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Impact of herbicides on biomolecular constituents of *Eisenia fetida*

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Abstract

The present study of 60 days has been carried out to analyze the impact of herbicides *viz.* Sulfuron and Pinoxaden on the biochemical constituents of *Eisenia fetida*. For this, the earthworms were exposed to Sulfuron and Pinoxaden for sixty days and the earthworm samples from the herbicides treated substrate were collected on 1st, 30th and 60th day of experiment. Dose dependent biomolecular reduction has been observed in herbicide treated earthworms. The impact of herbicides on carbohydrates, lipid and protein contents were quite significant. The decrease in carbohydrates (60.68%), lipid (45.39%) and protein (19.00%) has been observed in earthworms exposed to Pinoxaden (3.0 ml/kg of soil) as compared to control. Pinoxaden induced more biochemical degradation in earthworms as compared to sulfuron.

Keywords: Herbicides, biomolecules, stress, toxicity, *Eisenia fetida*

1. Introduction

Use of herbicides in developed countries account for more than 70% of the total pesticides use [1]. Amplified use of herbicides on crops like rice, tea, wheat, beans etc. poses serious threat to the non- target organisms present in soil ecosystems. Soil structure is vital for the mobilization of herbicides and the residual effects of herbicides are dependable on soil structure and environmental factors [3]. Nonetheless, invertebrates and other fauna present in soil ecosystems ingest the herbicides from soil and thus bioaccumulation occurs in the tissues of organisms that pose threat to their survival. Despite having lower toxicity, the herbicides still are detrimental to worms' health [2]. It has been establishes that being thin and moist, earthworms' skin act as significant route for contaminants' uptake from the soil while the activity of ingestion adds to it [4]. Earthworms may act as biological indicators for the presence of herbicide contamination [5].Their biological activities after exposure to chemical contamination can provide an early warning of deterioration in soil quality [6]. *Eisenia fetida* is one of the most widely used earthworm species used in northern India. Therein, the present study has been carried out to envisage the impacts of herbicide exposure on biomolecules of *E. fetida*.

2. Materials and methods

The study was carried out in March, 2015 in Department of Zoology, CCSHAU, Hisar.

2.1 Collection of earthworms

The test healthy and clitellated earthworms were collected from a Vermicomposting Unit of the Department of Zoology, CCSHAU, Hisar.

2.2 Collection of substrate

The earthworms were cultured on the predigested cowdung that was procured from the Biogas plant, Department of Microbiology, CCCSHAU, Hisar.

2.3 Experimental set up

After the release of forty five washed earthworms in predigested cowdung the different concentrations of herbicides as mentioned in table 1 were sprayed on them. The technical grades of pesticides were collected from Ag Chem Access Ltd. Five replicates were maintained for each treatment along with the control. It was ensured that all the worms have burrowed in cowdung and factors like moisture (50%) and temperature (20-25℃) were maintained at optimum levels.

Table 1: Following treatments (sprayed) were given to the test earthworm species along with control

Sr. No.	Treatment	Concentration mg/kg soil
1.	Control	No treatment of herbicides
2.	Sulfuron	0.4, 0.8, 1.5, 3.0 mg/kg soil
3.	Pinoxaden	0.4, 0.8, 1.5, 3.0 ml/kg soil

2.4 Biochemical assays
After 1st, 30 and 60 days of pesticides exposure, the earthworms were dissected and various organs were removed rapidly for biochemical analysis. Body tissue homogenate was prepared by macerating tissue in 20% cold trichloroacetic acid (TCA). These contents were then incubated at 70ºC for 20 minutes and the supernatant was used for further chemical estimations. The total body carbohydrate was analyzed by the standard Phenol sulphuric method [7]. Total body lipids were estimated by following the Soxhlet extraction method [8]. Lowry method [9] was followed to analyze the protein content of earthworms.

2.5 Statistical Analysis
The data recorded in the experiment was subjected to one way ANOVA to analyze the significant differences among various treatments.

3. Results
The effect of herbicides on the carbohydrates content of earthworm has been indicated in Table 2. Dose dependent decrease in carbohydrates level in pesticides exposed earthworms has been observed. Decrease upto 60.68% and 45.03% has been observed in earthworms exposed to pinoxaden (3.0 ml/kg soil) and sulfuron (3.0 mg/kg soil) respectively. The observations also showed gradual decrease in carbohydrates content with the increase in concentration of herbicides. As shown in Table 3 and 4, the dose and time dependent reduction in the levels of lipid and protein contents have also been observed. The decline in protein upto 19% and 16.15% has been observed in earthworms exposed to pinoxaden (3.0ml/kg soil) and sulfuron (3.0 mg/kg soil) respectively. Significant reduction in the levels of carbohydrate, lipid and protein contents has been recorded as compared to control on each day of observation.

Table 2: Effect of herbicides on the carbohydrates content of earthworm, *Eisenia fetida*

Treatments	Description	Carbohydrate concentration (µg/mg of body tissue)		
		1 DAS*	30 DAS	60 DAS
T1	Control	12.89±0.006	12.89±0.006	12.89±0.006
T2	Sulfuron (0.4 mg/kg soil)	11.89±0.007	10.72±0.007	9.14±0.007
T3	Sulfuron (0.8 mg/kg soil)	11.69±0.007	10.12±0.007	8.20±0.007
T4	Sulfuron (1.5 mg/kg soil)	11.13±0.007	9.75±0.007	7.27±0.007
T5	Sulfuron (3.0 mg/kg soil)	11.08±0.007	8.72ª±0.007	6.09±0.007
T6	Pinoxaden (0.4 ml/kg soil)	12.96±0.007	11.80±0.007	10.00±0.011
T7	Pinoxaden (0.8 ml/kg soil)	11.56±0.007	10.46±0.007	8.80±0.007
T8	Pinoxaden (1.5 ml/kg soil)	11.46±0.007	8.72ª±0.007	6.33±0.007
T9	Pinoxaden (3.0 ml/kg soil)	11.32±0.007	7.52±0.007	4.45±0.007
	C.D. (p=0.05)	0.020	0.020	0.022

Values with the same superscript do not differ significantly
*DAS: Days after spray

Table 3: Effect of herbicides on the lipid contents of earthworm, *Eisenia fetida*

Treatments	Description	Lipid concentration (µg/mg of body tissue)		
		1 DAS	30 DAS	60 DAS
T1	Control	12.19±0.009	12.198±0.009	12.19±0.009
T2	Sulfuron (0.4 mg/kg soil)	11.91±0.009	10.96±0.007	10.73±0.007
T3	Sulfuron (0.8 mg/kg soil)	11.86ª±0.010	10.43±0.007	9.50±0.014
T4	Sulfuron (1.5 mg/kg soil)	11.83±0.009	10.21±0.007	8.30±0.007
T5	Sulfuron (3.0 mg/kg soil)	11.80±0.010	9.12±0.007	7.09±0.007
T6	Pinoxaden (0.4 ml/kg soil)	11.95±0.007	10.30±0.007	9.59±0.007
T7	Pinoxaden (0.8 ml/kg soil)	11.89ª±0.007	10.54±0.007	8.09±0.007
T8	Pinoxaden (1.5 ml/kg soil)	11.75±0.007	9.66±0.007	7.49±0.007
T9	Pinoxaden (3.0 ml/kg soil)	11.72±0.007	8.00±0.007	6.40±0.007
	C.D. (p=0.05)	0.024	0.021	0.024

Values with the same superscript do not differ significantly

Table 4: Effect of herbicides on the protein contents of earthworm, *Eisenia fetida*

Treatments	Description	Protein concentration (µg/mg of body tissue)		
		1 DAS	30 DAS	60 DAS
T1	Control	28.96±0.009	28.96±0.009	28.96±0.009
T2	Sulfuron (0.4 mg/kg soil)	28.69±0.008	27.51±0.007	26.60±0.007
T3	Sulfuron (0.8 mg/kg soil)	25.53±0.005	26.23±0.007	25.41ª±0.007
T4	Sulfuron (1.5 mg/kg soil)	28.20±0.006	26.16±0.007	24.00±0.007
T5	Sulfuron (3.0 mg/kg soil)	27.86±0.007	25.21ª±0.006	23.36±0.008
T6	Pinoxaden (0.4 ml/kg soil)	28.00±0.007	27.82±0.009	25.42ª±0.005
T7	Pinoxaden (0.8 ml/kg soil)	27.02ª±0.007	26.55±0.008	24.39±0.006
T8	Pinoxaden (1.5 ml/kg soil)	27.01ª±0.007	25.00ª±0.007	23.26±0.008
T9	Pinoxaden (3.0 ml/kg soil)	27.00ª±0.007	24.12±0.008	21.87±0.008
	C.D. (p=0.05)	0.021	0.021	0.022

Values with the same superscript do not differ significantly

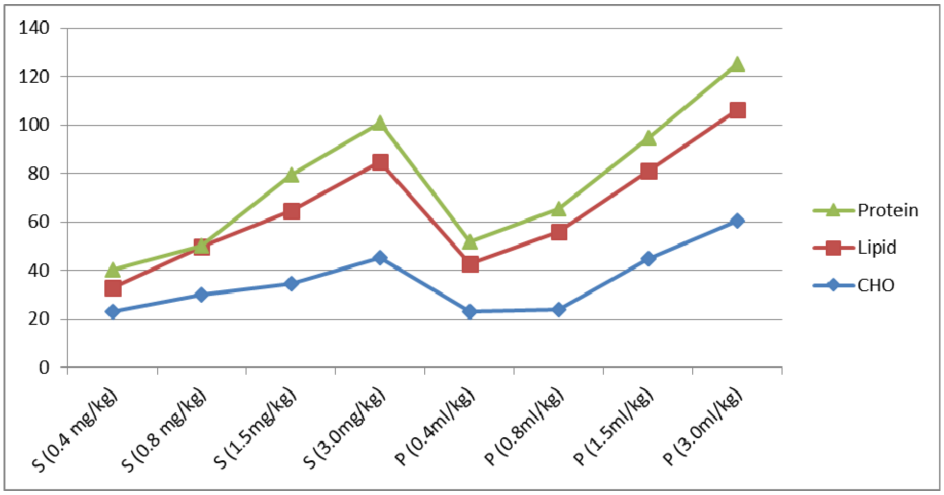

Fig 1: Percent decrease in biomolecular concentration of earthworm, *Eisenia fetida*

4. Discussion

Biochemical changes induced due to any pollutant act as vital indicators for the presence of contaminants in environment. However, this kind of studies help in establishing the threshold levels of pollution and lead to the better understanding of detrimental impacts of pollution. The carbohydrate level in earthworms exposed to Sulfuron (3 mg/kg) and Pinoxaden (3 mg/kg) decreased from 11.08 µg/mg to 6.09 µg/mg and from 11.32 µg/mg to 4.45 µg/mg respectively on 60th days after herbicide exposure. Gradual dose dependent decrease in total carbohydrates has been observed that can be supported by the findings of Dezwaan and Zandee, [10] and stated that pesticide exposure may lead to the occurrence of hypoxia that in turn increases the carbohydrate utilization. The principal immediate source of energy is represented in the form of carbohydrates [11] and the stress induced due to pesticide toxicity has resulted in increased carbohydrate catabolism. As indicated in Table 3, lipid levels fell to 7.09 µg/mg in earthworm treated with sufuron on 60 DAS while the control had about 12.19 µg/mg on the 60th day after treatment. Due to Pinoxaden (3 mg/kg) the lipid level fell to 6.40 µg/mg marking the depletion of lipid contents of earthworms. Dose and time dependent reduction in lipid content has also been observed in the present study that can be justified by the observations recorded by Padmajor and Rao [12] and Shukla and Kumar [13] who reported the stress induced declination in lipid contents of the earthworm. Lipids being the energy giving components may get mobilize for mitigating huge energy demands of the organism during stress condition [14]. Increased lipase activity can also be correlated with the decreased lipid contents [15]. Dose dependent significant decrease in protein content of pesticide exposed earthworms observed in present study may be stamped by the findings of Mosleh *et al.* [16]. The increased turnover of protein due to interference of toxicant may contribute to the decreased level of proteins. It has also been established previously that protein may act as alternate source of energy during prolonged stress condition that also advocates the present findings [17]. The stress may also induce structural deterioration at molecular level due to genotoxicity that may affect the total protein level of earthworm.

5. Conclusion

Significant decrease in carbohydrates, lipid and protein contents as compared to control have been observed. Pinoxaden was found to be more toxic as compared to the Sulfuron. The degrading effects of pesticides on the biomolecular composition of earthworms may be stamped by the present findings. Thus, pesticides poses threat to soil fertility by decreasing the survivability of earthworms in soil especially in pesticides exposed agro-ecosystems.

6. References

1. Mutthukaruppan G, Janardhanan S, Vijayalakshami GS. Sublethal toxicity of herbicide butachlor on earthworm *Periyonyx sensibaricus* and its histological changes. Journal of Soil Sediments. 2005; 5:82-86.
2. Zarea MJ, Ghalavand A, Mohammadi GE, Rejali F. Effects of clovers intercropping and earthworm activity on weed growth. Journal of Plant Protection Research. 2010. 50:469-469.
3. Zarea MJ, Karimi N. Effects of hearbicides on earthworms. Dynamic soil, Dynamic plant. 2012; 6(1):5-13.
4. Paton GI, Killham K, Weitz HJ, Semple KT. Biological tools for the assessment of contaminated land: Applied soil ecotoxicology. 2005; 21:487-499.
5. Zhang Q, Zhang B, Wang C. Ecotoxicological effects on the earthworm *Eisenia fetida* following exposure to soil contaminated with imidacloprid. Environmental Science and Pollution Research. 2014; 1:12345-12353.
6. Ali SA, Khan I, Ali AS. Friendly Earthworms. Science Reporter. 2006; 43(1):28-30.
7. Masuko T, Iwasaki N, Yamane S, Funakoshi T, Majima T, Minami A et al. Chitosan–RGDSGGC conjugate as a scaffold material for musculoskeletal tissue engineering. Biomaterials. 2005; 26:5339-5347.
8. Soxhlet F. Die gewichtsanalytische Bestimmung des Melcheftes. Journal Dingler's Polytechnisches. 1879; 232:462-465.
9. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with Folin Phenol Reagent. *Journal of Biology and Chem*istry. 1951; 193:265-275.
10. Dezwaan A, Zandee DI. The utilization of glycogen and accumulation of some intermediates during anaerobiasis in *Mytilus edulis.* Comparative Biochemistry and Physiology. 1972; 43:47-54.
11. Umminger BL. Physiological studies on super cooled fish *Fundulus heteroclitus*. Carbohydrate metabolism and survival at sub zero temperature. Journal of Experimental Zoology. 1970; 17:159-74.
12. Padmajor S, Rao JV. Toxic effect of chlorpyrifos on lipid

contents of earthworms. Ecotoxicology and Environmaental Safety. 1994; 54:296-301.

13. Shukla, V, Kumar P. Effect of endosulfan and cypermethrin to the earthworms. Biochemical Journal. 2004; 181:47-50.
14. Hochachka PW, Somero G. Biochemical Adaptation. 1984; Princeton University Press, Princeton, New Jersey.
15. West, P., Perandones, C.E., Illera, V.A., Peckham, F. and Ashman, R.A. Regulation of Apoptosis *in vitro* in Mature Spleen T Cells. Journal of Immunology. 1967; 151:3521-3529.
16. Mosleh YY, Ismail SMM, Ahmed MT, Ahmed YM. Comparative toxicity and biochemical responses of certain pesticides to the mature earthworm *Aporrectodea caliginosa* under laboratory conditions. Environmental Toxicology. 2003; 18:338-346.
17. Sturzenbaum SR, Winters C, Galay M, Morgan AJ, Kille P. Metal ion trafficking in earthworms. Journal of Biological Chemistry. 2001; 276(36):34013-34018.