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DERMAL TOXICITY OF PROFENOFOS ON *EISENIA FETIDA* (SAVIGNY) AND ITS HISTOLOGICAL EFFECTS

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Abstract

Earthworms are soil bioindicators, indicating soil stress conditions. Agrochemicals are key stress factors in agricultural soils. The ecotoxicity research employed profenofos, a pesticide widely used in Tamilnadu's delta areas. In this way, Profenofos dermal toxicity (LC50) on earthworms (Eisenia fetida) was evaluated and its impact on earthworm tissues was anticipated using histology research. Profenofos was found to be “Super toxic”, with an LC50 of 0.0088g/cm2. The earthworm epidermis, peritoneal epithelium, Chloragogen cells, longitudinal muscles, etc. were severely damaged histologically. The study indicated that Profenofos is very hazardous to earthworms and that additional research on acute and chronic toxicity is required.

Keywords: Ecotoxicity; Paper Contact Test; Eisenia Fetida; Profenofos; LC_{50}; Histology of Eisenia Fetida.

1. INTRODUCTION

A soil ecosystem's physical and chemical characteristics directly affect its sustainability. The progress of agriculture has increased the production and emission of agrochemicals, notably pesticides that end up in the soil. Pesticides' influence on soil micro- and macro-fauna has become a major worry for environmentalists. Pesticides are used in agricultural fields to control pests. But they harm soil biota, notably earthworms, reducing variety, development, and reproduction, organic matter breakdown, and soil fertility. Soil ecosystems are increasingly in need of ways to analyse pesticide side effects.

Increasing concern about environmental consequences of chemicals has led to the development of risk assessment approaches that include exposure and effect data (OECD, 1984). Recognizing the importance of soil animals in ecological and agronomic processes, most regulatory agencies consider their impacts when registering pesticides. In places like India, the impacts of agrochemicals on soil fauna are overwhelming. So there is a need to raise knowledge about chemical examination in soil ecosystems.

Pesticides' influence on earthworms is yet unknown, as is tropical agriculture (Van den Brink et al. 2003).

Earthworms are vulnerable to pesticides, according to Edwards and Bohlen (1993). Evaluating environmental risk assessment systems requires research on pesticide impacts on earthworms. Assessing such consequences is important for achieving sustainable development in tropical

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areas. As a first stage, the cutaneous toxicity of Profenofos on earthworm Eisenia fetida was calculated. Histological investigations demonstrated the action of Profenofos.

2. MATERIALS AND PROCEDURES

2.1 Test organism

Eisenia fetida (Oligochaeta, Lumbricidae) is listed as a potential test organism for ecotoxicological studies (OECD 1984a; 2003). For this work, *E. fetida* was obtained from the vermicomposting unit, A. V. C. College, Mannampandal.

2.2 Test chemical

Though various pesticides are used, Profenofos is the most widely used in India (Habiba and Ismail, 1992). Profenofos is a pyrethroid pesticide. It's a light yellow to amber liquid with a garlic-like smell. Profenofos' CAS number is 41198-08-7, its chemical name is O-(4-Bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate, and its molecular formula is C11H15BrClO3PS. In Mayiladuthurai, Tamil Nadu, India, we bought Profenofos (50 percent Effective Concentration).

2.3 Test solution preparation

Each concentration was prepared by measuring and dissolving Profenofos in deionized water. Profenofos is a dangerous substance, thus it must be metered carefully. So a stock solution was made up of dilutions and utilised for various test concentrations. The concentration range was 100-250 ppm. As a result, the ultimate concentrations were 100 ppm.

2.4 Experimental Procedure

2.4.1. Dermal Toxicity test – Paper Contact Method

Profenofos was tested for acute toxicity in earthworms using the OECD testing guideline no. 207. The test vial was a 10cm x 1.4cm glass petri plate. 10.5cm diameter round filter paper (Whatman No. 1) was cut to size and inserted such that sides were lined with filter paper. Each vial received 5ml test solution to moisten the filter paper. 5 ml deionized water was used as a blank. Each concentration included five duplicates, one earthworm per vial. The tests lasted 48 hours in the dark in a tropical climate. A modest mechanical stimulation to the front section of the earthworm after 48 hours checked for death. All laboratory experiments followed GLP (Good Laboratory Practice) guidelines (OECD 1998).

2.4.2. Dermal Toxicity Histology

The histology of earthworm clitellum and intestines was examined using paraffin (Humason 1979).

2.4.3. Statistical Analysis

Zar claims routine statistical analyses were used (1984). A Levene test was performed on the data prior to analysis. 0.05 was judged significant.

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2.4.3.1. Determination of toxicity

With this technique, Profenofos was classified as supertoxic (1.0µg/cm2), highly toxic (1–10µg/cm2), very toxic (10–100µg/cm2), moderately toxic (100–1000 g/cm2), or generally benign (>1000µg/cm2) (Roberts and Dorough 1984).

3. RESULT

3.1. Dermal toxicity test - Paper contact method

A basic paper contact toxicity test indicated that the chemical is likely to be hazardous to earthworms in soil and that further extensive testing in artificial soil is required. Although the basic paper contact approach ignores the input of soil components, it offers several benefits such as good repeatability and direct comparison. The deadly toxic concentration of Profenofos to Eisenia fetida was therefore determined to be 0.0088µg/cm2. According to Roberts and Dorough (1984), Profenofos is "Super toxic" to Eisenia fetida. Profenofos exposure to the earthworm at varied doses revealed harmful effects on filter paper as substrate material.

Figure 1: Acute Dermal toxicity of Profenofos on Eisenia fetida at 0.0088µg/cm2 Concentration

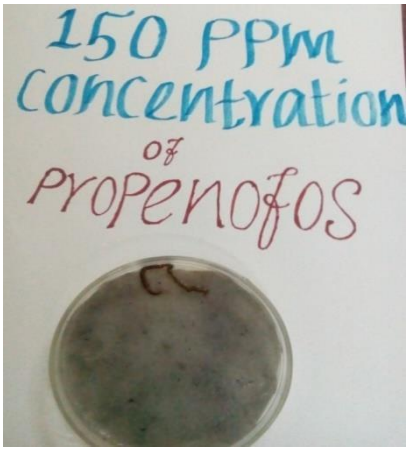

Figure 2: Detrimental effects like lesions, inflammations, and separation of body parts observed on Eisenia fetida

There were five geometric concentrations of diluted test solutions generated and tested. Earthworm death was reported at 0.0088, 0.0106, and 0.115 g/cm2 concentrations after 48, 16, and 8 hours, respectively. Only 100ppm/ml proved non-toxic to earthworms for 48 hours. Profenofos fatal concentration for Eisenia fetida was 150ppm/ml, or 0.0088µg/cm2 (Fig. 1). Profenofos caused ulcers, inflammations, and separation of the posterior body parts in earthworms (Fig. 1 and 2). After 40 hours, the worm lost its ability to move, and its body parts separated, killing it. Fig. 3 depicts the mortality graph at different concentrations.

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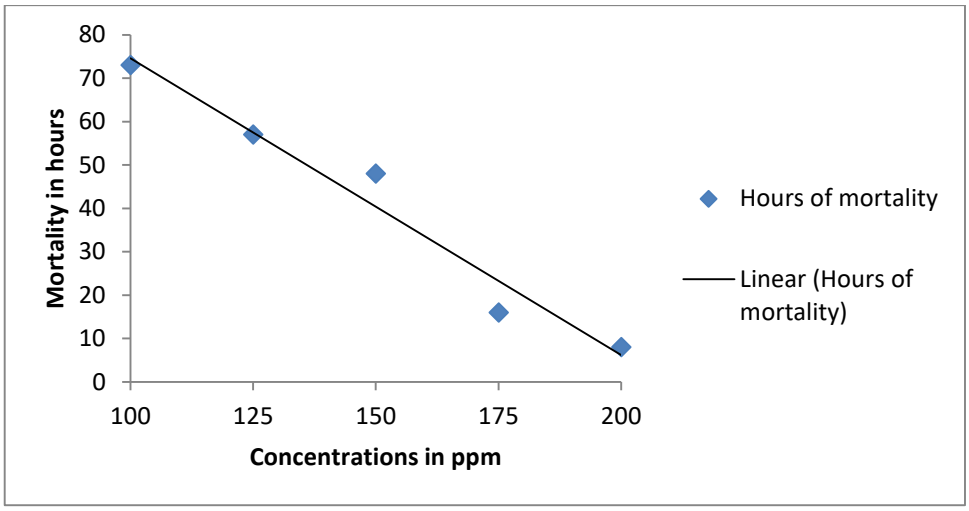

Figure 3: Chart showing the mortality of earthworms and the corresponding concentration

3.2. Dermal Toxicity Histology

Histology investigations show the chemical's effects on the organism's tissues. Thus, this paper studied the effects of Profenofos on earthworm tissues using histological methods. Clitellum and intestine were regarded crucial earthworm body components. Thus, transverse dissection was performed on these organs to assess damage. The findings demonstrated greater tissue damage as Profenofos levels raised.

3.2.1. Earthworm control histology:

The cuticle (C) and epidermis (E) of the clitellum and stomach area are visible in control earthworm tissues. The intestine's lumen (L) is not round, but the clitellum's lumen is. The circular and longitudinal muscles (CM and LM) are intact. The dorsal, ventral, and ventral nerve cords remain undamaged. Figure 4 displays the intestine's coelom and peritoneal epithelium. Extraperitoneal chloragogen layer (Cg) formed by main blood arteries. The control linking the longitudinal muscles and the ventral nerve cord (MS) (Fig 3).

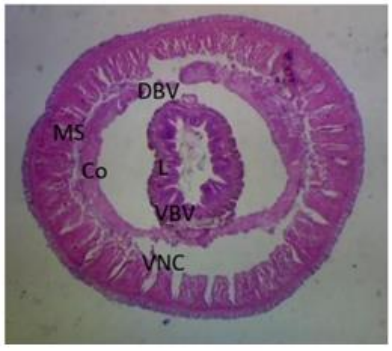

Figure 3: Transverse section of Clitellum region of *Eisenia fetida* in the control(4X)

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Figure 4: Transverse section showing the epithelium of *Eisenia fetida* in the control(10X)

Figure 5: Transverse section of Clitellum region of *Eisenia fetida* in the control(10X)

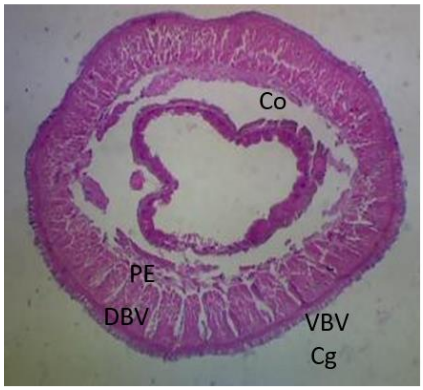

Figure 6: Transverse section of Gut region of *Eisenia fetida* in the control(4X)

***DBV – Dorsal Blood Vessel, VBV – Ventral Blood Vessel, VNC –Ventral Nerve Cord, Cg – Chloragogen cells, L – Lumen, Co – Coelom, E – Epithelium with secreting cells, C – Cuticle, CM – Circular Muscles, LM – Longitudinal Muscle, MS- Muscle Strand, PE- Peritoneal Epithelium**

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3.2.2. Profenofos treated earthworm histology

The tissues of earthworms treated with Profenofos at 100 and 125ppm are mildly impacted. Figure 7 illustrates the mild damages at 0.0055g/cm2. The cuticle is usually distinct with a few breaks. Light epidermis damage Both the ventral nerve cord and the dorsal and ventral blood arteries are severely injured. The cuticle is unaffected, and the longitudinal muscle loosens with little harm (Fig. 7). Circular muscles show little damage.

Several points on the peritoneal epithelium indicate disconnect. The chloragogen cells have crumbled and vacuolated (VCg) (Fig 7). Broken muscle strands. Lumen is contracted in clitellum and widened in gut. Inflamed ventral nerve cord. Some places show Chloragogen cell detachment from peritoneum (Fig 7).

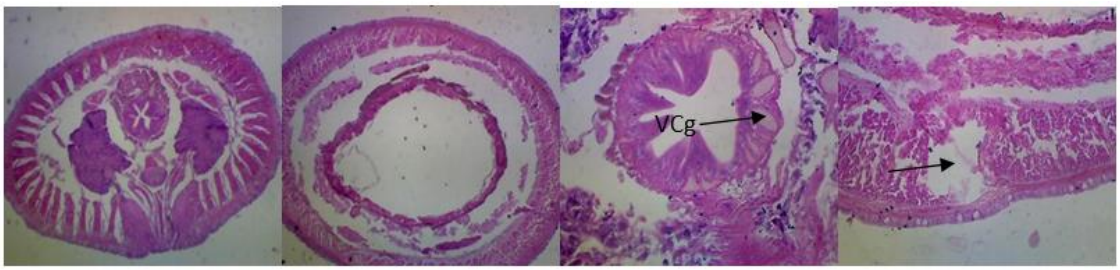

Figure7: Histology of Clitellum and Gut region of *Eisenia fetida* in 0.0055µg/cm^{2}concentration (4X and 10X); Arrow shows the damaged longitudinal muscles; VCg – Vacuolated Chloragogen cells

The cuticle of the earthworm stomach area and the epidermis underneath it are visible in 150, 175 and 200ppm treated earthworms. The epidermis has entirely disintegrated in 200ppm places. Some circular muscles show some damage. However, longitudinal muscles show greater degeneration and cracks. A considerable portion of the peritoneum is affected. The gut epithelial lining and lumen are also affected. The dorsal and ventral blood vessels are more severely injured. The ventral nerve cord is destroyed. In summary, increased acute Profenofos concentrations affect the histopathology of E. fetida. Some places show Chloragogen cell detachment from clitellum (Fig. 8). The clitellum coelom cavity is enlarged. Compared to the control, the stomach shape changes (refer Fig. 6 and 8).

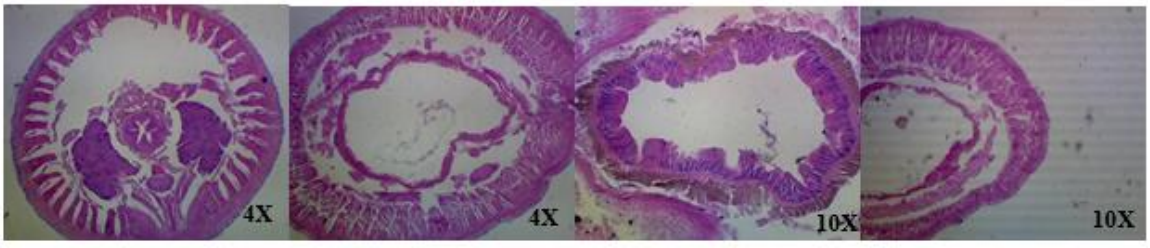

Figure 8: Histology of Clitellum and Gut region of *Eisenia fetida* in 0.007µg/cm^{2}concentration (4X and 10X)

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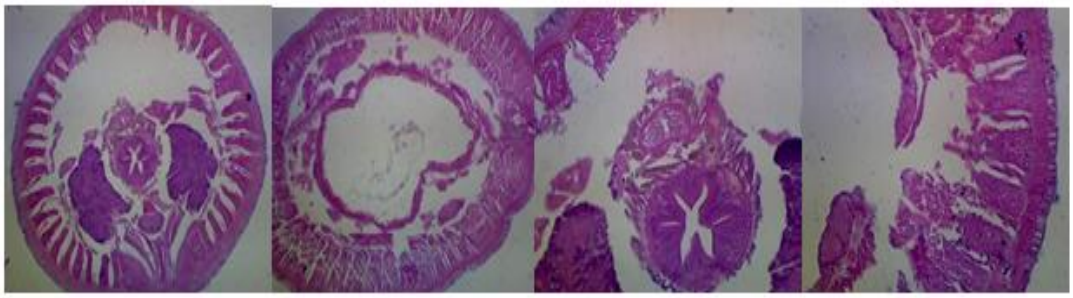

Figure 9: Histology of Clitellum and Gut region of *Eisenia fetida* in 0.0088µg/cm^2 concentration (4X and 10X)

When the clitellum is concerned, the peritoneal epithelium surrounding it is found necrosis. The coelom cavity is found increased and the other parts like dorsal and ventral blood vessels, ventral nerve cords are completely damaged (Figs.10 and 11). In the 0.0106µg/cm^2 concentration, the histology slide showed a drastic damage to the gut and other parts surrounding it, in particular, the longitudinal muscles are seen broken, the dorsal and ventral blood vessels are completely damaged, the peritoneal epithelium is broken in several places, the coelom cavity increases in size, vacuoles are formed and even the epidermal layer is damaged (Fig. 10 and 7). Profenofos accumulate in the chloragogen cells, which eventually separate from the intestinal wall and float freely in the coelomic fluid (Fig. 10).

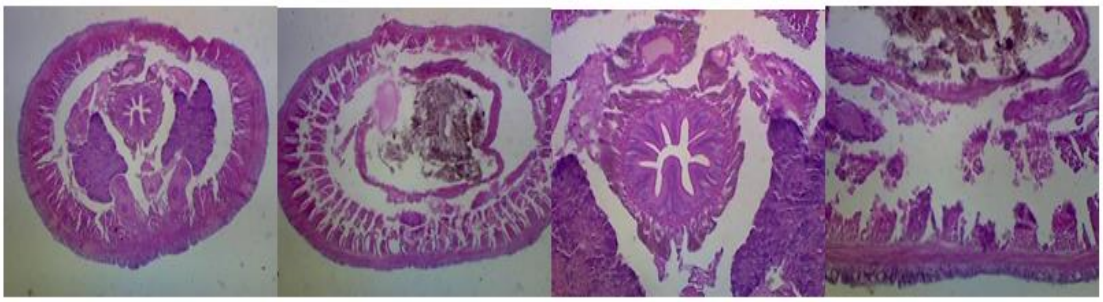

Figure 10: Histology of Clitellum and Gut region of *Eisenia fetida* in 0.0106µg/cm^{2}concentration (4X and 10X)

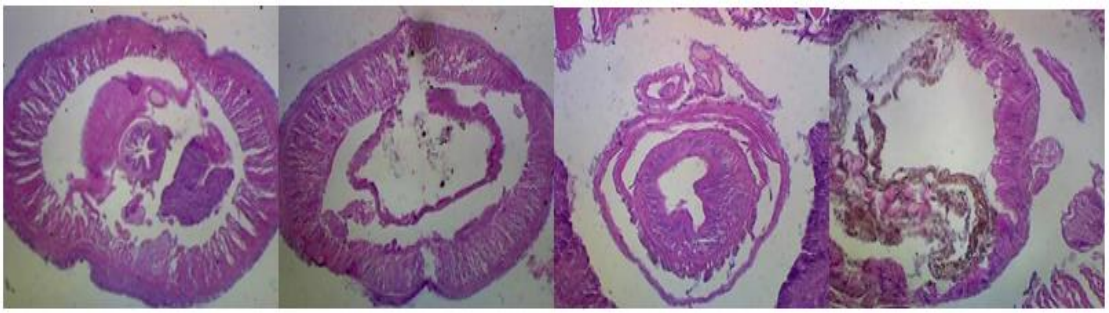

Figure 11: Histology of Clitellum and Gut region of *Eisenia fetida* in 0.115µg/cm^2 concentration (4X and 10X)

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The worm was only subjected for a few hours (i.e. 4 hours) to examine the tissue damage in greater concentrations. Because tissue inspection will be clearer and specimen preparation will be simpler.

Also, post-mortem tissue was severely injured, making it unsuitable for specimen processing and examination. Because mortality occurs in 8 hours, the material was exposed for 4 hours.

The increasing concentration wreaked havoc. The clitellum's visceral peritoneum and longitudinal muscle strands are both severely damaged (Fig.11). The gap between the longitudinal muscles was expanded. In Fig. 11, the epidermal layer is severed and the peritoneal epithelium lining the gut is injured. A 4-hour paralysis of the worm's movement. The posterior region has several lesions and cuts in the body wall.

4. DISCUSSION

To quantify pesticide risk to soil, laboratory toxicity data from single species testing (e.g., earthworm tests) must be estimated. The most widely used insecticide in India. Karanjkar and Nair (2010) explored Profenofos toxicity but not histological effects.

Their goal was to compare Profenofos cutaneous toxicity to soil acute toxicity. They showed that misuse of profenofos in agricultural areas may harm non-target soil species, particularly earthworms, which are important for soil fertility.

According to a literature review, only a few writers have studied the effects of chemicals on animal histology (Filipek-Mazur et al. 2000). Miyazaki et al. (2002) tested the toxicity of Chlorophenol on earthworm Eisenia fetida and compared it to freshwater species. But the tissue damage was ignored. So, this research looked at Profenofos' influence on earthworm histology. Experiments in this regard were done, and the shift in animal cell structure was described using cross sections of earthworms.

5. CONCLUSION

Chemical pollutants in agricultural fields may harm soil creatures from bacteria to higher trophic level vertebrates like humans. Earthworms are among the most immediately harmed. Earthworms are soil bioindicators. However, risk assessments of such compounds on tropical earthworms are uncommon.

Thus, assessing the impacts of agrochemicals, including pesticides, on soil ecosystems has become more important. Thus, this study was intended to investigate Profenofos' toxicity to earthworms. Profenofos is “Super toxic” to earthworms and causes tissue damage even at low dosages. These findings may be beneficial in assessing the ecological risk of agricultural operations such as overuse of Profenofos and preventing environmental harm.

Conflicts of interest - There are no conflicts of interest to declare

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