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Determination of LC_{50} of commonly used roundup herbicide on the earthworm, *Eisenia fetida*

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Abstract
Earthworms are invertebrates and contribute to soil fertility improvement and plant growth. Roundup is widely used herbicide in agriculture. *Eisenia fetida* has been suggested as a sensitive and standard species for ecotoxicological studies. *Eisenia fetida* were exposed to Roundup herbicide via soil. Acute toxicity evaluation of LC_{50} and the effect of Roundup herbicide on the earthworm, *Eisenia fetida* was studied under laboratory conditions for 24, 48, and 72 hours and the result indicated the acute toxicity of Roundup herbicide concentrations was 0·050, 0·034 and 0·024 ppm to be specific respectively. The aim of the present investigation was to assess LC_{50} values of Roundup herbicide was 0·04898, 0·03342 and 0·02350 and effect on survivality and mortality of earthworm, *Eisenia fetida*.

Keywords: roundup, toxicity, LC_{50}, survivality, mortality, earthworms

1. Introduction

Increasing population growth and urbanization, especially in a developing country like India, necessitates producing more food. Food crops require fertile soil to grow. For terrestrial ecosystems, soil serves as a medium of entry to the nutrients. The first pesticides started to be used in USA agriculture in the 1930s and their adverse effects were discussed in a Wildlife Conference in 1938. During this era, about 30 pesticides were in use (pyrethrum, nicotine, calcium arsenate, mercurial fungicides, dinitro-ortho-creasol) (Rattner, 2009). In the 1960s other organochlorine insecticides and fungicides became very common in agriculture, such as aldrin, dieldrin, chlordane, heptachlor and toxaphene. These pesticides caused extensive wildlife mortality, especially when they were used for budworm control and Dutch elm disease in forests, grasshopper, mosquitoes and fire ant control (Peterle, 1991; Dustman and Stickel, 1969). A selection of these toxicological studies of pesticides in earthworms (Sanchez-Hermandez, 2006). Continuous agricultural activity tends to decrease the soil fertility. However, the increasing application of herbicides and pesticides has also threatened the human environment and the ecosystems with dexterous consequences. Earthworms represent a great proportion of biomass of terrestrial invertebrates. They have been selected as suitable representative of soil organisms as they are key components of soil biota. They are found almost all over the world in the temperate and tropical regions wherever there is plenty of moisture in the ground. They prefer loamy or partly sandy soil rich in humus. *Eisenia fetida* is the standard test organism used in ecotoxicology because it can be easily bred on a variety of organic waste within short generation times. Earthworms contribute to soil fertility, improvement, plant growth and play a key role in converting an organic matter (Reynolds, 1994). Soil animals, especially earthworms, are one of the best bioindicators of pesticide contamination (Edwards and Thompson, 1973). Pesticides are mostly synthetic chemicals that are used in agriculture to control pests. Pesticides include herbicides, insectides, fungicides, nematocides and rodenticides. The dramatic increase of global use of pesticides in the 1960 and 1970s was the result of growing population and the need for increased food production. Earthworms constitute a major component in soil functioning, and they play an important role in chemical element transformations (Lee, 1985). Contamination by heavy metal can change the functioning of soil ecosystems qualitatively and quantitatively by disturbing the activities of soil fauna (Cortet, *et al.*, 1999) and can lead to contamination of the terrestrial food chain by, for example, the transfer to heavy metals to predators of the soil fauna (Abdul Rida and Bouche, 1994). Pesticides, because of their potential toxicity are known to produce morphological, behavioural and physiological changes in the vital organs such as reproductive, nervous, respiratory, osmoregulatory etc. of different animals (Fingerman, 1984). Thompson, (1971) reported that normal agricultural doses of endrin decreased earthworm populations, so it seems that endrin is a chemical that is relatively toxic to earthworms. Callahan, *et al.*, (1994) observed the concentration response (mortality) relationship of 4 species of earthworms, *Eisenia fetida, Allolobophora tuberculata, Eudrilus eugeniae*and *Perionyx excavates*. They tested 62 chemicals and reported chemical impact on organisms and all four species appeared to be similar in the range of tolerance to these toxic chemicals. Kuo and Haung, (1993) observed the lethal effects of benomyl, hymexazol, metalaxyl, methasulfocarb and carbofuran pesticides on the earthworm (Eisen). They reported that carbofuran and methasulfocarb were more toxic. Lethal effect of benomyl varied with the time of feeding. Mortality of the earthworms decreased as the time of feeding delayed. Several workers have also investigated the effects of organophosphate insecticides on the earthworm's populations. Azinphosmethyl did not affect earthworm populations (Hopkins and Krik, 1957). Senapati, *et al.,* (1987) observed the impact of malathion on the population of the earthworms, *Drawida willsi*and reported its stressful effect on earthworm in agroecosystem. Roundup herbicide is widely used in agriculture. The main aim of this ecotoxicological studies to get a more comprehensive understanding the toxic effects of Roundup herbicide on earthworms, *Eisenia fetida* and to provide informative data for use in ecolgical risk assessment on soil ecosystem. Hence, in the present study Roundup herbicide was selected for assessment of their toxic effect on earthworms, *Eisenia fetida*.

2. Materials and Methods

Eisenia fetida were selected for the experiments because which is widely used in the agriculture and it is cheap test species, easy maintain and readily available and found in India. The cultured adult earthworms, *Eisenia fetida* were used for the experiments. All worms were healthy, adult and fully clitellate and brought to the laboratory. Earthworms looking healthy and having approximately equal size were selected for all experiments. Earthworms were maintained under normal day night illumination for three days in a glass container containing with wet soil subjecting for acclimatization to laboratory conditions as described by Kulkarni, (1989). *Eisenia fetida* were exposed to Roundup herbicide via soil. Concentration series of Roundup herbicide were prepared by diluting stock solution. Effect of Roundup herbicide was studied for survivality, mortality and LC_{50} values in ppm of earthworms, *Eisenia fetida*. In order to ascertain the Lethal Concentration or LC_{50} (the concentration of pesticide which kill 50% of sample population) of herbicide for 24, 48 and 72 hours, were maintained separately in 250 ml beaker, containing 200 gm wet garden soil for their natural habitat, with desired concentrations were obtained by test solutions having different concentrations (ppm) of Roundup herbicide added in 30 ml water each beaker (200 gm soil + 30 ml test solution) thoroughly mixed and groups of 10 worms inserted. Its effect was studied in the laboratory.

3. Results

Present study was carried out to see the effect of Roundup herbicide on survivality and mortality of earthworms, *Eisenia fetida*. Study was conducted to investigate the acute toxicity evaluation for LC_{50} values and the effect of Roundup herbicide on earthworms, *Eisenia fetida* corrected % mortality under laboratory conditions. Moreover, the direct toxic action of Roundup herbicide on different end points of the earthworms (lower and higher % of mortality) was studied. *Eisenia fetida* of experimental group were exposed to different concentrations. Result was recorded after exposure to Roundup herbicide for 24, 48 and 72 hours. The result obtained after toxicity evaluation of *Eisenia fetida* are cited in Tables-1 to 3 and Fig. 1. Different concentrations of Roundup herbicide tested in ppm for LC_{50} values and percentage mortality. Such concentrations was 0·040, 0·045, 0·050, 0·055 and 0·060 ppm after 24 hours obtained mortality result in 30, 40, 50, 60 and 70% respectively. After 24 hours 50% mortality was found in the 0·050 ppm. The LC_{50} values was 0·04898 ppm.

Different concentrations of Roundup herbicide tested in ppm for LC_{50} values and percentage mortality. Such concentrations was 0·030, 0·032, 0·034, 0·036 and 0·038 ppm after 48 hours obtained mortality result in 30, 40, 50, 60 and 70% respectively. After 48 hours 50% mortality was found in the 0·034 ppm. The LC_{50} values was 0·03342 ppm.

Different concentrations of Roundup herbicide tested in ppm for LC_{50} values and percentage mortality. Such concentrations was 0·020, 0·022, 0·024, 0·026 and 0·028 ppm after 72 hours obtained mortality result in 30, 40, 50, 60 and 70% respectively. After 72 hours 50% mortality was found in the 0·024 ppm. The LC_{50} values was 0·02350 ppm. Calculation of LC_{50} values using probit analysis are summarized in Table-4. Roundup herbicide was moderately toxic to earthworms, *Eisenia fetida*.

4. Discussion

Although most herbicides are considered to exert little direct impact on earthworms (Edwards and Bohlen, 1996), the reduced weed cover resulting from their application obviously can render habitats less hospitable to earthworms. Laboratory tests have shown that the herbicides bentazon, bromphenoxin, bromoxynil, octaonate/ioxynil and atrazine are moderately toxic to earthworms (Pizl, 1988). Some large spectrum herbicides, e.g., glyphosate, are quite harmful to earthworms such as *Aporrectodea caliginosa* even at very low doses (Springett and Gray, 1992). Epigeic earthworms, *Allolobophora chlorotica* and endogeic *A. rosea* seem to be negatively affected in grasslands spread with atrazine and pentachlorophenol (PCP) (Conrady, 1986). Zhou, *et al,* (2006) reported that weight of the earthworms was a more sensitive index compared to the mortality in indicating toxic effect, observation was made on length and colour of worm. There were no changes in length and colour of worms exposed to insectides. Khan, *et al,* (2007) reported a significant reduction in earthworm biomass after exposure to different concentration of copper chloride and concluded abnormal functioning of major physiological systems such as digestion and absorption. Effect of pesticides on growth and reproduction of earthworm was studied by (Shahla and D'Souza, 2010). Correia and Moreira (2010) studied effect of glyphosphate on earthworms, *Eisenia fetida*. The LC_{50} values for different test animals are very useful in evaluating the extent of toxicity of pesticide; otherwise it is difficult to predict the physiological responses of the animal to the toxicants (Dowden and Bennet, 1965). Several researchers reported the effects of chemicals, metals and pesticides practiced on earthworms like that is chlorpyrifos, carbofuran, mancozeb and their formulations to the tropical earthworm for their toxicity (De Silva, *et al.,* 2010) and the effect of two organophosphates, chlorpyrifos and diazinon (Booth, *et al.,* 2000).

The study obviously revealed that as the LC_{50} values and acute toxicity of Roundup herbicide. Xiao, *et al,* (2006) suggested that growth can be regarded as sensitive parameters to evaluate the toxicity of acetachlor on earthworms. The determination of LC_{50} values is useful in the evaluation of safe level of tolerance of pollutant and moreover it provides fundamental data to design more complex disposal modes of toxicity to the exposed animals. It is suggested that the cronic test, aiming at sublethal effects is more sensitive and is a more realistic approach for the prediction of environmental effects because in the field, the exposure concentration of pesticides are usually quite low (Römbke, *et al*, 2007). Toxicity test are basic tools for ecological risk assessment of toxic compounds. Riepert, *et al*, (2009) reported that the acute earthworm test is part of the basic test set, but the earthworm reproduction test is considered ecologically more relevant.

Table 1: Calculation of probit regression line for some experiments in which worms, *Eisenia fetida* were exposed to different concentrations of Roundup herbicide in the Busvine / Nash technique for a period of 24 hrs.

Concentration of Roundup herbicide (ppm)	Number of worms used	% of mortality	Corrected % mortality P	Log X	Concentration of Roundup herbicideX 1000	Empricalprobit	Expected probit Y	Working probit Y	Weighing Probit coefficient	Weight W	WX	WY	WX²	WY²	WXY	Y'	X
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
0·040	10	30	30	1·6020	40	4·48	4·47	4·48	0·558	5·58	8·939	24·942	14·320	111·490	39·957	4·4823	1·6019
0·045	10	40	40	1·6531	45	4·75	4·78	4·74	0·616	6·16	10·183	29·444	16·833	140·742	48·674	4·7828	1·6530
0·050	10	50	50	1·6989	50	5·00	5·02	5·005	0·637	6·37	10·821	31·977	18·383	160·524	54·321	5·0521	1·6988
0·055	10	60	60	1·7403	55	5·25	5·34	5·252	0·626	6·26	10·894	33·428	18·958	178·505	58·173	5·2956	1·7402
0·060	10	70	70	1·7781	60	5·54	5·52	5·528	0·581	5·81	10·330	32·071	18·367	177·031	57·021	5·5179	1·7780
										30·18	51·167	151·862	86·861	768·292	258·146		
										∑W	∑WX	∑WY	∑WX²	∑WY²	∑WXY		

Table 2: Calculation of probit regression line for some experiments in which worms, *Eisenia fetida* were exposed to different concentrations of Roundup herbicidein the Busvine / Nash technique for a period of 48 hrs.

Concentration of Roundup herbicide (ppm)	Number of worms used	% of mortality	Corrected % mortality P	Log X	Concentration of Roundup herbicideX 1000	Empricalprobit	Expected probit Y	Working probit Y	Weighing Probit coefficient	Weight W	WX	WY	WX²	WY²	WXY	Y'	X
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
0·030	10	30	30	1·4770	30	4·48	4·48	4·48	0·558	5·58	8·241	24·998	12·171	111·991	36·919	4·5257	1·4769
0·032	10	40	40	1·5050	32	4·75	4·80	4·72	0·627	6·27	9·436	30·096	14·201	144·460	45·292	4·8078	1·5049
0·034	10	50	50	1·5314	34	5·00	5·12	5·00	0·634	6·34	9·709	32·460	14·868	166·195	49·710	5·0737	1·5313
0·036	10	60	60	1·5562	36	5·25	5·34	5·252	0·626	6·26	9·741	33·428	15·158	178·505	52·016	5·3236	1·5561
0·038	10	70	70	1·5797	38	5·54	5·54	5·528	0·581	5·81	9·178	32·187	14·498	178·315	50·846	5·5604	1·5796
										30·26	46·305	153·169	70·896	779·466	234·783		
										∑W	∑WX	∑WY	∑WX²	∑WY²	∑WXY		

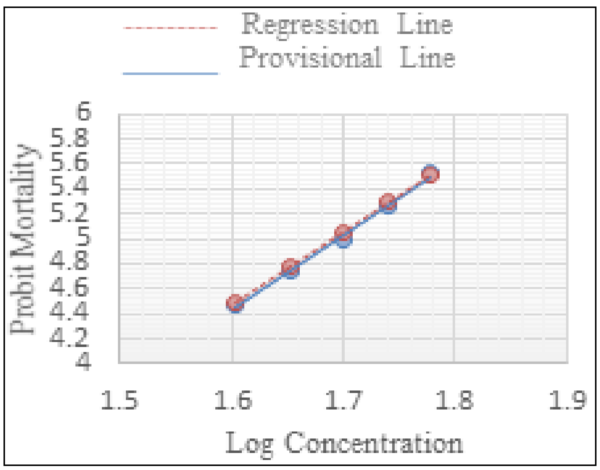
Table 3: Calculation of probit regression line for some experiments in which worms, *Eisenia fetida* were exposed to different concentrations of Roundup herbicidein the Busvine / Nash technique for a period of 72 hrs.

Concentration of Roundup herbicide (ppm)	Number of worms used	% of mortality	Corrected % mortality P	Log X	Concentration of Roundup herbicideX 1000	Emprical probit	Expected probit Y	Working probit Y	Weighing Probit coefficient	Weight W	WX	WY	WX²	WY²	WXY	Y'	X
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
0·020	10	30	30	1·3009	20	4·48	4·47	4·48	0·558	5·58	7·259	24·942	9·443	111·490	32·447	4·5002	1·3008
0·022	10	40	40	1·3423	22	4·75	4·78	4·74	0·616	6·16	8·268	29·444	11·098	140·742	39·521	4·7935	1·3422

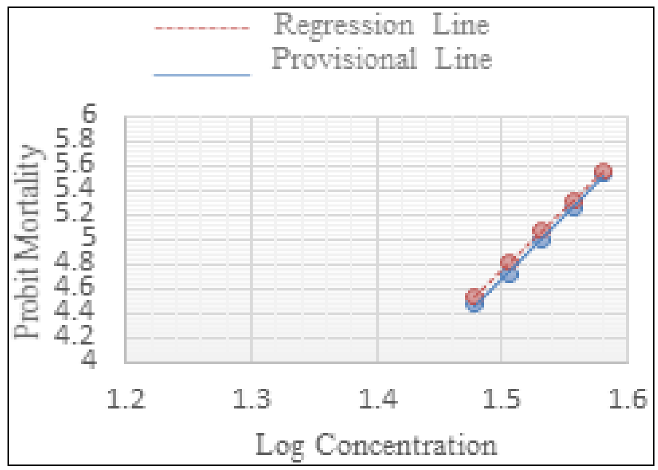
0·024	10	50	50	1·3801	24	5·00	5·09	5·005	0·637	6·37	8·791	32·423	12·132	165·033	44·746	5·0614	1·3800
0·026	10	60	60	1·4149	26	5·25	5·32	5·252	0·626	6·26	8·857	33·303	12·531	177·171	47·119	5·3079	1·4148
0·028	10	70	70	1·4471	28	5·54	5·53	5·528	0·581	5·81	8·407	32·129	12·165	177·673	46·490	5·5360	1·4470
										30·18	41·582	152·241	57·369	772·109	210·323		
										$\sum W$	$\sum WX$	$\sum WY$	$\sum WX^2$	$\sum WY^2$	$\sum WXY$		

Table 4: Calculation of LC_{50} values using probit analysis for the earthworm, *Eisenia fetida* after exposure to Roundup herbicide for 24, 48 and 72 hours.

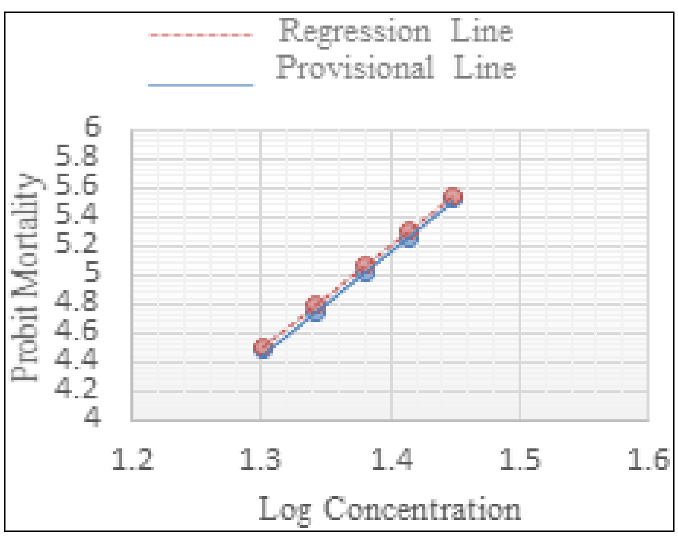
Sr. No.	Time of exposure	Regression equation $Y = \overline{Y} + b(X - \overline{X})$	LC_{50} value	CHI square	variance	Fiducial limits upto 95% confidence	
	(Hours)		ppm	f^2	V	M_1	M_2
1	24	5·0318 + 5·881 (1·6989 - 1·6953) = 5·0521	0·04898	0·0683	0·001	0·04246	0·05636
2	48	5·0617 + 10·075 (1·5314 - 1·5302) = 5·0737	0·03342	0·1008	0·001	0·02871	0·03811
3	72	5·0444 + 7·085 (1·3801 - 1·3777) = 5·0614	0·02350	0·0277	0·001	0·02032	0·02698



(a)



(b)



(c)

Fig. 1: Relation between probit mortality of the worm, *Eisenia fetida* and dose of Roundup herbicide showing mortality in probit/concentration regression line after exposure for (a) 24, (b) 48 and (c) 72 Hours.

Calculation of working probit are calculated from the formula, working probit (Y)

(1) $Y = YO + Kp$,

Where P = Percentage killed

YO and K are read from (Busvine Table-7) Table-10

= 3·58 + 0·0300 × 30

= 3·58 + 0·90

= 4.48

(2) 3·70 + 0·0260 × 40 = 4·74

(3) 3·75 + 0·0251 × 50 = 5·005

(4) 3·68 + 0·0262 × 60 = 5·252

(5) 3·54 + 0·0284 × 70 = 5·528

$$\bar{X} = \frac{\Sigma WX}{\Sigma W} = \frac{51 \cdot 167}{30 \cdot 18} = 1 \cdot 6953$$

$$\bar{Y} = \frac{\Sigma WY}{\Sigma W} = \frac{151 \cdot 862}{30 \cdot 18} = 5 \cdot 0318$$

Estimation of regression Coefficient 'b' is found by the formula:

$$b = \frac{\Sigma WXY - \bar{X}\,\Sigma WY}{\Sigma WX^2 - \bar{X}\,\Sigma WX}$$

$$b = \frac{258 \cdot 146 - 1 \cdot 6953 \times 151 \cdot 862}{86 \cdot 861 - 1 \cdot 6953 \times 51 \cdot 167}$$

$$b = 5 \cdot 881$$

Regression equation

$$Y' = \bar{Y} + b(X - \bar{X})$$

(1) $Y' = 5 \cdot 0318 + 5 \cdot 881(1 \cdot 6020 - 1 \cdot 6953) = 4 \cdot 4823$

(2) $Y' = 5 \cdot 0318 + 5 \cdot 881(1 \cdot 6531 - 1 \cdot 6953) = 4 \cdot 7828$

(3) $Y' = 5 \cdot 0318 + 5 \cdot 881(1 \cdot 6989 - 1 \cdot 6953) = 5 \cdot 0521$

(4) $Y' = 5 \cdot 0318 + 5 \cdot 881(1 \cdot 7403 - 1 \cdot 6953) = 5 \cdot 2956$

(5) $Y' = 5 \cdot 0318 + 5 \cdot 881(1 \cdot 7781 - 1 \cdot 6953) = 5 \cdot 5179$

Calculated X' Values

$$X' = \frac{Y - (\bar{Y} - b\bar{X})}{b}$$

$$(1)X' = \frac{4 \cdot 4823 - (5 \cdot 031 - 5 \cdot 881 \times 1 \cdot 6953)}{5 \cdot 881} = 1 \cdot 6019$$

$$(2)X' = \frac{4 \cdot 7828 - (5 \cdot 031 - 5 \cdot 881 \times 1 \cdot 6953)}{5 \cdot 881} = 1 \cdot 6530$$

$$(3)X' = \frac{5 \cdot 0521 - (5 \cdot 031 - 5 \cdot 881 \times 1 \cdot 6953)}{5 \cdot 881} = 1 \cdot 6988$$

$$(4)X' = \frac{5 \cdot 2956 - (5 \cdot 031 - 5 \cdot 881 \times 1 \cdot 6953)}{5 \cdot 881} = 1 \cdot 7402$$

$$(5)X' = \frac{5 \cdot 5179 - (5 \cdot 031 - 5 \cdot 881 \times 1 \cdot 6953)}{5 \cdot 881} = 1 \cdot 7780$$

Calculated LC_{50} Values for regression equation

$$Y = \frac{Y' - (\bar{Y} - b\bar{X})}{b}$$

$$X = \frac{Y' - (\bar{Y} - b\bar{X})}{b}$$

$$= \frac{5 - (5 \cdot 031 - 5 \cdot 881 \times 1 \cdot 6953)}{5 \cdot 881}$$

$$= \frac{9 \cdot 939}{5 \cdot 881}$$

$$= 1 \cdot 6900$$

$= 1 \cdot 6900$ Antilog $48 \cdot 98$ divided by $1000 = 0 \cdot 04898$

$LC_{50} = 0 \cdot 04898$ ppm.

Calculation of Standard error of LC_{50} as follows

$$V = \frac{1}{b^2}\left(\frac{1}{\Sigma W} + \frac{(m - \bar{X})^2}{\Sigma WX^2 - \frac{(\Sigma WX)^2}{\Sigma W}}\right)$$

$$V = \frac{1}{34 \cdot 586}\left(\frac{1}{30 \cdot 18} + \frac{(1 \cdot 69 - 1 \cdot 6953)^2}{86 \cdot 861 - \frac{(51 \cdot 167)^2}{30 \cdot 18}}\right)$$

$V = 0 \cdot 000963$ Antilog $1 \cdot 000$ divided by 1000

$V = 0 \cdot 001$

Calculation for Chisquer (f^2)

$$f^2 = (\Sigma WY^2 - \bar{Y}\,\Sigma WY) - b\,(\Sigma WXY - \bar{X}\,\Sigma WY)$$

$$f^2 = (768 \cdot 292 - 5 \cdot 0318 \times 151 \cdot 862) - 5 \cdot 881\,(258 \cdot 146 - 1 \cdot 6953 \times 151 \cdot 862)$$

$$f^2 = 0 \cdot 0683$$

$f^2 = 0·0683$ for n-2 i.e. (5–2) 2 degree of freedom the table value (Table – 5 original from Busvine 1971) at 0.05 level is 7.851 this shows that $P < 0.05$ indicating homogeneity.

Calculation for fiducial limit

$M_1 = m - 1 \cdot 96\sqrt{V}$

$M_1 = 1 \cdot 69 - 1 \cdot 96\sqrt{0 \cdot 001}$

$M_1 = 1 \cdot 628021$

$1 \cdot 6280$ Antilog $42 \cdot 46$ divided by $1000 = 0 \cdot 04246$

$M_1 = 0 \cdot 04246$

$M_2 = m + 1 \cdot 96\sqrt{V}$

$M_2 = 1 \cdot 69 + 1 \cdot 96\sqrt{0 \cdot 001}$

$M_2 = 1 \cdot 7519$

$1 \cdot 7519$ Antilog $56 \cdot 36$ divided by $1000 = 0 \cdot 05636$

$M_2 = 0 \cdot 05636$

Note: Likewise calculations for the tables 2 and 3 were also done.

5. Conclusions

It was observed that increased/more concentrations are increasing the percent mortality. It was reported that (Hanumante, 1975). In conclusion, Roundup herbicide is moderately toxic to earthworms, *Eisenia fetida*.

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