

***In vitro* Assessment of the Food Preference and Toxicity of Five Insecticides against The Land Snail *Eobania vermiculata* (Gastropoda; Helicidae)**

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Abstract. The land snail *Eobania vermiculata* is one of the most cosmopolitan and harmful agricultural pests, causing economic devastation to many crops. For this purpose, the choice and non-choice methods was used to determine the palatability of certain plants for *E. vermiculata*. Moreover, the vulnerability of the snail was assessed via its exposure to five common insecticides (spirotetramat, sulfoxaflor, chlorantraniliprole, spinetoram and fipronil) using leaf-dipping technique under laboratory conditions. The median lethal dose was determined for each compound while biomarkers, such as enzymatic activity levels of AST, ALT, total protein TP, and lipid TL were used to evaluate sublethal effects. The findings of the no-choice feeding trial revealed that *E. vermiculata* significantly consumed a higher amount of *Lactuca scariola* var. *sativa* leaves compared to other tested plants. *Cichorium cicorea* leaves were found to be the least preferred by *E. vermiculata*, with an average of 1.71 g after 5 days. On the other hand, the results of the free choice feeding trial revealed that *L. scariola* var. *sativa* and *Brassica oleracea* leaves were the most frequently consumed by *E. vermiculata*. Conversely, *E. vermiculata* exhibited the lowest preference towards *Brassica rapa* leaves. The results of the molluscicidal activity indicated that the mortality rate is dose-dependent. After one month of exposure to a concentration of 1,000 ppm per 100 mL, chlorantraniliprole caused 46.4% mortality, followed by sulfoxaflor and fipronil, which exhibited equal mortality values of 42.9%. The latter insecticides revealed LC₅₀ of 1,010.5, 2,501.9, and 1,444.7 ppm per 100 mL against *E. vermiculata*, respectively. Nevertheless, spinetoram and spirotetramat caused a lower mortality rate for *E. vermiculata*. The biochemical analysis results showed that the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total proteins (LP), and the lipid profile of *E. vermiculata* have increased by 50% in response to the insecticides. Compared to the control and other compounds, spirotetramat increased total

cholesterol by 33 mg dL⁻¹. The activity of ALT, AST, and triglycerides decreased after the application of spinetoram and fipronil treatment, with values reaching 13 u L⁻¹, 32 u L⁻¹, and 4 mg dL⁻¹ of TL, respectively. However, no substantial effects of insecticides were observed on TP, Total cholesterol, LDH, or LP levels after the exposure period. The study's findings indicate that chlorantraniliprole, a novel insecticide group, could be a promising approach for controlling the land snail *E. vermiculata*. Unlike other, more hazardous insecticides, chlorantraniliprole has not previously been used to control snails. Furthermore, it appears to be safe for non-target organisms and mammals, making it an excellent choice for snail management.

Key words: *Eobania vermiculata*, food preferences, insecticides, mortality, biochemical analysis.

INTRODUCTION

The land snail, *Eobania vermiculata* (Müller, 1774), is a significant representative of the Helicidae land snails and has successfully expanded its range worldwide thanks to human activities. Its adaptability and resilience make it a fascinating subject of study for those interested in the impact of human influence on the environment. The species is native to the Mediterranean area but has found its way to many other countries. Land mollusks are important members of gastropods, and they cause significant damage to vegetables, field crops, ornamental plants, fruit trees, and ecosystems. Due to their high reproductive potential, nocturnal activity, and feeding habits, several species of snails and slugs are considered pests in agroecosystems worldwide, resulting in crop damage and economic losses. *Eobania vermiculata* is a well-known circum-Mediterranean land snail with a cosmopolitan distribution, affecting various crops, vegetables, orchards, and ornamental plants (Carlsson et al., 2004; Ismail, 2004; Barbara & Schembri, 2008; Cilia, 2011; Desouky & Busais 2012; Puizina et al., 2013; Colonese et al., 2014; Mienis et al., 2016; Ronsmans & Van den Neucker, 2016; Routray & De, 2016; Ali, 2017; Chellat et al., 2018; Abd El-Atti et al., 2020; Camilleri et al., 2021; Cheriti et al., 2021; Das et al., 2020; Dedov et al., 2022; Bayoumi et al., 2023; Racevičiūtė-Stupelienė et al., 2023 and Bronne & Delcourt, 2024).

Despite the generalist nature of the majority of land snails, they are capable of exhibiting temporal and spatial variation in their dietary preferences (Ampuero et al., 2023). Several studies have been conducted on the dietary preferences and patterns of various land snails. For example, carrots, lettuce, and cucumber were the most preferred and consumed by land snails such as, *Thaba pisana*, and *E. vermiculata* (Keshta et al., 2006). In addition, Shoieb (2008) demonstrated that ornamental plants in public gardens in Port Said, Egypt, have been infested with land snails, specifically *E. vermiculata*. Moreover, Al-Akraa et al. (2010) stated that *Peganum* and *Hisbicus* leaves were the most consumed by *Eobania vermiculata* over the experimental course of five days. Furthermore, Mohamed (2016) demonstrated that cabbage and lettuce were more preferable to land snails such as *Monacha cartusiana* and *Helicella vestalis* in comparison to other plants. Furthermore, Valarmathi (2017) demonstrated that among the twelve food materials presented, land snails *Cryptozonia bistrialis* exhibited a strong preference for carrots, cabbage, cucumber, and chow chow. Additionally, Soha et al. (2020) displayed that *E. vermiculata* and *Monacha obstructa* preferred untreated lettuce leaves compared to treated plant leaves. Also, Bashandy & Awwad (2022) indicated that in the non-choice method, the leaves of cabbage and lettuce had the most palatability.

However, in the free-choice methods, land snails, *E. vermiculata* showed a preference for berseem leaves as their preferred food.

The use of synthetic pesticides or specific molluscicides is the most common method for regulating terrestrial gastropods (Radwan et al., 1992; El-Wakil & Attia, 1999; Moran et al., 2004; El-Shahaat et al., 2005; El-Shahaat et al., 2009; Eshra, 2014). Several studies have also been conducted to detect the molluscicidal effects of common molluscicides such as metaldehyde and methiocarb, which are applied as baits (Miller et al., 1988; Radwan, 1993). Unfortunately, because these compounds are hazardous, they cause toxicity in non-target organisms as well as harmful long-term effects on the ecosystem (Kenko & Ngameni, 2022; Kenko et al., 2022; Kenko Nkontcheu et al., 2023; Kenko et al., 2024).

Safe pesticides and molluscicides with distinct modes of action are critical. For instance, spirotetramat (Movento 10% SC), a spirocyclic tetramic acid derivative, is a fully systemic insecticide for sucking pests (Bretschneider et al., 2007). Spirotetramat is a lipid biosynthesis inhibitor (Nauen et al., 2006). Its mode of action leads to a reduction in the fecundity, fertility, and insect populations at all stages of pests. Furthermore, since its approval as an insecticide in 2010, sulfoxaflor (Transform 50%WG) has been used to safeguard a range of crops from a number of insect pests (Rossaro et al., 2018). Babcock et al. (2011) assert that sulfoxaflor's high efficacy and minimal cross-resistance with other pesticides make it an excellent substitute for some neonicotinoids, if not an improvement over them. Many authors have reevaluated the threats connected with sulfoxaflor to people, the environment, and non-target animals. They have stated that sulfoxaflor poses a major threat to honeybees and bumblebees but is only mildly hazardous to humans, birds, and most aquatic organisms (Bishop, 2015; Pan et al., 2017; Centner et al., 2018; Chakrabarti et al., 2020; Al Naggar & Paxton, 2021; Li et al., 2021; Bellisai et al., 2022; El-Din et al., 2022; Mundy-Heisz et al., 2022). With a license to control insects in a range of crops, the active ingredient of Coragen 20% SC, a new insecticide, is chlorantraniliprole (Kar et al., 2013; Bacca et al., 2021). According to Noha & Meligi (2019), Coragen, an insecticide, causes biochemical and histological changes in some important organs in male albino rats.

Spinetoram (Radiant 12% SC) is an insecticide belonging to the spinosyn category, known for its extended residual activity. It acts swiftly on the nervous system of insects through both contact and ingestion (Thompson et al., 2000). Spinosyns, a novel class of insecticides, exhibit high efficacy with minimal environmental impact. Their mode of action involves the breakdown of nicotinic acetylcholine receptors (Kirst, 2010). Spinetoram, which is chemically related to spinosad, a safe pesticide used in organic farming, effectively targets insects at low application rates while sparing beneficial insects. Its mechanism of action involves consistent stimulation of insect nicotinic acetylcholine receptors (Anonymous, 2014).

Fipronil is one of the highly effective pesticides that fall under the category of a new type of pesticide called phenylpyrazole pesticides. It functions by blocking GABA-gated chloride channels and glutamate-gated chloride (GluCl) channels in the target organism (Raymond et al., 2005). Under controlled laboratory conditions, the toxicity of fipronil against the land snail *E. vermiculata* was found to be greater than its toxicity against the land snails *Theba pisana* and *Helicella vestalis*, according to a study by Eshra et al. (2016). The treatment had the highest impact on the proportion of eggs hatched in adult

E. vermiculata snails when compared to snails that were not treated (Hussein & Sabry, 2019).

Considering the wide range of effects these chemicals have on pest control and the limited information available on their impact on sub-lethal biochemical markers in land snails, such as *E. vermiculata*, and the consumption of plants by these snails, we can conclude that there is a lack of research in these specific areas. Therefore, this study aimed to investigate the feeding behaviour of the chocolate-band snail, *E. vermiculata*, and its control under laboratory conditions. The study will first evaluate the palatability of certain plant leaves to the chocolate-band snail, *E. vermiculata*, using two methods: free-choice and no-choice. Second, evaluate the molluscicidal efficacy of five insecticides belonging to the Tetramic acid, Sulfoximine, Anthranilic diamide, Spinosad, and Phenylpyrazole classes against the land snail *E. vermiculata*. Furthermore, examine the impact of low concentrations of these pesticides, when sprayed on the leaves and dipped in, on the functioning of five crucial enzymes in these terrestrial snails.

MATERIALS AND METHODS

Chemicals

The insecticides used in this study (Table 1), were provided by the Central Agricultural Pesticide Laboratory (CAPL) in Dokii, Egypt.

Table 1. Displays the insecticides investigated in this study against land snails *E. vermiculata*

Trade name	Active ingredient	Chemical group	Rate of application	Manufacturer / Year
Movento 10% SC	Spirotetramat	Tetramic acid	40 mL per 100 L	Bayer AG, German, 2020
Transform 50% WG	Sulfoxaflor	Sulfoximine	125 g per 200 L	Dow AgroSciences, UK, 2021
Coragen 20% SC	Chlorantraniliprole	Anthranilic diamide	60 mL per 200 L	FMC Corporation, France, 2020
Radiant 12% SC	Spinetoram	Spinosad	10, 15 and 20 mL per feddan	Corteva Agriscience, USA, 2021
Fipronil 5%	Fipronil	Phenylpyrazole	500 mL per 100 L	MAC-GmbH, Germany, 2021

Collection of Land Snails *E. vermiculata*

Adult snails of *E. vermiculata* (Müller, 1774) (20.4 ± 1.1 mm in shell diameter and 2.4 ± 0.1 g in body weight) were hand-collected from the field of Citrus lemon trees in March 2022 from Beheira Governorate ($30^{\circ}30'43.9''N$, $30^{\circ}17'38.9''E$) (Bashandy, 2018; Hussein & Sabry, 2019). The snails were then placed in transparent sacks and transferred to the research center of the Zoology Agricultural and Nematology Department, Faculty of Agriculture, Al-Azhar University. After being washed with freshwater, the collected snails were kept in a glass cage ($60 \times 40 \times 30$ cm) with 100 individuals per cage. The cage was filled with a mixture of sterilized clay and sand in a 1:1 ratio. The snails were fed lettuce leaves for 15 days under laboratory conditions at RH $60\% \pm 5$ and a temperature of 22 ± 2 °C (Shetaia, 2005; Bashandy & Raddy, 2021).

Assessment of Feed Preferences of Land Snails *E. vermiculata*

No-choice Feeding for Land Snail *E. vermiculata*

Three plastic cups (10×14 cm) filled with blended soil to a depth of 5cm and with 60% moisture were used for the animals. Three replicates were used for each treatment. Each cup contained 10 healthy adult snails. A Known weight of plant leaves (Table 2) was given daily to *E. vermiculata*. The consumption of foodstuff by *E. vermiculata* was accounted daily for five days following the method previously published (Al-Akraa et al., 2010; Mohamed, 2016; Bashandy, 2018).

Free Choice Feeding for Land Snail *E. vermiculata*

Cabbage, Cos lettuce, Komatsuna, Chicory, Milky tassel, and London rocket leaves were used (Table 1). Three wooden boxes (50×40×20 cm) each were filled with mixed soil to a depth of 10 cm and maintained at 60% moisture content. Each box included 30 animals, which were placed in the center, and known weights of fresh leaf samples from each plant were placed around the snails on the box's sides. To eliminate partiality for a certain place, the food ingredients and their sides were changed regularly (Mohamed, Ghade, 2016). After being weighed, the leaf samples were replaced every day. For five days, the adjusted weight losses caused by *E. vermiculata* feeding were estimated.

Table 2. The plants used for feed preferences of land snails *E. vermiculata*

Family	Common name	Scientific name
Asteraceae	Chicory	<i>Cichorium cicorea Dumort.</i>
	Milky tassel	<i>Sonchus ciliatus Lam.</i>
	Cos lettuce	<i>Lactuca scariola var. sativa</i>
Brassicaceae	London rocket	<i>Arabis charbonnelii</i>
	Komatsuna	<i>Brassica rapa</i>
	Cabbage	<i>Brassica oleracea</i>

Determination of molluscicidal activity of five insecticides against land snail *E. vermiculata*

Pesticides evaluation experiments

Five insecticides were evaluated for their toxicity against adults of land snails (*E. vermiculata*) under laboratory conditions at 22 ± 2 °C and RH 60 ± 5 (Table 1). A series of seven concentrations of each compound (5, 10, 20, 50, 100, 300, and 1,000 ppm per 100 mL) was prepared by mixing an appropriate amount of each compound with one drop of Tween 80 and one drop of DMSO until the compounds became completely soluble. This was followed by the addition of the appropriate volume of water to create a homogeneous suspension (Gad et al., 2023). An appropriate size of lettuce leaf was dipped in each concentration and left for a minute, then taken out and left to dry, then served to snails. Ten snails were placed in a plastic box (15×10 cm) and supplied with a disc of lettuce. For each treatment, three replicates of ten land snails each were used. The boxes were covered with a muslin cloth and secured with a rubber band to prevent the snails from escaping. The remaining three replicates were provided with lettuce leaves immersed in water as a control). The plastic boxes were checked daily for a month. When needed, treated lettuce leaves were changed, and untreated animals were sprayed with only water to provide appropriate humidity. A lack of contraction indicated death, and dead snails were recorded and removed immediately.

Biochemical Analyses

The process of collecting and preparing tissues for sub-lethal biochemical tests

To investigate the effects of the five mentioned insecticides on *E. vermiculata* snails, their biochemical changes were examined by exposing them to a sub-lethal level of LC₅₀, as listed in Table 5, for seven days in a separate experiment. Each treatment involved triplicates, with each replicate consisting of ten snails. After seven days of treatment, the soft tissues of *E. vermiculata* snails were extracted from their shell and homogenized for one minute in 10 volumes (W/V) of 0.1 M phosphate buffer at PH 7.4 using a glass homogenizer. The homogenates were then centrifuged for 20 minutes at 1,000 xg in a cooling centrifuge (5417R) set to 4 °C. The supernatants were kept in a -20 °C freezer until they were used to determine the activities of alanine aminotransaminase (ALT), aspartate amino transaminase (AST), lactate dehydrogenase (LDH), total protein (TP), and total lipid (TL). The supernatant was employed as an enzyme substrate (Laila & Genena, 2011; Bislimi et al., 2013; Banaee et al., 2019).

Enzymatic and biochemical measurements

Enzymatic measurements

The activities of AST and ALT were determined according to Reitman and Frankel's method (1957). The enzyme activities were reported in Units L⁻¹. Lactate dehydrogenase (LDH) was determined using the colorimetric method described by Cabaud & Wroblewski (1958).

Biochemical measurements

The protein content (TP) was determined using Bradford's technique (1976). Total lipids (TL) were estimated according to Knight et al. (1972). All the biochemical measurements used in this study were based on the methodology established by Radwan et al. (2008), specifically for snails.

Statistical analysis

The data were subjected to the One-Sample Kolmogorov-Smirnov Test using the SPSS program (version 20). The corrected mortality for land snails due to lethal toxicity was computed using the Abbott formula (1925), using Ldp line computer software (Bakr, 2005). The sub-lethal of the tested compound's LC₅₀ values (LC₂₅), 95% confidence limits, and slope for the interval were calculated using Probit analysis as described by Finney (1971). Data were expressed as the mean ± standard error (SE). The data on food palatability and biochemical were analyzed by one-way ANOVA (Duncan's test) at a significance level of $p \leq 0.05$ using CoStat computer software, version 2.6 CoStat program (2002).

RESULTS

Feed preferences of land snails *E. vermiculata*

No-choice feeding for land snail *E. vermiculata*

Data in Table 3 indicated that leaves of Cos lettuce were the most preferred by *E. vermiculata*, with an average consumption of 4.8 ± 0.1 g. Furthermore, cabbage, komatsuna, and milky tassel were also preferred by *E. vermiculata* with consumption averages of 4.0 ± 0.1 , 3.3 ± 0.1 , and 3.5 ± 0.1 g after 5 days, respectively. On the contrary,

London rocket was moderately palatable with an average consumption of 2.5 ± 0.1 g, and chicory had the lowest palatable with an average consumption of 1.7 ± 0.0 g.

Table 3. Shows the *in vitro* no-choice consuming rate of the land snail *E. vermiculata* to fresh foliage of six plant species

Plants	Consuming rate/day					Mean \pm SE
	1 st	2 nd	3 rd	4 Th	5 Th	
Cabbage (<i>Brassica oleracea</i>)	3.9	3.9	4.5	3.8	3.9	4.0 ± 0.1^b
Cos lettuce (<i>Lactuca sativa</i>)	4.7	4.9	4.6	4.9	4.9	4.8 ± 0.1^a
Komatsuna (<i>Brassica rapa</i>)	3.1	3.3	3.4	3.3	3.2	3.3 ± 0.1^c
Chicory (<i>Cichorium cicorea</i>)	1.7	1.8	1.7	1.7	1.7	1.7 ± 0.0^e
Milky tassel (<i>Sonchus ciliates</i>)	4.0	3.6	3.3	3.3	3.3	3.5 ± 0.1^c
London rocket (<i>Arabis charbonnelii</i>)	2.7	2.8	2.4	2.4	2.4	2.5 ± 0.1^d

Means with the same letter are not significantly different ($p < 0.05$) according to Duncan's multiply range test at $0.05 = 0.26$, \pm SE = standard error ($n = 5$), and (30 land snails in three replicates).

Free choice feeding for land snail *E. vermiculata*

According to the statistically analyzed results of the data in Table 4, there was a significant difference ($p < 0.05$) in the average weight consumption of land snails *E. vermiculata*. Cos lettuce and cabbage were the most prevalently consumed, with values reaching 3.8 ± 0.2 and 3.4 ± 0.2 g, respectively. Chicory also exhibited a similar consumption rate, with an average consumption of 3.6 ± 0.1 g. Furthermore, there were no significant differences ($p < 0.05$) in the average intake of weight leaves between milky tassel and London rocket for land snails. On the other hand, komatsuna was the lowest consumed by snails during the 5-day experimental course, with an average amount of 1.6 ± 0.1 g.

Table 4. Shows the *in vitro* free-choice consuming rate of the land snail *E. vermiculata* on fresh foliage from six plant species

Plants	Consuming rate/day					Mean \pm SE
	1 st	2 nd	3 rd	4 Th	5 Th	
Cabbage (<i>Brassica oleracea</i>)	3.3	3.3	3.4	4.0	3.0	3.4 ± 0.2^b
Cos lettuce (<i>Lactuca sativa</i>)	4.7	3.5	3.8	3.7	3.5	3.8 ± 0.2^a
Komatsuna (<i>Brassica rapa</i>)	1.7	1.5	1.5	1.9	1.5	1.6 ± 0.1^d
Chicory (<i>Cichorium cicorea</i>)	3.5	3.5	3.7	3.8	3.7	3.6 ± 0.1^{ab}
Milky tassel (<i>Sonchus ciliates</i>)	2.4	2.4	2.8	2.3	2.4	2.5 ± 0.1^c
London rocket (<i>Arabis charbonnelii</i>)	2.1	2.9	2.3	2.34	2.6	2.5 ± 0.1^c

Means with the same letter are not significantly different ($p < 0.05$) according to Duncan's multiply range test at $0.05 = 0.39$, \pm SE = standard error ($n = 5$), and (30 land snails in three replicates).

Determination of molluscicidal activity of the five insecticides

The molluscicidal activity of five insecticides, when applied as lettuce leaf poison bait against *E. vermiculata*, is shown in Table 5. The data indicates that the mortality percentage increases with concentration and exposure period. From Table 5 and Figs 1 and 2, it was evident that the tested pesticides spirotetramat, sulfoxaflor, chlorantraniliprole, and spinetoram showed no lethal effect against the land snail *E. vermiculata* in the first six days of the trial. However, after three days, sulfoxaflor and fipronil at a concentration of 5 ppm exhibited mortality percentages of 7.1% and 10.7%,

respectively, against *E. vermiculata*. Meanwhile, other insecticides displayed a low mortality rate at the same concentration. One week later, the mortality percentage increased gradually for the tested insecticides. After one month of exposure to 1,000 ppm, chlorantraniliprole significantly overwhelmed other pesticides and showed a mortality of 46.4% with an LC₅₀ 1,010.5 ppm per 100 L. However, as the time elapsed to thirty days, sulfoxaflor and fipronil showed a gradual increase in the cumulative mortality percentage and exhibited a mortality percentage of 42.9% against *E. vermiculata* with lethal concentrations of 2,501.9 and 1,444.7 ppm per 100 mL, respectively.

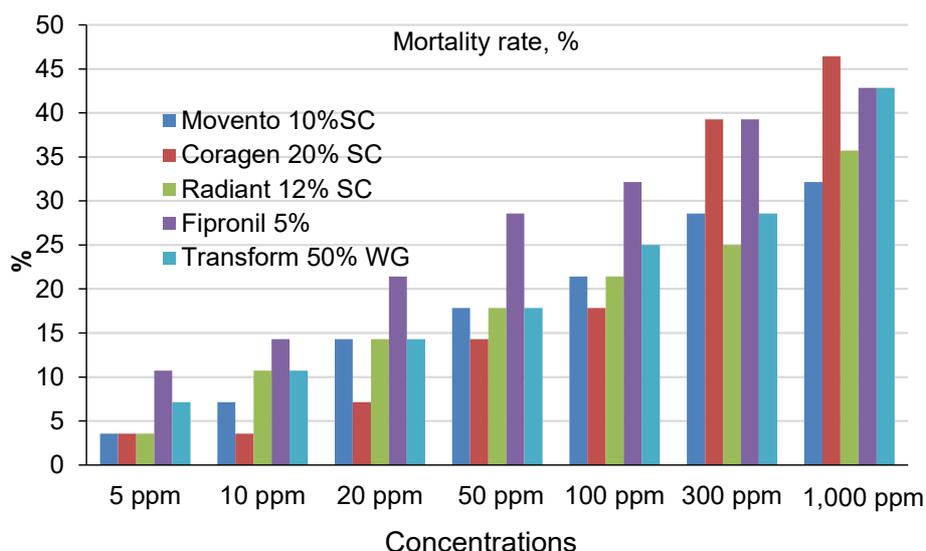


Figure 1. Mortality percentage of land snail, *E. vermiculata* by five insecticides after one month under laboratory conditions.

Table 5. Molluscicidal activity (LC₅₀ and LC₂₅) of five insecticides against *E. vermiculata* under laboratory conditions

Insecticides	LC ₅₀	Con. L.		Index	R	Slope ± SE	LC ₂₅	Con. L.	
		1	2					1	2
Chlorantraniliprole	1,010	465	4,211	100	1	0.83 ± 0.15	156.7	85.9	313.3
Fipronil	1,444	389	65,185	69.9	1.4	0.46 ± 0.13	47.9	12.7	129.3
Sulfoxaflor	2,502	649	96,843	40.4	2.5	0.53 ± 0.14	130.3	53.0	432.6
Spirotetramat	4,857	979	654,834.47	20.8	4.8	0.51 ± 0.14	228.0	89.7	127.2
Spinetoram	5,244	985	1,147,465	19.3	5.2	0.49 ± 0.19	214.1	81.5	1,292.8

The index compared with Chlorantraniliprole; Resistance Ratio (RR) compared with Chlorantraniliprole; Con. L. (Confidence limit), (1) Lower limit, (2) Upper limit, SE (standard error).

Furthermore, at the end of the trial, spinetoram and spirotetramat exhibited lower mortality of 35.71% and 32.14%, respectively, with LC₅₀ values of 5,244.45 and 4,857.20 ppm per 100 mL. No mortality was recorded during the experiment for snails fed on untreated control lettuce leaves. Therefore, the investigated insecticides can be arranged in descending order according to their mortality percentages as follows: chlorantraniliprole > sulfoxaflor > fipronil > spinetoram > spirotetramat.

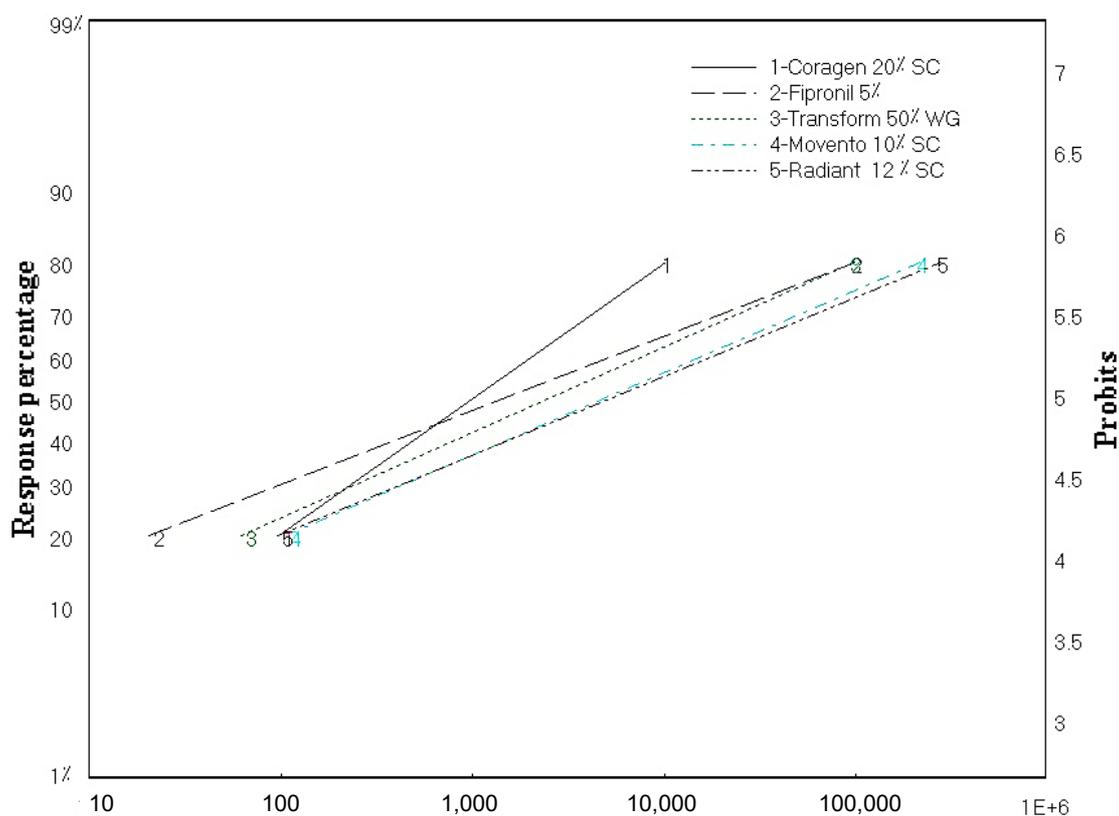


Figure 2. Probit regression lines representing the effect of insecticides leaf dipping against terrestrial snail, *E. vermiculata*.

Biochemical evaluation

The data in Table 6 show the enzymatic activity levels of AST, ALT, total protein TP, and lipid TL in the land snail *E. vermiculata* in response to five insecticides after seven days of exposure. When compared to other pesticides and controls, data showed that fipronil and sulfoxaflor increased ALT activity, with values reaching 120 u L^{-1} and 108 u L^{-1} , respectively. The *E. vermiculata* treated with spinetoram exhibited the lowest ALT value of 13 u L^{-1} . On the other hand, Spirotetramat and chlorantraniliprole revealed similar results, with control levels of 43 and 38 u L^{-1} , respectively. Radiant and spirotetramat significantly decreased AST activity on exposure days, with values 32 u L^{-1} and 73 u L^{-1} , respectively. On the other hand, Sulfoxaflor, fipronil, and chlorantraniliprole showed an increase in enzyme levels after 7 days of treatment, with values of 407 u L^{-1} , 333 u L^{-1} , and 164 u L^{-1} , respectively. When compared to untreated snails over the same period, these results indicated the least effectiveness on the activity of the AST enzyme. The data demonstrated that there was no significant difference ($p < 0.05$) among snails treated with insecticides and the control in terms of total protein levels. Furthermore, *E. vermiculata* treated with spinetoram, sulfoxaflor, spirotetramat, and chlorantraniliprole exhibited higher amounts of triglycerides: $4,728$, $2,064$, 798 , and 271 mg dL^{-1} , respectively. On the contrary, fipronil reduced the number of triglycerides to 4 mg dL^{-1} in total lipids. Seven days of spirotetramat exposure resulted in the largest rise in total cholesterol levels of the lipid profile, with an increase of 33 mg dL^{-1} compared to the

control (3 mg dL⁻¹) and other pesticides (1 mg dL⁻¹). There is no significant difference among chlorantraniliprole, spinetoram, fipronil, and sulfoxaflor in terms of total cholesterol. The data demonstrated that there was no significant difference between the pesticides and the control group in terms of LDH levels in the lipid profile after the exposure period.

Table 6. Shows the effect of LC₂₅ of five different pesticides on enzymatic levels in *E. vermiculata* tissues exposed to 5 ppm for seven days

Insecticides	ALT (u L ⁻¹)	AST (u L ⁻¹)	Total protein (g dL ⁻¹)	Lipid profile		LDH (u L ⁻¹)
				Total cholesterol (mg dL ⁻¹)	Triglycerides (mg dL ⁻¹)	
Spirotetramat	43 ^c	73 ^c	0.2 ^a	33 ^a	798 ^c	2 ^a
Chlorantraniliprole	38 ^d	164 ^d	0.3 ^a	1 ^c	271 ^d	1 ^a
Spinetoram	13 ^e	32 ^f	0.3 ^a	1 ^c	4,728 ^a	1 ^a
Fipronil	120 ^a	333 ^c	0.3 ^a	1 ^c	4 ^f	1 ^a
Sulfoxaflor	108 ^b	407 ^b	0.3 ^a	1 ^c	2,064 ^b	2 ^a
Control	37 ^d	448 ^a	0.2 ^a	3 ^b	6 ^e	2 ^a
<i>LSD</i> 0.05	1.97	1.78	0.178	1.03	1.78	1.26

Means with the same letter are not significantly different ($p < 0.05$) according to Duncan's multiply range test.

Accordingly, fipronil and sulfoxaflor increased the activity of ALT, while sulfoxaflor, fipronil, and chlorantraniliprole were found to increase the levels of the enzyme AST after 7 days of treatment. Spinetoram resulted in the lowest values of ALT and AST. Additionally, fipronil caused a reduction in triglycerides in the TL. However, there is no significant difference observed between the pesticides and the control group in terms of the levels of total protein, Total cholesterol, and LDH in the lipid profile after the exposure period.

DISCUSSION

Snails are polyphagous and feed on a variety of plant materials, such as leaves and fruits, as well as on decaying organic matter (Albuquerque et al., 2008; Ademolu et al., 2011). Feed preference studies (Iglesias & Castillejo, 1999; Chevalier et al., 2000; Chevalier et al., 2003; Ebenso & Adeyemo, 2011; Mohamed, 2016; Bashandy & Awwad, 2022) have demonstrated the capacity of snails to choose their food when given free choice feeding and to retain memories of preferred feeds. Furthermore, Ogbu et al. (2014) showed that the different species of land snails have preferences for different feedstuffs and exhibit differences in feeding behavior.

In our study, the results showed significant ($p < 0.05$) differences in the preference of different feedstuff by land snails *E. vermiculata*. The most appetent feedstuffs consumed were cos lettuce, followed by cabbage. Similarly, Arafa (1997) reported the mean daily consumption (mg/snail) of *Eobania* sp. over 7 days, with lettuce, sweet peas, cabbage, and nursery rocket being consumed at rates of 1.1, 1.2, 1.4, 1.3, 1.5, 1.4, and 1.9 mg/snail, respectively. Similar to our findings, Abd El-Hak (1997) discovered that *Eabania* sp. favored new lettuce leaves, followed by peas and cabbage. Furthermore, the detailed study of Eshra (1997) supported our results, with lettuce leaves being the most preferred, followed by cabbage leaves. On the other hand, the fruits of carrot and squash

were found to be the least favored. Additionally, Giant African land snails, such as *Archachatina marginata*, consume various vegetable plants including cabbage, pawpaw, pineapples, nuts, cherry, flowers, and potatoes (Okafor, 2001). On the other hand, Mahrous et al. (2002) found that *Monacha cartusiana* snails preferred cabbage and lettuce in larger quantities, while pepper, pea, and tomatoes were the least preferred. Additionally, giant African land snails, such as *A. marginata*, have been observed to favour approximately 500 different types of plants, including peanuts, beans, peas, cucumbers, and melons (Akintomide, 2004). Furthermore, Okonta, (2012) observed that *A. marginata* snails consumed a higher amount of palm fruits (*Elaeis guineensis*) compared to *Ipomea babatas* leaves. Additionally, the results of Mohamed-Ghada (2004, 2016) exhibited that cabbage and lettuce were the most palatable options for land snails, specifically *Monacha cartusiana*, and *Helicella vestalis*. with rates of 63.3% and 57.9%, respectively, for *Monacha cartusiana*, and 67.3% and 42.9%, respectively, for *Helicella vestalis*. Furthermore, the high consumption of two plants was (34.8, 41.5) and (30.9, 33.3) for two snails, respectively. Additionally, the study of Asran et al. (2016) revealed that *E. vermiculata* preferred lettuce, followed by squash, carrots, and potatoes. In contrast, Shoeib (1997) observed that *E. vermiculata* consumed more Dahlia leaves compared to cabbage and lettuce. Also, in the feed preference test by Nakhla & Tadros (1995), *E. vermiculata* showed a strong preference for banana plants. Moreover, Bashandy (2018) observed that cabbage was the most palatable, with an average consumption rate of 0.552 g, while London rocket and Snow thistle had the lowest consumption rates for the march slug, *Deroceras leave*, at 0.244 g and 0.215 g, respectively. Furthermore, according to Bashandy & Awwad (2022), in the non-choice method, cabbage and lettuce leaves were found to have the highest palatability, with consumption rates of 5.17 g and 3.76 g, respectively. However, in the free-choice method, berseem leaves had the highest food preference among land snails, *E. vermiculata*, with a consumption rate of 4.27 g over a period of five days.

The chemical control of *E. vermiculata* land snails through the application of pesticides is still the most effective approach, particularly over large areas (Asif, 2018). This study elucidated the efficacy of six insecticides at series concentrations in controlling *E. vermiculata* under laboratory conditions. Our findings revealed that chlorantraniliprole caused higher mortality compared to other chemicals used to control *E. vermiculata*. The results are similar to Liu et al. (2017), as they exhibited high toxicity levels of chlorantraniliprole to *Helicoverpa armigera* moths, resulting in a mortality of 86.67% during 24 h period at the concentration of 1 mg a.i. L⁻¹. Chlorantraniliprole, a key anthranilic diamide, is a novel chemical insecticide that has been reported as the most effective compound for controlling lepidopteran pests (Carscallen et al., 2019). It can induce feeding cessation and muscle paralysis, resulting in death by binding with ryanodine and promoting calcium release (Plata-Rueda et al., 2019). Moreover, sulfoxaflor and fipronil caused the death of less than 50 percent of land snails for one month. But Eshra et al. (2016) reported that fipronil had the highest toxicity against *E. vermiculata*, and mortality was 82.99–91.20% after 96 hrs. Also, Hussein & Sabry (2019) showed that the recommended field rate of Fipronil was very effective against the eggs of *E. vermiculata* at 22.7% compared with 96.3% in the control group. In this trial, spinetoram and spirotetramat had the lowest efficacy in terms of mortality for *E. vermiculata* snails. However, Sabry & Hussein (2022) demonstrated that spirotetramat in both conventional and nano formulations, revealed 100% and 53%

mortality with LC₅₀ values of 7.7 and 35% against *E. vermiculata*. Furthermore, Al Naggar & Paxton (2021) and Chakrabarti et al. (2020) reported that sulfoxaflor is moderately toxic to mammals and birds and slightly toxic to most aquatic species, but it poses a high risk to honeybees and bumblebees when they come into contact with spray droplets shortly after application. Some studies have reported the high toxicity of sulfoxaflor to bees. Chlorantraniliprole is an anthranilic diamide insecticide that exhibits a high degree of specificity towards insect ryanodine receptors (RyRs), which play a crucial role in insect muscle contraction (Lahm et al., 2019). According to Brugger et al. (2010), chlorantraniliprole exhibited selectivity towards several beneficial parasitoid wasp species, including *Aphidius rhopalosiphi*, *Trichogramma dendrolimi*, *Trichogramma chilonis*, *Trichogramma pretiosum*, *Aphelinus mali*, *Dolichogenidea tasmanica*, and *Diadegma semiclausum* (Brugger et al., 2010). Furthermore, this pesticide exhibited little toxicity towards both the larvae and adults of the predators *Harmonia axyridis* and *Chrysoperla sinica* (Liu et al., 2016).

Pesticides cause biochemical impairment and lesions of tissues and cellular processes, resulting in hundreds-fold increases in the activity of enzymes (AST), (ALT), (ALP), and (LDH) in *Monacha cantiana* and *Theba pisana*, two land snail species, as a consequence of organ cell injury (Ali, 2004; Mahmoud, 2006; Celik et al., 2009; Ghouri et al., 2010; El-Gohary & Genena, 2011; Khalil, 2016). Furthermore, Bakry et al. (2013) discovered that lipid peroxidase (LP) activity increased in *Bulinus truncatus* after two weeks of exposure to sublethal concentrations of glyphosate. Additionally, Bislimi et al. (2013) showed that a high rate of cholesterol and total protein in the hemolymph of garden snails, *Helix pomatia* L. in the contaminated regions. Moreover, several biological targets on *E. vermiculata* were altered by the chemical compounds that were investigated, potentially resulting in severe detrimental impacts on the metabolism and cells of snails (Mahal et al., 2015). According to Abdelmonem (2016), the LD₅₀ (102.32 µg/snail) of methomyl lannate inhibited AChE more in the brain ganglia than in the foot muscle of *E. vermiculata*. Except for ALP, there was a considerable elevation of hemolymph enzymes in snails exposed to 21.32 and 53.30 µg/snail for 48 hours via contact. Moreover, Esam (2023) showed that Bis-(1,2-diphenyl-2-(p-tolylimino)-ethanone decreased the activities of ALT, AST, and TP in *E. vermiculata* with mean values lower than the control, while treatment with a mixture of chemicals increased ALT, AST activities, and TP with mean values higher than the control. El-Bassouiny et al. (2022) demonstrated that spiroetramat (Movento) achieved a 24 h-LC₅₀ of 12.05 ppm against the cotton bollworm, *Earias insulana*, and caused a significant change in the activities of transaminase enzymes (AST and ALT), phenol oxidase, and acetylcholinesterase. It also caused a significant decrease in total protein and lipids.

According to Das et al. (2019), sulfoxaflor was toxic to adult bees and caused significant changes in antioxidative (SOD, CAT), lipid peroxidation (POD, LPO, MDA), detoxification (GST, GR, GSH), and signal transduction-related (AChE, ACh) enzymes or products in both larvae and adult honeybees in the laboratory over 96 hours. Therefore, chlorantraniliprole takes into account the well-being of parasitoids and natural enemies, chlorantraniliprole proves to be a good candidate for controlling land snail *E. vermiculata*.

CONCLUSIONS

In conclusion, the results showed the most food preference for land snails, specifically *E. vermiculata* was Cos lettuce and cabbage leaves in both the ‘no-choice feeding’ and ‘free-choice feeding’ methods. Additionally, based on the previous findings, it was possible to arrange the pesticides used based on the extent of their effect on land snails and their vital enzymes as follows: chlorantraniliprole > sulfoxaflor > fipronil > spinetoram > spirotetramat. Therefore, these substances could be useful for managing land snails. Incorporating these insecticides into a comprehensive management strategy to mitigate any negative effects of land snails while ensuring the overall well-being of the environment. The most likely route of action of these chemicals on land snails still needs more research.

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