Effect of Organophosphate Pesticide "Methyl Parathion" and "Phorate" on Earthworm Central Nervous System

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Research Article

Abstract: The research work compiles of collecting earthworm's species *Eudrilus eugeniae* and to study the effect of widely used organophosphate pesticides namely- 'methyl parathion' and 'phorate' on adult earthworms. The sub-lethal concentration of the pesticide was used to assess its effect on nervous system through histopathological study. Cholinesterase is used as potential biomarker. With the help of stereo research microscope and stage micrometer the CNS was studied and microphotographs were taken. Research work indicates that methyl parathion and phorate acts as neurotransmitter inhibitor and has disastrous effect.

Keywords: Earthworms, Organophosphate pesticide, Cholinesterase, Biomarker, Nervous system, Histopathology

Introduction

Earthworms has unavoidable beneficial role in the ecosystem. They act as aerator, crusher, mixer physically, degrader chemically and biologically as a stimulator in the decomposer system enhancing soil texture and fertility. The application of chemical fertilizers sprays and dusts can have a disastrous effect on the earthworm populations (Lawrence and London, 1997). Farming practices such as the application of superphosphates on pastures and a switch from pastoral farming to arable farming had a devastating effect on Giant Gippsland earthworm. Earthworms are classified into three main ecophysiological categories: leaf litter/compost dwelling worms (epigenic) - Eiseniafetida; topsoil dwelling worms (endogenic) and worms that construct permanent deep burrows, through which they visit surface to obtain food. E.g. - Lumbricusterrestris (Tisdall and McKenzie, 1999). Earthworms ingest through peristalsis (visceral muscle contraction) any other soil particles that are small enough including sand grains, into its gizzard wherein minute fragments of grit grind everything (Wang et al; 2004). When excreted, these excreta are made available in an accessible form. These annelids have the ability or simply the power of regeneration (regain the lost segments). Stephenson (1930) wrote a chapter of his great monograph while G.E Gates (1972) published few of his findings that: Eiseniafetida showed head regeneration including 23/24 segment. Central nervous system of earthworm plays a vital role. They act as tiny computers that form brain and ventral nerve cord. They analyze the incoming sensory information and send out appropriate instructions to perform further actions. Pesticides could cause ceasing earthworm activity. Application of these artificial chemical has disastrous effect, not only on humans but has greater impact on earthworms which are globally used as biomarkers for evaluating chemical environmental pollution (David and Hopkin, 1993). Therefore the most reliable way to maintain worm population is to avoid the use of pesticides, adding organic matter preferably as surface mulch, on a regular basis that creates optimum conditions of heat and moisture to stimulate their activity. Phorate and methyl parathion are organophosphate insecticide and acaricide with high level of toxicity. Presently effect of "Phorate" and "Methyl parathion" were studied for its deleterious effect on earthworms with respect to central nervous system.

Methods

Experimental organism

Earthworm (Eudrilus eugeniae) was used as test organism in present work.

Study areas

Earthworms were collected from GKVK (Bangalore) for rearing purpose. Adult worms were chosen from wetland areas as these are the topsoil dwellers. Alive worms collected in plastic bags along with suitable quantity of moist soil.

Techniques of rearing and culture

Adult earthworms were brought to the laboratory and kept at 25℃. They were reared in the same soil along with mixture of organic materials compost, manure etc.

Test sample and concentrations

Test sample used in the experiment was Methyl parathion- 0.12g and Phorate- 0.05g for the treatment of earthworms

Toxicity determination

1. **Method of Treatment**

Phorate at a concentration of 0.05g/500g of soil and Methyl parathion of 0.12g/500g of soil were taken in separate beaker for testing their inhibitory action on CNS, whereas one bowl with soil containing adult healthy earthworms were kept as control(uninfected by pesticides) to determine the environment effect. 5 adult worms of same size were released into each beaker containing pesticides separately and were left undisturbed for 3 days (acute toxicity method was adapted). Post treatment of pesticides, observations were made in the laboratory by procuring the worms.

2. **Histological studies**

After period of 3 days, worms were externally studied for their behavior. Adult worms were removed respectively from the beaker and were anaesthetized using chloroform. The worms were shifted to a petridish with a wax bed for the study of external morphology. After careful positioning of the worms, anterior and posterior regions were pinned on the wax tray using needle and forceps. A median dorsal opening was made using fine scissors on the body. After further cuts, the body cavity was exposed completely and was accordingly pinned on the wax bed. The CNS was carefully excised and observed. After the removal of central nervous system from the clitellarworms, they were immediately fixed in 70% alcohol fixative in a watch glass for further histological studies. Tissue wax preparations using routine laboratory procedures were followed. The brain of the earthworm was processed and slides were prepared. A detailed microscopic observation was done in stereo research microscope. The size variations and microphotographs of the CNS were taken.

Observation and Result

Observation

In the present work on adult earthworm, Eudrilus eugeniae , toxicities of Phorate and Methyl parathion and their effects were studied and histological differences between control and pesticide infected earthworms are compared and conclusion is drawn. The experiment study in which earthworm played a major role showed progressive signs and symptoms of toxicity such as coiling, curling and excessive mucous secretion with sluggish movements. Swelling of the clitellum was also seen. Extrusion of coelomic fluids resulting in bloody lesions within 48 hours of exposure was observed. Earthworms showed degenerative changes at the anterior part of nervous system. Disappearance of metameric segmentations and loss of pigmentations were observed. The control earthworms showed excellent burrowing movements and exhibited no extra ordinary behavior. This suggests that toxicity is associated with accumulation of Phorate (0.05g/ 500g of soil) and Methyl parathion (0.12g/500g of soil) showed inhibition of acetylcholine in the CNS of earthworm. This shows complete drain of utilizable levels of energy reserves and subsequent autolysis of its own tissues meet energy requirements, since protrusions were also seen due to bulging of clitellar regions which restricted their free movements.

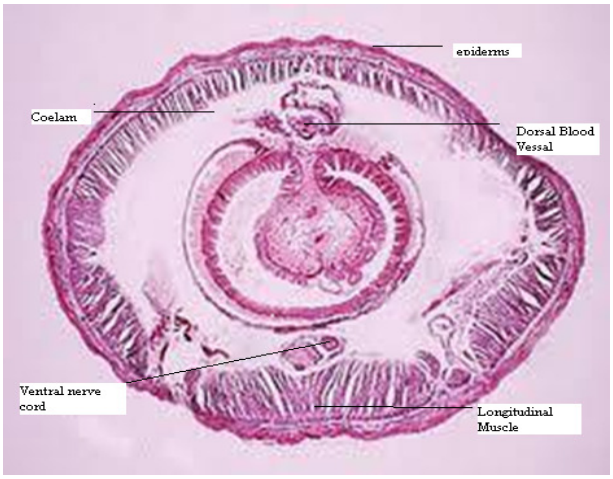

Figure 1: The Control crosssecion of CNS.

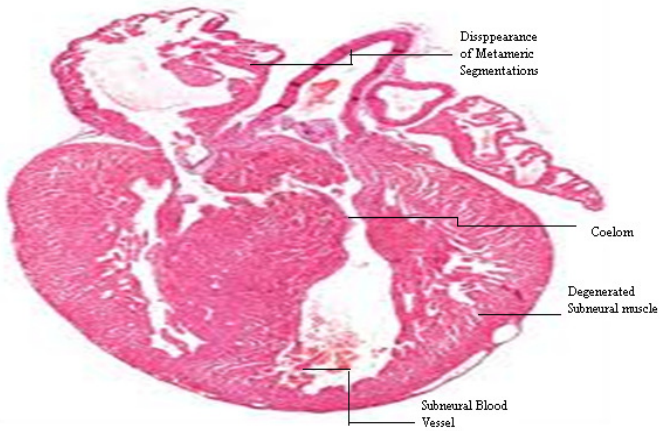

Figure 2: Pesticide affected section of CNS

Conclusion

In the present finding, Phorate and Methyl parathion mainly acts as neurotransmitter inhibitor. Predictable signs and symptoms such as sluggish movements and bloody lesions are due to the usage of artificial chemicals (pesticides) that harm earthworms. Chemistry indicates that these are acaricide that could pass to groundwater through its capability and accumulate in environment. They not only affect earthworms which are profitable investors, but also affect the ecosystem.

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