International Journal of Zoology Studies
ISSN: 2455-7269
Impact Factor: RJIF 5.14
www.zoologyjournals.com
Volume 3; Issue 2; March 2018; Page No. 96-98

Acute toxicity evaluation of chlorpyrifos on earthworm *Megascolex konkanensis* (Fedarb, 1898) by a simple paper contact method

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Abstract
The study was carriedout on the acute toxicity of chlorpyrifos on earthworm *Megascolex konkanensis* (Fedarb,1898) by a simple paper contact method. The filter paper contact test was performed for earthworm acute toxicity assessment based on OECD guidelines 207. The test substance chlorpyrifos is dissolved in acetone to give a range of known concentrations. The Whatsman No.1 filter paper of diameter 10cm was placed in 10 cm petridish. 1% of toxic test solution was prepared from selected pesticide. Test substance of desired concentration is pipetted into each petridish. The petriplates were incubated in the dark at room temperature for 48hrs. At the end of 48hrs mortality was recorded. The dose response test always included a control organism which is treated with acetone. 1% solution of Chloropyrifos sprayed in 10 set of petriplates and each set contain two number of earthworm. Therefore 0.7ml concentration of test solution showed very toxic to earthworm. Some clinical signs like pinching off coiling, curling and mucus secretion were observed.

Keywords: toxicity, chlorpyrifos, *Megascolex konkanensis*, filter paper

1. Introduction
Earthworms are the representative of soil fauna being largest invertebrate biomass of soil ecosystem and they are common in many soils and thus are vulnerable to various impacts on soil. Pesticides are widely used throughout the world in agriculture to protect crops and in public health to control diseases [1]. The soil not only acts as a substrate for organisms but also as a recipient medium for chemicals. The earthworm, *Eisenia foetida* is reported to be a suitable test species [2]. Pesticides occupy a unique position among the many chemicals that we encounter daily, in that they are deliberately added to the environment for the purpose of killing or injuring some form of life [3]. The ecotoxicology testing with earthworms routinely employ acute toxicity test in the laboratory tests in the laboratory and were developed based on the OECD Guidelines 207 [4]. Acute toxicity studies are designed to express the potency of the toxicant in terms of a median lethal dose LD50 causing death of 50% of the universal population of the species exposed under the defined conditions of the test [5]. These standardized earthworm toxicity experiments were mainly conducted on contact filter paper tests or artificial soil tests. Filter paper tests were used to understand the relative toxicity of a wide spectrum of chemicals to different earthworm species [4, 6]. Toxicity assessment of pesticides to earthworms is mainly based on *E. andrei* or *E. fetida* as the standard test species [4, 12]. As compost worms, *Eisenia* species have a limited ecological role compared to local mineral dwelling species. In addition, they mainly occur in temperate region [7]. Pointed out the use of tropical species in toxicity tests could contribute to a more relevant and reliable risk assessment of chemicals in these areas. The aim of the study is the determination of acute toxicity of chlorpyrifos on earthworm *Megascolex konkanensis* [8] by a simple paper contact method.

2. Materials and Methods
2.1. Requirements
Adult earthworms with clitellum (at least two months old) and individual weight 150-250mg, Whatman No.1 filter paper, Petridishes, Chlorpyrifos standards (Sigma Aldrich), Acetone, weighing balance, pipette,1ml pipette tips and distilled water.

2.2. Selection and Maintenance of earthworms
Megascolex konkanensis was adopted as the test species (fig:1) as a model organism for acute toxicity test has a short life span of 8-10 weeks easy to rear into a genetically homogenous culture hence meet the basic requirement for a species to qualify as a model for toxicity tests. *Megascolex konkanensis* [8] Megascolocideae are large family of earthworms which has native representatives in Australia, New-Zealand, both south-east and East Asia and North America. These are widely distributed in the tropical and temperate zones.

Fig 1: *Megascolex konkanensis*

Table 1: Taxonomic classification of *Megascolex Konkanensi*

Kingdom	**Animalia**
Phylum	Annelida
Class	Clitellata
Order	Haplotaxida
Suborder	Lumbricina
Family	Megascolicidae
Genus	*Megascolex*
Species	*Konkanensis*

Simple method was used for culturing earthworms. Hand sorting method was used for collecting earthworms. This method is widely used for sampling earthworms in India [9, 10]. The quadrate is provided on 20*20*30cm^2 and are gently broken and the worms are hand sorted. The collected worms were kept in a culturebed prepared following the method given by [11] Soil and litter were used for preparing culturebed. The worms were released gently to the surface of culturebed. Plastic net was used for covering the tray to avoid predators. Culturebed was set in a laboratory condition. Age synchronized adult earthworms, which possessed clitellum and had an individual wet weight of 250–350mg, were subjected to acute toxicity testing following guidelines for testing of chemicals for earthworms [4, 12].

2.3. Pesticides

The pesticide tested in the experiment was organophosphorus insecticides, chlorpyrifos. Chlorpyrifos was ordered from Sigma-Aldrich Chimie S.A.R.L. (Saint Quentin-Fallavier, France). Other chemicals of rigorously pure grade were also obtained from Sigma-Aldrich Chimie S.A.R.L.

2.4. OECD contact filter paper test

A modified filter paper contact test was performed for earthworm acute toxicity assessment based on OECD guidelines 207 [4]. Worms were kept on moist filter paper to depurate for three hours before subjecting to toxicity assay. They are then washed before use. The test substance, compounds chlorpyrifos (Sigma Aldrich) is dissolved in acetone to give a range of known concentrations. A Whatsman No.1 filter paper of diameter 10cm was placed in 10cm petridish.1ml of test substance of desired concentration is pipetted into each petridish and rotated horizontally to spread chlorpyrifos evenly and then the solvent was allowed to evaporate. The filter paper was remoistened with 1ml distilled water and each earthworm was weighed and transferred to petriplates corresponding to the different concentration. The petriplates were incubated in the dark at room temperature for 48hrs. At the end of 48hrs mortality was recorded. An earthworm was considered dead if it failed to respond to a gentle mechanical touch on the front end of the test. The dose response test always included a control organism which is treated with distilled water.

2.5. Statistical Analysis

The OECD filter paper contact test method, organism test based on the resulting 48h LC50 values,the fertilizer will be classified as supertoxic (<1 ml/1ml), extremely toxic (0.8–0.9 ml/1ml), very toxic (0.6–0.7 ml/1ml), relatively nontoxic (>0.5 ml/1ml) [13].

3. Result and Discussion

The OECD filter paper contact test organisms were subjected to the assessment of toxic effects of Chlorpyrifos on earthworm, *Megascolex konkanensis*. Experiment were set in three replicates, each replicate contain 10 set of petriplates with earthworm species as a test animals and one set of petriplates used as a control. 1% solution of Chloropyrifos sprayed in 10 set of petriplates and each set contain two number of earthworm shown in (fig-3). After 48hrs all individuals found to be dead in all the very toxic and supertoxic concentration of Chlorpyrifos and only control found alive. Since the organisms analyzed varied in their weight.

0.1ml–1ml test solution compare with control which was having 1ml distilled water. The test solution of 0.8ml, 0.9ml and 1ml of 1% chloropyrifos all earthworms were died and melt. This concentration was shown very toxic and supertoxic to earthworms. In the test solutions of 0.1ml and 0.3ml the earthworms were very active and moving. In test solutions of 0.4ml and 0.5ml the earthworm were slightly less active and moving and in 0.6ml and 0.7ml, concentration of test solution earthworm was live and less active was seen in this experiment.

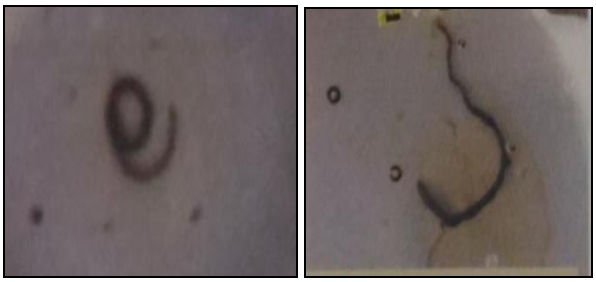

Fig 2

The LC50 of superphosphate recorded as 210mg/5ml (i.e.300 µg/cm 2) (Kovilpathu, *et al*., 2013). The LC50 mortality of chloropyrifos (Organophosphate) was found 0.4ml-0.7ml acetone in contact filter paper test method shown. Therefore 0.7ml concentration of test solution showed very toxic to earthworm. Some clinical signs like pinching off [13] coiling, curling and mucus secretion were observed [14] shown in (fig-4). The filter paper contact test remain as a quick and efficient method to convey first hand toxicity information of a compound. The logic behind the test is that earthworms have a cuticular layer comprising protein and polysaccharides which fascilitates absorption of lipophilic substances from the environment.

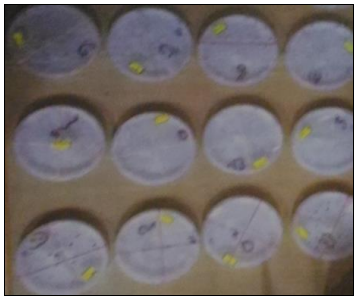

Fig 3: Filter paper contact test set up

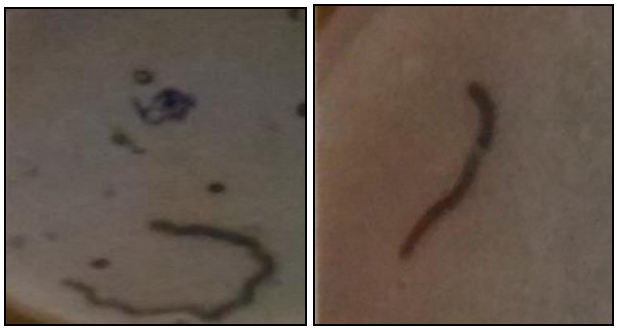

Fig 4: Observed sign of Chlorpyrifos toxicity in *Megascolex konkanensis*

Conclusion

From the present work conclude that the filter paper contact test method is the best method for assessment of insecticide. By this method, investegator found that LC50 mortality of chloropyrifos on earthworm. From the observation 0.7ml concentration of test solution shown LC50 mortality on earthworm *Megascolex konkanensis*. This species of earthworm were highly affected from test solution concentration 0.6ml to 1ml is super toxic.

References

1. Manoj K, Ashok K. Application and Health Effects of Pesticides commonly used in India. ECO Services International, Green pages, 2007.
2. Heimbach F. Comparison of laboratory methods for toxicity testing with earthworms. In: Earthworms in Waste and Environmental Management (Ed. C.A. Edwards and E.F. Neuhauser), 1988, 329-335.
3. Lucio GC. Toxic effects of pesticides. In: Casarett And Doull'S Toxicology The Basic Science of Poisons, Seventh Edition, Eds: Curtis D. Klaassen, McGraw-Hill Medical Publishing Division, 2008, 883-930.
4. Organization for Economic Co-Operation and Development. OECD guideline for testing of chemicals, Section 2. Test No. 207: Earthworm Acute Toxicity Test. Paris, 1984.
5. Edward CA. Earthworm ecology 2nd edn CRC Press LLC, Boca Raton, FL, USA, 2014, 12-23.
6. Wang Y. Comparative acute toxicity of twenty four insecticides to earthworm, Eisenia fetida. Ecotoxicology environment, 2012, 12-16.
7. De Silva PMCS, Pathiratne A, van Gestel CAM. Toxicity of chlorpyrifos, carbonfuran, mancozeb and their formulations to he tropical earthworm *Perionyx excavates*. Applied Soil Ecology. 2010; 44:56-60.
8. Fedrab. *Lumbricus corethrurus*, Busten schwanz. *Archivfür Naturge schichte*, 1856-1898; 23(1):113-116.
9. Edwards CA, Lofty JR. Biology of earthworms. Chapman & Hall, London, 1972, 283.
10. Reynolds JW. The earthworms (Lumbricidae and Sparganophilidae) of Ontario. Life Sci. Misc. Publ., Roy. Ont. Mus., 1977, 141.
11. Ismail SA. Vermicology: The biology of Earthworms. Orient Longman Limited, Chennai, 1997, 92.
12. Organization for Economic Co-Operation and Development. OECD guideline for testing of chemicals. Test No. 222: Earthworm Reproduction Test (*Eisenia fetida/andrei*). Paris, 2004.
13. Roberts B, Dorough. Relative toxicities of chemicals to the earthworm *Eisenia foetida*. Environmental toxicological and chemicals. 1984; 3:67-78.
14. Rao J, Kavitha P. Toxicity of azodrin on the morphology and aceyly cholinesrase activity of the earthworm Eisenia foetida. Environmental Research. 2004; 96:323-327.