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Effect of ampligo insecticide on caudal regeneration in earthworm, *Eisenia fetida*

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Abstract
Earthworms are invertebrates and contribute to soil fertility improvement and plant growth. Ampligo insecticide is commonly used by farmers in agriculture in Marathwada region. It is indirectly effects on non-target organism like Earthworm which is very useful for maintain the fertility of soil. In the present study, investigated the effect of Ampligo insecticide on caudal regeneration of earthworms, *Eisenia fetida*. Its effect on the regeneration of the earthworm, *Eisenia fetida* was studied in the laboratory. The worms were collected from the vermiculture tank and acclimated to the laboratory conditions for a period of three days. All worms were adult and fully clitellate. The amputated worms were exposed to 30 days sublethal concentrations of Ampligo insecticide 0.003, 0.004, 0.005, 0.006 and 0.007 ppm and observed their regenerating efficiency was in percentage these are 53.33%, 50.00%, 46.15%, 38.46% and 33.33% respectively. One group of controlled worms it shows 72.22% regenerating efficiency. Experiment was conducted for 30 days.

Keywords: ampligo insecticide, *Eisenia fetida*, caudal regeneration

Introduction
Earthworms the "Golden-bough of our agricultural history" are the terrestrial oligocheates of the class Chaetopoda and Phylum-Annelida. Earthworms, *Eisenia fetida* species found in India as well as many regions of the world. *Eisenia fetida* it is cheap test species, which is widely used in vermiculture, agriculture, etc. and it can be easily cultivated in the laboratory conditions. So, earthworms, *Eisenia fetida* species was selected for the present study.
Some researchers reported the effect of different chemicals and pesticides on the physiology of earthworms. Khan, *et al.*, (2007) [12] reported a significant reduction in earthworm biomass after exposure to different concentration of copper chloride and concluded abnormal functioning of major physiological systems such as digestion and absorption. Effect of pesticides on growth and reproduction of earthworm was studied by (Shahla and D'Souza, 2010) [17]. Corriea and Moreira (2010) [4] studied effect of glyphosphate on earthworms, *Eisenia fetida*. The LC_{50} 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 (Dowden and Bennet, 1965) [6]. Several researchers reported the effects of chemicals, metals and pesticides practiced on earthworms like that is chlorpyrifos, carbofuran, mancozeb and their formulations to the tropical earthworm for their toxicity (De Silva, *et al.*, 2010) [5] and the effect of two organophosphates, chlorpyrifos and diazinon (Booth, *et al.*, 2000) [2]. Xiao, *et al.*, (2006) [18] suggested that growth can be regarded as sensitive parameters to evaluate the toxicity of acetachlor on earthworms.
The main aim of this study are to get a more comprehensive under- standing on the toxic effects of Ampligo insecticide on the caudal regeneration of earthworms, *Eisenia fetida* and to provide informative data for use in ecological risk assessment on soil ecosystem.
The species *Eisenia fetida* is experimental animal recommended by (OECD, 1984) [15]. In the present experiment, Ampligo insecticide used test method for the assessment of toxic effects on earthworm, *Eisenia fetida*.

Materials and Methods
The experiment was conducted to see the effect of ampligo insectide concentrations on caudal regeneration of earthworms, *Eisenia fetida*. The wet soil collected from garden and used for experiments. The soil used for this experiment was taken from the chemical-free area. Concentrations of pesticide were prepared by mixing in water. The concentrations of pesticides were increased from 0·003 to 0·007 ppm. Different concentrations like 0·003, 0·004, 0·005, 0·006 and 0·007 ppm stock solution were separately made and used for experiments.
Initially all experimental group was maintained in 250 ml beaker and 30 ml stock solution thoroughly mixed with 200 gm wet garden soil. Five group maintained for (1) 0·003, (2) 0·004, (3) 0·005, (4) 0·006 and (5) 0·007 ppm concentration and the beaker was filled. Acclimatized 20 earthworms looking healthy and having approximately equal size and weight were selected. Usually 10 caudal segments amputated of each earthworm using fine sterilized scissor under dissecting microscope and inserted in beaker. For amputation the earthworms used were not anaesthetized. To avoid any escape of worms, prevent the moisture loss and prevent the entry of other organisms the beaker was sealed with transparent and small mesh size cotton cloth covered on a beaker with small holes to allow exchange of air. One group was served as control. A strip was stuck on each beaker and marked as experimental group, control group, pesticides concentrations, date of experiment, weight of substrate used,

size and number of earthworm loading, and days. Record of progress in each beaker was thus maintained. All the experimental beakers were kept in a laboratory and provide natural conditions. The moisture level was maintained throughout the study period by periodic sprinkling of adequate quantity of tap water. The experiment was carried out till 30 days. After 30 days to observed the effect of Ampligo insecticide concentrations on regeneration and their regenerating efficiency, development of new segments and physiology of earthworms, *Eisenia fetida*. The counting of regenerated segments is easy due to their vascularized state, transparency and dimensions. Such counting, however, is possible up to a period of 30 days, after which new segments assume normal dimensions and colour. For calculating the number of segments regenerated the earthworms were lightly anaesthetized (because of their extreme agility) in 0.5% ethanol (V/V) in tap water and were held on paraffin tray. The process of regeneration and development of new segments were assessed with the help of a Binocular Microscope, Dissecting microscope and hand lens were used to confirm body structures that could not easily be seen with naked eye during the identification process. Observations were made over a period of 30 days.

These differences most likely related to physiological adaptations associated with the modes of life of the earthworms, and could have serious implications when using earthworms as a biological monitor to assess contaminated soils and the toxicity of chemicals.

The percentage of regenerating efficiency was calculated using the given formula.

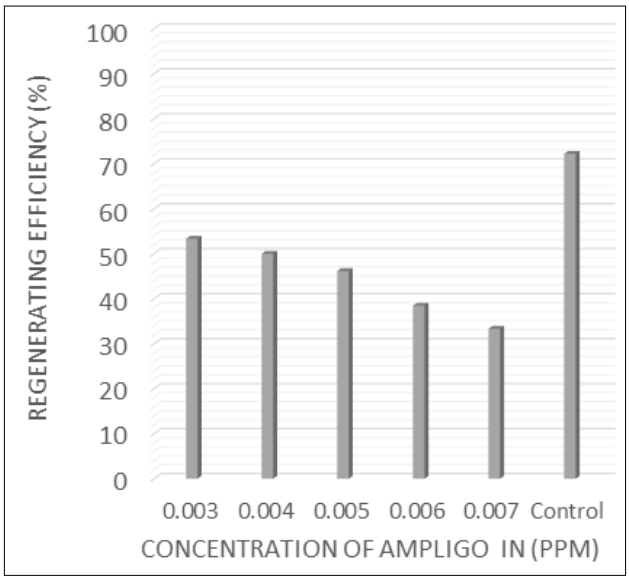
$$\% \text{ of Regenerating efficiency} = \frac{\text{Number of worms regenerating more than 50\% caudal segments}}{\text{Number of regenerants}} \times 100$$

Result

The Ampligo insecticide concentration-response relationship was also demonstrated in the caudal regeneration. The experiments were done below the LC_{50} values concentration. The experiments were maintained separately for 30 days exposure period in order to various concentrations at 0.003 ppm, 0.004 ppm, 0.005 ppm, 0.006 ppm, 0.007 ppm and the result of regenerating efficiency were 53.33%, 50.00%, 46.15%, 38.46% and 33.33% respectively in descending order. In control the regenerating efficiency was 72.22% (Table-1 and Graph-1). Number of earthworm's caudal regeneration after 30 days were calculated. Results revealed that an increasing Ampligo insecticide concentration, number of earthworm's formation caudal regeneration decreased. Also, exposure concentrations affected caudal regeneration formation in a linear manner as is shown in Table-1 and Graph-1. Maximum caudal segments formation occurred at lowest test concentration of 0.003 ppm. Number of earthworm's caudal segments formation was observed at concentration of 0.003 ppm. Also, caudal segments formation was found to be the maximum after 30 days. The effect of concentration and exposure time on earthworm resulted in the maximum reduction in caudal segments formation at highest concentration of pesticide after 30 days of exposure when compared to the control. A large difference in biomass between treated and non-treated test organisms was observed after 30 days of exposure (Table-1 and Graph-1). In conclusion Ampligo insecticide was toxic.

Table 1: Effect of Ampligo insecticide on the caudal regeneration in earthworm, *Eisenia fetida*

		Concentration of Ampligo insecticide (ppm)					
		0.003	**0.004**	**0.005**	**0.006**	**0.007**	**Control**
Number of worms used		20	20	20	20	20	20
Number of worms survival after 30 days		17	17	16	15	14	19
Number of worms displaying regeneration		15	14	13	13	12	18
Number of worms displaying non vascular pygidium		02	02	02	01	01	01
Number of worms displaying vascular pygidium		00	01	01	01	01	00
Number of worms regeneration segments.							
10 Segments amputated from each worm.	1	00	00	00	00	00	00
	2	02	02	02	02	02	00
	3	03	02	02	03	03	02
	4	02	03	03	03	03	03
	5	02	02	02	01	01	03
	6	02	02	01	02	01	03
	7	02	01	01	01	01	03
	8	01	01	01	01	01	02
	9	01	01	01	00	00	01
	10	00	00	00	00	00	01
Regenerating Efficiency		53.33%	50.00%	46.15%	38.46%	33.33%	72.22%



Graph 1: Showing the effect of Ampligo insecticide on the caudal regeneration of earthworm, *Eisenia fetida*

Discussion

The earthworms are farmer's friend. Widely used for maintain the fertility of soil. Higher percent of mortality occurred with increase in concentration and exposure period of Ampligo insecticide. Similar observations have also been reported by various workers using different toxicants and different test animals. Some of the investigators have studied bioaccumulation of pesticides in the annelids. Several researchers reported chemicals, chlorpyrifos and diazinon (Booth, *et al.,* 2000) [2]. Polivka, (1953) [16] reported that all the doses of Heptachlor tested decreased earthworm populations, and this has been confirmed by (Edwards and Arnold, 1966) [7].

In the present investigation it was also observed that the behavioral response of the earthworms was different in accordance with the concentrations, type of pesticide and period of exposure. The earthworms, may avoid harmful effects by producing mucus which leads to reduction in exposure of external body surface. Similar results are reported by number of workers. Zhou, *et al.,* (2006) [19] reported that weight of the earthworms was a more sensitive index compared to the mortality in indicating toxic effect, observation was made on length and colour of worm. There were no changes in length and colour of worms exposed to insectides.

The earthworm regenerating efficiency was found to be decreased with increasing concentrations of Ampligo insecticides. Several researchers reported that regeneration capacity varies in different animal species and in different parts of the same species. Almost all the annelids, both polychaetes and oligochaetes have the capacity for regeneration (Clarke and Clarke, 1962; Hay, 1966; Johnson, 1995; Kulkarni, *et al.,* 2005 and Zoran, 2010) [3, 10, 11, 14, 20]. The regenerating efficiency was decreased in Ampligo insecticides. These results concluded that Ampligo insecticides were more toxic to the earthworms. Regeneration of segments varied among treatment groups (Table- 1and Graph-1). Earlier many workers reported the effect of pesticides on earthworm's physiology (Hanumante, 1975; Kodarkar, 1979; and Bedre, 1986) [9, 1].

Neurosecretory cells secrete the regeneration promoting hormones (RPM) is required for regeneration but the polluted soil adversely affected on the physiology of earthworm, *Eisenia fetida*. Presence of nerve cord and its physiological state are some of the prerequisites of regeneration in annelids. Removal of nerve cord caused no regeneration amongst oligochaetes. Nerve depressants such as lithium, acetyl choline and neurotoxic pesticides like parathion, disulfoton were reported to have inhibitory effects on the regeneration (Edwards and Lofty, 1977) [8]. The results obtained in the present study explain that Ampligo insecticides were a suppressor of regeneration.

Conclusions

Some conclusions that can be made on the basis of the observations and findings on various factors, survival, caudal regeneration and their regenerating efficiency. In various concentrations at 0·003 ppm, 0·004 ppm, 0·005 ppm, 0·006 ppm, 0·007 ppm, the result of regenerating efficiency were 53.33%, 50.00%, 46.15%, 38.46% and 33.33% in Ampligo insecticide in descending order. The regenerating efficiency indicated that Ampligo insecticide was toxic to earthworm, *Eisenia fetida*. It might be due to greater residual property of Ampligo insecticide in the earthworms, *Eisenia fetida*. Annelids have tremendous capacity of biological accumulation. Hence, Ampligo insecticide became fatal to non-target orgamisms such as annelids.

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