

Toxic Effect of Endosulfan on The Caudal Regeneration (Posterior Region) of The Earthworm Oligochaete, *Eisenia fetida*.

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Abstract: Endosulfan is an organochlorine which is indiscriminately used in agriculture and has lethal effects on many organisms. The acute toxicity of Endosulfan (Organochlorine) pesticide to earthworms, *Eisenia fetida* was evaluated to determine uptake and effect environmentally relevant concentrations on survival, mortality, population, morphology, physiology and behavior, etc. Acute toxicity and lethal concentrations (LC₅₀) for 24, 48 and 72h were calculated in soil probits/concentrations regression equation and the LC₅₀ found to be 0.006, 0.007 and 0.008 ppm of Endosulfan respectively recorded.

The earthworm, *Eisenia fetida*, is capable of the regeneration of missing body parts. The impact of endosulfan on the caudal regeneration of the Indian earthworm *Eisenia fetida* tested. The main theme of the present study is to assess the effect of Endosulfan on the caudal regeneration of the earthworm, *Eisenia fetida*, were transected after amputated posterior portion in different concentrations like 0.003, 0.004, 0.005, 0.006, 0.007 and 0.008 ppm and thereafter inoculated into 250 ml beaker containing soil mixed with single agricultural dose of Endosulfan for 30 days and then the result of regenerating efficiency is 46.15, 42.85, 38.46, 33.33, 21.42, and 16.66% respectively.

INTRODUCTION:

Different types of pesticides are spread through all segments of the environment due to their indiscriminate usage and their potential toxicity play an important role in the environment and biodiversity. Decomposition is brought about by the whole range of micro-organisms, fungi and soil animals that live in soil. Earthworms are important contributors to the decomposition process. *Eisenia fetida* is the standard test organism used in terrestrial

ecotoxicology, because it can be easily bred on a variety of organic wastes with short generation times [11,12,18,19].

There are many reports on the toxic effects pesticides on the earthworms ecology, morphology, behavior, physiology, etc. Ecologically relevant species of earthworms, as stated earlier [6], and also to other soil fauna to get a comprehensive knowledge on the malfunction in the soil biological processes due to pesticide pollution and food chain can be

minimized [1, 24]. A number of studies have been conducted on the morphological changes such as organophosphate tested on earthworms and observed decrease in the body weight of treated worms [3, 7] and weight loss has also been reported for organochlorine pesticides [2, 4, 5, 9, 10, 15].

Several researchers observed the effects of pesticides on the physiology of earthworm such as growth and reproduction, regeneration etc. Xiao, *et. al.*, [28] suggested that growth can be regarded as sensitive parameters to evaluate the toxicity of acetachlor on earthworms. Whereas toxicity of chlorpyrifos, carbofuran, mancozeb and their formulations on the *Perionyx excavates* [6]. Not much information regarding the effect of pesticides on the caudal regeneration of earthworm is available. Hence the present study has been undertaken to determine the effect commonly used pesticide, endosulfan on the caudal regeneration of *Eisenia fetida* used as a model organism.

MATERIAL AND METHODS:

In the present study earthworms were taken for regeneration. The earthworms *Eisenia fetida* were collected from wet soil on the outskirts of Omerga, Dist. Osmanabad in the Marathwada region. The species were brought to the laboratory and they were maintained under normal day/night illumination for three days in glass troughs containing wet soil before subjecting for experimentation as described by Kulkarni, [13]. Earthworms looking healthy and having approximately equalled in size (6 to 7 cm long) and weight were selected for all experiments.

Acute toxicity of the pesticide, like endosulfan has been tested. In order to ascertain the lethal concentrations (LC_{50}) of pesticides for 24, 48 and 72 h, groups of 20 worms were maintained separately in 250 ml beaker, containing 200 g wet soil (200g soil+30ml water). The desired concentrations in the range of 0.006, 0.007 and 0.008 ppm of Endosulfan were obtained from stock and (30 ml water mixes with pesticide) mixed separately in 200g

soil and worms (20 in each set) were exposed. The data collected were then elaborated statistically by means of the probits method [8]. by transforming to toxicity curve (% mortality/concentration). The average LC_{50} for 24, 48 and 72 h were calculated (Table-1).

To study the effect of Endosulfan, on the regeneration of earthworm *Eisenia fetida*, the worms having approximately equal size (6 to 7 cm long) and weight, were divided into six groups of 20 worms in each and maintained in 0.003, 0.004, 0.005, 0.006, 0.007 and 0.008 ppm sub lethal concentrations of Endosulfan. One group of worms in normal soil served as control. Experiments lasted for 30 days. To see the effect on the potential of caudal regeneration, specific number of caudal segment (usually 10) were amputated using fine sterilized scissors. After the transactions worms were returned to the test soil. For amputation the worms were not anaesthetized. The counting of regenerated segment was facilitated due to their vascularization and transparency. For calculating the numbers of segments regenerated worms were lightly anaesthetized in 0.5% ethanol and were held on paraffin tray.

RESULTS:

The observed LC_{50} values obtained after exposing worms to the different concentration of endosulfan were ascertained to the 0.006, 0.007 and 0.008 ppm for 24, 48 and 72 h respectively are shown in (Table-1).

In posterior 10 segments of earthworm *Eisenia fetida* initially both in treated and untreated (Control) soil showed regeneration after 30th day. The present studies regeneration shows that as the concentration of endosulfan increased from 0.003, 0.004, 0.005, 0.006, 0.007 and 0.008 ppm and decreased the rate of caudal regeneration efficiency such as 46.15, 42.85, 38.46, 33.33, 21.42, and 16.66% respectively. It is compared to non-treated control worms (Table-2 and Graph-1). Caudal regeneration efficiency were calculated the following formula.

$$\% \text{ Regenerating Efficiency} = \frac{\text{Number of worms regenerating more than 50\% caudal segments}}{\text{Number of regenerants}} \times 100$$

DISCUSSION:

The LC₅₀ values for different test animals are very useful in evaluating the extent of toxicity of pesticide, otherwise it is difficult to predict the physiological responses of the animal to the toxicants [27]. Numerous reports have demonstrated the adverse effects of pesticides on earthworms [14, 17, 21, 23, 25]. The toxicity of pesticides to earthworms deals predominantly with recently some research has focused on measuring sublethal effect on the either of the animal physiology (Reproduction and weight loss) or behavior (avoidance) [22]. Neuhauser and Callahan [16] have observed the

Table-1: LC₅₀ values of Endosulfan on Eisenia fetida
Sr.No Time of exposure LC₅₀ values
(Hrs) ppm 1240.0062480.0073720.008

Table-1: LC ₅₀ values of Endosulfan on <i>Eisenia fetida</i>		
Sr.No	Time of exposure	LC ₅₀ values
	(Hrs)	ppm
1	24	0.006
2	48	0.007
3	72	0.008

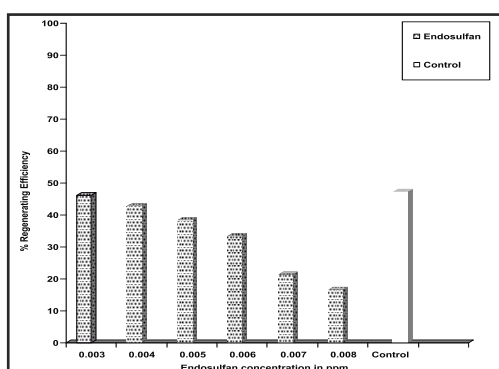
Table-2 : Effect of different concentration of Endosulfan on the caudal regeneration *Eisenia fetida*

	Endosulfan Concentrations (ppm)						Contro 1	
	0.003	0.004	0.005	0.006	0.007	0.008		
Number of worms used	20	20	20	20	20	20	20	
Number of worms surviving after 30 days	18	11	17	18	19	12	20	
Number of worms displaying regeneration	13	07	13	15	14	06	19	
Number of worms Non displaying non vascular pygidium	02	01	02	01	02	02	00	
Number of worms displaying non vascular pygidium	03	03	02	02	02	04	01	
Number of worms regenerating segments.								
10 Segments amputated from each worm.	1	00	00	00	02	03	01	02
		04	03	04	05	03	03	03
	2	00	01	01	02	03	01	02
		03	00	03	01	02	00	03
	3	01	01	01	02	00	01	02
		03	00	02	01	01	00	00
	4	01	01	01	01	01	00	03
		00	00	01	01	01	00	02
	5	00	00	00	00	00	00	00
		00	01	00	00	00	00	02
6	01	00	00	00	00	00	00	
7	01	00	00	00	00	00	00	
8	00	00	00	00	00	00	00	
9	00	00	02	00	00	00	00	
10								
12								
15								
20								
Regenerating Efficiency (%)	46.15	42.85	38.46	33.33	21.42	16.66	47.36	

recovery of growth and reproduction in *E. foetida* following short term exposure to sublethal concentration of Dendrin and Carbaryl. Panda and Sahu [20]. have reported, recovery of growth and reproduction of *D. willsi*. O. Espinoza-Navarro and E. Bustos-Obregón [7]. treated *Eisenia fetida* with malathion and observed decrease in the body weight of treated worms. Zhou *et al.*, [26]. have reported adverse effect of chlorpyrifos on fecundity in earthworm.

Earthworms can regenerate missing parts of its body. In the present experiment posterior segments treated with single endosulfan dose showed regeneration after 30th days and suggested as a response to the toxic effects of endosulfan. The aim of this work was to study caudal regeneration after 30 days amputated segments in *Eisenia fetida* observed and the previous study we also observed caudal regeneration of *Eisenia fetida* in different chemical fertilizers in different concentrations. This work also examined to endosulfan and its effect on regeneration of earthworm, *Eisenia fetida*. The importance of earthworms in promoting soil fertility and plant growth it is obvious that care should be exercised to select compounds that do not excessively damage earthworm population.

$$\% \text{ Regenerating Efficiency} = \frac{\text{Number of worms regenerating more than 50\% caudal segments}}{\text{Number of regenerants}} \times 100$$



Graph :- Showing the effect of endosulfan on the rate of caudal regeneration of *Eisenia fetida*.

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