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HISTOPATHOLOGICAL CHANGES IN TWO EARTHWORM SPECIES AFTER O, O-DIETHYL S-(ETHYLTHIO) METHYL PHASPHORODITL TOXICITY

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Abstract: Earthworm have an important impact on soil fertility and soil permeability, but it remains to be know about the factors that influence earthworm abundance and species diversity in agricultural soil and the impact of earth worm diversity on soil processes. Earthworms have a number of characteristics which make them one of the most suitable soil organisms to be used as key indicator organisms to assess soil pollution by chemicals. It is apparent that there is a need to measure the lethal effects of many toxic substances those commonly use in the field of agriculture and evaluate the effects on earthworms. Phorate (O, O-Diethyl S-(ethylthio) methyl phasphoroditl) a common broad spectrum systemic organophosphate (OP) used by the farmers in India, to protect the crops from chewing, sucking and boring insects. The study was undertaken to determine the morphological and histopathological changes by commonly used organophosphate (OP) pesticide, phorate on the body wall of *both* earthworms *Metaphire posthuma* and *Lampito mauritti*. The result of the study suggested that the toxic effects of phorate are mediated through its effect on the morphological and structural integrity of the tissues.
Keywords: Earthworm, Phorate, Histopathology, *Metaphire posthuma, Lampito mauritti*.

Introduction

Earthworms are important regulators of soil structure and dynamics of soil matter. They are a major component of soil fauna communities in most ecosystems and comprise a large proportion of micro fauna biomass. They are tube shaped, segmented animal, which feeds on live and dead organic matter. It conducts respiration through skin. Earthworm have an important impact on soil fertility and soil permeability, but it remains to be know about the factors that influence earthworm abundance and species diversity in agricultural soil and the impact of earth worm diversity on soil processes. Earthworms casting are rich in N, P, K, and other nutrients; this help soil to make, it more fertile and healthy. Vermicasting have led to significant reduction in pesticides use almost zero chemical fertilizer inputs.

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Expansion of agriculture is heavily relying on the use of pesticides; these are generally toxic to non-target soil organisms, by causing anatomical and physiological or morphological changes in the vital organs. Their fore requires information on the effect of pesticides on earthworms-beneficial soil organisms. Insecticide residues reach the soil in a variety of ways, causing toxicity to earthworm and they will suppress or nearly eliminate earthworm population. Pesticides residues enter the environment through industrial and agricultural activities, reaching the earthworm room soil and water. Extensive usage of organophosphorous (OP) compound in agriculture has resulted in a widespread distribution in the environment. Globally earthworms are used as biomarkers for evaluating chemical environmental pollution. Hence, in present study phorate was selected for assessment of their toxic effect on two indigenous earthworm species *Metaphire posthuma* and *Lampito mauritti* (Kinberg) was chosen as an indicator species for assessment of agro ecosystem contamination because of its widespared 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 to earthworm mortility, growth and reproduction were carried out. A very few studies have been reported on the histopathological effects of pesticides on earthworms. Histology is the most useful tool for determining the influence of agricultural pesticide, 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. Hence the present study has been undertaken to determine the morphological and histopathological changes by commonly used organophosphate (OP) pesticide, phorate on the body wall of *both* earthworms *Metaphire posthuma* and *Lampito mauritti*.

Material and Methods

Phorate (O, O-Diethyl S-(ethylthio) methyl phasphoroditl) a common broad spectrum systemic organophosphate used by the farmers in India, to protect the crops from chewing, sucking and boring insects. It is very odious and highly poisonous pesticide. It not only creates adverse effects to soil organisms but lethal to them too. The acute toxicity experiments were conducted by direct contact test (48h) through a filter paper method recommended by **OECD** Guideline, 207. The percent mortility of the *both* earthworms *Metaphire posthuma* and *Lampito mauritti* was recorded in different concentration of phorate after, the experiment were repeated three times in triplicate and the data were used to estimate the median lethal concentration (LC_{50}). Controls were also run parallel with water alone.

Prior to exposure, earthworms were placed on moist filter paper for 3h to adjust to the test environment under starvation. They were randomly divided into groups of 10 earthworms pretreatment and were exposed to different concentration of phorate. The histopathological studies may signal a damaging effect of organisms resulting from prior or ongoing exposure to toxic agents. The histology of the body wall of *Metaphire posthuma* and *Lampito mauritti* was studied adopting the routine paraffin method (Humason, 1979). All the reagents used in the present study were of analytical grade and used without any further purification. Tissues of earthworm dissected out control and experimental animals, were blotted free of mucus, washed thoroughly in physiological saline, cut in to piecs of desired size and fixed in bouins fluid fixative immediately after autopsy. Fixation was carried out at room temperature for 24hr, after which the tissue were transfer to 70% alcohol. Several changes of 70% alcohol were given until the yellow colour disappeared from the tissues. The tissue were then dehydrated by passing through ascending grades of alcohol, cleaned in xylene, infiltrated with molten paraffin, and finally embedded in paraffin wax(58 ^{0}C MP). Tissue section of 5 micro meter thick section were obtained, were stained in hematoxylene and eosin, dehydroxy phthalate xylol (DPX). The stained slides were observed under the olmypus research microscope (CKX-41).

Results

The toxic effect of phorate against both earthworms *Metaphire posthuma* and *Lampito mauritti* were recorded at 48h for the filter paper contact test. The present study reveals that the lower concentrations are enough to cause mortility. The earthworms showed progressive signs and symptoms of toxicity such as coiling, curling and excessive mucus secretion with sluggish movements and swelling. Morphological changes compare with (Fig.1.A & B) experimental worms such as constriction and swelling started appearing in the anterior regions with in 24h of exposure and degenerative changes appeared at the posterior end of the exposed earthworm after 48h of exposure: while in multiple ruptures at body length and oozing of coelomic fluid, nod like glandular appearance on the clitellum and posterior region in the earthworm *Lampito mauritti* (Fig.2.A) on the other hand changes in the earthworm *Metaphire posthuma* show coiling with release of copious amount of mucous with partial disruption of segment and degradation of body wall (Fig.2.B) .

Histopathological examination of body sections from control group revealed normal architecture of body wall and intact nature of circular and longitudinal muscles (Fig.3.A & B). Earthworms exposed to LC_{50} for 48h revealed loss of architecture, show a

tendency to develop excess changes in the body wall structure in the both the worms. Showed disintegration of ectodermal layer, neighboring cells in circular and longitudinal muscles appeared discontinuous (separated by narrow to large gap junctions) may be due to necrosis depending upon the effect of toxicant and also appeared enlargement of ectodermal cells and expansion of spaces between the longitudinal muscles with proliferation of glandular cells and erosion of ectodermal of body wall of *Lampito mauritti* (Fig.3.C). Damage in circular and longitudinal muscles and tissue erosion were prominent at 48h of exposure, which may lead to the fragmentation of the body with cloudy swelling of longitudinal muscles, damage in circular and longitudinal muscles and loss of structural integrity in longitudinal muscles and internal & ectodermal tissue erosion leading to total damage body wall of the *Metaphire posthuma* (Fig.3.D).

Discussion

Earthworms are standard test organism in soil toxicity testing. They have been broadly used to assess of environmental impact from heavy metal pollution; however, the knowledge on toxic effect from pesticides upon these organisms is still very limited **(Castellanos and Hernandez, 2007).** Earthworms are affected by pesticides either through skin contact or by feeding on contaminated litter in soil. Primarily, these toxicants passing through the skin throughout the body wall. Previous studies suggested that earthworm skin has direct contact to the contaminated soils and is considered as a significant route to uptake of toxicants (**Saxe** *et al.,* 2001; **Jager *et al.,* 2003**; **Vijver** *et al.,* 2003). Epidermis and cuticle represent a primary barrier that protects earthworm's body from the environment and are also responsible for the transport of ions, thus allowing/blocking xenobiotics to enter the body **(Clauss, 2001).** The current investigations on the earthworms *Lampito mauritti* & *Metaphire posthuma* revealed that the contact toxicity of phorate through its integument was increased with increasing concentration and /or exposure time of the given toxicant. It is evident from the results that phorate can be rated as highly toxic to these earthworms with LC_{50} value 20-40 µl/cm^2 at 48h of exposure. The present toxicant is comparatively several folds less toxic to *Eisenia fetida* in comparison to the earlier studies on other organophosphate (OP) insecticides (**Venkateswara Rao *et al.,* 2003b; Venkateswara Rao and Kavitha, 2004**). However work of (**Zang *et.al.* 2000)** revealed that these worms were highly sensitive to Chloronicotinoid insecticide, Imidacloprid with an LC_{50} of 0.1 and 0.034µg cm^{-2} after 24h and 48h of exposure, respectively.

The anatomical symptoms like coiling, abnormal swelling, fragmentation, rupture of body skin and loss of mucus etc., were noticed by the effect of phorate proved to be identical with the effects of lead acetate, tetra ethyl lead (TEL), methyl tetra-butyl ether (MTBE) (**Venkateswara Rao *et al.,* 2001, 2003a**) and it was found quite similar in other species of earthworms, *Perionyx excavates* and *Eisenia andrei* after exposure to MTBE and Imidacloprid **(Youn, 2005;Yvan *et al.,* 2005**). This happens when tissue in the body wall undergoes trauma or microscopic damage to an area by migration of toxicants or contaminations from the contact surface. During earthworm's exposure to phorate, epidermal cells underwent both hypertrophy and hyperplasia, increasing the thickness of the body wall. During earthworms exposure to DS, epidermal cells underwent both hypertrophy and hyperplasia, increasing the thickness of the body wall; these progressive changes, such as hyperplasia and hypertrophy, can be considered as general defense mechanism against toxicants **(Baynes and Hodgson, 2004),** which increase the distance between external environmental and the internal organs and thus serve as a barrier for the entrance of xenobiotice **(Poleksic *et al.* 2010).** After 48h exposure of phorate, a thinning of the earthworm body wall started due to atrophy, which ultimately led to necrosis and erosion of the entire body wall. Despite this, without establishing links between changes in the epidermis and muscle layers, it is impossible to assess the level of the cell reaction. This type of degeneration may indicate a complete drain of utilizable levels of energy reserves and subsequent autolysis of its own tissues to meet its energy requirements. A similar kind of autolysis from the posterior region was observed in earthworms, *Polypheretima elongate,* exposed to textile dyes **(Ramaswami and Subbram, 1992).**

The histopathological evaluation revealed that the cuticular membrane and ectodermal layers were completely disintegrated and profusion of glandular epithelium given a protection to the muscle layers of the body wall. It is evident from earlier reports that the morphological and histological changes were prominent when earthworms were exposed to different toxic metals and other OP pesticides **(Amaral *et al*., 2006).** Earthworms ingest large amounts of soil and are therefore elective non-target organisms exposed to pesticides through their external and internal surfaces. These findings indicate that tested organophosphate compound phorate have a potential to cause significant histopathological changes and act as a destructive element to the earthworms *Lampito mauritti* & *Metaphire posthuma.* Thus morphological or anatomical changes in these organisms are one of the suitable indicators for monitoring the effectiveness of soil pollutants.

In conclusion, the result of the study suggested that the toxic effects of phorate are mediated through its effect on the morphological and structural integrity of the tissues. Its effect on other biological indicators of stress and pollution **(Gobi *et.al.* 2004; Xiao *et. al.*, 2006**) could play a major role in its lethal effect. It further shows that the pesticide had adverse effect on non target organisms particularly the earthworms that are critical in evaluation of soil fertility.

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Fig.1A

Fig.1B

Fig.2A

Fig.2B

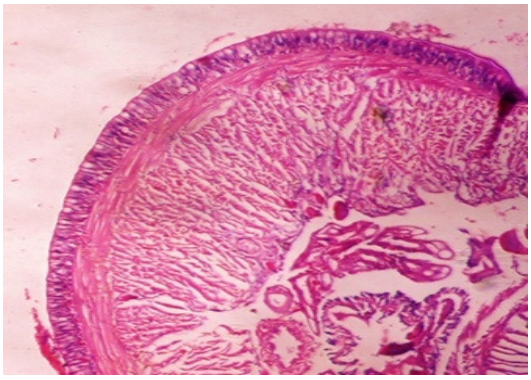

Fig.3A

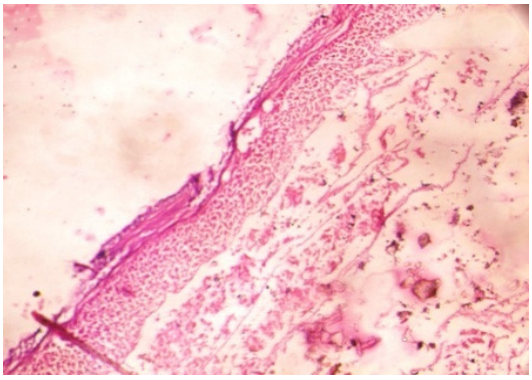

Fig.3B

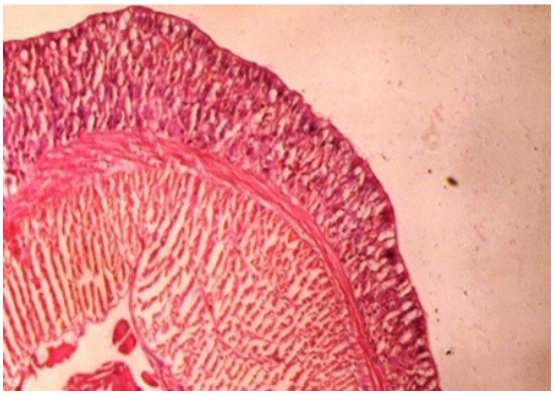

Fig. 3C

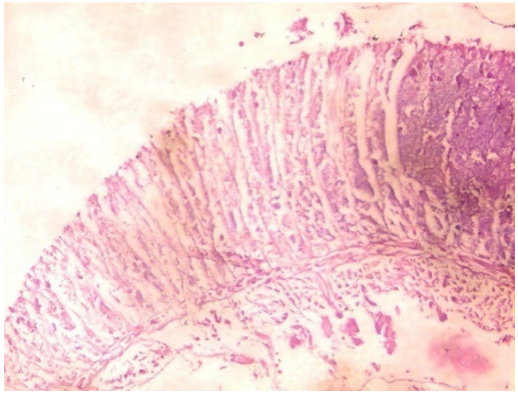

Fig.3D

EXPLANIATION OF FIGERS: - Morphology and histopathological changes of earthworms, *Lampito mauritti & Metaphire posthuma* during exposure (LC_{50} 48h) of phorate concentration on filter paper method.

Fig.1.A & B: Intact metameric segmentation in earthworm *Lampito mauritti & Metaphire posthuma* under controlled condition.

Fig.2.A: Multiple ruptures at body length and oozing of coelomic fluid, nod like glandular appearance on the clitellum and posterior region of earthworm *Lampito mauritti.*
Fig.2.B: Coiling of earthworm along with release of copious amount of mucous with partial disruption of segment and degradation of body wall in the earthworm *Metaphire posthuma.*
Fig.3.A & B: Intact nature of muscles and ectoderm layer in control earthworm *Lampito mauritti & Metaphire posthuma.*
Fig.3.C: Enlargement of ectodermal cells and expansion of spaces between the longitudinal muscles with proliferation of glandular cells and erosion of ectodermal of body wall of *Lampito mauritti.*
Fig.3.D: Enlargement of body wall, necrosis of glandular cells and partial damage with cloudy swelling of longitudinal muscles, damage in circular and longitudinal muscles and loss of structural integrity in longitudinal muscles and internal & ectodermal tissue erosion leading to total damage body wall.