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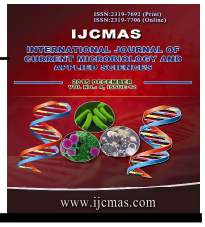

Original Research Article

Earthworms as biomarkers for the Assessment of Chlorpyrifos Effects on Soil Fauna

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Keywords

Chlorpyrifos, *Eudrillus euginae*, Microcosm, Bioassy test, Log probit analysis

A B S T R A C T

An incubation study was conducted to know the toxicity level of chlorpyrifos in soil fauna by using earthworm species *Eudrillus euginae* as a biomarker. The earthworms were incubated in the spiked soils, with concentrations of 200ppm, 100ppm, 50ppm, 25ppm and 10 ppm, for a period of 24 hours. From the study, 100 percent mortality was observed in 200 ppm and 100 ppm. In the case of 50 ppm and 25 ppm, it was 87.5 and 62.5 respectively. The LC50 and LC95 concentrations were calculated from that it was clear that chlorpyrifos is toxic to earthworms.

Introduction

Chlorpyrifos [O, O-diethyl-0-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate] is one of the most widely used organophosphate insecticides. It is effective against a broad spectrum of agricultural and household pests. The behaviors of chlorpyrifos in soil were studied intensely due to its long persistence in soil environments and harmful impacts upon organisms. Earthworms comprise the largest proportion of the biomass of the soil fauna and contribute to the process of cycling of nutrients in terrestrial ecosystems. They can accumulate soil-chemicals and are widely used as bioindicators of soil health and toxicity testing for chemicals (Lanno *et al*, 2004).

However, little is known about availability of chlorpyrifos to earthworms. Therefore, in this study the species of *Eudrillus eugineae* were used as soil model organisms to investigate the effect of chlorpyrifos. The aims of the present study were the bioassay of the chemical chlorpyrifos by using the Earthworm as the test organisms.

Materials and Methods

Pesticide

The technical formulation of chlorpyrifos (Lethal, EC 20%) a type of organophosphorus pesticide was selected for the study. The pesticide was purchased from

the local pesticide supplier (98.2 per cent purity Radar 20 EC from ISAGRO ASIA).This commercial formulation of chlorpyrifos was dissolved in sterile distilled water for amendments to soil samples.

Earthworm

Adult *Eudrilluseuginae*of equal size (10-15 cm) was purchased from vermicompostunit of college of agriculture Vellayani, Trivandrum. Identification of earthworm species was performed according to the methods described by Yan et al. (2000). All earthworms were allowed to acclimatize to laboratory conditions at for at least 4 days before use. Soils were mixed with decayed leaves and decomposed cow dung manure, and kept at room temperature (25^0 C). Soil water content was measured every week and moisture was adjusted to 35% of the maximum water-holding capacity by adding distilled water whenever necessary.

Soil Collection and Sample Preparation

Preparing stock

Prepared stock solution of chlorpyrifos (Radar 20EC) 10,000 ppm (50 ml chlorpyrifos in 950 ml distilled water).

Preparation of artificial microcosm for earthworms

Prepared artificial microcosm forearthworms (brought from the vermi-compost yard of Vellayanicampus) by mixing 1kg cow dung, 1 kg vermi-compost and surface soil (0~15 cm) collected from the field at Vellayani campus, Kerala Agriculture University. Soil was air dried at room temperature and passed through a sieve of 0.85 mm and mix these thoroughly (weighed 300g) and filled it into the 500 ml capacity flask. Water was added to adjust the soil moisture level to approximately 80% of water holding capacity (WHC).

Preparation of different ppm concentration in soil

Chose different concentration like 200ppm, 100ppm, 50ppm, 25ppm, 10ppm this concentration of solutions were prepared from the stock solution, and these are used for the bioassay test. The soil was mixed carefully with a glass rod.

Inserting worms to the flask containing pesticide

The flasks were then covered with muslin cloth and incubated at room temperature for 24 hours.

Bioassy test

For the purpose of bioassay test, earthworms from the vermipit (100gm) were used. For determining the LC50 selected 4 worms having similar size were inserted into each flask containing pesticide in different concentrations (ppm).All determinations were repeated twice for each concentration. A control set with equal number of Earthworm was also kept. Each flask was labeled was labeled with details such as concentration, replication, date and time of experiment. Observations on mortality were recorded at the time interval of 24 hours after treatment. Dead organisms were removed after taking observations at each time interval.Acute toxicity of chlorpyrifos was determined by estimating median lethal concentrations (LC50) with the help of log probit analysis (Finney, 1971).

Results and Discussion

LC_{50} of chlorpyrifos at 24 hrs =18.14083

LC_{95} of chlorpyrifos at 24 hrs =108.6499193

Log LC_{50} of chlorpyrifos at 24 hrs = 4.258657

Log LC_{95} of chlorpyrifos at 24 hrs = 5.036029

Table.1 Number of Earthworms Died in each Flask out of 4

No, of earthworms dead /4										
200ppm		100ppm		50ppm		25ppm		10ppm		
R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	C
4	4	4	4	3	4	2	3	1	1	0

Table.2 Percentage of Average Mortality of Earthworms

Average mortality (%)					
200ppm	100ppm	50ppm	25ppm	10ppm	control
100.0	100.0	87.5	62.5	25	0

Fig.1 Probit Mortality of Earthworms Towards Log Concentration of Chlorpyrifos

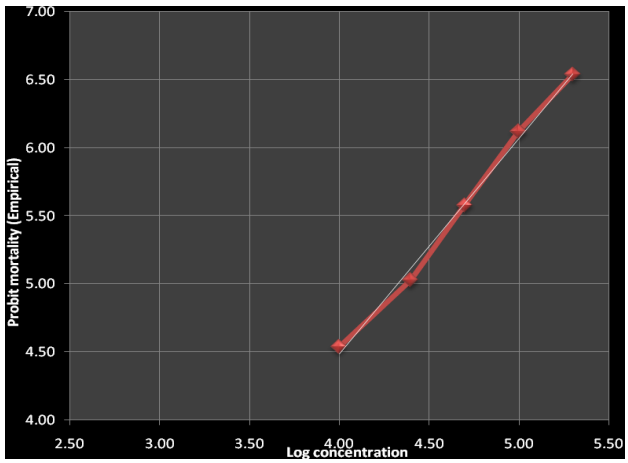
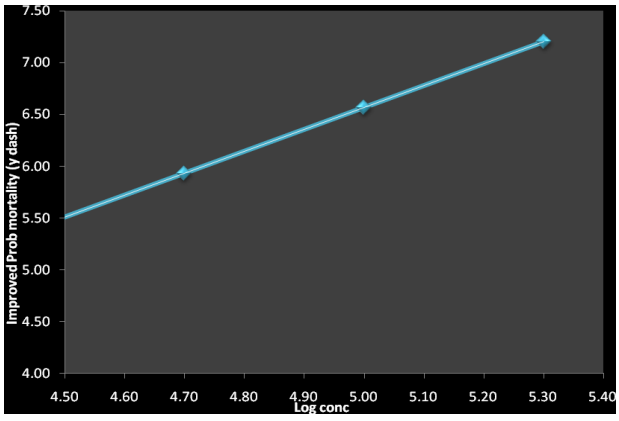


Fig.2 Improved Probit Mortality Towards Log Concentrations



The study revealed that the organophoshorus pesticide chlorpyrifos is toxic to earthworm species *Eudrilluseuginae* at a concentration of 100 ppm, which showed 100 per cent mortality within 24 hours (Table 1 and 2). Earthworm is one of the indicator organisms of macrofauna. From the probit analysis high level of LC value (Fig. I and II) can be seen so the study gives the information that the chlorpyrifos is toxic to earthworms at high concentrations levels from 100 ppm.

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