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RESEARCH ARTICLE

CHRONIC TOXICITY OF CYPERMETHRIN, A PYRETHROID INSECTICIDE TO EPIGEIC EARTHWORM *EISENIA FETIDA*

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ABSTRACT

Effects of sub-lethal doses of cypermethrin on biomass, reproduction and alkaline phosphatase activity of the epigeic earthworm, *Eisenia fetida* were investigated in natural grassland soil (pH-6.72±0.02, organic carbon-1.28±0.37) under laboratory conditions. *E. fetida* exposed to 0.006 to 0.025 mg/kg soil of cypermethrin, corresponding to 12.5 (T2) to 50 % (T4) of LC_{50} values of cypermethrin did not exhibit any significant reduction in biomass from control. Production of cocoons and juveniles remained significantly unaltered at T2 and T3 doses of the insecticide but was inhibited with exposure to the highest dose (T4) of cypermethrin. Alkaline phosphatase activity was found to remain significantly unaltered at T2 but increased both in T3 and T4 in comparison to control. Thus, though the LC_{50} value of cypermethrin was much higher than it's recommended agricultural dose indicating it's less hazardous nature, it can be concluded from the present study that sub-lethal doses of cypermethrin significantly affected reproduction and alkaline phosphatase activity of the test specimen and their widespread application in agro-ecosystems must be carefully monitored. The present study also indicated the potential use of alkaline phosphatase as a biomarker in assessment of insecticide pollution.

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INTRODUCTION

The urge for increasing agricultural yield to feed the increasing population has brought a large number of chemicals in protecting the crop against insect pests. However, a grave environmental problem have aroused because of the indiscriminate usage of these chemicals in agricultural fields (Tillman and Mulrooney, 2000; Zhu *et al*., 2004; Reinecke and Reinecke, 2007). Insecticides are known to be toxic to many non-target organisms and also cause serious sub-lethal effects (Nath *et al*., 1997; Suh *et al*., 2000; Yasmin, 2007) including increases and decreases in reproductive potential and growth rate (Takada *et al*., 2001; Willrich and Boethel, 2001; Yasmin and Dsouza, 2010) Cypermethrin is a pyrethroid insecticide that is used to control insect pests (Cox, 1996) and kills insects by disrupting normal functioning of the nervous system (Vijenberg & Bercken, 1990). Though it has been shown that they are harmful to beneficial insects, pyrethroids are effective at low rates and relatively inexpensive (Nowak *et al*., 2001; Xu *et al*., 2001). However the effects of pyrethroids on earthworms, a major group of non-target soil fauna has not been fully explored. Cypermethrin, one of the major pyrethroid insecticides currently used worldwide against insect pests (Usmani and Knoweles, 2001). Has been used in the present study to determine its effects on a non-target soil fauna *Eisenia fetida,* an epigeic earthworm known for its efficacy in nutrient recycling and widely used for vermicompost production throughout the world

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MATERIALS AND METHODS

Specimens of *Eisenia fetida* were collected from a local vermicompost unit around Midnapore town (W. Bengal, India) and were brought to the laboratory in plastic bags along with compost materials, identified and only pure species of *E. fetida* were used for culture. Specimens were cultured in large earthen pots (diameter 45.72 cm, mean depth 30.48 cm) with finely ground soil (collected from grassland that has never been used for any agricultural purpose and pest control; pH - 6.72 ± 0.02, organic carbon-1.28±0.37) and farmyard manure mixed in the ratio of 1:1 was used as the culture medium. The culture pots were covered with fine meshed iron nets and kept inside BOD incubators at 28±0.5◦ C. An approximate level of 60-70 % moisture was maintained by adding distilled water into the medium. Finely ground farmyard manure was added as feed every week during the entire period of culture. The cocoons were hand sorted, cultured in separate culture pots and were later used as test specimens following the guidelines for testing of chemicals for earthworm (OECD, 1984; 2004). The pH of the soil was measured using glass electrode pH meter (Systronics model 512SE) and organic carbon content was determined following the methods of (Walkley and Black, 1934). Cypermethrin ((RS)–a cyano-3phenoxy benzyl(1RS)-cis, trans-3 (2,2-dichlorovinyl)- 2,2- dimethylcyclopropane carboxylate) a synthetic derivative of Pyrethrum, a stomach and contact insecticide effective against a broad range of pests of cotton, fruit and vegetable crops was used as the test chemical. Experiments were conducted in small inert polythene boxes (total area, 192 cm^2) containing 600 g of finely ground grassland soil previously used in the culture medium as the test medium for the toxicity studies. Sub lethal doses of the insecticides were applied based on the previously established LC_{50} value of the insecticide on *E. fetida* (Dasgupta *et al*., 2011). The experimental boxes were exposed to control (T1) and different doses of insecticides (T2 to T4) representing 12.5, 25 and 50 % LC_{50} values of cypermethrin in five replicates (Table 1).

Sublethal doses of pesticides

Table 1. Doses (mg formulation /kg soil) of cypermethrin used in the sub lethal toxicity

Dose	Cypermethrin (mg/kg soil)
$T_{1(control)}$	0.000
T_2	0.006
T_3	0.012
T4	0.025

Age-synchronized adult specimens of *E. fetida* (average wet weight 300-350 mg) were collected and 10 such specimens were placed in each experimental box. Before introduction, the worms were rinsed with water, blotted dry on a filter paper and biomass per ten worms was determined. The boxes were maintained at 28 ± 0.5◦C inside a BOD incubator and 5 g (dry weight) of finely ground cow manure moistened to 50 % w/w was added each week to provide food for the growing worms. Additional food was given when all the food added was consumed. Moisture loss from the test soil was checked by weighing the test containers at weekly intervals and replenished if needed. The worms were weighed on the 28th day to determine the change in biomass and were removed from the test boxes. Reproductive success of the test specimens was determined from the rate of cocoon and juvenile production. For determination of rate of cocoon production the test medium was carefully examined with a magnifying glass every week and number of cocoons was noted. The boxes were left undisturbed after removal of the adults on the 28^{th} day till the 56^{th} day and were housed inside a BOD incubator. The temperature and moisture were maintained at 28 ± 0.5 ◦C and 60-70 % level respectively and food was provided as per OECD guidelines (2004). Hatchlings per box were counted on the 56th day to determine the number of juveniles.

The final endpoints studied were change in biomass on the 28th day, cocoons production per week and number of juveniles on the 56th day. Adult earthworm specimens removed from the test boxes on the 28^{th} day of the experiment were also used to determine the activities of the alkaline phosphatase and total protein of the earthworms following the methods of Walter and Schutt, 1974 and Lowry *et al*., 1951 respectively. The data was analyzed for single factor ANOVA followed by Least Significance Difference (LSD) test to determine significant variation between treatments at 5 % of probability (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

There was no mortality in any of the worms in control sets and in T2, T3. Average mortality recorded in T4 was 3.33 %. The control worms showed an increase of 68.1 ± 6.22 % of biomass from the initial value during the 4-weeks test period. The worms exposed to T2-T4 doses of the insecticides did not record significant alteration in change of biomass as compared to control. Worms recorded 63.2±3.09 increase in biomass when exposed to the lowest dose, i.e. T2 representing 12.5% of LC_{50} whereas an increase of 59.19 ± 4.89 % increase of biomass of the test worms was observed when exposed to T3 dose representing 25% of $LC_{50.}$ Earthworms exposed to T4 dose of cypermethrin (50% of LC_{50}) recorded 59.21 ± 5.26 % increases in biomass of the test worms from their initial value. This increase was again not significantly different from the change in biomass of the control worms (Fig 1).

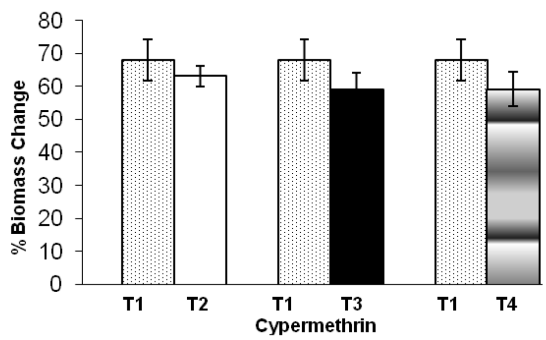

Fig. 1.

Number of cocoons produced per worm per week and juveniles produced in control and different treatments on *E. fetida* have been presented in Table 2.

Effects on reproductive parameters

Table 2. Cocoon production and juvenile production in *E. fetida* exposed to control (T1) and sub-lethal doses (T2, T3 & T4) of cypermethrin

Treatment	Cocoons/worm/week*	Juveniles (No. per ten worms)*
T1	1.04 ± 0.05^a	31.0 ± 1.58^a
T2	1.02 ± 0.04^a	$30.80 \pm 2.4\,0^a$
T3	0.99 ± 0.08^a	27.40 ± 0.01^a
T4	$0.\ 47 \pm 0.01^b$	$19.80 \pm 3.1\,0^b$

*Values are expressed as mean ± S.D; Superscripts in the same column denote significant difference at p<0.05

Mean number of cocoons produced per worm per week in the control was 1.04 ± 0.05. Mean number of juveniles produced in control was 31.0 ± 1.58. Sub-lethal doses of the insecticides did not significantly affect the reproductive performance of the worms except at the highest dose (T4) of the insecticide (0.47 ± 0.04, cocoons/worm/week and juvenile production decreased to 19.80 ± 3.1). Activities of the enzyme alkaline phosphatase of *E. fetida* were significantly elevated in all the treatments except in T2 where the increase was statistically insignificant in comparison to control (Fig 2).

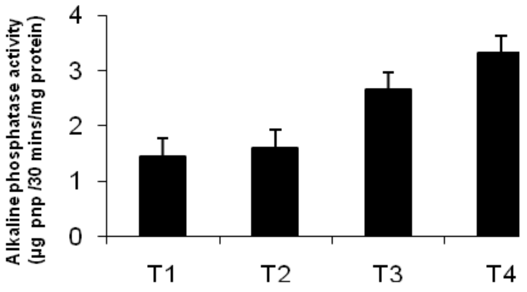

Fig. 2.

Ingesfield (1984) reported low toxicity of cypermethrin to earthworms and explained that this non toxicity can be due to the property of pyrethroids being adsorped onto the organic matter of the soil particles which renders part of the dose unavailable to the worms. Since the LC_{50} value was many times greater than the maximum recommended agricultural dose for the compound, it was concluded by Ingestfield (1984) that cypermethrin would not have adverse effects on natural populations of earthworms. Similar conclusion was made by Dasgupta *et al*., 2010. Paula *et al*., 2011 found that earthworms *Eisenia andrei* avoided the soils treated with cypermethrin at concentration 15 µ/g soil onwards but there was no dose-related response within the range of concentration tested. Growth has been reported to be a sensitive parameter to evaluate the toxicity of insecticides on earthworms (Xiao *et al*. 2006). The weight loss may indicate feeding inhibition, with the earthworms regulating the intake of insecticides by reducing consumption rate and thus affecting growth rate (Mosleh *et al*., 2002, 2003).

Reduction in growth of earthworm by sub-lethal doses of insecticides has also been observed in *Eisenia fetida* (Yasmin and Dsouza, 2007) exposed to several carbamate and organophosphate insecticides. However no significant alteration in the change of biomass of the earthworms could be detected in the present study as compared to control even with the exposure of the highest sub-lethal dose used, i.e., 0.25 mg/kg cypermethrin corresponding to 50% of the LC_{50} value of the insecticide indicating its less toxic nature in comparison to carbamates and organophosphates. Significant alterations in the reproductive performance of the test worms in terms of cocoons/worm/week and juvenile production per ten worms compared to control was detected when exposed to the highest sub lethal dose used, i.e., 0.25 mg/kg cypermethrin corresponding to 50% of the LC_{50} value of the insecticide in the present study indicating its possible hazardous nature. Various scientists have also reported similar result regarding insecticides influencing reproduction of earthworms (Mosleh *et al*., 2002; Booth *et al*., 2000; Yasmin and DSouza, 2007). The observed rise in alkaline phosphates activity in the present study may be indicative of an adaptive rise in enzyme activity to the persistent stress (Murphy and Porter 1966). It is established that insecticide exposure causes reduction in soluble protein and there is a relationship between growth rate, protein content and phosphatases and thus an increase in phosphatases may result. Again elevation of alkaline phosphatase has been reported to be related to resistance of the organism towards the insecticide and the level of pathological and physiological damage caused to the particular organism (Gupta *et al*. 1985; Srinivas *et al*. 2003).

Conclusion

Thus it can be concluded from the present study that sub-lethal dose of cypermethrin significantly affects reproductive performance and alkaline phosphatase activity of the test specimen and their widespread application in agro-ecosystems must be carefully monitored. The present study also indicated the potential use of alkaline phosphatase as a biomarker in assessment of insecticide pollution.

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