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Artificial Soil Test of Phorate on *Eisenia fetida*

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Abstract

Phorate an organophosphate insecticide is commonly applied on crops to kill the stem borers but major part of it reaches on the soil which is a pivotal component of the environment. The soil possesses diverse and numerous life forms amongst which earthworms are considered as major representative organisms. The level of toxicity of various pesticides is found different and is considered as one of the valuable parameters in killing the agricultural pests. In the present paper the acute toxicity (LC_{50}) of phorate was evaluated for the epigeic earthworm, *Eisenia fetida* and assessed by artificial soil test method as per proposed in OECD testing guideline No. 207 (1984). Results showed that acute toxicity (LC_{50}) of phorate was found to be 22.5 mg/kg at the end of 14 day artificial soil test, and is considered as 'extremely toxic' to the experimental worm. The purpose of the study was to ascertain its minimal dose to be applied to kill the pests but not the earthworms. These findings showed that a dose of less than its acute toxicity level may be applied on the crops that will not affect the earthworms.

Keywords: Acute toxicity; LC_{50}; Phorate; *Eisenia fetida*

Introduction

Phorate (O,O-Diethylthio S-ethylthio methyl phosphorodital), (trade name: Thimet-10G) a broad–spectrum organophosphorous compound, is a highly toxic pesticide used in agriculture against to control sucking and chewing insects like leafhoppers, leaf miners, mites, some nematodes and rootworms.

In eco-toxicological research, earthworms have been widely used as model terrestrial organisms to estimate the index of environmental pollution because of their importance in the structure and the function of the ecosystem of the soil. Earthworms are bioindicators of the contamination of soil and can be used to supply thresholds of security to watch degree of contamination of soil to examine the toxic effects of chemicals under laboratory conditions.

The main objective of the study was to evaluate the acute toxicity potential of phorate in an artificial soil on an epigeic earthworm species, *Eisenia fetida*. This test was standardized at the international level, being recognized and promoted by international organizations (OECD-organization of economical cooperation and development) to elaborate international guideline on environment quality assessment [1-5].

Methods

The study was designed to investigate the acute toxicity of phorate on one of the sensitive earthworm, *Eisenia fetida* (Savigny). The evaluation of acute toxicity of experimental test compound, Phorate on the experimental worm, *Eisenia fetida* was carried out as per OECD no. 207 (1984) guidelines for testing of the chemicals. All the worms were collected from the Worm House of the Department of Animal Science of the University. These were acclimatized for two months in the pre-decomposed cattle dung. Adult earthworms having fully developed clitellum with individual wet weight in the range of 300 -350 mg were segregated and selected for acute toxicity test. Phorate one of the widely used organophosphate insecticide by the farmers to protect their crops from chewing, sucking and boring insects, is very odious and highly poisonous pesticide [6-8].

In artificial soil test, soil consisted of 10% organic matter (peat moss), 20% kaolinite clay, 70% industrial quartz sand and calcium carbonate in order to adjust the pH in optimum range (6.0 ± 0.5). The dry components of the artificial soil were mixed thoroughly before water was added in order to achieve moisture content of about 35% dry weight. The physico chemical parameters were also determined. The soil was artificially contaminated by different concentrations of pesticide (dry weight basis). Phorate was added at 5, 10, 20, 40, 60 and 80 mg/kg soil. Each pesticide concentration was represented by five replicates and an additional five replicates for the control treatments. 600 adult *E. fetida* were segregated from the culture beds, washed with deionized water and kept in a trough on moist filter for three hours to devoid their gut contents. Prior to toxicity test, all selected earthworms were acclimatized in the artificial soil under test condition for 24 hours. After that 10 adult worms of same size and weight were released in to each tub separately. All the tubs were covered with a perforated plastic film and kept under laboratory conditions for 14 days. Mortality percentage was assessed after 7^{th} and 14^{th} days of experiment.

Dead worms were removed immediately from the tubs if any mortality was noted during the experimental period. The LC_{50} of phorate on *Eisenia fetida* was quantified using the log dose/probit regression line method (Figure 1). The average value of each concentration was plotted along X-axis and percent of mortality along Y-axis and the eye fitted curve was drawn. The LC_{50} values were calculated as per Finney on the basis of line drawn [9,10].

Results and Discussion

Log/probit analysis showed a clear-cut concentration-response relationship was observed. During the exposure period it was also noticed that earthworms mortility was increased with increasing dose of phorate concentration in each experimental tub; while in control tubs there was no mortality. In the tubs where phorate dose was 5 mg/kg no mortality occurred; while at the highest dose of phorate (i.e., 80 mg/

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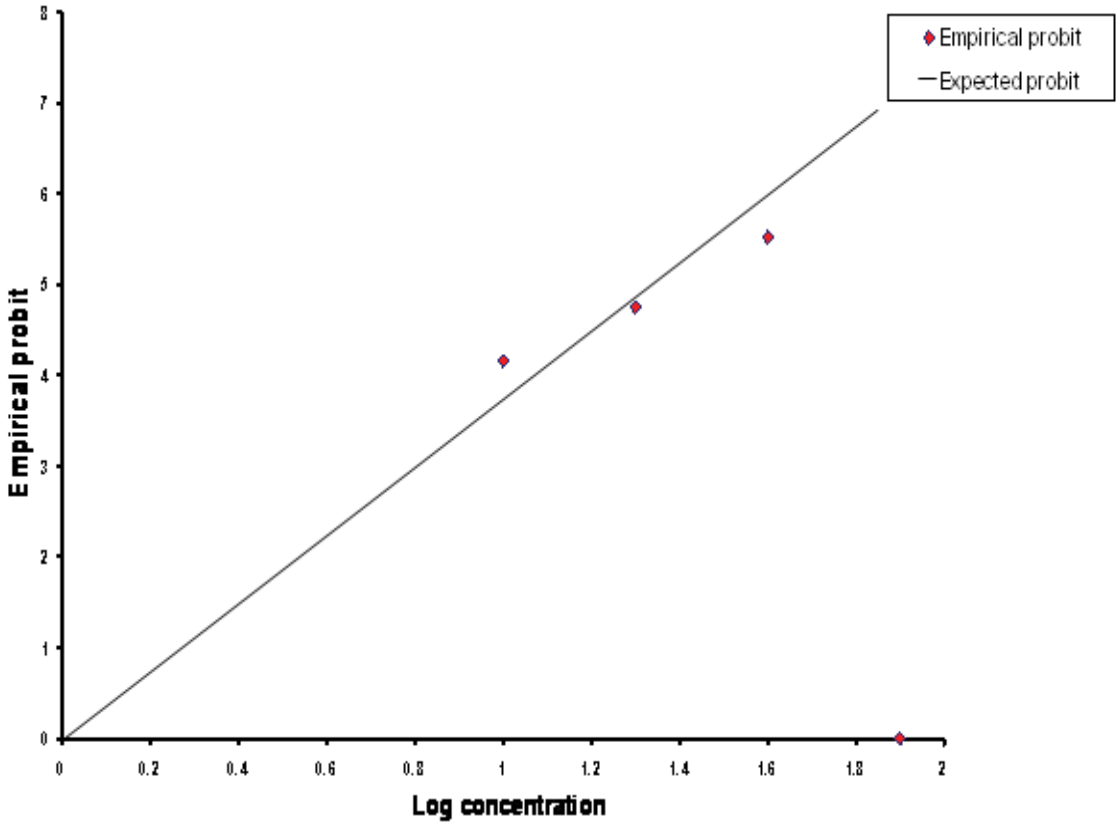

Figure 1: Showing regression equation to determine LC_{50} of phorate for *Eisenia fetida*.

kg) mortality was 100 percent. At 10 mg/kg dose of phorate, 20% of mortality and at 20 mg/kg dose 40% mortality of worms was noted. The mortality of worms was 70% at the dose of 40 mg/kg of phorate.

On the basis of 14 day LC_{50} results, the toxicity level of phorate may be considered as regulation of environment safety assessment test for agricultural pesticides. The suggested standard of toxicity is 'super toxic' when concentration of phorate was in the range of <40-80 mg/kg, extremely toxic in 20-40 mg/kg, very toxic in 10-20 mg/kg, moderately toxic in 5-10 mg/kg and relatively non-toxic in >1-5 mg/kg concentration. It may be concluded that acute toxicity (LC_{50}) of phorate was found to be 22.5 mg/kg at the end of 14 day artificial soil test, which is considered as 'extremely toxic' to the experimental worm, *Eisenia fetida*.

Although workers have reported similar trend of toxic effects of other organophosphates (OP). Brown [11] has stated that OP compounds intended for soil use have little lasting effect on earthworm population but some can cause heavy initial kills; while Bennuor [12] pointed out that formothion-an organophosphate is found toxic to *Aporrectodea caliginosa* causing moderate reduction in numbers and body mass. Bharati and Subba Rao [13] have reported LC_{50} of phosphamidon, monocrotophos and dichlorovos for earthworm, *Lampito mauritii*. Patnaik and Dash [14] exposed earthworms to different concentrations of pesticides for LC_{50} of monocrotophos for *Drawida calebi* and *Octochactona surensis* to be 14.79 ppm and 14.13 ppm and fenitrothion to be 15.67 ppm and 15.14 ppm, respectively. Reddy and Rao [15] observed LC_{50} for profenofos - an organophosphate to be 4.56 and 3.55 μL/cm^2, respectively for *Eisenia fetida*. Gupta et al. [16] found that cypermethrin was more toxic to *Perionyx excavates* (LC_{50} - 0.008 mg/kg) followed by endosulphan (LC_{50} - 0.03 mg/kg), carbayl (LC_{50} - 6.07 mg/kg), aldicarb (LC_{50} - 7.3 mg/kg) and monocrotophos (LC_{50}-13.04 mg/kg); while Mosleh et al. reported LC_{50} of endosolphon 12.29.5.82 and 3.36 ppm for 2, 7, and 14 day after exposure. Hu et al. observed that the LC_{50} of chlorpyrifos in *E. fetida* was 83.63 mg/kg in the 14 day artificial toxicity test. The toxicity of the OP compounds to earthworms is mainly through their ability to inhibit the acetylcholine esterase (AchE) - an enzyme responsible for the synthesis of the neurotransmitter, Acetylcholine which is found in the synaptic regions of the nervous system [17,18] and the role of neurotransmitter is considered as a vital process for usual functioning of the entire nervous system [19-21]. The chloropyrifos - an organophosphate compound have toxic effect on the earthworm, *Aporrectodea caliginosa* causing mortality on high dose [22,23]. Park et al. [24] assessed the effect of salinity on toxicity and metabolism of endosulphan in the worm, *Eisenia fetida*. Abbiramy and Ross [25] demonstrated that inorganic fertilizer urea was very toxic to earthworms when contacted directly and the lethal concentration (very toxic) was recorded 28 μL/cm^2 based on resulting LC_{50} value for urea. These studies suggest that care must be taken before using or applying any chemical pesticide on any crop.

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