ULTRA STRUCTURAL CHANGES IN CLITELLUM REGION OF EISENIA FOETIDA AFTER TREATMENT WITH MALATHION

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INTRODUCTION
To date there are lot of practices which are responsible for a great revolution in agriculture like introduction of pesticide to reduce the harmful effects, caused by different type of pests. Malathion is a one of insecticide which is widely used and has been reported to cause harmful effects (Stromborg, 1986, Espinoza et al., 2002 and Cabello et al., 2003).

Malathion act by inhibition of the enzyme acetyl cholinesterase, which produces accumulation of acetylcholine, responsible for the clinical manifestation of poisoning (Kwong, 2002).

Modern agriculture has become increasingly dependent on the use of pesticides that are beneficial but they also affect the structure of soil invertebrate populations. Earthworms represent a greater fraction of biomass of invertebrates as well as they play a variety of important role in managing soil structure and nutrient availability for plants, Sorour and Larink, 2001, Bustos-Obregon and Goicochea, 2002.

Eisenia foetida was chosen for this present study due to its easy availability as well as it is prescribed as a test organism for toxicity testing (OECD, 1984; Kula and Larink, 1998). Eisenia foetida is hermaphrodite and fertilizes its eggs inside a quinitious cocoons secreted by the clitellum.

The clitellum of Oligochaete worms consists of a band of glandular epidermal cells located in gonadal region and extending over a variable number of segments (Stephenson, 1930; Avel, 1959). A variety of functions have been attributed to these glandular cells. Among these functions which suggest a diverse ultrastructure are:

1. Secretion of the mucous slime tube formed during oviposition,
2. Secretion of the cocoon or egg capsule, and
3. Secretion of the albuminous content of the cocoon (Grove and Cowey, 1927, Hamilton and Hess, 1971, Vena et al., 1969)

The clitellum is a reproductive structural characteristic of the oligochaetes, which matures as a secondary sexual gland (Ruppert and Barnes, 1996; Siekierska, 2002). The present study unfolds the toxic effects of Malathion on clitellum region of Eisenia foetida, at ultra structural level.

MATERIALS AND METHODS
Experimental model
Earthworm, E.foetida was chosen to evaluate toxicity in present study because it was hardly easy to handle and it easy availability. Earthworms were procured from the vermicompost unit of Rajasthan College of Agriculture, Udaipur. They were maintained in the laboratory condition, after 15 days acclimatization they were used for further conducted experiment. The worms used in the experiment were of approximately same body weight and body length.

Chemical
The pesticide used in the experiment was Malathion (5%). It was purchased from the local market’s shop. The LD50 value of this pesticide for the E. foetida was evaluated by author first that is 16g/kg of soil and the result of this LD50 supported by the finding of Omar and Bustos (2004).

Experimental set-up
Present method of the experiment was based on the method
was used by Yasmin and Souza (2007). Dried soil (from nearby farmland) was crusted and filtered through a fine mesh sieve. One kg of fine soil was than poured in each plastic tub and water was added to moistened the soil and 250 mg dried powdered (3 week old) cow dung was also added to each plastic tubs. Cow dung was added to avoid starvation, as recommended by the International Workshop on Earthworm Ecotoxicology held in Sheffield in 1991 (IWEE1). 20 mature earthworms (some age group) were added to each plastic tubs. Each plastic tub was covered with muslin cloth. Thus one control set and one experimental set were prepared 3 replicated were used for each set.

Treatment of the worm with malathion

Higher sub lethal dose of Malathion (3/4 of LD50 value) was selected to study the toxicity of Malathion for *E. foetida*. Thus in experimental set higher sub lethal lose of Malathion was added and proper mix throughly in the plastic tub.

Regular water supply was done to maintained 60% to 70% Moisture level. After 96 hr of the treatment the worms were taken from the plastic tub and dissected out for desired part of the body.

Electron microscopy of clitellum

Dissected clitellum region from both group (control and experimental) were immersed in Karnovsky fixer (1% glutaraldehyde and 4% paraformaldehyde in 0.2 M cacodylate buffer at pH 7.4). Then washed in 0.2M Cacodylate buffer (pH 7.4) and post fixed in 1% Osmium tetroxide, similarly buffered, for 1h following dehydration in an acetone series, the tissue were embedded in resin. Ultrathin sections were stained with uranyl acetate followed by lead citrate, examined and photographed with a Morgagni 268D, Electron Microscope.

RESULTS

Developed clitellum shows sexual maturity.

Type-1 and type-2 cells are clearly seen (on their visual
appearance we designated these cells as type-1 and type-2 cells) in Fig. 1 and 2. Longitudinal and circular muscle cells are also visible, arranged parallel to each other (Fig. 3).

Type-1 cells: - This type of cells consist slender columnar shape, having a limiting membrane and a diameter of (Fig. 4) cytoplasm of in these cells bears numerous microtubule-containing granules. The cell organelles, like Golgy is also filling the free area of the cell. (Fig. 1).

Type-2 cells: - These cells bear large columnar shape and contain any secretary bodies that fill the entire inner portion of these cells. (Fig. 2)

After treatment with malathion

The clitellum region of *Eisenia foetida* shows some abnormalities (Fig. 8) after exposure to higher dose of Malathion. In type-1 cells the microtubules become distorted and secretary bodies become irregular in shape (Fig. 5).

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Presence of multivascular bodies indicating high endocytotic activity clitellar region. (Fig. 8). Disruption of muscle cell also observed. (Fig. 7)

DISCUSSION

The generalized distribution of chronically non-lethal environmental polluting agents has demonstrated multigenerational effects on the biological systems, altering reproductive, endocrine and immune systems, changes that can be registered by mean of simple organisms that are living in the same ecosystem (Lock *et al.*, 2002, Venkateshwara *et al.*, 2003).

Malathion has an ample use in agriculture houses and gardens, and has lethal effects on many living systems. A lot of work has been done to attempted toxicity of Malathion in living organisms. (Ronald *et al.*, 1983; Wali *et al.*, 1984; Blasiak *et al.*, 1999)
Malathion is an organophosphorus insecticide, which exert their toxicity by inhibition of acetyl cholinesterase, enzymes responsible for the degradation of acetylcholine (Koelle, 1994; Sidell, 1994).

Eight types of secretory cells are identified by Morris (1985) in the clitellum epithelium of Eisenia fetida, in which type-1 and type-2 cells have been observed to be affected by Malathion after exposure, as well as some muscle cells have also identified to be injured after Malathion exposure. This is confirming by the finding of Reddy and Rao (2008) because they also observe same toxic effects at muscle cells as Eisenia fetida.

The present study includes the information about the toxic effects of malathion on clitellar region of Eisenia fetida. To date many researchers have found that Malathion affect the reproduction as well as morphology of the Eisenia fetida. Malathion also cause skin rupture (Reddy and Rao, 2008) in Eisenia fetida, no abnormalities were reported in other cell types of the clitellum region. It can be concluded after this study that deformities in some cells types of clitellum region of Eisenia fetida can be due to the toxic effects of Malathion.

REFERENCES


