



Effect of Lindane on *Eudrilus Eugeniae*

KEYWORDS

Lindane, survival, morphology and *Eudrilus eugeniae*

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ABSTRACT Survival, locomotion and body morphology of *Eudrilus eugeniae* exposed to the soil, spiked with lindane @ of 15 mg/kg (at different time interval of 24 hr, 48 hr and 72hr) were measured and compared with that of the control (0 hr). At higher doses of lindane (25-35 mg/kg) the earthworm showed 100 % mortality. LD50 was estimated as 20 mg of lindane per kg of soil. *Eudrilus eugeniae* showed progressive signs and symptoms of toxicity of lindane such as on body weight, coiling behavior, curling and excessive mucus secretion with sluggish movements and swelling of clitellum, numerous protrusions and degenerative changes. On the basis of the present study it was concluded that lindane is highly toxic to *Eudrilus eugeniae* juveniles and adults.

INTRODUCTION

Persistent polluting chemicals are substances which have long life mainly due to their chemical stability. DDT, dieldrin, lindane, endosulfan are well known examples of chemicals which can be found in various compartments of the aquatic environment as well as in living organisms (Bro-Ramussen, 1996). Among different such insecticides lindane is an organochloride insecticide which has been used on a wide variety of crops as well as in public health to control insect borne diseases (with fungicides) and as seed treatment (Ortiz et al., 2001).

MATERIALS AND METHODS

Earthworm

Eudrilus eugeniae were purchased from Soil Chemist Office, Takatpur, Baripada, Mayurbhanj and kept in plastic tray (30cm x 25cm x 6.5cm) in the laboratory. Each tray contains soil covered with net and moist gunny cloth (maintain temperature and darkness). *Eudrilus* were acclimatized for seven days in the laboratory condition prior to the experiment.

Growth parameters

The biomass (dry weight) of the worms were weighed in an electronic balance and thereafter kept inside a hot air oven at 60° C temperature for different time intervals i.e., 0 hr, 5 hr and 10 hr. After oven dry the final weight of the worms was recorded. In this way a comparison of biomass at different time intervals was noted.

From the above culture (stock), few, healthy, mature adult earthworms (weighing an average of about 0.095g) having well developed clitellum were taken and placed in different plastic trays (30cm x 25cm x 6.5cm), labelled C for control, E for experimental, L for Lindane, filled with 1kg of soil covered with net and moist gunny cloth (maintain temperature and darkness).

Effect of lindane on the body weight at different time intervals and mean growth rate (mg/w/d) of *Eudrilus eugeniae* were determined before they were introduced into the experimental trays and thereafter for a time period of 720 hour (30 days). The worms were weighed in an electronic balance and the worm's growth rate (mg/w/d) for specific

periods was calculated using the formula:

$$\frac{W_2 - W_1}{t_1 - t_2}$$

Where W_1 and W_2 are the body weight at the beginning and end, respectively and t_1 and t_2 are the age of the worms at the beginning and end of the specific period of study.

Toxicity of lindane on the morphology of the earthworm *Eudrilus eugeniae*

Ten numbers of earthworms were transferred to C_L as control where as ten number of earthworms were transferred to other three trays (E_{L1} , E_{L2} , E_{L3}) as experimental. The soil in E_{L1} , E_{L2} , E_{L3} was spiked with lindane (15, 20 mg kg⁻¹). The test containers were covered with perforated plastic film to prevent the test organisms from escaping the test containers to retain the moisture content in the medium. The control medium was the same quantity of water without any additive agent. Testing was done in continuous light at 22 ± 2° C. The coiling behavior, morphological abnormalities and the percentage mortalities were recorded and photographed after 24 hr, 48 hr, 72 hr, 96 hr and after a month of exposure to lindane.

Physico-chemical tests of soil sample and used for soil bioassay

The soil sample was tested by using KO54 Soil Testing Kit of Himedia Laboratories Pvt. Limited.

Statistical analysis

One-way ANOVA and Post Hoc analysis was carried out to find out the level of significance between *Eudrilus eugeniae* treated with lindane over a period of 24 hr, 48 hr, 72 hr and in control. A difference was taken as significant when P was less than 0.05. Statistics is done with the help of software SPSS package 16.0.

RESULTS

Soil test result of soil sample used for soil bioassay

On the basis of the soil test results, the pH of the soil sample was 7.0 i.e., neutral (Table-1). Percentage (%) of

Oxidizable Organic Carbon (OC) was in between the range 0.407-0.500 i.e., low in soil . Amount of available Phosphate in soil Kg. per hectare as (P₂O₅) was nil i.e., absent in the soil sample. Quantity of available Potassium in soil Kg. per hectare was low, below 112 Kg/ha as (K₂O) because all the three lines are visible (Table-2). The quantity of Ammoniacal Nitrogen and Nitrate Nitrogen of the soil will change very quickly. If the Organic Carbon was low then the supply of nitrogen to the soil was also low. The value of Ammoniacal Nitrogen was low about 15 Kg/ha as (N) (Table-2) and that of Nitrate Nitrogen was high about 50 Kg/ha as (N) in soil (Table-2). The water holding capacity of the soil was about 75%.

During the soil sample analysis there was an abrupt increase in the mortality rate of *Eudrilus eugeniae* with increasing doses of lindane from 5 mg to 25 mg/kg of soil. At higher doses of lindane (25-35 mg/kg) the earthworm showed 100 % mortality. In untreated (controls) there was no mortality after 12 days of exposure to lindane. LD₅₀ was estimated as 20 mg of lindane /kg of soil to *Eudrilus eugeniae*.

Table-1: Physico-chemical tests of soil sample

Serial No.	Parameter	Before addition of lindane	After addition of lindane
1	pH	7.0 (Neutral)	
2	Organic Carbon (%)	0.407-0.500 (Low)	
3	Available Phosphate (P ₂ O ₅) Kg ha ⁻¹	Blank (Nil)	
4	Available Potassium (K ₂ O) Kg ha ⁻¹	Below 112 (Low)	
5	Ammoniacal Nitrogen Kg ha ⁻¹	About 15 (Low)	
6	Nitrate Nitrogen Kg ha ⁻¹	About 50 (High)	
7	Water Holding Capacity (%)	75	

Comparison of biomass at different time intervals

Biomass (g) of *Eudrilus eugeniae* at 60° C temperature were 0.791667 ± 0.182612 g at 0 hour (control), 0.225 ± 0.083217 g after 5 hour and 0.191667 ± 0.045977g after 10 hour (Fig. 2). In other words, biomass of *Eudrilus eugeniae* was highest at 0 hour (control) and gradually the biomass decreases after 5 hour and 10 hour Maximum decrease in biomass was noticed at 10 hour.

One way ANOVA revealed that the biomass is significant [F (2, 17) = 40.239, (P=0.00)] It was found that, the biomass in *E. eugeniae* in control and in those after 5 hours and 10 hours showed significant differences. Post Hoc analysis revealed that the biomass at 5 hr and 10 hr is significant (P≤0.05; LSD) with respect to control.

Toxic effect of lindane on body weight at different time intervals

Body weight (g) of *Eudrilus eugeniae* exposed to lindane (15 mg/kg soil) were 0.945714 ± 0.142112 g at 0 hour (control), 0.941429 ± 0.141566 g at 24 hour, 0.787143 ± 0.114107 g at 48 hour, 0.641429 ± 0.10162 g at 72 hour and 0.374286 ± 0.143214 g after 30 days (Fig. 3). In other words, body weight of

Eudrilus eugeniae was highest at 0 hour (unexposed) and gradually the body weight decreases during 1 day, 2 day, 3 day and after 30 days. Maximum decrease in weight was noticed at 30 days of lindane exposure.

One way ANOVA was performed in order to compare the body weight in *Eudrilus eugeniae* treated with lindane (15 mg/kg soil) at different time intervals. One way ANOVA revealed that the body weight in *Eudrilus eugeniae* is significant [F (4, 34) = 20.384, (P=0.00)]. Post Hoc analysis revealed that when treated with lindane, a significant, (P<0.05; LSD) increase in the body weight was observed at 48, 72 and 720 hours. The mean difference was not significant at 24 hours with respect to control.

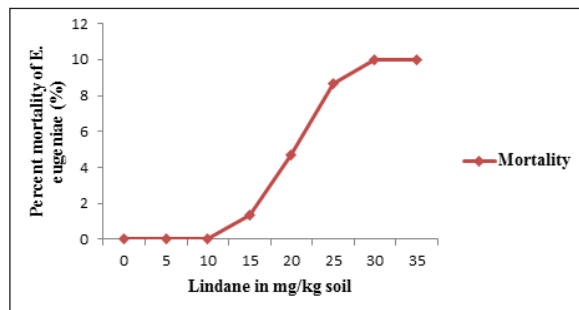


Fig. 1: Graph showing percent mortality of *Eudrilus eugeniae* in soil bioassay.

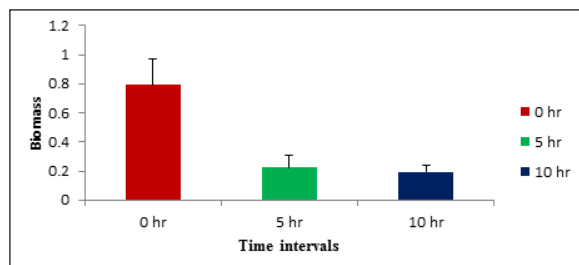


Fig. 2: Comparison of biomass at 60° C temperature in *Eudrilus eugeniae* at different time intervals.

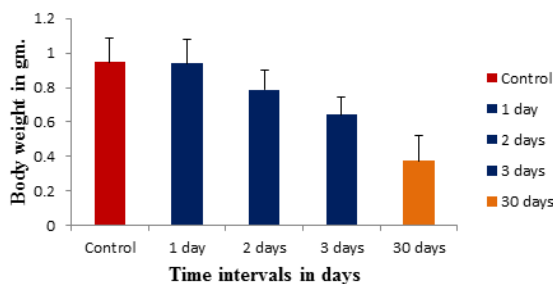


Fig. 3: Comparison of body weight in *Eudrilus eugeniae* treated with lindane (15 mg/kg soil) at different time intervals.

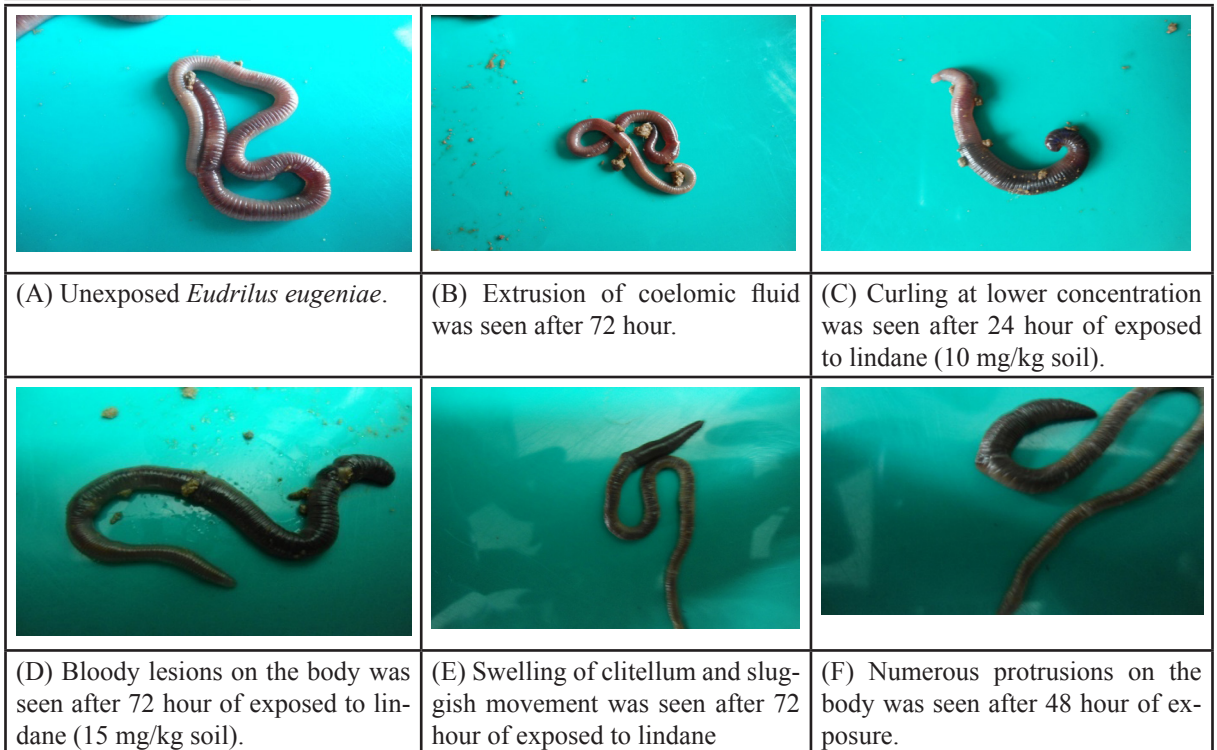


Fig. 4: (A-F) Morphological abnormalities in *Eudrilus eugeniae* after 24 hour of exposure to lindane (15 mg/kg soil).

Toxicity of lindane on the body morphology of *Eudrilus eugeniae*

The toxic effects of lindane against *Eudrilus eugeniae* was recorded at 0 hour, 24 hour, 48 hour and 72 hour. The present study reveals morphological symptoms when the length of exposure is increased from 72 hour to 96 hour.

Eudrilus eugeniae showed progressive signs and symptoms of toxicity of lindane such as coiling, curling and excessive mucus secretion with sluggish movements and swelling of clitellum at lower conc. of lindane (15 mg/kg soil) [Fig. 4 (A-F)]. While extrusion of coelomic fluid resulting in bloody lesions occurred at the higher conc. of lindane (20 mg/kg soil) [Fig. 4 (G)]. These symptoms were also recorded at dose of lindane (15 mg/kg soil) if the length of exposure was increased to 96 hour and more. Numerous protrusions was observed on the clitellated part of *Eudrilus eugeniae* exposed to lindane (15 mg/kg soil) [Fig. 4 (H, I)]. The earthworms exposed to lindane remained at the bottom of the test container (from day 2 onward), due to the bulging of the clitellar regions which might have restricted their free movement. Whereas the control earthworms exhibited an excellent borrowing movements in the middle of the test container and exhibited normal behavior.

The result of the present study indicates that lindane exerts toxic effects on *Eudrilus eugeniae*. Within 72 hour of exposure some type of degenerative changes appeared at the posterior end of the exposed earthworm [Fig. 4 (K, L)]. 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.

DISCUSSION

Numerous studies have been carried out using earthworms in recent years, but they mostly focus on single insecticide (Spurgeon et al., 2003). Single insecticide experiments fail to reflect field situations where multiple insecticides or in-

secticide mixtures are used (Zhou et al., 2011). A number of soil animals have been proposed as indicator organisms of soil pollution with both advantages and drawbacks (Zhu et al., 2008). However, earthworms have long been used as a key index of ecotoxicology diagnosis although they are becoming extinct in many agricultural soils (Xiao et al., 2004; Zhu et al., 2008). Chlorinated hydrocarbons are employed as part of the integrated farming practice to protect crops and animals from insects, weeds and diseases. The use of chlorinated hydrocarbons such as DDT, dieldrin and lindane as pesticides in agriculture are worldwide. The growth and reproduction of earthworm have also been important endpoints used in environmental ecotoxicity (Van Gestel et al., 1991, 1992; An and Lee, 2008). In addition, many other chemicals and insecticides have already been tested for their ability to induce avoidance on earthworms (De Silva and Van Gestel, 2009; Owojori and Reinecke, 2009; Santos et al., 2011). Furthermore, juvenile earthworms may be more sensitive to pollutants than adults (Zhou et al., 2008). Data from juvenile earthworms experiments should therefore also be considered when proposing a safe environment concentration for a specific insecticide (Booth and O' Halloran, 2001). Earthworms are affected by toxicants either through the skin contact or by feeding on contaminated litter in soil. Primarily, these toxicants passing through the skins reach the coelomic fluids and then transport throughout the bodies (Homa et al., 2007).

Biomass changes can be a good indicator of chemical stress, which may link chemical effects to energy dynamics and ultimately inhibit growth. A dose dependent decrease was observed in the growth of *Eisenia fetida* exposed to dieldrin at several sublethal concentrations. A significant growth inhibition on earthworm *Lumbricus rubellus* was also seen when exposed to PAH pyrene (Brown et al., 2004). The present investigation also showed similar result. Increase in treatment period decreased the biomass of *E. eugeniae*.

Growth inhibition of *Eisenia fetida* exposed to lindane and deltamethrin treated soil appeared to be positive and significantly higher than those of the controls. After 3, 7, 10, 14 days of exposure to lindane, the growth inhibition of earthworms treated with the higher dose (120 and 150 mg/kg) were significantly higher than those for treatments at low and medium concentrations (20, 50 and 80 mg/kg) (SNK test, $P < 0.05$). During the 14 day exposure period, the growth inhibition for all of the earthworms cultured in the deltamethrin-treated soil were positive and significantly different ($P < 0.001$) from those for the controls. The present investigation also showed similar result. Increase in treatment period decreased the body weight of *E. euginae*.

A similar kind of autolysis from the posterior region was observed in earthworms, *Polypheretima elongate*, exposed to textile dyes (Ramaswami and Subbram, 1992). The present investigation also showed similar result.

CONCLUSION

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