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Mortality (LC_{50}) By Standardization Artificial Soil Test Mathod For Estimation Of Toxic Effect Of Dieldrine And Cythion On Earthworm, Eisenia Foetida

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***Abstract:** In present data, we observed that cythion causes pathological changes in earthworm, Eisenia foetida that is one of the non-target species for pesticide. During experimentation, it was found that the rate of mortality and impact of selected pesticides on earthworm was dose and duration dependent. In 14-days exposure periods, the maximum mortality rates were reached 91.0% and 95.0%.of cythion and dieldrin respectively. The LC_{100} of the pesticide has been registered on the 14^{th} day with concentration of (cythion) 50ml/kg-1 and 100ml.kg-1(dieldrin). The LC_{50} of the pesticide cythion and dieldrin at 14^{th} days were 19.47ml/kg and 26.36ml/kg day respectively. This is explained by the fact that the median concentration causes 50% of mortality rate. With longer period of influence it occurs at lower concentration. The most common variable for assessing the acute toxicity of chemicals to animals is LC_{50} (lethal concentration to 50%). LC_{50} is used to suggest the average lethal dose of such chemicals to the mintages.*

***Keyword:** Dieldrine, cythion, esenia foetida, artificial soil test, LC_{50}*

I. INTRODUCTION

Earthworms are one of the most important organisms responsible for mechanical mixing of soil and play a major role in maintaining physical soil characteristics and processes such as aeration, water permeability and mineral turnover. Earthworms are key components in natural food chains providing a food source for many small mammals, birds, fishes and prawn. Pesticides are known to produce morphological, anatomical and physiological changes in the vital organs such as reproductive, nervous, respiratory and osmoregulatory of different non-target animals, such as earthworms and other beneficial organisms (Satchell, 1967 and Fingerman, 1984). Earthworms are, as a matter of fact important components of soils because they boot fine-tune femininity of soils its saturation (Baxter, *et al.*, 1975) and pH (Balthazor and Hallas 1985). But these organisms are destroyed (Arias-Estevem *et al.*, 2008) by interaction of industrial‘s pollutants, effluents, flues, at the boiling point wastes, municipal wastes, household wastes, hut wastes, artificial fertilizers, pesticides, insecticides, herbicides, particulates, corrosive gases, fog and global warming. These harmful substances were change physical, chemical and biological properties of soils. These substances are regularly acidic and basic in bias and they gave a pink slip constitute a microbiological corrosion prison (Chen, *et al.*,2007) by the whole of earthworms’ thus biological corrosion life starts mutually these species and in this by the number their morphology can be displaced which leads to the rack and ruin of these organisms in soils. Ponds, canals, rivers and reinforce raw material for irrigation of soils and this polluted water sources create a corrosive environment for earthworms (Feng, *et al.*, 2006).

Earthworms represent a significant, if not a dominant part of the soil biomass, and are regarded as soil engineers regulating important soil processes and are being broadly used to assess environmental impact of heavy metal pollution (Cao, *et al.*, 2013). In recent years there is a growing interest in the development of sub lethal earthworm biomarkers; one of them is neutral red retention assay, which measures the membrane

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stability of lysosomes within the coelomocytes of earthworms in response to contaminants. Thus, it can be used for evaluation of toxicity of a range of toxicants under different exposure conditions. Recently, from this laboratory, a series of publications have come up, where the effects of metals like lead, copper, aluminium and some pesticides have been studied on growth, reproduction and avoidance behaviour of earthworms, *E. foetida* and *Lumbricus terrestris*, have reported that the hazardous pollutants significantly affect the sensitive parameters of these soil organisms (Ali, 1997).

EXPERIMENTAL ANIMAL

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

EARTHWORM CLASSIFICATION

CLASSIFICATION

Kingdom: --------Animalia
Phylum: ----------Annelida
Class: -------------Clitellata
Order: ------------Haplotaxida
Family: -----------Lumbricidae
Genus: ------------Eisenia
Species: -----------*foetida*

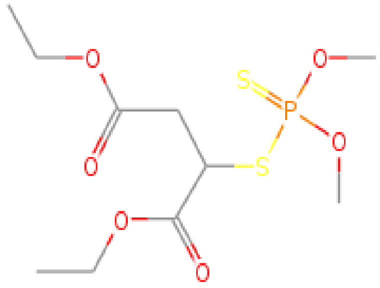
This annelids belongs to the family of Lumbricidae and genus *Eisenia*, which is experienced by other names such as the tiger worm, garlic worm (abdominal bad odour fluid when alarmed release the flavour of the garlic), flatworm, Cadillac worms and worm for fishing bait. It can be applied in a relative wide range of climates and is probably the most common worm used for composting. *E. fetida* is commonly called the composting worm, manure worm, red worm or red wigglers, which understandably leads to confusion. It is an epigeic earthworm that is typically found in the litter or decaying organic matter near the soil surface, including animal manure on the soil surface.

PESTICIDES

We had selected two pesticides i.e. cythion (Organophosphate) and dieldrin (Organochlorine) as a test chemical, purchased from the agro-pesticide market, Cotton market area, Amravati and the doses were prepared on the basis of lethal concentration (LC_{50}).

CYTHION

Commercial name: Cythion 50% E.C.
Chemical name: O.O-Dimethylphosphodithionate-diethyl mercapto succinate.
Chemical Formula: $C_{10}H_{19}O_6PS_2$
Structural Formula:



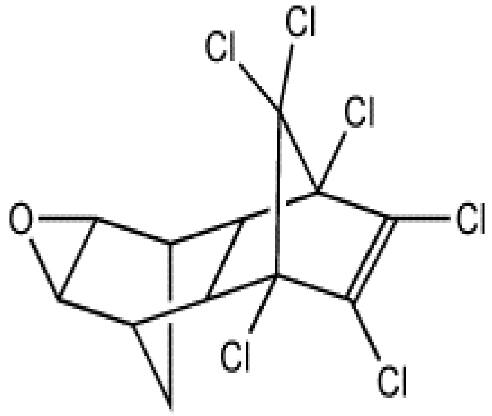
Physiological properties: Clear, brown coloured viscous soluble in water and most of the organic solvent 5% DP.50%EC.20% WP.
Manufacturer: Cyanamid India Ltd.P.O.Atul Valsad 396020 (Gujrat State).

BIOLOGICAL PROPERTIES

Cythion is an organophosphate base pesecticide wich has good activity against pest like stem borer, trips, vassids, leaf rolled, leaf miner, army worms, pest of cotton, sugar cane, tobacco, jawar, lady figure, vegetable etc. It has an effective controlling ecto-parasite of animal like earthworm.

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: $C_{12}H_8Cl_6O$
Structural Formula:



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.

BIOLOGICAL PROPERTIES

Dieldrin is the common names of two structurally similar compounds that were once used as pesticide. They are chemicals that are made in the laboratory and do not occur naturally in the environment. The abbreviation for the scientific name for dieldrin is HEOD. Technical-grade dieldrin contains not less than 85% dieldrin. The trade names used for dieldrin include Alvit, Dieldrix, Octalox, Quintox, and Red Shield. Dieldrin is white powders, while technical-grade aldrin and dieldrin are tan powders. Dieldrin slowly evaporates in the air. Dieldrin has mild chemical odours.

MORTALITY (LC_{50}) BY STANDARDIZATION ARTIFICIAL SOIL TEST

Mortality was assayed according to Buchatsky (*et al.*, 2007). The soil used in this test was the OECD artificial soil (OECD 1984). The pesticides cythion and dieldrine were added to the soil, which were 10mg, 20mg, 30mg, 40mg and 50mg/kg dry weight soil for cythion and 20mg, 40mg, 60mg, 80mg and 100mg/kg dry weight soil. 10 full clitellated mature worms were used and kept to each concentration as a treated group and another untreated as a control group. Each experimental jar covered with porous fabric plate to provide sufficient ventilation. After 24hr in laboratory condition removed from artificial soil, washed with water and kept on filter paper for absorption of execs water and finally placed in experimental jar for 14^{th}days. At the end the experiment the worms were counted and the result was calculated by the following formula.

$$LC_{50} = \frac{(a-b)\cdot(50-B)}{A-B} + b$$

Where A - Mortality rate exuding 50, 0%
a - rate causing A
B - Mortality rate below 50, 0%
b - rate causing B

EXPERIMENTAL DESIGN

Experimental animal (*Eisenia foetida*) were reared in the laboratory in artificial soil according to ISO (1993), the soil were mixed with shredded and decayed leaves, cow dung manure and virgin black soil of and were kept at room temperature 20± 2^{0}C. Before exposure of experimental animal earthworm, *Eisenia foetida* were acclimatized for one month in the laboratory as per the method of Rao and Kavitha (2004). We had used fully clitelled adult earthworms which were having above 250- 300mg of body weight (ISO, 1993) and placed in to 10 dose group and one control group which were exposed to different concentration of cythion i.e. 3mg, 6mg, 9mg, 12mg and 15mg /kg dry weight of soil and dieldrine 4mg, 8mg, 12mg, 16mg and 20mg/kg dry weight of soil. The control group was given the same quantity of water without any additive agent.

Exprimental Animal	Group	Category
Eisenia foetida	A	Control
	B	Cythion
	C	Dieldrine

Table 1: Experimental setup

Flowing parameters were analysed after the different period of experimentation. The different time intervals were fixed for each dose concentration which was 2, 4, 6, 8, 10 and 14^{th}days for acute test and 10, 20, 30, 40, 50 and 60 days for chronic test. Acute test used for survival and biomass and chronic test used for growth and reproduction. The method used for these tests were standardised artificial soil test.

II. RESULT AND OBSERVATION

ASSESSMENT OF MORTALITY (LC_{50}) OF CYTHION AND DIELDRINE BY STANDARDIZATION ARTIFICIAL SOIL TEST

Concentration (mg/kg) Cythion	Mean (± SE)	Percentage %
50	**9.1(±3.4)**	**91**
40	**8.7(±3)**	**87**
30	**5.7(±0)**	**57**
20	**5.1(±0.6)**	**51**
10	**3.2(±2.5)**	**32**

Table 1: Percent and mean earthworm mortality compared with the initial test of cythion at different concentrations 50 to 10 mg/kg soil for 14^{th} days

Concentration (mg/kg) Dieldrin	Mean (± SE)	Percentage %
100	**9.5(±2.2)**	**95**
80	**8.2(±1.2)**	**82**
60	**6.4(±0.8)**	**64**
40	**4.2(±2.8)**	**42**
20	**0**	**0**

Table 2: Percent and mean earthworm mortality compared with the initial test of dieldrine at different concentrations 50 to 10 mg/kg soil for 14^{th} days.

Preparation	Day Mortality	A	b	A	B	LC_{50} (mg/kg)
Cythion	**14**	**20**	**10**	**51**	**32**	**19.47**
Dieldrine	**14**	**60**	**40**	**64**	**42**	**26.36**

Table 3: Cythion and deldrine concentration (a, b) causing the upper and the lower (A, B) limits of the 50% earthworm mortality rate (LC_{50})

III. DISCUSSION

MORTALITY (LC_{50}) BY STANDARDIZATION ARTIFICIAL SOIL TEST

Mortality has been the most frequently used parameter to evaluate the chemical toxicity in earthworms (Van Gestel, and Van Dis, 1988; Robidoux, *et al.*, 1999). Preliminary toxicity tests were conducted to determine the LC_{50} value of monocrotophos to *lampito mauritii* under laboratory conditions, according to the method of the American Public Health Association, (2005). Mortality was calculated according to the guidelines proposed (OECD, 1984). It was calculated by two methods. 1) Filter paper contact test method and 2) Standardization artificial soil test. The endpoint "LC_{50}"

showed effects at relatively high pollutant concentrations and denotes the maximum damage liability to an organism. In the present study, LC_{50} of cythion and dieldrin were assessed and showed 19.47mg/kg and 26.36mg/kg, respectively, approximately reported by Hu *et al.,* (2004) that LC_{50} was 83.63 mg/kg soil. According to the regulation of environmental safety assessment test for agricultural pesticide, the suggested standard of toxicity is LC_{50}< 1 mg/kg for high-toxic pesticide, 1–10 mg/kg for mid-toxic pesticide,>10mg/kg for low-toxic pesticide. A soil test is more representative of the natural environment of earthworms and the pesticides is mainly absorbed by gut in this method (De Silva, *et al,* .2009; Udovic and Lestan, 2010). Therefore, the soil test is more adequate when the toxicity of pesticides to earthworms is evaluated (Wang *et al.*, 2012).

Malathion is one of the most widely used organophosphate pesticides (Giri, *et al.*, 2002). We had selected two pesticides cythion which was organophosphate and dieldrin which was organochlorine. They are very harmful to the earthworm life and production. Some researchers have observed that the skins of earthworms were adversely affected due to Malathion poisoning (Baker, *et al.* 1992; Mackinson, 1981; Kahn, *et al.* 1992 and Gosselin, *et al.*, 1994). Cythion and dieldrin showed maximum impacts on the skin and the body of an earthworm. Sabina has also reported that Malathion can change physical and chemical properties of membrane in rates (Sabina, *et al.* 2003). Once Malathion is introduced into the environment, it may caused severe metabolic disturbances in non-target species (Anonymous, 2005). In present data, we observed that cythion causes pathological changes in earthworm, *Eiseniafoetida* that is one of the non-target species for pesticide. During experimentation, it was found that the rate of mortality and impact of selected pesticides on earthworm was dose and duration dependent. In 14-days exposure periods, the maximum mortality rates were reached 91.0% and 95.0%.of cythion and dieldrin respectively. The LC_{100} of the pesticide has been registered on the 14^{th} day with concentration of (cythion) 50ml/kg-1 and 100ml.kg-1(dieldrin). The LC_{50} of the pesticide cythion and dieldrin at 14^{th} days were 19.47ml/kg and 26.36ml/kg day respectively. This is explained by the fact that the median concentration causes 50% of mortality rate. With longer period of influence it occurs at lower concentration. The most common variable for assessing the acute toxicity of chemicals to animals is LC_{50} (lethal concentration to 50%). LC_{50} is used to suggest the average lethal dose of such chemicals to the mintages. Granting to the regulation of environmental risk assessment for agricultural pesticides, the suggested measure of toxicity is LC_{50}< 1 mg/kg for highly-toxic pesticides, 1–10 mg/kilo for medium-toxic pesticides, and > 10 mg/kg for low-toxic pesticides (MEPPRC, 1990). Similarly with this standard, the acute toxicity of cypermethrin on adult and juvenile earthworms was found to be dispirited. Likewise, we are finding the LC_{50} for the (Organophosphate) cythion and (Organochlorine) dieldrin show LC_{50}, 19.47 mg/kg and 26.36 mg/kg in 1% solutions for contact standard soil test. Kovilpathu 2013 was recorded the LC_{50} of superphosphate as 210mg/5ml (I. e. 300 µg/cm^2) (Kovilpathu, *et al.*, 2013). Similarly toxicity tests were conducted to determine the LC_{50} value of cythion and dieldrin under laboratory conditions, according to the method of standardization artificial soil test. We had selected two pesticides cythion and dieldrin for evaluating the chemical toxicity on earthworms. These selected pesticides were produced dose dependent and duration dependent impact on earthworm. In this study, we found that cythion showed highly toxic effects than dieldrin. Kurawar, confirmed current results, as she reported ruptured cuticle, in *Eiseniafoetida* after exposure to pesticide Malathion (cythion) (Kurawar, 2009). We investigated that the organophosphate (cythion) showed major impact on the earthworm mortality.

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