International Journal of Agriculture, Environment and Bioresearch

Vol. 3, No. 01; 2018

ISSN: 2456-8643

EFFECTS OF PESTICIDE, CHLOROPYRIFOS ON AN ANECIC EARTHWORM LAMPITO MAURITII

Dr. AlivaPatnaik* and Maminee Panda
Assistant Professor ,School of Life Sciences, Sambalpur University, JyotiVihar, Burla-768019

ABSTRACT

Earthworm, the "Intestine of Earth" plays a vital role in providing nutrients to soil increasing its fertility potential. This study was done to study the impact of chloropyrifos pesticide on different biological parameters of LampitomauritiiKinberg. Subtoxic concentration of chloropyrifos such as 5ppm, 10ppm, 20ppm brought significant changes insurvivility, growth, feeding, regeneration, respiration and excretion as compare with 0ppm dose. The values of these parameters decreased significantly with the increasing dose of the input pesticide, when statistically observed

Keywords: Biodiversity, Bioindicator, Chloropyrifos, Ecotoxicological assessment, Organophosphate

INTRODUCTION

The correlation between soil and its biodiversity is strongly maintained by different biological activities played by its organisms which in turn display the physical and chemical composition of soil. Estimations by Ouellet*et. al.* (2008) and Jouquet*et. al.*(2010) indicate that earthworms may represent up to 60–80% of the total animal biomass in soil which help in increasing water infiltration, soil aeration, and the stabilization of soil aggregates, thus enhancing soil fertility. Abundant uses of pesticides and fertilizers not only hamper soil health but also get accumulated in the living bodies by biomagnifications. Moreover, simultaneous inoculation of different pesticides even causes 99% mortality of biodiversity than individual pesticides (Relyea, 2005) and also causing severe health impairment at their sub lethal concentration. In 2011, EPA estimated that, in the general US population, people consume 0.009 micrograms of Chlorpyrifos per kilogram of their body weight per day directly from food residue.

The objective of the work is to study the toxic impact of chloropyrifos on some physiological parameters like survivility, growth, feeding, regeneration, respiration and excretion of *Lampitomairitii* earthworm.

MATERIAL AND METHOD:

Lampitomairitii is an epi-anecicearthworm widely distributed in India which has greater importance in soil fertility. Chlorpyrifos is an organophosphate pesticide brings about the death of insects and worms belonging to Coleoptera, Diptera, Homoptera and Lepidoptera in agriculture. It acts on the nervous system of insects by inhibiting acetyl cholinesterase.

In order to accomplish the objectives the following methodologies were adopted.

Vol. 3, No. 01; 2018

ISSN: 2456-8643

Survivility: The survivility experiment was done for 240 hours by the method proposed by OECD (Organisation for Economic Co-operation and Development; 1984). It was found that the earthworm, Lampitomauritii ,Kinberg did not survive beyond 25ppm for 240 hours. So the subtoxic level was set at 5, 10 and 20 ppm of chlopyriphos and other experiments were conducted.

500g (300g soil and 200g cowdung) 2mm sieved, air dried soil was taken with moisture maintained at 20±2 g% by addition of distilled water in case control sets i.e. '0'ppm and respective Chloropyrifos solution in experimental sets i.e. 5, 10 and 20 ppm. 10 replicates of each concentration were taken to which about 1.5g of earthworms were inoculated after 5 days of moisture addition during which microbial activation in soil occurs. The observations were taken on 10^{th} ,20^{th} and 30^{th} day of exposure to various subtoxic levels of exposure to the Chloropyriphos and statistically analysed (Snedecor and Cochrane, 1967; Gupta, 1980).

Growth: The earthworms were exposed to prepared 0 ppm (control), 5 ppm, 10 ppm, 20 ppm of the Chloropyrifosin soil to observe its toxicity on the growth by noticing the changes in their gained biomass over initial biomass.

Feeding rate of the exposed organisms was done by the estimation of Carbon content of the stable aggregate by Walkley and Black Titration method (1934) and also from the amount of carbon, energy conversion was done (Remmert, 1980).

Respiratory metabolism was quantified by Alkali Absorption method (Witkamp, 1966).

Excretion: Spectrophotometric analysis (Kaplan, 1969) was carried out for determination of Ammonia excretion.

Regeneration: For estimation of regeneration ability, the number of regenerated segments was observed in the respective solutions.

RESULT:

Survivility:

Experimental result for percentage of survivility of *Lampitomauritii*indicates 100% mortality of the earthworm occurs at 180 hours, 168 hours, 132 hours when exposed to 5 ppm, 10 ppm and 20 ppm respectively. Likewise at 120 hours 100%, 77.5%, 67.5%, 5% of the total earthworms input survived under treatment with 0 ppm, 5 ppm, 10 ppm,20 ppm respectively as shown in Fig-1.

Vol. 3, No. 01; 2018

ISSN: 2456-8643

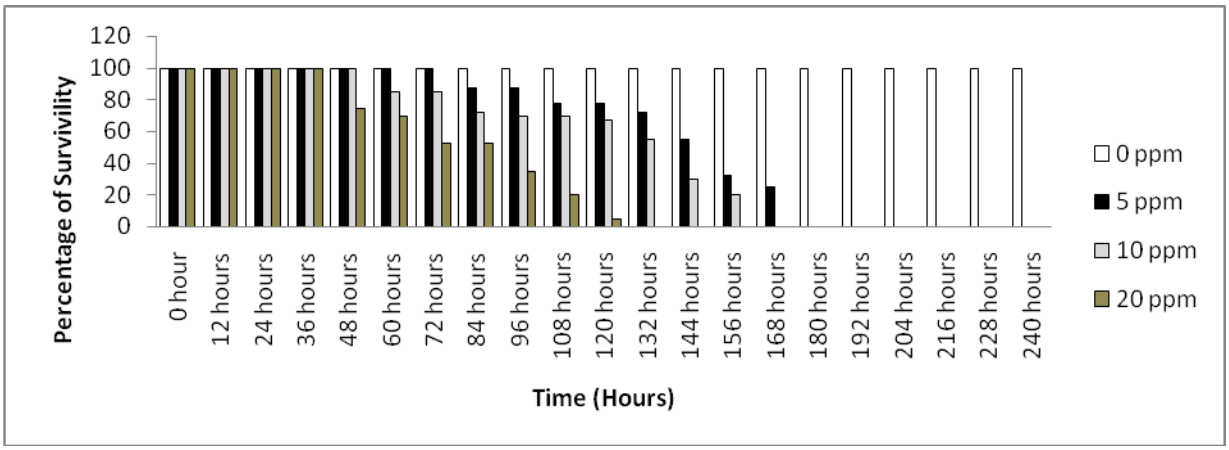

Fig-1: Percentage survivility of *Lampitomauritii* earthworm under the impact of Chloropyrifos in laboratory culture.

Growth:

Alteration in growth in earth has been presented in Fig-2.There is a significant depletion in the weight of the earthworms when observed after 10,20 and 30 days. The percentage in body weight of the earthworm increases to 5.29%, 7.95%, 23.18% at 0 ppm Chloropyrifos on 10^{th}, 20^{th}, 30^{th} day over 0^{th} day respectively. But the weight was reduced by 35.53%, 36.42%, 40.26% over initial weight on exposure to 5 ppm, 10 ppm, 20 ppm respectively on 30^{th} day.

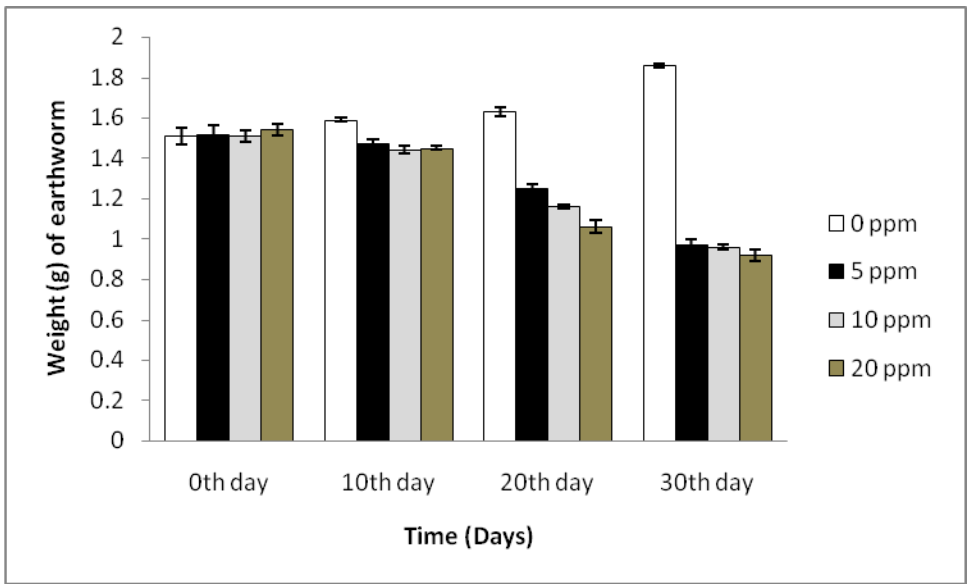

Fig-2: Change in weight of *Lampitomauritii* under the impact of Chloropyrifos in laboratory culture.

Vol. 3, No. 01; 2018

ISSN: 2456-8643

Feeding:

Fig-3 gives the information regarding the changes in the stable aggregate formation formed by the organisms under exposure to experimental solutionsover control solution at 10, 20 and 30 days. There was a significant decrease in the weight of stable aggregate formed also in the energy content of the produced stable aggregates in the respective concentrations over 0 ppm at at an interval of 10 days each (ANOVA at < 0.01 level of significance). After 10 days the percentage of stable aggregates declines to 34.06%, 49.61%, 51.32% when expose to 5 ppm, 10 ppm, 20 ppm over 0 ppm. Likewise after 30 days the percentage of stable aggregates reduces to 34.1%, 60.04%, 65.73%. Also the energy content amounted to 465.73, 271.59, 195.63 (KJ, Kg^{-1} soil, g^{-1} live tissue) in 5 ppm, 10 ppm, 20 ppm over the energy content 747.46 Kg^{-1} soil, g^{-1} live tissue of 0 ppm.

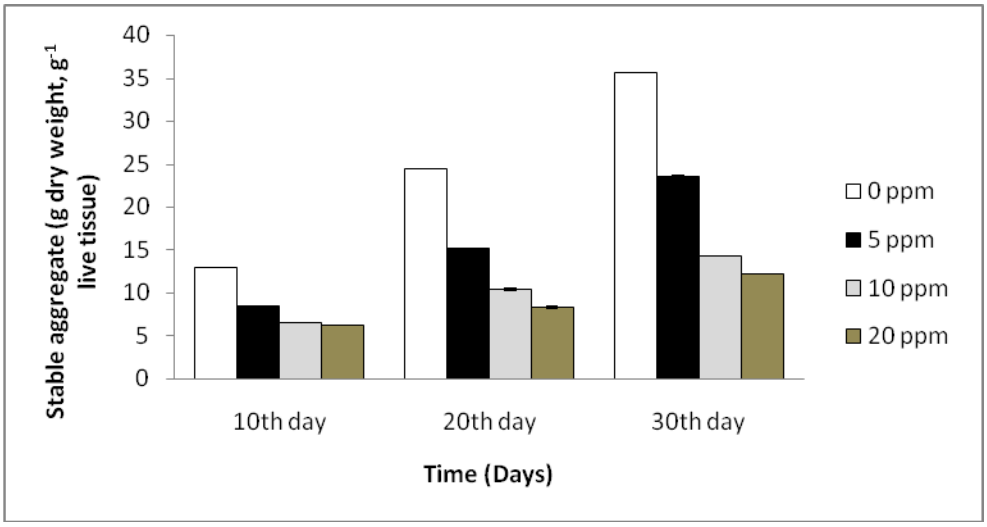

Fig-3: Stable aggregate formed by *Lampitomauritii* under the impact of Chloropyrifos in laboratory culture.

Respiration:

Rate of respiration increases with treatment duration and concentration of the pesticide as figured in Fig-5.It becomes elevated at 0^{th}, 10^{th}, 20^{th} and 30^{th} day when taken over 0 ppm. After 30 days the respiratory metabolic rate increases to about 13.71%, 20.42% and 39.60% at 5 ppm, 10 ppm, 20 ppm with respect to 0 ppm respectively.

Vol. 3, No. 01; 2018

ISSN: 2456-8643

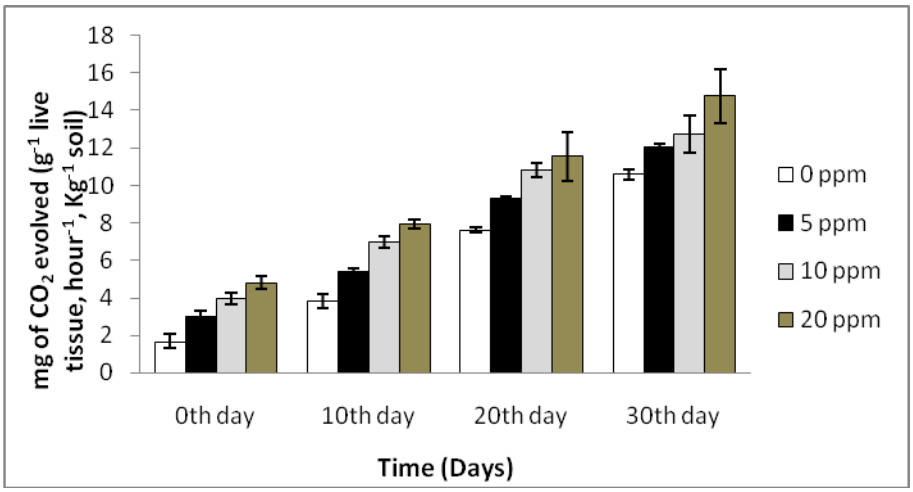

Fig-5: Respiratory metabolic rate of *Lampitomauritii* earthworm under the impact of chloropyrifos in laboratory culture.

Excretion:

Ammonia excretion was negatively influenced by the treatment of the earthworm with different concentrations with respect to durations as presented by Fig-6. Ammonia excretion after 10 days decreased by 21.76%, 34.54%, 52.65% 5 ppm, 10 ppm, 20 ppm when compared with 0 ppm respectively. Also reduction was observed in 20th day with a cut of 45.39%, 56.43%, 72.62% in all the respective solutions with comparison to the control one.

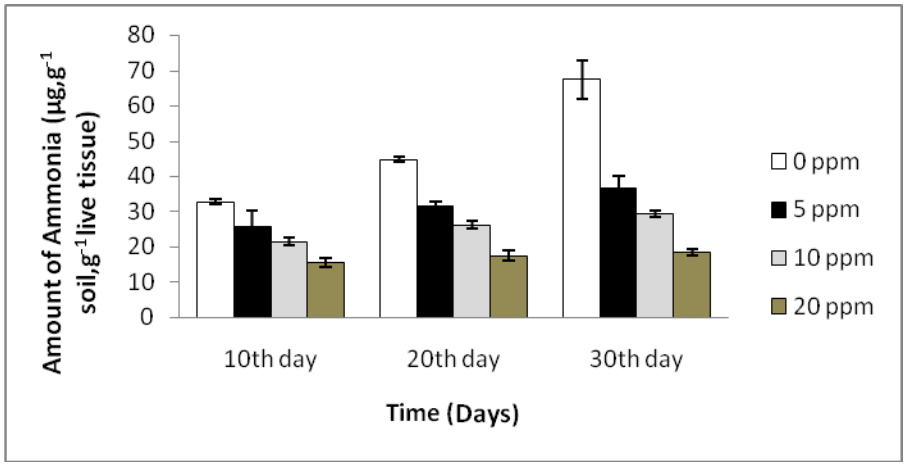

Fig-6: Ammonia excretion of *Lampitomauritii* earthworm under the impact of chloropyrifos in laboratory culture

Again when ANOVA at level of significance 0.01 was exercised for each parameter individually, significant alterations in their values were noted.

Vol. 3, No. 01; 2018

ISSN: 2456-8643

Regeneration:

Fig-4 clears about the decrease of regenerated segments at 5 ppm, 10 ppm, 20 ppm over 0 ppm at the rate of 19.57%, 44.57%, 60.87% at 10th day respectively. After 20 days, the percentage reduces to 18.98%, 48.61%, 63.43% in the respective treatment as compare to 0 ppm. Similarly on 30th day, about 14.83%, 36.92%, 54.36% decrease in the regenerated segments was seen in 5 ppm, 10 ppm, 20 ppm over 0 ppm respectively.

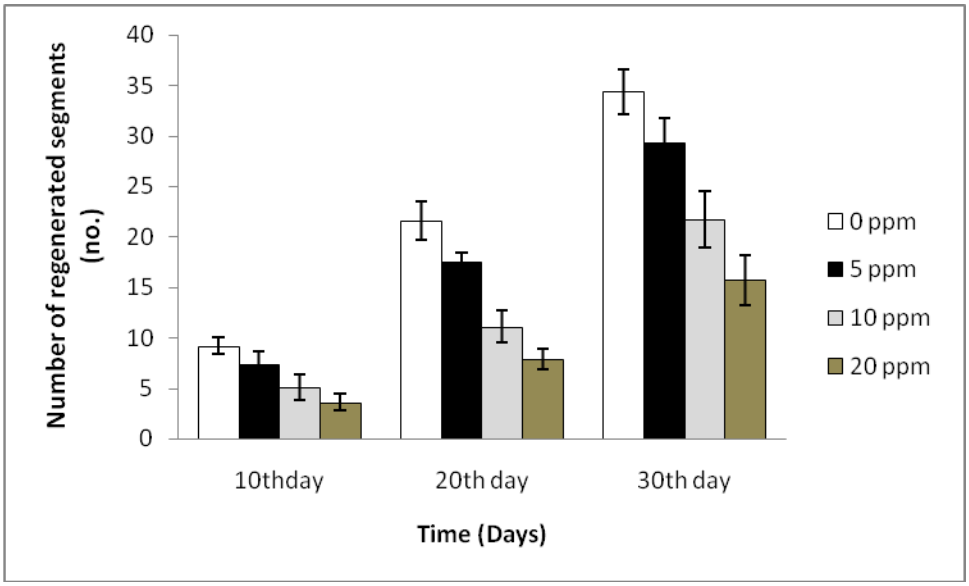

Fig-4: Percentage change in the number of regenerated segments of *Lampitomauritii* under the impact of chloropyrifos in laboratory culture.

DISCUSSION:

Survivility: Indiscriminate use of Pesticide in crop field has highly adverse and lethal impacts on almost all organisms. The extent of their survivility is interfered by the dose and concentration of the chemicals and duration of treatment with the biota. Study by Lapinski et.al. (2008)indicates cadmium and mercury ceases the survival rate of earthworms. Muhammad et.al.(2010) found Immidacloprid is toxic to earth wom*P. posthuma* with LC_{50} of 0.011ppm. Oluah et.al.(2010) reported that Atrazine had significant effect on earthworm survivility.These findings are similar with our present investigation when *L. mauritii* was exposed to Chloropyrifos. This may be due to the reduction in AChE and GST activities of pesticide-treated earthworms, *A. caliginosa* as was proved by Badawy*et. al*. (2013).Also coelomocytes showed a high level of DNA damage, by exposure to Chlorpyrifos treated soils as experimented by Piola*et. al.* (2009).

Growth: Biomass is a good indicator of the physiological requirements of tissues and material cycling (Thompson, 1970). Zhou et.al. (2007) found adverse effect of Chloropyriphos on the growth of earthworms and concluded that change in weight is a more sensitive index compared

Vol. 3, No. 01; 2018

ISSN: 2456-8643

to mortality in indicating toxic effects of acetochlor and methamidophos. Booth et. al. (2000) and Moslehet. al. (2003) found reduction in growth rate of pesticide treated *Aporrectodeacaliginosa.* Farrkh and Ali (2011) reported reduction in weight of various groups of earthworms when exposed to different concentrations of dichlorovos fumigant insecticide. Present study also shows significant decrease in biomass of *L. mauritii* on exposure to subtoxic levels of Chlopyriphos. Decrease in growth rate may be due to channelization of most of the energy towards respiration.

Feeding (Stable aggregate formation): Earthworms feed on organic matter according to their ecological category. They have a dramatic effect on aggregate size distribution and greatly increase the macro aggregate fraction and thus the overall carbon incorporation into the macro aggregates (Bossuytet.al., 2004). According to Tisdall and Oedes (1982) the feeding habit results in physical aggregation of the soil and earthworm activity creates structures, casts and galleries which modify the circulation and accumulation of water. They eject a significant amount of nutrients in their casts which is due to increase microbial activity in their gut and from their own metabolic activity. Reduction in surface casting, abundance of earthworms, biomass and increased litter accumulation when benomyl and related fungicides are sprayed (Wright, 1977). According to Dittbrenner*et. al.* (2010), significant decreases in cast production with respect to imidacloprid was found in *A. caliginosa* and *L. terrestris* which is similar to our study. Decline in stable aggregate formation by *Lampitomauritii* earthworm on application of different doses of chloropyriphos and thereby interfere in positive contribution of earthworms.

Soil metabolism (Respiration): Respiratory metabolism represents the energy loss of an organism. Soil respiration is being used for estimation of biological activity (Lundergarh, 1927). Earthworms on exposure to carbaryl and end osulfan showed an increased value of CO_2 evolution. Temperature stress results in 3 fold increase in the oxygen consumption in summer as compared to winter have been reported by Senapati and Dash (1983). In the study, enhancement in respiratory rate was found which also supports the study conducted by Yanchevaet. al. (2017) in Zebra mussel. This alterationin oxygen consumption may be due to respiratory distress as a consequence of impairment in oxidative metabolism as was observed in some fish by Marigoudaret. al.(2009).

Excretion: Mucus protein and nitrogenous metabolic wastes like ammonia and urea and free amino acids are the main nitrogenous compounds excreted by the earthworms. Present study indicates significant decrease in ammonia excretion on exposure to subtoxic level of Chloropyriphos which means the organism remain in the the ecosystem but it fails to contribute positively towards the system. Decrease in excretion by earthworms has been reported on exposure to sublethal dose of malathion by Senapatiet.al., (1992). Similar results were also reported by Patnaik and Senapati, (1996).

Regeneration: Regeneration of lost parts is proportional to growth (Stephenson, 1930). Kulkarni and Wakale (2012) studied that endosulfan dose showed regeneration after 30 days and commented this as toxic effect of endosulfan on earthworms. Regeneration is hampered on removal of nerve cord (Zhinkin, 1936). Kulkarniet. al. in 2012 stated that the rate of caudal

Vol. 3, No. 01; 2018

ISSN: 2456-8643

regeneration efficiency decreases with the increased concentration of endosulfan. In the present study regeneration rate has been significantly reduced by application of chloropyriphos at sub-lethal level.

CONCLUSION

Soil supports life through main five processes, biomass productivity, detoxification of pollutants, cycling of nutrients and water and also acts as a carbon sink which is hugely disturbed the vast application of pesticides and fertilizers to it. To overcome the problem, methods of biological pest control by application of pheromones, entomopathogenic micro-organisms, biopesticides to be implemented in place of agrchemicals.Geetha and Fulekar (2008) suggest that degradation of chloropyrifos is possible by the use of *Pseudomonasaeruginosa,*soil bacteria. Also according to (Ravi et. al., 2015) microbial consortium has the potential to degrade62.72 % of chlorpyrifos in pesticides contaminated soil.Therefore earthworms can be regarded as bioindicatorsin ecotoxicological assessmentto overcome soil pollution by increasing their healthy population.

REFERENCS

Badawy, M. E. I., Kenawy, A. and El-Aswad, A. F. 2013. Toxicity Assessment of Buprofezin,Lufenuron, and Triflumuron to the Earthworm *Aporrectodeacaliginosa.International Journal of Zoology,*Hindawi Publishing Corporation. Article ID 174523, 9 pages.

Booth, L. H., Heppelthwaite, V. J. and O'Halloran, K. 2000. Growth, development and fecundity of the earthworm *Aporrectodeacaliginosa*after exposure to two organophosphates. *New Zealand Plant Protection*, **53**: 221–225.

Bossuyt, H., Six, J. and Hendrix, P.F. 2004. Rapid incorporation of fresh residue-derived carbon into newly formed stable microaggregates within earthworm casts. *European Journal of Soil Science*,**55**: 393–399.

Dittbrenner, N., Triebskorn, R., Moser, I., Capowiez, Y. 2010. Physiological and behavioural effects of imidacloprid on twoecologically relevant earthworm species (*Lumbricusterrestris*and *Aporrectodeacaliginosa*). Ecotoxicology.

EPA (2011) U.S. EPA (2011-06-30). Chlorpyrifos Preliminary Human Health Risk Assessment for Registration Review (PDF) (Report).

Farrukh, S. and Ali, A.S. 2011.Effects of Dichlorovos Organophosphate on Growth, Reproduction, and Avoidance Behavior of Earthworm *Eiseniafoetida.* Iranian Journal of Toxicology, **5**(14): 495-501.

Geetha, M., Fulekar, M.H.2008. Bioremediation of pesticides in surface soil treatment unit using microbial consortia. Afr. J. Environ.Sci. Technol., **2**(2): 36-45.

Gupta, S.P.1980. Statistical Methods (14th eds.). *Sultan Chand and Sons, New Delhi*, pp. 1000.

Vol. 3, No. 01; 2018

ISSN: 2456-8643

Jouquet, P., Plumere, T., Thu, T. D., Rumpel, C., Duc, T. T., Orange, D.2010. Therehabilitation of tropical soils using compost and vermicompost is affected bythe presence of endogeic earthworms. Appl. Soil Ecol. **46**:125–133.

Kaplan, A.(1969). The determination of urea, ammonia and urease.In: Methods of Biochemical Analysis. Vol. 17. D. Glick (Edt.), John Wiley and Sons, 311-312.

Kulkarni, S. G., Wakale, A. S. 2012. Toxic Effect of Endosulfan on The Caudal Regeneration (Posterior Region) of The Earthworm Oligochaete,*Eiseniafetida*. International Journal of Basic and Applied Research.**02**(2):24-29.

Lapinski, S., Rosciszewska, M. 2008**.** The impact of cadmium and mercury contaminationon reproduction and body mass of earthworms. Plant SoilEnviron.,**54**(2): 61–65.

Lundergarh, H. 1927. Carbon dioxide evolution of soil and crop growth. Soil Sci., **23**: 417-453.

Marigoudar, S. R., Ahmed, R. N., David, M. 2009. Cypermethrin induced respiratory and behavioural responses of the freshwater teleost,*Labeorohita*(Hamilton).Vet. Arhiv.**79** (6), 583-590.

Mosleh, Y. Y., Ismail, S. M. M.,.Ahmed, M. T. and Ahmed, Y. M. 2003. Comparative toxicity and biochemical responses of certain pesticides to the mature earthworm *Aporrectodeacaliginosa* under laboratory conditions.Environmental Toxicology, **18**(5): 338–346.

Muhammad, F., and Farhanullah, M. K. 2010. Toxicity of imidacloprid (Nicotinoid) against earthworm *pheretimaposthuma*with reference to its effects on protein. Journal of Basic and Applied Sciences, **6**(1): 55 – 62.

O.E.C.D. (Organization for economic Co-operation and Development).1984. Earthworm, Acute toxicity text. OECD Guidelines for testing of chemicals No. 207, April 4 pp-194.

Oluah, N.S., Obiezue, R. N., Ochulor, A. J. and Onuoha, E. 2010. Toxicity and Histopathological effect of Atrazin herbicides on the earthworm *Nsukkadrilusmbae* under laboratory condition. Animal Research International, **7**(3): 1287-1293.

Ouellet, G., Lapen, D. R., Topp, E., Sawada, M., Edwards, M. 2008. A heuristic modelto predict earthworm biomass in agroecosystems based on selected management and soil properties. Appl. Soil Ecol. **39**, 35–45.

Patnaik **A. and** Senapati B.K. 1996. Impact of copper fungicide on the survivility, growth and regeneration of an anecic earthworm. Ecology, Environment and Conservation**.2**: 109-113.

Piola, L., Fuchs, J., Oneto, M. L., Basack, S., Giménez, R., Massaro, R., Papa, J. C., Kesten, E. and Casabé, N. 2009. Biomarkers for the assessment of chlorpyrifos effectson earthworms and on soil functional parameters.Pesq. agropec. bras. Brasília, **44**(8): 874-880.

Ravi, R. K.,Pathak, B. and Fulekar, M. H. 2015. Bioremediation of Persistent Pesticides in Rice field Soil Environmentusing Surface Soil TreatmentReactor. Int.J.Curr.Microbiol.App.Sci**., 4**(2): 359-369.

Relyea, R. A. 2005. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecological Applications*, **15**: 618–627.

Vol. 3, No. 01; 2018

ISSN: 2456-8643

Remmert, H. 1980. Ecology A Text Book, *Springer-Verlag, Berlin,* pp.289.
Senapati, B.K., Biswal,J., Pani, S.C. and Sahu, S.K. 1992.Ecotoxicological effects of malathion on earthworms.Soil Biol. And Biochem.(UK) **24** (12):1719-1722.
Senapati, B.K. and Dash, M.C. 1983. Energetic of earthworms population in tropical pastures from India. Proc. Indian Acad. Sciences*(Anim. Sci*) **92** : 315-322.

Snedecor, G.W. and Cochrane, W. G. 1967. Stastistical Methods. *Lowa Uni. Press, lowa,* U.S.A.

Stephenson, J. 1930. The Oligocheata. *Oxford Univ Press.*
Thompson, A.R. 1970. Effects of nine insecticides on numbers and Biomass of earthworms in pasture. Bull of Env. Cont. andToxicology, **5**: 577-586.
Tisdall, J.M. and Oedes, J.M. 1982. Organic matter and waste stable aggregates in soils. J. Soil Sci, **33**: 141-163.
Walkley, A., and Black, I.A. 1934. An examination of the Degtareff method for determination of organic carbon in soils: Effect of variations in digestion condition and of inorganic soil constituents. Soil Sci., 63: 251-263.

Witkamp, M. 1966. Rates of carbon dioxide evolution from the forest floor. Ecology, 47: 492-494.

Wright, M. A. 1977. Effects of benomyl and some other systematic fungicide on earthworms. Ann. Appl. Biol., 87: 520-524.

Yancheva, V., Mollov, I., Georgieva, E., Stoyanova, S., Tsvetanova, V., Velcheva, I.2017**.** Ex situEffects of Chlorpyrifos on the Lysosomal MembraneStability and Respiration Rate in Zebra Mussel, *Dreissenapolymorpha*(Pallas, 1771).Acta Zool. Bulg., Suppl. 8: 85-90.

Zhinkin, L. 1936. The influence of nervous system on the regeneration of Rhynchelmislimosella. J. Exp. Zool., 73: 43-65.