Faculty of Resource Science and Technology

Toxicity Test of Three Commonly Used Herbicides on Soil-Dwelling Ant, *Odontomachus simillimus* and Weaver Ant, *Oecophylla smaragdina* (Hymenoptera: Formicidae)

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DECLARATION

No portion of the work referred to in this dissertation has been submitted in support of an application for another degree qualification of this or any other university or institution of higher learning.

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LIST OF ABBREVIATIONS

CO_2	Carbon dioxide
LD_{50}	Median lethal dose 50
LD_{90}	Median lethal dose 90
LT_{50}	Median lethal time
LC_{50}	Median lethal concentration
m	Meter
mm	Millimetre
N/A	Not available
µL	Microliter
SDA	Soil-dwelling ant
WA	Weaver ant
%	Percentage

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Toxicity Test of Three Commonly Used Herbicides on Soil-Dwelling Ant, *Odontomachus simillimus* and Weaver Ant, *Oecophylla smaragdina* (Hymenoptera: Formicidae)

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ABSTRACT

A study on the toxicity test of three commonly used herbicides (glyphosate-isopropylamine, triclopyr butotyl and clethodim) against soil-dwelling ants, *Odontomachus simillimus* and weaver ants, *Oecophylla smaragdina* were tested using topical toxicity test. Different concentrations (5, 15, 25, 35, 45, 55 and 65%) with five replicates for each concentration including control were tested on both ants. Each ant was treated with one microliter (1 μL) of herbicide at the thorax. Mortality of both ants was recorded after 24 hours (2, 4, 6, 12 and 24 hours). Using probit analysis, LD_{50} and LD_{90} values of glyphosate-isopropylamine for soil-dwelling ants were 1.76% and 1.83%, triclopyr butotyl were 0.47% and 0.94%, and clethodim were 0.64% and 1.04%. LD_{50} and LD_{90} values of glyphosate-isopropylamine for weaver ants were 1.56% and 1.88%, and clethodim were 1.56% and 2.27%. However, LD_{50} and LD_{90} values of triclopyr butotyl for weaver ants were unable to determine as there is 100% mortality within the 24 hours observation. In comparison, for soil-dwelling ants, triclopyr butotyl was more toxic than clethodim, and clethodim is more toxic than glyphosate-isopropylamine and for weaver ants, triclopyr butotyl was more toxic than glyphosate-isopropylamine and glyphosate-isopropylamine was more toxic than clethodim.

Keywords: *O. simillimus*, *O. smaragdina*, herbicides, topical toxicity test and probit analysis

ABSTRAK

Kajian mengenai ujian ketoksikan tiga racun rumpai yang kerap digunakan (glyphosate-isopropylamine, triclopyr butotyl dan clethodim) terhadap semut hitam, Odontomachus simillimus dan kerangga, Oecophylla smaragdina telah diuji dengan menggunakan ujian toksik secara topikal. Kepekatan yang berlainan (5, 15, 25, 35, 45, 55 dan 65%) dengan lima ulangan untuk setiap kepekatan termasuk kawal telah diuji kepada kedua-dua semut. Setiap semut dirawat dengan satu mikroliter (1 μL) racun rumpai di bahagian toraks. Kematian kedua-dua semut direkodkan selepas 24 jam (2, 4, 6, dan 24 jam). Denggan menggunakan analisis probit, nilai LD_{50} dan LD_{90} glyphosate-isopropylamine untuk semut hitam ialah 1.76% dan 1.83%, triclopyr butotyl 0.47% dan 0.94%, dan clethodim 0.64% dan 1.04%. Nilai LD_{50} dan LD_{90} glyphosate-isopropylamine untuk kerangga ialah 1.56% dan 1.88%, dan clethodim 1.56% and 2.27%. Walaubagaimanapun, nilai LD_{50} and LD_{90} untuk triclopyr butotyl untuk kerangga tidak dapat dinyatakan kerana 100% kematian dalam tempoh 24 jam pemerhatian. Sebagai perbandingan,untuk semut hitam, triclopyr butotyl lebih toksik daripada clethodime, dan clethodim lebih toksik daripada glyphosate-isopropylamine dan untuk kerangga triclopyr butotyl lebih toksik daripada glyphosate-isopropylamine dan glyphosate-isopropylamine lebih toksik daripada clethodim.

***Keywords**: O. simillimus, O. smaragdina, racun rumpai, ujian toksik secara topikal dan analisis probit*

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Herbicides such as 2,4-D dymethylamine and paraquat are widely used to kill weedy plants. The weedy plants competing for water, light, soil nutrients, space and carbon dioxide (CO_2) with benefit plants (Lingenfelter & Hartwig, 2007). At the same time, herbicides were also exposed to organisms that beneficial to plants and soil such as ants. In general, little is known about the non-target impacts of these chemicals on native species Crone *et al.* (2009) and Greenslade *et al.* (2010) stated that no effect was observed on Formicidae. However, some studies show that certain concentrations of herbicide can cause mortality of ants.

Although herbicide is applied at an adequate rate to undesirable plants, however according to Lingenfelter & Hartwig (2007), herbicide use also carries risks that include ecological, environmental and human health effects. On the other hand, the application of the herbicides to the plants is widespread without realizing their toxicity effect on organisms including ants that lives near the plants.

In this study, glyphosate-isopropylamine, triclophyr butotyl and clethodim used against soil-dwelling ants and weaver ants. These three herbicides are easily found in stores such as hardware shops around Kuching. According to Lingenfelter & Hartwig (2007), glyphosate-isopropylamine is herbicide that act systemically (kill weeds without large damage to desired plants) from glycine group that control weeds in the garden. Triclopyr butotyl is a certain kind of systemic poison from pyridine carboxylic acid group that absorbed into leaves and bought to the rest of the tree while clethodim is from cyclohexanedione group that control weed like prevent biosynthesis lipid (Lingenfelter & Hartwig, 2007).

Ants belong to the order Hymenoptera and classified in the family Formicidae. Both ant species, *Odontomachus simillimus* from the genus *Odontomachus* (Subfamily: Ponerinae) and *Oecophylla smaragdina* from genus *Oecophylla* (Subfamily: Formicinae) are from predatory genus.

The soil-dwelling ants, *O. simillimus* are also known as trap-jaw ants as it have large mandibles. The distribution of ant genus *Odontomachus* is pantropical and includes more than a hundred species which live in a variety of different habitats, ranging from rainforest to semi-arid environment (Brown, 1976). The soil-dwelling animal depicted as animal transporting material from within the ground to the surface as their role in biotubation by excavate and to maintain their nests (Halfen & Hasiotis, 2010b). In other words, the ant has importance in nutrient cycling which it gives benefits to plants. Besides that, trap-jaw ants are predatory and most are preying a variety of arthropods and dietary generalists (Suarez & Spagna, 2009). Based on its dietary, it can act as biological control in agriculture.

On the other hand, weaver ants, *O. smaragdina* are highly visible and readily recognisable by their reddish to orange-brown bodies which make the ant known as a common red tree-dwelling weaver ant (Leong & D'Rozario, 2012). Weaver ants, whose leaf nests are held together with larval silk, are dominant ants in open forest in Southeast Asia, China, India and Australia (Schluns *et al.*, 2009). The weaver ant actively patrols canopies and preys upon a wide range of potential pests, effectively protect tropical tree crops which make them as biocontrol agents (Van Mele, 2008).

The nest locations of these two ants' species always have contact with plants, usually with desirable plants. Basically, useful plants give natural protection to ants in order to protect their nest. As social insects, they work together to immediately and without selfishness defend and maintain their large colonies (Craig, 2009). Based on the location of the nest, both ants species are most exposed to herbicides.

Test of contact toxicity with topical application used in this study to see the effect of herbicides against soil-dwelling ants, *O. simillimus* and weaver ants, *O. smaragdina*. This test is used to see the different toxicity effect of the ants against different concentration or dose of the herbicide. Three different herbicides (glyphosate-isopropylamine, tryclopyr butotyl and clethodim) will be treated against the ants.

The common analysis in toxicity studies is probit analysis which was introduced by Chester Ittner Bliss in 1934. Probit Analysis is used to determine the relative toxicity of chemicals to living organisms and commonly used in toxicology (Vincent, n.d). It is also a method to convert percentage of mortality of the sample into probability. By using probit analysis, the concentration that causes the death or injuries to samples can be determined.

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1.2 Problem Statement

The application of herbicides is commonly used nowadays to kill grass like weeds that competing basic requirement such as nutrients with benefit plans in order to live. Without realizing it, the use of herbicides often exposed to other organisms such as ants that always have contact with plants. This has an impact on these organisms and indirectly disrupts the stability of ecosystems in an area. That is why this study is important to know the concentration of herbicides that can affect organisms so the stability of ecosystems is not disturbed.

1.3 Objective

The objectives of this study are:-

- to determine whether three commonly used herbicides has any toxicity effect on soil-dwelling ant, *O. simillimus* and weaver ant, *O. smaragdina*.
- to compare the toxicity effect of three commonly used herbicides between two species, *O. simillimus* and *O. smaragdina*.

1.4 Hypothesis Statement

- H_O : There is no toxicity effect of herbicide against ants.
- H_A : There is toxicity effect of herbicide against ants.

CHAPTER 2

LITERATURE REVIEW

2.1 Genus *Odontomachus*

According to Molet *et al.* (2007), the genus *Odontomachus* consists of 64 species that occur from forests to semi-desert areas, nesting in the ground under logs, rotten logs or stones. Collingwood & Harten (2001) stated that *O. simillimus* is a large predatory species found mostly on open ground among spread trees. However, Wilson (1962) has collected these ants in a wide range of habitats; open cultivated places areas, sandy grass-plain near shore, low vegetation near lake, young forest at cultivated areas (which was about three meter high) and rainforest.

The genus *Odontomachus* in average about six millimeters long and it is easily to be spotted on the ground. This genus has big head with big, powerful mandibles which is usually used to hunt prey. All the *Odontomachus* species are carnivorous, even if they can capture a large variety of small soil-dwelling arthropods and most other predatory ants with their powerful sting, they are typical predatory ants as they mostly specialized on termites (Mora *et al.*, 2008).

As residents of forest ecosystems, ants are important both in terms of ecosystem function and biomass as they have varied roles as predators, scavengers, herbivores and granivores (Guiseppe *et al.*, 2006; Ward, 2006). According to Halfen &

Hasiotis (2010a), the soil-dwelling ants has important role in soil bioturbation and soil pedogenesis. This is based on the movement of the ants carrying sediment upward and downward from its nest. Characteristics and behaviour of the ants give many benefits to the plants and soil which it can be used as biological control in agriculture.

Though the genus *Odontomachus* is well symbolize in the tropics and neotropics, few studies have focused on its predatory behaviour, less on the flexibility of this behaviour depending on prey characteristics and little studies on its contact with herbicides (Mora *et al.*, 2008).

2.2 Genus *Oecophylla*

Two humid-tropics species, *O. longinoda* in Africa and *O. smaragdina* in Asia and Australia, can be treated as one as they have so similar in biologies (Way & Khoo, 1992). According to Bluthgen & Fiedler (2002), the genus *Oecophylla* is among the most important and dominant ants in tree canopies of Africa (*O. longinoda* (Latreille)), Australia, South-East Asia, and western Pacific islands (*O. smaragdina* (Fabricius)).

According to Leong & D'Rozario (2012), weaver ant colonies live in a widely dispersed network of aerial nests which are constructed from leaves that woven together. Usually, plants with medium to large leaves are being used such as *Caryota mitis* (family Arecaeae) and the fish tail palm, which have flexible and broad leaves to provide shelter and shade.

Genus *Oecopylla* in average three to nine millimeters long and were easily can be spotted as these ants are highly visible and readily recognizable by their reddish to orange-brown bodies (Leong & D'Rozario, 2012). This genus has triangle head with short mandibles uses to killing pest or other animals that enter their area.

This weaver ant is well known for the construction of complex arboreal nests as well as an usually aggressive predator (Cesard, 2004). According to Crozier *et al.* (2010) the activities of the ants make it as beneficial insects as they controlling pest species and they are also human food in some areas such as Indonesia and China. Way & Khoo (1992) stated that weaver ants managed to kill or chase away pests throughout in their area due to thier extensive foraging and aggressive behaviour,.

Furthermore, besides manage to controlling pest in an area, weaver ants also have many other importances in ecosystem. According to Schluns *et al.* (2009), these ants are important in research on communication, territoriality, biological control, and colony integration. Although weaver ants have benefits to crops, however, these ants are easily exposed to herbicides.

2.3 Chemical Composition of Herbicides

Herbicides can be defined as crop-protecting chemicals used to kill undesirable plants or weedy plants that interrupt normal plant growth (Lingenfelter & Hartwig, 2007). Many commonly used herbicides are broadleaf herbicides that suppress forbs or kill depending on concentration and species-specific sensitivity (Crone *et al.*, 2009). According to Guiseppe *et al.* (2006), the current controversy involves the effects of herbicides on non-target species mostly relates to the indirect effects of herbicide applications.

The application of herbicide is based on the category of the unwanted plants and it can be either selective or non-selective. Selective herbicides are used to kill weeds without major damage to desirable plants while non-selective herbicides injure or kill all plants present if applied at an adequate rate (Lingenfelter & Hartwig, 2007).

Each herbicide contains different chemical compositions depend on the herbicide effectiveness in controlling plants' growth or killing the plants. Cautions always needed when handling herbicides to prevent any things that should not happen. Material Safety Data Sheet (MSDS) is used to determine the properties of each herbicide as shown in Table 2.1.

Table 2.1: Summary of Material Safety Data Sheet (MSDS).

Chemical	**Summary of Material Safety Data Sheet (MSDS)**
Glyphosate-isopropylamine	Form: Liquid Colour: Clear, viscous, yellow solution Odour: Ammoniacal odour Vapour pressure: N/A Specific density: 1.22 ± 0.01 Flashpoint: Non flammable Flammability limits: Non flammable Solubility in water: Completely soluble
Triclopyr butotyl	Form: Liquid Colour: Amber Odour: Aromatic odour Boiling point (^oC): >200 ^oC Vapour pressure: N/A Flashpoint: 95 ^oC Flammability limits: Non-flammable Specific Density: 1.20 ± 0.01 Water solubility: Forms emulsion
Clethodim	Form: Liquid Colour: Amber liquid Odour: Aromatic pH: 3.6 in 5% solution Melting point (^oC): N/A Boiling point (^oC): N/A Specific Gravity: 0.96 Vapour Pressure: N/A Flammability Limits: N/A Solubility: Soluble in hydrocarbon solvents. Emulsifiable in water Viscosity: 2.05 cps @ 25 ^oC

(Data Source: http://www.kenso.com.au)

According to Lingenfelter & Hartwig (2007), to stop the growth of desired plants, it depend on the enzyme produced by the herbicide as each herbicide has different effect on types of plants. Glyphosate-isopropylamine, triclopyr butotyl and clethodim are effective on different types of plants.

Lingenfelter & Hartwig (2007) stated that glyphosate-isopropylamine is from class Plant Growth Regulators (PGRs) which has no effect on grasses or sedges but effective on annual and parennial broadleaf plants. The herbicide produce artificial enzyme called auxins to interfere with normal growth of plants (Lingenfelter & Hartwig, 2007).

Triclopyr butotyl is from class amino acid biosynthesis inhibitors which mostly effective on annual broadleaves and some herbicides from this group are effectives on grasses, parennial plants and nutsedge (Lingenfelter & Hartwig, 2007). According to Lingenfelter & Hartwig (2007), the herbicides in this class are interfering with enzymes that produce specific amino acid in the plants and indirectly shutdown the metabolic activity which lead the plants to death.

Clethodim is from class fatty acid (lipid) biosynthesis inhibitors which has no effect on broadleaves plants but effectives on grasses (Lingenfelter & Hartwig, 2007). The herbicides are translocated to the growing points of the plants where they prevent meristematic activity which almost immediately stopping the plants’ growth (Lingenfelter & Hartwig, 2007).

2.4 Bioassay Test against Hymenoptera (Family: Formicidae)

According to Finney (1971), bioassay (biological assay) is the measurement of the potency of any reactions including chemical or biological, physical, stimulus, physiological or psychological which it produces in living matter. One of the bioassay methods is topical toxicity test. Several studies have researched the effect of herbicides on ants (Formicidae).

The test of the effect of herbicides; bromoxynil ($C_7H_3Br_2NO$) and hoegrass (diclofop-methyl) on surface-dwelling Collembola (springtail) and surface-dwelling Formicidae (ant) has been done by Greenslade *et al.* (2010). In their studies, after the herbicide treatment, two of 14 species of Collembola were detected but no effect was observed on Formicidae. Therefore, Greenslade *et al*. (2010) concluded that herbicides have a minimal effect on most species of surface-active arthropods although Collembola were more affected than Formicidae.

Costa & Rust (1999) used untreated control, diazinon and fipronil against Argentine ants, *Linepithema humile*. Treatment of soil-mix fipronil killed ≥ 93% in one week, application of fipronil killed a large number of ants after two weeks and killed almost all ants by week five. About 40% of the ants were killed by diazinon in one week but the percentage did not increase after that. Costa & Rust (1999) believed that the higher concentration of fipronil took longer to kill ant colonies and they suggesting it may have been more repellent to ants than lower concentrations.

By using oral toxicity of boron compounds; disodium octaborate tetrahydrate, boric acid, and borax to Argentine ant, *Linepithema humile*, Klotz *et al.* (2000) showed that time required to kill 50% of the ants (median lethal time - LT_{50}) decrease with increasing concentration of the three boron compounds.

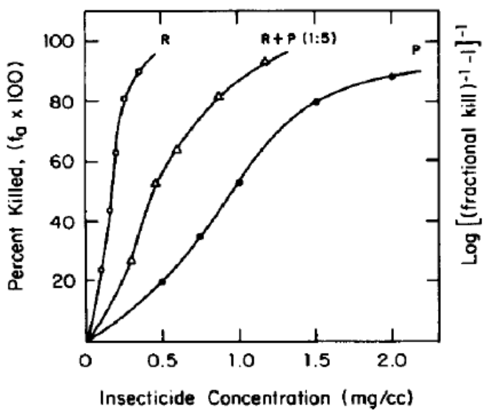
Wiltz *et al.* (2009) used four chemicals against Argentine ant, *Linepithema humile*; chlorfenapyr, bifenthrin, fipronil and thiamethoxam. Befenthrin has the highest mortality of ants which is 80% followed by chlorfenapyr (18%), fipronil (11%) and thiamethoxam (12%). The median lethal time (LT_{50}) for chlorfenapyr is 118.8 min (110.9-126.9), fipronil is 168.7 min (162.5-174.6) and thiamethoxam is 54.7 min (52.4-57.9). However, LT_{50} of bifenthrin could not determine as 98.5% of ants were dead before the first observation was made.

A study by Oi *et al.* (1996) found the median lethal concentration, LC_{50} and LC_{90} of bifenthrin and tefluthrin in potting soil for female elates of the red imported fire ants, *Solenopsis invicta*. LC_{50} and LC_{90} for bifenthrin were 1.1 and 5.2 ppm and for tefluthrin were 8.5 and 19.0 ppm.

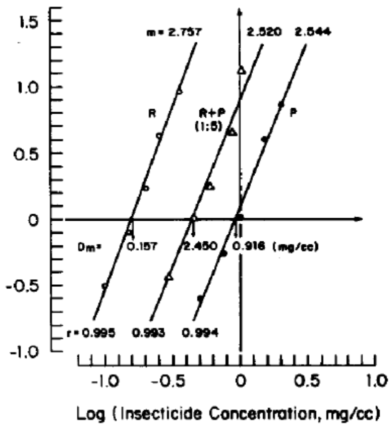
2.5 Statistical Analysis (Probit Analysis)

According to Finney (1971), probit analysis is a specialized regression model of binomial response variables and it is used to determine the relative toxicity of chemicals on living test specimens. It transforms the sigmoid dose-response curve to a straight line that can then be analyzed by regression either through least squares or maximum likelihood (Vincent, n.d).

By using probit analysis, median lethal dose LD_{50} can be estimated. LD_{50} is refers to a concentration that can cause 50% death of the specimens. The transformation of sigmoid dose-response curve to a straight line is as following:



(a) Sigmoid curve of dose-respond



(b) Straight line of dose-respond

Figure 2.1: Graphs of dose-respond. (a) sigmoid curve of dose-respond before using probit table to change percentage of concentration to probit, (b) straight line of dose-respond after using probit table. (Chou & Talalay, 1984)

The regression formula is as following:

$$\mathbf{y = \alpha + bx}$$

Where:-

y = predicted value of dependent variable-Y

x = observed value of independent variable-X

a = constant (point at which the line crosses Y axis when X = 0)

b = coefficient representing the slope line

Means of both variables ($\bar{y}$ and) and value of **b** is determined first before **a** is calculated. To calculated **b**, the following formula is used:

$$\mathbf{b} = \frac{\quad}{\quad}$$

Research made by Klotz *et al.* (2000) and Wiltz *et al.* (2009) used probit analysis to determine median lethal times (LT50) for each concentration of chemicals used against Argentine ant, *Linepithema humile*. However, the mortality data by Klotz *et al.* (2000) were corrected with Abbott's (1925) formula and mortality data by Wiltz *et al.* (2009) were not corrected as the control mortality was low and some of the time periods contained zero mortality values.

On the other hand, Oi *et al.* (1996) used probit analysis to determine median lethal concentration (LC_{50} and LC_{90}) on red imported fire ants, *Solenopsis invicta*.