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Poll Res. 34 (1) : 47-51 (2015)
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ISSN 0257–8050

COMPARATIVE TOXICITY STUDIES OF FOUR PESTICIDES ON THE EPIGEIC EARTHWORM *PERIONYX EXCAVATUS* IN TWO DIFFERENT SOIL MEDIA

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(Received 26 June, 2014; accepted 1 August, 2014)

ABSTRACT

The LC_{50} values of four insecticides were recorded at 96 hours, under suitable laboratory conditions, on a non-target epigeic earthworm *Perionyx excavatus* in two different Soil media, viz, the natural garden soil and the artificial OECD (Organisation for Economic Co-operation and Developement) soil, composed as per OECD guidelines. The physiochemical parameters of both the soil media, viz, pH and Organic carbon content were measured and the temperature and moisture content were kept constant. The Pyrethroid pesticide, Cypermethrin was found most toxic in both natural garden soil (LC_{50} value-0.012 mg/kg) as well as Artificial OECD soil (LC_{50} value-0.013 mg/kg) followed by Pendimethalin, Dimethoate and Pretilachlor.

KEY WORDS : Artificial soil, Dimethoate, *Perionyx excavatus,* Pretilachlor

INTRODUCTION

Concern for pesticide contamination in the environment in the current context of pesticide use has assumed greater importance (Zhu *et al.*, 2004). Pesticides are toxic products designed to kill a target organism (WHO 2003). The indiscriminate use of toxic chemicals like pesticides in agriculture to increase the productivity of crops often leads to toxic hazards to beneficial soil organisms like earthworms. Thus the indiscriminate use of pesticides has contaminated our environment as the agro chemicals used effect the non-target organisms in soil and water and can cause serious damage to ecosystems (Reinecke and *et al.*, 2007). Chemicals that are applied in tropical and subtropical countries are transported over long distances by global air circulation (Ongley, 1996). Miller (2004) reported that pesticide use contributes to pollinator decline, destroys habitat (especially for birds), and threatens endangered species.

Soil biota plays a crucial role in influencing soil structure and related soil physical properties. Earthworms, through their burrowing and feeding activities influence particle size distribution, organic matter content and location, soil aggregation, aggregate stability and tensile strength, soil roughness, and water infiltration (Blanchart *et al.*, 2004). Earthworms constitute more than 80% of the invertebrate biomass (Macfaydan, 1961). Their contribution to complex processes such as litter decomposition, nutrient cycling and soil formation is very important; hence the presence of earthworms is beneficial to agro-ecosystems (Edward and Fletcher, 1988). 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). More than 17 species of earthworms are available in West Bengal (Chanda and Chakravorty, 2003). Among these species, the epigeic earthworm *Perionyx excavatus* has been widely used to evaluate the toxicity of pesticides (Dasgupta *et al.*, 2010).

In the present study, experiments are carried out to evaluate the toxic effects of four pesticides on the epigeic earthworm *Perionyx excavatus*, as well as to

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determine a comparative study in two different soil media, the natural garden soil and artificial OECD soil (OECD, 1984) on the earthworms and their potential to damage soil ecosystem. The pesticides used in the study include one organophosphate (Dimethoate), one pyrethroid (Cypermethrin) and two herbicides (Pendimethalin and Pretilachlor).

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 meshed iron nets and kept inside Environmental Chamber at 28 ± 0.5ºC. An approximate level of 60-65% 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).

Direct toxicity studies were performed with age synchronized specimens (250–300 mg). Experiments were conducted in small inert polythene boxes (16 X 12 X 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 (Joy and Chakravorty, 1991). Finally the experimental boxes were kept in an Environmental Chamber at a constant temperature of 28 ± 0.5°C and 60-65% relative humidity.

Similar experiment was carried out using artificial soil as the test medium. OECD artificial soil consists of 10% finely ground sphagnum peat, 20% kaolinite clay, approx. 69% quartz sand and approximately 1% of $CaCO_3$ to adjust pH to approximately 6.0. Although extrapolation of toxicity data generated through artificial soil to field conditions is sometimes difficult,it remains a popular substrate in soil ecotoxicology due to the existence of a large data base for a wide range of xenobiotics, such as pesticides, metals and industrial chemicals, and the easy interpretation and easy comparison of the results.Recently, however, it has become evident that sphagnum peat is scarce and completely unavailable in many regions, including the tropics. Locally available coco peat (composted and non-composted), paddy husk and saw dust might be suitable substrates for replacing sphagnum peat in artificial soil (de Silva *et al.*, 2009). We used paddy husk in our experiments since sphagnum peat was unavailable. Paddy husk is also a very common material in the tropics, where rice is the main agricultural commodity. It is a biodegradable material resulting from processing paddy into rice. Currently it has no commercial value but it has been used in agriculture as a fertilizer and as an energy source (de Silva *et al.*, 2009).

The physiochemical parameters of both the soil media, viz, pH and Organic carbon Content were

Table 1. The insecticides used in the present study along with their commercial name and recommended agricultural doses

Pesticide Group	Technical name	Commercial Name	RAD* (mg/kg)
Herbicides	Pendimethalin, Pretilachlor	Dhanutop, Racer	0.008, 0.002
Organophosphate	Dimethoate	Rogorin	0.002
Pyrethroid	Cypermethrin	Ustaad	0.002

*RAD- Recommended Agricultural Dose

Table 2. Physiochemical parameters of the two different test soil media

Soil Parameters	Natural Soil	Artificial Soil
pH	7.17	6.6
Organic Carbon Content	0.86%	0.78%
Moisture	61.2%	62%

measured following the method of Piper (1942) and the temperature and moisture content were kept constant (Table 2).

Different levels of the insecticides based on their recommended agricultural doses (RAD) (viz RAD, 1/2X-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 setup 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 (Dasgupta *et al.*, 2010).

RESULTS

The findings of the laboratory experiments are summarized in Tables 3 and 4. In the case of natural garden soil, Cypermethrin with the LC_{50} value of 0.012 mg/kg was found most toxic to *Perionyx excavatus*, followed by pendimethalin (LC_{50}- 0.016 mg/kg), dimethoate (LC_{50}- 0.017 mg/kg) and pretilachlor (LC_{50}- 0.052 mg/kg) (Table 3).

Similar results were found in the case of artificial soil where Cypermethrin (LC_{50}- 0.013 mg/kg) was found most toxic to *P. excavatus*, followed by pendimethalin (LC_{50}- 0.018 mg/kg), dimethoate (LC_{50}- 0.022 mg/kg) and pretilachlor (LC_{50}- 0.064 mg/kg) (Table 4).

In case of natural garden soil as well as artificial soil, all the four pesticides tested in the current experiments show LC_{50} values more than their respective Recommended Agricultural Dose (RAD), so all the pesticides were considered to be ecologically safe, where the order of toxicity was found to be Cypermethrin> Pendimethalin> Dimethoate>Pretilachlor with respect to their LC_{50} values. The LC_{50} values of all the four pesticides was lower in natural soil, than in comparison to artificial soil, so more toxicity was noted in natural soil but the LC_{50} values of natural soil and artificial soil are not statistically significant. So, it can be concluded that the degree of toxicity of all the four pesticides used in the experiment are more or less similar in both the soil media.

DISCUSSION

Based on the LC_{50} values obtained from the above experiments it was seen that pyrethroid pesticide cypermethrin was most toxic to the earthworm *P. excavatus*. Dasgupta *et al.*, 2010, reported cypermethrin to be very toxic to *P. excavatus* in natural soil.Dasgupta *et al.*, 2011 also reported that *E.*

Table 3. LC_{50}values of the different groups of pesticides in natural garden soil

Pesticide Group	Technical name	LC_{50}(96 hour) (mg/kg) Natural Soil	
		LC_{50}	Confidence Limit
Herbicides	Pendimethalin, Pretilachlor	0.016, 0.052	0.01-0.04, 0.04-0.07
Organophosphate	Dimethoate	0.017	0.01-0.04
Pyrethroid	Cypermethrin	0.012	0.01-0.03

Table 4. LC_{50}values of the different groups of pesticides in artificial OECD soil

Pesticide Group	Technical name	LC_{50}(96 hour) (mg/kg) Artificial Soil	
		LC_{50}	Confidence Limit
Herbicides	Pendimethalin, Pretilachlor	0.018, 0.064	0.01-0.08, 0.05-0.13
Organophosphate	Dimethoate	0.022	0.01-0.23
Pyrethroid	Cypermethrin	0.013	0.01-0.04

fetida was found highly susceptible to the pyrethroid insecticide cypermethrin (LC_{50} – 0.054 mg kg^{-1}), the value was higher than its RAD.But Ingesfield (1984) exposed *Eisenia fetida* to 0.1, 1.0, 10 and 100 mg/kg of cypermethrin and noted a maximum of 7.5% mortality concluding that it was not toxic to earthworms. He explained that this non toxicity can be due to the property of pyrethroids being adsorbed onto the organic matter portion of the soil particles which renders part of the dose unavailable to the worms. Saxena *et al.*, 2014, has reported that Cypermethrin was least toxic to *Eisenia fetida*. Toxicity studies using cypermethrin on *P. Excavatus* in artificial soil has not been reported. The herbicide Pendimethalin comes next in the order of toxicity after cypermethrin. No toxicity studies have been reported on *P. excavatus* in artificial soil. Despite heavy usage and environmental persistence, little information is available regarding the effects of pendimethalin in non-target soil organisms (Belden *et al.*, 2005). The organophosphate Dimethoate comes next which showed less toxicity compared to cypermethrin and pendimethalin. Toxicity of dimethoate (insecticide) to an earthworm (*Aporrectodeaca liginosa tuberculata*) was studied in three different soil types (artificial soil, clayey soil, and humus sandy soil). Parameters measured were survival and biomass change of the earthworms. The biomass reduction of the earthworms occurred at lower concentrations than reduction in survival (Martikainen, 1996). Kula and Larink (1997), showed the effects of dimethoate (insecticide) are investigated in two standard test soils, the OECD artificial soil and the LUFA 2.2 soil. Acute and sublethal effects of dimethoate and on the standard test organism *Eisenia fetida* as well as *Eisenia andrei* were investigated in order to select suitable test conditions. Acute effects of dimethoate to *Eisenia fetida, Aporrectodeaca liginosa, A. rosea* and *Allolobophora chlorotica* showed only small differences within one order of magnitude. Whalen (2012) reported that laboratory bioassays showed the lethal and sub-lethal concentrations of dimethoate to adult *Eisenia fetida* (Savigny) and their offspring. The most sensitive toxicity endpoint was adult *E. fetida* growth rates. Lastly, the herbicide pretilachlor showed least toxicity among the four pesticides on *P. excavatus.* No toxicity studies using pretilachlor on *P. excavatus* has been reported so far.

Finally, it can be concluded from the above study of toxicity of different groups of pesticides on *P. excavatus* that artificial soil can be used as a potential test soil media in toxicity studies.

ACKNOWLEDGEMENT

We thank the Head, Department of Zoology, University of Kalyani and Principal, Raja N.L. Khan Women's College for providing necessary laboratory facilities. S.S, P.P.C and R.D thankfully acknowledge UGC for their financial support.

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