THE EFFECT OF 2, 4-D (2, 4-Dichlorophenoxyacetic Acid) AND GLYPHOSATE (N-phosphonomethyl glycine) TOXICITY ON STINGLESS BEE, Trigona Itama USING DIRECT SPRAY AND BIOASSAYS METHODS

MOHD HAIRUL FAZLI BIN MD AMIN

FP 2018 14

THE EFFECT OF 2, 4-D (2, 4-Dichlorophenoxyacetic Acid) AND GLYPHOSATE (N-phosphonomethyl glycine) TOXICITY ON STINGLESS BEE, *Trigona Itama* USING DIRECT SPRAY AND BIOASSAYS METHODS

BY

MOHD HAIRUL FAZLI BIN MD AMIN

FACULTY OF AGRICULTURE
UNIVERSITI PUTRA MALAYSIA
SERDANG, SELANGOR DARUL EHSAN
2017/2018

THE EFFECT OF 2, 4-D (2, 4-Dichlorophenoxyacetic Acid) AND GLYPHOSATE (N-phosphonomethyl glycine) TOXICITY ON STINGLESS BEE, *Trigona Itama* USING DIRECT SPRAY AND BIOASSAYS METHODS

BY

MOHD HAIRUL FAZLI BIN MD AMIN

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

Faculty of Agriculture

Universiti Putra Malaysia

2017/2018

CERTIFICATION PAGE

This project report entitled "**THE EFFECT OF 2, 4-D (2, 4-Dichlorophenoxyacetic Acid) AND GLYPHOSATE (N-phosphonomethyl glycines) TOXICITY ON STINGLESS BEE, *Trigona Itama* USING DIRECT SPRAY AND BIOASSAYS METHODS**" is prepared by Mohd Hairul Fazli bin Md Amin and submitted to the Faculty of Agriculture in fulfilment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Agricultural Science.

Students's name: Student's signature:

MOHD HAIRUL FAZLI BIN MD AMIN _____

Certified by:

_____

(DR. NORIDA MAZLAN)

Project Supervisor

Department of Agriculture Technology

Faculty of Agriculture

Universiti Putra Malaysia

Date: _____

ACKNOWLEDGEMENT

Assalamualaikumwarahmatullahiwabarakatu,

Firstly, I wish to express my thankfulness to Almighty Allah S.W.T because of His bless and guidance to me from the beginning until my project end. Apart from my effort on doing this project, most it comes from my family, my friends, supervisors and many other. The success of this project depends on the encouragement and guidelines from those that I already mention earlier.

Firstly, I would like to express my appreciation to my project supervisor. Dr. Norida Mazlan for her welcoming me in carrying this project and her invaluable advice, encouragement and ideas to make sure this project run successfully. I also like to express my thankfulness to the postgraduate students, Nur Adibah Ishadi and Engku Ahmad Khairi bin Engku Ariff for always helping me during the experiment.

Secondly, I also would like to thank to my family especially my father, Md. Amin Bin Md Said and mother, Jamaiah Binti Misran that support me, pray, giving advice, motivation to complete my project smoothly. I also would like to thank to my friends especially Nurul Sahirah Saroni and Aisyah Binti Mahamed because helping and support me to fulfil my project until successful. Without their support and advice, I will not be able to achieve this stage.

Lastly, I would like to express my thankful to all the staff in Department of Agriculture Technology especially for the laboratory staff, En. Jarkasi Sarbini, En Johari and En. Hishammudinn Zainudin for the advice and guidance to handle the experiment in the laboratory.

TABLE OF CONTENT

CHAPTER 2

LITERATURE REVIEW

CHAPTER 3

METHODOLOGY

CHAPTER 4

RESULT AND DISCUSSION

CHAPTER 5

LIST OF TABLES

Table

LIST OF FIGURES

Figures

LIST OF APPENDICES

Appendix

ABSTRACT

Stingless bee (*Trigona itama*) is the largest group of eusocial insects on earth, simply called as meliponines. The most common genus of the stingless bees are *Melipona* and *Trigona*. Stingless bees are the main pollinators of many flowering plants in tropics. Among various honey in Malaysia, the honey produced from *Trigona* contains high level of potassium, magnesium, iron and zinc. However, because this insect used as a pollinator, there are some issues that relate to the herbicide uses towards the pollinator insect especially bees. Herbicides are the most widely used pesticide in agriculture worldwide both in terms of the volumes used and the areas treated. Although it is used to killed weeds, however, it could killed stingless bees directly or indirectly. The objective of this study is to determine the mortality and toxicity level of two most commonly used herbicide on stingless bee. This experiment were conducted at two different places which are the Field 10, Faculty of Agriculture, Universiti Putra Malaysia and Laboratory C, Department of Plant Protection, Faculty of Agriculture, UPM. Direct spray method and filter paper bioassay method were used to determine the mortality and toxicity level of herbicides on stingless bee. For direct spray method, a recommendation rate of 2, 4-D Amine and Glyphosate were sprayed directly to stingless bee. The second experiment is filter paper bioassay method, where four concentrations of 2, 4-D amine and Glyphosate and were tested to find the LC_{50} value of the herbicides. Both experiments were replicated four times with 10 stingless bees in each replication. On direct spray method shows that these herbicides does have toxicity effect on stingless bee as after 24 hour, all stingless bee died in both treatments. The toxicity study shown 2, 4-D herbicide are more toxic than Glyphosate. LC_{50} Value of 2, 4-D amine for 24 and 48 hr are 296.153 ppm and 137.128 ppm respectively while

Glyphosate in 24 and 48 hour are 483.509 ppm and 315.423 ppm respectively. Thus 2, 4-D Amine has higher toxicity level compare to Glyphosate.

ABSTRAK

Lebah kelulut (*Trigona itama*) adalah kumpulan serangga yang paling aktif di dunia, yang dipanggil sebagai meliponin. Genus lebah yang paling biasa adalah Melipona dan Trigona. Lebah kelulut merupakan agen pendebungaan utama tumbuhan berbunga di kawasan tropika. Antara madu lebah di Malaysia, madu yang dihasilkan dari kelulut mengandungi tahap kalium, magnesium, zat besi dan zink yang tinggi. Walau bagaimanapun, kerana serangga ini digunakan sebagai agen pendebungaan, terdapat beberapa isu yang berkaitan dengan penggunaan racun herba terhadap serangga pendebungaan terutama lebah. Racun rumpai adalah racun yang paling banyak digunakan di dalam sektor pertanian di seluruh dunia dari segi jumlah yang digunakan dan kawasan yang dirawat dengan racun herba. Walaupun ia digunakan untuk membunuh rumpai, secara langsung atau tidak langsung racun ini mampu membunuh lebah. Terdapat dua tujuan eksperimen ini dijalankan kerana kita menggunakan dua jenis racun rumpai yang berbeza. Yang pertama adalah untuk mengkaji tahap kematian menggunakan racun 2, 4-D amine dan Glyphosate isopropylammonium pada lebah kelulut dan untuk menentukan tahap ketoksikan 2, 4-D amine dan glyphosate isopropylammonium pada lebah. Eksperimen ini akan dijalankan di dua tempat yang berbeza iaitu di Ladang 10, UPM dan Makmal C, Jabatan Perlindungan Tumbuhan, Fakulti Pertanian, UPM. Kaedah semburan secara langsung dan kaedah bioesei menggunakan cakera kertas penapis yang akan digunakan untuk menentukan tahap kematian dan ketoksikan racun rumpai pada lebah kelulut. Untuk kaedah semburan secara langsung, kadar semburan yang disyorkan untuk Glyphosate dan Hextar 2, 4-D amine akan disembur terus ke lebah. Eksperimen kedua adalah kaedah bioesei menggunakan kertas cakera, di mana empat kepekatan

iaitu Glyphosate dan 2, 4-D Amine akan diuji untuk mencari nilai LC_{50}racun rumpai tersebut. Kedua-dua eksperimen ini akan diulang empat kali dengan 10 ekor lebah kelulut dalam satu ulangan. Pada kaedah semburan langsung menunjukkan bahawa racun herba ini mempunyai kesan ketoksikan pada lebah tanpa selepas 24 jam, semua lebah mati dalam kedua-dua rawatan. Kajian toksisiti yang ditunjukkan menunjukkan bahawa racun rumpai 2, 4-D lebih toksik daripada Glyphosate. Pada kaedah semburan langsung menunjukkan bahawa selepas 24 jam, lebah mati di sangkar. Nilai LC_{50} 2, 4-D amine selama 24 dan 48 jam masing-masing adalah 296.153 ppm dan 137,128 ppm manakala Glyphosate dalam masa 24 dan 48 jam masing-masing adalah 483.509 ppm dan 315.423 ppm. Oleh itu 2, 4-D Amine mempunyai tahap ketoksikan yang lebih tinggi berbanding dengan Glyphosate.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Pollination is the most important insect activity that keeps the ecosystem maintain and it can increase food security and improve live hoods. Many insect including bees and stingless bees are the main pollinator of the flowering plants. Herbert et al., (2014) stated that *Apis mellifera* is the main pollinator in agricultural environment and is a well-known model insect for behavioural research. Honey bees and other bees are also accurate biosensors of environmental pollutants and their appetite behavioural response is a suitable tool to test sub-lethal effect of agrochemicals (Herbet et al., 2014) .Stingless bees has small size compare to honey bees. A very important role-played by stingless bees is to pollinate a flowering plant resulting in increased in quantity and quality of fruit and seeds. Stingless bee is also important to human especially on the healthcare that come from the honey, beebread and propolis.

Among various honey in Malaysia, the honey produced from Trigona contains high level of potassium, magnesium, iron and zinc (Moniruzzaman, 2014). The activity to culturing this stingless bees are called meliponiculture. In Malaysia, the most common genus of the stingless bees are Melipona and Trigona and the species that used by the farmers for meliponiculture is *Trigona itama* snd *Trigona thoracica*. In stingless bee colony they have three different division which is the queen, workers and drone, each division has their own function and role. The most important part in stingless bee's body is the hind legs which use are for collecting and transporting the pollen.

In food security, there is a need to ensure enough food production for the people. Chemical such as herbicides, insecticide, fungicides are used to protect the crop from the pest and also to maintain the quality of the food. However, these chemicals are harmful to beneficial insect especially the pollinator insect such as stingless bees and honey bees. Stingless bees are exposed to pesticide in two ways either tropical or oral exposure. The oral exposure when they consume the flower or the pesticide itself exposed directly on the bees. When they return to hive, they will bring the pesticide residue to the colony and brood. As for the larvae, pollen is the main food sources, and if it contains pesticide residue, then it can cause mortality (Gill & Raine, 2014). Although the herbicides are used to kill weeds, however they could also killed stingless bees directly or indirectly.

Example of herbicides that are mostly being used in the agricultural sector to kill the weed is Glyphosate and 2, 4-D amine. Glyphosate is a broad-spectrum herbicide used for weed control (Herbert et al., 2014). Glyphosate is the herbicide that kills all the weed type and can potentially kill the crops. This herbicide mode of action inhibit the aromatic amino acids tryptophan, tyrosine, and phenylanine synthesis, which are all needed for protein synthesis or for biosynthetic pathway that leading to growth. The 2, 4-D amine herbicide is selective to broadleaf plant such as *Ageratum conyzoides, Clidemia hirta, Amaranthus sp*. and many others. This herbicide is addition of synthetic Indole Acetic Acid (IAA) that results in an imbalance of the growth regulating hormone. This herbicide is mostly kill the pollinator by the inert ingredient of the herbicides and not the active ingredient of the herbicide. The research on the sensitivity of herbicide is lacking because usually herbicides is used to kill the weeds and not to kills the pollinator. Therefore the objective of this research is to determine the mortality and effects of 2, 4-D amine and glyphosate on stingless bee.

REFERENCES

Aktar , M., Sengupta, D., & Chowdhury, A. (2009). Impact of pesticides use in agriculture:. *Interdisciplinary Toxicol*, 1-12.

Arcaute, C. D., S. Soloneski, & Larramendy, M. (2018). Synergism of mixtures of dicamba and 2,4-dichlorophenoxyacetic acid herbicide formulations on the neotropical fish Cnesterodon decemmaculatus (Pisces, Poeciliidae). *Environmental Pollution*, 33-39.

Florencia, F., Carolina, T., Enzo, B., & Leonardo, G. (2017). Effects of the herbicide glyphosate on non-target plant native species from Chaco forest (Argentina). *Ecotoxicology and Environmental Safety*, 360-368.

García-Espiñeira, M., Tejeda-Benitez, L., & Olivero-Verbel, J. (2018). Toxicity of atrazine- and glyphosate-based formulations on Caenorhabditis elegans. *Ecotoxicology and Environmental Safety*, 216-222.

Gill, R., & Raine, N. (2014). Chronic impairment of bumblebee natural foraging behaviour induced by sublethal pesticide exposure. *Functional Ecology*, 1459-1471.

Herbert,T.,. Vázquez, L., E, Arenas, D. &. Farina, W. M (2014). Effects of field-realistic doses of glyphosate on honeybee appetitive bahaviour . *The Journal of Experimental Biology*, 217, 3457-3464.

Jaapar, M., Jajuli, R., & Mispan, M. R. (2016). *Lebah Kelulut Malaysia, Biologi Dan Penternakan*. Institut Penyelidikan dan Kemajuan Pertanian (MARDI).

Jones A. T. P. (1964) Effect on honey bees of 2, 4-D, *New Zealand Journal of Agricultural Research*, 7:3, 339-342

Kwapong, P., Aidoo, K., Combey, R. & Karikari, A. 2010. Stingless Bees Importance, Management and utilisation A Training Manual for Stingless Beekeeping. *Unimax Macmillan Ltd*, Ghana. Pp 1-28.

Lipok, J. (2009). Dual action of phosponate herbicides in plants affected by herbivore-Model study on Black bean aphid Aphis Fabae rearing on broad bean Vicia faba plants. *Ecotoxicology and Environmental Safety*, 1701-1706.

Lambert, O., Piroux , M., Puyo, S., Thorin, C., Larhantec, M., Delbac, F., & Pouliquen, H. (2012). Bees, honey and pollen as sentinels for lead environmental contamination. *Envirnmental Pollution*, 254-259.

Mullin A, C., Franzier, M., L. Frazier, J., Ashcraft, S., Simonds, R., & VanEngelsdorp, D. (2010). High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health. *PLoS One 5 (3)*, e9754. http://dx.doi.org/10.1371/journal.pone.0009754.

Mullin A, C. (2015). Effects of 'inactive' ingredients on bees. *ScienceDirect*, 194-200.

Milan, M., Rovere, G., Smits, M.., Ferraresso, S.. Pastore, P Marin, M.,. and Motozzo, V. (2018). Ecotoxicological effects of the herbicide glyphosate in non-target aquatic species: Transcriptional responses in the mussel Mytilus galloprovincialis. *Environmental Pollution*, 442-451.

Moniruzzaman, M.,YungAn,C.,Rao, P.V., Hawlader,M.N.I.,Azlan, S.A.B.M., Sulaiman, S.A. & Gan, S.H., 2014. Identification of phenolic acids and flavonoids in monofloral honey from Bangladesh by high performance liquid chromatography: determination of antioxidant capacity. *BioMed Res. Int*., 2014

Moffet, O.J., & Morton, L.H. (1975). How Herbicides Affect Honey Bees. *American Bee Journal Vol. 115(5)*, 178,179 and 200.

Rao, P. V., Krishnan, K. T., Salleh, N., & Gan, S. H. (2016). Biological and therapeutic effects of honey produced by honey bees and stingless bees: a comparative review. *Brazilian Journal of Pharmacognosy*, 657-664.

Shackleton, K., Toufailia, H. Al, Balfour, N. J., Nascimento, F. S., Alves, D. A., & Ratnieks, F. L. W. (2015). Appetite for self-destruction: Suicidal biting as a nest defense strategy in Trigona stingless bees. *Behavioral Ecology and Sociobiology*, 69(2), 273–281.

Tuksitha, L., Sophia Chen, Y.-L., Chen, Y.-L., Wong, K.-Y., & Peng, C.-C. (2018). Antioxidant and antibacterial capacity of stingless bee honey from Borneo (Sarawak). *Journal of Asia-Pacific Entomology*, 563-570.

USEPA, 1974. United States Environmental Protection Agency. Compendium of Registered Pesticides U.S. Government Printing Office, Washington, DC.

Wu J.Y, Aneli. C. & Sheppard W.S. (2011). Sub-Lethal Effects of Pesticide Residue in Brood Comb on Worker Honey Bee (*Apis mellifera*) Development and Longevity. *PLoSONE 6(2): e14720*. https://doi.org/10.1371/journal.pone.0014720