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Research Article

Effect of Butachlor Herbicide on Earthworm *Eisenia fetida*—Its Histological Perspicuity

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With the advent of the Green Revolution, there has been a quantum leap in the use of synthetic herbicides and pesticides throughout the world to sustain high yielding crop varieties. Continuous use of these synthetic chemicals leads to loss of soil fertility and soil organisms. To explore the effect of exposure to commercial herbicide (Butachlor) on the life history parameters (biomass, clitellum development, and cocoon production) and the histological changes in the earthworm *Eisenia fetida* over 60 days, the dried cow dung was contaminated with 0.2575 mg kg^{-1}, 0.5150 mg kg^{-1}, and 2.5750 mg kg^{-1} of butachlor based on the LC_{50} value, and a control was maintained. The mean earthworm biomass was found to be decreased with increasing herbicide concentration. Similarly, cocoon production was also reduced by the increasing herbicide concentration. A possible explanation is an increased demand for energy, needed for the regulation and detoxification of herbicide. All earthworms in the exposed group were found to have glandular cell enlargement and to be vacuolated.

1. Introduction

Earthworms are often used as test organisms because of their important function, for example, as decomposer [1] and their sensitive reactions towards environmental influences. Earthworms are ecologically very important because there may be a risk secondary poisoning through feeding on worms contaminated by toxic substances. This could occur whether or not the worms themselves suffer any adverse effects. However, for most chemicals doses, which are toxic to birds or mammals and toxic to worms too, in such poisoning incidents, dead worms are found with affected predators [2, 3]. For that reason, earthworms are regarded as a reference compartment to observe soil contaminant bioavailability [4]. They are used to evaluate the lethal and sublethal effects of chemical contaminants and pollutants. Therefore they are useful to assess the contaminant fractions which may act on all organisms getting in touch with soil. Earthworms have been shown to be affected by the fate of herbicides in soil. Earthworms directly influence the persistence of herbicides in soil by metabolizing a parent compound in their gut [5, 6], by transporting herbicides to depth and increasing the soil bound (non extractable herbicides) fraction in soil or by absorbing herbicide residues in their tissues. The OECD earthworm toxicity test number 207 is widely used for terrestrial ecotoxicological test which is applied both in prospective and increasingly in retrospective ecotoxicological research. The present work reveals clearly that sublethal effect of herbicide butachlor on earthworm *Eisenia fetida* that provide fresh impetus concerning the environmental biology of its histology sheds light on the damage done to the intestinal region of earthworm.

2. Materials and Methods

2.1. Chemical. A commercially available Butachlor (2-Chloro-2′, 6′-diethyl-N-(butoxymethyl) acetanilide) (label content Butachlor) was used. Butachlor is used for the control of undesirable grasses and broadleaf weeds in transplanted direct seeded paddy and barley fields. It is a member of the chloroacetanilide class of chemistry.

2.2. Acute Toxicity Test. Tests were conducted using laboratory-bred adult earthworms of *Eisenia fetida*. The LC_{50} value of a commercially formulated butachlor was determined using the method recommended by OECD guideline

Table 1: Influence of different concentrations of herbicide on the growth of *E. fetida* over 60 days.

Days	Control	0.2575 mg kg^{-1}	0.5150 mg kg^{-1}	2.5750 mg kg^{-1}
1st day	0.020 ± 0.0003	0.019 ± 0.0004	0.0193 ± 0.0002	0.019 ± 0.0004
15th day	0.094 ± 0.0026	0.080 ± 0.0035	0.097 ± 0.0050	0.094 ± 0.0039
30th day	0.324 ± 0.0141	0.314 ± 0.0234	0.357 ± 0.0453	0.339 ± 0.0284
45th day	0.318 ± 0.0212	0.349 ± 0.0267	0.339 ± 0.0269	0.265 ± 0.0164
60th day	0.365 ± 0.0194	0.3534 ± 0.0278	0.3626 ± 0.0404	0.342 ± 0.0203

Mean ± SE; Significant at $P < .001$; Tukey test confirms Significant at $P < .05$ between days and treatments.

number 207 [7]. Different amounts of test substances had been mixed homogenously. The selected earthworm species for toxicity test were exposed to different concentrations of herbicides (equivalent to 100 g dry weight) by amendment method for 96 hours. Each concentration level was tested where five replicates in lieu of level was tested with five replicates. Finney's [8] probit method using graphical analysis was followed to calculate the LC_{50} value. In this study, the LC_{50} value was 0.515 mg kg^{-1} dry weight of medium.

2.3. Sublethal Toxicity Test. The substrate used was urine-free cattle manure that was sun dried, ground and sieved to a particle size of 500–1000 μm. Butachlor was diluted in solvent and mixed into the substrate to give moisture content of 75%. Prepared substrate was left for 24 hours to evaporate the excess solvents mixed in test chemicals. One group served as control, and three groups were exposed to a concentration of 0.2575 mg kg^{-1}, 0.5150 mg kg^{-1}, and 2.5750 mg kg^{-1}, respectively. Three replicates of each concentration, each vessel containing ten animals, were set up, and ten-day-old earthworms were inoculated. Biomass was determined over 60 days by removing the worms from substrate, washing them with distilled water and drying them on paper towels. They were then weighed fortnightly in a preweighed water-filled boats. This was done to prevent the worms from drying out and dying.

2.4. Clitellum Development and Cocoon Production. Worms were observed closely every two days starting from four weeks after they hatched. Worms were classified as juvenile, preclitellate, and clitellate using the rather subjective criterium clitellum and absence, partial development of the clitellum, and the presence of a fully developed clitellum. The culture medium of troughs was thoroughly searched for cocoons every second day, starting from 35th day. Cocoons were transferred to multicell containers to be incubated in distilled water. The containers were kept in the dark place and the water contents were replaced weekly to prevent bacterial growth.

2.5. Histological Study. After completion of life cycle, earthworms from each concentration were taken and washed with distilled water after which 50 mL jars were filled with 30 mL of 1.5% agar gel prepared with deionized water. After getting cooled and solidified, this gel in the jars was taken out and cut into small pieces. The earthworms were transferred separately into jars containing agar pieces and kept for 96 hours to remove all the soil from their gut. After removing the gut contents, earthworms were narcotized and cut into pieces and transferred to Zenkar's fixative for 12 hours and washed with running tap water for 12–24 hours. The worm samples were mounted weekly and stained with Iron Haemtoxylin stain for histological observation.

3. Results and Discussion

3.1. Influence of Herbicide on the Growth of E. fetida. The worms gained weight up to the last day of the experiment (see Table 1). The earthworm (15th day) in the control group had a mean biomass of 0.094 ± 0.0026 g and those in the exposure group 0.3534 ± 0.0278 g in 0.2575 mg kg^{-1}, 0.3626 ± 0.0404 g in 0.5150 mg kg^{-1}, and 0.342 ± 0.0203 g in 2.5750 mg kg^{-1}. On the termination of experiment, earthworms (60 days old) in the control group had mean biomass of 0.365 ± 0.0194 g. But in the exposure group it was 0.3534 ± 0.0278 g in 0.2575 mg kg^{-1}, 0.3626 ± 0.0404 g in 0.5150 mg kg^{-1}, and 0.342 ± 0.0203 g in 2.5750 mg kg^{-1}; the difference was significant at $P < .001$ levels. Further, Tukey test confirms the significant deference between days and treatments. At the end of experiment there was no difference between the mean biomass of control group and the exposure group.

3.2. Influence of Herbicide on the Clitellum Development of E. fetida. The rate at which the worms attained maturity differed in *E. fetida* exposed to different concentrations of butachlor. The percentage of mature specimens (expressed in terms of clitellate specimens) is shown in Figure 1. All specimens of *E. fetida* started developing clitellum from 29th day and on 33rd day clitellum development completed. The percentage of clitellum development decreased with increasing concentration of butachlor. The maximum clitellum development was observed in control on 30th day (40%), 43.3% for 0.2575 mg kg^{-1} at 29th day, 33.3% for 0.5150 mg kg^{-1}, and 26.6% for 2.5750 mg kg^{-1} concentration. Analysis of Variance (ANOVA) shows that the percentage of clitellum development of *E. fetida* differed significantly at ($P < .01$) in between days and it was not significant between treatment.

3.3. Influence of Herbicide on the Cocoon Production of E. fetida. The mean number of *E. fetida*'s cocoon production in different concentrations of butachlor is listed in Table 2. The maximum number of cocoons laid by control worm was 79.66 ± 2.603 on the 65th day. The worms exposed

Table 2: Cumulative cocoon production of earthworm *E. fetida* in different concentrations of herbicide.

Days	Control	0.2575 mg kg^{-1}	0.5150 mg kg^{-1}	2.5750 mg kg^{-1}
45th	28.33 ± 1.763	26.00 ± 2.081	22.00 ± 1.154	18.00 ± 1.732
55th	51.00 ± 2.081	44.00 ± 1.000	41.66 ± 2.333	35.00 ± 3.055
60th	79.66 ± 2.603	73.00 ± 2.309	62.33 ± 1.763	48.33 ± 3.480

Mean ± SE; Significant at $P < .001$; Tukey test confirms Significant at $P < .05$ between days and treatments.

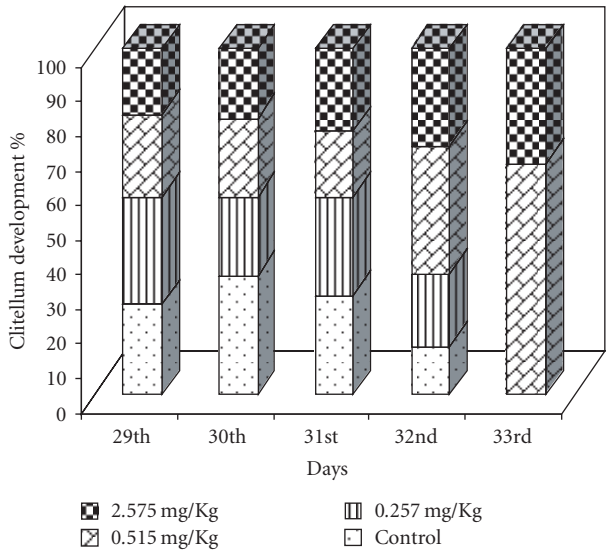

Figure 1: Percentage of clitellum development in *E. fetida* after exposure to different concentrations of herbicide.

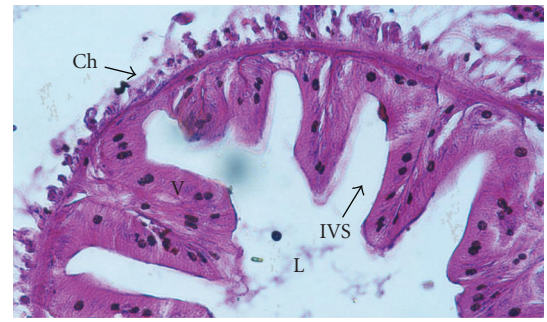

Figure 2: Cross section of earthworm *E. fetida* normal intestine and chloragogen tissue at control. L: Lumen; V: Villi; Ch: chloragogen tissue; IVS: Inter villious space.

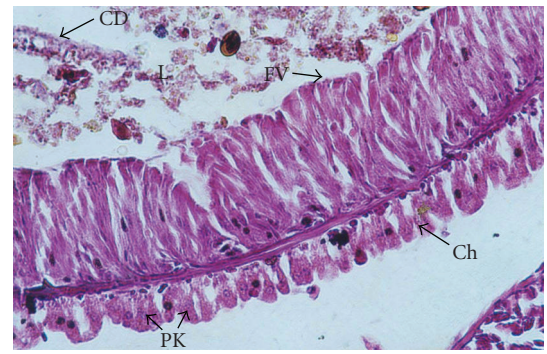

Figure 3: Cross section of earthworm *E. fetida* showing intestine and chloragogen tissue at 0.2575 mg kg^{-1} concentration of herbicide. PK: pyknotic nuclei; CD: Cellular Debris; L: Lumen; FV: Fused Villi; Ch: chloragogen tissue.

to 0.2575 mg kg^{-1} concentration exhibited the second peak of cocoon production over this period, which amounted to 73.00 ± 2.309 on 65th day. The minimum number of cocoon production was recorded in the 2.5750 mg kg^{-1} concentration (18.00 ± 1.732) on 45th day and maximum amount was 48.33 ± 3.480 on 65th day. The mean number of cocoon produced in the remaining concentration (0.5150 mg kg^{-1}) was found to be 62.33 ± 1.763 on 65th day. An ANOVA on the cocoon production shows significance ($P < .001$) between growth and treatment. Further, Tukey test confirms the significant ($P < .05$) difference between days and treatments.

3.4. Histopathological Changes on the Earthworm E. fetida. The intestine in control worm of *E. fetida* (Figure 2) consists of normal epithelial layer, the intermediate layer of longitudinal and circular muscle and blood vessels, and the other chloragogenous layer. Figure 3 revealed the histological changes of *E. fetida* at 2.5750 mg kg^{-1} concentration. The epithelial layer structure was grossly destroyed; fused and extra villous growth was pertained. Cell debris originated due to necrotic cell rupture and was found disseminated. Pyknotic nuclei had expressed. The chloragogen tissue was completely devastated with weak reserve inclusion.

In 0.5150 mg kg^{-1} concentration (Figure 4), the epithelial layer of villi was fused. A distinct cavitation was developed and pyknotic nuclei were observed in epithelial layer. The vaculoation could be seen inside the testis, testis sac, and vas deferens. The size of chloragogen tissue was reduced. However, 0.2575 mg kg^{-1} concentration (Figure 5) also shows the fused villous growth in the epithelial layer. There was no intervillous space between villi. In contrast, the pyknotic nuclei were found in many cells and cavitation was seen in chloragogen tissue layer.

4. Discussion

The biomass results of *E. fetida* revealed that earthworms had no inhibitory effect on the biomass. This result on growth was contradictory to that of Muthukaruppan et al. [9] who exposed *Perionyx sansibaricus* to the same herbicide butchlor and found that there was significant biomass difference. This is due to species sensitivity of earthworm.

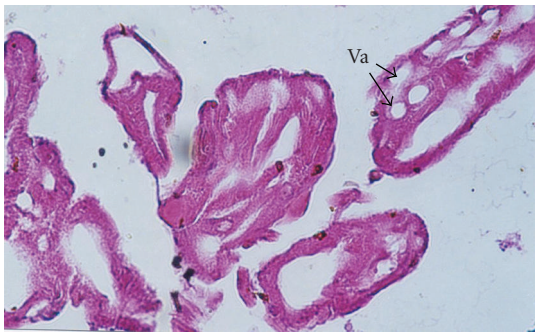

Figure 4: Cross section of earthworm *E. fetida* showing ovary at 0.5150 mg kg^{-1} concentration of herbicide. Va: Vacuoles.

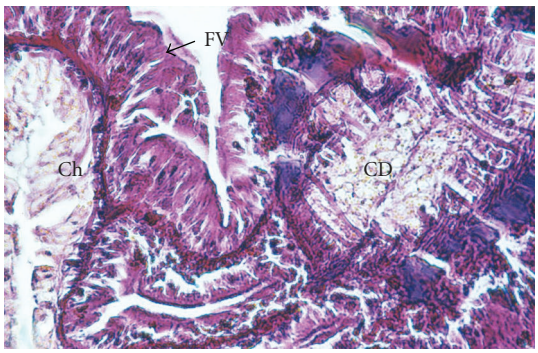

Figure 5: Cross section of earthworm *E. fetida* showing intestine and chloragogen tissue at 2.5750 mg kg^{-1} concentration of herbicide. Va: Vacuoles.

The maturation rate could not, therefore, be considered as a sensitive parameter to evaluate the effect of herbicide butachlor on this species.

In the present study, earthworm *E. fetida* produced cocoons which showed decreasing trend when the concentrations of butachlor were increased. Similarly [10] observed that the fungicide copper oxychloride reduced cocoon production with increased concentration of fungicide in *Eisenia fetida*. The present study confirms that the ability to resist a toxicant physiologically may be expensive interms of energy and other resources. This could involve a diminution of the ability to invest in other processes; for example, the energy available for reproduction is reduced. In the present study, epithelial tissue of earthworm *E. fetida*, exposed to butachlor, was severely affected. The present study confirms to the findings [11] that extreme nuclear swelling resulting in more than 2-fold volume increase of the average minimum could yet be observed only on the effect of sublethal paraquat toxication.

5. Conclusion

Earthworms are useful as test organisms to assess the toxicity of herbicidal contaminated soils, because of their sensitive changes occurred in biomass and cocoon production, and histological changes in tissues. The results clearly indicate that herbicide butachlor can retard growth and cocoon production and cause damage to epithelial tissue. The histological findings throw light on epithelial tissue damage especially fusion of intestinal villi that leads to the reduction of nutrient absorption area from food. Simultaneously, intoxication process consumes reserve energy from chloragogen tissue. This leads to reduced production of biomass and cocoon production The described methods and endpoints might help to understand the histopathological changes of earthworms towards herbicides and lead to an adapted test methodology. So histological-based end point provides more information about earthworm toxicology.

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