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Histopathological Effects of Sublethal Dose of Dichlorvos on Nerve Cord and Chloragogen Tissue of *Eisenia fetida*

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Abstract:
Dichlorvos or DDVP is an organophosphate pesticide which is used in agriculture rampantly, as well as in households and stored grain houses as an insecticide. While application, non target organisms in the soil may also get exposed to Dichlorvos. The persistence of residues of the pesticides is also a serious concern on the health of soilbiota. In present study, the effect of sublethal dose of the Dichlorvos was studied in earthworm *Eiseniafetida.* The worms were exposed to two sublethal doses of the pesticide for 7 and 14 days, subsequently, the worms were sacrificed and processed to prepare sections afterstaining and the changes in the nerve cord and chloragogen tissue were analyzed. By general observation, many behavioral changes were observed. Worms looked sluggish and showed excessive coiling. Histological changes in the peritoneum lining of the nerve cord were observed in the pesticide exposed worms. Changes in location of the giant fibres were also seen. Worms in control showed normal placement of the giant fibres, while in pesticide exposed worms, giant fibres appeared displaced. The muscle layer and epineurium also showed many changes compared to the control. Under exposure of Dichlorvos, Erosion of chloragogen tissue was seen. Significant vacuolization was also observed. The intestinal villi also were found to be eroded. Even at a sublethal dose, dichlorvos and other organophosphates can hamper the normal physiology of the soil biota such as earth worms and this can have detrimental effects on their survival and reproduction.

Keywords:
Diclorvos, *Eiseniafetida*, epineurium, giant nerve fibres, chloragogen tissue.

INTRODUCTION

Dichlorvos, abbreviated as DDVP is an organophosphate pesticide. The mechanism of action of such pesticides is by inhibiting the activity of the enzyme, acetylcholinesterase. The enzyme acetylcholinesterase is responsible for rapid degradation of the neurotransmitter acetylcholine[5]. Organophosphate pesticides, such as dichlorvos thus hamper the relay of nervous signals by inhibiting acetylcholinesterase. When used in agricultural setup, inhibition of the enzyme leads to

mortality of many agricultural insect pests, thus keeping their number low, but resistance in the agricultural pests are continuously reported[8].

Application of the organophosphates also exposes soil fauna to it. In an agricultural field, earthworms form majority of the soil fauna. Earthworms are considered good bioindicators of toxicity in soil ecosystem, as they are scavengers and composters and are consumed directly by many animals, including birds[21]. Epigeic earthworms such as *Eisenia fetida* are surface feeders and thus they are most prone to the exposure of the chemical pesticides which are directly sprayed on the crop in agricultural fields[3]. Earthworms can be dynamically used to study mechanism of neuronal pathology induced by various chemicals present in the atmosphere[19]. Many behavioral changes are observed in the earthworms under pesticide exposure, such as coiling, constriction in the body, swelling in body, blister formation, etc[1]. Degeneration of reproductive tissues, vacuolization, necrosis and reduction in spermatic follicles has been observed after exposure to organophosphates[9]. Necrosis in the cells of ectoderm and muscle layer has been observed due to an organophosphate pesticide, profenofos[15]. Rupture of cuticle and bloody lesions in *Eisenia fetida* were observed at sub lethal doses of chlorpyrifos[14]. Pyrethroid pesticide, cypermethrin and organophosphate pesticide, chlorpyrifos individually and in combination have been shown to reduce growth and reproduction rates in earthworms[22]. Chlorpyrifos has been shown to have negative effects on fecundity of earthworms, cocoon production severely declined[23]. Cellular dysfunction, protein catabolism and increase in reactive oxygen species (which is a marker of stress) was observed in earthworms exposed to mixture of organophosphate pesticides[18].

Nerve cord represents part of the central nervous system and plays important role in the growth, reproduction and other physiological processes of the worm.

Chloragogen tissue surrounds the outer gut wall of earthworm, it is an important center for metabolism of amino acids and urea and storage of glycogen. The chloragogen tissue acts as a homeostatic device when it breaks off and distributes material throughout the coelom. Thus, it plays and important role in physiology of earthworms. Adult healthy earthworms would require the distribution of important lipids and glycogen in the reproductive organs.

Since, all the processes of physiological importance are related to the nervous system and chloragogen tissue , it is imperativeto study the pathological changes in the nerve cord histology and chloragogen tissue, more so under sub lethal levels of pesticides.

Earthworms are hermaphrodite, they reach maturity in a short duration and the appearance of clitellum is an identification mark of a reproductively active mature worm. Soon enough, they start laying cocoons, which hatch, and a juvenile emerges from it. In the present study the compost worm, *Eiseniafetida* was utilized. They reach sexual maturity within 60-90 days after hatching from cocoon. They can be grown in artificial soil augmented with cattle manure. They remain reproductively active for a long period and number of juveniles from a cocoon vary from 1 to 9[20]. A variety of organic waste, domestic waste, agro industrial waste can be composted using earthworms such as *Eisenia fetida*[17].

Sub lethal toxic effect studies are important as they impact parameters such as growth, reproduction and other physiological aspects of the organism. Earthworms play important role in maintaining the soil structure and are vital soil fauna. At sub lethal levels pesticides can hamper their reproductive capability and thus altering the invertebrate biomass distribution.

MATERIALS AND METHODS

Earthworms (*Eisenia fetida*) were procured from Bhoojeevan organics private limited, Delhi. Worms were cultured in lab in earthen pots lined with wet shredded paper and cardboard at the base for bedding and Tropical artificial soil as culture medium (10 % Non-decomposed Coconut coir dust/ coconut peat, 20 % kaolin clay, 70% fine industrial sand/silica. Calcium carbonate was added, and pH was adjusted to 6 ± 0.5)[2], further supplemented with cow dung and kitchen waste as food for the

worms. Commercially available Dichlorvos (label name Newban- Dichlorvos 76% EC, active agent, Dichlorvos- 92%w/w) was used. LC50 (83.17mgkg^{-1}) of Dichlorvos was calculated by acute toxicity tests according to the OECD guidelines 207. Two sub lethal doses were selected 40 and 60 mg kg^{-1}of dry soil. Adult worms, with well-developed clitellum and weight in 400-500mg range, were selected and washed with water, cleaned with blotting paper and were released in test pots with treated medium. Ten adult clitellated worms in replicates of four were exposed to the two concentrations. After a period of 7 and 14 days, worms were collected and processed for histological preparations. Worms were fixed in 10% formalin fixative and dehydrated with increasing alcohol concentration, cleared by xylene and fixed in paraffin wax. Sections were cut by microtome (Radical, RMT-30A) and Haemotoxylin and Eosin staining was done.

RESULTS

At initial observation worms were found coiling and showing irregular movement and irregular jumpingbehavior. Worms were moving in peripheries of the treated media; excessive coiling and mucous release was also observed. Analysis of the histological sections of the ventral nerve cord showed marked differences. In *Eisenia fetida*, nerve cord is double and located ventral to the digestive tract. It is composed of an outer peritoneum, a layer of muscle fibres and connective tissue, within which lie the two nerve cords, giant nerve fibres are also located within the connective tissue at the dorsal region, there is one median giant fibre, two lateral giant fibres and sub median giant fibre (Fig 1 A). At the region of segmental ganglion, the two nerve cordsjoin, and segmental nerves arise (Fig 1 B).

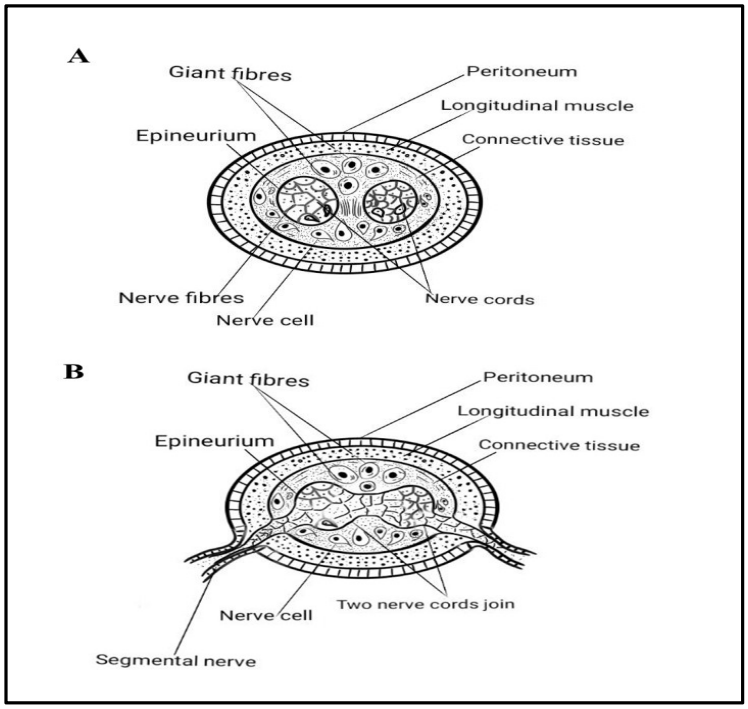

Figure 1: Nerve cord *Eisenia fetida* (A) Diagrammatic representation of Transverse Section of nerve cord of *Eisenia fetida*, showing two separate nerve cords embedded in muscle and covered by a layer of peritoneum, covering of individual nerve cords is referred as epineurium (B) Diagrammatic representation of nerve cord at the level of segmental ganglion, here two nerve cords coalesce in the middle, also at the level of segmental ganglion, segmental nerves arise

In control worms the basic nerve cord structure was well established (Fig 2 A&B). At sublethal dose of Dichlorvos (60mg kg^{-1}), after 7 and 14 days of treatment, significant thickening of peritoneum could be observed, also the outer peritoneum and connective tissue stained darker. The giant fibres also

seem to have lost their usual state. They appear swollen and vacuolated, and all the giant fibres could not be distinguished from each other (Fig 2 C , D, E & F). The nerve cells found at the ventral part stained darker at both the sublethal doses of 40 and 60mg kg^{-1}. Both at 7 and 14 days of treatment similar observations were observed. At sub lethal dose of 60 mg kg^{-1}soil, the nerve fibers or the two nerve cords seem too dispersed as compared to the control (Fig 2 C& D). At the level of segmental ganglion as well, similar observations can be seen (Fig 3 A, B and C).

Under sublethal exposure of DDVP, there was significant vacuolization observed in the chloragogenous tissue. The control worms showed normal histology of chloragogen tissue (Fig 4). The thickness of the tissue reduced, and excessive erosion of the tissue could be seen. Even the muscle layers separating the intestinal mucosa and the chloragogen tissue appeared to have lost normal morphology. Spaces between muscle fibres could be seen in many histological preparations. There was significant erosion to the intestinal villi as well (Fig 5 and 6).

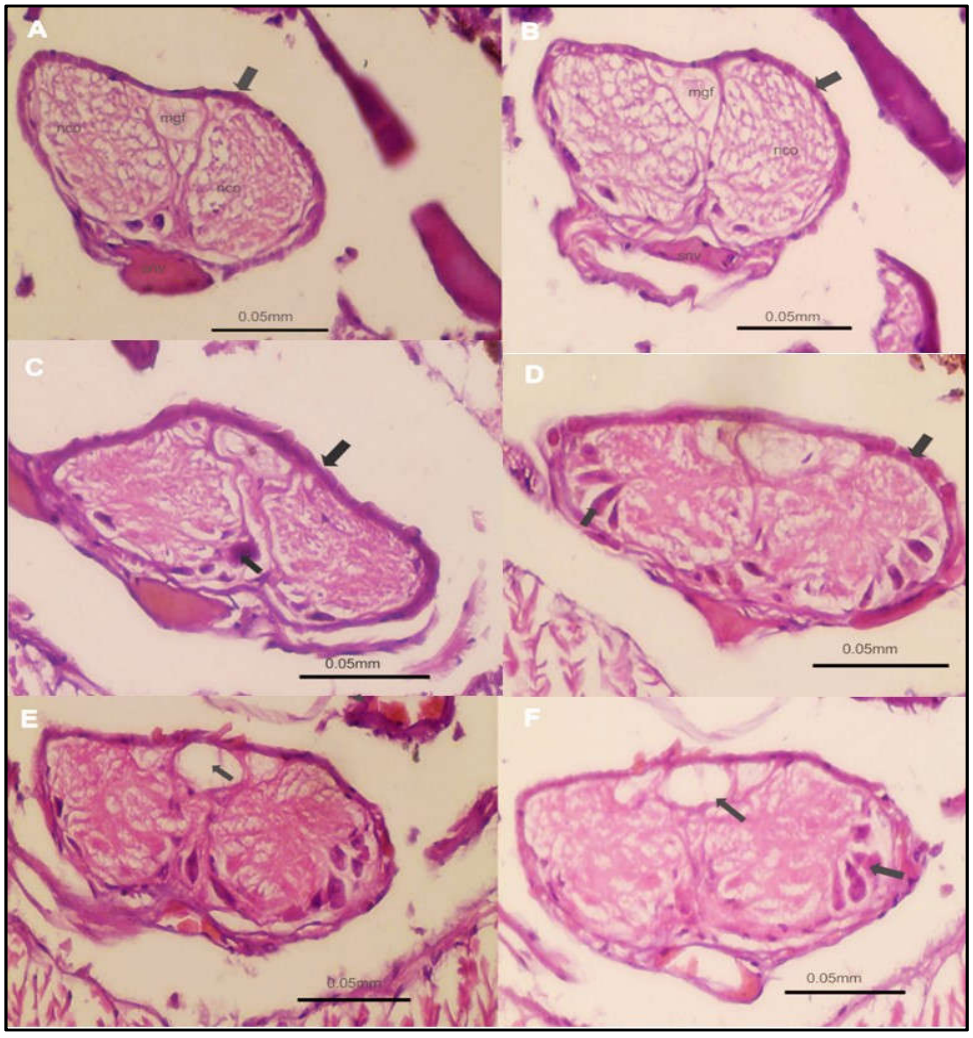

Figure 2: Histological sections of nerve cord (A)Control at 7 days, showing normal physiology of peritoneum and normal placement of giant fibres. (B) Control at 14 days, no change is observed, normal tissue morphology seen. (C) At DDVP conc. 60 mg/kg for 7 days, thickening of peritoneum is observed, nerve cells stain darker, shown by arrows. (D) DDVP conc. 60 mg/kg for 14 days, thicker peritoneum, nerve cells more and darker. (E) & (F) DDVP 42 mg/kg for 7 & 14 days respectively, mgf appears larger.

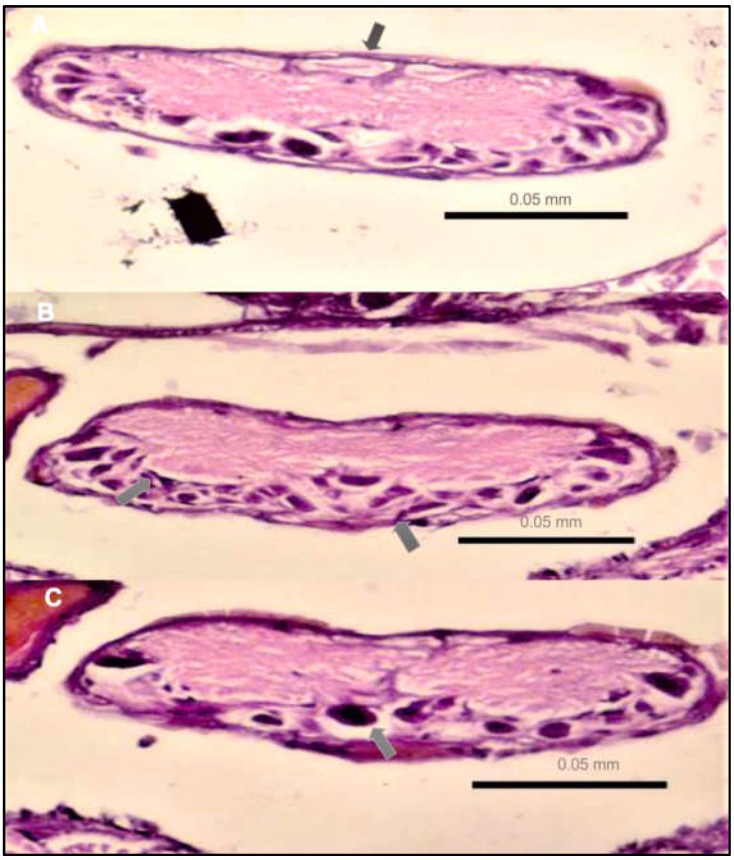

Figure 3: Histological sections of nerve cord at segmental ganglion. (A) Control showing normal placement of giant fibres, shown by arrow. (B) At Conc. DDVP 60mg/kg numerous nerve cells are observed on the ventral side, shown by arrows. (C) At DDVP Conc. 40mg/kg, nerve cells stained darker.

Figure 4: Histological section showing Intestinal villi and chloragogen tissue separated by muscle layers of control worms.

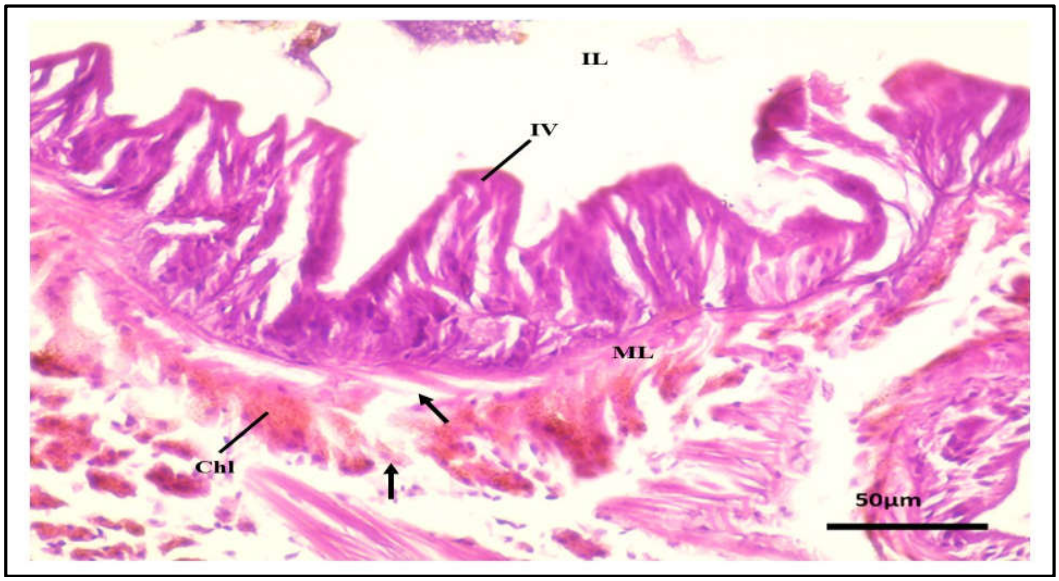

Figure 5: Damage to chloragogen tissue at 60 mg/kg DDVP, shown by arrow, also the muscle layers showed damage.

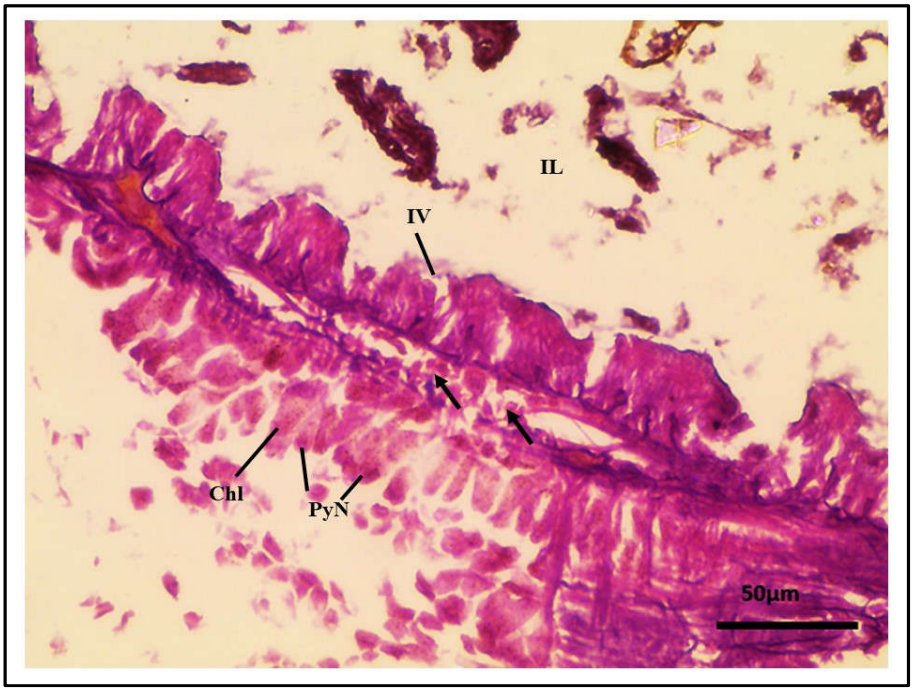

Figure 6: Vacuolization seen in chloragogen tissue, Damage to muscle layers and development of pyknotic nuclei (PyN) observed after exposure to 40 and 60 mg/kg DDVP.

DISCUSSION

The nerve cord plays important role in relaying the signal to the reproductive organs, it is responsible for the evasive reflexes of the worm, nervous system relays the information to epidermis to release mucous. Organophosphate pesticides show dose dependent changes, accumulation of pesticides and inhibition of acetycholinesterase causes pathological changes such as extrusion of coelomic fluid and excessive mucous production[13].

Excessive mucous production and coiling due to the toxic effects of the pesticide hinders the ability of the worm to mate and thus lay viable cocoons. Lesser number of cocoons laid would impact the population size. When the stocking density of the worms is too low then the vermicompost produced is not of adequate quality[10]. In natural conditions also, lower density of the earthworms would hamper the overall soil health of the ecosystem.

Chloragogen tissue acts as a mobile liver[16], the tissue helps in maintaining required levels of substances in coelomic fluid and blood by its movement[4]. Damage to tissue has detrimental effects on survival of earthworms and even in production of cocoons.

Eventual aim of soil ecotoxicological studies is to understand the long-term impact of different environmental pollutants and chemicals present in the soil ecosystem. It requires better understanding of how the chemicals can affect soil dwelling organisms, specifically the non target organisms, at sub lethal concentrations.

CONCLUSION

Organophosphates such as Dichlorvos, can negatively affect the non target soil biota even at sub lethal concentrations. Under exposure to concentration which are near NOEC, despite no mortality observed, significant histopathological changes in nerve cord and chloragogen tissue could be seen. So, sub lethal doses of these pesticides have serious impacts on reproductive and physiological health of earthworms and likewise can affect other soil biota.

ABBREVIATIONS

NOEC- No observed effect concentration, OECD- Organization for economic cooperation and development, DDVP- 2, 2-dichlorovinyl dimethyl phosphate or Dichlorvos, mgf- median giant fibre, EC- Emulsifiable concentrate.

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