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Dr.T. Determination Of Lc50 Values Of Three Detergents And Its Effect On Biochemical Compounds Of Eisenia Fetida

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ABSTRACT

The adult earthworms of EIsenia fetida are exposed to two sublethal concentrations such as 1/10th LC50 and 1/20th LC50 doses of three detergents. Biochemical components were studied in the total body tissues. The ANOVA results showed earthworms treated with detergents significantly differed in their biochemical components ($p<0.05$) than control group. Total Sugars, Total Cholesterol, Total Protein levels are significantly ($p<0.05$) reduced in the earthworms group treated with Arasan and Rin detergents, whereas increased Urea, transaminase (GOT and GPT) and phosphatase (ALP and ACP) levels found in treated group organs. The results demonstrated that exposure of earthworms to detergents through soil disturbed the basic biochemical compounds in tissues and their damages are indicated by increased enzyme levels.

Keywords: Detergents, Earthworm, Biochemical compounds, Enzyme studies, suggestions.

INTRODUCTION

Earthworms are major component of soil fauna in a wide variety of soils and climates and are involved directly and indirectly in biodegradation, stabilization through humus formation and various soil processes (MunnoliPrakashMallappa *et.al*. 2010). Earthworms represent the greater fraction of biomass of invertebrates in the soil as soil macro fauna and play a vital role in structuring and enhancing plant nutrients and hence they can be successfully used as bioindicators for the evaluation of toxic risks of xenobiotics in terrestrial ecosytems (Reinecke and Reinecke 2004). This is important for protecting the health of natural environments, and of increasing interest in the context of protecting human health (Beeby, 2001).

Earthworm population is influenced by various factors (soil, temperature, moisture and pH) and the availability of organic matter, which may come from plant residues and animal or human waste applied to the land (Baker et.al. 1992). The abundance of earthworms in soils represents the health of soil ecosystems and the level of environmental safety (Edwards *et.al.* 1995). Soil texture can influence earthworm population because of its effects on other soil properties, such as soil moisture relationships, nutrient status and cation exchange capacity, all of which can have important influences on earthworm population.

A detergent is a surfactant or a mixture of surfactants with cleaning properties in dilute solutions. Detergents figure in an extensive array of industrial and home cleaning applications, including laundry and freshwater detergents. They are also used in pesticide formulations and for dispersing oil spills at sea. Linear alkylbenzene sulphonate (LAS), a major detergent and corrosion inhibitor ingredient is poorly broken down in rivers and soils and may be toxic to soil organisms (Lightowlers, 2004).

Uncontrolled discharge of detergents into water and arable soil may cause reduced biological production of these ecosystems, which can have adverse ecological and economic effects. Soil microorganisms, as a biological soil component, can affect their biodegradation to a certain extent, converting them into less toxic or, often, energetically important sources of nutritive elements (Stojanovic et al., 1990). The adverse effects of laundry greywater on the environment, as well as avoiding the occurrence of eutrophication in the water bodies which received these wastes can be limited by the introduction of phosphate-free detergents Jacob and Wirtschaftsforschung (2005).
The present study was designed to find out the LC50 values of three detergents (Arasan, Rin and VIP Soap) on Earthworm *Eisenia fetida* and its effect on the Biochemical components of the Earthworm.

MATERIAL AND METHODS

Earthworms

Eisenia fetida, the common earthworm found in agricultural land, was procured from Agricultural College, Killikulam and cultured for three months. They were all cultured in vermiculture bed under the Laboratory conditions in A.P.C.Mahalaxmi College for Women, Thoothukudi, under controlled conditions. Adult and matured earthworms with clitellae and individual wet weight of 100±15 mg were selected for LC50 determination and additional experiments.

Soil toxicity test

The artificial soil consisted of 10% ground coco peat (<0.5 mm), 20% kaolinite clay (>50% kaolinite), and 70% fine sand (OECD, 1984). A small amount of calcium carbonate was added to adjust the pH to 6.0 ± 0.5. In the toxicity tests, the water content was adjusted to 35% of the dry weight. For each tested concentration, the desired amount of detergent was dissolved in 10 ml distilled water and mixed with a small quantity of fine quartz sand. The sand and the detergent were mixed well and then mixed thoroughly with the pre-moistened artificial soil in a household mixer. The final moisture contents of the artificial soil were adjusted to the described level by the addition of distilled water. A total of 100g of artificial soil was placed in a 500 ml plastic box and 5 adult earthworms were added to each box. Controls were prepared similarly but only with 10 ml distilled water and no detergent. The boxes were loosely covered with its lids to allow for air exchange and stored at 20±1°C with 80–85% relative humidity under 400–800 lx of constant light. Mortality was assessed at 7 and 14 days after treatment. A range of concentrations from 1mg to 10mg/kg dry soil was used in the pre-trials to determine the concentrations that produced 0–100% mortality. Exact triplicates were maintained for the experiment.

Animal Preparation and Exposure

The earthworm *Eisenia fetida* were separated into four groups in plastic tubs of 25 litre capacity. The plastic tubs were filled with 5kg of soil. Experimental soil is prepared by mixing cow dung and dried neem leaves with 4.3% organic matter and pH 5. The soil is sieved through a 5mm sieve and transferred to plastic tubs. Two different sublethal concentrations ($1/10^{th}$ LC50and $1/20^{th}$Lc50) of detergents were prepared to study the impacts on biochemical components. The prepared soil is mixed with various concentrations of detergents and left undisturbed for 24h in room temperature. After 24h, mature earthworms were introduced into the prepared tubs and observed. The control soil with worms and without detergents was also maintained.

Biochemical studies

After 45 days of experimental duration, the earthworms were collected and dissected. The gut contents were removed by feeding filter paper. The total body muscles were used for Biochemical analysis. Total sugar, Protein, Cholesterol and Urea levels are analyzed by standard spectrophotometer methods such as Anthrone method (Loewus, F. A. 1952), Lowry *et al.,* (1951) method, Zollner and Kirsch (1962) method and Diacetyl Monoxime (DAM) method (Beale and Croft, 1961) respectively.

Tissue samples were homogenized in 0.1M cold phosphate buffer (pH 7.2) and then centrifuged at 10,000g for 20mins at 4°C. The clear supernatant was used for the determination of GOT, GPT, ALP and ACP activities (Song *et al* 2009). The enzyme studies were done in the Diagnostic laboratory, DCW Ltd, Sahupram, Thoothkudi**.**

RESULTS AND DISCUSSION

It has been demonstrated that surfactants present in detergents can negatively affect living cells in different ways by damaging cell membranes attaching to proteins, and affecting cell physiological and biochemical processes (Azizullah et al. 2012).

Soil toxicity tests of three detergents to the earthworm in different concentrations showed various toxic effects (Table 1). The mortality of earthworms was observed from 4mg/100g soil to10 mg/100g soil concentrations after 8 days in Arasan and Rin detergents. Mortality of earthworm was observed from the concentration of 6mg/100g soil after 10 days in VIP detergent. Only the 1mg/100mg soil to 4mg/100mg soil concentrations were non-toxic to earthworm after 14 days.

The toxicity of the three detergents was first manifested in sluggish movement, enlargement of clitellum and discolouration of earthworms on the seventh day. Amala Thomas et al 2019 observed significant changes in weight, length and diameter of tested earthworms after exposure to high doses of detergent. The weight of earthworms exposed to high concentration of detergent decreased steadily. The sharp decrease in the weight of earthworms revealed that high concentration of detergent was detrimental to the worms. However, at low concentration of detergent there was no significant change in morphological parameters like weight, length and diameter of the earthworms (Amala Thomas et al 2019).

Significant reduction ($p<0.05$) of biochemical parameters like total sugars, total cholesterol and total proteins were observed in $1/10^{th}$ and $1/20^{th}$ LC50 doses of Arasan and Rin detergents and non significant reduction of these parameters were noticed in both the concentrations of VIP detergent (Table 2 and 3). Vaidya (2016) recorded the depletion of protein level in worms after 5 days of exposure to sub lethal dose of mercuric chloride in all the tissue. Decrease in protein content may be due to degradation of proteins in to amino acids to be utilized for gluconeogenesis (Begum and Dharni, 1996) to mitigate the stress. During stress, organisms need more energy to detoxifying, biotransforming and to excrete the toxicants with the view of minimizing the toxic effects. This is achieved by the use of carbohydrate, the principal and immediate energy source (Umminger 1977). Thedepletion of protein fraction in organisms may be due tothe degradation of carbohydrate (Khalil 2016). These facts could be the reasons for significant reduction of total sugars after the treatment of earthworms with detergents for 45 days.

In the present study significant ($p<0.05$) reduction of total cholesterol was observed in $1/20^{th}$ LC 50 doses of three detergents. Baghul *et al* (2017) observed reduction of triglyceride of worms exposed to 3/4th sub lethal concentration of Cypermethrin (22.4 %) and Oxyfluorfen (35.8 %). During the time of low availability of carbohydrates, lipids serve as a source of energy for supporting the physiological functions of the body. Hence, the decline in triglyceride content was due to the utilization of lipids for meeting the energy demand under the detergents stress. (Somaiah *et al* 2015) supported the present work that the triglyceride level was changed when worm exposed to toxicants like Cypermethrin and Oxyfluorfen.

Urea was significantly ($p<0.05$) increased in $1/10^{th}$ and $1/20^{th}$ doses of Arasan and Rin detergents and the urea level was unaltered in both the doses of VIP detergent in the present study. Urea is the end product of the protein metabolism and it is synthesized in the liver from the ammonia produced by the catabolism of amino acids. Increased urea level indicates renal diseases, shock, and congestive heart failure in the treated organism. Decreased protein content in the ZnO NPs treated earthworm is due to the increased protein assimilation resulted in increased urea production in the

earthworm. Various research indicates that GOT and GPT can be used as biomarkers of cellular damage in blood plasma, protein degradation and organ damage (Sesha Srinivas 2007).

GOT, GPT, ALP and ACP are the functional enzymes known for their role in the utilization of proteins and carbohydrates (Table 4 and 5). Transamination reactions are prominent under stress conditions (Harper et al 1978) and these enzymes are released during cellular damage or lysis and hence used to measure the stress intensity developed by the exposed toxicant to the organisms (Nuchumbeni *et al* 2007).

Significantly altered enzyme levels in the earthworms treated with three detergents in $1/10^{th}$ and $1/20^{th}$ LC5 doses are due to the disturbed physiological condition resulted from decreased biochemical composition in the organs. Similar to the results obtained after the treatment of earthworms with detergents, increased transaminase levels, increased acid and alkaline phosphatase enzymes with respect to decreased protein content in different pollutant exposed earthworms are reported by Habiba and Ismail (Habiba et al 1992). During the exposure to pollutants, earthworms are capable to reduce the toxic effects of the chemical product by adjusting their internal biochemical responses before the growth is affected. So, the biochemical reactions are rather important to evaluate the potential adverse effects of chemicals on the environment Gao et al., (2007).

CONCLUSION

It can be concluded that discharge of laundry greywater into the soil alter the soil parameters, produces toxic effects on soil flora and fauna. Biodetergents with new biochemical substances and suitable disposal methods of laundry water at least in houses are some suggestive measures to conserve the soil biodiversity. Earthworms are the so called “Farmers friend” and their abundance in the soil should be preserved for soil wealth.

List of Tables

Table 1 LC50 values of three detergents

Detergents	LC50 values mg/100mg soil	$1/10^{th}$ LC50 mg/100g soil	$1/20^{th}$ LC50 mg/100g soil
Arasan	6.5	0.65	0.33
Rin	7.2	0.72	0.36
VIP	8.7	0.87	0.44

Table 2. Total Sugars and Cholesterol of control and experimental Earthworms

Groups	**Total Sugars (mg/dL)**		**Total Cholesterol (mg/dL)**	
	1/10	**1/20**	**1/10**	**1/20**
Control	5.35±0.11	5.35±0.11	14.63±0.16	14.63±0.16
Arasan	3.25±0.12[a]	3.61±0.06	11.65±0.12	12.03±0.18
Rin	3.63±0.09[a]	4.01±0.13	11.90±0.15	13.23±0.17
VIP	4.95±0.11	5.10±0.09	12.45±0.12	13.80±0.14

Table 3. Total Urea and Protein of control and experimental Earthworms

Groups	**Total Urea (mg/dL)**		**Total Protein (mg/dL)**	
	1/10	**1/20**	**1/10**	**1/20**
Control	5.48±0.22	5.48±0.22	850±16	850±16

Arasan	7.60±0.18	6.50±0.38	570±24	640±30
Rin	6.43±0.24	6.13±0.34	690±56	720±46
VIP	5.36±0.32	5.83±0.26	770±68	810±54

Table 4. Total GOT and GPT of control and experimental Earthworms

Groups	**GOT(IU/L)**		**GPT(IU/L)**	
	1/10	**1/20**	**1/10**	**1/20**
Control	56.33±2.12	56.33±2.12	214.40±1.49	214.40±1.49
Arasan	98.60±1.35	97.67±2.50	262.50±3.30	233.43±2.14
Rin	88.47±2.20	76.13±2.42	226.13±1.78	186.30±2.44
VIP	72.70±1.64	61.47±1.33	196.00±2.56	191.17±3.35

Table 5. Total ALP and ACP of control and experimental Earthworms

Groups	**ALP(IU/L)**		**ACP(IU/L)**	
	1/10	1/20	1/10	1/20
Control	251.67±2.43	251.67±2.43	185.5±3.45	185.5±3.45
Arasan	356.43±2.54	318.43±2.23	285.30±3.54	228.38±3.46
Rin	323.17±3.42	283.48±12.93	254.60±1.80	213.50±2.45
VIP	284.40±1.75	272.92±3.27	218.45±1.73	193.42±2.78

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