Toxicological Effects of Organophosphate Pesticide on Ceolomocytes Viability of Earthworm *E. Foetida* Using NRRA

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ABSTRACT

Background: In recent years, there has been a growing interest in the development of sub-lethal earthworm biomarkers as they are relevant indicators of environmental change and they are among the five key indicators for ecotoxicological testing of industrial chemicals determined by the OECD. In the present study, the effects of an organophosphate pesticide dichlorovos on lysosomes of coelomocytes of earthworm *E. foetida* are studied using Neutral Red Retention Assay (NRRA).

Methods: Earthworms were exposed to three sub-lethal concentrations of the pesticide for 7, 14, 21, and 28 days and neutral red retention assay was done following the method employed by Weeks and Sevendsen and Booth *et al.*

Results: It was observed that the pesticide significantly affected the coelomocyte viability within 28 days of exposure. The neutral red retention time of lysosomal membrane significantly decreased at all concentrations when compared with well-matched controls.

Conclusion: After the analysis of results, it was concluded that the neutral red retention time assay in earthworms can be used to link changes in the permeability of lysosomal membranes to ecologically relevant life cycle effects caused by such toxic substances.

Keywords: Coelomocytes, Dichlorovos, Lysosomes, Neutral Red Retention Assay, Toxicity.

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INTRODUCTION

Earthworms have received more attention among other soil invertebrates because of their ecological importance. Earthworms represent a significant, if not a dominant part of the soil biomass, and are soil engineers regulating important soil processes. They have been regarded as standard test organisms for soil toxicity testing and are being broadly used to assess environmental impact from heavy metals pollution [1]. However, the existing body knowledge on the toxic effects of pesticides upon these organisms is still limited. Various studies have shown that earthworms are useful bioindicators of land use, soil fertility, and soil ecosystem health. Studies have also shown that worms can accumulate high concentrations of pesticides, and, therefore, can be used as bioindicators of soil pollution by metals and pesticides [2].

In recent years, there has been a growing interest in the development of sub-lethal earthworm biomarkers. They are relevant indicators of environmental change and are included among the top five key indicators for ecotoxicological testing of industrial chemicals determined by the OECD [3]. Some researchers have suggested the use of certain biomarkers for studying the effects of toxic substances on earthworms; one of these is Neutral Red Retention Assay which measures the membrane stability of lysosomes within the coelomocytes of earthworms in response to contaminants [4, 5]. Thus, it can be used for evaluation of the toxicity of various toxicants under different exposure conditions.

Farrukh and Ali [6] studied the effects of dichlorovos, belonging to the organophosphate group of pesticides, on growth, reproduction, and avoidance behavior of earthworm *E. foetida* and found that the pesticide significantly affected these parameters. Ongoing with further investigations, the present study was undertaken to study the effects of dichlorovos on the coelomocyte viability of earthworm *E. foetida* using Neutral Red Retention Assay (NRRA).

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MATERIALS AND METHODS

Selection and Culture of Earthworms

E. foetida was selected as test species, because it is suggested as a sensitive and standard species for ecotoxicological testing by OECD [3]. The worms were provided from MPCST Nursery Obedullahganj (District Raisen) M.P. All worms were acclimatized prior to exposure 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 *et al* [7].

Exposure to Pesticide

For exposure to the pesticide, DDVP adult earthworms were sorted out with full clitellum, and mature earthworms of 1.2 to 1.5 g bodyweight were used. After calculating the proper LC50 as per the method of Rao *et al*., 2004, the earthworms were exposed to the pesticide dichlorvos whose three sub-lethal doses in log concentrations were found to be 19 mg (D1), 38 mg (D2), and 76 mg (D3)/kg dry weight of soil. Earthen pots of 20 cm height and 12 cm diameter were used for exposing the earthworms to the pesticide; as many as 10 earthworms were used for each concentration for varying periods of exposure ranging from 7 to 28 days. Each pot was filled with 2 kg of dry soil mixed with respective doses of the pesticide for both control and dichlorvos exposed groups. The pots were then covered with perforated filter paper sheets to minimize the loss of water by evaporation as well as movement of earthworms out of the pots. Soil temperature was maintained between 25ºC and 30ºC, with soil pH of 6.0 ± 0.5.

Neutral Red Retention Time Assay

A neutral red retention time assay was done according to the method described by Weeks and Sevendsen [4] and Booth *et al* [8]. A neutral red working solution of 80 mg/ml was prepared in earthworm physiological ringer solution [9]. Coelomic fluid was collected from earthworm by inserting a needle containing 20 microlitres of ringer into the coelomic cavity posterior to the clitellum allowing it to fill by intra coelomic pressure, after gentle drawing action on the syringe. The coelomic fluid was extracted from the control and the treated worms. They were placed on to a clean slide and mixed with 20 microlitres of neutral red solution, before a cover slip was placed on top of it. The slides were scanned for two minutes at five-minute intervals and the number of stained and unstained cells was counted. The cells were counted until 50% of the cells turned red or for 60 minutes. This time was recorded as neutral red retention time.

RESULTS

In the present investigation, coelomocyte viability was measured by neutral red retention assay. According to the data presented in Table 1a and Figure 1b, a marked decrease was observed in mean NRRTs of the exposed worms in all the dichlorovos exposed groups (i.e. D1, D2, and D3). The data presented in Table 1a and Figure 1b show that the mean NRRT of coelomocytes of earthworms was 43.45±1.84 minutes at the dose of 19mg/kg after seven days of exposure. It further decreased to 36.91±0.97 min after 14 days of exposure. In addition, it was 46.82±0.48 minutes after 28 days of exposure. At the same time, the corresponding time for control was 54.09±0.90 min.

Similarly, based on Table 1a and Figure 1b, NRRTs decreased to 35.73±0.98 min at the dose of 38 mg/kg, and it was found to be 21.27±1.83 min after 28 days at the same dose. At 76mg/kg, a significant decrease was found as 15.09±0.69 min after seven days of exposure and no remarkable changes in the values were further observed during the period of 28 days of exposure. All values obtained from exposed groups were found to be significantly different from their relevant controls (P<0.001).

Table 1. The effects of dichlorovos on neutral red retention time in coelomocytes of earthworms *Eisenia foetida* during 7, 14, 21, and 28 days of exposure (values marked with asterisks are significantly different from control at P< 0.001).

Dosage	7 days	14 days	21 days	28 days
D0(control)	54.09±0.90	58.82±0.88	55.82±0.72	54.91±1.15
D1(19mg/kg)	43.45±1.84	36.91±0.97	40.82±0.35	46.82±0.48
D2(38mg/kg)	35.73±0.98	22.73±0.74	21.55±1.86	21.27±1.83
D3(76mg/kg)	*15.09±0.69	*14.45±0.37	16±0.81	16.73±1.82

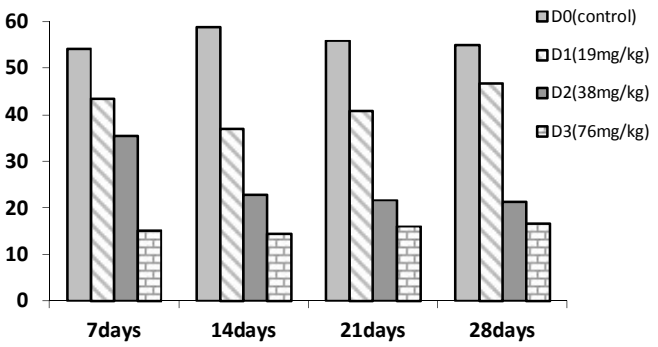

Figure 1. The effects on neutral red retention time in coelomocytes of earthworms *E. foetida* during 7, 14, 21, and 28 days of exposure (values marked with asterisks are significantly different from control at P< 0.001).

DISCUSSION

In the present study, sublethal doses of pesticide dichlorovos caused a significant decrease in the mean NRRT of coelomocytes of earthworms *E. foetida* (P<0.001). The decrease in NRRT was found to be dose-dependent. The percentage of reduction in mean NRRT at different doses of dichlorovos as compared to control was found to be 20, 44, and 73% at 19, 38, and 76mg/kg dry weight doses of soil after seven days of exposure and during study period (28 days). No change or restoration of NRRT values was observed after 28 days except for the lowest dose of 19mg/kg dry weight of soil. The results showed that NRRT of coelomocytes of *E. foetida* is very sensitive, even to the lowest sub-lethal doses of the pesticide dichlorovos and responded equally in a dose-dependent manner. Therefore, it can be concluded that coelomocyte viability can be used as a sensitive parameter for assessing the toxicity of soil pollutants such as pesticides. It has been reported that pathological alterations in lysosomes is especially useful in the identification of adverse environmental impacts on organisms [10] with much evidence for aquatic organisms, but with rather limited evidence for terrestrial organisms [11]. The findings of the present study are in agreement with the findings of Svendsen and Weeks [4] that indicated significant reductions in neutral red retention time of lysosomes of the coelomocytes of *L. rubellus* with increasing external copper concentrations. Booth and Halloran [8] reported a significant reduction in NRRT of lysosomes when *A. caliginosa* was exposed to two organophosphate pesticides i.e. chlorpyrifos and diazinon. The response of neutral red retention assay to environmental contaminants occurs sooner at sub-cellular levels than at physiological or other levels making it a useful biomarker to serve as an early warning system of stress.

CONCLUSION

In the present investigations, progress was made in showing that the neutral red retention time assay in earthworms can be used to link changes in the permeability of the lysosomal membranes to ecologically relevant life cycle effects caused by certain substances. The inclusion of the biomarker approaches in these laboratory experiments will be helpful in predicting test outcomes in terms of pesticide bioavailability and its sublethal effects.

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REFERENCES

1. Cao X, Song Y, Fan S, Kai J, Yang X, Chen L. Optimization of Ethoxyresorufin-O-deethylase Determination in the Microsomes of Earthworms and Its Induction by Polycyclic Aromatic Hydrocarbons. CLEAN–Soil, Air, Water. 2014;42(8):1121-5.

2. Farrukh S, S Ali A. Effects of Dichlorovos Organophosphate on Growth, Reproduction, and Avoidance Behavior of Earthworm Eisenia *foetida*. Iranian Journal of Toxicology. 2011; 5(14):495-501.
3. OECD. 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.2007.
4. Weeks JM, Svendsen C. Neutral red retention by lysosomes from earthworm (Lumbricus rubellus) coelomocytes: a simple biomarker of exposure to soil copper. Environmental Toxicology and Chemistry. 1996; 15(10):1801-5.
5. Booth L, Heppelthwaite V, O Halloran K, editors. Growth, development and fecundity of the earthworm Aporrectodea caliginosa after exposure to two organophosphates. Proceedings of the New Zealand plant protection conference; 2000: New Zealand Plant Protection Society; 1998.
6. Farrukh S, Ali AS. Effects of Endosulfan, an organochlorine pesticide on growth, reproduction and avoidance behavior of earthworm Eisenia foetida. Biosci.Biotech.Res.Comm.Vol.4, No.1:84-89
7. 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.
8. 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.
9. Speed FM, Smith JB.Investigation in behaviour and elementary Neurobiology. 1975.p.181-2.
10. Rodríguez-Castellanos L, Sanchez-Hernandez JC. Earthworm biomarkers of pesticide contamination: current status and perspectives. Journal of Pesticide Science. 2007; 32(4):360-71.
11. Moore MN. Cellular responses to pollutants. Marine Pollution Bulletin. 1985; 16(4):134-9.