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Assessment of Mortality (Lc_{50}) Of Cythion and Dieldrine by Filter Paper Contact Test on Earthworm *Esenia Feotida*

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Abstract: ***We used filter paper contact test method for obtaining LC_{50} for cythion and dieldrin. This Organophosphate is very poisonous, which affects the body and Growth of Earthworm. We had seen in the test solution of 0.8mg/cm^2, 0.9m g/cm^2 and 1mg/cm^{2}of 1% dieldrin, all earthworms died and melt. Similarly, cythion 0.3 and 0.27 showed the same result. This concentration was shown supertoxic to earthworms. In the test solutions of 0.1mg/cm^2 and 0.2mg, the earthworms were very active and not stationary. In test solutions of 0.3mg/cm^2 and 0.4mg/cm^2 the earthworm was slightly less active, and in 0.5mg/cm^2, the concentration of test solution earthworm was live and less active in this experiment. Similarly the 0.03m g/cm^2, 0.06 mg/cm^2 and 0.09 mg/cm^2 concentration showed same effect for cythion. We used three replica test set for the better resulting and calculated mortality. At the end of the experiment, we observed that 0.5 mg/cm^{2}concentration of dieldrin and 0.15m g/cm^2 for cythion was shown LC_{50} value of mortality. In some set like 1 mg/cm^2, 0.9 mg/cm^2 was very highly affected on earthworm they had been broken, melt and the body had destroyed. It was shown directly effect on the earthworm's body and growth.***
Keywords: Filter Paper, Cythion, Dieldrine, Esenia Feotida.

INTRODUCTION

Earthworms (Annelid) play important roles in the growth of plants. The earthworms give an important role in agriculture and food production. Among the organisms with their support activity in the filth, the earthworms are recognized for their significant role involving the betterment of physical and chemical features of land, and so increasing its fertility (Abdul Rida and Bouche, 1997). The soil is fundamental to the diverse communities of microbes, plants, and invertebrate and vertebrate animals that comprise the terrestrial ecosystem, and it is significant to consider the effects and hazards of polluted soil sites in relation to these ecological receptors (Ann, *et al.,* 1999). Pesticide applied to control, turf diseases or insect pests may severely affect earthworm. Malathion is considered one of the most abundantly used organophosphate pesticide, induced adverse effects in a non-target organism like an earthworm (Wali, *et al.,* 1984). *Eisenia fetida* is the standard test organism used in terrestrial Ecotoxicology, because it can be easily kept on a variety of organic wastes with short generation times (ISO, 1993). Its susceptibility to chemicals resembles that of true soil organisms. Sensitivity tests of multiple earthworm species have revealed that *Eisenia fetida* is comparatively less sensitive (Fitzgerald, *et al.,* 1996).

Vermicompost as organic manure was identified to put plant available forms, for example, nitrates, phosphates, exchangeable calcium and soluble potassium (Orozco, *et al.,* 1996). It was identified to play a critical part in the outgrowth and maturation of a mixture of plants (Edwards, 1998). A broad scope of plants, for example, grain and legumes, vegetables, decorative and flowering plants (Edwards and Burrows, 1988) and field crops (Arancon, *et al.,* 2006) are known subsequently influenced variously every action of vermicompost. A great proportion of biomass of terrestrial invertebrates is represented by earthworms which play an important role in increasing the nutrient content of the soil. Earthworms play a vital role in the maintenance of soil structure, functions, and fertility. Their activities modify soil aeration, drainage, and availability of nutrients for plants generally integrates soil organic and mineral elements to improve soil structure. Earthworms have been selected as a suitable representative of soil organism as they are key components of soil biota and contribute to the overall productivity of agricultural soils through their feeding, casting and burrowing activities (Culy and Berry, 1995). Crawlers are not practically killed by toxic chemicals, but their magnification, coming age, and bearing are aside from physically contacted on. They also accumulate several chemicals in their tissues at higher calibres than that of the substrate anywhere they await whatever these properties figure them first-rate for surveys as environmental pollution. Crawlers have a considerable influence on the physical construction of the soil by their active burrowing and ingestion of the land. This effect in the blending of the surface and sub-surface soils and enhance nutrient dynamics, because by reducing organic matter into its components, they liberate nutrients usable by other organisms (Lee, 1987; Werner, *et al,.* 2005). Their presence or absence in any soil, and the overall species composition may also reflect environmental changes that are not easily made out using the physical or chemical substance. This offers a sensitive measure of land contamination.

MATERIAL AND METHODS

Experimental Animal

Earthworm, *Eisenia foetida* (Savigny, 1826) is a recommended earthworm test species by Organization for Economic Co-operation and Development (OECD, 1984a) and European Economic Community (EEC, 1985).

Animal Collection

Earthworm, *Eisenia foetida* brought from commercial suppliers, Nursery Department of Forest, Wadali, Amravati and adopted as the test species, recommended by (OECD, 1984) guideline for testing of chemicals no. 207, earthworm, and acute toxicity tests.

Biology of Earthworm

Earthworms are invertebrates composed of many parts. They don't have bones and move by contracting and relaxing the body segments in chronological sequence. They also have little bristle like organs that help them adhere to slippery surfaces. The most earthworms have both male and female organs. Typically, even then, they still require a mate to multiply. When earthworms mate, they lay side by side in reverse side. At that moment they exchange sperm. The sperm stored in small chambers called spermathecal apertures. These are located in front of the egg-producing organs. After matching, the swollen external gland called the clitellum produces egg cases called cocoons. From 3 to 1,000 cocoons can be raised per year, depending on species and environmental conditions. Typically, an earthworm will produce 20 to 30 cocoons per year, with each cocoon containing 1 to 10 eggs. Peak cocoon production is in the spring or early summer. The eggs in the cocoons hatch when conditions are right. Under ideal conditions, it may take from 1 to 5 months for the eggs to incubate it. It may then lease from 3 to 12 months before these worms are sexually mature. Worms typically live only a few months because of the many environmental threats they face. They have been observed to hold out for 10 years in a protected environment. In a favorable environment previously without earthworms, earthworm populations increased 80-fold in 4 years after presentation. Insect Taxonomy (type), Anatomy, Morphology (appearance) and Biology (life cycle). Taxonomy is the science of identifying and classifying living organisms. While there are reportedly over 6000 species of earthworms, only 6 or 7 are used for vermin composting. The type of worm used depends on in part on the climate and temperature range. Worm selection varies from tropical (to a lesser extent than 23 degree latitude) to temperate climates. *Eisenia fetida, Lumbricus rubellus, Eisenia hortensis,* and *Eudrilus Eugenia* are the scientific names of worms commonly used for vermin composting. These worms can also be utilized for bait worm production (www.earthwormbiology.com).

Crawlers do not have lungs, instead, oxygen is obtained through the body skin; thus water moisture is important for gas exchange and dynamic development. A bedding moisture content of 60 to 90% is recommended. Insects can live on for several weeks in very wet or flooded systems if there is adequate oxygen. The sizes of *E. fetida* vary (small to large) depending on the food available and moisture content of the environment. A commonly provided figure is 1000 "average" size *E. fortitude* earthworms per pound with a range of from 500 large or 2000 small worms per pound. An *E. fetida* earthworm emerging from a cocoon will be 0.5 to 0.75 inches in length. The mature size can range from 2 to 6 inches with an average more like 3 to 4 inches. The mouth opening or orifice is small and there are no teeth like structures to physically cut the size of the material ingested. There is an internal "crop" or "gizzard" that assists in physical decomposition. The earthworms primarily consume decaying organic matter that has already been broken down by microorganisms such as bacteria, fungi, protozoa, etc. Both living and dead microorganisms that are providing the decomposition are also eaten.

The biological activity and a food web to provide decomposition are important to the success of the worms. The microorganisms are too influenced by moisture, temperature and oxygen availability. There is experimental evidence that the presence of earthworms increases the variety of micro organisms' present and biological decay.

Environmental factors affecting earthworm production

Earthworms have certain minimum care requirements that must be met on a regular schedule. The key environmental factors affecting earthworm growth, reproduction, and health are temperature, moisture, and aeration.

Temperature

Live and breed at temperatures between 55 and 85 degrees Fahrenheit. For commercial earthworm production, the ideal temperatures for growth and activity range from 60° to 80°. Bed temperatures should be between 60° and 70° to facilitate intensive cocoon production and hatching. If bed temperatures rise too high, they may be lowered by adding water, activating fans in or near the system, and reducing the amount of feedstock applied.

Moisture

Earthworms need adequate moisture to help them breathe through their skin. Beds need to sustain a moisture range of 60 to 85 percent and feel crumbly-moist, not soggy-wet. They should be sheltered from direct sunlight so they do not dry out and overheat. One method of increasing cocoon production after worms are fully established is to stop watering the beds for several days or until the top 1 or 2 inches are scarcely moist. Then dampen the beds enough to restore them to their recommended moisture content.

Chemical

Cythion

Commercial name: Cythion 50% E.C.

Chemical name: *O.O-Dimethylphosphodithionate-diethyl mercapto succinate.*

Chemical Formula: $C_{10}H_{19}O_6PS_2$

Physiological properties: Clear, brown colored viscous soluble in water and most of the organic solvent 5% DP.50%EC.20% WP.

Manufacturer: Cyanamid India Ltd.P.O.Atul Valsad 396020 (Gujrat State).

Dieldrin
Commercial name: Quinalphose 25% EC
Chemical name:
12341010-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8-8a-octahydroendo-1,4-exo-5,8-dimethanonaphthalene(HEOD).
Molecular formula Dieldrin: $C_{12}H_8Cl_6O$
Pysiological properties: Dieldrine is one of the more stable chlorinated hydrocarbons.
Solubility: Soluble in water 0.195 mg/L at temp 25°C
Manifacturer: Shell oil Company Gujrat.

Mortality (LC_{50}) by Filter Paper Contact Test
Experimental test was performed by following the method described in the OECD (1984) guideline for testing of chemicals no. 207. This is a simple screening test to identify the toxic potential of the chemical to an earthworm for cythion and dieldrin. The test vials were a plastic round box which was transparent of 14cm diameter and 2cm height. Round filter papers (What man No. 1) were cut to the suitable size and located in such a manner that all sides were lined with filter paper. The test solution was prepared and make up the volume of test solution from 0.03mg/cm^2 to 0.3 mg/cm^2 of cythion diluted in 100ml distilled water. From this prepared test solution 1ml sprayed to each 10 filter paper and kept on this filter paper in 10 round plastic box. One blank test was prepared with 1ml of deionised water and applied as a control. For each treatment, 3 replicates were used, each consisting of one earthworm per vial. Adult earthworms, which possessed clitellum and had an individual wet weight of 250–350mg, were selected for testing. Earthworms were washed briefly with deionised water, and were kept on moist filter paper for 3h to devoid the gut content, after which it was rinsed again with deionised water, blotted on the filter paper and placed in a test vial. An earthworm was introduced per vial and the vial was covered with a plastic film that had been plugged with small holes using needles. Trials were made out in the dark at 28±2 ^{0}C for 48 h. After 48 hours the earthworm was monitored for mortality by a gentle mechanical stimulus to the front orifice.

Similar experimental test was also used for dieldrine. The test solution was prepared and made up the volume of test solution from 0.1 mg/cm^2 to 1 mg/cm^2 of dieldrin diluted in 100ml distilled water.
Result and observation.

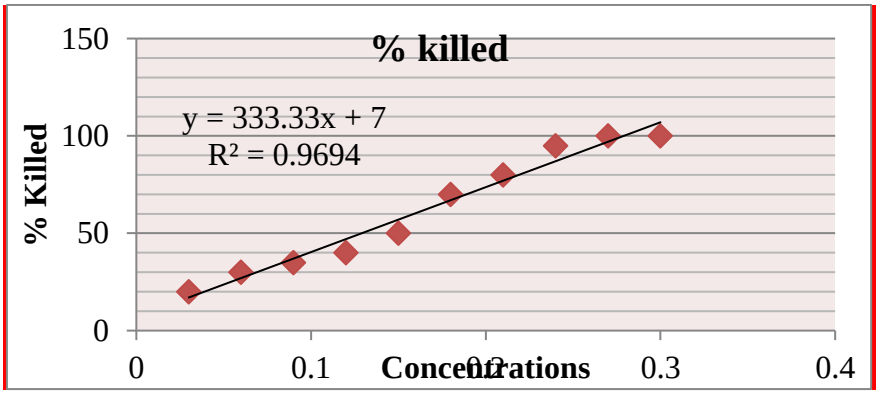
Assessment of mortality (LC_{50}) of cythion and dieldrine by filter paper contact test

Mortality (LC_{50}) of cythion
Different parameters were analysed concern to the toxic effect of two selected pesticides, dieldrin (organochlorine) and cythion (organophosphate) on an earthworm. These selected pesticides were showed dose and duration dependent impact on an earthworm. In this study, we found that cythion showed highly toxic impact than dieldrin. Mortality was calculated by plotting probit analysis curve . Filter paper contact test method was used for mortality and it was observed that the LC_{50} of cythion in filter paper contact test method was 0.15mg/cm^2.

Table 4.2.1 Percent mortality of earthworm (*Eisenia foetida*) treated with cythion.

No	Earthworm exposed	Concentrations (ppm)	% Killed	S.D
1	3	0.03	20	0.17321
2	3	0.06	30	0.24495
3	3	0.09	35	0.3
4	3	0.12	40	0.34641
5	3	0.15	50	0.3873
6	3	0.18	70	0.42426
7	3	0.21	80	0.45826
8	3	0.24	95	0.4899
9	3	0.27	100	0.51962
10	3	0.3	100	0.54772
11	3	Control	0	0

Figure: 4.2.1. Probit analysis curve showed LC_{50} of cythion



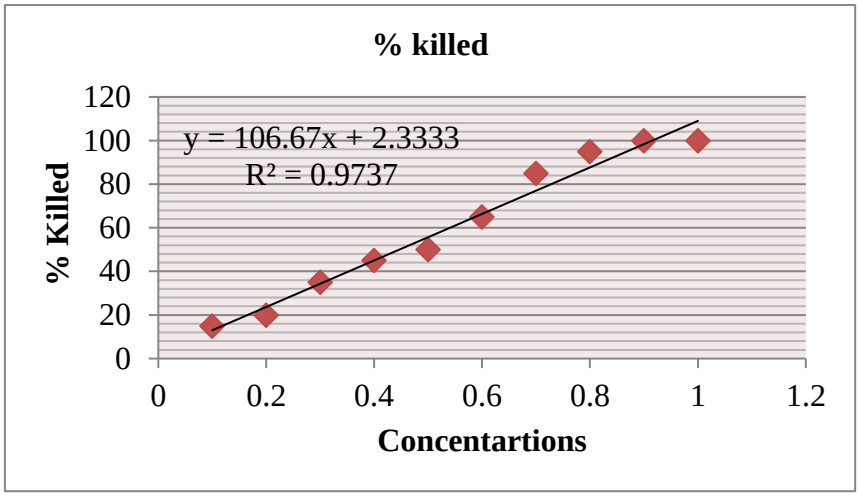
Mortality (LC_{50}) of dieldrin

The filter paper contact test method would be carried out for the finding of the LC_{50}. LC_{50} of dieldrin in filter paper contact test method was 0.5mg/cm^2. The above concentration of 0.5 mg/cm^2 concentration showed highly effected impact on earthworms and their body was bending, melted and died. The 0.8, 0.9 and 1mg/cm^2 concentration showed super toxic and 0.6 and 0.7 mg/cm^2 concentration determined less toxic impact.

Table: 4.2.2 Percent mortality of earthworm (*Eisenia foetida*) treated with dieldrine.

No	Earthworm expose	Concentrations	% Killed	S.D
1	3	0.1	15	0.31623
2	3	0.2	20	0.44721
3	3	0.3	35	0.54772
4	3	0.4	45	0.63246
5	3	0.5	50	0.70711
6	3	0.6	65	0.7746
7	3	0.7	85	0.83666
8	3	0.8	95	0.89443
9	3	0.9	100	0.94868
10	3	1	100	1
11	3	control	0	0

Figure: 4.2.2 Probit analysis curve showed LC50 of dieldrine



DISCUSSION

Mortality (LC_{50}) by Filter Paper Contact Test

The earthworms are farmer's friend and widely used for maintaining the fertility of the soil. The species *Eisenia foietida* is experimental animal recommended by (OECD, 1984). For the present work, we used filter paper contact test method for the assessment of toxic effects of cythion and dieldrin on the earthworm, *Eisenia foietida*.We had observed that the LC_{50} mortality of cythion and dieldrin was found 0.15mg/cm^2 and 0.5mg/cm^2 respectively in the contact filter paper test method. Therefore 0.15mg/cm^2 and 0.5mg/cm^2 concentration of test solution showed a very toxic impact on earthworm as a body of earthworm was banding seen and at higher concentration earthworm was died in all three replicate.

Acute toxicity of earthworm is an efficient tool in assessing ecological risks of contaminated soils (Lukkari, *et al.*, 2005) and the end point is mortality (Dean-Ross, 1983). Assessment of the toxic effect of two selected pesticides by using two different test methods of OECD (48 hours contact filter paper test and fourteen days standardization artificial soil test). The result of this study reveals that the two selected pesticides cythion and dieldrin were toxic to earthworms (*E.feotida*) based on LC_{50} values from 48 hours contact filter paper test, while they also showed a different degree of toxicity profile in fourteen days soil test based on pesticide classes. Contact filter paper test is an initial screening technique to assess the relative toxicity of chemicals to earthworms, where pesticides are mainly absorbed by the skin; however, it represents the situation in the soil ecosystems (Miyazaki, *et al.*, 2002; Grumianx, *et al.*, 2010; Tripathi, *et al.*, 2010). The result of this study also revealed that pesticides were toxic to earthworms than herbicides and fungicides. 48 hours LC_{50} of filter paper contact test method showed that pesticide cythion and dieldrin were highly toxic to earthworms (*E. feotida*) with LC_{50} values of these pesticides were 0.15mg/cm^2 for ctythion and 0.5mg/cm^2 for dieldrin. Some data were observed that the Lamda Cyhalothrin, Immidacloprid, Dimethoate and Termicot (chlorpyrifos) ranges between 0.000 ml to 0.001 ml, the toxic nature of these insecticides based on 48 hours filter paper contact test (Wang, *et al.*, 2012), who reported the acute toxicity of Neonicotinoid (Imidacloprid) as super toxic to earthworms (*Eisenia fetida*), Pyrethroid (Cypermethrin and Lamda Cyhalothrin) and Organophosphate (Termicot) as very toxic.

Zhou, *et al*., (2006) has reported that the weight of the earthworms was a more sensitive index compared to the mortality in indicating toxic effects of acetochlor and methamidophos. The highly toxic impact was observed at 0.9 mg/cm^2 concentration, at this concentration the body of the earthworm was broken, melts and ravaged. In this experiment we had selected 10 different concentrations of Organophosphate (cythion) from 0.30 mg/cm^2 to 0.300 mg/cm^2, some test animal had been active at 0.150 mg/cm^2 and comparatively dieldrin concentration at 0.1ml to 0.3 mg/cm^2 had shown less impact on earthworm they had been active and body morphology was not changed at this concentration. From 0.120 mg/cm^2 to 0.150 mg/cm^2 of cythion was also showing some impact on morphological level earthworm as they had compared with control. In the concentration of 0.180 mg/cm^2 to 0.240 mg/cm^2 was very effected on earthworm body. The body of an earthworm was folding and died where as some part of body of earthworms was broken down. At the concentration above 0.240 mg/cm^2 to 0.300 mg/cm^2 was super toxic which affect on the earthworm. The earthworms had been melting and died and tail region of earthworm had been broken. Similar response was observed by Muhamoud, *et al.*, (1984) Immidacloprid as toxic to the earthworm. Posthuma with LC_{50} of 0.011 ppm also with symptoms of poisoning including tiredness, twitching, cramps, and muscles weakness (Muhamoud, *et al.*, 1984). According to the literature, the increasing Imidacloprid (neonicotinoid) concentration causes significant linear decrease in burrow volume and burrowing behaviour in two earthworm species *Aporrectodea caliginosa* and *lumbricus terrestris* (Dittbrenner, *et al.*, 2011).

In the present data on the toxicity of the (Oraganochlorine) dieldrin and (Organophosphate) cythion, the avoidance responses of earthworm for the toxicated filter paper with both pesticides at all concentrations were observed. At the highest dose of of both pesticides clearly showed that the pesticides had a detrimental impact on the earthworm. No mortality was observed at 0.1, 0.2 and 0.mg/cm^2 concentration of dieldrin during the intoxication period of 48 hrs. But 100% mortality was observed at 0.8, 0.9 and 1mg/cm^2 concentrations of dieldrin. Similar data documented by Muhammad and Farhanullah, (2010) when the two doses of imidacloprid were revealed that the LC_{50} for the two considered types was between no mortality and 100% mortality. Similar result was also observed in cythion exposed earthworm which was higher than the dieldrin. 100% mortality was found at 0.21, 0.24, 0.27 and 0.30 mg/cm^2 concentration of cythion and 0% mortality was observed at 0.03, 0.06 and 0.09 mg/cm^2 concentrations of cythion. The above report showed the highest LC_{50} value of cythion i.e. 0.15mg/cm^2 that could be due to sensitivity of species and stress response as cythion is highly toxicants than dieldrin.

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