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# Studies on Sub Lethal Effect of Cypermethrin and Oxyfluorfen on Biochemical Parameters of Earthworm Species, *Eisenia foetida* Savigny, 1826

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**ABSTRACT:** Present piece of work deals with sub lethal effect of pesticides viz., Cypermethrin and Oxyfluorfen on biochemical parameters of earthworm species, *Eisenia foetida*. The results revealed that the sub lethal concentration of both pesticides significantly reduce the biochemical content of experimental worms over the control. Decreased values of protein, triglyceride and glycogen were dose dependent and found to be 11.1 % & 4.9 %; 35.8 % & 22.4 % and 13.6 % & 22.7 % in worm exposed to 3/4<sup>th</sup> sub lethal concentration of Cypermethrin and Oxyfluorfen respectively.

**KEY WORDS:** Pesticides, Cypermethrin, Oxyfluorfen, Protein, triglyceride and glycogen etc.

### I. INTRODUCTION

Herbicides and insecticides have become inseparable part of modern agricultural production and technology. But it can adversely affect on beneficial soil organisms and there by contribute to environmental concern. This necessities permanent monitoring of their use and effects on the growth and development of the soil macro and micro flora and fauna. Particularly earthworms. Earthworms are highly susceptible to changes of ecological factors [1]. Globally, Cypermethrin is one of the most widely used synthetic pyrethroid for agricultural and domestic purposes. Most of the pesticides used in the agriculture end up as residues in the soil, making soil dwelling organisms, especially earthworms more susceptible to pesticides intoxication. Cypermethrin is known to be a neurotoxicant to many model organisms, including mammals and insects, but such type of toxicity evidence is not available for invertebrate system like earthworms [2]. The use of Cypermethrin occupies 4<sup>th</sup> position globally in pest application. The usage is more than one million pounds per year in the US only as an active ingredient for agriculture necessities [3]. However, despite its usage and knowledge on the toxicity of Cypermethrin, Surprisingly very less work have been carried out on earthworm to evaluate the effects of this insecticide [4].

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, by transporting herbicides to depth and increasing the soil bound (non extractable herbicide) fraction in soil or by absorbing herbicide residues in their tissues. [5]. *Eisenia fetida* is an earthworm of high abundance in compost upper layer of soil. It has been used as a biomonitor for heavy metal and insecticide ecotoxicology and potential spin-off for the use of earthworm data in ecotoxicological study risk assessment [6]. Earthworm have been exposed the soil spiked with the herbicide precursor, Cloroacetamide, which was lethal to the animals [7]. Butachlor herbicide causes histological changes [8].

The abundance use of herbicides does not affect only selected weeds, but also destroy non-target species in the agricultural fields as well as and also affect the texture and physiochemical properties of soils [9]. The use of specific herbicide, fungicides, and insecticides in the agricultural field can be highly toxic to earthworm population [10]. Correira and Moreira [11] reported the effect of herbicide on growth, survival and reproduction rate of earthworm

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species, *Eutyphoens waltoni*. Sublethal concentration of Cypermethrin shown significant responses on fatty acid, sugars and amino acids in worm species, *Metaphire posthuma* [2]. Earthworms have been studied as a readily available, easily maintainable and cheap test species for assessing chemical pollution. The present piece of investigation aims to find out the 1/4<sup>th</sup> and 3/4<sup>th</sup> sub lethal effect of Cypermethrin and Oxyfluorfen on biochemical parameters viz., protein, triglyceride and glycogen content of the worm, *Eisenia foetida*.

### II. MATERIAL AND METHODS

**BIOLOGICAL MATERIAL:** Earthworm species *Eisenia foetida* were obtained from state Government agricultural nursery, Sakri (Dhule) M.S. They were maintained in the laboratory as per OECD [12] guidelines. Only healthy adult worms having well developed clitella were used for the experiment.

**CHEMICAL MATERIAL:** Two chemicals i.e. commercially available Cypermethrin (10% EC) an insecticide belongs to synthetic pyrethroid and selective herbicide, Oxyfluorfen (23.5% EC) commonly called “Goal” were purchased from local pesticide shop. The quality soil and a month old cow dung were collected from agriculture field and cow shed respectively.

**EXPERIMENTAL SET-UP:** The experiment was performed in plastic tough having five kg capacities. A dried quality soil was ground and sieved. In a tough 800 g of fine soil thoroughly mixed with 200 g Cow dung and appropriate amount of water was added to moisten the mixture.

**TREATMENT:** The LC<sub>50</sub> values of both the pesticide in earthworms were already estimated in our previous study, which was 2.240 ml/kg for Cypermethrin and 0.420 ml/kg for Oxyfluorfen. The sub lethal concentrations i.e. 1/4<sup>th</sup> and 3/4<sup>th</sup> doses of Cypermethrin is 0.56 and 1.68 ml/kg respectively. While the sub lethal doses (i.e. 1/4<sup>th</sup> and 3/4<sup>th</sup>) of Oxyfluorfen is 0.105 and 0.315 ml/kg respectively. The dose concentrations of both pesticides were separately added in 100 ml of distilled water that was mixed in the experimental groups only. On next day, 20 mature worms were added to each tough. The tough was covered with perforated lid and kept for 45 days. The experimental set up was prepared in triplicate for each treatment.

### BIOCHEMICAL MEASUREMENT:

**ESTIMATION OF PROTEINS:** At the end of the experiment of about 45 days, earthworms were removed from vermibed and fasted for 24 h, so that their digestive tract was completely empty. For biochemical analysis, the worms were cut into pieces and mixed with ice cold 0.86% invertebrate ringer solution, the mixture was homogenized and centrifuged. The resulting supernatant was used for the determination of protein. It was estimated according to the method suggested by Lowry [13]. Triglycerides were estimated from the earthworm homogenate by GPO/PAP method [14] and glycogen was estimated by method suggested by Goel [15]. Results were tabulated and statistically analyzed.

The per cent change in biochemical contents were determined by applying following formula i.e.

$$\% \text{ change} = \frac{\text{Control} - \text{Experimental}}{\text{Control}} \times 100$$

### III. RESULTS AND DISCUSSION

The biochemical contents of control and experimental worms exposed to sublethal concentration of both pesticides is presented in table-1. This table shows that there is significant and dose dependent decline in biochemical contents like protein of the worm exposed to 1/4<sup>th</sup> and 3/4<sup>th</sup> sub lethal concentration of Cypermethrin and Oxyfluorfen is 7.4 % & 11.1 % and 1.2 % & 4.9 % respectively. In case of triglyceride content, the values are 28.4 % & 35.8 % and 13.4 % & 22.4 % respectively. Whereas glycogen content were found to be 4.5 % & 13.6 % and 18.2 % & 22.7 % respectively under both pesticide stresses.

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**PROTEINS:** Proteins are very important organic substance. In present study, the results show maximum reduction (11.1 %) in protein content was found in worm exposed to 3/4<sup>th</sup> dose concentration of Cypermethrin than Oxyfluorfen exposed worms. The decrease in protein content could also be attributed to spontaneous utilization of amino acids inside the organism by various catabolic reactions in order to combat the stress condition. Similar results were reported by Jha and Verma [16] in fish species, *Clarias batrachus* exposed to pesticide mixture. Baruah [17] reveal study on effect of paper mill effluents of muscle proteins in fish, *Heteropneustus fossilis*. In chronic toxicity, fish were exposed to atrazine herbicide for 30 days. The results showed significant decrease in plasma total protein, albumin, glucose, cholesterol and triglycerides [18]. Somaiah [19] reported significant decline in total glycogen, protein, lipids, carbohydrates and free amino acids etc were observed in freshwater fish, *Labeo rohita* exposed to sublethal concentration of organophosphate pesticides, Phenthoate.

**TRIGLYCIRIDES:** Triglyceride content has decreased in the present report. It is observed that maximum (35.8 %) decreased was found in worm exposed to 3/4<sup>th</sup> sub lethal concentration of Cypermethrin than Oxyfluorfen (22.4 %). During the time of low availability of carbohydrates, lipids serve as a source of energy for supporting the physiological functions of the body. Hence, the decline in triglyceride content was due to the utilization of lipids for meeting the energy demand under the pesticide stress. Similar results [19, 20] support the present work that the triglyceride level was changed when worm exposed to toxicants like Cypermethrin and Oxyfluorfen.

**GLYCOGEN:** The Carbohydrates (Glycogen) are the main source of energy in the cells and play a vital role in cellular metabolism by acting as fuel and providing energy to the body cells. In present study maximum glycogen depletion was observed in worm exposed to the higher sub lethal concentration of herbicide, Oxyfluorfen (i. e. 22.7%) than insecticide, Cypermethrin (i. e. 13.6 %). The change in carbohydrate metabolism that would meet the changing energy demand may be subjected to stress. These alterations supports that carbohydrate metabolism in animals is effected by the toxicants [19, 21, 22]. Nesrine and Soltani [23] revealed reduction in the amount of glycogen content in pupae of *Drosophila melanogaster* exposed to sub lethal concentration of an insecticide, Spiromesifen. Our work is corroborating with Nagaraja [24], they studied oxidative stress in freshwater fish, *Labeo rohita* exposed to sublethal concentration of Chlorantraniliprole (Coragen). They revealed that protein as well as glycogen and total lipid content of various organs were decreased significantly. Epoxiconazole a persistent widely used pesticide affect on lipid and glycogen content after 28 days of exposure [25].

**Table-1: Biochemical content of *Eisenia foetida* treated with pesticides**

Treatment	Dose concentration	Biochemical parameters (mg/ dl)		
		Protein	Triglyceride	Glycogen
Control	--	40.50 ± 2.0	33.50 ± 1.50	11.00 ± 0.5
Cypermethrin	1/4 <sup>th</sup>	37.50 ± 1.0 (7.4) *	24.0 ± 2.0 (28.4) **	10.5 ± 0.5 (4.5) NS
	3/4 <sup>th</sup>	36.00 ± 1.5 (11.0) *	21.50 ± 1.5 (35.8) ***	09.50 ± 0.5 (13.6) *
Oxyfluorfen	1/4 <sup>th</sup>	40.0 ± 1.0 (1.2) NS	29.0 ± 1.5 (13.4) *	9.0 ± 0.5 (18.2) **
	3/4 <sup>th</sup>	38.50 ± 1.5 (4.9) NS	26.50 ± 1.5 (22.4) **	08.50 ± 1.0 (22.7) **

Significant values: \*P<0.05, \*\*P<0.01, \*\*\* P<0.001.

Values in the parenthesis are % change over control. CD (Mean ± SEM, n = 3)

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## IV. CONCLUSION

The present study indicates that both pesticides i.e. Cypermethrin and Oxyfluorfen cause alterations in all the biochemical parameters of earthworm, *Eisenia foetida*. The significant reduction in levels of protein, triglyceride and glycogen contents were found to be dose dependent over the control worm. It might be caused by intoxication of pesticidal stress in the intermediary metabolism of the earthworm.

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