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TOXICITY EFFECT OF CHLOROACETAMIDE ON EARTHWORM (*EISENIA FOETIDA*)

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ABSTRACT

Chloroacetamide was studied at different concentrations against the earthworm, *Eisenia foetida* in artificial soil medium and the pH of the medium was 6.5. The initial moisture content of the medium was 35% and final moisture content on three trials were 32.9%, 33.0% and 32.7% respectively. The average weight of the worms were 0.34 – 0.57 g on initial day and on 14th day the average weight (0.30 – 0.49 g). The median lethal concentrations were 41.86, 42.46 and 49.69 mg/kg dry weight of the soil on 1st, 2nd and 3rd trials respectively. The average LC_{50} value was 44.67 mg/kg dry weight of soil. In the present study suggested that chloroacetamide was toxic to earthworm and will be reduced to use in the agriculture ecosystem.

Keywords: Earthworm, Toxicity, Lethal concentration, Average weight, Moisture content.

INTRODUCTION

Many inorganic fertilizers and agrochemicals are used to enrich the soil as well as crop production. The application of different agrochemicals such as fertilizers, pesticides and herbicides accumulates in the soil. In each year, many tonnes of fertilizers are utilized to improve soil quality for food production [1]. Soil ecosystems are contaminated by the usage of xenobiotic compounds and it affects the organisms exist in the soil. Chloroacetamide is a versatile compound and is used for control the grasses and weeds, it harm to crop plants growth and affect the human erythrocytes of hemolysis, lipid peroxidation and catalase activity [2].

Metolachlor is family of Chloroacetamide which affect the growth of diatom, *Fragilaria rumpens*, *Fragilaria ulna*, *Encyonema silesiacum* and *Gomphonema parvulum* [3].The biomass of the algae were reduced by the effect of alachlor, during 21 day treatment [4]. A common chloroacetamide herbicide, acetochlor was widely used and it contaminates the soil [5-6].

Earthworms are the important organism occurs in the wide diversity of soils and it contribute the maintenance of soil structure with biodegradation [7]. The population of earthworm in a particular ecosystem depends on factors such as soil, temperature, moisture, pH and occurrence of organic waste [8]. It dwells into the soil and provides nutrients to plants directly and indirectly by producing vermicompost, soil ventilation by burrowing and enhances the availability of atmospheric nitrogen. Earthworms are considered as a bioindicator [9]. Thus the evaluation of environmental toxicity effects on earthworm becomes essential. Among the different earthworm species, *Eisenia foetida* is used for the ecotoxicology studies for the reason that it can be bred and maintained easily in short period [10]. Hence the present study was aimed to evaluate toxic effect of chloroacetamide on *E. foetida*.

MATERIALS AND METHODS

Earthworm Culture

The Earthworm, *E. foetida* was bred and maintained in Bioscience Research Foundation (BRF), Chennai, India was used for toxicity test and

performed according to OECD guideline 207. The earthworms were cultured using sphagnum peat and cow dung in a wooden box of 10 liter capacity. The earthworm culture was maintained at 20±2 ºC. The worms were held healthy by periodical removal of vermicompost and providing adequate substrate with moisture content to medium. The individual adult worms older than two months with clitellum and 300 – 600 mg weight are selected for the test [11].

Artificial Soil Preparation

Artificial soil was used as the substrate for the toxicity test. The ingredients to formulate the artificial soil are 70% finely sieved industrial soil of 200 micron size, 20% kaolin clay and 10% well ground sphagnum peat. The major constituent of artificial soil preparation for the experiment is pH and moisture content. The pH of the soil should be 6.0 ± 0.5 and initial moisture content of the soil ought to be 35%. The initial moisture content of 35% was attained by calculating the constant dry weight of the mixed proportion of artificial soil at 105 ^{o}C and based on the result adequate amount of water was used to make the substrate [11]. The pH of the soil medium was analyzed by stirring 1M Potassium chloride solution to the small quantity of the dried soil mixture for 5 minutes and left for 2 h. Then the supernatant was used to analyze the pH of the soil [12].

Toxicity Test

The artificial soil was prepared and the earthworms are acclimatized for 24 h before the exposure period. The healthy, mature earthworms with clitellum of 300 – 600 mg weight was selected from the culture and released into the artificial soil for acclimation. The five different concentrations with 4 replications of chloroacetamide such as 20, 40, 60, 80 and 100 mg/kg dry weight of soil were prepared in deionized water and mixed with 750 g of artificial soil. The appropriate amount of water was used to attain the initial moisture content of 35%. The 750 g of prepared exposure medium with chloroacetamide was transferred to each glass container of 3 liter capacity and artificial soil without chloroacetamide was considered as control (4 replications: Number: 40) and the entire experiment was performed in 3 trials. The acclimatized earthworms were released into the exposure medium of treated and control (10 worms in each container). The glass containers was covered with perforated thin films and arranged under continuous light illumination. The exposure period was 14 days and the worms were observed for mortality by emptying the container in a tray on 7th and 14th day. On 7th day of the observation, the content was replaced back into the container for 14th day of observation. At the end of the test, moisture content of the exposure medium was calculated [11].

Statistical analysis

The average weight of the worms and mortality (%) were presented in Mean ± SD. The Lethal concentration was calculated by using NCSS software.

Table 1. Effect of Chloroacetamide on *Eisenia foetida* in three different trials

Dose (mg/kg dry weight of the soil)	7th day mortality			14th day mortality		
	1st	2nd	3rd	1st	2nd	3rd
Control	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
20	2.5±5.0	0.00±0.0	0.00±0.0	17.5±9.5	15.0±12.9	0.00±0.0
40	17.5±12.5	15.0±5.7	12.5±9.5	45.0±12.9	40.0±8.1	32.5±9.5
60	37.5±5.0	37.5±9.5	37.5±5.0	57.5±12.5	62.5±9.5	60.0±8.1
80	50.0±8.1	60.0±14.14	60.0±14.14	85.0±12.9	90.0±8.1	90.0±8.1
100	90.0±14.14	87.5±15.0	77.5±17.07	100.0±0.0	100.0±0.0	100.0±0.0

Mean ± SD (Number of worm - 40/ concentration), 1st, 2nd and 3rd trial.

Table 2. Average weight of worms in three different occasions after treatment of Chloroacetamide

Dose (mg/kg dry weight of the soil)	0th day			14th day		
	1st	2nd	3rd	1st	2nd	3rd
Control	0.40±0.00	0.35±0.02	0.49±0.06	0.40±0.01	0.35±0.02	0.49±0.06
20	0.38±0.01	0.36±0.04	0.45±0.02	0.37±0.01	0.35±0.04	0.45±0.02
40	0.41±0.01	0.36±0.01	0.45±0.03	0.40±0.01	0.35±0.01	0.45±0.03
60	0.36.±0.04	0.37±0.05	0.49±0.03	0.37±0.01	0.36±0.05	0.49±0.03
80	0.41±0.01	0.34±0.01	0.57±0.03	0.30±0.20	0.33±0.01	0.37±0.25
100	0.41±0.01	0.38±0.01	0.49±0.04	-	-	-

Mean ± SD; 1st, 2nd and 3rd trial.

Table 3. Effective concentration (mg/kg dry weight of the soil) of chloracetamide on *Eisenia foetida* after 14 day of treatment

Trials	LC_{50}	95% confidential limit		LC_{90}	95% confidential limit	
		Lower limit	Upper limit		Lower limit	Upper limit
1st	41.86	38.88	44.84	189.59	154.88	224.3
2nd	42.46	39.45	44.94	160.15	135.51	184.79
3rd	49.69	47.27	52.11	122.62	109.55	135.69
Average	44.67	-	-	-	-	-

RESULTS AND DISCUSSION

The pH of the medium was 6.5 on 0th day. The mortality of the earthworm was observed on 7th and 14th day of the test period. The total mortality was observed at 100 mg/kg dry weight of the soil on 14th day of all the trials. No mortality was observed in control group. The minimum mortality of 2.5% and 15% was observed at 20 mg/kg dry weight of the soil on 7th and 14th day respectively. The highest mortality of 90% was recorded on 7th day at 100 mg/kg dry weight of soil. The total mortality of 100% was seen on 14th day at 100 mg/kg dry weight of the soil (Table 1). The average weight of worms was recorded on initial and final day of the experiment. The average weight of the worms was 0.34 – 0.57 g on initial day and 0.30 – 0.49 g at the end of the test. There was no change in the weight of the worms in control on 0th and 14th day. Thereafter weight of the worms were reduced from initial and final day as the concentration increases (Table 2). The final day moisture content of the artificial soil on three different (1st, 2nd and 3rd) trials were 32.9%, 33.0% and 32.7% respectively. The LC_{50} values were 41.86, 42.46 and 49.69 mg/kg dry weight of the soil and the average of LC_{50} value was 44.67 mg/kg dry weight of the soil (Table 3). Similarly, Chloroacetamide, Cd (chloride), 3,4- Dichloroaniline and Pentachlorophenol were studied against *E. foetida* and the LC_{50} value were 40, >1000, >180 and 28.5 mg/kg respectively [13]. Buchatsky also studied A-8 insecticide and chloroacetamide on *E. foetida* for 14 days and the LC_{50} value were 6.5 and 47.5 mg/kg respectively. The LC_{50} value for chloroacetamide was similar with previous study. Whereas Guzyte [14] has tested salinity effect of Sodium chloride on *E. foetida* and total mortality was found at 6000 and 8000 mg/kg soil. The LC_{50} value was 5623 mg/kg was reported after 28 days of test period. Many different agrochemicals were usually studied against *E. foetida*. The toxicity of zinc on *E. foetida* was evaluated by Spurgeon and Hopkin [15] and the results revealed that decline in survival and cocoon production of worms. *E. foetida* was studied using Polychlorinated biphenyls contaminated soil and the results revealed that the cocoons production was inhibited [16]. An oganophosphate insecticide, Malathion was treated against *E. foetida* and found that it affects the neuromuscular function and coiling of the tail [9]. The reproduction capacity of earthworms was affected by the usage of inorganic fertilizers, insecticides, molluscides, bactericides, fungicides and nematicides [17]. The avoidance behavior of *E. foetida* was tested with benomyl, carbendazim and lambda-cyhalothrin. The EC_{50} values of compounds were 28.2 for benomyl, 127.4 for carbendazim and 3.3 for lambda-cyhalothrin respectively [18].

The worldwide used nitrogen fertilizer; urea was tested for 48 h against *E. foetida* and evaluated the lethal concentration as 28µg/cm^2 [1]. Fertilizers are most commonly used to enrich the soil artificially and it affects the fertility of the soil. Potash is a mineral fertilizer used globally and its lethal concentration to *E. foetida* was estimated by Abbiramy and Ross [19] as 57µg/cm^2. The effect of organic and inorganic fertilizers were studied and the outcome of this experiment showed that inorganic fertilizer affects the earthworm and organic fertilizer had optimistic effect [7]. Pawar and Shahezad [20] studied that chloropyriphose of 0.1, 0.2, 0.3, 0.4 and 0.5 mg/kg dry soil against *E. foetida* for 35 days and found that the concentrations, 0.3, 0.4 and 0.5 mg had affected the growth and weight of the worm.

CONCLUSION

In the present study, the LC_{50} values of the Chloroacetamide were 41.86, 42.19 and 49.69 mg/kg dry weight of soil for 1st, 2nd and 3rd trials respectively. The average LC_{50}value was 44.67 mg/kg dry weight of the soil and there was not much different between 1st and 2nd trials. The obtained results are comparatively similar with other authors report. In this study clearly showed Chloroacetamide was toxic to earthworm, therefore reduce the usage and protect the agricultural environment.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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