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EFFECTS OF TWO HERBICIDE FORMULATIONS ON AVOIDANCE BEHAVIOUR OF THE EARTHWORM (*Eisenia Andrei*) IN TWO DIFFERENT SOIL TYPES

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ABSTRACT

Herbicides are most commonly used pesticides in agriculture in Sri Lanka. Earthworms are dominant soil fauna among soil dwelling species. Excessive use of herbicides is known to affect earthworms. In this study, the earthworm avoidance tests were performed to investigate the effects of some selected herbicides on earthworm avoidance behavior. MCPA and Propanil were used as the test substances and OECD (Organization for Economic Co-Operation and Development) artificial soil and natural soil were used as test substrates. The experimental process was performed based on ISO (International Standard Organization) guidelines for earthworm avoidance studies (ISO/DIS 17512). Net avoidance response LOEC and NOEC values were determined. Results showed that significant avoidance behavior in contaminated soils with Propanil followed by MCPA as compared to untreated. Higher concentrations (100mg *a.i*/kg and 300 mg *a.i*/kg) had significant avoidance as well as reduced habitat functions. Higher toxicity was recorded in natural soil than artificial soil. Overall results of this study indicated that avoidance test could be used as an initial screening test to investigate the herbicide effects on earthworms.

INTRODUCTION

Ever-growing human population demands increasing food production and intensification of agricultural activities in tropical countries including Sri Lanka which depend heavily on agrochemical use. Pesticides may pose always non beneficial to environment. Discriminate use of pesticides usage may lead to environmental imbalance too. Use of herbicides in Sri Lanka has been increased to alarming levels in recent years. Direct application of herbicides to soil may affect beneficial soil organisms. The edaphic fauna, specifically earthworms, are important soil organisms inhabiting in the soil ecosystem (Edwards and Bohlen, 1996; Lavelle and Spain, 2001). Earthworms represent greater proportion (80%) among the soil invertebrates and are considered as most useful group to evaluate ecotoxicology tests either on field or laboratory. The epigeic species *Eisenia andrei* was, and still remains, as the favored eco toxicological test species mainly because of their universal distribution. In addition, they have life cycles with short duration, adaptability to wide temperature ranges as well as moisture tolerance range and flexible body structure. They can move easily and quickly within deep soil strategies exposing to contaminated soils with herbicides. The most common endpoints in evaluating toxicity of herbicides to earthworm are mortality, changes in body growth and cocoon production. In addition, earthworm avoidance test has been used as a simple risk assessment tool in soil ecotoxicology, especially for tropical regions. Therefore earthworm avoidance tests were performed to investigate some selected herbicides on earthworms.

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MATERIALS AND METHODS

Test Organisms and Test Conditions

The tested earthworm species was *Eisenia andrei.* Adult *Eisenia andrei* were randomly selected from synchronized stock cultures established at the Department of Zoology, University of Ruhuna, Matara. Cow dung was used as the feeding material.

Test Substrates

A standard artificial soil was developed based on OECD guidelines (1984). Artificial soil(PH 4.5,WHC 66%,Organic matter 10.6%) was prepared by a mixture of 69 % of No. 70 mesh silica sand, 20% kaolin clay and 10% coco peat with a small amount of calcium carbonate added for pH adjustment. Coco peat was used instead sphagnum peat. In addition, natural soil was also used as the substrate for this experiment. Natural soil (sandy clay loam soil) was collected from University premises and sieved (pH 6.2, WHC 45%, organic matter 9%). Deionized water was used to adjust the water holding capacity up to 50%.

Test Substances

Commercially available MCPA and Propanil formulations were used as test substances of which the detailed information is given in Table 1.

Table 1. Detailed information of herbicides formulations tested

Trade name	Chemical name	Molecular weight	Water solubility	Badge number	Reg: number	Shelf life
MCPA	(4-chloro-2-methylphenoxy) acetic acid	200.62	825 mg/L	6131095	007	2 Yrs
Propanil	3',4'-dichloropropionanilide	218.08	225 mg/L	AK12BB2	FF 15405	2 Yrs.

Avoidance Behavior

Pesticides concentrations of MCPA and Propanil (1, 3, 10, 30,100,300,900 mg *a.i* /kg dry soil) were prepared by dissolving in deionized water. The plastic trays (20 x 10 x 6 cm size) were used as test containers with two sections. One side was filled with pesticides treated soil and other side filled with control soil (about 500 g dry weights).Weight of the soil in both side was adjusted to 50% WHC. Adult *Eisenia andrei* (300-350 mg, n=10)were randomly selected from synchronized stock cultures, cleaned and were placed into the middle groove of each container. This procedure was performed for all tested concentrations of each herbicide and consisted of five replicates. The two sides were separated from using a rough cardboard piece to avoid mixing of soils in respective sides. Trays were kept under lights to facilitate the movements of the introduced earthworms into the soil and thin black colour perforated polythene sheets were used to cover the test containers. These sheets prevented worms escaping during each exposure period and also facilitated air exchange between the medium and the environment. Test trays were kept for a 48 hours incubation period and the worms in both sides were counted by locating the direction of the head (Figure 1).

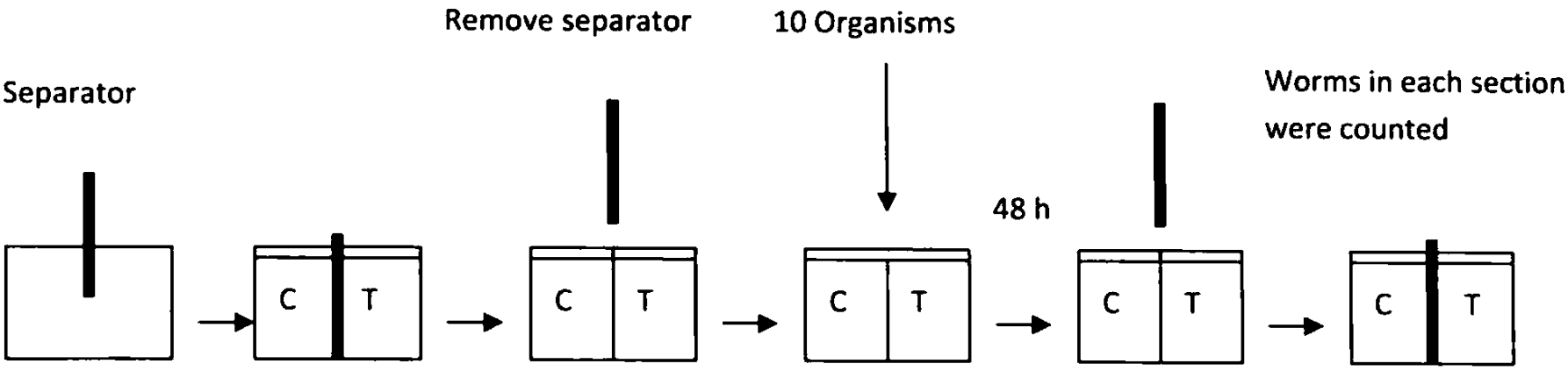

Figure 1: Experimental procedure for avoidance test for earthworms (C = control soil and T = treatment soil)

DATA ANALYSIS

Results of the avoidance tests were calculated as percentages. Percentage avoidance was calculated using following equation:

$$\text{Avoidance percentage} = \{(C-T)/N\} \times 100$$

C= Number of worms in the control soil,
T= Number of worms in the polluted soil,
N= Total number of worms at the start of the test.

Data were recorded as positive and negative avoidance percentages. A positive percentage indicates avoidance of the polluted soil, a zero indicates no avoidance, and a negative percentage indicates an attraction for the pesticide-treated soil. Avoidance response for each chemicals exposed concentrations with five replicates was also used to determine significance of avoidance. Lowest Observed Effect Concentration (LOEC) and No Observed Effect Concentrations (NOEC) were analyzed using one way ANOVA and Dunnett's t tests.

RESULTS AND DISCUSSION

In the study, the effective test procedure ensured a homogenous distribution of earthworms in both sides when control soil is in both compartments. The untreated soil in both sides did not show any significant preference to one side ($P > 0.05$). Differences of homogenous distribution of earthworms in each soil were not evident due to their physical characters, organic matter content, *etc.*

Effect of MCPA on the avoidance behavior of *Eisenia andrei* in artificial soil is summarized in Table 2. Low concentration such as 1, 30mg *a.i*/kg did not show significant avoidance behavior compared to the other concentration and did not indicate reduced habitat function. But, a higher concentration *i.e.* 100, 300mg *a.i*/kg had significant avoidance as well as reduced habitat function. LOEC (Lowest Observed Effect of Concentration) was 100mg *a.i*/kg and NOEC (No Observed Effect of Concentration) was 30mg *a.i*/kg per MCPA.

Table 2. Effects of MCPA on the avoidance behavior of *Eisenia andrei* in artificial soil.

MCPA concentrations	Distribution of earthworms (%)		Net response (%)	Toxicity evaluation
	Control	Treated		
1	44	56	-12^{NS}	NRHF
3	60	40	20^{NS}	NRHF
10	66	34	32^{NS}	NRHF
30	74	26	48^{NS}	NRHF
100	86	14	72^{**}	RHF
300	100	0	100^{ND}	RHF

(*P<0.05, **P<0.01, ***P<0.001, NS: Not Significant at P>0.05, NRHF: No Reduced Habitat Function was considered when 20% of worms in treated soil and toxic =< 20% of worms in treated soil and RHF: Reduced Habitat Function, ND: Not Determined due to 100% avoidance or mortality)

According to Table 3 concentrations of 1mg *a.i*/kg ,3mg *a.i*/kg and 10mg *a.i*/kg did not show significant avoidance (P>0.05) and <20% were observed in the control sides indicating no reduced habitat function. But, MCPA concentration 30mg *a.i*/kg showed significant avoidance behavior (p<0.05) in natural soil and this is contradictory with the artificial soil. Highest concentration100mg *a.i*/kg, 300mg *a.i*/kg recorded significant avoidance (p<0.001) .These two concentrations also showed reduced habitat function. NOEC value and LOEC value per mcpa in natural soil was 10mg *a.i*/kg and 30mg *a.i*/kg respectively. MCPA is a slightly toxic compound and a general Use Pesticide. MCPA and its formulations are rapidly degraded by soil microorganisms and it has low persistence, with a reported field half-life of 14 days to 1 month, depending on soil moisture and soil organic matter (Wauchope *et al.*, 1992). Previous work did not report avoidance behavior on earthworms with reference to MCPA but reported acute and chronic toxicity of MCPA to other animals.

Table 3. Effects of MCPA on the avoidance behavior of *Eisenia andrei* in natural soil.

MCPA concentrations	Distribution of earthworms (%)		Avoidance response (%)	Toxicity evaluation
	Control	Treated		
1	60	40	20^{NS}	NRHF
3	76	24	20^{NS}	NRHF
10	60	40	20^{NS}	NRHF
30	74	26	48^{*}	NRHF
100	98	2	96^{***}	RHF
300	100	0	100^{ND}	RHF

(*P<0.05, **P<0.01, ***P<0.001, NS: Not Significant at P>0.05, NRHF: No reduced habitat function is considered when 20% of worms in treated soil and toxic =< 20% of worms in treated soil and RHF: Reduced Habitat Function, ND: Not Determined due to 100% avoidance or mortality).

Effects of Propanil on the avoidance behavior of *Eiseniaandrei* in artificial soil are reported in Table 4. All the concentrations [1, 3 mg *a.i*/kg ($p<0.01$), 10 mg *a.i*/kg ($p<0.001$) and 30,100 mg *a.i*/kg] had significant avoidance. In concentartion1 to 30 mg *a.i*/kg reduced habitat function was not reported. >20% of the worms were recorded in treated side. At higher concentrations used 30mg *a.i*/kg and 100mg *a.i*/kg 100% avoided was recorded but only 100 concentrations. NOEC value for Propanil in artificial soil could not be determined as all the tested concentrations caused significant avoidance behavior.

Table 4. Effects of Propanil on the avoidance behavior of *Eisenia andrei* in artificial soil

Propanil concentrations	Distribution of earthworms (%)		Avoidance response (%)	Toxicity evaluation
	Control	Treated		
1	66	34	32^{**}	NRHF
3	60	40	32^{**}	NRHF
10	58	42	72^{***}	NRHF
30	74	26	100^{ND}	NRHF
100	98	2	100^{ND}	RHF

(*P<0.05, **P<0.01, ***P<0.001, NS: Not Significant at P>0.05, NRHF: No Reduced Habitat Function is considered when 20% of worms in treated soil and toxic =< 20% of worms in treated soil and RHF: Reduced Habitat Function, ND: Not Determined due to 100% avoidance or mortality).

Effects of Propanil on the avoidance behavior of *Eisenia andrei* in natural soil were given in the Table 5. The lowest concentrations tested 1mg *a.i*/kg reported the earthworms were attracted to the treated side. The reasons that caused this attraction can not be explained. Earthworms and showed significant avoidance at 3mg *a.i*/kg ($p<0.01$) and 10mg *a.i*/kg ($p<0.001$). Earthworms in 30mg *a.i*/kg and 100mg *a.i*/kg concentration were attracted to the control side indicating higher toxicity of Propanil in natural soil. Reduced habitat was recorded at 10, 30 and 100mg *a.i*/kg. A NOEC and LOEC value per Propanil in natural soil was 1mg *a.i*/kg and 3mg *a.i*/kg respectively. Propanil is an acetanilide post-emergence herbicide with no residual effect and also it is used against weedy grasses and broad-leaved weeds in rice, potatoes, and wheat. This is a low persistency chemical (Wauchope *et al.*, 1992). The field half-life is 1 to 3 days (Wauchope *et al.*, 1992). Propanil has higher water solubility and it adsorbs only weakly to soil particles as well as rapidly broken down in the soil by microorganisms. Propanil shows highest activity under moist and wormy conditions.

The overall results of the studies conducted with MCPA and Propanil suggested that the toxicity of Propanil was higher than MCPA in both natural and OECD artificial soils. In addition, toxicity in natural soil was always recorded higher than in the artificial soil. This indicates that low organic matter content in natural soil affects bio availability of these herbicides resulting in higher uptakes of herbicides.

Table 5. Effects of Propanil on the avoidance behavior of *Eisenia andrei* in natural soil

Propanil concentrations	Distribution of earthworms (%)		Avoidance response (%)	Toxicity evaluation
	Control	Treated		
1	46	54	-8^{NS}	NRHF
3	74	26	42^{**}	NRHF
10	86	14	72^{***}	RHF
30	100	0	100^{ND}	RHF
100	100	0	100^{ND}	RHF

(*P<0.05, **P<0.01, ***P<0.001, NS: Not Significant at P>0.05, NRHF: No Reduced Habitat Function is considered when 20% of worms in treated soil and toxic =< 20% of worms in treated soil and RHF: Reduced Habitat Function, ND: Not Determined due to 100% avoidance or mortality).

CONCLUSION

Avoidance behavior is a very valuable and simple tool for environment toxicity assessment of pesticides on earthworms. Accordingly, it can be used to investigate the effects of herbicides on earthworms in contaminated soil. The toxicity of herbicides was higher in natural soil than OECD artificial soil. Therefore it is recommended to perform acute and chronic toxicity tests with these chemicals to achieve a better understanding on non target effects on soil fauna, specifically earthworms.

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