AVOIDANCE BEHAVIOUR OF *Eisenia foetida* AS A TOOL FOR ECO-TOXICOLOGICAL TESTING OF PESTICIDES

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Abstract:

To feed the growing human population, there is need of mechanization and extensive use of pesticides and inorganic fertilizers. Earthworms act as "friends of farmers" by increasing the fertility of soil. Earthworm avoidance test involves use of "dual compartment system" to analyse avoidance of chemicals for 48 hours by *Eisenia foetida* (ISO, 2005). A significant avoidance was recorded at high concentration of chlorpyrifos while a non-significant avoidance of glyphosate was observed in all concentration by *Eisenia foetida*. This avoidance was attributed to rupture of chemoreceptor pathway of earthworm.

Key words: *Eisenia foetida*, chlorpyrifos, glyphosate, avoidance behaviour, soil.

Introduction:

The soil acts as a support system for human development. India is facing the problems of soil erosion, soil pollution, population explosion and lack of agricultural land. To feed Indian population, there is a need to restore soil quality. Earthworms being abundant in biomass and diverse in nature can be utilised for soil restoration. However, fewer efforts were made to explore earthworms for soil restoration.

Eisenia foetida (Savigny, 1826) is the most commonly used composting species due to its short life cycle, easiness to maintain and composting speed. Earthworms serve variety of ecosystem services like conversion of organic waste to humus, p^H maintenance, increasing the water holding capacity and porosity of soil etc.

There is an increased tendency towards use of agrochemicals to increase the yield. However, these agrochemicals are poisons which kill the organisms coming in contact with them irrespective of whether they are pests or beneficial organisms like earthworms or honeybees. Chlorpyrifos and glyphosate are the most commonly used organophosphate insecticide and herbicide respectively used throughout the world to control the pests.

Materials and methods:

The earthworm avoidance test was performed according to guidelines of ISO (2005) which involves use of a "dual compartment system" and *Eisenia foetida* to study the avoidance. The earthworms were exposed simultaneously to both naive and spiked soil for 48 hours. The avoidance of earthworm was evaluated by measuring the number of earthworms in each compartment and analysing the results by using one way ANOVA followed by *post voc* Bonferroni's multiple comparison test.

The thick paper boxes lined with plastic (20 X 10X 10 cm) were used for experiment. The pesticides (viz. chlorpyrifos and glyphosate) were mixed in dry soil sieved through 3 mm mesh size at a concentration of 1-5 mg/kg of dry soil. Chlorpyrifos was hand mixed while glyphosate was dissolved in distilled water and spread uniformly.

The container was divided into two equal halves using a separator. One compartment was filled with 1 kg of dry soil spiked soil while other was filled with 1 kg of dry soil naive soil. The control was maintained in which both the compartments contained naive soil. The moisture was maintained at 30% by using distilled water.

The test was performed in triplicates. Ten adult clitellated *Eisenia foetida* were placed exactly at the centre of container by removing the separator. The separator was again placed at the centre after 48 hours and number of earthworms per compartment was measured. Earthworms at the centre were assigned to a compartment where their head was pointed. Net response was calculated according to method of Antunes *et al.* (2008).

Results:

Application of one way ANOVA followed by post voc Bonferroni's multiple comparison shows that the there was a non-significant avoidance of chlorpyrifos at low concentrations (1 mg/kg, 2mg/kg, 3 mg/kg of dry soil). Significant avoidance was observed only in higher concentration (4mg/kg and 5 mg/kg) (Figure-1; Table-1).

Avoidance of glyphosate by Eisenia foetida was non-significant in all concentrations when one way ANOVA followed by Bonferroni's multiple comparison test was applied (Figure-2; Table-2).

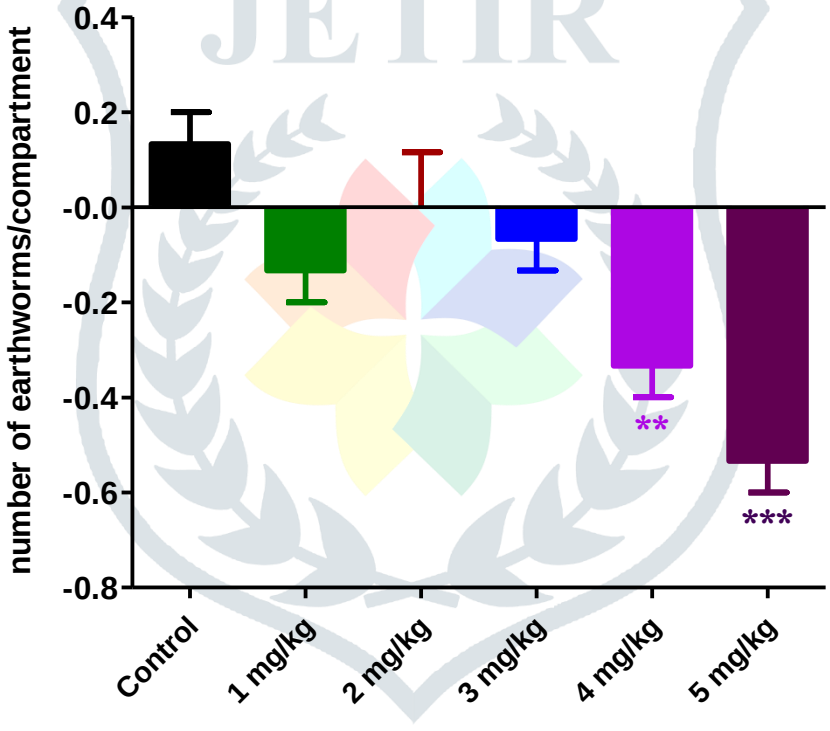

Figure-1: Effect of chlorpyrifos on avoidance behaviour of *Eisenia foetida*.

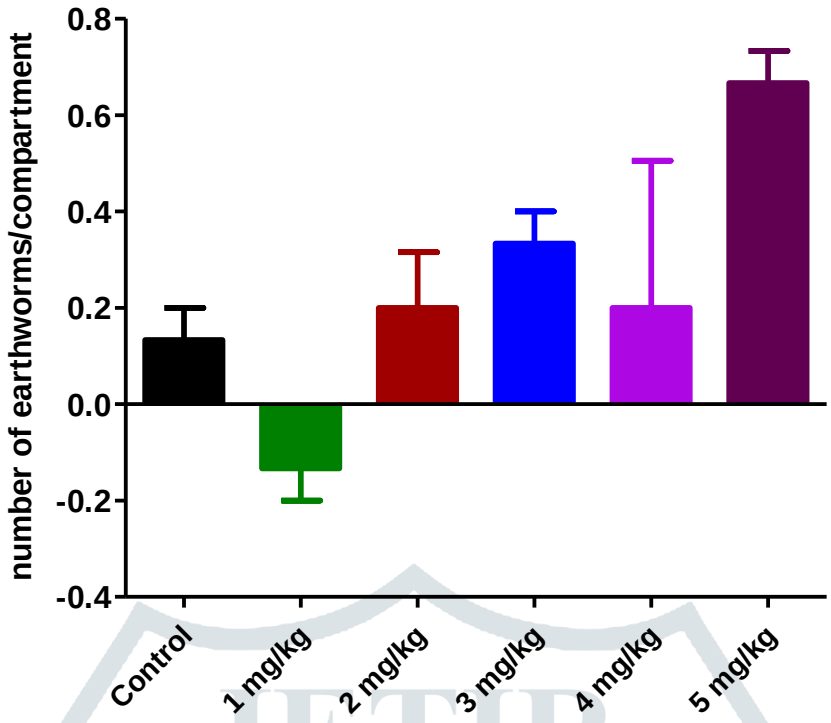

Figure-2: Effect of glyphosate on avoidance behaviour of *Eisenia foetida*

Table-1: Effect of chlorpyrifos on avoidance behaviour of *Eisenia foetida*

Sr. No.	Concentration (gm/kg)	Number of earthworms	Net response= (C-T)/N	mean	SE
1	Control	4	0.2	0.1333	0.0667
		5	0		
		4	0.2		
2	1	6	-0.2	-0.133	0.0667
		6	-0.2		
		5	0		
3	2	5	0	0	0.1155
		4	0.2		
		6	-0.2		
4	3	5	0	-0.067	0.0667
		5	0		
		6	-0.2		
5	4	7	-0.4	-0.333	0.0667
		7	-0.4		
		6	-0.2		
6	5	8	-0.6	-0.533	0.0667
		7	-0.4		
		8	-0.6		

Table-2: Effect of glyphosate on avoidance behaviour of *Eisenia foetida*

Sr. No.	Concentration (gm/kg)	Number of earthworms	Net response=(C-T)/N	mean	SE
1	Control	5	0.2	0.1333	0.0667
		5	0		
		4	0.2		
2	1	6	-0.2	-0.133	0.0667
		6	-0.2		
		5	0		
3	2	4	0	0.2	0.1155
		4	0.2		
		3	0.4		
4	3	3	0.4	0.3333	0.0667
		3	0.4		
		4	0.2		
5	4	3	-0.4	0.2	0.3055
		2	0.6		
		3	0.4		
6	5	2	0.6	0.6667	0.0667
		2	0.6		
		1	0.8		

Discussion:

There was a reduction in biodiversity and organic matter content in agricultural soil due to soil erosion, pesticide and heavy metal residues in soils. Earthworms avoid contaminated soil by using chemoreceptors and sensory tubercles present (Reinecke *et al*., 2002). Due to its speed, ecological relevance and sensitivity, earthworm avoidance test is most commonly used ecotoxicological test (Hund-Rinke and Wiechering, 2001).

A non-significant avoidance was observed at low concentrations of chlorpyrifos and all concentrations of glyphosate. Similar results were obtained by Capowiez and Berard (2006) in *Aporrectodea nocturna* and *Allolobophora icterica* exposed to sublethal doses of imidacloprid. Bengtsson, Nordstrom and Rundgen (1983), however, recorded a significant avoidance of extractable metals.

However, due to non-avoidance or slight attraction of earthworm towards neurotoxic compounds like organophosphates and irritants, earthworm avoidance was used as repellence rather than toxicity test (Capowiez *et al*., 2003). Care must be taken while using avoidance test since avoidance behaviour was determined by chemical to be tested, species used and soil type (Reinecke et al., 2002; Hund-Rinke and Wiechering, 2001).

Earthworm avoidance behaviour could be a promising endpoint for toxicity testing when coupled with specific biomarkers (Morcillo et al., 2013). It could also be used for evaluation of hazardous waste sites (Yeardley, Lazorchak and Gast, 1996).

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