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Effect of Sulfentrazone 500 g/L SC on the Growth and Reproductive Output of Earthworm Eisenia fetida in Artificial Soil

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Abstract
The chronic toxicity effect of Sulfentrazone 500 g/L SC was studied in Earthworm, *Eisenia fetida* to assess the growth, mortality, and reproductive output under laboratory conditions. The test concentrations of 50, 100, 250, 500 and 1000 mg/kg dry artificial soil were selected for the experiment and adult earthworms were exposed for 35 days. No mortality or abnormal behaviour were observed in adult earthworms and there was no significant difference observed in the biomass of adult worms treated with all test concentrations (50 - 1000 mg/kg dry artificial soil) when compared with the worms in the control (Untreated artificial soil). The juveniles were counted after 60 days in the control and treated groups. The average number of juveniles were significantly decreased in the highest test concentration 1000 mg/kg dry artificial soil when compared with control group. There was no significant change observed in the average number of juveniles in the other treated groups (50 - 500 mg/kg dry artificial soil) when compared with control group. Similarly, the biomass of juveniles was significantly decreased in the highest treatment 1000 mg/kg dry artificial soil when compared with control group. The present findings revealed that there was an adverse effect on reproduction and growth of Juveniles at highest test concentration of Sulfentrazone 500 g/L SC under controlled environmental conditions.

Keywords: Earthworms, Eisenia Fetida, Sulfentrazone 500 G/L SC, Juveniles

Introduction

Worldwide, herbicides are used in agriculture to control weeds for enhancing the crop yield. Hence, it remains important as they can significantly impact on the yield and prevent emerging of harmful weeds in the crop growing area. During application, there is a possibility of seepage of herbicides, enter into the soil ecosystem, and may affects the living things which are present in the ecosystem by way of affecting their feeding behaviour, reproductive systems etc., Earthworm is one of the major beneficial organism in the soil eco-system, as it is a key member of soil food webs [1] affected by various herbicides which leads to their weight loss and reproductive capacity. Earthworm mortality depends on the nature, concentration

and the duration to which earthworms are exposed to the herbicide. Earthworms play an important role in the biomass of macrofauna which is present in the soil [2]. The distress of earthworms is due to the herbicides usage in the soil and earthworms have been used for the estimation of lethal and sub lethal consequences of chemical contaminated pollutant [3].

Sulfentrazone is a selective soil-applied herbicide for control of annual broadleaf weeds in a variety of crops including soybean, sunflower, dry bean, dry peaand suppresses some grass weeds. This herbicide is commonly applied as pre-emergent treatments and possesses long residual effects and is registered for controlling weeds in various crops globally [4]. Earthworms are widely used in toxicology experiments that investigate their behaviour and biology and thus consider earthworms an environmental factor that influences soil properties and biological interactions [5]. Earthworms are exposed typically to agricultural pesticides and their soil degradants present within the soil profile. *Eisenia fetida* is one of the representatives of soil fauna and an easily cultured earthworm species and amenable to good experimental design which is recommended by OECD test guideline 207 for evaluation of safety usage of agrochemicals.

The aim of this present research is to assess the effect of Sulfentrazone 500 g/L SC on mortality and growth of the earthworm *Eisenia fetida* after 35 days and on reproductive output after 60 days under controlled environmental conditions.

Materials and Methods

Earthworm *Eisenia fetida* at the age between four to six months old were selected for the experiment. The worms were acclimatized under laboratory conditions for one day in the artificial soil which is used in the test. Based on OECD 207 guidelines, artificial soil was prepared with ingredients of 10% Sphagnum-peat, 20% Kaolin clay and 70% fine sand. The glass containers were used as test chambers and filled with 550 g of dry artificial soil for each replicate. The test item stock solution was prepared by weighing known quantity of test item and dissolved in known volume of deionized water. The test concentrations of 50, 100, 250, 500 and 1000 mg/kg dry artificial soil were prepared and achieved in the soil from a stock solution.

Sulfentrazone 500 g/L SC was applied in the soil at the rate of 50, 100, 250, 500 and 1000 mg/kg dry artificial soil in two batches consisting of 2 replicates per batch (approximately 1.1 kg dry soil/batch) for each concentration and control. The known quantity of test solution per batch was applied to the soil and mixed thoroughly using a laboratory mixer with the required quantity of deionised water. There were four replicates (10 worms/replicate) altogether at each concentration level and for control. The light intensity and test room temperature were maintained in the range of 400 – 800 LUX and 18 - 22^{o}C. Earthworms were washed with tap water, blotted carefully with filter paper, individually weighed, and then released into the test medium of each test container. The test containers were covered with perforated plastic lids after the release of earthworms.

Behavioral abnormalities were assessed after 15 minutes of earthworm release on day 0. Adult worms were exposed to treated soil for 35 days and after the completion of 35 days, worms were removed from the soil and weighed. Five gram of cow manure per container was carefully mixed into the artificial soil

to feed the juveniles. No additional food was given for the remaining study period.

Collection of worms

All live adult earthworms in each test container were weighed at the test start (day 0) and on the 35th day of exposure. The mean body weight change was calculated from the mean difference between replicate body weights at the test start and on day 35.

Adult worms

Juveniles

The experiment was terminated on day 60 and the artificial soil of each container was emptied on to a sieve and sieved manually. The number of juveniles in the soil present on the sieve was transferred to a plastic tray and counted using a hand tally counter. The remaining soil of the container was transferred to an enamel tray, and it was placed in a hot water bath at about 60^{o}C. All wriggled out juveniles were removed and counted. The collected juveniles in the control and treated groups were weighed and average body weight of juveniles were calculated. The body weight of juveniles from the treated worms were compared with control worms by statistical analysis.

Results and Discussion

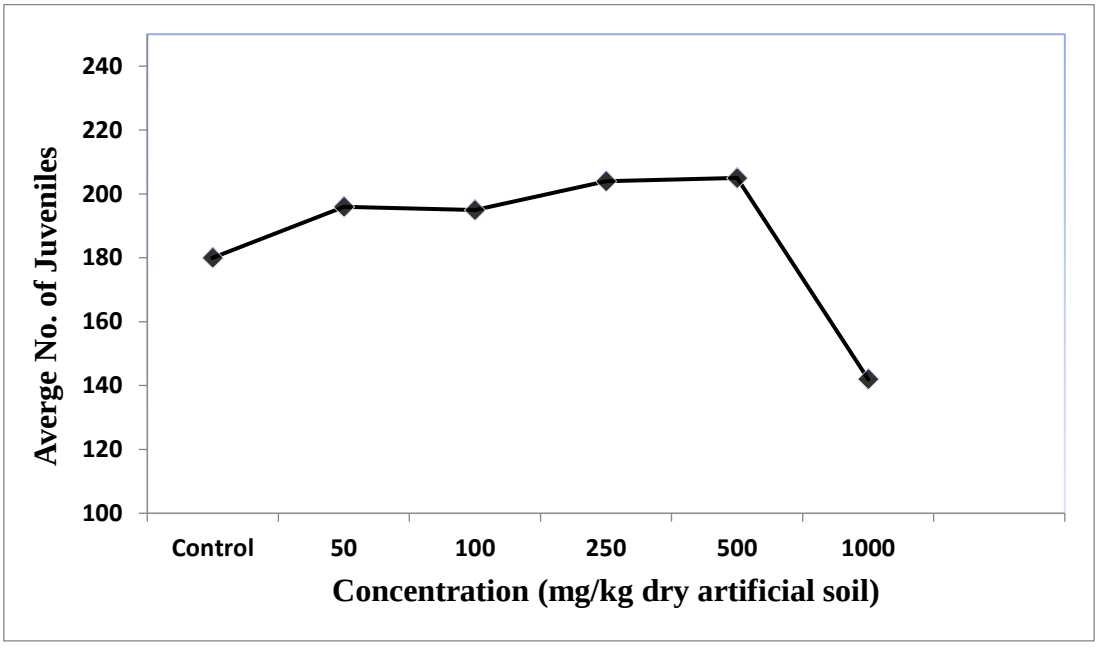
At the end of 35 days, there was no mortality and behavioral abnormalities were observed in all the control and test item treated earthworms. The biomass changes of the worms exposed to Sulfentrazone 500 g/L SC ranged from – 2.84% to 6.37%. The biomass change of earthworms in the control was 9.02%. There was no significant change observed in the biomass of adult worms treated with all test concentrations (50 - 1000 mg /kg dry artificial soil) when compared with the control worms. The average no. of juveniles in the control was 180. The average no. of juveniles exposed to Sulfentrazone 500 g/L SC ranged from 142 to 196. The average number of juveniles were significantly decreased in the highest test concentration 1000 mg/kg dry artificial soil when compared with control group. Similarly, the weight of juveniles was

significantly decreased belongs to treated group of 1000 mg/kg dry artificial soil when compared with control group. There was no significant decrease observed in the average number of juveniles and weight in the other treated groups (50 - 500 mg/kg dry artificial soil) when compared with control group.

Table 1 Biomass Changes of Adult Earthworms exposed to Sulfentrazone 500 g/L SC

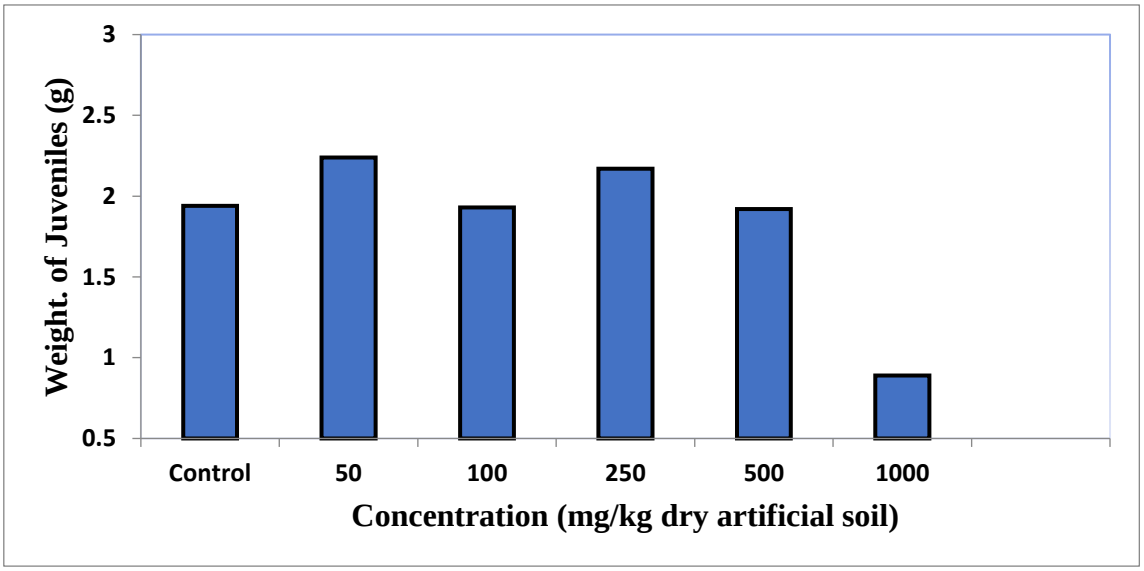
Treatment Group (mg/kg)	Mean body weight per earth worm (mg)		Biomass change (%)
	At start	After 35 days	
Control	403.4	367.1	-9.02
50	424.0	411.6	-2.84
100	393.4	418.1	6.37
25	399.5	402.6	0.78
500	391.7	389.0	-0.66
1000	414.6	422.1	1.87

Figure 1 Average no. of juveniles produced from adult earthworms exposed to Sulfentrazone 500 g/L SC



*Significantly decreased from control

Figure 2 Average weight of juveniles produced from adult earthworms exposed to Sulfentrazone 500 g/L SC



*Significantly decreased from control

In agriculture, judicious application of pesticide causes threat to ecosystems and even when used in small quantities, their toxicity and persistence have an adverse effect on ecological system [6]. Sulfentrazone is practically less toxic to non-toxic for aquatic and terrestrial organisms on an acute basis [7]. Although toxicity data on earthworm for other herbicides are available, for Sufentrazone there is not much data about its reproductive effect. In one of the similar types of research, the reproduction of *E. fetida* was not affected at low concentrations of acetochlor herbicide [8] but the toxicity at highest concentration of 1000 mg/kg soil is unknown. Mostly adult earthworms are designed to estimate the effects of chemicals on survival and reproduction based on guidelines such as EEC and OECD [9]. But juvenile earthworms are often more sensitive to pollutants than adults which is indicated in few studies [10] which was on par with our current investigation, where the juvenile production and biomass were affected at highest concentration. Sulfentrazone herbicide not only affects the reproduction in earthworms, but also to zebra fish embryos. Although Sulfentrazone is non-toxic to zebra fish at juvenile stage, it was found to be toxic at embryo stage in long-term study [11]. At the onset, it can also be quoted that, the said indices can be used as a tool in bioassessment to monitor toxicity risks of Sulfentrazone to the earthworms further correlating the possibilities of similar conditions in other organisms in the environment as well. Hence Sulfentrazone herbicide formulation may cause long-term adverse effects to organisms present in the aquatic and terrestrial ecosystem and further investigations are required to establish more toxicological data.

Conclusion

The current research findings revealed that the Sulfentrazone 500 g/L SC does not have any effect on mortality and biomass change in adult earthworms after 35 days of exposure, but it affects the reproductive output and biomass of juveniles at the higher concentration after 60 days of exposure. Thus Sulfentrazone 500 g/L SC have long-term effect on earthworms which inhibits the juvenile production and its growth.

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