

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

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ABSTRACT

The objective of the present communication is to screen the effect of a single dose of a commonly used insecticide, Malathion on clitellar region of *Eisenia foetida*. The ultra structure observations clearly reveal that exposure of a single dose (12gm/kg soil) of Malathion (for a period of 96 h.) imparts a toxic effect on some cells types like T₁, T₂ and muscles cells found in the clitellum. Thus this study authentically proves that Malathion adversely affects the clitellum which plays a major role in cocoons production, consequently causes low reproductive capacity in *Eisenia foetida* which can be very harmful to the earthworm population. The electron micrographs clearly exhibit the extent of damage caused at intracellular level.

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 quitinous 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 ultra structure 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 (IWEET). 20 mature earthworms (some age group) were added to each plastic tub. 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 throughoughtly 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

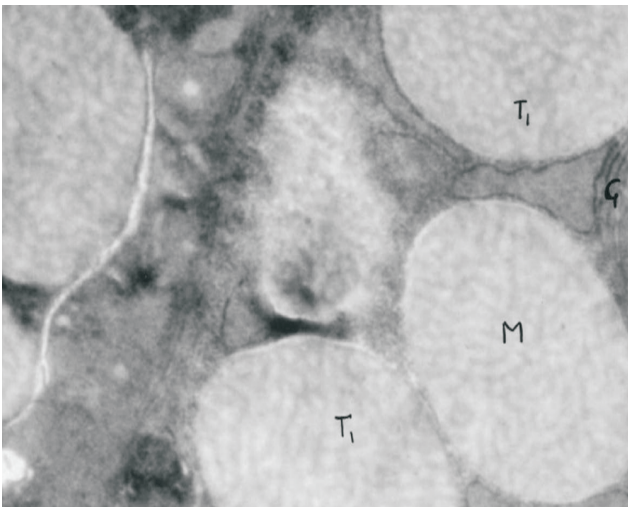


Figure 1: The microtubules of cell type -1 are shown here almost in cross section. The microtubules arranged regularly within the granules. The golgi also clearly seen associated with these cells. 6300x
T₁ (type 1 cell); G(golgy); m (mitochondria)

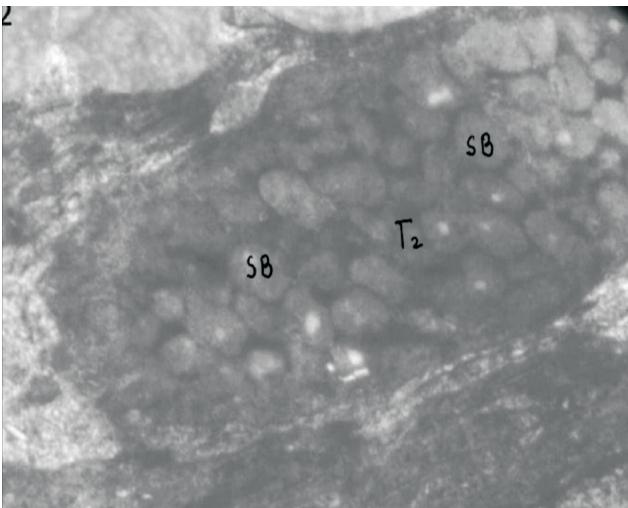


Figure 2: Type 2 cell are clearly visible in this micrograph with secretory bodies. 5000x
T₂ (type 2 cell); SB (secretary bodies)

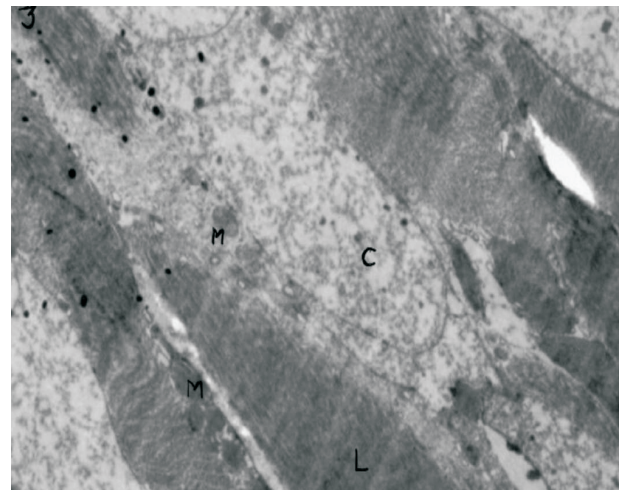


Figure 3: Longitudinal and circular muscles cells are seen in this micrograph, arranged parallel with each other. 1600x
M (microtubules); C(circular muscle); L(longitudinal muscle)

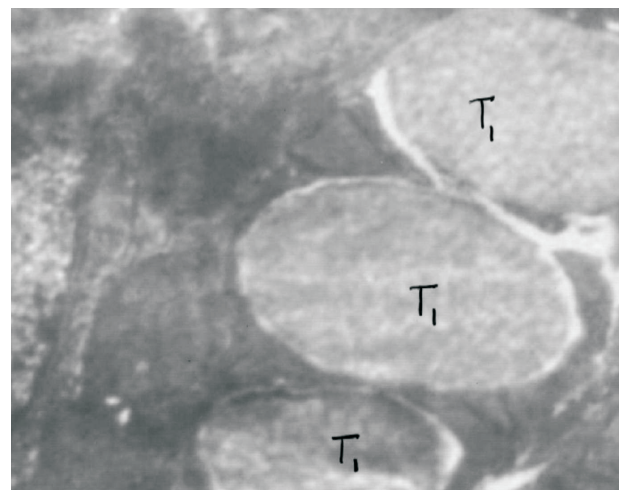


Figure 4: Type -1 cells are clearly seen in different diameter. 4000x
T₁ (type 1 cell)

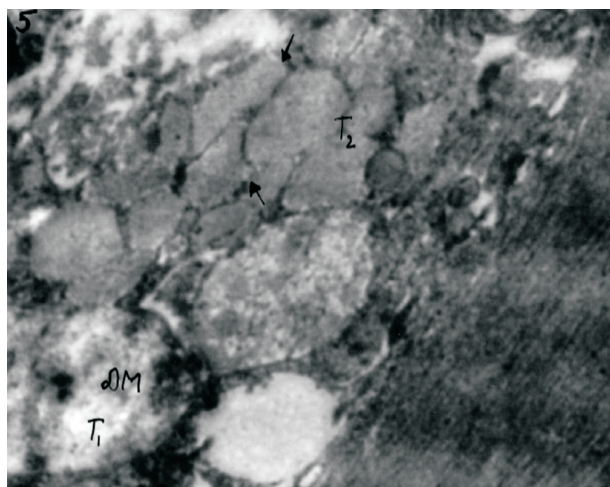


Figure 5: Degenerated part of micro tubules of cell type-1 and irregular shaped secretory bodies are indicated by arrows after treatment with single dose of Malathion. 8000x

T₁ (type 1 cell); T₂ (type 2 cell); DM (degenerated microtubules)

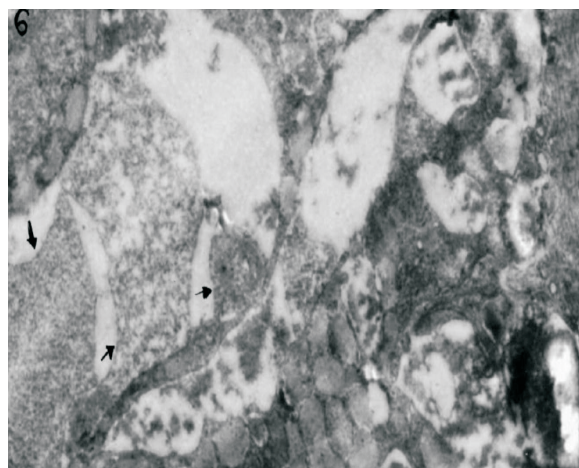


Figure 6: Different sizes of vacuoles are clearly visible (showing by arrows) in this micrographs after treatment with single dose of Malathion. 4000x

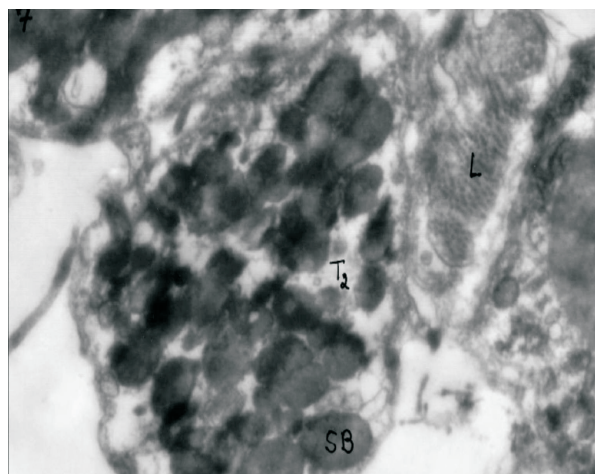


Figure 7: This micrograph showing irregular, deshaped secretory bodies within cell type -2 and ruptured longitudinal muscle cell after treatment with single dose of Malathion. 5000x

T₂ (type 2 cell); SB (secretary bodies); L (longitudinal muscle);

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 secretory 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 secretory bodies become irregular in shape (Fig. 5).

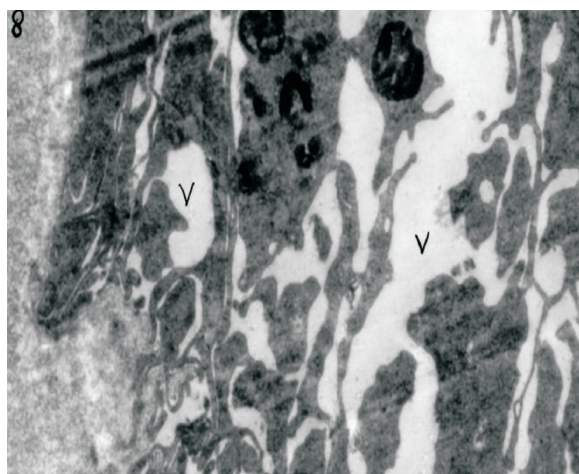


Figure 8: Highly vacuolized portion of clitellum region after treatment with single dose of Malathion. 2500x

V (vacuolised region)

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 clitellar epithelium of *Eisenia foetida*, in which type-1 and type-2 cells have observed to be been 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 foetida*.

The present study includes the information about the toxic effects of malathion on clitellar region of *Eisenia foetida*. To date many researchers have found that Malathion affect the reproduction as well as morphology of the *Eisenia foetida*.

Malathion also cause skin rupture (Reddy and Rao, 2008)

In *Eisenia foetida*, 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 foetida* can be due to the toxic effects of Malathion.

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