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HISTOPATHOLOGICAL CHANGES IN THE INTESTINE OF THE EARTHWORM *Lampito mauritii* (Kinberg) EXPOSED TO SUBLETHAL CONCENTRATION OF MONOCROTOPHOS

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ABSTRACT

The present study has investigated the toxic effect of monocrotophos on the intestine of the earthworm *L. mauritii* in a laboratory experiment. A sub-lethal concentration of monocrotophos (1/3rd of 96 h LC_{50} value - 0.311 ppm) was applied for 30 days. The changes such as vacuolization, degenerated nuclei, damaged epithelial lining of villi and congestion of blood sinuses were observed in the intestine of 1st and 5th day of monocrotophos exposure. In the 30th day of exposure, slight damages were observed. These results suggest that monocrotophos could severely affect the intestine of 1st and 5th day of *L. mauritii's* when compared to 30th day. This study indicates the importance of histopathological bioassay as an indicator of environmental pollution.

INTRODUCTION

Earthworms represent a major component of the soil biomass. They can contribute extensively to soil formation through consumption of dead plant and animal matter, mixing of the particles during digestion, depositing their casts throughout the soil column, and improving aeration and drainage of the soil by burrowing. They are also important contributors to the recycling of carbon and nitrogen in the ecosystem. This makes them one of the most suitable bioindicator organisms for testing chemicals in soils (Callahan, 1988; Goats and Edwards, 1988).

Use of specific herbicides, fungicides and insecticides in the agricultural field can be highly toxic to earthworms and they will suppress or nearly eliminate earthworm population (Chris Williamson, 2000). Monocrotophos is one of the organophosphate insecticide, owing to its high insecticidal properties is used most widely for agriculture. Hence, in the present study monocrotophos was selected for assessment of their toxic effect on earthworm *L. mauritii*. In India *L. mauritii* was chosen as an indicator species for assessment of agroecosystem contamination because of its widespread occurrence in aerable and fasture lands and its consequent vulnerability to surface applied pesticides.

In recent years, through a number of studies for assessing the toxicity of pesticides and herbicides to earthworm mortality, growth and reproduction were carried out. A very few studies have been reported on the histopathological effects of pesticides on earthworms (Mohssen Morowati, 2000; Gobi *et al.*, 2005 and Bansiwal and Rai, 2010). Histology is the most useful tool for determining the influence of agricultural pesticides, industrial pollutants, organic wastes etc., at tissue level of an organism as it provides useful information concerned with the growth, damage and disorganization of tissues. Apart from the role of earthworms in preparing and absorbing nutrients, the intestine is the first line of defense against chemical insults through the oral route. Hence, an attempt is made to study the histopathological changes in the intestine of Indian earthworm *L. mauritii* when exposed to sub-lethal concentration of monocrotophos.

MATERIALS AND METHODS

Earthworm

L. mauritii were collected from agricultural farm of Annamalai University. They were maintained in the laboratory condition for two weeks in cowdung media at 28 ± 2^0 C with 50 – 60% moisture. The

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worms used in the experiment were adults with well developed clitellae.

Chemical

The pesticide used in the experiment was monocrotophos (manufactured by Coromental Fertilisers Ltd., Secunderabad). It was purchased from local pesticide agency.

Acute toxicity test

For assessing acute toxicity, in plastic troughs, selected concentration of monocrotophos was mixed well with 1 kg of soil substrate using 300 ml of water. The sun dried and powdered cowdung (earthworm's nitrogen rich natural food) was mixed with soil (low in nitrogen) in the ratio of 1:3 (vol / vol) and used as soil substrate. 10 adult L. mauritii were introduced into each experimental media. The mortality of earthworms were observed and recorded after 24, 48, 72, 96 and 120 h of exposure. The LC_{50} values were calculated by Probit Analysis method (Finney, 1971). From the 96 h LC_{50} value (0.933 ppm), a sub-lethal concentration ($1/3^{rd}$ of 96 h LC_{50} value – 0.311 ppm) was selected to assess the effect of monocrotophos on L. mauritii's intestine.

Histopathological study

For the experimental media preparation, 2 plastic troughs each filled with 1 kg of soil substrate designated as C (Control) and T (Treatment). The control was mixed only with water. For treatment, the sub-lethal concentration of monocrotophos was added and mixed with soil substrate using 300 ml of water to ensure homogenous mixture and required moisture. 10 clitellated *L. mauritii* were introduced into each experimental media. The troughs were covered by nylon net. It was maintained at room temperature 28 ± 2^{o} C with 60 – 70% moisture. The duration of study was 30 days, during which media were watered regularly to avoid dryness. The experiments were repeated twice.

On 1^{st}, 5^{th} and 30^{th} day of experiment, three treated and control worms were removed from the experimental media and kept in plain water overnight to clear their intestines. Then to minimize their movement while dissecting them, they were kept in a freezer for about one hour before dissection. Later the animals were dissected and small pieces of the intestine (ranging from about 20 – 100 segments) were removed and fixed for 24 h in Bouin's fixative. After tissue processing paraffin sections of intestine were cut at five micrometer thickness and stained with hematoxylin – eosin method for microscopic examination.

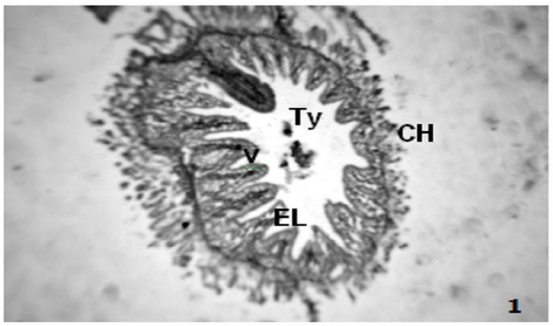

Fig.1: Cross section of intestine of control *L. mauritii* showing villi (V) covered by epithelial layer (EL), typhlosole (TY) and chloragogen cells (CH). X 100

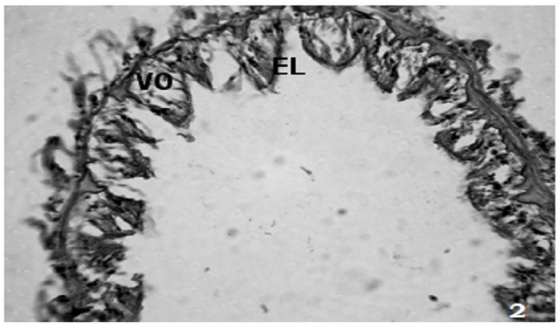

Fig.2: Cross section of intestine of *L. mauritii* exposed to sublethal concentration of monocrotophos for 1st day showing damaged villi and vacuolations in cells of epithelial lining (VO). X 120

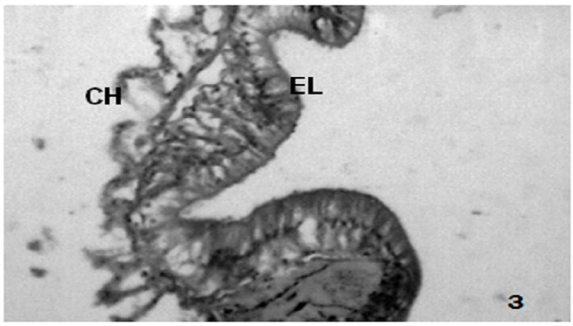

Fig. 3: Cross section of intestine of *L. mauritii* exposed to sublethal concentration of monocrotophos for 5th day showing damaged chloragogen cells (CH) and epithelial lining (EL). X 150

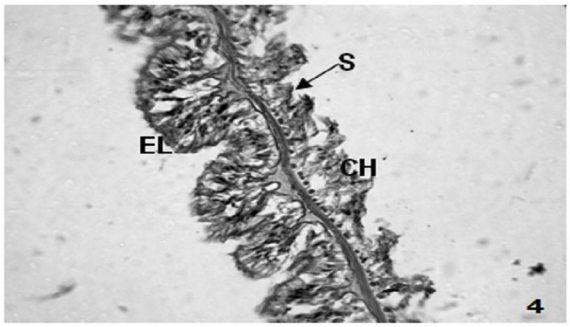

Fig. 4: Cross section of intestine of L. *mauritii* exposed to sublethal concentration of monocrotophos for 30th day showing renewed epithelial lining (EL), chloragogen cells (CH) and normal blood sinuses (S) X 120

RESULTS

Histology of the intestine of control *L. mauritii*

The intestine is made up of four layers. An outer layer is called visceral peritoneum. The most of the cells of this layer are chloragogen cells. They play an important role in excretory as well as chief centre for synthesis and storage of glycogen and fat. Next to this, two muscle layers – longitudinal and circular muscle fibers are found. The fourth layer is the epithelial lining of columnar cells. They are mostly glandular and ciliated to form villi. The blood sinuses lie outside the epithelium (Fig.1).

Histopathology of the intestine of *L. mauritii* exposed to monocrotophos

The first day of exposure, the earthworm showed loss of compact structure of epithelial lining, damaged villi, fusion of cells and pyknotic nuclei in some regions (Fig.2). After fifth day of exposure, there were vacuoles in the cytoplasm, degenerated nuclei, damaged epithelial lining of the villi, space formation and congestion of blood sinuses. The extent of intestinal damage is more severe in fifth day of exposure (Fig.3).

At the end of 30th day of exposure renewed epithelial cells and more or less compact arrangement of the epithelial layer were observed (Fig.4). In the present study, the degree of histopathological changes in intestine is not found to be severe at the end of 30th day when compared to 1st and 5th day of exposed *L. mauritii.*.

DISCUSSION

The intestinal cells of the experimental earthworms showed disruption of the cell membrane from 1st to 5th day of exposure to monocrotophos. Cell death or necrosis was observed in this period. According to Bowen and Lochshin (1981) cell death is not a single entity but heterogenous structure, mechanism and biological function. Cell death or necrosis is characterized by pyknotic nuclei, cytoplasmic swelling and mitochondrial damage which results from failure in osmotic regulation caused by loss of cellular energy supplies. By the 30th day of exposure there was observed recovery of the epithelial lining. According to Stephenson (1930) recovery could be brought by the chloragogen cells. These cells are known to migrate to the wound or lost tissue and regenerate them. It is well known fact that earthworms have a great power of regeneration (Leblond and Walker, 1956 and Hammana *et al*., 1995).

Gobi *et al.* (2005) were found the glandular cell enlargement and vacuolization in the intestine of the earthworm *Perionyx sansibaricus* exposed to sublethal concentration of herbicide butachlor. An extreme (2 fold) nuclear swelling has been reported in *E. fetida* exposed to herbicides (Fischer and Molnar, 1992). Mohssen Morowati (2000) has reported that *Pheretima elongata* exposed to herbicide glyphosate showed loss of epithelial cell structure in intestine, lacking regeneration of the cells and total loss of chromatin from first week to the third week of exposure and a marked regeneration of the cells in the fourth week of exposure. Bansiwal and Rai (2010) observed that sublethal dose of organophosphate insecticide malathion has induced marked pathological changes in the body wall such as ruptured cuticle, with distortion of the shape of longitudinal muscle cells.

Daane and Haggblom (1999) have suggested that the microflora may influence the survival of earthworms exposed to toxic chemicals. The earthworms are known to have efficient detoxification capacity with a large number of aerobic and anaerobic bacteria (Karsten and Drake, 1995). Kavitha *et al.* (2008) observed that *L. mauritii's* gut bacterial and fungal species such as *K. pneumonia, E. aerogens, E. cloacae, B. subtilus, A. fumigatus, A. niger* and *A. flavus* were able to survive and degrade endosulfan after 30 days of exposure. So, the earthworms gut microbes might have played a major role in the biodegradation of pesticides. This is one of the main reasons for recovery of intestinal epithelial lining in the 30th day of monocrotophos exposure. This study indicates the importance of earthworm histopathological bioassay as an indicator of environmental pollution.

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