



Effect of chlorpyrifos on survival, growth and reproductive performance of *Eudrilus eugeniae* (Kinberg)

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Abstract: The present laboratory study was conducted to determine the effect of chlorpyrifos on growth and reproduction of the earthworm *Eudrilus eugeniae*. Chlorpyrifos was mixed with soil substrate at four different dose formulations i.e. D1 (2.5ml/1000ml); D2 (5.0ml/1000ml); D3 (7.5ml/1000ml); D4 (10.0ml/1000ml). Soil substrate without chlorpyrifos served as control. Growth and survival rates were determined till four weeks and effects on reproduction are assessed after eight weeks of exposure. A non significant decrease ($p > 0.05$) was observed in body weight of earthworms which was recorded on day 1, 2, 3, 7, 10 and 14. A dose dependent effect on cocoon production and survivability of hatchlings was observed in all treated groups. The effect of chlorpyrifos on life cycle of earthworm was more in dose D3 (54.25 days) when compared to control (28.75 days) while no worm survived in D4 dose. The present study revealed that chlorpyrifos at high dose levels (7.5ml/1000ml and 10.0ml/1000ml) affect the reproduction and growth of earthworms, primary bioindicators of soil fauna, whereas the base dose (5.0ml/1000ml) may be considered as safe for soil applications.

Keywords: Chlorpyrifos, *Eudrilus eugeniae*, Growth, Reproduction

INTRODUCTION

With increasing demand for food and fiber, agriculture has to depend on the use of pesticides and fertilizers for high yields which leads to environmental contamination varieties. The use of pesticides has increased with the increasing awareness of their utility in agriculture, animal husbandry, and post harvest technology and in public health worldwide (Muthuviveganandavel *et al.*, 2008). Chlorpyrifos is a broad spectrum organophosphate insecticide effective against a large number of agricultural and household pests. In India, it is widely recommended for seed treatment against white grub and termites and persists in the soil for 3-4 months (Kurawar, 2010). The wide spread use of pesticide protect the floral diversity against the particular pest but also affect the non target fauna (Muir *et al.*, 2004). Earthworms are common soil organisms in most environments and play an important role in improving structure and fertility of soil ecosystems (Georgescu and Werber, 2007; Bartlett *et al.*, 2010) by modifying soil organic matter both chemically and physically, facilitating the formation and stabilization of soil aggregates and improving soil porosity. Earthworms have sensitive receptors on their body surfaces with which they sense chemicals in the soil (Haque and Ebing, 1983) and thus are good bioindicators of soil providing safety thresholds for insecticide applications (Suthar *et al.*, 2008; Lourenco *et al.*, 2011). Earthworms constitute about 60-80% of total soil biomass

(Ouellet *et al.*, 2008; Jouquet *et al.*, 2010) and when exposed to pesticides and heavy metals may experience physiological disturbances such as a reduction in biomass, population size and reproduction as well as lysosome deficiencies (Ricketts *et al.*, 2004; Spurgeon *et al.*, 2004). *Eudrilus eugeniae* (African night crawler) is used extensively for composting in tropics, especially in Africa and India. This species is more productive in terms of rates of growth than other species of earthworm. Changes in growth and reproductive parameters of earthworms exposed to agro-pesticide loaded soils seem to be useful indicators of soil pollution. The present study was carried out to determine the effect of chlorpyrifos on survival, growth and reproduction of *Eudrilus eugeniae* (Kinberg).

MATERIALS AND METHODS

Stock: *E. eugeniae* was cultured in cement concrete rings containing farmyard manure (FYM) substrate in the Department of Zoology, PAU, Ludhiana. The FYM was collected from the livestock farm, located at GADVASU, Ludhiana. Earthworms in pre-clitellar and clitellar phase of life cycle were used during the study.

Experimental dose and collection of soil sample: Considering the dose recommended by PAU as base (5.0ml/1000ml) different doses viz D1 (2.5ml/1000ml), D2 (5.0ml/1000ml), D3 (7.5ml/1000ml) and D4 (10.0/1000ml) were prepared in water to access the toxicity of chlorpyrifos on

growth and reproduction. The soil sample collected from the experimental fields of PAU campus as control consisted of 80% soil and 20% farm yard manure and sterilized in an autoclave as per standard procedure.

Effect on growth and reproductive performance:

E. eugeniae were added to 500 mg of soil mixed with different doses of chlorpyrifos described above in plastic trays to study the effect on growth and survival. To avoid moisture loss the plastic trays were covered with perforated plastic lids/moist cloth. The experiment was carried out for 14 days and worm's survival/ activity was evaluated on days 1, 2, 3, 7, 10 and 14. Reproductive performance of adult *E. eugeniae* was estimated by placing one pair of earthworm in plastic tray containing 250-300 gm of the soil substrate sample treated with chlorpyrifos. Rate of reproduction was assessed on the basis of the number of cocoons produced per adult worm at each dose treatment following the method of Sahu and Senapati (1988).

Statistical analysis: The experiments were replicated in triplicate and the data was statistically analyzed by Analysis of Variance (ANOVA) using computer software CPCS1.

RESULTS

Body weight of worms exposed to chlorpyrifos at different days is shown in Table 1. A non significant decrease ($p>0.05$) in body weight was noted in control and treated groups. The effect of chlorpyrifos on cocoon production rate was dose dependent as indicated by the decrease in value (Table 2) in different doses: D1 (1.34±0.04); D2 (1.5±0.08); D3 (1.36±0.06); D4 (1.3±0). A similar trend was observed in hatchling production rate and hatchling per cocoon per week with the hatchlings emerging from cocoons in the

fourth week in control (Table 3). The dozed substrates had hatchlings only in the 5th week which is a delayed occurrence. Interestingly, no hatchlings were observed in the 7th week in doses D2, D3 and D4, while low hatchability was observed in D1 and control. The values of percentage survivability of hatchlings are the strong pointers that this effect is dose dependent and not species specific (Table 4). Maximum survivability was observed in D1 dose when compared with control where as no worm survived in D4 dose. A significant increase ($p<0.05$) in the body weight was observed in 8th week of experiment in control, D1 and D3 dose where as in D2 maximum weight gain was observed during 6th week of the experiment (Table 5). No worm survived in D4 beyond one week of exposure. Total time taken by *E. eugeniae*, from the emergence of hatchlings to reach adult stage with a fully developed clitellum was recorded to be 28.75±0.82; 43.25± 0.82; 45.75± 0.82; 54.25± 0.82 days in control, D1, D2 and D3 respectively. No worms survived in D4 dose application (Table 6).

DISCUSSION

As earthworms play an important role as engineers in many soils, they have become standard test organisms in ecotoxicology due to their easy handling and culturing. Reduction in body weight of worms indicates the physiological reaction of the organism to insecticide exposure and the changes in earthworm's body mass are thought to be of ecological relevance. It has been assumed by earlier workers that high losses in body mass may lead to negative effects on reproduction and survival (Capoweiz et al., 2005; Olvera-Velona et al., 2008) and is relevant in the observations recorded dur-

Table 1. Changes in body weight (g) of *E. eugeniae* in different doses of chlorpyrifos exposed on different days.

Doses	Days					
	1 st day	2 nd day	3 rd day	7 th day	10 th day	14 th day
D1	0.45±0.01	0.44±0.02	0.42±0.02	0.42±0.03*	0.40±0.03*	0.40±0.007*
D2	0.44±0.04	0.43±0.03	0.39±0.03	0.36±0.08*	0.33±0.08*	0.33±0.07*
D3	0.37±0.02	0.36±0.05	0.37±0.02	0.41±0.01*	0.44±0.005*	0.39±0.005*
D4	0.42±0.02	0.35±0.05	0.34±0.05	0.30±0.05*	0.34±0.05*	0.34±0.04*
Control	0.43±0.02	0.42±0.03	0.44±0.02	0.33±0.04	0.33±0.04	0.32±0.01

D1, D2, D3 and D4 are the chlorpyrifos dose samples i.e. 2.5, 5, 7.5, 10ml/1000ml; Values are Mean±S.E of four replicates; *Values are significant at 5% level of significance

Table 2. Cocoon production rate (cocoon/worm/week) and total number of cocoons produced by *E. eugeniae* different doses of chlorpyrifos and control samples.

Doses	Number of Weeks					
	1	2	3	4	5	6
D1	0	0.67±0*	1.34±0.04*	1.36 ±0.10*	1.12±0.03*	0.6 ±0*
D2	0	0	1.5±0.08*	1.37±0.05*	0.60±0.03*	0
D3	0	0	1.36±0.06*	1.25±0.11*	0.62±0.08*	0
D4	0	0	1.3±0*	1.20±0*	1±0*	0
Control	1.7±0.06	1.9±0.07	2.5±0.13	3.4±0.07	1.6±0.17	1.0±0

D1, D2, D3 and D4 are the chlorpyrifos dose samples i.e. 2.5, 5, 7.5, 10ml/1000ml; Values are Mean±S.E of four replicates; *Values are significant at 5% level of significance

Table 3. Juvenile production rate (juveniles/ cocoon/ week) for *E. eugeniae* in different doses of chlorpyrifos.

Doses	Number of Weeks						
	1	2	3	4	5	6	7
D1	-	-	-	-	0.85±0.13*	0.87±0.09*	0.5*
D2	-	-	-	-	0.72±0.18*	0.64±0.09*	-
D3	-	-	-	-	0.73±0.07*	0.58±0.06*	-
D4	-	-	-	-	0.60±0*	0.5±0*	-
Control	-	-	-	1.35±0.024	1.29±0.28	1.39±0.05	1.8±0.10

D1, D2, D3 and D4 are the chlorpyrifos dose samples i.e. 2.5, 5, 7.5, 10ml/1000ml; Values are Mean±S.E of four replicates; *Values are significant at 5% level of significance

Table 4. Effect of chlorpyrifos on survival and growth *E. eugeniae* hatchlings.

Doses	Number of Weeks							
	1	2	3	4	5	6	7	8
D1	100	100	100	100	100	100	100	75
D2	100	100	100	100	75	75	50	50
D3	100	100	75	75	50	50	50	50
D4	100	-	-	-	-	-	-	-
Control	100	100	100	100	100	100	100	100

D1, D2, D3 and D4 are the chlorpyrifos dose samples i.e. 2.5, 5, 7.5, 10ml/1000ml

Table 5. Weekly changes in body weight (g) of hatchlings of *E. eugeniae* in chlorpyrifos.

Dose Samples	Number of Weeks							
	1	2	3	4	5	6	7	8
D1	0.047 ± 0.01	0.050 ± 0.01	0.070 ± 0.02	0.085 ± 0.02	0.090 ± 0.03	0.14 ± 0.04*	0.14 ± 0.04*	0.16 ± 0.04*
D2	0.035 ± 0.01	0.050 ± 0.01	0.045 ± 0.014	0.060 ± 0.02	0.065 ± 0.025	0.22 ± 0.03*	0.13 ± 0.02*	0.13 ± 0.03*
D3	0.035 ± 0.00	0.053 ± 0.01	0.060 ± 0.02	0.20 ± 0.05	0.18 ± 0.05	0.22 ± 0.04*	0.22 ± 0.04*	0.30 ± 0.04*
D4	0.033 ± 0.00	-	-	-	-	-	-	-
Control	0.050 ± 0.013	0.05 ± 20.01	0.060 ± 0.01	0.070 ± 0.02	0.149 ± 0.02	0.19 ± 0.02*	0.35 ± 0.03*	0.40 ± 0.007*

D1, D2, D3 and D4 are the chlorpyrifos dose samples i.e. 2.5, 5, 7.5, 10ml/1000ml; Values are Mean±S.E of four replicates; *Values are significant at 5% level of significance; All the hatchlings died in the 4th dose samples after one week of rearing

Table 6. Duration of life span of juveniles of *E. eugeniae* in chlorpyrifos and control sample.

Dose Sample	Number of days
D1	43.25 ± 0.82
D2	45.75 ± 0.82
D3	54.25 ± 0.82
D4	All died after 1 st week
Control	28.75 ± 0.82

ing the present investigations. Similar reduction in body weight by increased concentration of malathion on *E. fetida* has been reported (Espinoza-Navarro and Bustos-Obregon, 2005). The loss of weight along with signs and symptoms of toxicity such as coiling, swollen body, sluggish movements and discharge of coelomic fluid was observed in *Lumbricus terrestris* when exposed to pesticides (Cyren, Ridomil, Triplen and Mamba) for 4 weeks (Ahmed, 2013). Fecundity in earthworms is sensitive to insecticides even though population densities of earthworms may not be immediately affected, there can be reproductive changes which may reduce populations in the longer term. Reduction in fecundity was observed in *E. fetida* on exposure to neonicotinoids (Wang et al., 2015). Low

reproduction of earthworm was seen in finely sieved soil as compared to sandy soil indicating that porosity of soil may influence earthworm mobility and gaseous exchange, thus affecting its life cycle.

In the present study, as minimum numbers of cocoons were collected from D3 and D4 dose relating the reduction to the high concentration of insecticide. It is further inferred that exposure of earthworm *Eudrilus* to insecticide chlorpyrifos severely impairs the cocoon production and is dose-independent. Contaminated dose samples have delayed cocoon production and it has been suggested that cocoon production rates were particularly sensitive during early period of reproduction (Sprugeon and Hopkin, 1999). Similarly, exposure of worms to higher sub lethal dose of malathion produced lesser number of cocoons and hatchling than the worms exposed to lower sub lethal concentration of malathion (Rai and Bansiwai, 2009). Reproduction toxicity was also observed in case of *E. fetida* following exposure to deltamethrin and fenvalerantoin soil (Song et al., 2015).

The lesser number of hatchlings produced in each chlorpyrifos treated sample as compared to control in the present study, is due to the elevated levels of insecticide.

ticide. Percentage of high insecticide influences the reproduction parameters i.e. cocoon production, mean and maximum number of hatchlings per cocoon, and a longer incubation period of worms in a dose dependent manner as observed by earlier workers (Booth and O'Halloran, 2000; Gupta and Saxena, 2003; Yasmin and D' Souza, 2007). The results of the present study correlate strongly and suggest that there being a direct relationship between dose treated and emergence of hatchlings from each cocoon.

High mortality percentage of juveniles shows the sensitivity of juveniles to elevated levels of insecticide. The estimation of various biochemical parameters was not in the preview of the present experimental design, but it is hypothesized that since newborn and younger animals are more sensitive to toxicants than older or mature animals (Rozman *et al.*, 2001; Singh and Rishi 2005) they are affected more acutely and within a shorter exposure period. The detoxification mechanisms of juvenile animals in general may also not be as well developed as those of adult animal. It is assumed for earthworms, there may be a biochemical basis for increased sensitivity of juveniles (Matsumura, 1975).

Decrease in biomass seems to be a sensitive endpoint because it was possible to detect decreases in biomass even at low concentrations of insecticides. Our study suggests that weight loss in earthworms occurred due to some exclusive factors like inactivity, as direct response to the insecticide or as a mechanism of avoidance. The observation (Capoweiz *et al.*, 2005) was similar to our results and this leads to prospective conclusion that weight loss in earthworms was a sensitive biomarker for exposure to insecticides even at low concentrations. It has been observed that earthworms gain more weight when exposed to sub lethal dose of malathion in comparison when exposed to higher sub lethal dose of malathion (Rai and Bansiwala, 2009). Thus the exposure to different insecticides leads to similar weight gain options.

High concentration of chlorpyrifos strongly accounts for the longer duration of days to reach maturity this is due to the physiological stress on the animal leading to a decreased energy level for maintenance, growth and the two reproductive processes *i.e.* differentiation to reach maturity and cocoon (egg) production (Kooijman, 1986). Our study revealed that high dose of insecticide caused the increase in duration of life span of juveniles in all the chlorpyrifos treated samples as compared to control prolonging the life cycle of the earthworm. Selection of life history parameters can be of relevance when populations are exposed to factors (such as pollutants) that reduce survival and lower reproductive effort (Charlesworth 1980). Slow maturation in earthworms exposed to contaminated soil samples has also been observed earlier (Sprugeon and Hopkins, 1999; Sprugeon *et al.*, 2004).

Conclusion

The earthworm is the major soil mega fauna to be exposed to soil contaminants and their presence is an indicator of soil health. The present study investigated the effect of chlorpyrifos on survival, growth and reproductive potential in earthworms. The higher dose (7.5ml/1000ml and 10.0ml/1000ml) of chlorpyrifos was detrimental to the growth and reproductive performance in the earthworms while low and recommended doses could be used for soil application. However, to investigate the effects on higher trophic levels we need to acquire more knowledge on the chemical nature, mode of action, and means of degradation of pesticides in soil.

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