

# **Sub Lethal Effects of Cypermethrin and Oxyfluorfen on Stress Enzyme Activities of Earthworm Species, *Eisenia foetida* Savigny, 1826**

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**ABSTRACT:** Present study aims to assess the effect of Sublethal dose of the commercial formulations of Cypermethrin (insecticide) and Oxyfluorfen (herbicide). Laboratory study was conducted in a natural Soil and Cow Dung in order to measure effect of these chemicals on activities of stress enzymes viz., Glutathione, Catalase (CAT) and LPO/MDA for a period of 90 days. The activity of Glutathione and CAT was significantly decreased whereas LPO/MDA activity significantly increased as compare to control. These results confirm the toxic effect of insecticide and herbicide on earthworm and pollution indicator under long term exposure.

**KEY WORDS:** Sublethal, Cypermethrin, Oxyfluorfen, Pollution indicator, stress enzymes.

## **I. INTRODUCTION**

Soil pollution has enormously increased during the last 3-4 decades due to the intensive use of pesticides and fertilizers in agriculture, industrial activities, urban waste and atmospheric deposition. It causes decrease in soil fertility, alteration of soil structure, disturbance of the balance between flora and fauna residing in the soil contamination of the crops, groundwater pollution and threat for living organisms [1].

Earthworms are common soil organisms in moist environment and play an important role in improving structure and fertility of soil ecosystems [2]. It has been indicated that earthworms may represents up to 60-80% of the total animal biomass in soil [3]. Unlike many other soil organisms that are protected by thick cuticle on the exterior of their bodies, earthworms are protected by thin cuticle and therefore these are particularly susceptible to soil chemicals [4]. The bioaccumulation of insecticides in earthworms may not lead to significant effects to the animal itself, but may produce serious damages to higher tropic levels [5]. Therefore earthworms are suitable bio indicators of soil contamination and can be used to provide safety thresholds for insecticides applications and also used as key index of eco toxicology diagnosis [6,7].

The earthworm skin is extremely permeable to water [8] and it represents a main route for contaminant uptake [9,10] Secondly, these organisms ingest large amount of soil, therefore they are continuously exposed to contaminants absorbed to solid particles through their alimentary tract [11]. The acute toxicity test for earthworm requires 14 days [12] and it only measures lethality, it may be insufficient for predicting long term effect [13]. Chronic test, based on the inhibition of earthworm reproduction [14] provides a more ecological relevant sublethal end point than lethality but it may requires a longer exposures period (at least 50 days) for accurate assessment [15]. Antioxidant defenses are the most used biomarkers due to their crucial role in cell homeostasis preventing toxic action of chemicals [16]. Therefore, earthworms is receiving increasing attention for its potentially in soil pollution monitoring and assessment. Biomarkers specific to oxidative stress, recommended for biomonitoring the quality of the aquatic environment, including malondialdehyde (MDA) which is derived from lipid peroxidation of polyunsaturated fatty acids in cell membranes

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during oxidative stress [17,18,19]. Reduced glutathione (GSH) involved in the antioxidant defense system [20] and Catalase (CAT) which is the first line of defense against oxidative stress [21].

Cypermethrin is a synthetic pyrethroid insecticide, an analogue of Pyrethrins. It is extensively used for pest control programs in domestic, industrial and agricultural sector [22]. On other hand Oxyfluorfen is a selective pre and post emergent contact herbicide used to control certain annual broad leaf and grassy weeds in vegetables, fruit, cotton, ornamentals and on non-crop areas. In India, both pesticides i.e. Cypermethrin and Oxyfluorfen are very common and extensively used in agriculture [23,24].

Very little information is available regarding effects of insecticide and herbicide on earthworm. It promoted us to undertake this work. Hence, our aims to assess the effect of sublethal dose of a commercial formulation of Cypermethrin and Oxyfluorfen on earthworm, *E.foetida* Savigny, 1826.

## II. MATERIALS AND METHODS

**BIOLOGICAL METHOD:** Earthworm species *Eisenia foetida* were obtained from state Government agriculture nursery, Sakri, Dist- Dhule (M.S). As per guideline (OECD, 1984) they were maintained in the laboratory. Only healthy adult worms having well developed clitella were used for the experiment.

**CHEMICAL METHOD:** Two chemicals i.e. a commercially available Cypermethrin (10% EC) an insecticide belongs to synthetic pyrethroid and selective herbicide, Oxyfluorfen (23.5% EC) commonly called 'Goal' are used. Both pesticides were purchased from local agro market.

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 5 litre capacities. A dried quality soil was ground and sieved. In a tough 800 g of fine soil thoroughly mixed with 200 g cow dung (CD) and appropriate amount of water was added to moisten the mixture. On next day 20 mature worms were added to each tough. The tough was covered with perforated lid. The experimental set up was prepared in triplicate for each treatment.

### TREATMENT OF THE WORMS

The LC<sub>50</sub> Values of both the pesticides in earthworm were already estimated in our previous study, which was 0.240 ml/ kg for Cypermethrin and 0.420 ml/ kg for Oxyfluorfen. A higher sublethal (3/4<sup>th</sup>) dose i.e. 0.180 and 0.315 ml/ kg of Cypermethrin and Oxyfluorfen respectively was added in 100 ml of water and that was mixed thoroughly in the experimental groups only. To maintain 75 ± 5 % moisture level water was sprinkled at the interval of 3 to 4 day. The entire tough was kept for 90 days under shade at room temperature.

### ENZYMATIC MEASUREMENT

At the end of the experiment, earthworms were fasted for 24 hours so that their digestive tract was completely empty. Prior to 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 activities of enzymes like Glutathione, CAT and MDA. The Glutathione was realized by [25], the activity of CAT was measured according to the method of [26] and lipid peroxidation level (MDA) was assessed by measuring the TBARS formation as described by [27].

## III. RESULTS AND DISCUSSION

Antioxidant activities of Glutathione, CAT and MDA are presented in table-1. It shows that the activity of Glutathione was slightly or non significantly decreased whereas it was significantly decreased in CAT. Similarly the MDA activity was found to be significantly increased for both pesticides group over the control.

Earthworms were hardly studied in past and their use was mainly concentrated on the effects of heavy metals [28,29] whereas very few studies in past have included research on the impact and effect of organic pollutants such as

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pesticides on the earthworm. [30,31]. During the exposure to pesticides, earthworms are capable to reduce the toxic effects of the chemical product by adjusting their internal biochemical responses. So the biochemical reactions are rather important to evaluate the potential adverse effects of chemicals on the environment [32]. Our results showed decreased Glutathione and CAT and increased LPO/MDA activities. The inhibition of CAT activity may be the result of increased H<sub>2</sub>O<sub>2</sub> in the cell which can be generated directly by divalent reduction of O<sub>2</sub> or indirectly by univalent reduction of O<sub>2</sub> followed by dismutation of oxygen [33].

The decrease of the rate of GSH, is due to the use of the GSH to thwart the installation of the oxidative stress induced by the ROS generated by the exposure to the insecticide used. Our results are corroborated with [34] and [35] who revealed reduction in Glutathione in earthworm treated with insecticide like methonyl.

The increased MDA was a good correlation with the generation of ROS [36,37]. Many studies have indicated that the level of the peroxidation of lipids was an important parameter assessing the level of the oxidant efforts in the living organisms. Induced an increase of the rate of MDA. This agreement with the previous work of [38,39]; suggested that one of the most damaging effect of ROS and their products in cells is the peroxidation of membranes lipids, who can be indicated by the detection of MDA. Our results also corroborated with work done by Zeriri *et al* (2012) on potential toxicity of an insecticide on earthworm, *Octadrilus complanatus* an increase of catalase activity and the rate of MDA after exposure of earthworms to the methonyl insecticide. Similar work i.e. treatment of Malathion to bivalve snail (mollusca) was studied by [40]. They showed that the activity of acetyl cholinesterase was strongly inhibited whereas the activity of catalase increased. Enhanced LPO/MDA levels in fishes indicated development of oxidative stress [41]. Depleted catalase activity can suggest increased oxidative stress and increased MDA activity may be indicative of free radical tissue injury [42].

**TABLE-1**  
**Antioxidant activities of stress enzymes (Glutathione, CAT and MDA) in earthworm exposed to Sublethal concentration of Cypermethrin and Oxyfluorfen.**

Sr. No.	Pesticide	Group	3/4 <sup>th</sup> Sublethal dose (ml/kg)	Antioxidant activities		
				Glutathione (mg/g)	CAT (mg/g)	MDA (N mole/g)
1	Cypermethrin	Control	--	19.34 ± 2.20	0.480 ± 0.020	40.56 ± 2.50
		Experimental	0.180	18.16 ± 1.76 (-6.10) NS	0.348 ± 0.020 (-25.5) **	65.52 ± 3.20 (+ 38.09) ***
2	Oxyfluorfen	Experimental	0.315	16.70 ± 1.58 (-13.65)*	0.276 ± 0.020 (-42.50) ***	90.56 ± 3.40 (+55.21) ***

\* Significant value: P<0.05, \*\* P<0.01, \*\*\* P<0.001. Values in the parenthesis are per cent change over control. CD (Mean ± SEM, n = 3).

#### IV. CONCLUSION

In nutshell, earthworms are the sentinel animals in pollution by Cypermethrin and Oxyfluorfen. Similarly, this commercial formulation causes change in MDA, Catalase (CAT) and Glutathion enzyme activity. The increased MDA/LPO activity may be indicative of free radical tissue injury of oxidative stress. Decreased glutathione and CAT activity may be due to the failure of detoxification phenomenon of pesticide and development of oxidative stress. The depleted CAT can also suggest increased oxidative stress.

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