

Assessment of the acute toxicity of agrochemicals on earthworm (*Aporrectodea caliginosa*) using filter paper contact and soil mixing tests

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Abstract. El-Aswad AF, Fouad MR, Aly MI. 2023. Assessment of the acute toxicity of agrochemicals on earthworm (*Aporrectodea caliginosa*) using filter paper contact and soil mixing tests. *Asian J Agric* 7: 14-19. Earthworms are suitable bioindicators of chemical contamination in terrestrial ecosystems. Therefore, adult earthworms (*Aporrectodea caliginosa* Savigny, 1826), which are common in Egyptian rice fields were used to study the side effects of fenitrothion and thiobencarb. Also, it was used two common Egyptian soil types; alluvial soil (clay soil) and the calcareous soil (sandy clay loam soil). Two tests were used; filter paper contact test at 24, 48 and 72 h, and the soil mixing test at 5 and 10 days. The effect of both pesticides on mortality of earthworm was insignificant at 24 h with a low mortality percentage. LC₅₀ value of fenitrothion and thiobencarb were 83.16, 288.26, 24.67, and 39.98 µg a.i mL⁻¹ at 48 and 72 h, respectively. Based on the LC₅₀ values, fenitrothion was 3.5 and 1.6 times more toxic than thiobencarb at 48 and 72 h, respectively. At 5 and 10 days after treatment, respectively, the LC₅₀ for fenitrothion in the soil mixing test decreased from 334.27 to 55.45 in clay soil and from (415.90) to (25.00) in sandy soil. Regarding to fenitrothion in soil mixing test, the LC₅₀ was reduced from 334.27 to 55.45 in clay soil and from 415.90 to 25.00 in sandy soil at 5 and 10 days after treatment, respectively. Also, the LC₅₀ of thiobencarb in clay soil was decreased from 0.93 to 0.41 and from 55.28 to 10.65 at 5 and 10 days, respectively. In general, a positive relation was recorded between the tested pesticide toxicity on earthworm and concentrations, and also exposure time. Fenitrothion was more toxic on earthworm in contact filter paper test than thiobencarb. While, in clay soil, fenitrothion was least toxic to the earthworm in soil mixing test, this could be attributed to the slow degradation in the worms and subsequently less elimination of the metabolites as well as attributed to its higher adsorption and lower desorption in soil.

Keywords: Earthworm, fenitrothion, soil, thiobencarb

INTRODUCTION

Earthworms may be the most significant members of the soil biota because they play major role in the functioning of the soil ecosystem by enhancing soil structure and the decomposition of organic materials. Despite not being numerically dominant in soils, earthworms contribute significantly to overall biomass because of their size. Earthworms play a vital function in structuring and their activities are such that they are incredibly important in sustaining soil fertility in a variety of ways. They create a bigger percentage of biomass (> 80%) of terrestrial invertebrate creatures (Yasmin and D'Souza 2010). The first person to pay attention to the function of earthworms in aerating the soil was Aristotle. In the fauna of soil-dwelling invertebrates, earthworms are more significant organisms (Fouad et al. 2023). Several studies from a long time ago have reported on their favourable effects in the agricultural soils (Badawy et al. 2013; Fouad 2021, et al. 2023).

On the other hand, earthworms are a very significant and helpful species for determining the extent of harmful substance pollution in the soil (Whitacre 2008). As a result, they can serve as appropriate bioindicators of chemical pollution in the soil in terrestrial ecosystems, giving an early warning of a decline in soil quality. It is crucial to do this to safeguard the health of the ecosystem, which is

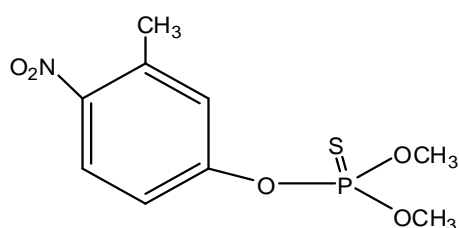
becoming increasingly critical in light of the need to safeguard human health as well as that of other terrestrial vertebrate animals that eat earthworms (Beeby 2001; Yasmin and D'Souza 2010). The primary reason earthworms are used as bioindicators of soil toxicity is that they consume a significant amount of the manure, decomposing litter, and other organic materials deposited in soil, aiding in the conversion of that material into rich topsoil. Additionally, research has revealed that an important pathway for pollution absorption occurs through the epidermis of earthworms (Lord et al. 1980), the earthworms play a crucial part in the bio-magnification process of many soil pollutants since they are a popular prey item for many terrestrial vertebrate species, including birds and small mammals. Therefore, it may be useful to investigate earthworm biomarkers for risk assessment in ecological systems (Yasmin and D'Souza 2010).

Earthworms may be killed, temporarily impaired, or have their behavior and activities inhibited by agrochemicals. Acute, expressed as (LC₅₀ or LD₅₀), and chronic toxicities of pesticides on earthworms can be described in laboratory, semi-field, or field circumstances. Additionally, there was no evidence of an influence on the median lethal values, and the time effect (LT₅₀) and concentrations are helpful in determining the negative impact. The calculation of the median lethal time can be used to identify some pesticides with chronic toxicity, such

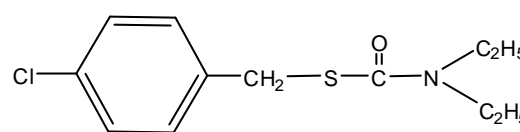
as poisonous after a prolonged exposure. While polluted soils with higher pollutant concentrations (mortality) can be easily analyzed using the acute test, those with lower pollutant concentrations (sublethal) call for more delicate test methods, such as the reproduction test in the risk assessment (Yasmin and D'Souza 2010).

The harmful affects of various pesticides, including imidacloprid, chlorpyrifos, phoxim, and lambda-cyhalothrin, on earthworms was researched individually and in combination. In the filter paper contact test, imidacloprid, lambda-cyhalothrin, and phoxim were shown to be the most toxic to earthworms, while chlorpyrifos was found to be the least hazardous. The results of the 14-day soil toxicity test revealed that chlorpyrifos and imidacloprid still had the highest levels of toxicity. Lambda-cyhalothrin was found to be comparatively less harmful, while phoxim was shown to be the least poisonous (Cang et al. 2017). Earthworms were exposed to 2,4-D in feed material and in different soil types. The toxic effect of 2,4-D on the earthworm was dose and time dependent. This compound caused 100% mortality of earthworm in soil treated with either 500 to 1000 mg/kg (Singh and Singh 2015). There were no juveniles or cocoons in glyphosate-treated soil. Nevertheless, the soil still contained living earthworms (Correia and Moreira 2010; Singh and Singh 2015).

In this paper, the acute toxicity of the two pesticides (fenitrothion and thiobencarb) commonly used on the rice crop in Egypt on earthworms is studied using the filter paper method and the method of mixing with the soil to reach the best agricultural practices while preserving the environment from pollution.



Fenitrothion



Thiobencarb

Figure 1. Chemical structures of fenitrothion and thiobencarb

Table 1. Physicochemical properties of soils

Chemical properties	Alluvial soil	Calcareous soil
Texture class	Clay	Sandy clay loam
Water holding capacity (mL/100g)	46	38
EC (m mhos/cm) at 25°C	1.32	5.03
Soil Ph	8.25	8.15
Organic matter content (%)	3.31	1.54
Total carbonate (%)	7.87	44.64
Soluble cations conc. (meq/L)	18.70	50.30
Soluble anions conc. (meq/L)	13.30	50.20

MATERIALS AND METHODS

Pesticides

Fenitrothion; UPAC name: O, O-dimethyl O-4-nitro-m-tolyl phosphorothioate, Chemical formula: $C_9H_{12}NO_5PS$, Chemical structure: Figure 1, Molar mass: $277.2 \text{ g}\cdot\text{mol}^{-1}$, Solubility: Water 0.038 g/L, Product: Technical 97.0% a.i., Production Company: Shandong Chuangying Chemical Co., Available formulations: EC 50%; ULV 100%; WP 25, 40, 50%; G 5%; D 2, 2.5, 3, 5%.

Thiobencarb; UPAC name: S-4-chlorobenzyl diethyl thiocarbamate, Chemical formula: $C_{12}H_{16}ClNOS$, Chemical structure: Figure 1, Molar mass: $257.8 \text{ g}\cdot\text{mol}^{-1}$, Solubility: Water 0.030 g/L, Product: Technical 97.0 % a.i., Production Company: Shandong SanYoung Industry Co., Ltd, Available formulations: EC 50%; G 10%.

Soils

Two types of soil were used, alluvial soil and calcareous soil from Alexandria, Egypt. Soil samples were collected from different places of the surface layer. The physical and chemical properties of the soil were estimated and presented in Table 1 (Badawy et al. 2017; Fouad and El-Aswad 2018; El-Aswad et al. 2019; Aly et al. 2021; Fouad et al. 2021; Fouad 2022, 2023).

Earthworms

The type of earthworm used in this study is one that is found abundantly in rice fields (*Aporrectodea caliginosa* Savigny, 1826). Individual worms were gathered from nearby areas in the Alexandria Governorate and raised in big plastic containers with synthetic soil. Before the studies, the earthworms were kept in the synthetic soil for 15 days (Fouad 2021, et al. 2023).

Experiments

Filter paper contact test

Following the contact toxicity protocol, filter papers were treated with pesticide in acetone (0.01, 0.1, 1, 10, 50, 75, 100, and 150 $\mu\text{g/mL}$) to yield fenitrothion and thiobencarb final concentrations of 7.5, 5, 3.75, 2.5, 0.5, 0.05, and 0.0005 $\mu\text{g/cm}^2$. After acetone evaporation, four worms were added to each replicate and three replicates to each concentration in addition to the control. The mortality rate was recorded for 24, 48 and 72 hours and the LC_{50} calculated (Fouad 2021; Fouad et al. 2023).

Soil mixing test

In a lab setting, synthetic soil was used to adapt the earthworms. Next, aqueous (35% moisture) solutions of various pesticide formulations were applied to plastic boxes (5 x 10 cm) containing 200 g of the tested soils to produce 200, 100, 10, 1.0, and 0.1 $\mu\text{g/g}$ soil. Then, ten mature earthworms that had been cleaned and aired were added to each box. After 5 and 10 days, the mortality percentage was evaluated. Additionally, using LdP line software, the LC_{50} values for fenitrothion and thiobencarb were determined (Fouad 2021; Fouad 2023).

Statistical evaluation

The Ldp line software ran a statistical analysis on experimental data that was displayed as LC_{50} (Fouad 2021; Fouad et al. 2023).

RESULTS AND DISCUSSION

Testing the toxicity of fenitrothion and thiobencarb on earthworms using filter paper

The findings of an experiment measuring the acute toxicity of fenitrothion and thiobencarb on filter paper showed that the toxicity to earthworms was highly variable (Table 2). The obtained data demonstrated that thiobencarb at a dose of $\leq 1 \mu\text{g mL}^{-1}$ caused 0% mortality at 48 h and $\leq 50 \mu\text{g mL}^{-1}$ of both tested pesticides caused 0% mortality at 24 h. The effect of both compounds fenitrothion and thiobencarb on the mortality of earthworm was insignificant with a low mortality percentage at 24 h, suggesting a very weak effect on earthworm. In addition, the concentration of 50 $\mu\text{g mL}^{-1}$ for the tested pesticides caused lower than 50% mortality. The highest tested concentration 150 $\mu\text{g mL}^{-1}$, gave only 33% and 17% at 24 h for fenitrothion and thiobencarb, respectively. Higher LC_{50} values are less toxic because greater concentrations are required to produce 50% mortality in organisms. Fenitrothion demonstrated the greatest intrinsic toxicity to the worms over at 48-h period, with an LC_{50} value of 83.16 $\mu\text{g a.i mL}^{-1}$ (Table 3). At 72-h interval, the toxicity of thiobencarb increased to the earthworms with an LC_{50}

value of 39.98 (22.88-73.61) $\mu\text{g a.i mL}^{-1}$ (0.42 $\mu\text{g cm}^{-2}$), however, fenitrothion still exhibited the highest toxicity with an LC_{50} value of 24.67 (17.95-33.90) $\mu\text{g a.i mL}^{-1}$ (0.26 $\mu\text{g cm}^{-2}$). There is a positive association between pesticide toxicity and exposure time, as evidenced by the fact that the toxicity rose as the exposure time grew. Based on the LC_{50} values, the toxicity of fenitrothion at 72 h was 3.4 times more than its toxicity at 48 h and the toxicity of thiobencarb at 72 h was 7.2 times more its toxicity at 48 h. In addition, based on the LC_{50} values, fenitrothion was 3.5 and 1.6 times more toxic than thiobencarb at 48 and 72 h, respectively. From comparing the efficacy of fenitrothion and thiobencarb according to their toxicity indices LC_{50} , it was found that fenitrothion has relative toxicity = 100, with thiobencarb has relative toxicity index of 28.85 and 61.71, compared to fenitrothion at 48 and 72 h, respectively. This is how the relative toxicity was determined;

$$\text{Relative toxicity} = \frac{\text{LC}_{50} \text{ for the most effective pesticide}}{\text{LC}_{50} \text{ for the other pesticide}} \times 100$$

The slope values did not significantly differ between the two tested pesticides. This was due to a similarity in sensitivity of the earthworm individuals to both tested pesticides. The Chi-Square values were 10.35, 65.69 for fenitrothion and 2.76, 17.82 for thiobencarb at 48 h and 72 h, respectively. The p-value ranged from 0.01 to 0.37 for two tested pesticides (Table 3).

It was reported that to calculate LC_{50} , concentration needed to cause 50% mortality after a given period of exposure (Simon-Delso et al. 2018). Therefore, the lethal toxicity and its parameters were calculated only at 48 and 72 h for tested pesticides. However, all of the fenitrothion and thiobencarb concentrations tested caused the earthworm mortality in a concentration-dependent manner. In general, according to system proposed by Roberts and Dorrough (1984), which indicated that based on the resulting LC_{50} values of earthworms that exposed to deposits of the chemicals on filter paper for 48 h. It can be stated that fenitrothion would be classified as supertoxic (LC_{50} at 48 h = 0.88 $\mu\text{g cm}^{-2}$) whereas thiobencarb would be classified as moderately toxic (LC_{50} at 48 h = 303 $\mu\text{g cm}^{-2}$). In contrast, it has been illustrated that many organophosphates such as fenitrothion, diazinon, azinphos-methyl and malathion are only slightly toxic or may be not toxic to earthworms (Griffiths et al. 1967). Several pesticides, considered only (moderately or relatively nontoxic to mammals), were extremely or very toxic to earthworms; among these compounds were cypermethrin, carbaryl, malathion and benomyl, indicating the unpredictability of pesticide toxicity to different animal species, a fact which complicates the environmental risk assessment to one or more species based on data attained with another (Roberts and Dorrough 1984).

Table 2. Toxicity of tested fenitrothion and thiobencarb (Mortality % \pm SE) on earthworm by filter paper contact test

Conc. (ppm)	Fenitrothion			Thiobencarb		
	24h	48h	72h	24h	48h	72h
0.01	0.00	0.00	0.00	0.00	0.00	8.33 \pm 8.33
0.1	0.00	0.00	8.33 \pm 8.33	0.00	0.00	16.67 \pm 8.33
1	0.00	0.00	16.67 \pm 8.33	0.00	0.00	25.00 \pm 0.00
10	0.00	8.33 \pm 8.33	25.00 \pm 14.43	0.00	8.33 \pm 8.33	33.33 \pm 8.33
50	0.00	25.00 \pm 14.43	41.67 \pm 22.05	0.00	16.67 \pm 8.33	41.67 \pm 8.33
75	8.33 \pm 8.33	41.67 \pm 8.33	50.00 \pm 14.43	8.33 \pm 8.33	25.00 \pm 14.43	50.00 \pm 14.43
100	25.00 \pm 8.33	58.33 \pm 8.33	83.33 \pm 8.33	8.33 \pm 8.33	33.33 \pm 8.33	58.33 \pm 22.05
150	33.33 \pm 8.33	75.00 \pm 14.43	91.67 \pm 8.33	16.67 \pm 8.33	41.67 \pm 8.33	75.00 \pm 14.43

Table 3. Tests on evaluated pesticides' toxicity characteristics using contact with filter paper on earthworms

Pesticide	Time (h)	LC ₅₀ ^a (μ g/mL)	Confidence limits at 95%	Slope ^b	χ^2 ^c	P	Relative toxicity ^d
Fenitrothion	48	83.16	71.20-97.16	1.81 \pm 0.04	10.35	0.306	100
	72	24.67	17.95-33.90	0.75 \pm 0.04	65.69	0.309	100
Thiobencarb	48	288.26	163.82-512.94	1.04 \pm 0.04	2.76	0.367	28.85
	72	39.98	22.88-73.61	0.41 \pm 0.00	17.82	0.007	61.71

Note: a: Concentration causing 50% mortality. Results of LC₅₀ are expressed as mean of three replicates \pm standard error (SE). b: Slope of the concentration-mortality regression line \pm SE. c: Chi square value. d: Relative toxicity = (LC₅₀ level for the most effective pesticide/LC₅₀ level for the other pesticide) \times 100

Toxicity of tested pesticides on earthworm by soil mixing test

The soil mixing test is more accurate in simulating the natural environment of earthworms. Table 4 shows the mortality percentages of earthworms exposed to fenitrothion (EC 50%) and thiobencarb (EC 50%) at concentrations of 0, 0.1, 1, 10, 100 and 200 μ g/g soil at time 5 and 10 days after treatment in clay and sandy clay loam soil. The highest mortality percentage for fenitrothion at 200 μ g/g soil, was 68.00 and 88.00 % at 10 days in clay soil and sandy clay loam soil, respectively. When the exposure time was extended, the LC₅₀ values for the effects of fenitrothion and thiobencarb on earthworms rose. At 5 and 10 days after treatment, the LC₅₀ of fenitrothion was decreased in clay soil from 334.27 to 55.45 and in sandy soil from 415.90 to 25.00. Additionally, at 5 and 10 days, respectively, the LC₅₀ of thiobencarb in clay soil reduced from 0.93 to 0.41 and from 55.28 to 10.65. The toxicity of tested pesticides was greater (lower LC₅₀) in clay soil at both time intervals than that in sandy clay loam soil, except fenitrothion at 10 days. Thiobencarb showed the highest toxicity to *A. caliginosa* at 5 and 10 days in clay and sandy soil with the lowest LC₅₀ values compared to fenitrothion which exhibited the least toxicities to the worms with the highest LC₅₀ values (Table 5). Meanwhile, fenitrothion showed a relatively less toxicity with an LC₅₀ value of 55.45 μ g/g soil for clay soil and 25.00 μ g/g soil for sandy soil. On the other hand, based on the LC₅₀ values, the toxicity of thiobencarb in clay soil at 5 days was 360 times and at 10 days was 135 times more than the toxicity of fenitrothion. In addition, thiobencarb in sandy clay loam soil was 7.5 and 2.5 times more toxic than fenitrothion at 5 and 10 days, respectively. From comparing the efficacy of the tested pesticides according to the relative toxicity,

thiobencarb has relative toxicity = 100, with fenitrothion has relative toxicity of 0.28 and 0.74 in clay soil and 13.29 and 42.60 in sandy clay loam soil compared to thiobencarb at 5 and 10 days, respectively. It should be noted that fenitrothion was least toxic to the earthworms, this could be attributed to the slow degradation in the worms and subsequently less elimination of the metabolites. Moreover, the slope value was $<$ 0.8 at all probability levels. The Chi-Square ranged from 6.62 to 32.17 for fenitrothion and from 7.91 to 51.25 for thiobencarb (Table 5).

It was reported that fenitrothion could be slightly toxic or not toxic to earthworms and it was found that thiobencarb has moderate toxicity to earthworms, its LC₅₀ is 874 μ g/mL (EC 50%) at time 14 day on earthworms. Several herbicides (acetochlor, anilofos, flutamon, pretilachlor, S-metolachlor, and terbutryn) were very toxic in contact toxicity but were low toxic in soil toxicity testing. Therefore, an ecotoxicological assessment of the effects of herbicides on earthworms should be carefully evaluated. Several workers reported that herbicides have adverse effect on the survival of earthworms, as well as its growth and reproduction (Correia and Moreira 2010). Studies conducted by Monsanto researchers reported that no adverse effects were observed when earthworms were exposed to glyphosate residues in soil at rates equal to or greater than labeled rates. Application of simazine and isoproturon have no toxic effect on earthworms (Lydy and Linck 2003; Mosleh et al. 2003). Toxicity of tested pesticides depends on different factors such as soil type (Raj and Syriac 2017), pesticide type (Roberts and Dorough 1984), tested concentrations (Martin 1982), exposure time (Singh and Singh 2015), effects by dermal exposure or ingestion and effects by contact toxicity or soil mixing toxicity (Mosleh et al. 2003; Araneda et al. 2016).

Table 4. Toxicity of fenitrothion and thiobencarb on earthworm (Mortality % \pm SE) by soil mixing test

Conc. (ppm)	Clay soil		Sandy clay loam soil	
	5 th day	10 th day	5 th day	10 th day
Fenitrothion				
0.1	6.67 \pm 6.67	20.00 \pm 11.55	0.00	13.33 \pm 6.67
1	13.33 \pm 6.67	26.67 \pm 6.67	6.67 \pm 6.67	20.00 \pm 0.00
10	20.00 \pm 11.55	33.33 \pm 13.33	13.33 \pm 6.67	26.67 \pm 6.67
100	33.33 \pm 6.67	46.67 \pm 17.64	26.67 \pm 13.33	53.33 \pm 13.33
200	55.00 \pm 6.67	68.00 \pm 6.67	48.00 \pm 6.67	88.00 \pm 6.67
Thiobencarb				
0.1	33.33 \pm 6.67	40.00 \pm 11.55	6.67 \pm 6.67	13.33 \pm 6.67
1	46.67 \pm 6.67	53.33 \pm 6.67	13.33 \pm 6.67	20.00 \pm 0.00
10	66.67 \pm 6.67	80.00 \pm 11.55	20.00 \pm 0.00	26.67 \pm 6.67
100	86.67 \pm 13.33	93.33 \pm 6.67	46.67 \pm 6.67	73.33 \pm 6.67
200	100.00 \pm 0.00	100.00 \pm 0.00	80.00 \pm 11.55	100.00 \pm 0.00

Table 5. Toxicity indices and their parameters for fenitrothion and thiobencarb on earthworm by soil mixing test

Pesticide	Soil	Time (day)	LC ₅₀ ^a (ppm)	Confidence limits at 95%	Slope ^b	χ^2 ^c	P	Relative toxicity ^d
Fenitrothion	Clay	5	334.27	133.57-900.03	0.47 \pm 0.03	6.88	0.021	0.28
		10	55.45	23.86-151.71	0.35 \pm 0.02	8.67	0.134	0.74
	Sandy	5	415.90	198.45-933.77	0.66 \pm 0.07	6.62	0.342	13.29
		10	25.00	15.41-41.53	0.59 \pm 0.04	32.17	0.130	42.60
Thiobencarb	Clay	5	0.93	0.52-1.53	0.62 \pm 0.03	14.16	0.438	100
		10	0.41	0.22-0.69	0.67 \pm 0.04	7.91	0.491	100
	Sandy	5	55.28	34.51-91.12	0.66 \pm 0.04	23.71	0.214	100
		10	10.65	7.27-15.58	0.78 \pm 0.04	51.25	0.413	100

Note: a: Concentration causing 50% mortality. Results of LC₅₀ are expressed as mean of three replicates \pm standard error (SE). b: Slope of the concentration-mortality regression line \pm SE. c: Chi square value. d: Relative toxicity = (LC₅₀ level for the most effective pesticide/LC₅₀ level for the other pesticide) \times 100

In general, the acute toxicity test has been traditionally applied to assess the toxicity of soil contamination to earthworms (Fouad 2021; Fouad et al. 2023). The use of mixed pesticides is becoming increasingly popular in agriculture owing to their high efficiency, rapid action and convenience. Results from single-pesticide experiments did not actually show field situations in which multiple pesticides or pesticide mixtures are tested (Zhou et al. 2011). Therefore, more studies on the long-term effects of insecticides on earthworms are needed for adequate environmental risk assessment. Furthermore, juvenile earthworms may be more sensitive to contaminants than adults (Zhou et al. 2008). Estimating ecotoxicological risk using toxicity data from adults and single-pesticide experiments may lead to underestimation of the effects of soil pollutants on invertebrate populations. In addition, terrestrial ecosystems may not be adequately protected by the current environmental quality criteria based on exposure to a single insecticide and significantly different from those in the realistic environment, the conditions of laboratory tests are often controlled (Piola et al. 2013; Cang et al. 2017). The toxicity assays on earthworm could be used to evaluate the toxic potential of new agrochemicals or determine toxicity in contaminated soils (Fouad 2021; et al. 2022, 2023; El-Aswad et al. 2022; Shamsan et al. 2023).

In conclusion, the results of this work showed that, the concentrations of $\leq 50 \mu\text{g mL}^{-1}$ for fenitrothion and

thiobencarb caused 0% mortality at 24 h. Also, both compounds fenitrothion and thiobencarb at all tested concentrations had a limited effect on earthworm *A. caliginosa* within 24 hours exposure ($< 50\%$ mortality). Therefore, LC₅₀ was calculated only at 48 and 72 h. A positive correlation between the tested pesticide toxicity and exposure time or concentration was recorded. Based on the LC₅₀ values, the toxicity of fenitrothion at 72 h ($0.26 \mu\text{g cm}^{-2}$) was 3.4 times more than its toxicity at 48 h ($0.88 \mu\text{g cm}^{-2}$) and the toxicity of thiobencarb at 72 h ($0.42 \mu\text{g cm}^{-2}$) was 7.2 times more its toxicity at 48 h ($3.03 \mu\text{g cm}^{-2}$). Fenitrothion was 3.5 and 1.6 times more toxic than thiobencarb at 48 and 72 h, respectively. Fenitrothion would be classified as supertoxic (LC₅₀ at 48 h = $0.88 \mu\text{g cm}^{-2}$) whereas thiobencarb would be classified as moderately toxic (LC₅₀ at 48 h = $303 \mu\text{g cm}^{-2}$). The toxicity of thiobencarb in clay soil at 5 days was 360 times and at 10 days was 135 times more than toxicity of fenitrothion. In addition, thiobencarb in sandy clay loam soil was 7.5 and 2.5 times more toxic than fenitrothion at 5 and 10 days, respectively. It should be noted that fenitrothion was least toxic to the earthworms in soil mixing test, this could be attributed to the slow degradation in the worms and subsequently less elimination of the metabolites as well as attributed to the higher adsorption and lower desorption of fenitrothion compared to thiobencarb.

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