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Studies on Relative Toxicities of Six Insecticides on Epigeic Earthworm, *Perionyx excavatus*

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Abstract The 96 h LC_{50} values of six insecticides were determined on a non-target epigeic earthworm *Perionyx excavatus* under laboratory conditions. Cypermethrin was found most toxic to *P. excavatus* (LC_{50}-0.008 mg/kg), followed by endosulfan (LC_{50}-0.03 mg/kg), carbaryl (LC_{50}-6.07 mg/kg), chlorpyrifos (LC_{50}-7.3 mg/kg), aldicarb (LC_{50}-10.63 mg/kg) and monocrotophos (LC_{50}-13.04 mg/kg). When these LC_{50} values were compared with their respective recommended agricultural doses, aldicarb and carbaryl appeared more dangerous than other pesticides because of their lower LC_{50} values than their respective recommended agricultural dose. Mean lethal time to cause 50% mortality at recommended agricultural dose (LT_{50}) also indicated that aldicarb achieved the fastest LT_{50} (26 h) followed by endosulfan (38 h) and carbaryl (44 h) indicating the danger of these pesticides to *P. excavatus.*

Keywords *Perionyx excavatus* · LC_{50} · LT_{50} · Environmental hazard

Earthworms are considered as ecosystem engineers (Lavelle et al. 1998) as they produce pronounced effects on soil structure by their burrowing activities, ingestion of soil and production of castings (Lavelle and Spain 2002).Pesticides, that are applied in agriculture fields, may affect the non-target soil organisms including earthworms and significantly damage the ecosystem (Reinecke and Reinecke 2007). Since earthworms are preferred as food by amphibians (Lescure 1966), reptiles (Catling and Freedman 1980), birds (Harlin 1977) and mammals (Churchfield 1979) there is a possible risk of these pesticides reaching higher trophic levels (Marino et al. 1992). Several researchers have advocated the use of earthworms as ecotoxicological model for risk assessment and bioassay of pesticides (Edwards 1990; Edwards and Bohlen 1992).

In the present study experiments were made to evaluate the effects of six insecticides on an epigeic earthworm *Perionyx excavatus*. This species is widely distributed in India and is considered as an ecologically important soil organism for its role in increasing soil fertility and for it's potential of being used in the production of vermicompost (Ismail 1997). The insecticides tested in the present study include two carbamates (carbaryl and aldicarb), two organophosphates (monocrotophos and chlorpyrifos), one organochlorine (endosulfan) and one pyrethroids (cypermethrin). The purpose of these experiments was to evaluate the comparative toxicity of these insecticides on *P. excavatus* and their potential to damage the soil ecosystem.

Materials and Methods

Specimens of *Perionyx excavatus* were collected from the grasslands, around Midnapore town (West Bengal, India), that has never been used for any agricultural purpose and pest control. The specimens were brought to the laboratory and were cultured in large earthen pots. Finely grinded soil (collected from the same grasslands) and farmyard manure mixed in the ratio of 1:1 was used as the culture medium (Ismail 1997). The culture pots were covered with fine

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Table 1 Insecticides used in the study

Insecticide	Formulation	Source of procurement	Recommended agricultural dose (g/ha)
Carbaryl	50 WDP[a]	Bayer CropScience Ltd, India	2,000
Aldicarb	50 EC[b]	M/S Union Carbide Corp, USA	3,500
Chlorpyrifos	20 EC	Dow AgroScience, USA	500
Monocrotophos	36 EC	DeNocil Crop Prod, India	600
Endosulfan	35 EC	Crop Care Ltd, India	2.50
Cypermethrin	10 EC	United Phosphorous Ltd, India	0.75

[a] Wet dispersible powder; [b] Emulsifiable concentrate

meshed iron nets and kept inside BOD incubators at 28 ± 0.5°C. An approximate level of 50% moisture was maintained by adding distilled water into the medium. 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 guidelines for testing of chemicals for earthworm (OECD 1984, 2004).

The insecticides used in the present study along with source of procurement and recommended agricultural dose are summarized in Table 1.

Direct toxicity studies were performed with age synchronized specimens (250–300 mg). Experiments were conducted in small inert polythene boxes (16 × 12 × 1 cm; total area, 192 cm^2) containing soil, collected from the same grasslands from where the mother earthworm specimens were collected, as the test medium. Soil samples were dried, grinded and sieved to get a particle size of 0.25 mm before laying in the experimental boxes. The moisture content of the soil was measured by Infrared Torsion balance moisture meter [Adair Dutt, Kolkata] (Joy and Chakravorty 1991). Finally the experimental boxes were kept in a BOD incubator at a constant temperature of 28 ± 0.5°C and 50% moisture.

Different levels of the insecticides based on their recommended agricultural doses (RAD) (viz RAD, $^1/_2$X-RAD, 2X-RAD and 3X-RAD) were administered into the test boxes with a micropipette (Lofs-Holmin 1983). The amount of an insecticide required was determined from the total area of the experimental box and was converted into mg per kg soil taking into consideration the total amount of soil (200 g) contained in one box. The experiment was set up with three replicates for each level of the insecticide and control. The boxes were then left undisturbed for about 30 min for uniform spreading of the chemical in the soil medium. Five numbers of age synchronized specimens of *Perionyx excavatus* were then transferred into the boxes. Observations were made every 24 h. Those individuals, who showed no apparent sign of life, even when poked with a needle, were considered dead and were removed. The total mortality obtained after 96 h of exposure were subjected to probit analysis by EPA probit analysis program, version 1.5(US EPA 2006) to determine LC_{50} value and 95% confidence limit of each insecticide. The entire experiment was repeated three times.

The LT_{50} values (time taken to achieve 50% mortality) of the insecticides at recommended agricultural dose were determined for *P. excavatus* following the methods of Chakravorty (1990). Required dose of an insecticides (determined as mg/kg soil) were applied into the test boxes, which were then left undisturbed for about 30 min for uniform spreading of the insecticide in the soil medium. Five numbers of age synchronized specimens of *P. excavatus* were transferred into the boxes. Observations were made every hour and the dead individuals were removed. The experimental boxes were kept in a BOD incubator at a constant temperature of 28 ± 0.5°C and 50% moisture. The time taken to achieve 50% mortality was noted and expressed as LT_{50} of the insecticide.

Results and Discussions

The findings of the laboratory experiments are summarized in Tables 1 and 2. Cypermethrin with the LC_{50} value of 0.008 mg/kg was found most toxic to *P. excavatus*, followed by endosulfan (LC_{50}-0.03 mg/kg), carbaryl (LC_{50}-6.07 mg/kg), chlorpyrifos (LC_{50}-7.3 mg/kg), aldicarb (LC_{50}-10.63) and monocrotophos (LC_{50}-13.04 mg/kg) (Table 2). However, aldicarb and carbaryl were found more dangerous than the other insecticides when the LC_{50} values were compared with the respective RAD for an equivalent amount of soil (200 g/192 cm^2). Thus LC_{50} values of aldicarb and carbaryl were much less than their respective RAD (3,500 and 2,000 g/ha equivalent to 35 and 20 mg/kg soil, respectively), while those of the endosulfan and cypermethrin were slightly lower and chlorpyrifos and monocrotophos higher than their respective RAD (2.5, 0.75, 500, 600 g/ha equivalent to 0.025, 0.005, 5.75, 4.8 mg/kg soil, respectively).

Table 2 96 h LC_{50} values (mg/kg soil) of different insecticides on *P. excavatus*

Insecticide	96 h LC_{50} (mg/kg soil)	95% Confidence limit
Carbaryl	6.07	0.12–10.80
Aldicarb	10.63	0.21–18.97
Chlorpyrifos	7.30	5.06–10.18
Monocrotophos	13.04	10.53–20.73
Endosulfan	0.03	0.01–0.05
Cypermethrin	0.008	0.003–0.08

Table 3 LT_{50} (at recommended agricultural dose) of different insecticides on *P. excavatus*

Insecticide	LT_{50} (at recommended agricultural dose)
Carbaryl	44 h
Aldicarb	26 h
Chlorpyrifos	Not achieved within 96 h
Monocrotophos	Not achieved within 96 h
Endosulfan	38 h
Cypermethrin	Not achieved within 96 h

Table 3 depicts the mean lethal time to cause 50% mortality at RAD (LT_{50}) which also show that aldicarb achieved the fastest LT_{50} (26 h) followed by endosulfan (38 h) and carbaryl (44 h) indicating the danger of these pesticides to *P. excavatus*. Cypermethrin, monocrotophos and chlorpyrifos did not produce 50% mortality within 96 h when recommended agricultural dose of the respective insecticide was applied.

Results of the present investigation indicate that environmental hazard caused by an insecticide to a soil organism can be assessed properly only when it is compared with the quantum of the insecticide applied in the field. Based on the LC_{50} values it was revealed that *P. excavatus* was most susceptible to the pyrethroid insecticide cypermethrin followed by the conventional insecticides endosulfan, carbaryl, chlorpyrifos, aldicarb and monocrotophos. However, the carbamate insecticides aldicarb and carbaryl possessed more ecological risks to *P. excavatus* than the other insecticides because LC_{50} values of these were about one-third of the level of these insecticides applied in the field (RAD). The risk of these insecticides was also evident from their LT_{50} values with RAD (26 and 44 h, respectively). The implication is that earthworms are likely to be eliminated from the fields when these insecticides are applied for control of insects. Many workers have reported high toxicity of carbamates to earthworms. Edwards (1990) confirmed that carbaryl could be rapidly bioconcentrated in the body of earthworms causing immobility, rigidity and ultimately death. Potter (1990) also reported that carbaryl treatment could reduce earthworm population by 90% within 96 h of treatment.

LT_{50}(RAD) value of cypermethrin could not be established within 96 h. Cypermethrin is a photodegradable pyrethroid insecticide and is quickly degraded when exposed to light (Saha and Kaviraj 2008).The organophosphate insecticide, chlorpyrifos showed a LC_{50} value, which was higher than its RAD. Moreover, RAD of chlorpyrifos did not produce 50% mortality within 96 h of treatment. Thus it was revealed that chlorpyrifos was moderately toxicity to *P. excavatus* and environmentally less hazardous than the carbamates, endosulfan and cypermethrin. Monocrotophos, another organophosphate insecticide, also recorded LC_{50} value, which was much higher than its RAD, and 96 h LT_{50} of RAD could not be achieved. Thus monocrotophos was considered as the least toxic of the six insecticides tested in this study. Other research findings also indicate that most of the organophosphate insecticides are not severely toxic to earthworms (Edwards and Bohlen 1992). Endosulfan, however, has been reported to be severely toxic to different species of earthworm (Bostrom and LofsHolmin 1982; Mahanthaswamy and Patil 1990).

Thus, it is concluded from the present study that the carbamate insecticide aldicarb and carbaryl and the organochlorine insecticide endosulfan are more dangerous to soil organisms than the pyrethroid insecticide cypermethrin and the organophosphate insecticides chlorpyrifos and monocrotophos.

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