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Impact of Pendimethalin on Growth and Reproduction of *Eisenia fetida*

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ABSTRACT
Earthworms play a crucial role in soil quality and agricultural practices, and their populations can be influenced by various factors, including the presence of pesticides like pendimethalin used in soybean crop. This study aims to evaluate the toxicity of pendimethalin on *Eisenia fetida*, a common naturalized exotic earthworm species found in Kota, India. Five sets of 10 worms were exposed to different concentrations of pendimethalin (ranging from 0.474 to 47.4 mg/kg dry weight), with one set of control soil. After four weeks, the growth rate and reproduction of *E. fetida* were significantly reduced by pendimethalin exposure, highlighting the potential negative impact of this herbicide on soil ecosystems.

Key words: Exposure, Dosage, Juveniles, Correlation, Confidence interval

INTRODUCTION

In modern times, agrochemicals have become crucial in meeting the growing demand for food by enhancing productivity and protecting crops from pests and weeds. Pesticides, which are primarily utilized for this purpose, exhibit toxicity to several non-target organisms that play a critical role in the crop and ecosystem's health. It was also found a trade-off between herbicide amounts and toxicological hazards to humans and these non-target organisms (Cech et al. 2022). These non-target organisms include beneficial insects, non-target plants, fish, and birds. While insecticides are typically the most acutely toxic pesticides, herbicides also pose a significant risk to non-target organisms. Studies have shown varying levels of toxicity from different agrochemicals on earthworm species like *Eisenia fetida* a naturalized exotic in India and other earthworm species. Fungicides like copper oxychloride (Helling et al. 2000) and mancozeb (Vermeulen et al. 2001) had moderate negative effects on growth and reproduction at recommended field doses. The herbicides acetochlor (Xiao et al. 2006) and butachlor (Gobi and Gunasekaran 2010) were found to reduce biomass and cocoon production as their concentrations increased.

Insecticides displayed a wider range of toxicity levels. Carbamates like carbofuran and organophosphates like chlorpyrifos (Reinecke and Reinecke 2007), malathion (Rai and Bansiwal 2009) and methyl parathion (Suthar 2014) endosulfan and dichlorovos (Farrukh and Ali 2011a, b) were moderately to highly toxic. Yasmin and D'Souza (2007) also evaluated the impact of pesticides on the reproductive output of *E. fetida*. Pyrethroids like deltamethrin (Shi et al. 2007) were very toxic. Neonicotinoids like imidacloprid (Wang et al. 2012), guadipyr (Wang et al. 2015a) were found to be extremely toxic - more so than current herbicides like glyphosate and pendimethalin (Anshu et al. 2020).

The banned organochlorine insecticide DDT was significantly toxic at soil concentrations over 200 mg/kg (Shi et al. 2016). Li et al. (2015) and Wang et al. (2015b) also conducted similar studies. Species like the tropical earthworm *Perionyx excavatus* proved more sensitive than standard test species (DeSilva et al. 2010). Factors like feeding niche and habitat also influenced susceptibility across earthworm species (Suthar 2014).

To assess the risk of soil pollution, eco-toxicity tests on earthworms are useful. In the present study, we evaluated the impact of the herbicide Pendimethalin on the growth and reproduction of *E. fetida*. We chose to use this chemical due to its extensive use in soybean (Kharif) crops in Kota (Rajasthan). The objective of our study was to determine the effect of this chemical on the disturbance of the eco-cycle in agricultural fields, which may lead to adverse consequences, such as the breakdown of the eco-cycle.

MATERIALS AND METHODS

The soil used in this study was obtained from an uninhabited area in Kota, India where no farming practices were carried out and no pesticides were known to have been used. The physicochemical properties of the soil were analyzed and provided by the Nanta agricultural farm, under the supervision of the Project Director for crops in the Kota district. The soil was identified as clay loam (Vertisols) and had a pH of 8.33, a conductivity of 0.291 m Mhos/cm, and an organic carbon content of 0.03%. The color of the soil was brown.

E. fetida, obtained from Krishi Vigyan Kendra in Borkhera, Kota were used in this study. Adult worms with a clitellum and weighing between 200 and 300mg were selected for all tests. The earthworms were bred in cow dung at temperatures ranging from 15-30°C, and mass cultures of *E. fetida* were established under a light cycle of 16/8 hrs. Finely ground cow dung was used as their food at intervals of 7 to 10 days. Selected worms were acclimatized in the soil of interest 24 hrs before the start of the experiment.

The test chemical used in this study was Pendimethalin, which is an herbicide belonging to the dinitroaniline class. In this study, Pendimethalin was tested as Panida 30EC (250 ml, Rallis India Ltd). It is commonly used to control annual grasses and certain broadleaf weeds by inhibiting their cell division and elongation in various crops, including wheat, barley, corn, soybeans, rice, potato, legumes, fruits, vegetables, nuts, and ornamental plants. The concentration levels used in this experiment were based on the recommended dose for Kharif crops in Kota, Rajasthan (India), as advised by the Department of Agriculture, Govt. of Rajasthan, Kota Division office. The recommended dose for Pendimethalin was 1 kg/ha.

In this study, five different concentrations of Pendimethalin were used, with a multiplicative factor of 3.167 times applied between each concentration. As a result, every alternate concentration was either 10 times higher or lower than the previous concentration. The concentrations tested were 0.474, 1.50, 4.74, 15, and 47.4 mg/kg of dry soil weight. One concentration was intentionally selected to be lower than the recommended dose, which was based on the observed strong avoidance behavior of earthworms towards a concentration of 31.6 mg/kg of Pendimethalin in this experiment (Khunteta and Singh 2017). The avoidance behavior demonstrated by the earthworms at certain concentration levels indicated the need to avoid very high doses of chemical in our experiment.

A mixture of soil and cow dung powder in a ratio of 2:1, with a dry weight of 500 gms, was used. Five sets of transparent plastic containers with a 1-liter capacity were used for the experiment. The chosen concentrations of Pendimethalin were added to the soil to create the treatments and control without the addition of Pendimethalin. Ten adult earthworms weighing between 2 to 3 gm were added to each container and monitored for four weeks for growth rate.

Before adding the earthworms were sorted out of the soil, washed with fresh water, and left on blotting paper to dry before their weight was measured using an electronic balance. Once the earthworms were added to the soil surface, they immediately burrowed themselves into the soil. Moisture content was closely monitored and maintained at 50% by adding water as needed. The amount of water added was determined by measuring the weight difference of the container compared to the weight at the time of sampling.

After 28 days, adult earthworms were removed from treated and control soil and weighed to observe the impact of biomass growth rate. In addition, the number of juvenile earthworms was also counted after eight weeks to evaluate the effect of the chemical on reproduction.

Biomass growth rate is calculated as per the following formula:

$$\text{Growth rate of earthworms} = \frac{\text{average weight after 28 days of exposure}}{\text{average weight at begining of incubation}} \times 100$$

To investigate the impact on reproduction, cocoons were left in the soil for an additional four weeks. After this period, the number of young worms in the soil was counted to determine number of juveniles produced per earthworm in our experiment.

RESULTS AND DISCUSSION

The results of the experiment indicated that Pendimethalin has a significant adverse impact on the growth rate and reproduction of *E. fetida*. The following observations summarize the results obtained, along with the range of variation for the 95% confidence interval (CI) of the mean.

Effect on growth

After 28 days, the growth rate of earthworms in the control soil was found to be 153%±20.9%. In contrast, the growth rates of earthworms in soil treated with different concentrations of Pendimethalin (0.474 mg/kg, 1.5 mg/kg, 4.74 mg/kg, 15 mg/kg, and 47.4 mg/kg) were significantly lower, with values of 38.88%±11.45%, 25.48% ±2.29%, 20.54% ±4.70%, 19.68%± 6.35%, and 10.2% ±4.99%, respectively. Figure 1 shows the graphical representation of this effect.

The growth rate was significantly reduced at concentrations of 4.74, 15, and 47.4 mg/kg. Statistical analysis revealed that the growth rate was not significant for Pendimethalin-exposed earthworms within a 95% confidence interval (CI) of the mean. The correlation value for this chemical is -0.7947, indicating an adverse effect on the growth rate (Table 1).

Our findings on growth inhibition align with several previous studies on the effects of pesticides on earthworms. Anshu et al. (2020) reported similar results due to Pendimethalin toxicity and its negative effect on survival and growth parameters of *E. fetida* over 90 days. Rai and Bansiwal (2009) found that Malathion reduced growth in *E. fetida*. Gobi and

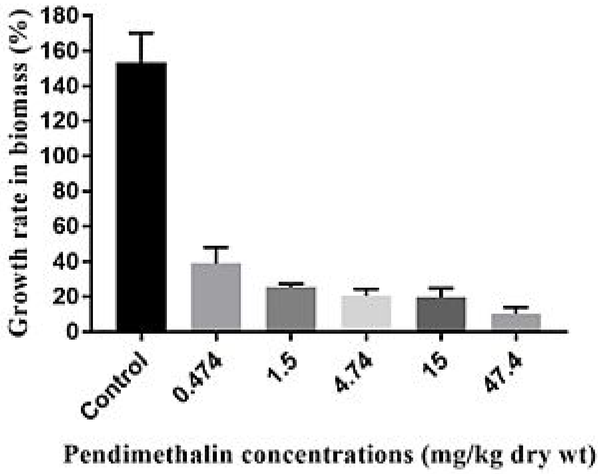

Figure 1. Growth rate for different concentrations of Pendimethalin

Gunasekaran (2010) observed decreased biomass in *E. fetida* with increasing concentrations of the herbicide Butachlor. Farrukh and Ali (2011a, b) reported that endosulfan and dichlorovos organophosphate decreased the weight of *E. fetida*. These studies collectively support our findings that pesticides, including Pendimethalin, have a negative impact on the growth of earthworms.

Effect on reproduction

The study found that after 56 days of exposure, Pendimethalin caused a sharp decline in the number of juveniles produced per earthworm compared to the control soil. The control soil had an average of 20.2 ± 3.21 juveniles per earthworm, while the concentrations of Pendimethalin at 0.474, 1.5, 4.74, 15, and 47.4 mg/kg had 5.84 ± 0.69, 4.1 ± 0.36, 3.28 ± 0.87, 2.82 ± 1.23, and 1.38 ± 0.78 juveniles per

Table 1. Effect of different concentrations of Pendimethalin on biomass growth of *E. fetida*

Chemical concentration (mg/kg)	**Mean of biomass growth (%) μ**	**Std. 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
0.474	38.88	9.218	4.122	27.43 and 50.33
1.5	25.48	1.843	0.824	23.19 and 27.77
4.74	20.54	3.787	1.694	15.84 and 25.24
15	19.68	5.117	2.289	13.33 and 26.03
47.4	10.2	3.874	1.732	5.39 and 15.01

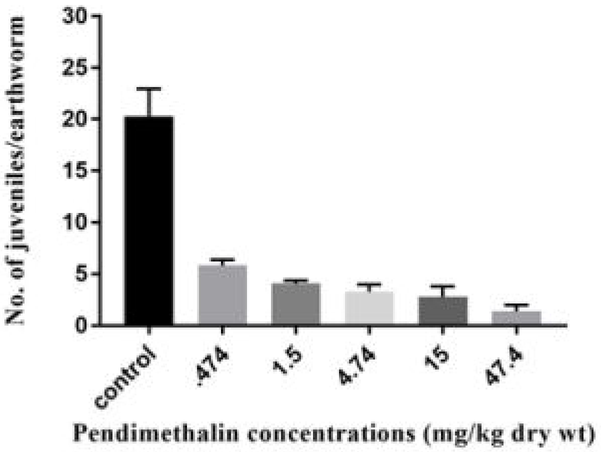

Figure 2. No. of juveniles produced per earthworm for different concentrations of Pendimethalin

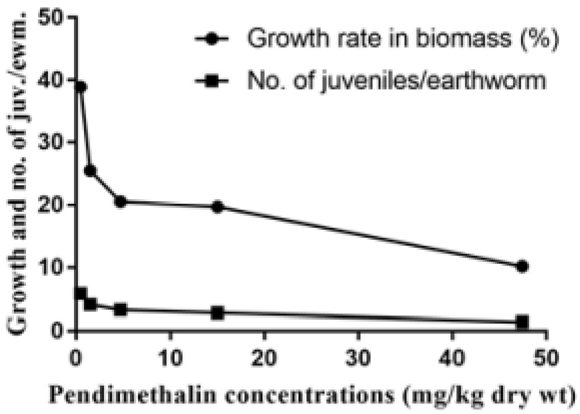

Figure 3. Correlation between growth rate and no. of juveniles produced per earthworm for different concentrations of Pendimethalin

earthworm, respectively (Fig. 2).

The number of juveniles produced per earthworm under the influence of different concentrations of Pendimethalin is not significant within a 95% CI of the mean. The correlation value between the numbers of juveniles produced per earthworm and the concentration values is 0.8403, indicating an adverse effect (Table 3). Interestingly, there was no clear dose-response relationship between the substrate concentrations and the mean number of juveniles produced per earthworm (Table 2).

Our results on reproduction effects are consistent with previous research. Anshu et al. (2020) noted negative effects on fecundity of *E. fetida* due to Pendimethalin exposure. Rai and Bansiwal (2009) observed reduced cocoon production in *E. fetida* exposed to Malathion. Gobi and Gunasekaran (2010) found decreased cocoon production in *E. fetida* with increasing Butachlor concentrations. Farrukh and Ali (2011a, b) reported that endosulfan and dichlorovos organophosphate significantly affected reproduction in *E. fetida*.

Figure 3 depicts the correlation between the growth rate of earthworms and the number of juveniles produced per earthworm for different concentrations of Pendimethalin. This correlation further emphasizes the overall negative impact of Pendimethalin on both growth and reproduction.

CONCLUSIONS

To summarize, the results of the study indicate that even at the recommended dose, Pendimethalin has a negative impact on the growth rate and reproduction of earthworms. The statistical analysis shows that

Table 2. Effect of different concentrations of Pendimethalin on reproduction of *E. fetida*

Chemical concentration (mg/kg)	**Mean no. of juvinile /earthworm** μ	**Std. deviation (SD)** s	**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
0.474	5.84	0.555	0.2482	5.151 and 6.529
1.5	4.1	0.2915	0.1304	3.738 and 4.462
4.74	3.28	0.6979	0.3121	2.413 and 4.147
15	2.82	0.9834	0.4398	1.599 and 4.041
47.4	1.38	0.6301	0.2818	0.5977 and 2.162

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

Parameter under study	Correlation value	95% CI	$R_{squared}$	P (two-tailed)	Significant (alpha= 0.05) Confidence (95%)
Concentration v/s Biomass growth (%)	-0.7947	-0.9858 to 0.2930	0.6316	0.1082	No
Concentration v/s no. of juvenile/earthworm	-0.8403	-0.9892 to 0.1622	0.7061	0.0747	No

there is a significant correlation between the concentration of Pendimethalin and the growth rate and number of juveniles produced per earthworm. These findings suggest that the use of this herbicide can harm the soil ecosystem and may have long-term implications for soil health and agricultural productivity. Therefore, it is crucial to explore alternative methods of pest control that do not have adverse effects on the soil biota. Further research is needed to determine the impact of different environmental conditions on earthworms and other soil organisms, as well as to identify the mechanisms by which Pendimethalin affects earthworms. Overall, the study highlights the need for sustainable agricultural practices that take into account the health and wellbeing of the soil ecosystem.

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Authors' contribution: AKK did all the field work, experimentation and research paper writing. AS guided during experimentation and contributed valuable suggestions in paper editing.

Conflict of interest: Authors declare that there is no conflict of interest.

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