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Research Article

SUBLETHAL EFFECT OF ATRAZINE ON THE INTESTINE OF AN INDIAN EARTHWORM *LAMPITO MAURITII* (KINBERG) (ANNELIDA; OLIGOCHAETA)

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ABSTRACT

The present study has investigated the toxic effect of Atrazine on the intestine of the earthworm *L. mauritii* in a laboratory experiment. A sub-lethal concentration of Atrazine (1/5th of 96 h LC_{50} value-1.99 mg/kg) 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, 5th and 15th day of Atrazine exposure. In the 30th day of exposure, slight damages were observed. These results suggest that Atrazine could severely affect the intestine of 1st, 5th and 15th day of exposure when compared to 30th day. Histopathological study in *L. mauritii's* intestine is a suitable parameter for detection of soil contamination by application of pesticides in agricultural field.

Keywords: Atrazine, Earthworm, Intestine, Soil, Toxicity.

INTRODUCTION

Increasing population growth and urbanization, especially in a developing country like India, necessitates producing more food. Food crops require fertile soil to grow. For terrestrial ecosystems, soil serves as a medium of entry to the nutrients. Continuous agricultural activities tend to decrease the soil fertility. However, the increasing application of herbicides and pesticides has also threatened the human environment and the ecosystems with deleterious consequences. Insecticide residues reach the soil in a variety of ways, causing toxicity to beneficial organisms. Earthworm represent the greater fraction of biomass of invertebrates in the ground (>80%). They can play a variety of important roles in agro ecosystems. Their feeding and burrowing activities incorporate organic residues and amendments into the soil, enhancing decomposition, humus formation, nutrient cycling, and soil structural development (Kladivko *et al*., 1986). Therefore, earthworms can be used as bio indicators to detect pesticide contamination in agricultural soil. A pesticide is a chemical intended to kill, or disrupt the population of pest organisms. Pests are unwanted insects, mites, plants, disease causing organisms, and other organisms that interfere with health or commerce. Pesticides are classified into insecticides, herbicides, fungicides, nematicides, rodenticides, etc. based on their action on type of pest. Insecticide target insects, herbicides target plants, fungicides target disease causing fungi, nematicides target nematodes and so on. The most common of there are herbicide which account for approximately 80% of all pesticide use. Atrazine is one of the most commonly used herbicides to prevent pre and post emergence broadleaf weeds in crops such as sugarcane and maize and on turf, such as golf courses and residential lawns. Atrazine is prepared from cyanotic chloride, which is treated sequentially with ethylamine and isopropylamine. The chemical name of atrazine is 2-chloro-4-ethylamino-6-isopropylamino-s-triazine. It is colourless solid and soluble in water.

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. Histopathological studies may signal a damaging effect of organisms resulting from prior or ongoing exposure to toxic agents. Earthworms have been shown to be affected by the

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fate of pesticides in soil. Earthworms directly influence the persistence of pesticides in soil by metabolizing a parent compound in their gut (Gilman & Vardanis, 1974), by transporting herbicides to depth in soil, or by absorbing herbicide residues in their tissues (Edwards & Lofty, 1982).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. The circulatory system is closely associated with the intestinal tract and therefore, entry into the capillaries is rapidly affected. A major factor favouring absorption in the intestine is the presence of microvilli and in the case of earthworms the typhlosole, which increases the surface area. This makes the absorption greater in this area of gastro-intestinal tract. The epithelial cell lining of the intestinal wall plays a major role in nutrient uptake. On exposure to any type of chemicals and pesticides, cellular enlargement and the loss of chromatin materials in the nuclei is to be observed in earthworms (Muthukaruppan *et al*., 2005).

A very few studies have been reported on the histopathological effects of pesticides in the intestine of earthworm species. Morowati, (2000) has studied about the histopathology of intestine of earthworm (*Phereti maelongata*) exposed to a field dose of the herbicide glyphosate. The sublethal toxicity of herbicide butachlor on the intestine of the earthworm of the *Perionyxex cavatus* was studied by Muthukaruppan *et al.* (2005). Reddy & Rao, (2008) have investigated the effect of organophosphorus pesticide, profenofos on the histological changes in the body wall of the earthworm, *Eisenia fetida.* Histopathalogical effect of pesticides in south Indian earthworm *Lampito mauritii*is was not studied by any researchers. Now a day, herbicides are widely used to control weeds in the agricultural field. Atrazine is one of the most used herbicides in the major crop of sugarcane filed in south India. Hence in the present study, atrazine was selected to observe their toxic effect on *L. mauritii's* intestine.

MATERIALS AND METHODS

Selection of *Lampito mauritii*

The native earthworm, *L. mauritii* has been selected for the following reasons. It is widely distributed in south India, commonly available throughout the year, and easily maintainable in the laboratory on cow dung. This earthworm is responsive to a wide range of toxicants and routinely used for toxicological studies. It is an efficient decomposer of organic wastes (Dash & Dash,1999) and suitable for vermicomposting (Senapati *et al*., 2002). It is anecic in nature and inhabiting up to 20 cm depth in the top soil, feeding mainly upon organic wastes and highly adaptable to environmental factors like temperature, moisture etc. It has high rate of fecundity and reproduction with moderate biomass production together a long period of survival and reproductive capacity.

Collection and maintenance of stock earthworm

Specimens of *L. mauritii* were collected from the Garden of Government College for women (A), Kumbakonam. The worms were stocked in plastic trough contained sundried and powdered cow dung. It was used as substrate to maintain the adult worm, and hatchlings, since cow dung was deemed as a highly suitable natural feed for worms (Hatanaka *et al*., 1983; Lee, 1985). Moisture content about 70-80% was continuously maintained by sprinkling water. This stock culture was covered with iron mesh and maintained at room temperature (27 ± 2°C) inside the laboratory. Every 15 days, the top layer of substrate was removed and filled with fresh cow dung.

Collection and procurement of materials

Soil was collected from Garden of Government College for women at Kumbakonam. It was sundried and stored. The soil was clay loam in nature. Fresh cow dung was collected from cow houses in Nachiyarkovil village. It was sundried, powdered and stored in jute bag. Atrazine was purchased from agro centre at Kumbakonam and used for the experiment.

Preparation of soil substrate (Soil – Cow dung mixture)

In nature, earthworm *L. mauritii* can move through the soil and collect their food materials-organic debris. In the experimental soil substrate, due to confined area and limited soil volume, the worm may not get enough food material. Further, in soil the nitrogen content is very low. Hence, in the present study the dried and powdered natural nutrition's food cow dung (CD) was mixed with soil in the ratio of 1:3 (CD: soil) (Vol/Vol) and used throughout the study as soil substrate.

For histopathological study the sublethal concentration of atrazine was selected from 96h LC_{50} value (1.99 mg/kg). For the experimental media preparation, 2 plastic troughs were 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 Atrazine was $1/5^{th}$ of 96h LC_{50} value – 0.4 mg/kg added and mixed with soil substrate using required amount of water to ensure homogenous mixture. 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 ºC 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.

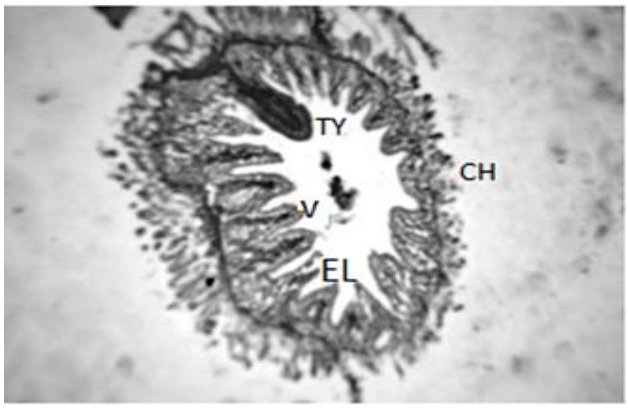
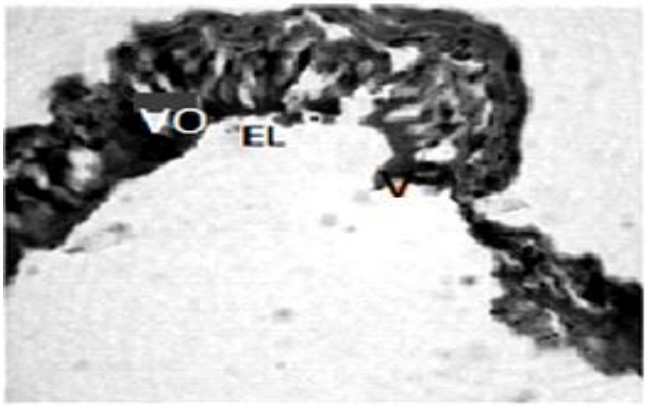


Figure 1



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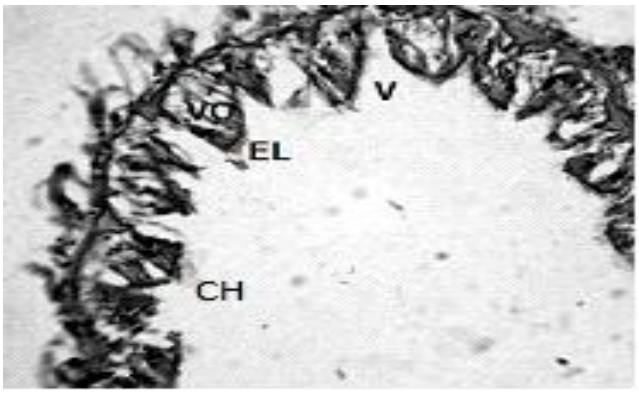

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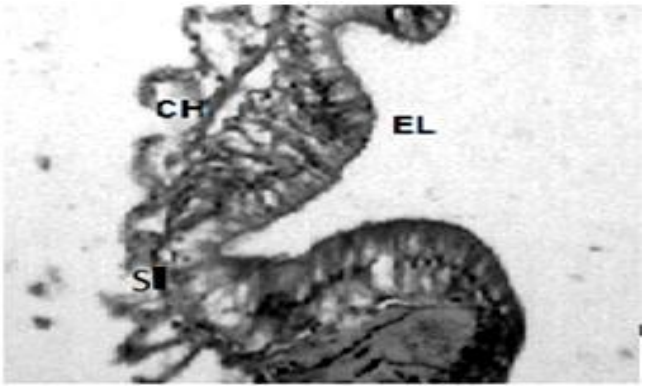

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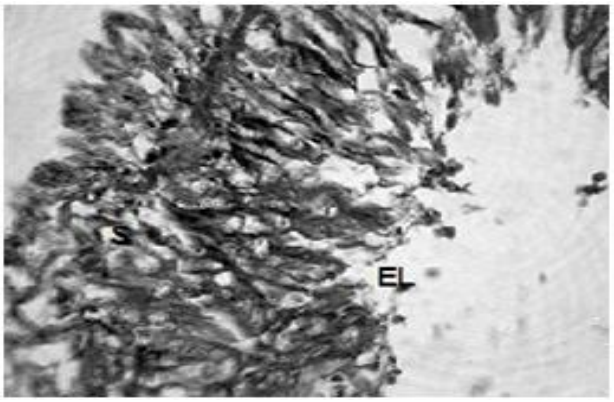

Figure 5

Figure 1. Cross section of intestine of control *L. mauritii* showing Villi (V) covered by Epithelial Layer (EL), Typhlosole (TY) and Chloragogen cells (CH). X120.

Figure 2. Cross section of intestine of *L.mauritii* exposed to sublethal concentration of Atrazine for 1st day showing damaged Villi and Vacuolations in cells of Epithelial Lining (VO).

Figure 3. Cross section of intestine of *L.mauritii* exposed to sublethal concentration of Atrazine for 5th day showing damaged Chloragogen cells (CH) and Epithelial Linng (EL).

Figure 4. Cross section of intestine of *L.mauritii* exposed to sublethal concentration of Atrazine for 15th day showing damaged Epithelial Lining (EL) and Blood Sinuses (S).

Figure 5. Cross section of intestine of *L.mauritii* exposed to sublethal concentration of Atrazine for 30th day showing renewed Epithelial Lining (EL), Chloragogen cells (CH) and normal Blood Sinuses(S). X120.

On 1st, 5th, 15th and 30th day of experiment, two 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 easily 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.

RESULT AND DISCUSSION

The intestine is made up of four layers; an outer layer of visceral peritoneum which forms the outermost covering layer of intestine. The most of the cells of this layer around the stomach and intestine are modified and called chloragogen cells or chloragocytes. Chloragogen cells are believed to be of vital importance in the metabolism and they play a role similar to that of liver in vertebrates. These cells are excretory as well chief centre of synthesis and storage of glycogen and fat. Next to this, two muscle layer-outer layer of longitudinal muscle fibres and inner layer of circular muscle fibres are found. Both these muscle layers are well developed around the pharynx and oesophagus but poorly development around the stomach and intestine. The longitudinal muscle fibres are absent in gizzard. All the muscles of the gut wall are involuntary and un striped. The last or fourth layer is the pharynx, the stomach and intestine. It is mostly glandular and ciliated to form Villi in the intestine. In the gizzard, it mostly secretes cuticle. The intestine blood sinus or plexus lies immediately outside the epithilum (Figure 1). 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 (Figure 2). After 5th 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 (Figure 3).

After 15th day of exposure, damaged intestinal villi (V) and vacuolations in cells of Epithelial lining (EL) was observed. The extent of intestinal damage is more severe in 15th day exposure (Figure 4). At the end 30th day of exposure renewed epithelial cells and more or less compact arrangement of the epithelial layer were observed (Figure 5). 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, 5th and 15th day of atrazine exposed *L. mauritii*.

After exposure to atrazine, disruption of the cell membrane was observed in the intestine at 1st, 5th and 15th day. Cell death or necrosis was observed during the experimental period. According to Bowen and (Lee, 1985) cell death is not a single entity but it is heterogeneous in structure, mechanism and biological function. Cell death or necrosis is characterized by pycnotic nuclei, cytoplasmic swelling and mitochondrial damage, which is in keeping with the hypothesis that it 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 (Kaster, 1980) 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 the well known fact that earthworms have a great power of regeneration (Hamana *et al*., 1995; Leblond & Walker, 1956).

Muthukaruppan *et al.* (2005) have reported the glandular cell enlargement in the intestine of the earthworm exposed to sublethal toxicity of herbicide butachlor and they have further observed that changes in the intestinal region may massively affect food intake and which inturn may indirectly inhibit earthworm reproductive capacity. An extreme (2–fold) nuclear swelling has been reported in *E. fetida* exposed to herbicides under different experimental conditions (Molnar, 1992). (Gupta & Sundararaman, 1991) have reported the swollen nuclei and loss of chromatin material in carbaryl intoxicated *Pheretima posthuma.* (Morowati, 2000) has reported that *Pheretima elongata* exposed to a field dose of 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 & 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. Oluah *et al.* (2016) have been statedthat after exposure to atrazine in the earthworm *Nsukkadeilus mbae*, damages were observed in chloragogenous layer, epithelial tissues, glandular enlargement of the epithelial tissues, prominent vacuolations and pylenotic cells.

Alternatively, Daane & 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 & Drake, 1995). During prolonged exposure (30 days) the increased microbial number and activity in the gut of *L. mauritii* might have resulted in accelerated degradation of pesticides as a consequence, the impact of pesticides on the gut of *L. mauritii* were minimized. Kavitha *et al.* (2011) was observed that *L. mauritii's* gut bacterial and fungal species such as *K. pneumonia, E. aerogens, E. cloacae, B. subtilus, A. fumigatus,* A. *nigar* 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. It is also strongly supported the results for recovery of intestinal epithelial lining in the 30th day of atrazine exposure.

CONCLUSION

The present study has investigated the toxic effect of Atrazine on the intestine of the earthworm *L. mauritii* in a laboratory experiment. A sublethal concentration of Atrazine ($1/5^{th}$ of 96h LC_{50} value –1.99 mg/kg) 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 1^{st}, 5^{th} and 15^{th} day of Atrazine exposure. In the 30^{th} day of exposure, slight damages were observed. These results suggest that Atrazine could severely affect the intestine of 1^{st}, 5^{th} and 15^{th} day of exposure when compared to 30^{th} day. Histopathological study in *L. mauritii's* intestine is a suitable parameter for detection of soil contamination.

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