Effects of Dichlorovos Organophosphate on Growth, Reproduction, and Avoidance Behavior of Earthworm *Eisenia foetida*

*Sameena Farrukh [1], Ayesha S. Ali [2]**

Received:27.08. 2011 *Accepted:20.09.2011*

ABSTRACT

Background: The indispensable use of agrochemicals has created serious threats for earthworms abundance and their population; therefore, several earthworm protocols have gained acceptance for use in tests to assess the effects of soil chemicals on soil organisms. Dichlorovos, an organophosphorus pesticide, is one of the widely used pesticides in agricultural fields of M.P., Central India. The aim of the present investigation was to assess the effects of the dichlorovos on growth, reproduction, and avoidance behavior of an earthworm, *Eisenia foetida.*

Methods: After evaluating LC_{50}, artificial soil substrate was prepared using an evenly blended mixture of 68% mesh silica sand, 20% kaolin clay, and 10% sphagnum peat. Different concentrations after LC_{50} probit analysis of the pesticides were prepared in 1000ml of distilled water and mixed with 1 kg of the soil to expose the earthworms in a dose-dependent manner.

Results: A decrease was observed in the weight of earthworms at all concentrations of the pesticide used, whereas reproduction and avoidance behavior which are sensitive parameters were found to be significantly affected by all three doses of dichlorovos.

Conclusion: Based on the findings of this study, it can be concluded that growth, reproduction, and avoidance behavior can be taken as useful indicators for assessing the sub-lethal effects of pesticides on non-target soil organisms such as earthworms.

Keywords: Ecotoxicology, Eisenia Foetida*,* Growth, Organophosphorus*,* Two-Chamber Avoidance Test.

INTRODUCTION

Earthworms play a vital role in the maintenance of soil structure, functions, and fertility. Their activities modify soil aeration, drainage, and availability of nutrients for plants and generally integrate soil organic and mineral elements to form aggregates and improve soil structure (1). The indispensable use of agrochemicals has caused serious threats for earthworms abundance and population; therefore, several earthworm protocols have gained acceptance for use in tests to assess the effects of soil chemicals on soil organisms (2,3).

Venter and Reinecke 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 (4). Parameters such as growth and fecundity must be investigated to observe the sub-lethal effects of pollutants (5). The reduction of population size due to mortality or reduced reproduction is an ecological consequence of exposure to chemicals in soil. However, behavioral changes, such as substrate avoidance can also be relevant since they have direct impacts on soil ecosystem as reduction in earthworm population in an area may affect the number and distribution of their vertebrate predators (6). The presence of earthworms in a wide range of soils and their high contribution to soil biomass make them suitable to determine the effects of soil pollutants such as pesticides (7).

1.PhD Student, Postgraduate Department of Biotechnology, Saifia Science College, India.

2.Postgraduate Department of Biotechnology, Saifia Science College, India.

* Corresponding Author Email: drayeshariqueali@yahoo.com

Dichlorovos is an organophosphorus, contact fumigant insecticide with quick knockdown action, which controls chewing and mining insects of paddy sugarcane, groundnut and vegetable crops, and is widely used in the agricultural fields of M.P., Central India. However, its effects on non-target soil organisms such as earthworms have been totally ignored. Therefore, the present study was done to evaluate its toxic effects on growth, reproduction and avoidance behavior of *Eisenia foetida*.

MATERIALS AND METHODS

Acclimatization and culture of earthworms

E. foetida was selected as test species because it has been suggested as a sensitive and standard species for ecotoxicological studies by OECD (2). The worms were obtained from M.P Council of Science and Technology (MPCST) Nursery Obedullahganj (District Raisen, M.P). Prior to exposure, all worms were acclimatized for one month in an uncontaminated soil medium which was a mixture of cow dung manure and virgin black soil, as for the method of Rao and Kavitha, in laboratory *(*2004) (8).

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, dichlorovos (organophosphate), were formulated (dichlorovos 76% EC, Syngenta India limited) and prepared in 1000 ml of distilled water, mixed with 1 kg of the soil (dry weight) described above, and placed into earthen pots. The pH level 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 dichlorovos (15, 30, 45,60,75,90,105, and 120 mg/kg dry weight of soil). The control medium was the same quantity of water without any additive agent. Testing was done in continuous light at $20±2^0$C and mortality was recorded after 14 days of exposure. The concentration versus percentage mortality was subjected to probit analysis using Statplus (2009) portable software for calculating the median of lethal concentration (LC50) of the test substance during the exposure period.

Selection of sub-lethal doses of the pesticides

After calculation of LC50 which was found to be 83.80 mg/kg dry weight of soil, the sub-lethal doses of dichlorovos were selected as 19, 38, and 76 mg/kg dry weight of soil and coded as D0(control), D1(19 mg), D2(38 mg), and D3(76 mg) groups.

Assessment of the growth and reproduction of earthworm (Eisenia foetida)

Following Spurgeon's full clitellate method, 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 the soils and counting the number of surviving worms (9). Weight change was assessed by comparing mean final weight to mean initial values for each container.

By applying Spurgeon's method, the cocoon production was assessed by wet sieving the soil and collecting all cocoons (9). The number of cocoons produced during the test was compared with survival data to obtain cocoon production rates (cocoon/worm/week). Cocoon viability was assessed using Van Gestel's method. The cocoons were poured into a bowl filled with water as the empty cocoons were floating on the water surface, and non-floating cocoons were inspected to see whether they were non-viable or had not yet emerged (10). Fertile but not yet hatched cocoons were emptied to count their numbers and were expressed as the percentage of viable cocoons. Cocoons (only viable ones) were, then, placed into Petri dishes containing wet filter paper, maintained at 20^0C, rinsed with fresh water every other day to prevent the growth of mould, and incubated for 28 days to monitor hatching success. Tsukamato and Watanabe found an average incubation time of 23-28 days for the incubation of cocoons of *Eisenia foetida* on wet filter paper at 20^0C. likwise, this was chosen for the present study (11).

Avoidance Behavior

Avoidance behavior was studied using 'two-chamber avoidance test' according to Hund-Rinke and Weichering (12). In this system, two square plastic containers (20×20×10 cm) were filled with test substrates up to a height of 7 cm (1600 g 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 center line on the soil surface. After entrance of the worms to the soil (D_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 determined to be 48 hours based on the method utilized by Yeardley *et al*. (1996) (13). After the 48-hour incubation time, the two soils within a test unit were separated with an inserted separator and the number of worms in each test substrate was 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 level (P<0.05, P<0.001) using NCSS statistical software (2007) version 7.1.14.

RESULTS

Effects of dichlorovos on the growth of earthworms (Eisenia foetida)

In the present study, data presented in Figure 1 clearly show that dichlorovos caused a decrease in the weight of all groups of earthworms, when they were exposed to different concentrations of dichlorovos (i.e. D1, D2, and D3) except for the control group D0 in which the weight was found to increase over the 28-day period. The initial fresh weight biomass of sexually mature earthworm was found to be 845.6±18.27. Earthworms of group D1 (i.e. 19 mg/kg DDVP) exhibited an initial decline in their weight after seven days from the initial value of 845mg to 748.9 mg with an increase thereafter. The 19 mg dose showed a gradual increase in weight over days 14, 21, and 28 of exposure; however, compared with the control earthworm, the net weight showed a decline after 28 days; the value being 815.2 mg from the initial value of 845 mg.

The next two high doses, 38 and 76 mg/kg, brought about a further decline in the weight, in all periods of exposure days (7, 14, 21, and 28). At the 38 mg dose, there was a decrease in the mean weight of earthworms from the initial value of 845 mg to 578.65 mg, whereas the highest dose of 76 mg brought down the mean weight of earthworms from the initial value of 845 mg to 454.9 mg which was highly significant (P< 0.05) and almost half the weight of control worms (Figure 1).

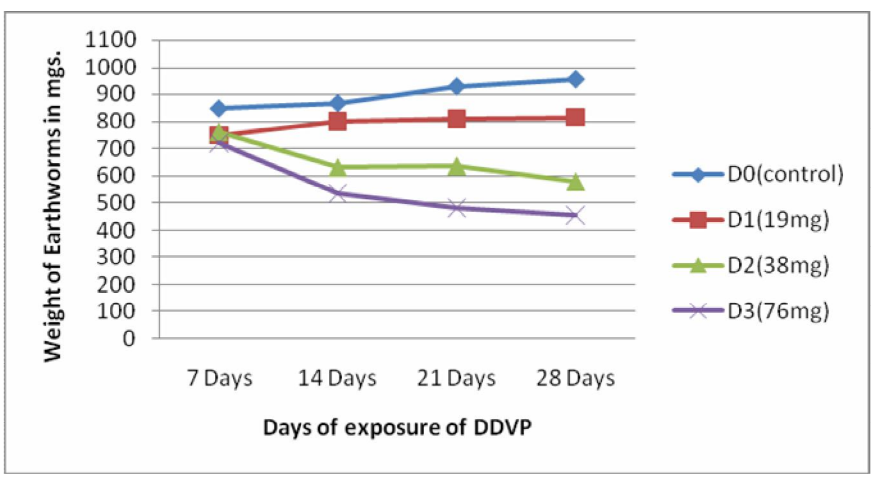

Figure 1. The change in biomass of earthworms during 7, 14, 21, and 28 days of exposure to different concentrations of dichlorovos with well-matched control earthworms

Effects of dichlorovos on cocoon production

Analysis of cocoon production rate as (number of cocoons/worm/week) in treated worms (*Eisenia foetida)* indicated dose-dependent changes. At 19 and 38 mg /kg doses, there were no significant differences compared with the obtained control values. At the 76 mg/kg dose, a decrease in the rate of cocoon production was observed after 7 days of exposure while at 19 mg/kg, the mean cocoon production rate (number of cocoons/worm/week) after 7 days of exposure was 0.33±0.20 which increased up to 2.16±0.19. At 38 mg/kg, the mean rate was 0.18±0.12 which reached 1.49±0.21 after 28 days while at 76 mg/kg, it was 0.1±0.04 and reached 1.2±0.24. The rate of cocoon production of control worms maintained in the same conditions were found to be 0.57±0.20 after 7 days of exposure and reached approximately 2.76±0.25 (Table 1).

Table 1. The effects on cocoon production of *Eisenia foetida* earthworms during 7, 14, 21, and 28 days of exposure to dichlorovos (Number 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
Control	0.57±0.20	1.29±0.21	2.79±0.28	2.76±0.25
D1(19mg/kg)	0.33±0.12	1.22±0.32	1.36±0.19	2.16±0.19
D2(38mg/kg)	0.18±0.12*	0.84±0.20	1.02±0.21	1.49±0.21
D3(76 mg/kg)	0.1±0.04*	1.07±0.29	1.01±0.26	1.27±0.24*

Cocoon viability and hatching success of cocoons

The viability of cocoons produced during the test period is expressed as percentage (%) of viable cocoons. At 19, 38, and 76 mg/kg doses, the viability values of the cocoons were found to be 29.8± 4.22, 22.4±3.30, and11±1.89 %, respectively while the viability value for the control was found to be 36±4.45%. The values obtained at 76 mg/kg showed the maximum decrease in the viability of cocoons compared with that of the control (Table 2).

The percentage of cocoons hatched at 19 mg/kg dry weight dose of soil was found to be 21.6±3.07%. At 38 mg/kg, this was 14.6±4.34% and for the 76 mg/kg dose it was 12.9±1.91% while that of the control was 56.3±2.75%. No statistically significant decreases were observed in the values obtained at all three doses of pesticide in comparison with that of the control (Table 2).

Effects of dichlorovos on avoidance behavior of earthworm (Eisenia foetida)

During the 48-hour period of the avoidance test, a mean 80±3.16% of worms preferred uncontaminated soil, whereas 22±3.74 (%) preferred the soil containing 19 mg/kg of dichlorovos and 20±3.16 (%) preferred the soil contaminated with 38 mg/kg and only 6±4.00 preferred the soil containing 76 mg/kg dichlorovos (Table 3).

Table 2. The percentage of viable cocoons and cocoons hatched in worms exposed to dichlorovos for 28 days as well as in control worms

Dosage	% of viable cocoons	% of cocoons hatched
D0(control)	36±4.45	56.3 ± 2.75
D1(19mg/kg)	29.8±4.22	21.6 ± 3.07
D2(38mg/kg)	22.4±3.30	14.6 ± 4.34
D3(76mg/kg)	11±1.89*	12.9 ± 1.91

Table 3. The substrate preference, mean (% of total numbers n=40worms) ±S.E

Dosage	% of worms preference for substrate
D0(control)	80±3.16
D1(19mg/kg)	22±3.74
D2(38mg/kg)	20±3.16
D3(76mg/kg)	6±4.00

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 dichlorovos. At 19 mg/kg dose, a decrease in the weight (mg) of worms was observed after 7 days of exposure, but during the 21 days of exposure, a slight increase in weight was observed which shows that the worms got acclimatized to the soil contaminated with low concentrations of dichlorovos. This may also be due to the degradation of pesticide residues caused by microbial activity or the earthworm activity itself. The degradation of pesticides could mainly be due to chemical and microbial processes which can be favored by the higher temperature in tropical conditions (14). Singh reported the significant contribution of soil microorganisms to the degradation of organophosphorus insecticides in natural soil (15). A higher microbial activity in natural soils under tropical conditions can result in faster biodegradation resulting in lower sub lethal toxicities.

In addition, no mortality was observed at 19 mg/kg soil during the entire period of experiment which indicates that earthworms are able to survive at lower concentrations of dichlorovos but the effects of such doses on reproductive parameters and avoidance behavior showed that even low doses can hinder the population and abundance of earthworms. At concentrations of 38 and 76 mg/kg/dry weight of soil a significant decrease in the weight of worms was observed. There was no weight loss in control worms; in fact, they showed a normal growth with an increase in weight (mg) during the entire period of experiment. Such large differences observed in weight loss of treated worms might be due to the utilization of available resources for detoxification and repair function required to increase survival possibilities and ,therefore, hindering the growth rate. Khan reported a significant reduction in earthworm biomass after exposure to different concentrations of copper chloride and concluded that dysfunction of major physiological systems such as digestion and absorption with consequent disturbed metabolism resulted in biomass reduction (16). Similar effects were observed by Booth and O'Halloran where growth found to be significantly reduced in *A.caliginosa* by exposure to two OP pesticides, diazinon and chlorpyrifos, at 60 and 28 mg/kg doses (17).

Although the number of cocoons produced by dichlorovos treated worms was clearly affected and the percentage of viability of cocoons produced showed a significant decrease at 76 mg/kg dose, no significant decreases were observed in the percentage of cocoons hatched compared with the control ($P>0.05$). This may imply that the quality of ova is not affected by dichlorovos treatment within 28 days of exposure. Nevertheless, long lasting exposure under field conditions can produce chronic effects resulting in lower reproduction rates and could be the contributing factor causing low earthworm densities. Similar effects on cocoon production, cocoon viability, and hatching success in *E.foetida* on exposure to commercial parathion at doses of 444, 739, and 1478 mg/kg soil were observed by Bustos-Obreg and Goicochea (18).

In the present investigation, avoidance responses of earthworms for soil contaminated with different concentrations of dichlorovos (19, 38, and 76 mg/kg) and their preference for uncontaminated soil showed that worms made the preliminary selection on substrates as they often explored different test units before they finally decided on which substrate to enter (6).

Avoidance response test may be useful in predicting the lower threshold effects of pollutants on earthworm population. However, the behavioral symptoms are difficult to relate to chemical classes because of their general nature. De Silva found earthworm biomass and abundance to be significantly reduced during 1-3-month studies conducted on the field plots using OP pesticide chlorpyrifos and correlated it with the presence of pesticide residues in the soil; where earthworm abundance was not found to be affected, chlorpyrifos rapidly disappeared from the soil (19). On the other hand, Reinecke and Reinecke reported no significant differences in the density of earthworms in orchards and adjacent areas after chlorpyrifos application but predicted adverse chronic effects (20).

CONCLUSION

Based on the findings of the present study, it can be concluded that the lethal effects of chemicals such as pesticides is not a necessary consequence and sub-lethal effects might be produced. Dichlorovos significantly affected the growth, reproduction, and avoidance behavior of earthworm *Eisenia foetida*. Therefore, these parameters can be useful to assess the sublethal effects of organophosphate pesticides on nontarget soil organisms.

REFERENCES

1. Butt K, Frederickson J, Morris R. Effect of earthworm density on the growth and reproduction of Lumbricus terrestris L.(Oligochaeta: Lumbricidae) in culture. Pedobiologia (Germany). 1994.
2. Luo Y, Zang Y, Zhong Y, Kong Z. Toxicological study of two novel pesticides on earthworm Eisenia foetida. Chemosphere. 1999;39(13):2347-56.
3. OECD. guidance document on the breakdown of organic matter in litter bags. Paris;France.2007.
4. Venter J, Reinecke A. The life-cycle of the compost worm Eisenia fetida(Oligochaeta). S AFR J ZOOL/S-AFR TYDSKR DIERKD. 1988;23(3):161-5.
5. Yasmin S, D'Souza D. Effect of Pesticides on the Reproductive Output of Eisenia fetida. Bulletin of environmental contamination and toxicology. 2007;79(5):529-32.
6. Schaefer M. Behavioural endpoints in earthworm ecotoxicology. Journal of Soils and Sediments. 2003;3(2):79-84.
7. De Silva PM, Pathiratne A, van Gestel CAM. Influence of temperature and soil type on the toxicity of three pesticides to Eisenia andrei. Chemosphere. 2009;76(10):1410-5.
8. Rao JV, Kavitha P. Toxicity of azodrin on the morphology and acetylcholinesterase activity of the earthworm Eisenia foetida. Environmental research. 2004;96(3):323-7.
9. Spurgeon DJ, Svendsen C, Rimmer VR, Hopkin SP, Weeks JM. Relative sensitivity of life cycle and biomarker responses in four earthworm species exposed to zinc. Environmental toxicology and chemistry. 2000;19(7):1800-8.
10. Van Gestel C, Van Dis W, Van Breemen E, Sparenburg P. Comparison of two methods for determining the viability of cocoons produced in earth worm toxicity experiments. Pedobiologia. 1988;32(5):367-71.
11. Watanabe H, Tsukamoto J. Seasonal change in size, class and stage structure of lumbricid Eisenia foetida population in a field compost and its practical application as the decomposer of organic waste matter. Rev Ecol Biol Sol. 1976;13:141-6.
12. Hund-Rinke K, Wiechering H. Earthworm avoidance test for soil assessments. Journal of Soils and Sediments. 2001;1(1):15-20.
13. Yeardley Jr RB, Gast LC, Lazorchak JM. The potential of an earthworm avoidance test for evaluation of hazardous waste sites. Environmental toxicology and chemistry. 1996;15(9):1532-7.
14. Racke K, Skidmore M, Hamilton D, Unsworth J, Miyamoto J, Cohen S. Pesticides Report 38. Pesticide fate in tropical and the soils (Technical Report). Pure and Applied Chemistry. 1997;69(6):1349-72.
15. Singh BK, Walker A, Morgan JAW, Wright DJ. Effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium. Applied and environmental microbiology. 2003;69(9):5198.
16. Iram Khan, Ayesha S, Sharique A. Biomass and behavioral responses of earthwotm, Lumbricus terrestris to copper chloride. Iranian Journal of Toxicology. 2007;1(2).
17. Booth LH, O'Halloran K. A comparison of biomarker responses in the earthworm Aporrectodea caliginosa to the organophosphorus insecticides diazinon and chlorpyrifos. Environmental toxicology and chemistry. 2001;20(11):2494-502.

18. Bustos-Obregón E, Goicochea RI. Pesticide soil contamination mainly affects earthworm male reproductive parameters. Asian journal of andrology. 2002;4(3):195-200.
19. De Silva PM, Pathiratne A, van Straalen NM, van Gestel CAM. Chlorpyrifos causes decreased organic matter decomposition by suppressing earthworm and termite communities in tropical soil. Environmental Pollution. 2010.
20. Yasmin S, D'Souza D. Effect of Pesticides on the Reproductive Output of Eisenia fetida. Bulletin of environmental contamination and toxicology. 2007;79(5):529-32.