Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(5), 2465-2469 | ISSN:2251-6727

Effect of Chlorpyrifos on Morphological Phenomenon of Earth Worm Lampito Mauritii (Kinberg)

Mrs.Vedavathi Bonela1, Dr.Mani Gudivada2, Mrs.Aruna.M3

Lecturer in Zoology,KVR Govt. College for women,Kurnool,Cluster University,Kurnool, Andhra Pradesh.India.

Lecturer in Zoology,Andhra University,Visakhapatnam, Andhra Pradesh, India

Lecturer in Zoology,JMJ college for women, Tenali,Andhra Pradesh, India

(Received: 25 August 2025 Revised: 27 September 2025 Accepted: 14 October 2025)

KEYWORDS

Chlorpyrifos, toxicity,LC50 ,

Kurnoolsoil, Lampito mauritii(Kinberg).

ABSTRACT: The study aimed to assess the toxicological effects of organophospate pesticide chlorpyrifos on Lampito mauritii(Kinberg) .The lethal concentrations (LC50) were measured experimentally by exposing the earthworms in artificial soil test. The study revealed that chlorpyrifos was found to be the most hazardous insecticide at a 48-hour period, with a LC50 value of severe toxic 1to 10µgcm^{-2} against the earthworms. Few morphological signs like curling and mucus secretion bulging of clitellum were observed.

Introduction: Earth worm Lampito mauritii(Kinberg) is an hermaphrodite invertebrates belonging to the phylum Annelida, family lumbricidae and dominant in the temperate and tropical soilareasof Andhra Pradesh,India.. Earthworms create burrows in the soil these burrows improves soil prosity helpful in increased aeration and water filteration property, which increases the soil fertility.(Edward at.al1999) Earthworms, exhibit pronounced sensitivity to insecticide toxicity which allows scientists to use them in risk assesments to evaluate the chemical pollution. (Fang et al., 2018), The earthworm population decline due to over exposure of different insecticides for a prolonged period can lead to decrease in soil fertility and nutrient cycling and pedogenesis (Julka JM .2001.). The present research study carried on Lampito mauritii(Kinberg) widely seen in Kurnool soils of Andhra Pradesh, India, 518001. In Kurnool local language Lampito marutii is called as "Erralu". Because of their appearance in light pink colour. Earthworm study was based on evaluation of soil contamination with Chlorpyrifos chemicals (Edwards CA,1992) .

Lampito mauritii is extensively available throughout Andhra Pradesh, India, due to its ability to improve soil fertility and is known as an ecologically significant soil organism. In recent years usage of vermicompost has increased in all areas of world. Earth worms also acts as common prey for many vertebrates animals. They play a key role in environmental toxic studies as earthworms are the food source of many terrestrial vertebrate classes like Fishes, Amphibians, Aves, and small mammals. Chlorpyrifos is an organophosphate [97.2% technical product (TC)] is a chemical, very frequently used for agricultural crops by the local farmers. The chemical has been widely found in the soil environment as a result of frequent sprying of pesticides. Chlorpyrifos reminents were identified in the Joharapuram bazra crop fields which could be one of the causes for reduction of Earthworm population .

In Kurnool one of the major pesticides used by the farmers is Chlorpyrifos .In this regard our team studied the toxicity study on Chlorpyrifos pesticide on species Lampito mauritii(Kinberg) which is a common soil earworm in Kurnool crop fields. The study was conducted in crop fields at Joharapuram village, Kurnool district of Andhra Pradesh, 518002, is a state in peninsular part of southern India. The earthworm species Lampito mauritii(Kinberg) was used as a model organism for Contact toxicity by filter paper method estimated the toxic levels and lethality of different pesticide concentrations for 24 and 48 hrs.. (According to Goulson (2013). To increase yielding capacity synthetic pesticides are frequently applying in cultivation fields through spraying and soil mixing. (Goulson D2013) This

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(5), 2465-2469 | ISSN:2251-6727

practice of farmers adversely affecting the non target species biology.The laboratory experiment our team followed the OECD guidelines in conducting the laboratory experiment. As per the recommended agricultural doses (RAD), The earthworms Lampito mauritii(Kinberg) were exposed to the artificial soil contaminated with different concentrations of the tested pesticide Chlorpyrifos. The soil of pesticides was recognized as ecologically more dangerous to Lampito mauritii(Kinberg).

The filter paper contact test is easy to perform and gives accurate results with the sensitive animals like Earthworms. The artificial soil test gives toxicity data coincides with the field earthworms. The 50% lethal concentration (LC 50) values used in the experiment of Chloripyrifos pesticides were little bit more than with the crop field values. It means 50% mortality can be seen in the LC 50 test. A long-time exposure of the pesticides has shown toxicity on various parameters of the Earthworm life cycle. The laboratory experiment results have shown that over and long term usage of pesticides are leads to severe toxicity to Lampito mauritii(Kinberg population. (Garcia. M. 2011) In understanding the field experiment data more information about the toxicity effects on Kurnool soil earthworms are needed, which typically show variability in interpretation.

MATERIALS AND METHODS

Insecticide of formula grade Chlorpyrifos (purity >98%) was obtained from local vendors. Reagents used in the present study were of analytical grade purchased from Hanuman fertilizers, old market, Kurnool, used without further purification. Lampito mauritii with (weighing 300-500 mg), well-developed clitella (2month old) are randomly selected from Joharapuram, sudireddypalli crop fields They were carefully brought to the laboratory immediately and kept in the breeding media. Other equipment for this study are Whatman No.1 filter paper, Petridishes, Acetone, weighing balance, pipette,1ml pipette tips and distilled water.

BREEDING MEDIUM

For the Lampito mauritii laboratory experiment, we selected breeding medium containing cow manure and coconut peat, as they have low ionic conductivity. The dry constituents are blended and mixed thoroughly in a large-scale laboratory mixer. Wooden breeding boxes of about 50 x 50 x 15 cm are used . Three layers of contaminated free loam soil which is collected from the Sunkesula barraige region kept at the bottom as base soil, a thin layer of mixure of cocopeat (pH 5.8) finely crushed leaves and wet powder of cow dung with soft soil (5.0 milli-Siemens) layered in thin manner with a ratio of in a 50:50 mixture of medium proportion. Wooden breeding boxes of about 50 x 50 x 15 cm with tight lids are used for optimum production at a pH of 7.0. Wet jute clothes were placed as a cover on the boxes to avoid earthworm escaping. Earthworms Lampito mauritii were kept in the breeding media, laboratory temperature was at a temperature of 27±3 °C. These worms were acclimated for 7 days under artificial soil conditions for the toxicity tests.

Calculation of LC50

The test solution was prepared for 1000ml with ionized water . acute toxicity was tested for 48 hr by using no.1 Whatman filter paper contact toxicity test to understand the lethal concentration (LC), different concentrations of Insecticides were applied to earthworms Lampito mauritii in the filter paper contact test (OECD 1984) . The Petridishes are covered with filter paper at all sides. The test chemical dissolved in Chloroacetamide to create varied concentrations. 3hours prior to experiment the petridishes were sprayed with1.06 ml of test chemical and allowed for drying. After drying, the glass plates were remoistend by adding 3ml of de-ionized water,

Earthworms(300 to 400mg weight) were placed (one earthworm on glass-plate) and were divided into 10 groups with pesticide concentrations 0.99,0.152,0.230, 0.349 and 0.530µg/cm^2 were kept in the dim light for 24 hours at 27±2 °C. Here in this laboratory experiment Chloroacetamide was the control substance. Experiment done for four times, group mortality rate of Lampito mauritii reported following a 48- hour exposure period for 14 days. The morphological ariations were also identified when earthworms were indirect exposure to Chloropyrifos (Reinecke. SA. 2007) At final stage our

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(5), 2465-2469 | ISSN:2251-6727

team examined the results of earthworms at different test periods and concentrations .

Results

The filter paper contact test observations showed that the contact toxicities of the various pesticides to Lampito mauritii differed greatly. In the present study the observations showed that treated groups have lesser weight and growth than the control vial groups. Chlorpyrifos exhibited utmost toxic nature at a 24-hour period, with an LC50 value of 6.49 (4.03~30.17)mg.The data of pesticide toxicity from Laboratory tests explained a definite concentration- dependent relationship, and longer exposure periods resulted in higher death rate of the earth worms(Rico A, Sabater.2016) Surviving worms also had reduced body weight, and in some cases, morphological changes. Lethality rate was categorized as relatively no evidence of toxic >1000 µg cm^{-2},medium toxic 100-1000 µgcm^{-2}, high toxic10-100µgcm^{-2},severe toxic 1-10µgcm^{-2}. The calculations of LC_{50} values were made followed by the standard procedures using probit analysis. Data represented as (n=10) . The LC_{50} values are calculated by one-way ANOVA analysis with Duncan's Multiple Post Hoc Range Test(DMRT).The results were estimated as significant when the $p < 0.05$ values and confidence levels were 94.9%. . The quantitative analysis as showed in the table 1.

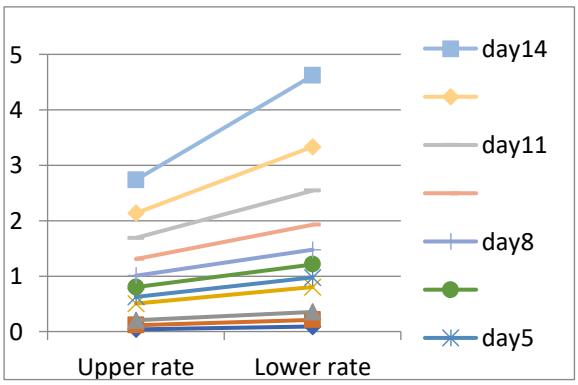
Table -1 Mean Mortality rate of earthworm *Lampito mauritii* .Each value is the mean ± SE individual observations.

Valuesarestatisticallysignificantat$P$<0.05.

Lethal concentrations(LC1-99) Chloropyrifos on earthworm Lampito mauritii at 48hr exposure			
Lethal concentrations	Chloropyrifos	Lower rates	Upper rates
LC1	0.06	0.039	0.091
LC5	0.098	0.074	0.119
LC10	0.12	0.091	0.143
LC15	0.135	0.106	0.15
			9
LC20	0.16	0.121	0.174
LC40	0.205	0.177	0.232
LC50	0.224	0.204	0.267
LC80	0.394	0.3	0.45
LC90	0.45	0.382	0.615
LC95	0.559	0.447	0.792
LC99	0.797	0.599	1.282
Slope±SEM	4.367±0.590	3.199	5.53
Intercept ± SEM	2.760±0.350	2.351	3.148
Chi-SquareValue	7.028		
Pvalue	<0.05		

Discussion

The toxic effect of Chlorpyrifos was greatly observed in 48hr LC50 values to earthworms (Wang . 2015). By this controlled experiment Chlorpyrifos, has a severe Toxic effect and hazardous insecticide, and have shown significant toxological effect on morphology of Lampito mauritii(Kinberg). The LC_{50} of Chlorpyrifos after 48hr exposure was found to be 0.224 µg/cm^2(Confidence interval: 0.204-0.266).



Graph-1 Lethal observations of Chlorpyrifos toxicity in Lampito mauritii, concentrations in mg/kg

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JCHR (2025) 15(5), 2465-2469 | ISSN:2251-6727

It is clearly observed that the morphological and behavioral abnormalities occurred in contaminated earthworms after 12hr of exposure when treated with Chloropyrifos (0.532 µg/cm^2- 0.105 µg/cm^2) symptoms like clitellum bulging and secretion of coelomic fluid from the cuticle, release of mucus were observed. In some earthworms body curling started at the highest and lowest concentration. (Wang at.ail2015). By the filter paper contact test we can easily detect the variations in tissues and external appearance, and the test is very much useful in eco toxicological studies especially in earthworm fauna(LavelleP 2002). The earth worms became restless due to the toxicity of Chloropyrifos. Chlorpyrifos highly effects on the nervous system by inhibiting acetyl cholineesterase (a neurotransmetor) enzyme secretion. After12hr external body changes like high mucus secretion, body coiling observed at concentration level 0.529 µg/cm^2- 0.100 µg/cm^2. tissue shrinkage appearance found at the low rlevel of concentration after exposure 6h of (0.049 µg/cm^2- 0.052 µg/cm^2)(Kathireswari P.2016). Morphological changes were not identified in the control group. when 48h exposure to the pesticide the earthworms recorded 50% Mortality at 0.023µg/cm^2 consequently 90% mortality seen at concentration of 0.051µg/cm^2.(Sizmur, T.2011.) The earthworm species Lampito mautitii was highly effected from the insecticide test concentration severe toxic 1to 10µgcm^{-2}.

Conclusion:

This study highlights the toxological reaction to chlorpyrifos on Lampito mauritii. Chloropyriphos frequently used in the crop fields of joharapuram region Kurnool, with a permitted dose of 2.5 ml l-1. When the earthworm Lampito mauritii exposed to the concentration of lethal dose the maphological alteration can occur, these changes could directly impact on the burrowing nature and soil porosity in the ecosystem, which contribute negative soil fertility. By this study we advise the kurnool farmers to use biofertilizers with no side effects instead of synthetic chemicals to maintain healthy earthworm population in the ecosystem.

ACKNOWLEDGMENTS

The authors are thankful to the the Vice chancellor, Cluster University, Kurnool for providing the necessary facilities for research work. and encouragement during the course of work

Ethics declaration: Not applicable.

Conflict of interest: No conflict of interest declared.

Funding Declaration: No funding received for this research.

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JCHR (2025) 15(5), 2465-2469 | ISSN:2251-6727

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