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Effect of Pesticides on the Reproductive Output of *Eisenia fetida*

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Abstract We investigated the effects of three different pesticides (carbendazim, dimethoate, and glyphosate) and their mixture on the growth and reproduction of the earthworm species, *Eisenia fetida*. The study was conducted following the suggestion of the International Workshop on Earthworm Ecotoxicology. The results showed that the pesticide treatment had a marked negative impact on the growth and reproduction of earthworms. Carbendazim and dimethoate were found to cause greater harm to the selected earthworm species than glyphosate.

Keywords *Eisenia fetida* · Pesticides · Weight · Reproduction

The indiscriminate use of pesticides has contaminated our environment. In response, environment risk assessments are conducted to test the toxicity of various pesticides on organisms that exist in soil and water environments. Among soil organisms, earthworms are particularly apt subjects for the assessments because of the potential role these organisms play in vermi-composting and nutrient cycling and also because they constitute up to 92% of the invertebrate biomass in the soil. Earthworms are among the most suitable animals for use as key bioindicator organisms for testing the toxicity of chemicals in soils (Callahan 1988; Goats and Edwards 1988; Bouche 1992). They have also been adopted as standard organisms for ecotoxicological testing by the European Union (EEC 1984) and the OECD (1984, 2000). Furthermore, among the various earthworm species, *Eisenia fetida* (*E. fetida*) is especially appropriate for the toxicity tests because it can be easily bred on a variety of organic wastes with short generation times. Its susceptibility to chemicals resembles that of true soil organisms. Sensitivity tests have revealed that *E. fetida* is comparatively less sensitive than other species of earthworm (Ma and Bodt 1993; Fitzgerald et al. 1996; Kula 1995).

The International Workshop on Earthworm Ecotoxicology held in Åarhus, Denmark in 2001 (IWEE III) was less supportive of the role of acute tests as a screening tool and suggested that research efforts focus on the development of sublethal reproductive tests instead. Neuhauser and Callahan (1990) reported that earthworms exposed to 50 mg/kg carbaryl showed no mortality. The exposure, however, led to a 50% reduction in cocoon numbers, which would cause a significant reduction in earthworm population two to three generations down the line. According to Brunninger et al. (1994), fecundity in earthworms is sensitive to pesticides; even though the earthworms may not be immediately impacted, changes resulting in the reduction of populations in the longer term might occur.

The soil environment often consists of mixtures of chemicals, and additional research is needed to provide more insight on the joint action of these chemical mixtures (IWEE III). However, only a few studies describing the toxicity impact of chemical mixtures on earthworms have been published thus far, all of which focus on metals (Condor and Lanno 2000; Khalil et al. 1996a, b; Marinussen et al. 1997; Weltje 1998). In contrast, our study investigated the effect of a fungicide (carbendazim),

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an insecticide (dimethoate), and a herbicide (glyphosate) on the fecundity of earthworm species *E. fetida*. We investigated the effects of a single dose (1×) and tetra dose (4×) of each chemical and the effects of the mixture of the three chemicals, in accordance with the recommendation of the International Workshop on Earthworm Ecotoxicology (IWEE).

Materials and Methods

E. fetida was chosen for the study because it was hardy, easy to handle, and easily available from the vermicompost unit of the college.

The mixture for the culture of worms was created using 75% soil and 25% cow dung. The dry black soil was powdered and filtered through a fine mesh sieve. The sieved soil was then moistened, and 30 adult worms were added to it. The cow dung, which was 3 weeks old, was added for food, and the system was covered with hay. After 28 days, the adult worms were removed from the system. We observed a 100% survival rate of the earthworms. During the 28 days in the system, the worms had reproduced and laid cocoons. After the worms were removed, the system was left undisturbed for 2 months. Only water was sprinkled to keep the soil moist. After the 2 months elapsed, worms of the same age were obtained from the system. These new worms were then used for the experiment.

The pesticides used in the experiment were Bavistin (carbendazim 50%), Glycel 41% S.L. (glyphosate 41% S.L.), and Rogor 30% E.C. (dimethoate).

The concentrations of pesticides to be applied were calculated as follows:

According to the available literature, the application rate of carbendazim is 0.18–0.6 kg/ha. The maximum recommended application rate is 0.6 kg/ha, which equals 600 g/ha. Assuming that the chemical would disperse into the top 5 cm of soil when the soil density is 1.5 g/cm^3, 1× for the experiment was calculated to be 0.8 mg/kg of soil, with 4× being 3.2 mg/kg of soil.

Similarly, 1× for glyphosate was calculated to be 2 mg/kg of soil (application rate = 2–3 L/ha; containing 480 g/L a.i.). Four times for glyphosate was 8 mg/kg of soil.

Dimethoate has a specified application rate of 1,000 mL/ha, with 300 g a.i. Based on this, 1× was calculated to be 0.4 mg/kg of soil, with 4× being 1.6 mg/kg of soil.

Our study was based on the reproductive test proposed by Kula and Larink (1997). We used earthen pots for our experiment. Dried black soil was crushed and filtered through a fine mesh sieve. One kilogram of fine soil was added to each earthen pot and moistened with water. Ten earthworms weighing between 3.0 and 3.5 g were taken from the cultured worm stock and released onto the soil surface. They immediately buried themselves into the soil. These earthworms were then exposed to 1× and 4× of pesticides for 28 days and were allowed to reproduce. The pesticides were added to the soil surface, and food (dried cow dung) was also scattered on the soil surface to avoid starvation, as recommended by the International Workshop on Earthworm Ecotoxicology held in Sheffield in 1991 (IWEE I). After 28 days in the pesticide-treated soil, the adult earthworms were removed and weighed, while the cocoons were left to remain in the soil for four additional weeks. After these 4 weeks, the young worms were removed from the system and counted. The system was then left undisturbed for another 4 weeks, so that the remainder of the cocoons could hatch. At the end of these 4 weeks, we counted new young worms. In this way, the effect of pesticides on the reproduction of earthworms was assessed by taking into account the number of young worms in the soil after 8 weeks of treatment with each pesticide and also with the mixture of the three pesticides.

One time of the mixture consisted of 0.8 mg of carbendazim, 2 mg of glyphosate, and 0.4 mg of dimethoate, per kilogram of soil. Four times of the mixture consisted of 3.2 mg of carbendazim, 8 mg of glyphosate, and 1.6 mg of dimethoate, per kilogram of soil. The experiment for each pesticide, and for their mixtures, was performed in triplicates. In addition, we kept aside three groups of worms as control.

The statistical analysis of the count data was performed using ANOVA. Because normality of data is a prerequisite for ANOVA, the count data were log-transformed to ensure normal distribution. When the final F test in ANOVA indicated that there were significant differences between the means of the counts of the young worm groups, the Tukey test was performed to determine exactly which group differed most significantly from the control group. The weights of the treated worms were compared to that of the control group with the help of the paired t test.

Results and Discussion

The experiment provided strong evidence that pesticides induced negative effects on the weight and reproduction of earthworms (Tables 1, 2). There was a sharp decline in the weight of the treated earthworms compared to the weight of the control worms (Table 1). Earthworm weight was reduced due to exposure to 1× of carbendazim ($p < 0.05$) and also to the mixture of the three pesticides ($p < 0.05$). Further, earthworm weight was significantly reduced due to its exposure to 4× of carbendazim ($p < 0.02$), glyphosate ($p < 0.05$), dimethoate ($p < 0.05$) and to the mixture of the

Table 1 Effects of pesticides and their mixture on the weight of *Eisenia fetida*

Names of pesticides	Weight (g) of ten worms after 28 days of exposure		
	Control (n = 3)	1× Dose (n = 3)	4× Dose (n = 3)
Carbendazim	6.0 ± 0.2	3.5 ± 0.1*	3.4 ± 0.1**
Glyphosate	6.0 ± 0.2	3.2 ± 0.3	2.9 ± 0.2*
Dimethoate	6.0 ± 0.2	3.4 ± 0.03	3.0 ± 0.03*
Mixture of pesticides (carbendazim, glyphosate, and dimethoate)	6.0 ± 0.2	3.5 ± 0.1*	2.9 ± 0.1***

Values are mean ± SE

* $p < 0.05$, ** $p < 0.02$, *** $p < 0.01$ for comparison with controls

three pesticides ($p < 0.01$). In a study by Espinoza-Navarro and Bustos-Obregon (2005), malathion-treated worms also showed significant reduction in body weight in a dose-dependent manner.

The count of young worms after 8 weeks of treatment provided an index to the reproductive output of the worms. A decline in the number of young worms in pesticide-treated soils compared to the control worms was observed (Table 2). There was a significant reduction in the number of young worms due to being exposed to 1× of carbendazim ($p < 0.05$) and to the mixture of the three pesticides ($p < 0.01$). The reproductive output was also significantly impacted due to exposure to 4× of carbendazim ($p < 0.05$), dimethoate ($p < 0.05$), and to the mixture of the three pesticides ($p < 0.01$) (Table 2). In addition, Arrate et al. (2002) found that carbendazim caused a reduction in the number of juveniles per adult of the worm *Enchytraeus coronatus*. Furthermore, Holmstrup and Martin (2000) also found a drastic decrease in the reproductive rates of two earthworm species, *Aporretodea longa* and *A. rosea*, in relation to the amount of benomyl in the grassland ecosystem. These reproductive rates were statistically significant when benomyl was found in its greatest amounts.

The pesticide concentration used in this study did not directly lead to the mortality of earthworms. However, carbendazim, dimethoate, and the mixture of the three pesticides (carbendazim, dimethoate, and glyphosate) had a significant negative impact on the weights and reproductive output of the earthworms. In contrast, glyphosate did not have a significant effect on the reproduction of *E. fetida*. Bustos-Obregon and Goicochea (2002) also found a reduction in the number of cocoons, young worms, body weight, and survival rates of earthworms due to exposure to parathion. In addition, Helling et al. (2000) found a significant reduction in the growth and cocoon production of *E. fetida* due to exposure to copper oxychloride (a fungicide). According to Bunemann et al. (2006), among all the effects of pesticides on soil organisms, only a few significant negative effects of herbicides have been documented, whereas the negative effects of insecticides and fungicides have been more commonly studied and documented.

Earthworms are considered bioindicators of soil fertility, and they have also been shown to improve the soil quality of degraded land (Marashi and Scullion 2004). Because earthworms play such an important role in soil processes, any practice that creates a favorable environment for earthworms will, in the long run, enhance soil fertility and conserve biodiversity. Unfortunately, earthworms, along with other beneficial soil microorganisms, have become the non-target recipients of pesticides. Moreover, earthworms accumulate pesticide residues and can potentially transfer these residues to predators higher up in the food chain (Spurgeon et al. 2003). *E. fetida* (Neuhauser and Callahan 1990) and *Apporrectodea caliginosa* (Booth et al. 2000) showed recovery in their weights only about 8 weeks after their removal from pesticide-treated soil. In real life, however, earthworms cannot be removed from soil exposed to pesticides. Instead, they would continuously be exposed to chemicals until the chemicals degraded. Furthermore, the chances of subsequent additions of pesticides to the soil cannot be ruled out. In conclusion, the present study has shown that the treatment of pesticides,

Table 2 Effects of pesticides and their mixture on the reproductive output of *Eisenia fetida*

Names of pesticides	Number of worms (after 8 weeks of exposure)[a]		
	Control (n = 3)	1× Dose (n = 3)	4× Dose (n = 3)
Carbendazim	56.0 ± 5.8 (1.8 ± 0.4)	48.3 ± 2.9* (1.7 ± 0.03)	39.3 ± 2.9* (1.6 ± 0.03)
Glyphosate	56.0 ± 5.8 (1.8 ± 0.4)	51.7 ± 5.2 (1.8 ± 0.04)	43.3 ± 2.4 (1.6 ± 0.02)
Dimethoate	56.0 ± 5.8 (1.8 ± 0.4)	48.7 ± 6.7 (1.7 ± 0.1)	37.3 ± 1.5* (1.6 ± 0.02)
Mixture of pesticides (carbendazim, glyphosate, and dimethoate)	56.0 ± 5.8 (1.8 ± 0.4)	43.7 ± 5.9** (1.6 ± 0.1)	35.0 ± 4.9** (1.5 ± 0.1)

Values are mean ± SE

* $p < 0.05$, ** $p < 0.01$ for comparison with controls

[a] The count data were log-transformed for statistical analysis (ANOVA). The log-transformed data are given in parentheses

individually or as a mixture, is detrimental to the growth and reproduction of earthworms. In addition, this study gives rise to many thought-provoking questions about the effects of pesticide treatment on other organisms, agriculture, and even human life. However, further studies are needed to elucidate the effects of pesticide treatment on the soil ecosystem as well as to expound on its associated effects on predatory organisms and the environment at large.

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