



The Effects of Glyphosate and 2, 4-D Herbicides on Viability of *Eisenia Foetida* Earthworms

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Abstract: The study aimed to examine the effects of glyphosate and 2,4-D herbicides on the reproduction of in-vitro *eiseniafoetida* earthworms. This species was chosen because of its easy in-vitro reproduction. The experiment followed a randomized complete block design with four replications. The study included a control treatment, a factory recommended dose treatment (1.5 L/ha 2,4-D, 5 L/ha glyphosate), two overdose treatments (2.25 L/ha 2,4-D, 7.5 L/ha glyphosate), and two underdose treatments (.75 L/ha 2,4-D, 2.5 L/ha glyphosate). The herbicide treatments caused weight loss in *eiseniafoetida* earthworms. Higher doses of each herbicide resulted in more weight loss. The weight loss effect of the herbicides degraded with time. This was observed for both herbicides in all treatments. After the fifth week, there was a significant difference between the control and the experimental groups. However, there was no significant difference between the experimental groups themselves, which can be related to herbicide degradation. The 2,4-D treatment caused more weight loss compared to the glyphosate treatment because it is a more toxic herbicide. Preliminary analysis indicated that behavioral tests could be used to quickly examine polluted soils. They can also be used as confirmatory analysis to assess the extent of the pollution.

Key words: Herbicides, Earthworms, Viability

Introduction

A greater proportion (80%) of biomass of terrestrial invertebrates is represented by earthworms which play an important role in structuring and increasing the nutrient content of the soil. Therefore, they can be suitable bioindicators of chemical contamination of the soil in terrestrial ecosystems providing an early warning of deterioration in soil quality (Bustos-Obregón and Goicochea, 2002). Earthworms are boneless animals with a soft body that stretches more than 3 cm. They digest dead plants and other organic matter and produce vermicompost. They also dig holes in the soil which allow for air and water to seep through and improve plant growth. *Eiseniafoetida* is the most commonly researched member of this family (Correia et al., 2002). 2,4-D is a low-cost herbicide common in world. This herbicide is weak in terms of biological transformation and is usually found in water. The use of this herbicide can cause metabolic changes and tissue death in non-target organisms and important members of the food chain such as fish (Dynes, 2003). Researchers have found that 2,4-D rarely affects earthworms unless it is administrated repeatedly. They have also observed that the worms are mostly affected in pastures and prairies. An in-vitro toxicology study examined the effect of 2,4-D dimethyl amine salt on *Eiseniafoetida* (Corriea et al., 2010). So, Herbicide effectiveness could be strengthened according to the target weed and application dosage and repetition. Neuhauser and Callahan (1990) suggested that more consideration should be given to evaluation of sublethal effects under field conditions. The present study examined the effects of glyphosate and 2,4-D on the reproduction of *eiseniafoetida* earthworms.

Materials and Methods

The experiment was carried out in ShirazCity, Fars Province, Iran, following a randomized complete block design with four replications in the year 2015. The worms were bought from ParsWormBreedingCenter in Estahban. The herbicides were bought from Zarrin Sam Company. Seven treatments were implemented

including a control treatment, a factory recommended dose treatment (1.5 L/ha 2,4-D, 5 L/ha glyphosate), an overdose treatment (2.25 L/ha 2,4-D, 7.5 L/ha glyphosate), and an underdose treatment (.75 L/ha 2,4-D, 2.5 L/ha glyphosate). The pots were 100 cm in length, and 40 cm in both width and height. They were filled with sand, clay, compost, and leaf soil. The temperature and moisture were fixed at 30 °c and 50%. Each week, for eight weeks, the worms were weighed using a digital scale. At the beginning, the worms in each pot weighed 300 g.

Statistical analysis was done using the SAS software, and charts were drawn using Microsoft Excel. To compare mean scores, Duncan's multiple range test was run ($p = .05$).

Results and Discussions

As table 1 shows, the experimental treatments significantly affected the worms' weights at either $p = .05$ or $p = .01$ for all eight weeks. The table demonstrates the weekly results.

The First Week: Based on the variance analysis, it was found that applying the herbicide treatments significantly affected weight at $p = .05$ (Table 1). Examining the mean scores through Duncan's test indicated the control treatment to have the highest weight value (301 g). The lowest weight value belonged to the 2.25 L/ha 2,4-D treatment. Other treatments also indicated a significant difference with the control treatment and were categorized in a different statistical group. The 1.5 L/ha 2,4-D, the 5 L/ha glyphosate, the .75 L/ha 2,4-D, the 2.5 L/ha glyphosate, the 2.25 L/ha 2,4-D, and the 7.5 L/ha glyphosate treatments caused 30, 22, 20, 20, 65, and 40% weight loss in the worms, respectively (Figure 1). During this week, overdosing both herbicides caused more weight loss.

The Second Week: Based on variance analysis, it was found that applying the herbicide treatments significantly affected weight at $p = .01$ (Table 1). Examining the mean scores through Duncan's test indicated the control treatment to have the highest weight value (640 g). The lowest weight value belonged to the 2.25 L/ha 2,4-D treatment (225 g). The 1.5 L/ha 2,4-D, the 5 L/ha glyphosate, the .75 L/ha 2,4-D, the 2.5 L/ha glyphosate, the 2.25 L/ha 2,4-D, and the 7.5 L/ha glyphosate, treatments caused 25, 21, 26, 16, 64, and 43% weight loss in the worms respectively (figure 2).

The Third Week: Based on variance analysis, it was found that applying the herbicide treatments significantly affected weight at $p = .01$ (Table 1). Examining the mean scores through Duncan's test indicated the control treatment to have the highest weight value (765 g). The lowest weight value belonged to the 2.25 L/ha 2,4-D treatment. Although the experimental treatments still significantly differed from the control, the difference showed a decrease compared to the first two weeks. The 1.5 L/ha 2,4-D, the 5 L/ha glyphosate, the .75 L/ha 2,4-D, the 2.5 L/ha glyphosate, the 2.25 L/ha 2,4-D, and the 7.5 L/ha glyphosate, treatments caused 20, 16, 14, 13, 52, and 32% weight loss in the worms respectively (Figure 3).

The Fourth Week: Based on variance analysis, it was found that applying the herbicide treatments significantly affected weight at $p = .01$ (Table 1). Examining the mean scores through Duncan's test indicated the control treatment to have the highest weight value (892 g). The lowest weight value belonged to the 2.25 L/ha 2,4-D treatment (509 g). The 1.5 L/ha 2,4-D, the 5 L/ha glyphosate, the .75 L/ha 2,4-D, the 2.5 L/ha glyphosate, the 2.25 L/ha 2,4-D, and the 7.5 L/ha glyphosate treatments caused 16, 11, 10, 09, 42, and 27% weight loss in the worms respectively (figure 4).

The Fifth Week: Based on the variance analysis, it was found that applying the herbicide treatments significantly affected weight at $p = .01$ (Table 1). Examining the mean scores through Duncan's test indicated the control treatment to have the highest weight value (1038 g). The lowest weight value belonged to the 2.25 L/ha 2,4-D treatment. The 1.5 L/ha 2,4-D, the 5 L/ha glyphosate, the .75 L/ha 2,4-D, the 2.5 L/ha glyphosate, the 2.25 L/ha 2,4-D, and the 7.5 L/ha glyphosate, treatments caused 10, 09, 03, 03, 34, and 20% weight loss in the worms respectively (figure 5).

The Sixth Week: Based on the variance analysis, it was found that applying the herbicide treatments significantly affected weight at $p = .05$ (Table 1). Examining the mean scores through Duncan's test indicated the control treatment to have the highest weight value (1151 g). The lowest weight value belonged to the 2.25 L/ha 2,4-D treatment. The 1.5 L/ha 2,4-D, the 5 L/ha glyphosate, the .75 L/ha 2,4-D, the 2.5 L/ha glyphosate,

the 2.25 L/ha 2,4-D, and the 7.5 L/ha glyphosate, treatments caused 07, 04, 02, 06, 29, and 16% weight loss in the worms respectively (figure 6). On the whole, the difference between the control and the experimental treatments was reduced.

The Seventh Week:Based on the variance analysis, it was found that applying the herbicide treatments significantly affected weight at $p= .05$ (Table 1). Examining the mean scores through Duncan’s test indicated the control treatment to have the highest weight value (1026 g). The lowest weight value belonged to the 2.25 L/ha 2,4-D treatment. The 1.5 L/ha 2,4-D, the 5 L/ha glyphosate, the .75 L/ha 2,4-D, the 2.5 L/ha glyphosate, the 2.25 L/ha 2,4-D, and the 7.5 L/ha glyphosate, treatments caused 15, 06, 02, 01, 30, and 10% weight loss in the worms respectively (figure 7).

The Eighth Week: Based on the variance analysis, it was found that applying the herbicide treatments significantly affected weight at $p= .05$ (Table 1). Examining the mean scores through Duncan’s test indicated the .75 L/ha 2,4-D to have the highest weight value (1275 g). The lowest weight value belonged to the 2.25 L/ha 2,4-D treatment. The 1.5 L/ha 2,4-D, the 5 L/ha glyphosate, the 2.5 L/ha glyphosate, the 2.25 L/ha 2,4-D, and the 7.5 L/ha glyphosate, treatments caused 04, 05, 01, 14, and 07% weight loss in the worms respectively (figure 8).Yasmin and Doris (2010) evaluated Effects of Pesticides on the Growth and Reproduction of Earthworm and they reported negative effects on Earthworm.

The Weight Change Process of Live Worm: Based on Chart 9, the worms gained weight through time. The least weight gain was determined for the 2.25 L/ha 2,4-D treatment (figure 9). This result is in order to result of CorreiaandMoreira (2010).

Table 1: Square mean of the worm weight during the eight weeks

	df	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Treatment	6	14.7084*	15.5187**	46.367**	33.488**	40.2698**	43.6836*	40.2864*	52.2123*
Error	21	4.76	3.87	11.65	5.98	8.87	12.34	11.99	15.87
CV		14.5	11.3	5.4	8.3	6.3	10.2	11.1	12.1

* and ** indicate significance at .05 and .01 respectively

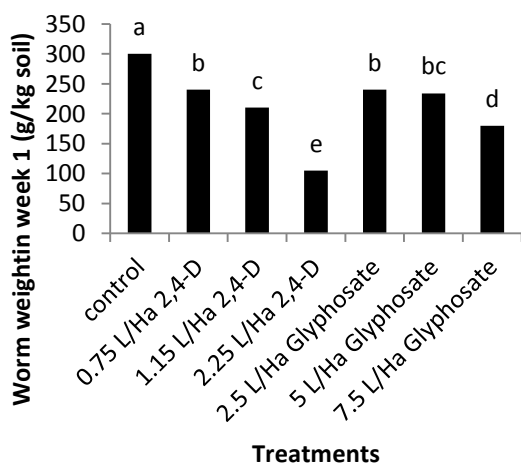


Figure 1 - The effect of treatments on worm weight in week 1

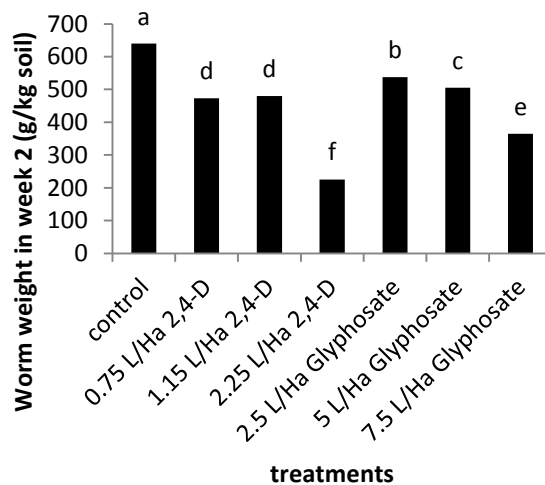


Figure 2 - The effect of treatments on worm weight in week 2

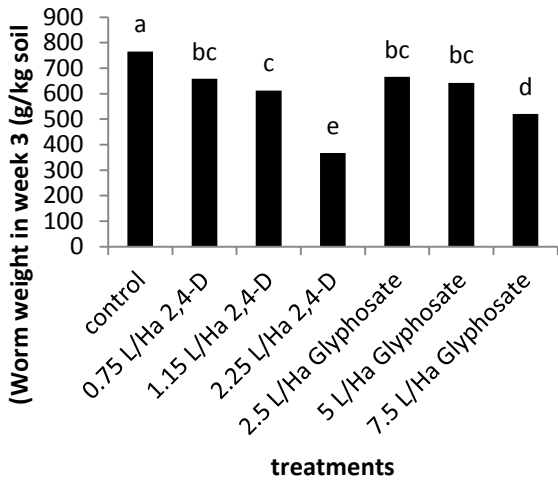


Figure 3 - The effect of treatments on worm weight in week 3

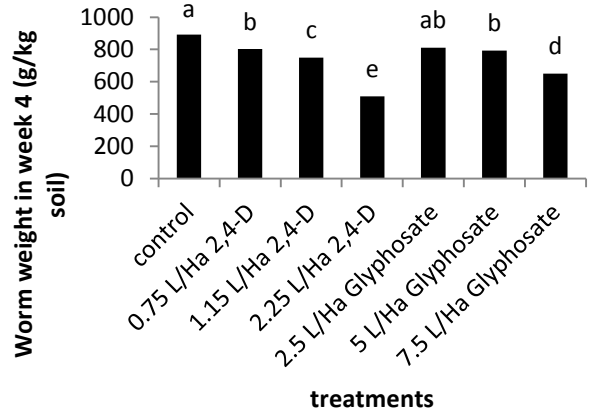


Figure 4 - The effect of treatments on worm weight in week 4

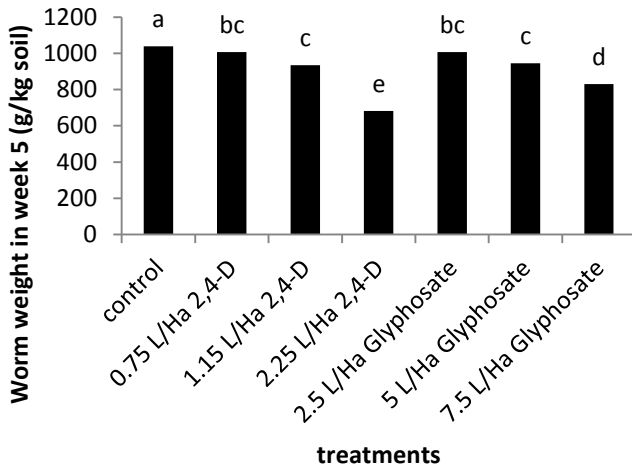


Figure 5 - The effect of treatments on worm weight in week 5

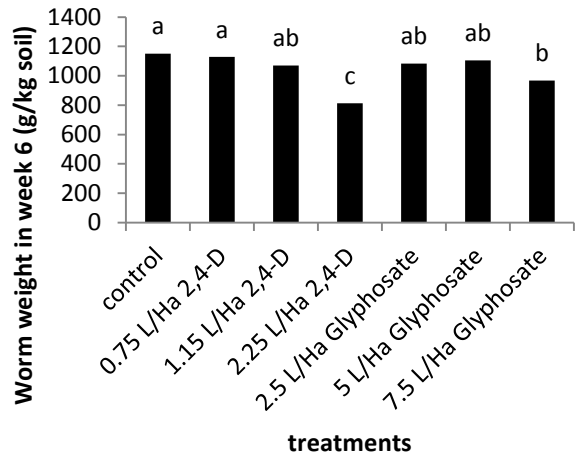


Figure 6 - The effect of treatments on worm weight in week 6

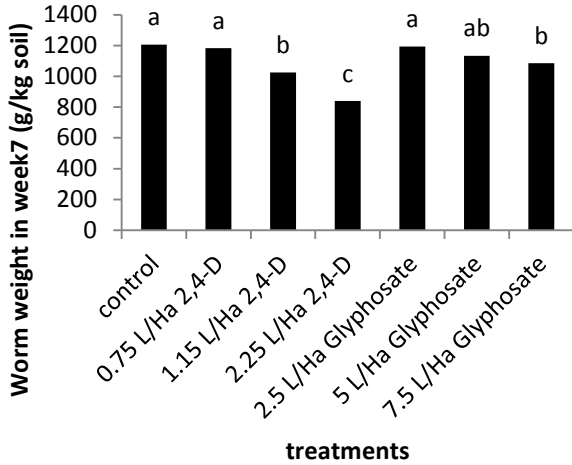


Figure 7 - The effect of treatments on worm weight in week7

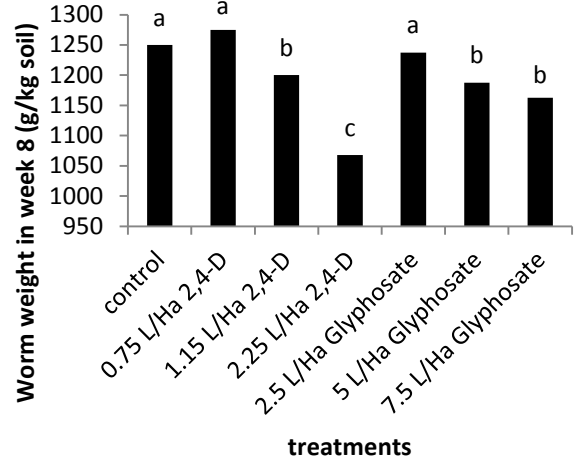


Figure 8 - The effect of treatments on worm weight in week 8

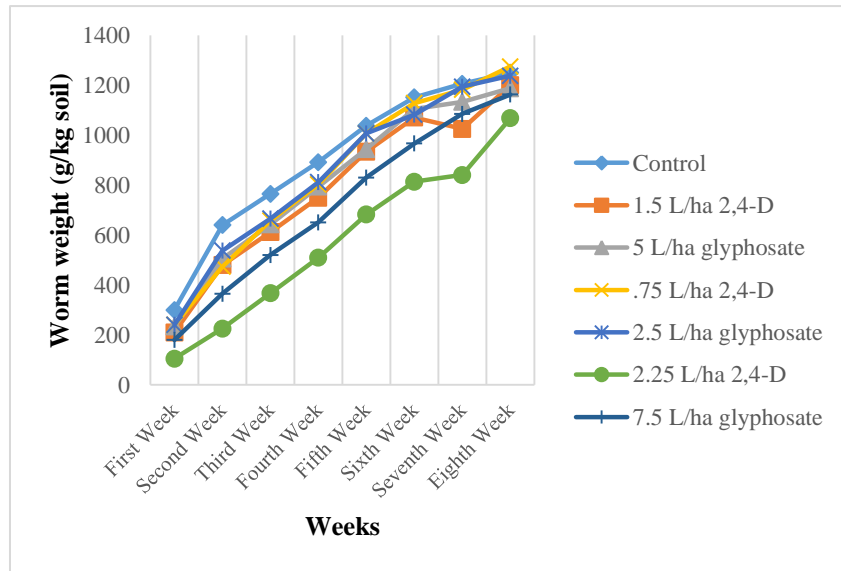


Figure 9: The weight change process of live worm in 8 weeks

Conclusion

Based on the results, it can be concluded that the herbicide treatments caused *Eiseniafoetidato* lose weight in soil. However, there are a few points to consider. Firstly, higher doses of each herbicide caused more weight loss. Secondly, the negative effect of both herbicides on the earthworms' weight decreased through time for all doses. In essence, a significant difference was found between the control and the experimental treatments after the fifth week; nevertheless, little variation was observed between the experimental treatments

themselves. This observation could be attributed to the degradation of herbicides through time. Lastly, the 2,4-D treatment caused more weight

Resources

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