

## ORIGINAL ARTICLE

# Acute toxicity of Phorate to *Eisenia fetida* using Simple paper Contact Method

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### ABSTRACT

Acute toxicity ( $LC_{50}$ ) of phorate was evaluated for the epigeic earthworm, *Eisenia fetida*. It was assessed by paper contact method as per proposed in OECD testing guidelines no. 207 (1984). It was  $20 \mu\text{l}/\text{cm}^2$  at the end of 48 hrs and considered as 'very toxic' to the experimental worm. The purpose of this study was to ascertain its minimal dose to be applied to kill the pests but not the earthworms.

**Keywords:** Acute toxicity, Earthworm, *Eisenia fetida*, Phorate.

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## INTRODUCTION

The potentiality of the farming lands is declining at a fast pace due to indiscriminate and excessive usage of chemical fertilizers and pesticides for the crop yield. These fertilizers boost up the growth of crops on one side and disbalance the nutritional status of the soil on the other ; while chemical ,pesticides kill the crop pests as well as transform the soil full of toxins. This causes killing of soil organisms which have a prime role in improving, maintaining and sustaining the soil fertility [19]. Earthworms, the known major augmenters of soil fertility, have also been seriously affected by the application of such toxic compounds in the cropping systems. Phorate- an organophosphate applied by the farmers to protect the crops from chewing, sucking and boring insects, is very odious and highly poisonous pesticide. It not only creates adverse effects to the soil organisms but fatal to them [13]. Ecological toxicity of phorate on earthworm, *E. fetida* was thus studied using a simple paper contact method. It is a screening test adopted by soil toxicologists to prove the toxic potential of chemicals to earthworms [14, 20, 22 and 23].

Looking in to such atrocious state, an attempt has been made to evaluate the acute toxicity of phorate for an epigeic species of earthworm, *Eisenia fetida* to identify its usual minimal dose that might affect normal functioning of these worms.

## MATERIALS AND METHODS

### Test organism: Earthworm

The laboratory study was designed to investigate the acute toxicity of phorate on the epigeic earthworm, *E. fetida* (Savigny, 1826). 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 were segregated with individual wet weight in the range of 300 - 350 mg and selected for acute toxicity test.

### Pesticide and experimentation

Acute toxicity test was performed on the selected earthworm, *E. fetida* to identify toxicity potential of phorate as proposed in OECD (1984). In the test, glass petriplates, (sized 14 cm diameter) earthworms were taken and the inner base of each petriplate was covered with Whatman filter paper No.1. Five different concentrations of phorate were prepared by dissolving phorate in 5 ml of deionised water in

geometric series of 5, 10, 20, 40 and 80 mg. 5 µl concentration of 5 mg phorate was pipetted in to a test petriplate having a wet filter paper with deionised water. Similar process was carried out for remaining concentrations of 10, 20, 40 and 80 mg of phorate except for those petriplates used for the control set. Five replicates were made for each treatment. Mature *E. fetida* were segregated from the culture beds, washed with deionised water and kept in a trough on moist filter paper for three hours to devoid their gut contents. These worms were rinsed again with deionised water, blotted on a filter paper and placed one in each petriplate. Each petriplate was covered with a perforated plastic film. Test petriplates were kept at laboratory temperature of  $20 \pm 2^\circ\text{C}$  for 48 hours and worm's mortality was observed by giving a gentle mechanical stimulus to their prostomial part.

## RESULTS AND DISCUSSION

It may be noted that phorate was toxic to the worm, *E. fetida*. Mortality was observed following its exposure to moderately high dose (20 mg / 5ml concentration of phorate). The acute toxicity ( $\text{LC}_{50}$ ) of phorate for the worm, *E. fetida* was found 20 µl /cm<sup>2</sup> at the end of 48 hrs and is considered as 'very toxic'. As per the standard toxicity values, the phorate may be classified as super toxic (<1.0 µl/cm<sup>2</sup>), extremely toxic (1-10 µl/cm<sup>2</sup>), very toxic (10-100 µl/cm<sup>2</sup>), moderately toxic (100-1000 µl/cm<sup>2</sup>) or relatively non toxic (>1000 µl/cm<sup>2</sup>) on the basis of results of 48 h  $\text{LC}_{50}$  values [20]. Earlier workers have reported similar trend of toxic effects of other organophosphates (OP) on earthworms. Phorate was found extremely toxic to earthworms [5, 11, and 23] and the worms were died from many soils even at its normal agricultural dose. It has been stated that OP compounds intended for soil use have little lasting effects on earthworm population but some can cause heavy initial kills [4]. Formothion- an organophosphate was noted toxic to *Aporrectodea caliginosa* causing moderate reduction in their numbers and body mass [2]. Phosphamidon, monocrotophos and dichlorovos showed toxicity to the earthworm, *Lampito mauritii* after 96-h  $\text{LC}_{50}$  study [3]. Exposure of different concentrations of monocrotophos for 24 and 48 hrs, the  $\text{LC}_{50}$  for *Drawida calebi* and *Octochactona surensis* was recorded 14.79 and 14.13 ppm respectively and for fenitrothion it was 15.67 and 15.14 ppm. Toxicity of profenofos - an organophosphate after 24 and 48-h  $\text{LC}_{50}$  was 4.56 and 3.55 µl/cm<sup>2</sup> for *E. fetida* [19]. It was also recorded that the cypermethrin was more toxic to *Perionyx excavates* ( $\text{LC}_{50}$  - 0.008 mg / kg) followed by endosulphan ( $\text{LC}_{50}$  - 0.03 mg / kg), carbaryl ( $\text{LC}_{50}$  - 6.07 mg / kg), aldicarb ( $\text{LC}_{50}$  - 7.3 mg/kg) and monocrotophos ( $\text{LC}_{50}$ -13.04 mg/kg) (8). The toxicity of 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 [20, 12] and the role of neurotransmitter is considered as a vital process for usual functioning of the entire nervous system (5, 13 & 9). The chloropyrifos- an organophosphate compound had toxic effect on the earthworm, *A. caliginosa* causing mortality on high dose [21&18]. Urea- a common nitrogenous fertilizer was found very toxic to the earthworm, *Eisenia fetida* as  $\text{LC}_{50}$  for urea was 28 µl/cm<sup>2</sup>[1]. In our opinion this test is an initial screening technique to assess the relative toxicity of chemicals to soil biota including earthworms in which the chemicals are absorbed by their skin that proves the toxicity of chemicals to these worms. Though, the method fails to represent the situation of the chemicals in the soil but it is important to know the toxic status of a particular pesticide, whether it is toxic or not. If the pesticide proved to be toxic, further extensive study on soil test could be of immense importance and be carried out as it may cause behavioral changes or mortality to soil micro and macro fauna and flora that play significant role in maintaining, sustaining and improving the physico-chemical and biological fitness of the soil. Earthworms are one of them. In general, most organophosphate insecticides are not very toxic to earthworms [6] but present study conducted for the first time for the said organophosphate disproved it. The findings showed that all the pesticides are not safe to these beneficial soil animals. Moreover, even the low as well as the sub-lethal dosages of most of the pesticides can lead to worm's mortality or worm's starvation due to behavioral changes and refusal to feed on the contaminated soil. The study also provides a clue that how much dose of phorate will not affect earthworms - the bio-engineers of the soil.

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