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ASSESSMENT OF MALATHION TOXICITY IN CERTAIN ORGANS OF EARTHWORM, *EISENIA FOETIDA*

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ABSTRACT
The present study reveals the toxic effects on integument and ventral nerve cord of *Eisenia foetida* after treatment of Malathion. A higher sub lethal dose of Malathion (3/4th of LD_{50} that is 16000mg/Kg soil) was used to evaluate the toxicity of Malathion on the integument and ventral nerve cord. These changes include ruptured cuticle, deformed circular and longitudinal muscles in integument and distorted ventral nerve cord. It is suggested that Malathion at 12000mg/Kg soil for 96 hr has caused histopathological changes in integument and ventral nerve cord that will ultimately affect the proper functioning of *E. foetida*. Thus it can be said that Malathion is not only a neurotoxin agent.

INTRODUCTION

Since the removal of organochlorine insecticides from use in agriculture, the organophosphate insecticides have become the most prevalent insecticides today.

More than forty of them are currently registered, although all run the risk of acute and sub acute toxicity. Organophosphates are used in agriculture, in the home lawn, in gardens and in veterinary practice etc. All apparently share a common mechanism of cholinesterase inhibition and can cause similar symptoms of toxicity since they share this line of action, exposure to the same organophosphate by multiple routes can lead to serious additive toxicity or synergistic effect (Savage *et al.,* 1988; Sullivan and Blose, 1992 and Bardin *et al.,* 1994).

Earthworms, called the "Intestine of Earth" by Aristotle, are very important soil organisms that aid in the decomposition of plant litter, such as the thatch layer, and in recycling of nutrients. Their tunnels in the soil help oxygen and water to enter the soil more easily and their casting (Waste) enrich the soil.

Pesticide applied to control turf diseases or insect pests may severely affect earthworms. Organophosphates are frequently used in pesticide due to their low half life. Malathion is considered one of the most abundantly used organophosphate pesticide, induced adverse effects in non-target organism like earthworm, rats and human etc. Because of indiscriminate use of this pesticide, it is toxic to earthworms, mouse, fishes, human being and many other living organisms (Mishra and Jain, 1962; Wali *et al.,* 1984; Senapati *et al.,* 1992; Contreras and Bustos, 1999; Omar and Bustos, 2005).

Although toxicant impairs the metabolic and the physiological activities of the organism, physiological studies alone do not provide complete understanding of pathological conditions of tissues under toxic stress. Hence, it is useful to have an insight into histological analysis, as they act as biological markers to assess the toxicity condition (Jayantha *et al.*, 1985; Tilak *et al.,* 2001; Srivastava *et al.,* 2008).

Hence an attempt is made to study the histopathological changes in tissue of earthworm *Eisenia foetida* when exposed to sub lethal dose of Malathion.

MATERIALS AND METHODS

Experimental model

Earthworm, *E. foetida* was chosen to evaluate toxicity in present study because it is easy to handle and 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% in dust form). It was purchased from the local market's shop.

Experimental set-up

Experimental method was based on the method used by Yasmin and Souza (2007). Plastic tub was used for our experiment and dried soil (from nearby farmland) was crushed

and filtered through a fine mesh sieve. One kg of fine soil was then poured in each plastic tub and then water was added to moistened the soil followed by 250 kg dried powdered (3 week old) cow dung, as recommended by the International Workshop on Earthworm Ecotoxicology held in Sheffield in 1991 (IWEE1). 20 mature earthworms (same age group) were added to each plastic tub. Each plastic tub was covered with muslin cloth. The experimental sets were prepared in triplicate for each treatment.

Treatment of the worms with Malathion

A higher sub lethal dose (12000mg/kg soil, 3/4th of LD_{50} value) of Malathion (5% in dust form) was selected to study its toxicity for *E. foetida*. A higher sub lethal dose of Malathion was added and mixed thoroughly in the treatment group. To maintain up-to 70 percent moisture level water was supplied regularly. After 72 hr desired parts of the worm were fixed in Bouin's fixative. After tissue processing paraffin sections of each tissue were cut at five micro meter thickness and stained with haematoxylin and eosin for microscopic examination.

RESULTS

Histopathological changes in integument of *Eisenia foetida*

Histologically integument of earthworm *Eisenia foetida* is differentiated into cuticle, epidermis, (a single layer of cells) and a double layer of muscle fibers (Fig. 1).

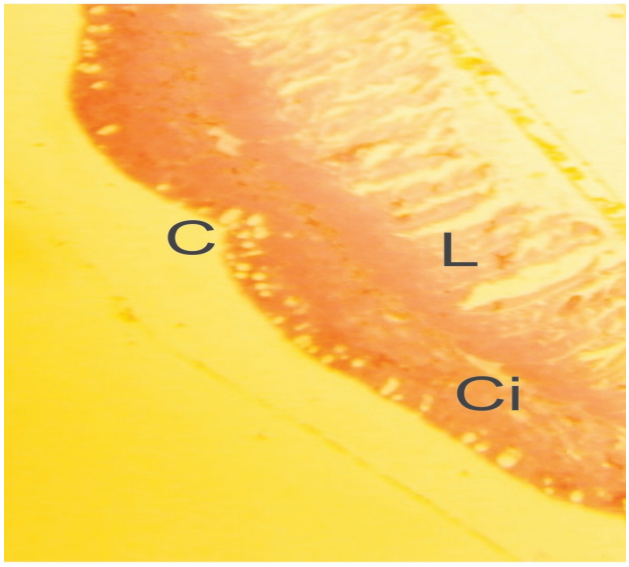

Figure 1: Integument of control earthworm, *Eisenia Foetida,* (with cuticle(C), circular muscles (Ci) and longitudinal muscles (L)). H and E. 100x

The body wall is covered externally by a thin, pervious and flexible cuticle. The cuticle is supported by the underlying epidermis. Sub lethal dose of Malathion (5%) has induced marked pathological changes in the body wall (Fig. 2). The changes include ruptured cuticle, with distortion of the shape of longitudinal muscle cell.

Histopathological changes in ventral nerve cord of earthworm *Eisenia foetida*

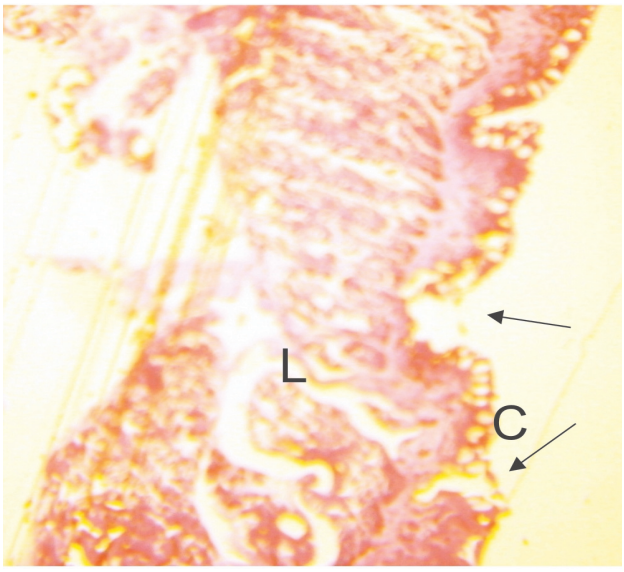

Figure 2: Integument of earthworm, *Eisenia Foetida,* exposed to Malathion (showing deshaped longitudinal muscles (L) and ruptured cuticle (C)). H and E. 100x

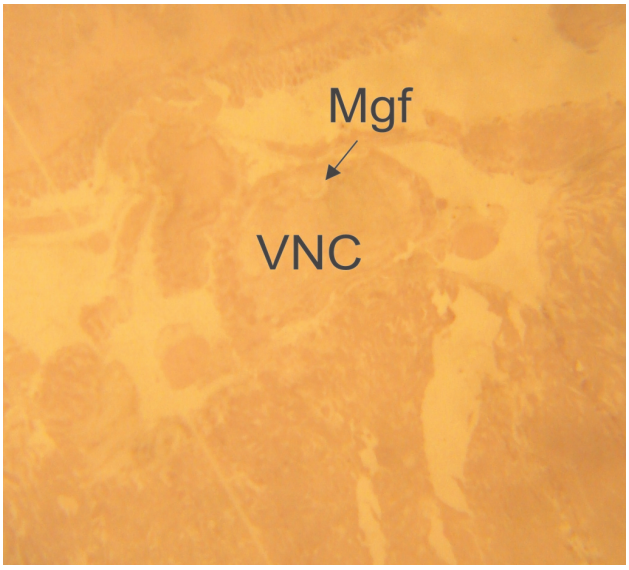

Figure 3: Ventral nerve cord (VNS) of control worm, *Eisenia Foetida* (Showing median giant fiber (Mgf) within V.N.S.). H and E. 100x

The ventral nerve cord of *Eisenia foetida* essentially consists of a sheath. In ventro-lateral region, ventral nerve cord contains bi or tripolar nerve cells, while mid and dorsal regions consist of nerve fibers (Fig. 3).

Sub lethal dose of Malathion (5%) has induced discrete pathological changes in the ventral nerve cord of *Eisenia foetida* (Fig. 4). These changes include distortion of sheath, degeneration of nerve cell and nerve fiber and formation of vacuoles within ventral nerve cord.

DISCUSSION

Organophosphoruses are primarily recognized for their

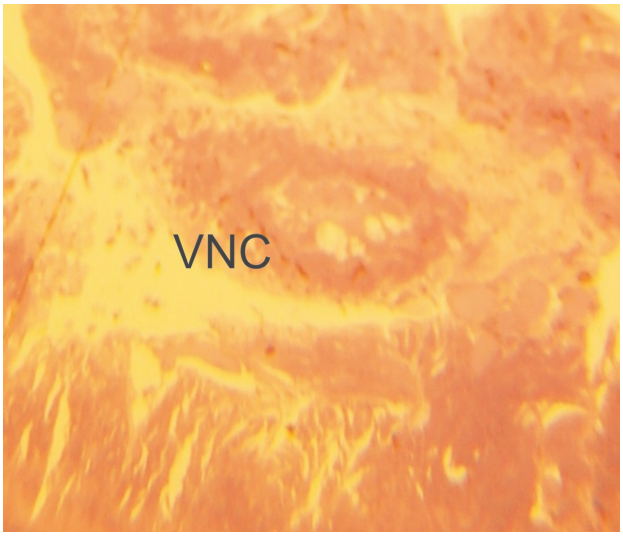

Figure 4: Ventral nerve cord (VNS) of worm, *Eisenia Foetida* exposed to Malathion. H and E. 100x

neurotoxic effects in mammals through inhibition of acetylcholine in the synaptic cleft (Hazarika *et al.,* 2003).

Malathion is one of the most widely used organophosphate pesticide (Giri *et al.,* 2002) in agriculture, therefore representing high potential risks to environmental pollution and human health and other living organisms (Ahmed *et al.,* 2002). Malathion shows strong insecticidal properties accompanied by low acute toxicity for vertebrates. The metabolism of Malathion in vertebrates and invertebrates is complex, and its metabolites present varied toxicity (Tos-Lofty *et al.,* 2003). Several of them can inhibit acetyl cholinesterase (AChE), leading to accumulation of acetylcholine (ACh) (Banerjee *et al.,* 1999; Hazarika *et al.,* 2003).

In the present study it is reported that due to toxic effect of Malathion the cuticular layer have ruptured. Kurawar, (2009) confirmed current results, as she reported ruptured cuticle, in *Eisenia foetida* after exposure to pesticide Malathion. Baker *et al.* (1978); Mackinson (1981); Kahn *et al.* (1992) and Gosselin *et al.*, (1994), observed adverse skin effects were found due to of Malathion poisoning, Sabina *et al.* (2003) have also reported that Malathion can change physical and chemical properties of membrane in rates.

Once Malathion is introduced into the environment, it may cause severe metabolic disturbances in non-target species (Anonymous, 2005). In present piece of study, it is also reported that Malathion cause histopathological changes in earthworm, *Eisenia foetida,* that is one of the non-target species for pesticide Malathion.

The present finding is supported by the finding of Desi *et al.* (1975); Stephens *et al.* (1996); Yokoyama *et al.* (1998); Cabello *et al.* (2001) and Kwong (2002), they have also observed neurological effects in rats following various exposure to Malathion.

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