

Assessment of Reproductive Toxicity of Malathion on the Earthworm, *Eisenia foetida* under Chronic Exposure Conditions.

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Abstract - The present study assessed the reproductive toxicity of Malathion on the earthworm *Eisenia foetida* using a 56-day chronic reproduction test. Based on a preliminary field survey in the Amravati region, Malathion was selected as a commonly used agricultural insecticide. Adult earthworms were exposed to sublethal malathion concentrations with three replicates for each treatment. Juvenile emergence was assessed after 56 days as the principal indicator of reproductive toxicity. Malathion exposure resulted in a clear concentration-dependent reduction in juvenile production. Juvenile emergence decreased from 58.00 in the control to 11.66 ± 0.58 at 30 mg/kg, while reproductive inhibition increased from 8.04% to 79.88%. ANOVA showed a highly significant effect of malathion concentration on juvenile emergence ($p < 0.001$). A very strong negative correlation was observed between malathion concentration and juvenile emergence ($r = -0.996$, $p < 0.001$), whereas reproductive inhibition showed a strong positive correlation ($r = 0.996$, $p < 0.001$). These findings indicate that malathion significantly impairs the reproductive performance of *E. foetida* and highlight the usefulness of juvenile emergence as a sensitive endpoint for assessing chronic pesticide toxicity in soil organisms.

Key Words: *Eisenia foetida*, Malathion, reproductive toxicity, juvenile emergence, reproductive inhibition, soil ecotoxicology.

1. INTRODUCTION (Size 11, Times New roman)

Earthworms are important components of soil ecosystems and play a vital role in maintaining soil structure, nutrient cycling, organic matter decomposition, and overall soil fertility. Among soil-dwelling organisms, *Eisenia foetida* (Savigny, 1826), commonly known as the red wiggler earthworm, is widely used as a model organism in soil ecotoxicological studies because of its ease of culture, short life cycle, high reproductive capacity, and sensitivity to environmental contaminants. Changes in earthworm survival, growth, biomass, and reproduction can therefore provide valuable information regarding the ecological effects of chemical contaminants present in soil.

The extensive use of synthetic pesticides in modern agriculture has contributed to increased crop productivity and effective control of agricultural pests. However, repeated and excessive application of pesticides can result in their accumulation and persistence in soil, potentially affecting non-target organisms. Soil-dwelling organisms may be exposed to pesticides through direct contact with contaminated soil, ingestion of contaminated organic matter, and absorption through the body surface. Such exposure can

interfere with physiological and biochemical processes and may ultimately affect important life-history characteristics, particularly growth and reproduction.

Reproduction is one of the most sensitive biological endpoints for assessing the chronic effects of pesticides on earthworms. Unlike mortality, which indicates an acute toxic response, reproductive impairment may occur at concentrations that do not produce immediate mortality. Exposure to pesticides may affect cocoon production, cocoon viability, hatching success, and juvenile production, thereby reducing the ability of earthworm populations to maintain themselves over successive generations. Consequently, assessment of reproductive responses provides a more ecologically relevant indication of long-term pesticide toxicity.

In *Eisenia foetida*, cocoon production and juvenile emergence are particularly useful indicators of reproductive performance. A reduction in the number of cocoons produced by exposed earthworms compared with untreated controls can indicate reproductive stress and may be used to calculate the percentage of reproduction inhibition. Such responses can reveal sublethal effects of Malathion that may remain undetected when toxicity is evaluated solely on the basis of mortality.

Therefore, the present study focuses on the assessment of reproduction inhibition in *Eisenia foetida* following exposure to different concentrations of Malathion. The study evaluates changes in reproductive performance, particularly cocoon production and related reproductive parameters, in comparison with an untreated control. The findings are expected to contribute to a better understanding of the chronic effects of Malathion on earthworms and provide useful information for evaluating the ecological risks associated with pesticide contamination of agricultural soils.

2. MATERIAL AND METHODS

Selection of pesticide

A preliminary field survey was conducted among farmers and agricultural input suppliers in the Amravati region to identify insecticides that are commonly used and readily available for crop protection. Information collected from farmers and local agricultural input outlets indicated that Malathion 50 EC is one of the commonly used insecticide formulations in the region. Based on its local availability and frequent agricultural use, Malathion 50 EC was selected as the test insecticide for the present study.

Preparation of Test Soil

Artificial soil was prepared as the exposure medium for evaluating the sublethal effects of Malathion on *Eisenia foetida*. A concentrated stock solution of Malathion was prepared using distilled water, and the required treatment concentrations were subsequently prepared from the stock

solution. Based on the previously determined LC_{50} value, preliminary range-finding observations, and the expected relative toxicity of the insecticide, seven treatment concentrations of 0, 5, 10, 15, 20, 25, and 30 mg/kg dry soil were selected for the chronic exposure experiment.

For each treatment, a predetermined quantity of dry artificial soil was accurately weighed, and the required volume of the respective malathion solution was added gradually. The solution was distributed uniformly over the soil while the soil was continuously mixed using a glass rod and mechanical blender. Thorough mixing was performed to ensure homogeneous distribution of the insecticide throughout the soil and to minimize the formation of localized areas with excessively high or low toxicant concentrations. The control treatment was prepared in the same manner but received an equivalent volume of distilled water instead of malathion solution.

Following treatment, the prepared soils were allowed to equilibrate for 24 h under controlled laboratory conditions to facilitate uniform distribution and stabilization of the insecticide within the soil matrix. After the equilibration period, the treated soils were used for the introduction of earthworm for subsequent chronic toxicity and reproductive assessment. The soil preparation and exposure procedure was conducted with reference to the principles described in OECD (1984).

Experimental Setup

The exposure concentrations were selected based on the previously determined LC_{50} value from the artificial soil toxicity test. Sub lethal concentrations were used to ensure adequate survival of adult earthworms throughout the exposure period and to allow reliable assessment of reproductive responses and inhibition.

For each treatment, 1 kg of treated artificial soil was transferred into earthen pots/tray culture containers. Ten healthy, mature, clitellated earthworms were carefully introduced into each container. Three replicates were maintained for each concentration, along with an untreated control. The containers were covered with perforated cotton cloth to provide adequate aeration while reducing moisture loss.

All experimental containers were maintained in an environmental chamber at $20 \pm 2^\circ\text{C}$ under continuous illumination, following the recommended conditions for earthworm chronic toxicity studies (OECD, 1984). Soil moisture was maintained close to the initial level by periodic addition of deionized water. A small quantity of finely powdered cattle manure was provided weekly as supplementary food to support normal physiological and reproductive activity during the exposure period (Edwards and Lofty, 1977).

The chronic reproduction experiment was conducted for 56 days. The first 28 days represented the adult exposure and cocoon-production phase, during which cocoon production was recorded weekly. The subsequent 28 days were allowed for cocoon incubation and juvenile development. At the end of Day 56, the number of newly emerged juveniles was recorded to evaluate the reproductive effects of Malathion.

Juvenile Emergence and Reproduction Inhibition

Following the removal of adult earthworms, the experimental containers were maintained for a further 28 days to allow adequate incubation of cocoons and development of juveniles. On Day 56, the soil from each container was carefully transferred onto sorting trays, and the emerged juveniles were separated and counted manually. The total number of juveniles produced was considered the principal endpoint for evaluating the reproductive effects of malathion.

A decrease in juvenile production compared with the control group was interpreted as evidence of reproductive inhibition resulting from Malathion exposure.

The percentage reproductive inhibition was calculated using the following formula:

$$\text{Reproductive Inhibition (\%)} =$$

$$\frac{\text{Number of juveniles in control} - \text{Number of juveniles in treatment}}{\text{Number of juveniles in control}} \times 100$$

100

Throughout the experimental duration, soil moisture and pH were monitored periodically to maintain stable exposure conditions. Moisture loss was compensated by addition of deionized water whenever necessary. At the termination of the experiment, final soil moisture and pH values were recorded (OECD, 1984).

Sr. No.	Malathion concentration (mg/kg)	Juvenile emergence (Mean $\pm$ SD)	Reproductive inhibition (%)
1	0 (Control)	58.00 $\pm$ 2.00	0
2	5	53.33 $\pm$ 1.53	8.04
3	10	46.33 $\pm$ 3.06	20.11
4	15	35.67 $\pm$ 2.08	38.5
5	20	26.33 $\pm$ 1.53	54.59
6	25	19.67 $\pm$ 1.15	66.09
7	30	11.67 $\pm$ 0.58	79.88

Table 1.1: Reproduction inhibition % of Earthworms exposed to different sublethal concentrations

Figure 1.1 : Effect of Malathion on Juvenile emergence in *Eisenia foetida*

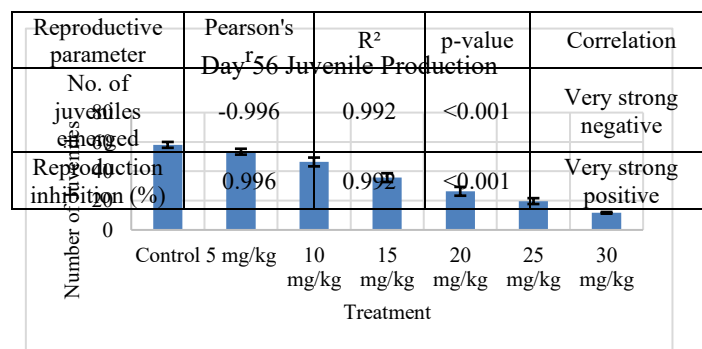


Table 1.2: Pearson correlation between Malathion concentration and juvenile emergence of *Eisenia foetida*.

The effect of Malathion exposure on the reproductive performance of *Eisenia foetida* was evaluated by determining the number of juveniles emerging from cocoons after the exposure period. The results revealed a clear concentration-dependent decline in juvenile emergence with increasing Malathion concentration. The control group recorded a mean juvenile emergence of 58.00 $\pm$ 2.00. This number progressively decreased to 53.33 $\pm$ 2.00, 46.33 $\pm$ 3.00, 35.66 $\pm$ 3.00, 26.33 $\pm$ 3.00, 19.66 $\pm$ 2.00, and 11.66 $\pm$ 0.58 juveniles at 5, 10, 15, 20, 25, and 30 mg/kg, respectively (Table 1.1 and Figure 1.1).

At the lowest concentration of 5 mg/kg, juvenile emergence showed a slight reduction to 53.33 $\pm$ 2.00 juveniles, corresponding to 8.04% reproductive inhibition compared with the control. At 10 mg/kg, juvenile production

declined further to 46.33 ± 3.00 , resulting in 20.11% reproductive inhibition. A more pronounced reduction was observed at 15 mg/kg, where the mean number of juveniles decreased to 35.66 ± 3.00 , corresponding to 38.50% reproductive inhibition. These findings indicate that increasing Malathion concentration progressively impaired juvenile production and reproductive success in *Eisenia foetida*.

At higher concentrations of Malathion, a marked reduction in juvenile emergence was observed. At 20 mg/kg, the mean number of juveniles emerged was 26.33 ± 3.00 , corresponding to 54.59% reproductive inhibition relative to the control. Juvenile emergence further declined to 19.66 ± 2.00 at 25 mg/kg, resulting in 67.81% reproductive inhibition. The maximum inhibitory effect was recorded at 30 mg/kg, where juvenile emergence decreased to 11.66 ± 0.58 , corresponding to 79.88% reproductive inhibition compared with the control.

Overall, juvenile emergence exhibited a clear concentration-dependent decline with increasing Malathion exposure. The mean number of juveniles decreased from 58.00 in the control group to 11.66 at 30 mg/kg, whereas reproductive inhibition increased progressively from 8.04% at 5 mg/kg to 79.88% at 30 mg/kg. These findings indicate that increasing Malathion exposure adversely affected reproductive output in *Eisenia foetida*, with higher concentrations causing substantially greater inhibition of juvenile production.

One-way analysis of variance (ANOVA) demonstrated a highly significant effect of Malathion concentration on juvenile emergence ($p < 0.001$). The significant F-value indicated that the number of juveniles differed significantly among the tested Malathion concentrations. The progressive decline in juvenile emergence with increasing exposure concentration further demonstrated the concentration-dependent reproductive toxicity of Malathion. The highest concentration tested (30 mg/kg) produced the greatest reduction in juvenile emergence and the highest reproductive inhibition (79.88%) relative to the control.

Pearson's correlation analysis was performed to assess the relationship between Malathion concentration and juvenile emergence in *Eisenia foetida*. A very strong and statistically significant negative correlation was observed between Malathion concentration and the number of juveniles emerged ($r = -0.996$, $R^2 = 0.992$, $p < 0.001$) as shown in table 1.2. This strong negative association indicates that juvenile production decreased consistently as Malathion concentration increased.

In contrast, reproductive inhibition showed a very strong positive correlation with Malathion concentration ($r = 0.996$, $R^2 = 0.992$, $p < 0.001$). The positive relationship indicates that reproductive inhibition increased progressively with increasing Malathion exposure. Thus, the correlation analysis supports the ANOVA findings and confirms that increasing Malathion concentration was strongly associated with reduced juvenile emergence and enhanced reproductive inhibition in *Eisenia foetida*.

DISCUSSION

The present study demonstrated that exposure to Malathion adversely affected the reproductive performance of *Eisenia foetida*, as evidenced by the concentration-dependent reduction in juvenile emergence. Juvenile production is an important indicator of reproductive fitness in earthworms and provides a sensitive measure of chronic and sublethal toxicity. In the present experiment, juvenile emergence progressively declined with increasing Malathion concentration, from 58.00

juveniles in the control group to only 11.66 ± 0.58 juveniles at 30 mg/kg. The corresponding increase in reproductive inhibition from 8.04% at 5 mg/kg to 79.88% at 30 mg/kg indicates that Malathion substantially impaired reproductive output.

The reduction in juvenile emergence at higher concentrations may be associated with the physiological stress caused by Malathion exposure. As an organophosphate insecticide, Malathion primarily interferes with cholinergic function through inhibition of acetylcholinesterase activity. Prolonged exposure may consequently disturb normal neuromuscular activity, feeding behaviour, metabolism and energy allocation. Under pesticide-induced stress, energy that would normally be directed towards growth, gamete production, cocoon formation and embryonic development may instead be diverted towards detoxification and maintenance processes. Such physiological disturbances could ultimately reduce successful reproduction and juvenile production.

The progressive decline observed at 20, 25 and 30 mg/kg suggests that reproductive impairment became increasingly severe as exposure concentration increased. At 20 mg/kg, juvenile emergence was reduced to 26.33 ± 3.00 , while at 25 mg/kg it declined further to 19.66 ± 2.00 . The lowest juvenile emergence was recorded at 30 mg/kg, indicating that the highest exposure level exerted the greatest reproductive stress. The corresponding reproductive inhibition of 54.59%, 67.81% and 79.88% at these concentrations further demonstrates the severity of the response.

The highly significant ANOVA result ($p < 0.001$) confirms that the observed differences in juvenile emergence among Malathion treatments were statistically significant. This finding supports the interpretation that the decline in juvenile production was associated with increasing Malathion exposure rather than being attributable to random variation among treatments. The concentration-dependent pattern also indicates that juvenile emergence can serve as a useful endpoint for evaluating the chronic reproductive toxicity of Malathion in *E. foetida*.

The Pearson correlation analysis provided additional evidence for this relationship. A very strong negative correlation was observed between Malathion concentration and juvenile emergence ($r = -0.996$, $R^2 = 0.992$, $p < 0.001$). The high coefficient of determination indicates that approximately 99.2% of the variation in juvenile emergence was associated with the variation in Malathion concentration within the experimental range. Conversely, reproductive inhibition exhibited a very strong positive correlation with Malathion concentration ($r = 0.996$, $R^2 = 0.992$, $p < 0.001$), demonstrating that reproductive impairment increased consistently with increasing pesticide exposure.

The observed reproductive effects may also result from indirect effects on cocoon development and embryonic viability. Even when adult earthworms survive pesticide exposure, sublethal physiological disturbances can interfere with cocoon production, fertilization, embryo development and hatching success. Therefore, the substantial reduction in juvenile emergence observed in the present study suggests that Malathion may affect multiple stages of the reproductive process rather than merely causing direct mortality of adult earthworms.

Overall, the findings indicate that reproductive endpoints were highly sensitive to Malathion exposure. The marked reduction in juvenile emergence and the corresponding increase in reproductive inhibition at higher

concentrations demonstrate that Malathion can negatively affect the reproductive potential of *E. foetida* even under conditions where the primary focus is not adult mortality. The strong concentration-response relationship observed in the present investigation highlights the importance of incorporating reproductive parameters such as cocoon production and juvenile emergence into the ecotoxicological assessment of pesticides. These results further suggest that repeated or prolonged exposure to Malathion-contaminated soil could potentially impair earthworm population sustainability and, consequently, affect the ecological functions performed by earthworms in soil ecosystems.

CONCLUSION

The present study demonstrated that Malathion exposure produced a pronounced, concentration-dependent adverse effect on the reproductive performance of *Eisenia foetida*. Juvenile emergence progressively decreased with increasing Malathion concentration, declining from 58.00 juveniles in the control group to 11.66 ± 0.58 juveniles at 30 mg/kg. Correspondingly, reproductive inhibition increased from 8.04% at 5 mg/kg to 79.88% at 30 mg/kg, indicating substantial impairment of reproductive output at higher exposure concentrations.

Statistical analysis confirmed the significance of these effects, with one-way ANOVA showing a highly significant difference among treatment groups ($p < 0.001$). Pearson correlation analysis further established a very strong negative relationship between Malathion concentration and juvenile emergence ($r = -0.996$, $R^2 = 0.992$, $p < 0.001$), while reproductive inhibition showed a very strong positive correlation with Malathion concentration ($r = 0.996$, $R^2 = 0.992$, $p < 0.001$).

Thus, the findings clearly indicate that increasing Malathion exposure significantly reduces juvenile production and reproductive success in *E. foetida*. The results emphasize that reproductive parameters, particularly juvenile emergence, are sensitive indicators of sublethal pesticide stress and should be considered in the ecological risk assessment of Malathion. Prolonged exposure to elevated concentrations of Malathion may adversely affect earthworm population renewal and, consequently, important soil ecosystem processes. Overall, the study highlights the potential ecological consequences of Malathion contamination and supports the need for its judicious use and appropriate management to minimize adverse effects on beneficial soil organisms.

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