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Research Article

IMPACT OF TRIAZOPHOS, CHEMICAL USED IN SOYBEAN CROP, ON THE GROWTH AND REPRODUCTION OF *EISENIA FETIDA*

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ABSTRACT

Earthworms are crucial for maintaining soil quality and supporting agricultural practices. Soil conditions, such as nutrients, toxicants, moisture, fertilizers, and pesticides, can impact earthworm populations, growth, and reproduction. Of particular concern is the potential negative effects of pesticides on higher ecological levels. This study aims to evaluate the toxicity of Triazophos, a commonly used insecticide during Soybean cultivation, on the earthworm species *Eisenia fetida* in Kota, Rajasthan, India. In this experiment, five sets of 10 earthworms were exposed to concentrations of Triazophos ranging from 0.158 to 15.8 mg/kg dry weight, while one set served as the control. The growth of earthworms was monitored after four weeks, and the number of juveniles was counted after eight weeks. The results showed that the growth rate and reproduction of *Eisenia fetida* were significantly reduced by exposure to Triazophos. These findings highlight the potential ecological risks associated with the use of Triazophos in agricultural settings.

Keywords: *Eisenia fetida*, Vertisols, Earthworm growth, Reproduction, Triazophos.

1. INTRODUCTION

Agrochemicals have become essential for meeting the increasing demand for food by enhancing productivity and protecting crops from pests and weeds. While pesticides are effective at killing insects and weeds, they can also be toxic to other organisms that are beneficial to crops and ecosystems, such as birds, fish, beneficial insects, and non-target plants. Many researchers have studied the toxicity of different agrochemicals on various life parameters of earthworm species, including *Eisenia fetida*, and found that these chemicals have a negative impact on earthworms. Helling et al. [1] observed that copper oxychloride concentrations had a definite negative effect on the growth rate of juveniles and cocoon production of *E. fetida*. In contrast, Vermeulen et al. [2] concluded that the fungicide mancozeb had no significant detrimental effect on either the growth or reproduction of *E. fetida* at the recommended dose or at an estimated environmental concentration. Landrum et al. [3] evaluated the effects of perchlorate on survival and reproductive success in *E. fetida,* using a range of concentrations to simulate different environmental scenarios. Xiao et al. [4] studied the effects of the herbicide acetochlor on *E. fetida* in artificial soils (OECD soil).

Biomarker tests conducted by Reinecke and Reinecke [5] showed a clear dose-related response between chlorpyrifos exposure concentration and biomass change in earthworms. Shi et al. [6] found that lindane and deltamethrin were toxic or even lethal to Eiseniafetida under acute exposure. Yasmin and D'Souza [7] investigated the effect of pesticides on the reproductive output of *Eisenia fetida*. Rai and Bansiwal [8] found that malathion reduced biomass and cocoon production in *Eisenia fetida*. Butachlor was found to reduce biomass and cocoon production in earthworms in the study by Gobi and Gunasekaran [9].

DeSilva et al. [10] studied the toxic effects of chlorpyrifos, carbofuran, mancozeb, and their formulated products on the survival, growth, and reproduction of the earthworm *Perionyx excavates*. The authors found that *Eisenia andrei* or *E. fetida* is less sensitive than *P. excavatus*. Farrukh and Ali [11, 12] investigated the effect of endosulfan and dichlorovos organophosphate on growth, reproduction, and avoidance behavior of *Eisenia fetida*. Wang et al. [13]

compared the acute toxicity of 24 insecticides to earthworms and concluded that neonicotinoids were super toxic, pyrethroids were very toxic, and insect growth regulators were moderately toxic. Wang et al. [14] and Li et al. [15] also conducted similar studies.

Suthar [16] investigated the toxicity of methyl parathion on the growth and reproduction of three ecologically different tropical earthworms. The author found that methyl parathion caused significant mortality in all tested species, and the niche structure and feeding habitats of test species directly affect the sensitivity of earthworms against methyl parathion. According to Wang et al. [17], guadipyr showed low toxicity to earthworms and did not elicit an effect on earthworm reproduction or growth in artificial soils at concentrations of 100 mg/kg. Shi et al. [18] found that contamination of DDT higher than 200 mg/kg was significantly toxic to both the survival and the growth of earthworms. Miglani and Bisht S.S. [19] concluded that the use of pesticides in agriculture systems results in many ecological problems.

Here, we aim to investigate the impact of Triazophos, a commonly used insecticide during Soybean cultivation, on the growth and reproduction of *E. fetida* in Kota, Rajasthan, India. By conducting experiments to assess the toxicity of Triazophos on *E. fetida*, we hope to contribute to the growing body of literature on the potential ecological risks associated with the use of agrochemicals.

2. MATERIAL AND METHODS

2.1. Test soil

The natural soil was collected from an abandoned area in Kota (India) where no agricultural activity was done and which was an area with no known history of pesticides use. The physico-chemical characterization of soil was provided by Nanta agricultural farm (office of Project Director, crop) in the Kota district. Sample soil was clay loam (Vertisols) having pH 8.33, conductivity 0.291 m Mhos/cm and organic carbon content 0.03 %. Soil color was brown.

2.2. Test organisms

The earthworms *Eisenia fetida*, were taken from Krishi Vigyan Kendra, Borkhera, Kota. The animals were bred in cow dung as a food at temperature ranging 15-30°C. For all tests, only adult worms with clitellum having weight between 200 and 300mg were taken. In addition, mass cultures of *E. fetida* were established where the cultures were kept in room temperature at a light cycle of 16 h/8 h. Finely ground cow dung, free of any chemical contamination, was used to fed the earthworms with the interval of 7 to 10 days. Acclimatization of selected worms was done in the selected soil 24 h before the onset of the experiment.

2.3. Test chemical

Triazophos 40 % ECis an Organophosphates and it is Acetylcholinesterase (AChE) class of inhibitor. Triazophos was tested as Trizocel (250 ml, Excel Crop Care Ltd). It is a broad-spectrum insecticide which acts upon insects through contact and stomach action. It penetrates deeply into plant tissues. It is effective for control of stem borer, leaf folder, green leaf hopper, Aphids and Bollworms in Soybean.

The concentrations used in present experiment are taken on recommended dose of this chemical for Kharif crops in Kota, Rajasthan (India). The recommended dose is 0.5mg/kg for Triazophos as per the recommendation of Department of Agriculture, Govt. of Rajasthan, Kota Division office.

2.3.1. Triazophos dose calculation

- Recommended dose 800 ml/hectare (v/w), now converted to (w/w) as per formulation gives 0.320 kg/hectare.
- Assuming that the chemical would disperse into the top 5 cm soil (as per literature), then volume of 1 hectare soil may be calculated as V = 9997.36 x 10^4 x 5 cm^3 (1 hectare = 9997.36 x 10^4 cm^2).
- Test soil density calculation: 1 kg of dry powdered soil contained 750 ml of volume in a container, so density 'd' of test soil is = 1000/750 = 1.33 gm/cm^3.
- Now weight of soil in 1 hectare area (for 5 cm soil depth) is
  - V.d = 9997.36 x 10^4 x 5 cm^3 x 1.33 gm/cm^3 = 6664824440 gm.
- Recommended dose calculation in mg/kg, for dry soil, can now be obtained by using dose of 0.320 kg/hectare. This comes out to be 0.5 mg/kg.

In the present study, five different concentrations were used with a multiplicative factor of 3.167 times. This led to the scenario where every alternative concentration is either 10 times or factored by 10. The concentrations tested for Triazophos were 0.158, 0.50, 1.58, 5, 15.8 mg/kg dry soil weight. In this set of experiment, one concentration is taken lower than the recommended dose. This had been done due to observed strong avoidance behaviour of earthworm at 10 mg/kg in Triazophos in present experiment [20]. Strong avoidance behaviour at certain concentration levels indicated regarding avoiding very high dose of chemicals in our experimentation.

2.4. Earthworm growth and reproduction test

Five sets of different concentrations were prepared to evaluate the growth of earthworms (in four weeks) and no. of juveniles (after eight weeks) to test the efficacy of pesticides. To do this, initially soil and cow dung powder were taken in the ratio of 2:1. Dry weight of this mixture was 500 gm. for each sample. For experimentation, transparent plastic container of 1 litre capacity was taken. The soil was artificially contaminated by Triazophos by adding chosen concentrations of pesticide as stated above. In addition to this, one set of experiment was conducted by taking control soil (no contamination). Ten worms were added in each container. Initially, all worms were sorted out of the soil, washed with fresh water and left over the blotting paper for some time before measuring their weight by using electronic balance. Now, ten earthworms having weight between 2 to 3 gm. were selected and left them over to the soil surface. They immediately buried themselves into the soil. In present study, close watch has been done on moisture content of the soil by checking it weekly and maintaining it at 50% by adding water, as required. Quantity of the water to be added was decided by measuring the difference in the weight of the container as compared to weight at the time of sampling. For growth rate observation, experiment lasted for 28 days.

Adult earthworms were removed from the chemical-treated soil after 28 days and weighed to observe the impact of the biomass growth rate.

Biomass growth rate is calculated as per the following formula:

Growth rate of earthworms = (average weight after 28 days of exposure/average weight at beginning of incubation) x 100

For the study of effect on reproduction, cocoons were left to remain in the soil for four additional weeks. After the completion of the period, young worms present in the soil were counted. This count is providing the parameter of no. of juveniles reproduced per earthworm from our experiment. In our work, we took ten earthworms initially for each set of experiment. So, dividing total number of juveniles by ten provides the second important parameter of our study.

3. RESULTS AND DISCUSSION

The results of the experiment indicate that pesticides have a significant negative impact on the growth rate and reproduction of earthworms. The findings are summarized below, along with the range of variation for the 95% confidence interval (CI) of mean.

3.1. Effect on growth

After 28 days, the growth rate of earthworms in the control soil was found to be 153% $\pm$ 20.9%. However, in the soil treated with Triazophos at a concentration of 0.158 mg/kg, the growth rate reduced to 97.94% $\pm$ 4.04%. At other concentration values of 0.50 mg/kg, 1.58 mg/kg, 5 mg/kg, and 15.8 mg/kg, the growth rates were 93.6% $\pm$ 12.6%, 69.6% $\pm$ 4.53%, 40% $\pm$ 4.73%, and 13.6% $\pm$ 7.58%, respectively. The graphical result of the effect of Triazophos on the growth of *Eisenia fetida* is shown in Fig. 1.

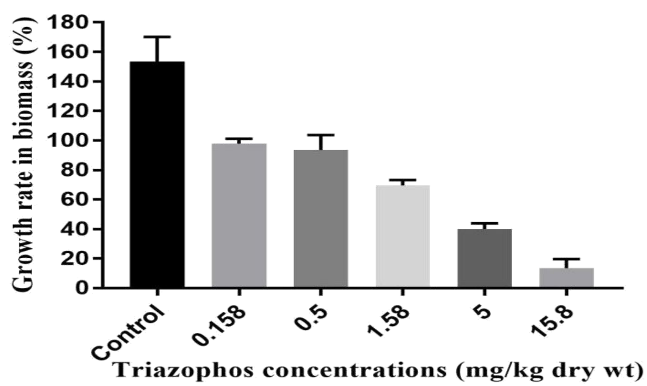

Fig. 1: Growth rate for different concentrations of Triazophos

Table 1: Effect of different concentrations of Triazophos on biomass growth of *Eisenia fetida*

Chemical concentration (mg/kg)	Mean of biomass growth (%) μ	Standard deviation (SD) σ	Std. error of mean (SE_{μ})	Lower and upper 95% CI of mean
Control soil	153.3	16.81	7.52	132.4 and 174.2
0.158	97.94	3.251	1.454	93.9 and 102
0.5	93.6	10.14	4.534	81.01 and 106.2
1.58	69.6	3.647	1.631	65.07 and 74.13
5	40	3. 808	1.703	35.27 and 44.73
15.8	13.6	6.107	2.731	6.017 and 21.18

Table 1 shows the statistical data of growth rate under influence of this chemical. Correlation value for this chemical is -0.9172. This ascertains the adverse effect on growth rate due to this chemical.

3.2. Effect on reproduction

After 56 days of exposure, the number of juveniles produced per earthworm in the control soil was found to be 20.2 ± 3.21. However, in the soil treated with a concentration of 0.158 mg/kg of Triazophos, the number of juveniles reduced to 8.68 ± 1.41. At other concentration values of 0.50 mg/kg, 1.58 mg/kg, 5 mg/kg, and 15.8 mg/kg, the numbers of juveniles produced per earthworm were 7.5 ± 0.47, 5.9 ± 0.8, 4.04 ± 0.45, and 2.1 ± 0.53, respectively. The graphical result of the effect of Triazophos on the reproduction of *Eisenia fetida* is shown in Fig. 2.

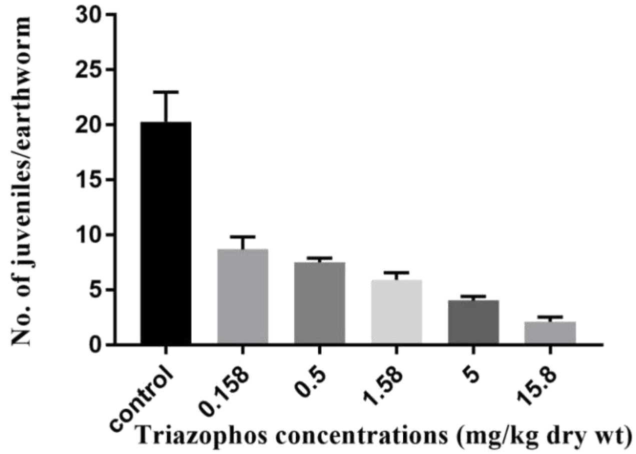

Fig. 2: Number of juveniles produced per earthworm for different concentrations of Triazophos

Table 2: Effect of different concentrations of Triazophos on reproduction of *Eisenia fetida*

Chemical concentration	Mean No. of Juvenile/ earthworm μ	Standard deviation (SD) σ	Std. error of mean (SE_μ)	Lower and upper 95% CI of mean
Control soil	20.2	2.586	1.156	16.99 and 23.41
0.158	8.68	1.139	0.5093	7.266 and 10.09
0.5	7.5	0.3808	0.1703	7.027 and 7.973
1.58	5.9	0.6519	0.2915	5.091and 6.709
5	4.04	0.3647	0.1631	3.587 and 4.493
15.8	2.1	0.4301	0.1924	1.566 and 2.634

Table 2 contains statistical data on the number of juveniles produced per earthworm under the influence of Triazophos. The results show that the number of juveniles produced per earthworm at different concentrations of Triazophos is significant, with a 95% CI of mean. The correlation value between the number of juveniles produced per earthworm and concentration values is -0.9005, confirming the adverse effect of Triazophos on the number of juveniles produced per earthworm.

The present study shows that at the recommended dose for agricultural use of Triazophos (0.5 mg/kg), the growth rate and number of juveniles per earthworm were drastically reduced. Fig. 3 shows the combined plot demonstrating the correlation between the growth rate of earthworms and the number of juveniles produced per earthworm at different concentrations of Triazophos. The graph indicates that Triazophos caused a gradual fall in growth rate. Experimentation on the number of juveniles produced per earthworm showed a sharp decline as compared to the control soil, but there was no clear dose-response relationship between substrate concentrations and the mean number of juveniles produced per earthworm.

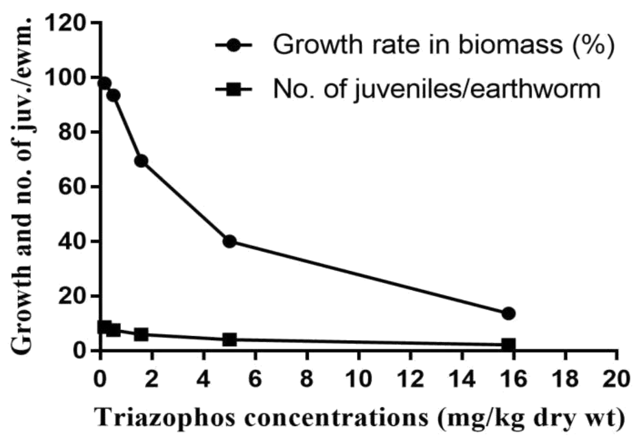

Fig. 3: Correlation between growth rate and no. of juveniles produced per earthworm for different concentrations of Triazophos

Table 3 shows that most of the P (two-tailed) values are closer to zero. This result ascertains negative effect of chemical over important life history parameters of earthworm. While $R_{squared}$ values are nearer to one. This shows that data is closer to the fitted regression line around its mean.

Interestingly, while previous research has explored the negative effects of chemicals like malathion and butachlor on *Eisenia fetida*, this study is the first to report the negative effects of Triazophos on this species. Rai and Bansiwal [8] found that malathion also showed negative effect on growth and reproduction of *Eisenia fetida*, as we observed in present study. Similar observations were reported by Gobi and Gunasekaran [9] while studying effect of herbiside (butachlor) on *Eisenia fetida*. Biomass and cocoon production were decreased with increased herbicide concentration. Sofia Lammertyn et al. [21] reported that the presence of a higher concentration (10 mg Kg −1) of the herbicide, atrazine, reduced one third of the number of total cocoons, affecting the reproduction.

The similarity of these observations across different chemicals suggests a common thread in their toxicity to earthworms, reinforcing the importance of limiting their use and exploring alternative, more sustainable agricultural practices.

Table 3: Statistical data of growth rate and no. of juveniles produced per earthworm under the influence of Triazophos

Parameter under study	Correlation value	95% CI	$R_{squared}$	P (two-tailed)	Significant (alpha= 0.05) Confidence (95%)
Concentration v/s Biomass growth (%)	-0.9172	-0.9946 to -0.1832	0.8413	0.0282	Yes
Concentration v/s no. of Juvenile/earthworm	-0.9005	-0.9935 to -0.0887	0.8109	0.0371	Yes

4. CONCLUSION

In conclusion, the results of this study indicate that the use of the insecticide Triazophos has a negative impact on both the growth rate and reproduction of earthworms. Even at concentrations lower than the recommended agricultural dose, the toxicity of this chemical was still evident. These findings confirm that Triazophos can be detrimental to the health and well-being of earthworms, which are critical organisms in soil ecosystems.

In addition, the results of this study demonstrate the importance of careful monitoring and regulation of chemical usage in agriculture. Given the significant role of earthworms in soil ecosystems, the negative impact of Triazophos on their growth and reproduction could have far-reaching consequences for agricultural productivity and the environment as a whole. It is therefore essential that policymakers and agricultural stakeholders work together to identify and implement more sustainable practices that minimize the use of harmful chemicals and promote the health of soil ecosystems.

In summary, this study highlights the negative impact of Triazophos on the growth and reproduction of earthworms, adding to the growing body of evidence on the toxicity of chemicals on soil organisms. By shedding light on this issue, we hope to encourage further research and advocacy for more sustainable agricultural practices that prioritize the health of our ecosystems and the planet.

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Conflicts of interest

Authors have no conflict of interest in this research work.

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