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SHORT COMMUNICATION

Toxicity Testing of Three Commonly Used Herbicides on Soil-Dwelling Ant (Family: Formicidae - *Odontomachus simillimus*)

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ABSTRACT

Toxicity evaluation of three commonly used herbicides by public consumer in Kuching district, namely: glyphosate-isopropylamine, triclopyr butotyl and clethodim were conducted against soil-dwelling ants, *Odontomachus simillimus*. Different concentrations (5, 15, 25, 35, 45, 55 and 65%) of selected herbicides with five replicates for each concentration with addition of one negative control were tested in this study. The ants were treated with 1 µL of herbicides at the thorax and the observations of mortality were recorded within 24 hours. By using probit analysis, LD_{50} and LD_{90} values of three selected herbicides were obtained: glyphosate-isopropylamine 1.76% and 1.83%, triclopyr butotyl 1.56% and 1.88%, and clethodim 0.47% and 0.94%, respectively. Triclopyr butotyl was found as the most toxic herbicide followed by clethodim and glyphosate-isopropylamine based on the study conducted.

Keywords**:** Herbicides, *Odontomachus simillimus*, probit analysis, topical toxicity test

Herbicides are chemicals which can be used to protect the desired crop plantations and at the same time the application of them could kill undesirable weeds that competing water, light, soil nutrients, space and carbon dioxide with plants of interest (Lingenfelter & Hartwig, 2007). As the consumer concern to protect their crop plantations by using herbicides, they have to understand that without any proper management, the excessive use of these chemicals would carry risks that include ecological, environmental and human health effects (Lingenfelter & Hartwig, 2007).

In Malaysia, herbicides are commonly used in agriculture sectors and public consumers to control weeds that infesting crops including oil palm (*Elaeis guineensis* Jacq.) and rubber (*Hevea brasiliensis*) plantations, vegetable field, orchards and also housing areas (Chuah *et al*., 2008; Zain *et al*., 2013). The reason why herbicides are used is mainly because of their effectiveness in controlling the propagation of undesirable weeds (Lingenfelter & Hartwig, 2007). This study was conducted to differentiate the toxicity level of three selected herbicides against the soil-dwelling ants. Those herbicides were glyphosate-isopropylamine 41.0% w/w, triclopyr butotyl 32.1% w/w and clethodim 25.0% w/w, and all of them can be obtained from local hardware stores in Kuching and Kota Samarahan districts. Each herbicide has speciality to control different types of weeds. Lingenfelter and Hartwig (2007) had discussed about the effectiveness of glyphosate-isopropylamine, triclopyr butotyl and clethodim on different types of plants, grasses and weeds.

The usage of herbicides often exposed to other organisms those are beneficial to plants and soil, which it is the current controversy involves the effect of herbicides on non-target species mostly relates to the indirect effect of herbicide applications (Guiseppe *et al*., 2006). Some studies showed that certain concentrations of herbicide can cause mortality to ants (Costa & Rust, 1999; Klotz *et al*., 2000; Oi & Williams, 1996; Wiltz *et al*., 2009).

In this study, the soil-dwelling ant was selected because this particular species is important in maintaining the soil quality needed by the crop plantations. The ant is

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basically involved in the nutrient cycles and act as a pest control. This ant is also important both in terms of ecosystem function and biomass as they have varied roles as predators, scavengers, herbivores and carnivores which they can act as biological control in agriculture (Guiseppe *et al*., 2006; Ward, 2006). Soil-dwelling ant is carnivorous and preying a variety of arthropods, mostly specialized on termites (Mora *et al*., 2008; Suarez & Spagna, 2009).

Tests of contact toxicity with topical application were conducted to determine whether the selected herbicides have any toxicity effect on soil-dwelling ant, and to compare the toxicity effect of these three herbicides on soil-dwelling ants as this study has never been conducted before. Probit analysis was carried out base on Finney (1947) and the concentration that causes the death to the ant was determined. Probit analysis is known as a specific regression model of binomial response variable which it is use to verify the relation of chemicals on organisms (Finney, 1947).

The nest of soil-dwelling ants was excavated and the ants were collected by hand-picking. Medium-sized aquarium filled with quarter of moist soil was prepared to maintain the humidity before the ants were placed into the aquarium. The colonies were provided with dead insects (e.g. grasshopper) as their food source and moisture cotton to provide water. Methods used on diet of soil-dwelling ants were referred from Klotz *et al*. (2000), Choe and Rust (2008), and Wiltz *et al*. (2009).

Test for contact toxicity by topical application on ants were conducted according to Oi and Williams (1996) and Hassan *et al*. (2010). The concentrations (5, 15, 25, 35, 45, 55 and 65%) of three herbicides: glyphosate-isopropylamine, triclopyr butotyl and clethodim were prepared by dilution with distilled water. 1.0 µL of diluted herbicide was applied to the thorax of the ant using a micropipette and it was observed at 2, 4, 6, 12 and 24 hours with five replicates and one negative control. The experiment was repeated by using different concentrations of herbicides following the same procedures as above.

Two median lethal doses, LD_{50} and LD_{90} were calculated using probit analysis. Finney's table was used to estimate the probit. Mortality rate of soil-dwelling ants versus different concentrations of herbicides and probit mortality of soil-dwelling ants versus log concentration of herbicides were plotted based on the recorded data.

There was no mortality of soil-dwelling ants recorded in 5 to 45% concentration of glyphosate-isopropylamine after twenty four hours observation. At 55 and 65% concentration, the mortality of ants was recorded 100% after treated with the herbicide. Triclopyr butotyl shows no mortality at 5% concentration, 67% mortality at 15% concentration and 100% mortality at 25 to 65% concentration. As for clethodium, the mortality rate of ants was recorded 67% for concentration 15 to 35% and 100% mortality at concentration of 45 to 65%. Table 1 shows the summary of the overall observations, and Table 2 shows the mortality of ants increase as the concentration of the herbicides increase.

Based on Table 2, graph of semi-log concentration of herbicides versus probit mortality of soil-dwelling ants was plotted as shown in Figure 1. Probit table was used to change the percentage of mortalities into probit values and the herbicides concentrations were changed into log concentration. The regression analysis was performed to get the regression values and to calculate LD_{50} and LD_{90} of median lethal dose of the ants. LD_{50} and LD_{90} for soil-dwelling ants were 1.76% and 1.83% for glyphosate-isopropylamine, 1.56% and 1.88% for triclopyr butotyl, and are 0.47% and 0.94% clethodim, respectively.

Each herbicide has different mortality rate onto the ants at different concentrations, and the result shows that the mortality of ants increase when the concentration of herbicides increased. This indicates these ants have their own resistance mechanisms in order to survive if when there were exposed to the herbicides (Kranthi, 2005; Liu *et al*., 2006; Prather *et al*., 2000). It was also described by World Health Organization (WHO) (1957) that each insects has resistant genes which allowing them to survive even after exposed to insecticides.

Table 1. Toxicity effect of herbicides against soil-dwelling ants.

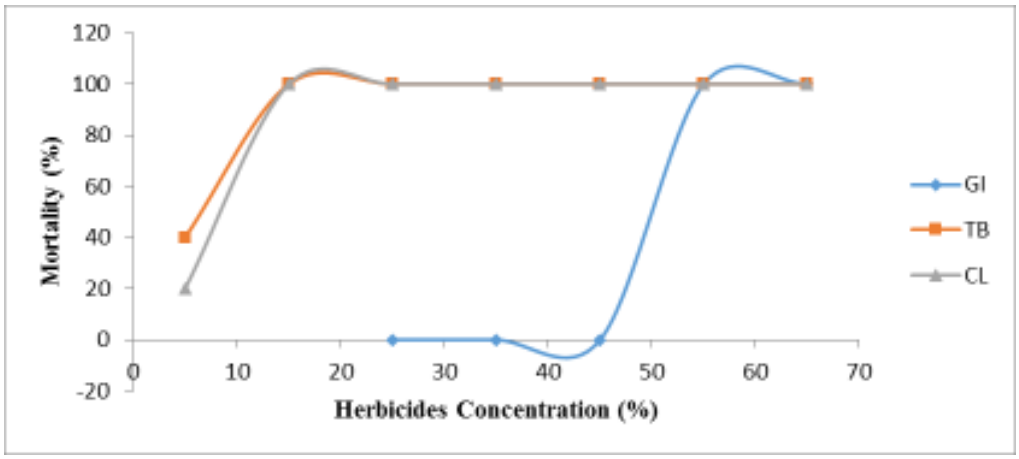
Treatment	Concentration (%)	Time of Death (hours)				
		0-2	2-4	4-6	6-12	12-24
Glyphosate-isopropylamine	Control	+	+	+	+	+
	5	+	+	+	+	+
	15	+	+	+	+	+
	25	+	+	+	+	+
	35	+	+	+	+	+
	45	+	+	+	+	+
	55	+	+	-	-	-
	65	+	-	-	-	-
Triclophyr butotyl	Control	+	+	+	+	+
	5	+	+	+	+	+
	15	+	-	-	-	-
	25	-	-	-	-	-
	35	-	-	-	-	-
	45	-	-	-	-	-
	55	-	-	-	-	-
	65	-	-	-	-	-
Clethodim	Control	+	+	+	+	+
	5	+	+	+	+	+
	15	+	-	-	-	-
	25	+	-	-	-	-
	35	+	-	-	-	-
	45	-	-	-	-	-
	55	-	-	-	-	-
	65	-	-	-	-	-

(+) Alive; (-) Dead

Table 2. Topical toxicity test of glyphosate-isopropylamine, triclophyr butotyl and clethodim against soil-dwelling ants.

Herbicides	Concentration (%)	No. of Ants Exposed	No. of Ants Dead	Percentage Mortality (%)	Log Concentration	Probit Value
Glyphosate-isopropylamine	25	5	0	0	1.3979	-
	35	5	0	0	1.5441	-
	45	5	0	0	1.6532	-
	55	5	5	100	1.7404	8.09
	65	5	5	100	1.8129	8.09
	Control	5	0	0	-	-
Triclopyr butotyl	5	5	2	40	0.6990	4.75
	15	5	5	100	1.1761	8.09
	25	5	5	100	1.3979	8.09
	35	5	5	100	1.5441	8.09
	45	5	5	100	1.6532	8.09
	55	5	5	100	1.7404	8.09
	65	5	5	100	1.8129	8.09
	Control	5	5	0	-	-
Clethodim	5	5	1	20	0.6990	4.16
	15	5	5	100	1.1761	8.09
	25	5	5	100	1.3979	8.09
	35	5	5	100	1.5441	8.09
	45	5	5	100	1.6532	8.09
	55	5	5	100	1.7404	8.09
	65	5	5	100	1.8129	8.09
	Control	5	0	0	-	-

A



B

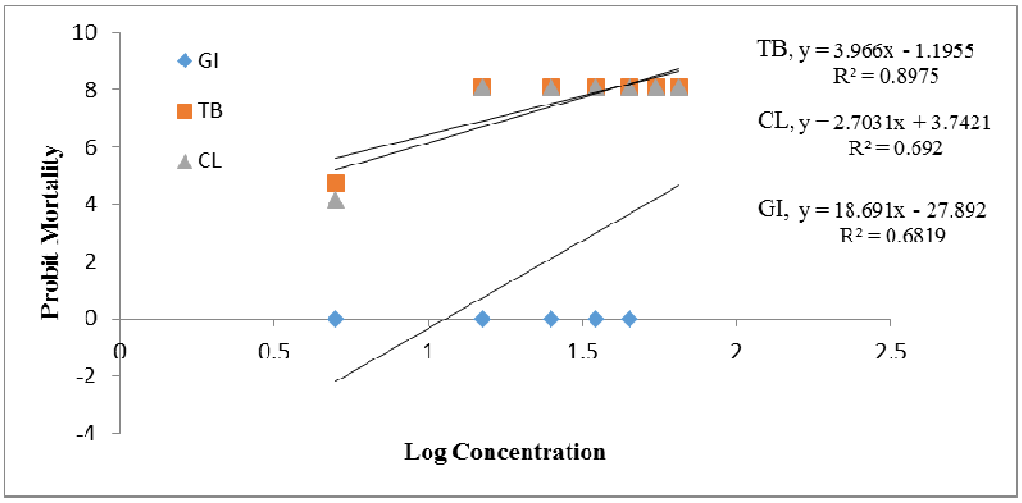


Figure 1. Mortality curve of soil-dwelling ant under the effect of herbicides. (A) Graph of mortality (%) versus herbicides concentration (%) before using probit table, sigmoid curve of concentration-respond; (B) Graph of probit analysis versus log concentration after using probit table. Straight lines show the increasing probit mortality of three herbicides, straight line of concentration-respond of triclophyr butotyl (TB), clethodim (CL) andglyphosate-isopropylamine (GI).

The triclophyr butotyl and clethodim were fast-acting chemicals as they caused mortality to ants within seconds while glyphosate-isopropylamine was slow-acting chemical as it caused mortality to ants after several hours. Similar research was carried out by Wiltz *et al.* (2009) which used four different types of chemicals; bifenthrin, chlorfenapyr, fipronil and thiamethoxam against Argentine ant, *Linepithema humile*. In their study, instead of using median lethal dose, they used median lethal time (LT_{50}) to estimate mortality of ants. The LT_{50} for chlorfenapyr was 118.8 min (110.9-126.9), fipronil was 168.7 min (162.5-174.6) and thiamethoxam was 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. Wiltz *et al.* (2009) stated that the fasting acting chemicals are more effective as a barrier and causes the mortality to ants while the slow acting chemicals allow the chance for ant-to-ant transfer.

The chosen herbicide concentration has to provide optimum affects to kill weeds but at the same time gives minimum effects to soil organisms including ants. It was found that in this research, the three chosen herbicides can kill weeds without causing any death to soil-dwelling ant parallel with the research that have been made if the consumers are aware of the maximum concentration of these three herbicides that can cause mortality to the ants. Glyphosate-isopropylamine caused mortality to soil-dwelling ants at concentration 55% and the permitted use based label on the herbicide bottle is between concentrations 2.2-55%. Therefore, it is recommended that the usage of this herbicide must not exceeding 55% concentration because this will cause mortality to the ants. The other two herbicides, both triclopyr butotyl and clethodim cause mortality to soil-dwelling ants at concentration 15%. Considering the permitted use based label, triclopyr butotyl recommended concentration between 0.8-5.8% and clethodim between 6-10%, and this inferred that these herbicides can work effectively with low concentration.

All herbicides used show toxicity effect on soil-dwelling ants. The probit mortality of the soil-dwelling ants at LD_{50} and LD_{90} in twenty four hours was determined. The LD_{50} and LD_{90} for glyphosate-isopropylamine were 1.76% and 1.83%, triclopyr butotyl were 1.56% and 1.88%, and clethodim were 0.47% and 0.94%. Thus, this study concluded that the most toxic herbicide towards soil-dwelling ant, *O. simillimus* was triclopyr butotyl, followed by clethodim and glyphosate-isopropylamine.

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