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RESEARCH ARTICLE

Eudrilus eugeniae - A Potent Bioremediator in Controlling Herbicide and Pesticide Pollution

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ABSTRACT

Eudrilus eugeniae were collected from vermibiotechnology laboratory and cultivated inside the campus of Manonmaniam Sundaranar University, Tirunelveli. They were acclimatized to the laboratory conditions at 28±1^{0}C for one month. The objective of this study was to evaluate *Eudrilus eugeniae* species as biological indicators of soil pollution with herbicide and pesticide. In addition to this, to identify the monocrotophos (pesticide) and glyphosate (herbicide) stress induced protein from an earthworm by using SDS-PAGE. From this study, the biomass of the earthworm was found to decrease on using monocrotophos. About 20±3.60 young ones and 20±2.08 cocoons produced by earthworms were analysed on control compared to glyphosate and monocrotophos exposed pot. Determination of preference of most earthworms was observed to be of neutral to slightly acidic when incorporated into glyphosate, monocrotophos soil. Among the herbicides and pesticides, the concentration of 0.44±0.02 mg/g of glyphosate accumulated in the different regions of earthworms which were analysed during the study. As agricultural land contains herbicides and pesticides contamination though not applied from exterior, higher concentration of herbicide of about 3.0±1.52 mg/g was determined in the tail of the earthworm. The maximum significant study of this experiment was observed in an extra band of protein in the head region of the worm treated with herbicide by using SDS-PAGE electrophoresis.

Key words: *Eudrilus eugeniae*, Glyphosate, Monocrotophos, Stress protein, Polyacrylamide gel, SDS-PAGE analysis.

INTRODUCTION

Earthworm is the common name for the largest members of Oligochaeta in the phylum Annelida. The basic body plan of an earthworm is a tube, the digestive system, within a tube, the muscular slimy and moist outer body. Most earthworms are decomposers feeding on undecayed leaf and other plant matter, others are more geophagous. *Eudrilus eugeniae* is a species of earthworm native to tropical West Africa and now widespread in warm regions both wild and under vermiculture, also called the African night crawler. Fecundity, growth, maturation and biomass production were all significantly greater at 25^{0}C than 15^{0}C, 20^{0}C or 30^{0}C. Its life cycle, *Eudrilus eugeniae* grew much more rapidly than *Eisenia fetida*, in similar environmental conditions [1].

The biomarker of an earthworm is defined as a "biochemical, cellular, physiological or behavioral variations that can be measured in tissue or body fluid samples, or at the level of whole organisms to provide evidence of exposure and/or effects from one or more contaminants" [2]. Among soil invertebrates earthworms are relevant organisms for soil formation and organic matter breakdown in most terrestrial environments.

They contribute to pedogenesis and soil profile, affect the physical, chemical and microbiological properties of soil and contribute to improve soil fertility [3]. In particular earthworms may increase mineralization and humification of organic matter by food consumption, respiration and gut passage [4]. They may indirectly stimulate

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microbial mass and activity as well as the mobilization of nutrients by increasing the surface area of organic particles and by their casting activity [5]. The role of earthworms in the decomposition of organic matter and subsequent cycling of nutrients has raised the interest of their use as indicator organisms for the biological impact of soil pollutants. This in turn has led to a large body of work on earthworm eco-toxicology [6].

Earthworms are able to accumulate various organic and inorganic contaminants present in the soil [7]. They accumulate certain metals such as cadmium, copper, zinc and lead from soils efficiently and tolerate high tissue metal concentrations using a variety of sequestration mechanisms [8] [9]. Earthworms appear to have well-developed trafficking and storage pathways for heavy metals, particularly essential trace metals such as copper and zinc [10]. The posterior alimentary canal of the earthworm of the body includes the intestine and the related chloragogenous tissue that separates the absorptive epithelia from the coelomic cavity [11]. The chloragogenous tissue is composed of pedunculated cells and its main functions are synthesis of hemoglobin, homeostasis of cation composition in the blood and coelomic fluid, maintenance of a balanced pH level, storage of nutrients and waste, and uptake and detoxification of toxic cations [12]. Therefore, chloragogenous tissue represents a major metal sink [[13]].

Overall, the use of biomarkers in earthworm is becoming increasingly important for the evaluation of effects of contaminants on soil organisms. Acetyl - cholinesterase, metallothionein, biotransformation enzymes and antioxidant defenses are among the most used biomarkers due to their crucial role in the neurocholinergic transmission and in cell homeostasis preventing toxic action of chemicals [14].

Bioaccumulation of an earthworm refers to the accumulation of substances such as pesticides or other organic chemicals in an organism. Bioaccumulation occurs when an organism absorbs a toxic substance at a rate greater than that at which the substance is lost. Thus, the longer the biological half-life of the substance the greater the risk of chronic poisoning, even if environmental levels of the toxin are not very high [15]. Biotransformation can strongly modify bioaccumulation of chemicals in an organism.

The present study investigates on the accumulation of toxic substances in different parts of the earthworm body. Our results expels that monocrotophos is found to be more toxic than the glyphosate in the body of the earthworm and bioaccumulation is at higher level in the part of below clitellum than the head region of the earthworm.

MATERIALS AND METHODS

Collection and maintenance of test animal:
Eudrilus eugeniae is a species of earthworm native to tropical West Africa and now widespread in worm regions, both wild and under vermiculture, also called the Africa night crawler [16].

For the present study, healthy individuals of *Eudrilus eugeniae* were used as the test animal and they were collected from Vermi Biotechnology laboratory of Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu by hand sorting method. These were grown with moist soil along with decayed leaves and the earthworms were acclimatized to the laboratory conditions for about 30 days at $28^0C \pm 1^0C$ [17, 18].

Preparation of stock solution:
The stock solution of pesticide (monocrotophos) and herbicide (glyphosate) was prepared by dissolving 0.005 ml and 0.025 ml of pesticide and herbicide in 1ml of sterile distilled water. This stock solution was used for further analysis of the earthworm study.

Determination of growth conditions of the earthworm:
For the present study, uniform weight of the earthworms were taken and they were grown separately in plastic containers containing equal amount of moist soil and one ml of the different varying concentrations of 0.05% pesticide solution and 0.1% of herbicide solution were added [19] [20]. A control was maintained without pesticide and herbicide addition. The biomasses of the earthworms (g/worm) were determined [21-23].

Determination of weight changes and reproduction conditions of the earthworm:
To assess the effect of the herbicide and pesticide of varying concentrations on the growth of an earthworm, the changes in the weight of the earthworms taken in different containers were determined. The reproductive capacity of the herbicide and pesticide treated animal was also

observed. Then earthworms were removed from the artificial soil and stored for 3 hours in Petri dishes on damp filter paper to avoid gut contents [24, 25]. The growth rate and the pH of the herbicide and pesticide treated soil were also determined.

Determination of toxicity tolerance test of an earthworm:

Tests were conducted to determine the toxicity of the selected pesticides and herbicides on mature earthworms were conducted by using the procedures described by [26] as modified by [27]. Different concentrations of each tested pesticide and herbicide were prepared in 50 ml of sterile distilled water in a separate container and then mixed well. Then prewashed and ventilated mature earthworms were introduced into each container. These were placed in incubation at relative humidity [28].

Spectrophotometric assay of herbicide and pesticide residues of an earthworm:

Earthworms were taken and cut into small pieces separately then crushed well in a sterile mortar and pestle. To the crushed sample, 100 ml of alcohol was added, stirred well and then filtered. After filtration, the collected filtrate was taken in a separate china dish and the alcohol was subjected to vaporization in a water bath and the residue obtained was dried in the oven. A small piece of sodium was taken in a fusion tube, till it melts. One of the above residues was added to the fusion tube and heated till red hot. The hot fusion tube in a china dish containing 10 ml of sterile distilled water was placed. The tube was broken and the contents of the china dish were boiled for about 5 minutes, cooled and filtered. To the collected filtrate, 1ml of freshly prepared ferrous sulphate solution was added and the contents were warmed. Then 2 to 3 drops of ferric chloride solution was added and acidified with dilute hydrochloric acid. The presence of pesticide and herbicide residues of the sample, coloured precipitate was obtained and absorbance was measured at the intensity of the colour was read at 620 nm in UV/Vis spectrophotometer.

Calculation:

The amount of herbicide and pesticide was estimated using the following formula:

Amount of herbicide and pesticide present in the sample =

$$\frac{\text{OD of the sample} \times \text{Concentration of standard}}{\text{OD of the standard} \times \text{Volume of the sample}}$$

Protein extraction by SDS-PAGE analysis:

Crushed earthworm crude sample was centrifuged at a maximum speed for about 10 minutes and the supernatant was taken. The protein content of the supernatant was confirmed by running a gel electrophoresis of 0.85% agarose gel. After completion of the electrophoresis observance of blue coloured bands will be visualized through our naked eyes.

RESULTS AND DISCUSSION

Cultivation of the earthworm:

Eudrilus eugeniae is a species of an earthworm native to tropical West Africa and now wide spread in warm regions, both wild and under vermiculture. For the present study, healthy individuals of *Eudrilus eugeniae* were collected and cultured with moist soil along with decayed leaves, monocrotophos and glyphosate. The test animals were taken care to reduce physical injuries and were acclimatized to the laboratory conditions (**Figure 1 & 2**).

Determination of growth conditions of the earthworm;

Eudrilus eugeniae were collected and maintained in earthen pots. The effect of pesticide and herbicide were evaluated in this experiment. The worm cultured without the exposure to the herbicide and pesticide were used as a control.

Previous studies have shown that, earthworms are the natural fertilizer factories which serve as bio-catalytic agents to enhance the soil fertility through physical, chemical and biological processes. Earthworms are one of the most important organisms among soil invertebrates owing to their beneficial effects on soil environment such as modification of soil physical properties and impact on decomposition of soil organic matter.

Monocrotophos in 0.05% and glyphosate in 0.1% dilution was used in the culture medium. Biomass of monocrotophos exposed earthworm was 0.475 g/worm, the glyphosate exposed earthworm was 0.646 g/worm and the control (without monocrotophos and glyphosate) worm was 0.667 g/worm. The biomass of the earthworm decreases due to the prolonged exposure to monocrotophos as compared to control and glyphosate (**Table 1**).

Determination of weight changes and reproduction of the earthworm:

In this study, the pesticide has produced prominent changes in the growth and reproduction of the earthworms exposed to the monocrotophos and glyphosate. The growth and reproduction rate of the earthworms decreases because of prolonged exposure to monocrotophos as compared to the control. About 20 young ones and 20 cocoons were produced in the control and glyphosate exposure pot compared to 5 young ones and 5 cocoons in the monocrotophos exposure pot (**Figure 3**). In the previous report, the weight of the earthworms was a more sensitive index compared to the mortality in indicating toxic effects [29]. Toxic effects affected the growth and reproduction of the earthworm also the biomass was different in between the earthworms.

From this study, the pH variation also affected the growth of the species. The slightly acidic pH only favours the growth of the earthworm. The pH of the control pot was 7.9. The pH of the herbicide used medium was 7 and the pesticide used medium was 7.8. The change of the pH affected the growth of an earthworm. But most earthworms favors neutral to slightly acidic soil for their growth (**Figure 4**).

Determination of toxicity tolerance test of the earthworm;

The toxicity tolerance tests were conducted to determine the toxicity of the selected pesticides to mature earthworms. At low concentrations of 0.1 mg/g and 0.2 mg/g of monocrotophos the earthworm *Eudrilus eugeniae* (**Table 2**) tolerated whereas it indicated a mortality in 30 minutes on exposure to 5 mg/g. The test earthworms tolerated to glyphosate at the concentrations of 1 mg/g and 2 mg/g which showed mortality at 5 mg/g in 12 hrs of exposure. The monocrotophos was found to be more effective than the glyphosate as the earthworms tolerated the less amount of pesticide (**Table 3**).

From the tolerance tests, agricultural land polluted with glyphosate and monocrotophos at a concentration of below 2 mg/g soil can be remediated using earthworm as the earthworm has a good capability to accumulate more amounts of glyphosate and monocrotophos in the tail region.

Spectrophotometric assay of herbicide and pesticide residues of the earthworm:

The earthworm has a high accumulation capacity. *Eudrilus eugeniae* accumulated the monocrotophos and glyphosate in different regions. Bioaccumulation occurs when an organism absorbs a toxic substance at a greater rate.

Generally agricultural lands contain herbicide and pesticide to their contamination. In the control sample (without herbicide and pesticide) bed contains 0.23 mg/g of herbicide/pesticide. The herbicide and pesticide inoculated bed contains the concentration of 0.44 mg/g and 1.12 mg/g respectively (**Table 4**).

According to previous report, several earthworm biomarkers have been developed [30] and applied [31]. Earthworms play a major role in the functioning of the soil ecosystem by participating in organic matter cycles and modifying soil structure.

Trace amount of chemical substances accumulated in the control worm. Herbicide accumulated in the different parts of the earthworm. In this study, the concentration of herbicide in the head region was 0.3 mg/g, in clitellum 1.0 mg/g and the tail region was 3.0 mg/g respectively (**Table 5**). The earthworm present a high bioaccumulation capacity of several metals such as cadmium, zinc and copper [32] [33] [34]. Bioaccumulation is often used as a general term to describe the process whereby a substance is taken up by living organisms from their environment and diet and stored in the body. The tail part of an earthworm can accumulate the high amount of herbicide and pesticide than the other region.

The concentration of pesticide monocrotophos was tested. Pesticide is highly toxic than the herbicide. In this testing, high amount of toxic substance was accumulated in the earthworm than the glyphosate. The concentration of pesticide in the head part was 0.7 mg/g, clitellum was 0.8 mg/g and the tail part contains 1.0 mg/g respectively (**Table 6**).

The accumulation of glyphosate was also tested in the earthworm with the same procedure of monocrotophos. The amount of glyphosate accumulated in the head region of the earthworm was about 1.0 mg/g, the clitellum region was 4.2 mg/g and the tail region was 8.1 mg/g respectively (**Table 7**).

From this study, glyphosate has been used as a test herbicide and the earthworm as a model. The earthworm have been growing, facilitating an easy dosage of glyphosate. The main objective has been to detect a biomarker in earthworm exposed to sublethal concentration of glyphosate. Glyphosate partly due to its wide spread use worldwide [35] because the mode of action for glyphosate is well described. In this analysis, the concentration of glyphosate was determined in the different parts of the earthworm. The concentration of glyphosate in the head region

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was 1.0 mg/g, in clitellum 4.2 mg/g and the tail of 8.1 mg/g respectively.

In the current study, the monocrotophos is an organophosphate insecticide. It is acutely toxic to animals. The total amount of accumulated monocrotophos was estimated in different parts of an earthworm. The concentration of monochrotophos of head region was 1.2 mg/g, clitellum region was 3.9 mg/g and the tail region was 7.5 mg/g (**Table 8**). Monochrotophos is a widely used and extremely dangerous insecticide. Its low cost and many applications will present a challenge to users looking for safe alternatives or which will protect health. The monochrotophos was accumulated in tiny amounts in the head regions of the earthworm.

Stress protein analysis by using SDS-PAGE electrophoresis:

According to the present report, the expression of stress protein in these species represents an early as biomarker of soil contamination. The earthworms are very important organisms for soil formation and organic matter breakdown in most terrestrial environments and traditionally they have been considered to be convenient indicators of land use impact and soil fertility. The SDS-PAGE analysis of the head regions samples of the control worm, herbicide treated worms and pesticide treated worms produced a extra band of protein in the head region of the worm treated with the herbicide (**Figure 5**). From the findings reported, the heat shock proteins, particularly HSP 60 and HSP70 have been shown to play a key role in the maintenance of normal cellular functions help in the recovery and survival environmental stressors including heat shock.

From the above reports, we conclude that the earthworm can accumulate the pesticide and herbicide from the soil. So that it can be used in agricultural fields to remediate pesticide and herbicide contamination. The earthworm can be used as a bioindicator and a bioremediator for polluted soil.

Table 1: Biomass and Reproduction of the Earthworm *Eudrilus Eugeniae*

Sample	Concentration	Biomass (g/worm)	Number of young ones	Number of cocoons
Control	-	0.667±0.029	20±3.60	20±2.08
Monocrotophos	0.05%	0.475±0.026	5±1.52	5±0.57
Glyphosate	0.1%	0.646±0.036	20±2.51	20±1.52

Table 2: Tolerance Test For Monocrotophos In Earthworm *Eudrilus Eugeniae*

S. No	Concentration of pesticide (mg/g)	Time until mortality (hrs)
1	0.1	-
2	0.2	-
3	0.3	48
4	0.4	36
5	0.5	24
6	1.0	8
7	2.0	6
8	3.0	4
9	4.0	1
10	5.0	0.5

Table 3: Tolerance Test For Glyphosate In Earthworm *Eudrilus eugeniae*

S. No	Concentration of herbicide (mg/g)	Time until mortality
1	1.0	-
2	2.0	-
3	3.0	2 days
4	4.0	1 day
5	5.0	12 hours

Table 4: Concentration Of Glyphosate And Monocrotophos In Soil

S. No	Sample	Concentration (mg/g)
1	Control	0.23±0.03
2	Glyphosate	0.44±0.02
3	Monocrotophos	1.12±0.02

Table 5: Concentration Of Herbicide In Control Worm Of *Eudrilus Eugeniae*

S. No	Sample	Concentration (mg/g)
1	Head	0.3±0.25
2	Clitellum	1.0±1
3	Tail	3.0±1.52

Table 6: Concentration Of Pesticide In Control Worm Of *Eudrilus Eugeniae*

S. No	Sample	Concentration (mg/g)
1	Head	0.7±0.2
2	Clitellum	0.8±0.20
3	Tail	1.0±4.04

Table 7: Concentration Of Glyphosate In Different Regions Of Earthworm *Eudrilus Eugeniae*

S. No	Sample	Concentration (mg/g)
1	Head	1.0±3.05
2	Clitellum	4.2±0.32
3	Tail	8.1±0.4

Table 8: Concentration Of Pesticide Monocrotophos In Different Regions Of Earthworm *Eudrilus Eugeniae*

S. No	Sample	Concentration (mg/g)
1	Head	12±0.35
2	Clitellum	3.9±0.41
3	Tail	7.5±0.20

Figure 1: *Eudrilus eugeniae* – Test animal

Figure 2: Earthworm culture in cement tank

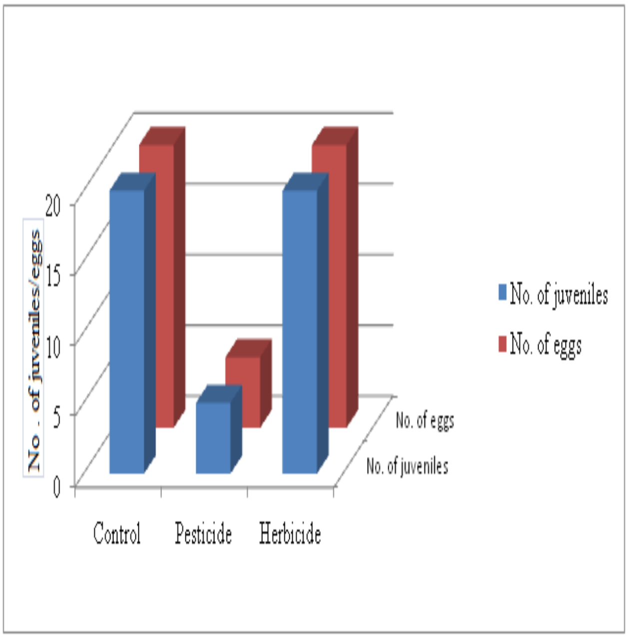

Figure 3: Fecundity status of *Eudrilus eugeniae*

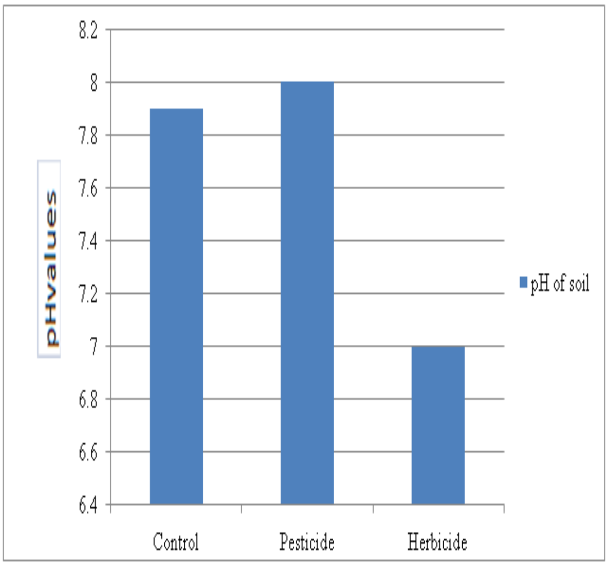

Figure 4: pH of the soil treated with pesticide and herbicide

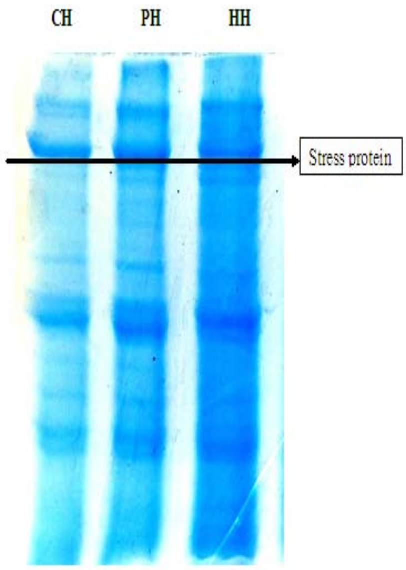

Figure 5: Stress protein from head regions of *Eudrilus eugeniae*

CH – Head part of control worm
PH - Head part of pesticide treated worm
HH – Head part of herbicide treated worm

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