Biosci. Biotech. Res. Comm. Vol. 4, No. 1, June, 2011(84-89)

Effects of Endosulfan, an organochlorine pesticide on growth, reproduction and avoidance behavior of earthworm *Eisenia foetida.*

Sameena Farrukh and Ayesha S. Ali*
Postgraduate Department of Biotechnology,Saifia College of Science Bhopal, 462001,India
*Corresponding Author

ABSTRACT :
In the present study, growth, reproduction, and avoidance behavior of earthworm Eisenia foetida have been investigated in response to the exposure of an organochlorine pesticide endosulfan for a period of 28 days. Growth was found to be significantly affected at all three concentrations of pesticide used, whereas, the earthworms of control group showed normal increase in growth during the entire period of experiment. Reproduction seemed to be deliberately affected and the avoidance test of 48hrs was also found to be a sensitive parameter in evaluation of the toxic chemical.

Key words; *organochlorine, endosulfan, Eisenia foetida, avoidance test, reproduction.*

INTRODUCTION

Earthworms are the common prey of many invertebrates such as birds and shrews, and therefore, they play a key role in the biomagnifications process of several soil pollutants and in the occurrence of indirect effects on terrestrial vertebrates from soil pollution (Dell'Omo *et al.,* 1999) Considering their leading role in the monitoring of soil pollution changes in earthworm abundance or species richness have been positively correlated to point sources to the level of soil degradation by agricultural activity (Spurgeon *et al.,*1999). In addition these organisms are suitable indicators for monitoring the effectiveness of polluted soil remediation procedures (Lock *et al.,*2003) The presence of earthworms in wide range of soils and their high contribution to soil biomass makes them suitable to determine the effects of soil pollutants such as pesticides (De Silva *et al.,* 2009).

Earthworm biomarkers of xenobiotics exposure have experienced a significant progress in the last decade as they are common organisms in agro ecosystems with direct beneficial effects on plant growth and soil functioning, but unfortunately very few studies have assured the impact of agricultural pesticide applications on earthworm populations through the use of biomarkers. Laboratory experiments are therefore needed to examine to what extent abiotic variables influence the normal fluctuations of earthworm biomarkers. (Ali *et. al.,* 2010)

Venter and Reinecke (1988) suggested that acute toxicity is insufficient to predict environmentally acceptable concentrations of chemicals as they do not reveal sub lethal effects of low concentrations on growth, behavior, and reproduction. The parameters such as growth and fecundity must be investigated to observe the sub lethal effects of pollutants, (Phillips and Bolger 1998, Yasmin and D'Souza 2007).The reduction of population size due to mortality or reduced reproduction is an ecological consequence of exposure to chemicals in soil. Worms are highly mobile in soils and hence have the ability to detect and avoid areas of contamination, which would have significant ecological implications (Yeardley *et al.,* 1996). Thus behavior seems to be a promising biomarker in earthworm studies since it can give different end points, that could linked, at least theoretically, to soil functioning (Annette, 2009).

Endosulfan is a pesticide belonging to the organochlorine group of pesticides. It is used against a broad spectrum of insects and mites in agriculture and allied sectors. It acts as contact and stomach poison and has a slight fumigant action. It is used in vegetables, fruits, paddy, cotton, cashew, tea, coffee and timber crops. It is one of the most extensively used pesticides in the agricultural fields of M.P., Central India. But its toxicological effects on nontarget soil organisms like earthworms have been totally ignored and therefore present studies have been undertaken to evaluate its effects on growth reproduction and avoidance behavior of earthworm, Eisenia foetida.

MATERIAL AND METHODS :

E. foetida has been selected as test species because it has been suggested as a sensitive and standard species for ecotoxicological studies by OECD (2007). These were brought from M.P Council of Science and Technology (MPCST) nursery Obedullahganj (District Raisen, M.P.). Prior to exposure all worms were acclimatized for one

Received 31st March accepted after revision 28th June 2011
BBRC ISSN : 0974 - 6455 A Soc Sci Nat India Publication

month in the uncontaminated soil medium in the laboratory which was the mixture of cow dung manure and virgin black soil, as per the method of Rao and Kavitha, (2004).

After calculation of LC50 according to the OECD guidelines No.207 (1984), artificial soil test substrate was prepared (using an evenly blended dry weight mixture of 68%, mesh silica sand, 20% kaolin clay and 10% sphagnum peat. Different concentrations of the pesticide that is endosulfan (organochlorine) formulated as (endosulfan35%EC,Hyderabad Chemicals Limited, India) were prepared in 1000 ml.of distilled water and mixed with 1 kg of the soil (dry weight) described above and placed into earthen pots .The pH was maintained at 6.9±0.5 and moisture content was maintained at 35%.

Batches of 40 adult earthworms of approximately (9.52±0.25cm) length and weight (845.6±18.27) were divided into four replicates of 10 earthworms, each batch was exposed to each concentration of Endosulfan i.e. (0.5, 1, 1.5, 2, 2.5,3, 3.5,4, 4.5 and 5mg/kg dry weight of soil). The control media was the same quantity of water without any additive agent. Testing was done in continuous light at 20±2^{0}C.The mortality was recorded after 14 days of exposure. The concentration verses percentage mortality was subjected to Probit analysis using Stat plus (2009) portable software for calculating the median lethal concentration (LC50) of the test substance during the exposure tenure.

After calculation of LC50 which was found to be 3.57 mg/kg dry weight of soil the sub lethal doses of Endosulfan were selected as 0.5, 1 and 2 mg/kg dry weight of soil and were coded as E0(control), E1(0.5mg/kg), E2(1mg/kg) and E3(2mg/kg).

Assessment of the growth and reproduction of earthworm (*Eisenia foetida*).
Following the method of Spurgeon *et al.,* (2000) full clitellate, mature worms were sorted from the soil, weighed individually and added to the relevant test containers, three replicates of each concentration were taken, and 10 worms were added to each treatment replicate by sorting soils and counting the number of surviving worms. Weight change was assessed by comparing mean final weight with mean initial values for each container. By applying the method of Spurgeon *et al.,* (2000) the cocoon production was assessed by wet sieving the soil and collecting all cocoons. The number of cocoons produced during the test was compared to survival data to allow cocoon production rates i.e. (cocoon/worm/week). Cocoon viability was assessed using the method of Van Gestel *et al.,* (1988) cocoons were poured into a bowl filled with water, empty cocoons were floating on the water surface, and non floating cocoons were inspected to find out whether they are non viable or have not yet emerged. Fertile but not yet hatched cocoons were emptied to count the number and were expressed as percentage of viable cocoons. Cocoons (only viable) were then placed into Petri dishes containing wet filter paper and maintained at 20^{0}C, rinsed with fresh water after every second day to prevent the growth of mould and were incubated for 28 days to monitor hatching success.Tsukamato and Watanabe (1977) found an average incubation time of (23-28) days for the incubation of cocoons of *Eisenia foetida* on wet filter paper at 20^{0}C, hence it was selected in the present study.

Avoidance behavior was studied using 'two-chamber avoidance test' according to Hund-Rinke and Weichering (2001). In this system, two square plastic containers (20×20×10 cm) were filled with test substrates up to a height of 7 cm (1600g moist substrate in total). One section of the test vessel was filled with the uncontaminated reference soil, separated by a plastic separator from the contaminated test substrate The separator was removed and 10 worms were placed on the centre line on the soil surface. When the worms had entered the soil (E_0) the substrate choice was noted and the containers were covered with a plastic lid allowing sufficient aeration, unhindered migration was possible between the two test substrates. The incubation time was kept for 48 hrs, as per the method of Yeardley *et al.,* (1996). After the incubation time of 48 hrs, the two soils within a test unit were separated with an inserted separator and the number of worms in each test substrate were sorted, counted and recorded. Four replicates were run for each test.
Statistical significance of all the values observed during the test was analyzed by comparing the values with their relevant controls at 95% confidence interval at significance of $p < (0.05)$ and $p < (0.001)$ using NCSS,(2007) version 7.1.14 statistical software.

RESULTS

Effects of Endosulfan on the growth of earthworm *(Eisenia foetida)*
In the present study the data of Fig No.1 clearly shows that endosulfan caused a gradual decrease in weight of all the groups of earthworms when they were exposed to different concentrations of endosulfan i.e. E1, E2 and E3 except the control group E0 in which the weight was found to increase over the period of 28 days. The initial fresh weight of sexually mature adult earthworm was found to be 852.35±18.17mg. Earthworms of group E1 i.e. 0.5mg/kg endosulfan exhibited the decline in weight from an initial fresh weight of 852.35mg to

608.25±16.36mg over the period of 28 days.

Similarly, the next two doses i.e. 1 and 2mg/kg brought about a further decline in the weights in all the periods of exposure i.e. 7, 14, 21 and 28 days .At the dose of 1mg/kg there was decrease in mean weight of earthworms from an initial value of 852.35±18.17mg to 427.05±14.08mg where as, the highest dose of 2mg/kg brought down the mean weight of earthworms from an initial value of 852.35±18.17mg to 425.75±25.85mg and when these values were compared with their relevant controls a statistical differences were observed at ($p<0.05$) which is highly significant and clearly indicate that growth rate was hindered in treated worms while for control worms, increase in weight indicated a normal growth. (Fig No.1)

Effects Of Endosulfan On Cocoon Production

Analysis of cocoon production rate as (no. of cocoons/worm/week)in treated worms *(Eisenia foetida)* indicated dose dependent effects .At the dose of 0.5 mg/kg there the rate of cocoon production was 0.30±0.14 which reaches up to 0.37±0.07 and found to be significantly low in comparison to its relevant control. At 1mg/kg a significant decrease in rate of cocoon production at ($p<0.05$) was observed over the entire period of exposure. At 2mg/kg the mean cocoon production rate as (no. of cocoons/worm/week) after 7 days of exposure was 0.32±0.06which was significantly low in compared to control and no cocoon production was observed in 3rd and 4th week of exposure at this dose, while the rate of cocoon production of control worms maintained at same conditions were found as 1.04±0.19 after 7 days of exposure and reaches up to 2.59±0.22. (Table No.1)

Cocoon Viability And Hatching Success Of Cocoons

The viability of cocoons produced during the test period is expressed as percentage (%) of viable cocoons at the dose of 0.5mg/kg the viability of cocoons found 14.67± 1.62(%), at 1mg/kg it was found as 18.11±2.97 (%) while at the dose of 2mg/kg no viable cocoons were observed where as, for the control it was found to be 36±4.45 (%). (Table No.2)

The percentage of cocoons hatched at the dose of 0.5mg/kg dry wt. of soil was found as 16.11±2.47 and at 1mg/kg it was 7.44±0.75 (%) while that of control it was found to be 56.3±2.75. Statistically significant decrease was observed at ($p<0.05$) when the values obtained compared with that of control. (Table No.2)

Effects of Endosulfan on avoidance behavior of earthworm (Eisenia foetida).

During the 48 hours of avoidance test the mean 85±3.87 (%) of worms preferred uncontaminated soil, while 20±3.16 (%) preferred the soil containing 0.5 mg/kg of Dichlorovos and 14±4.00 (%) preferred the soil contaminated with 1mg/kg while there were no earthworms found in the soil containing 2mg/kg endosulfan and therefore 100% avoidance was observed at this dose.

DISCUSSION

In the present study the change in biomass of earthworm *Eisenia foetida* was found to be dose dependent during 28 days of exposure to endosulfan. A gradual decrease in biomass of earthworms was observed at all concentrations of pesticides i.e. 0.5, 1 and 2mg/kg dry weight of soil and the highest weight loss was observed at highest concentrations of 2mg/kg and found to be significantly different at ($p>0.05$) from that of control worms where there was gradual increase in weight over the entire period of experiment. The gradual decline in weight of endosulfan treated earthworms even after 3 weeks of exposure indicates the persistent toxicity of the pesticide in the soil as well as in the tissues of worms. Similar effects on growth rate of *Lumbricus terrestris* were observed by Mosleh *et al.,* (2003) and they also detected the residues of endosulfan up to 40% after 2, 7, 15 days of exposure and concluded that concentration in the soil decreased faster at a higher initial concentration. Whereas, Antonious *et al.,*(1998) reported the differences in half lives of α and β isomers of endosulfan and therefore the weight loss may indicate a feeding inhibition situation, with the earthworm regulating the intake of the pesticides by reducing consumption rate. This strategy is commonly used to avoid poisoning with heavy metals and pesticides (Ribiero *et al.,* 2001).

The findings of the present study show that the sublethal doses of organochlorine pesticide endosulfan altered the reproduction process of earthworm *Eisenia foetida* during exposure period of 28 days. The number of cocoons produced by endosulfan treated worms was clearly affected and there was a significant decrease at ($p>0.01$) in the rate of cocoon production at highest dose of 2mg/kg. Moreover, the rate of cocoon production was found to be completely seized after 21 and 28 days in the worms exposed at this dose. The percentage of viability of cocoons and percentage of cocoons hatched were found to be significantly affected as compared to control which implies that endosulfan caused deleterious effects on reproduction of *Eisenia foetida* during the period of experiment. The reduction in rate of cocoon production may be due to reduction in the number of sperms or ovarian cells or due to change in morphology of sperms as suggested by the studies of Pandey *et al.,*(1990) endosulfan harms the reproductive system by affecting sperm count, spermatogonical cells and sperm morphology. Similarly studies of Picard *et al.,* (2002) suggest that exposure of organochlorine pesticides to

animals whether vertebrates or invertebrates reaching sexual maturation can severely affect their fertility and lineage.

In the present investigation the avoidance responses of earthworms for the soil contaminated with endosulfan at all concentrations were observed. A 100% avoidance of earthworms was observed for the soil contaminated with the highest dose of 2mg/kg which clearly showed that the pesticide has detrimental effects on earthworm population. Although no mortality was observed at all concentrations during the avoidance test period of 48hrs.but 80% earthworms avoided even the lowest dose of 0.5mg/kg which indicates that *Eisenia foetida* is very much sensitive to this pesticide. Detection of a noxious chemical substance is likely to result in an avoidance reaction (Christensen and Mather, 2003) which may be followed by an escape response. Under natural conditions, if juvenile and mature individuals exhibit similar behavioral patterns with respect to migration from pesticide contaminated soil, it probably reflects a phase of dispersal in relation to the decrease in population at a living site (Stephenson *et al.,* 1998). Similar responses were observed by Slimak *et al.,* (1996) when earthworm *Lumbricus terrestris* were exposed to ten different pesticides including organochlorine pesticides and found that avoidance occurred for all pesticides studied and increased as concentration increased.

Fig No. 1 showing the change in biomass during 7, 14, 21 and 28 days of exposure to different concentrations of endosulfan with well matched controls.

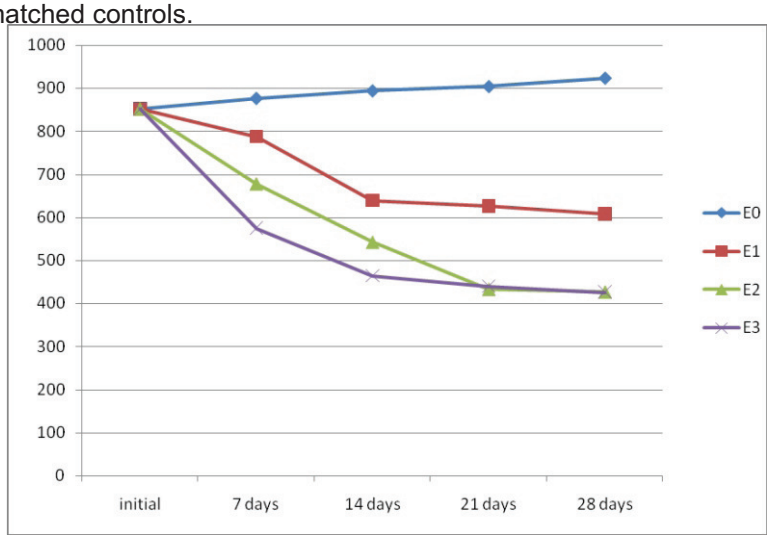

Table No. 1 Showing the effects on cocoon production of earthworms *Eisenia foetida* during 7,14,21 and 28 days of exposure to Endosulfan (No. of cocoons/worm/week, where n=9)(the values marked with asterisks are significantly different from control at $p < 0.05$)

Dosage	7 days	14 days	21 days	28 days
E0(Control)		1.69±0.13	2.21±0.14	2.59±0.22
E1(0.5mg/kg)	0.30±0.14	0.26±0.10	0.43±0.15	0.37±0.07*
E2(1mg/kg)	0.09±0.04*	0.08±0.04*	0.12±0.07	0.13±0.06*
E3(2mg/kg)	0.32±0.06*	0.34±0.16	0*	0*

Table No. 2 showing the percentage of viable cocoons and percentage cocoons hatched in worms exposed to Endosulfan for 28 days as well as incontrol worms.(The values marked with asterisks are significantly different from control at $p < 0.05$)

Dosage	% of viable cocoons	% of cocoons hatched
E0(control)	35.89±3.19	55.11±2.64
E1(0.5mg/kg)	14.67±1.62	16.11±2.47
E2(1mg/kg)	18.11±2.97	7.44±0.75*
E3(2mg/kg)	0*	0*

Table No.3 Showing the Substrate preference, mean (% of total numbers n=40worms) ±S.E.

Dosage	% of worms preference for substrate
E0(control)	85±3.87
E1(0.5mg/kg)	20±3.16
E2(1mg/kg)	14±4.00
E3(2mg/kg)	0

REFERENCES:

Ali A.S. *et al.,* (2010) Earthworms as Bio Markers of environmental pollution. Discovery publication New Delhi.Bustos-Obregon, E.and Goicochea, R. (2002)"Pesticide soil contamination mainly affects earthworm male reproductive parameters. Asian J. Androl. 4; 195-199.

C. A.M.Van Gestel, W.A.Van Dis, E.M. Van Breemen and P.M. Sparenburg (1988). "Comparison of two methods for determining the viability of cocoons produced in earthworm toxicity experiment". Pedobiologia, 32, 367-371.

D.R.Phillips and T.Bolger (1998) "Sublethal toxic effects of aluminium on the earthworm *Eisenia foetida".*

David J. Spurgeon., Claus Svendsen, Viv.R.Rimmer., Stephen P.Hopkin and Jason M.Weeks.(2000) "Relative sensitivity of life cycle and Biomarker responses in four earthworm species exposed to zinc".Envt.Toxicol and chem. Vol.19 No.7,p.p1800-1808.

Hund-Rinke K, Weichering H (2001). "Earthworm Avoidance test for soil Assessment". Jss- J Soils and Sediments 1, 15-20.

J. Venkateswara Rao and P.Kavitha, (2004). "Toxicity of azodrin on the morphology and acetyl cholinesterase activity of the earthworm *Eisenia foetida*". Environmental research 96, 323-327.

Lynn H. Booth and Kathryn O'Halloran (2001) "A comparison of biomarker responses in the earthworm *Aporrectodea caliginosa* to the organophosphorus insecticides diazinon and chlorpyrifos". Envt.Toxicol and chem. Vol.20, No.11, 2494-2502

Maike Schaefer, (2003) "Behavioral endpoints in earthworm ecotoxicology". J Soils and Sediments, 3(2)79-84.

OECD, (2007), guidance document on the breakdown of organic matter in litter bags. In; OECD series on testing and assessment no.56 Organization for Economic Co-operation and Development, Paris, France.

P. Mangala C.S.De Silva, Asoka Pathiratne, Cornelis, A.M. Van Gestel (2009) "Influence of temperature and soil type on the toxicity of three pesticides to *Eisenia andrei"* Chemosphere 76 (2009) 1410-1415.

P. Mangala C.S.De Silva, Asoka Pathiratne, Cornelis,Nico M.Van Straalen, A.M. Van Gestel (2010) "Chlorpyrifos causes decreased organic matter decomposition by suppressing earthworm and termite communities in tropical soil". Environmental Pollution 158, 3041-3047.

Reinecke, S.A., Reinecke, AJ. (2007). "The impact of organophosphate pesticides in orchards of on earthworms in Western Cape South Africa". Ecotoxicology and Environmental Safety 66. 244-251.

Andre Picard, Golbahar Palavan, Stephanie Robert, Danielle Pesando, and Brigitte Ciapa. (2003) "Effects of organochlorine pesticides on maturation of starfish and mouse oocytes." Toxicological Sciences 73, 141-148.

Saterbak A,Joy Rj, Wong DCL, Mc Main BJ,Williams MP, Dorn PB,Bruzy LP, Chai EY, Salanitro JP(1999) "Ecotoxicological and analytical assessment of hydrocarbon contaminated soils and application to

ecotoxicological risk assessment."Environmental Toxicology and Chemistry 18, 1591-1607.
Venter JM, Reinecke AJ. (1988). "The life cycle of the compost worm *Eisenia foetida* (Oligochaeta)". S.Afr J Zool 23; 161-165.

Watanabe,H., and Tsukamoto, (1976). "Seasonal change in size class and stage structure of Lumbricid *Eisenia foetida* populations in field compost and its practical application as the decomposer of organic waste matter". Rev.Ecol. Biol. Sol.13, 141-146.

Yasmin, S. and Souza, D.D (2007) "Effects of pesticides on reproductive output of *Eisenia foetida".*Bull. Env. Toxicol, 79:522-532.

Yeardley RB Jr., Lazorchak JM, Gast LC (1996) "The potential of earthworm avoidance test for evaluation of hazardous risk waste sites". Environmental Toxicology and Chemistry 15, 1532-1537.
Yu Luo, Yu Zang, Yuan Zhong and Zhiming Kong (1999) "Toxicological study of two novel pesticides on earthworm *Eisenia foetida"*. Chemosphere, Vol.39, No.13, pp.2347-2356.

Pandey N, Gunderia F, Prem AS, Ray PK, (1990) " studies on the genotoxicity of endosulfan, an organochlorine insecticide in mammalian germ cells". Mutant Res.vol.242 ISS1, P-1-7.

Cikutovic MA, Fitzpatrick LC, Venables BJ, Goven AJ (1993) "Sperm count in earthworms *(Lumbricus terrestris)* as a biomarker of environmental toxicology:effects of cadmium and chlordane." Environ Pollut 81:123-125.