. It is because of the rapid acting, readily available and also highly reliable in controlling the pest population in the farm. However, the use of the synthetic insecticides has been discovered to give a threat to human health and the environment which may lead to the undesirable effect, pollution, development of insecticide resistance and negative effects to the non- target organisms (Medina et al., 2004).

Other than that, synthetic insecticides are costly and leave residues on the crops that could affect the consumer. In order to solve this problem, botanical insecticide has been chosen as the driving forces for changes of insecticide usage in insect pest management. There are many botanical insecticides introduced nowadays, for examples rotenone, neem, garlic oil and andrographolide. The main reason in using insecticides in the field is to control the pest population that attacks the crops. However, the use of insecticides in the field may also affect other non- target organisms such as beneficial insects. The beneficial insect existence in the field can help to suppress insect pest population.

Green lacewing, *Chrysoperla nipponensis*, is one of the important beneficial insects in Malaysia. It is commonly found to control the insect pest population in the vegetables and fruit fields. The adults of *C. nipponensis* feed on pollen, sweet nectar and honey and they do not kill insect pest. However, their larvae do kill insect pest and the important predator of the Homopteran such as the aphids, whiteflies and mealybugs. The control of insect pest by using synthetic insecticide in the field could cause harmful effect to the population of beneficial insects in the field. Botanical insecticides as the alternative for environmentally- friendly method of insect pest control since they have no effect to non- target organisms, environmental pollution and health hazards. However, there is not much research conducted on the analysis of lethality of botanical insecticide on the larvae of *C. nipponensis*.

Therefore, this experiment was conducted based on objectives to: 1) measure immediate effects of botanical insecticide exposure on *C. nipponensis* larvae 2) identify the recovery and mortality rates of individuals over a 5 day period and 3) establish the relative efficacy of all tested botanical insecticides based on the objectives (1) and (2) properties. The outcome from this experiment will enable us to plan the best solution in controlling the insect pest population, which at the same time the beneficial insect population in the field will not be disturbed.

REFERENCES

- Amarasekare, K. G., & Shearer, P. W. (2013). Comparing effects of insecticides on two green lacewings species, *Chrysoperla johnsoni* and *Chrysoperla carnea* (neuroptera: Chrysopidae). *Journal of Economic Entomology*, 106(3), 1126-1133. doi:10.1603/EC12483
- Amarasekare, K. G., & Shearer, P. W. (2013). Life history comparison of two green lacewing species *Chrysoperla johnsoni* and *Chrysoperla carnea* (neuroptera: Chrysopidae). *Environmental Entomology*, 42(5), 1079-1084. doi:10.1603/EN13070
- Bharadwaj A., Hayes L. E. and Stafford K. C. 2015. Effectiveness of Garlic for the Control of *Ixodes scapularis* (Acari: Ixodidae) on Residential Properties in Western Connecticut, *Journal of Medical Entomology*, 52 (4): 722- 725.
- Biochemical biopesticides: Insect growth regulators. (2015). Retrieved from <http://www.biopesticideindustryalliance.org/biochemical-biopesticides/insect-growth-regulators/>
- Castrique, E. What is rotenone? Retrieved from <http://www.chm.bris.ac.uk/motm/rotenone/page2.htm>
- Charleston, D. S., Kfir, R., Dicke, M., & Vet, L. E. M. (2005). Impact of botanical pesticides derived from melia azedarach and azadirachta indica on the biology of two parasitoid species of the diamondback moth. *Biological Control*, 33(2), 131-142. doi: <http://dx.doi.org/10.1016/j.biocontrol.2005.02.007>
- Chiam, W. Y., Huang, Y., Chen, S. X. & Ho, S. H. (1999) Toxic and antifeedant effects of allyl disulfide on *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Sitophilus zeamais* (Coleoptera: Curculinidae). *Journal of Economic Entomology*, 92, 239- 245
- Choi, M., Mochizuki, A., & Henry, C. S. (2015). The green lacewing, *Chrysoperla nipponensis* in nature and in an insectary population in korea: Song types and mitochondrial COI haplotypes. *Journal of Asia-Pacific Entomology*, 18(2), 151-155. doi: <http://dx.doi.org/10.1016/j.aspen.2014.12.009>
- Corzo-Martinez, M., N. Corzo, and M. Villamiel. 2007. Biological properties of onions and garlic. *Trends Food Sci. Technol.* 18: 609-625

- Duso, C., Malagnini, V., Pozzebon, A., Castagnoli, M., Liguori, M., & Simoni, S. (2008). Comparative toxicity of botanical and reduced-risk insecticides to mediterranean populations of *Tetranychus urticae* and *Phytoseiulus persimilis* (Acari tetranychidae, phytoseiidae). *Biological Control*, 47(1), 16-21. doi: <http://dx.doi.org/10.1016/j.biocontrol.2008.06.011>
- El-Wakeil N.E. (2013). Botanical pesticides and their mode of action.65(4), 125--149. doi:10.1007/s10343-013-0308-3
- Esmaeily, S., Samih, M. A., Zarabi, M., & Jafarbeigi, F. (2014). Sublethal effects of some synthetic and botanical insecticides on *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Journal of Plant Protection Research*, 54(2), 171-178. doi:10.2478/jppr-2014-0027
- Flint, H. M., Parks, N. J., Holmes, J. E., Jones, J. A & Higuera, C. M. (1995) Test on garlic oil for the control of the silverleaf whitefly, *Bemisia argentifolia* Bellows and Perring (Homoptera: Aleyrodidae) in cotton. *Southwestern Entomologist*, 20, 137-150.
- Giger, M. (2002). The neem tree. Retrieved from <http://www.gigers.com/matthias/engmala/neemtree.htm>
- Gurusubramanian, G. & Krishna, S.S. (1996) The effects of exposing eggs of four cotton insect pests to volatiles of *Allium sativum* (Liliaceae), *Bulletin of Entomological Research*, 86, 29- 31.
- Hodgson W. E, Trina J. (2008). Beneficial insects: Lacewings and antlions. Retrieved from: https://extension.usu.edu/files/publications/factsheet/beneficial-insects_lacewings-and-antlions.pdf
- Isman M. B. (2005). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world.(51), 45-66.
- Isman, M. B. 2000. Plant essential oils for pest and disease management. *Crop Prot.* 19: 603- 608.
- Isman, M. B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.* 51: 45- 66
- Jarial, M.S. (2001) Toxic effect of garlic extracts on the eggs of *Aedes aegypti* (Diptera: Culicidae): a scanning electron microscopic study. *Journal of Medical Entomology*, 38, 446- 450
- Jones, A.M. & C. Pagan, 1949. A further toxicological comparison of Derris and Lonchocarpus. *Journal of Agricultural Research*, 77(9): 10, Washington D.C.

- Kazem, M. G. T., & Shereifa, A. E. H. N. E. (2010). Toxic effect of capsicum and garlic xylene extracts in toxicity of boiled linseed oil formulations against some piercing sucking cotton pests. *8*(4), 390-396.
- Khaliq, A., Khan, A. A., Afzal, M., Tahir, H. M., Raza, A. M., & Khan, A. M. (2014). Field evaluation of selected botanicals and commercial synthetic insecticides against thrips *Tabaci lindeman* (Thysanoptera: Thripidae) populations and predators in onion field plots. *Crop Protection*, *62*, 10-15. doi:10.1016/j.cropro.2014.03.019
- Khan, H. H., Kumar, A., Habil, D., & Yogi, K. (2014). Effects of botanical and chemical insecticides on the percentage mortality of third instar larvae of *Helicoverpa armigera* (hubner). *Pestology*, *38*(3), 35-39.
- Green lacewing, (2015). Retrieved from: http://www.dirtdoctor.com/garden/Lacewing-Green_vq878.htm
- Lanzotti, V. 2006. The analysis of onion and garlic. *J. Chromatogr.* 1112A: 3 22.
- Leskey, T. C., Lee, D., Short, B. D., & Wright, S. E. (2012). Impact of insecticides on the invasive halyomorpha halys (hemiptera: Pentatomidae): Analysis of insecticide lethality. *Journal of Economic Entomology*, *105*(5), 1726-1735. doi:10.1603/EC12096
- Malczewska, M., Gelman, D. B., & Cymborowski, B. (1988). Ecdysone: From biosynthesis to mode of action effect of azadirachtin on development, juvenile hormone and ecdysteroid titres in chilled *Galleria mellonella* larvae. *Journal of Insect Physiology*, *34*(7), 725-732. doi:[http://dx.doi.org/10.1016/0022-1910\(88\)90084-4](http://dx.doi.org/10.1016/0022-1910(88)90084-4)
- Medina, P., Budia, F., Del Estal, P., & ViÑ±uela, E. (2004). Influence of azadirachtin, a botanical insecticide, on *Chrysoperla carnea* (stephens) reproduction: Toxicity and ultrastructural approach. *Journal of Economic Entomology*, *97*(1), 43-50. doi:10.1603/0022-0493-97.1.43
- Mordue (Luntz), A. J., M. S J. Simmonds, S. V. Ley, W. M. Blaney, W. Mordue, M. Nasiruddin, and A. J. Nisbet. Action of azadirachtin, a plant allelochemical against insects. *Pesticide Sci* 1998. *54*:277–284
- Mordue(Luntz), A. Jennifer, & Nisbet, Alasdair J.. (2000). Azadirachtin from the neem tree *Azadirachta indica*: its action against insects. *Anais da Sociedade Entomológica do Brasil*, *29*(4), 615-632. Retrieved October 28, 2015, from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0301-80592000000400001&lng=en&tlng=en
- Mustafizur, R.G.K.M., and N. Motoyama. 2000. Repellent effect of garlic against stored product pests. *Nippon Noyaku Gakkaishi* *25*: 247- 252

- Natural pest control with green lacewings. (2015). Retrieved from <http://www.gardeninsects.com/greenlacewings.asp>
- Pandey, S., Singh, B. K., & Gupta, R. P. (2013). Effect of neem based botanicals, chemicals and bio-insecticides for the management of thrips in onion. *Indian Journal of Agricultural Research*, 47(6), 545-548.
- Parmar, B.S., S. Walia, O. Koul & G.S. Dhaliwal, 2001. Prospects and problems of phytochemical biopesticides. *Phytochem. Biopestic.* 9:192-223
- Pesticide information profile: Azadirachtin. (2008). Retrieved from <http://pmep.cce.cornell.edu/profiles/extoxnet/24d-captan/azadirachtin-ext.html#5>
- Pesticide News. (2001). Factsheet- rotenone. Retrieved from <http://www.pan-uk.org/pestnews/Actives/rotenone.htm>
- Rivlin R. S. 2001. Historical perspective on the use of garlic. *J Nutr.* 131: S951- S954
- Saeed, R. M. A., A. A. Zayed, A. H. El Namaky, H. M. Ismail, and H. Y Mady. 2010. Biochemical studies on *Culex pipiens* (L.) (Diptera: Culicidae) exposed to *Allium sativum*, Citrus limon and *Bacillus thuringiensis israelensis* with reference to assessment of the biosafety on albino mice. *Global Vet* 4: 22- 33.
- Saiful Irwan Zubairi, Mohamad Roji Sarmidi and Ramlan Abdul Aziz. A Study of Rotenone from Derris Roots of Varies Location, Plant Parts and Types of Solvent Used. *Adv. Environ. Biol.*, 8(2), 445-449, 2014
- Shepard HH. 1951. *The Chemistry and Action of Insecticides*. New York: McGraw- Hill. 504 pp.
- Singh, D. K., Verma, T. C., Aswal, S., & Aswani, G. (2014). Effect of different botanical pesticides against thrips tabaci on garlic crop. *Asian Agri-History*, 18(1), 57-61.
- Syafique Ahmad, Postgraduate student, Fakulti Pertanian, Universiti Putra Malaysia, personal communication, Mei 2015.
- Tassan, H. (2014). Identification: Natural enemies gallery (green lacewings). Retrieved from http://www.ipm.ucdavis.edu/PMG/NE/green_lacewing.html
- Weathersbee, A. A., & McKenzie, C. L. (2005). Effect of a neem biopesticide on repellency, mortality, oviposition, and development of *Diaphorina citri* (homoptera: Psyllidae). *Florida Entomologist*, 88(4), 401-407. doi:10.1653/0015-4040(2005)88[401:EOANBO]2.0.CO;2

White J., J. D. (2013). Vendors of microbial and botanical insecticides and insect monitoring devices. Retrieved from <http://www2.ca.uky.edu/entomology/entfacts/ef124.asp>

Woolfolk SW1, Smith DB, Martin RA, Sumrall BH, Nordlund DA, Smith RA. (2007).

World Health Organisation(WHO), Recommended classification of pesticide by hazard, WHO/PCS/01.4 (2000-01)

Yang, M. M., and J. H. Huang. 2004. A new record of *Cacopsylla* species (Hemiptera: Psyllidae) from pear orchards in Taiwan. *Formosan Entomol.* 24: 213-220

Zhao, N. N., Zhang, H., Zhang, X. C., Luan, X. B., Zhou, C., Liu, Q. Z., . . . Liu, Z. L. (2013). Evaluation of acute toxicity of essential oil of garlic (*Allium sativum*) and its selected major constituent compounds against overwintering *Cacopsylla chinensis* (hemiptera: Psyllidae). *Journal of Economic Entomology*, 106(3), 1349-1354. doi:10.1603/EC12191