https://dx.doi.org/10.4314/ejppri.v7i4.5

Egypt. J. Plant Prot. Res. Inst. (2024), 7 (4): 488 - 495

وزارة الزراعة واستصلاح الأراضي
Ministry of Agriculture and Land Reclamation
مركز البحوث الزراعية
Agricultural Research Center

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Comparative toxicity of certain compounds against terrestrial snails *Monacha cantiana* and *Succinea putris* (Gastropoda: Hygromiidae: Succineidae) under laboratory and field conditions

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ARTICLE INFO

Article History

Received: 10/10/2024

Accepted: 15/12/2024

Keywords

Toxicity, terrestrial snails, tolfenpyrad, emamectin benzoate and indoxacarb.

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Abstract

Monacha cantiana (Montagu) and *Succinea putris* (L.) (Gastropoda: Succineidae: Hygromiidae) are among the most significant harmful land snails affecting various economic crops in Kafr El-Sheikh Governorate, Egypt. This study compared the toxicity of different concentrations of certain pesticides against these two snail species using two methods under both laboratory and field conditions. The results indicated that, under laboratory conditions, the poison bait method was more effective than the leaf-dipping method. Among the pesticides tested, indoxacarb proved to be the most effective against *M. cantiana* when applied using the poison bait and leaf-dipping methods, followed by emamectin benzoate and tolfenpyrad, with LC_{50} values of (2.70, 8.29, and 87.92 ppm) and (1.62, 12.03, and 95.31 ppm) for the two methods, respectively. For the land snail *S. putris*, emamectin benzoate showed the highest effect, followed by indoxacarb and tolfenpyrad, the LC_{50} values were (2.79, 3.23, and 62.69 ppm) and (3.33, 4.19, and 67.14 ppm), for the poison bait and leaf-dipping methods, respectively. Conversely, under field conditions, the toxic baits of indoxacarb, emamectin benzoate, and tolfenpyrad achieved mortality rates of 99.5%, 85.83% and 83.07% for the land snail *M. cantiana*, respectively, after 21 days of application. Therefore, control programs targeting these land snails can effectively use these compounds, particularly indoxacarb, as molluscicides.

Introduction

Terrestrial gastropod mollusks, such as slugs and snails (Mollusca: Gastropoda), represent a significant threat to agriculture, as they are responsible for considerable yield reductions across a variety of crops globally (Barua *et al*., 2021). These crops include oilseed rape, vegetables, legumes, cereals, and fruits (Barker, 2002). In many Egyptian governorates, harmful land molluscs infest many economic agricultural crops (Mohammed, 2015; Shahawy *et al*., 2018; Ibrahim *et al*., 2021; Abo-Hashem *et al*., 2022 and Ibrahim and Ali, 2024). In the Kafr El-Sheikh governorate, land snails *Monacha cantiana* (Montagu) and *Succinea putris* (L.) (Gastropoda: Succineidae: Hygromiidae) are considered agricultural pests that seriously damage several crops (Gazzy *et al*., 2019). The use of insecticides is an essential approach in pest control programs, especially when the pest population increases. However, it causes many serious damages to the environment and biodiversity (Meunier *et al*., 2024), in addition to the resistance that these pests acquire. Therefore, it was necessary to strive to evaluate other compounds that are more effective and easily available so that they can be included in the integrated control programs for land snails. Avermectins, a group of natural product homologues with a 16-membered macrocyclic lactone structure, are produced by the soil microorganism known as Streptomyces avermitilis MA-4680 (NRRL 8165). Emamectin benzoate (MK-0244) represents a novel semi-synthetic derivative of the natural product abamectin. Regarding its mode of action, avermectin primarily inhibits the transmission of electrical signals in the muscles and nerves of invertebrates. This is achieved by enhancing the effect of glutamate on chloride channels that are specifically gated in invertebrates (Bloomquist, 2003; Subbanna *et al*, 2020 and El-Saber *et al*., 2020). Tolfenpyrad is a pyrazole insecticide that exerts its pesticidal effects by inhibiting complex I of the mitochondrial respiratory electron transport chain. This inhibition disrupts ATP production, leading to cellular energy deficits and ultimately causing the death of the target pests. Tolfenpyrad is effective against various pests at different life stages, including eggs, larvae, nymphs, and adults, and is utilized on a wide range of crops (World Health Organization, 2013). Indoxacarb, a member of the oxadiazine pesticide group, is a widely used and relatively new pesticide for pest control, which works as a sodium channel blocker, leading to the paralysis and death of targeted pests (Li *et al*., 2022).

The purpose of this study was to evaluate, in both the lab and the field, the molluscicidal activity of several novel compounds as potential substitute agents for controlling *M. cantiana* and *S. putris,* terrestrial snails.

Materials and methods

1. Collection of experimental animals:

Adult individuals of the land snails *S. putris* and *M. cantiana* were gathered from sugar beet-cultivated fields in Abofreen village, Sidi Salim district, Kafr El-Sheiekh Governorate, and then brought to the laboratory in plastic sacs for our studies. Before the bioassay trials, the snails were given fresh green lettuce leaves (*Lactuca sativa* L.) for two weeks to help them acclimate. They were housed in an aired glass container in the laboratory that was filled with damp, sandy, loamy soil covered with muslin cloths. Snails that were dead were removed immediately, and healthy individuals only were kept for use in an experiment (Ibrahim *et al*., 2022).

2. Tested Compounds:

2.1. Core (Emamectin Benzoate 5.7% WG):

Trade name: Core 5.7% WG
Active ingredient (Common name): Emamectin Benzoate
Molecular formula: $C_{56}H_{81}NO_{15}$
Chemical group: Avermectins
Application rate: 60gm/feddan

2.2. Agrovent (Indoxacarb 10% SC):

Trade name: Agrovent 10% SC
Active ingredient (Common name): Indoxacarb
Molecular formula: $C_{22}H_{17}ClF_3N_3O_7$
Chemical group: Oxadiazine
Application rate: $25cm^3$/100 L.

2.3. Tolficide (Tolfenpyrad 15% SC):
Trade name: Tolficide 15% SC
Active ingredient (Common name): Tolfenpyrad
Molecular formula: $C_{21}H_{22}ClN_3O_2$
Chemical group: pyrazole
Application rate: 100cm/100 L

3. In vitro toxicity tests:

Using two approaches, laboratory tests were conducted to assess the toxic effects of three compounds as molluscicides against *M. cantiana* and *S. putris* snails. These chemicals included emamectin benzoate (5, 10, and 20 ppm), indoxacarb (4, 12, and 20 ppm), and tolfenpyrad (80, 100, and 120 ppm).

Using the leaf-dipping technique, an experiment was conducted to investigate the effects of certain compounds under laboratory conditions. Different concentrations of the tested compounds were prepared using distilled water. Fresh lettuce leaves were soaked in the prepared solutions for 90 seconds and then allowed to dry under standard laboratory conditions (Helmy *et al*., 2022). Once dried, the leaves were placed in plastic containers filled with 3–5 cm of clay soil, each housing ten adult snails from the target species. The containers were covered with muslin cloth secured by rubber bands to prevent the snails from escaping. For each concentration, five replicates were conducted, while untreated lettuce discs served as the control group. Mortality rates were recorded after 1, 3, 7, and 10 days.

In the poisonous baits test, we combined the concentrations of the examined chemicals, prepared in appropriate amounts, with 5g of sugarcane syrup as an attractant. We supplemented the mixture with wheat bran until it reached a total weight of 100g, and then dampened it with modest quantities of water to form toxic bait. We did not add any toxicant to the control treatment. We provided each box filled with moist soil with a total of five grams of bait for the snails. A total of ten mature snails were allocated into three separate containers for each experimental condition. The containers were then covered with muslin cloth netting and tightly fastened with a rubber band to ensure that the snails could not escape. The boxes that were subjected to testing were inspected on days 1, 3, 7, and 10 in order to record and remove dead animals.

4. Toxicity tests in the field infested:

Due to the terrestrial snail *M. cantiana* being one of the most spreading snails on sugar beet fields in Kafr El-Sheikh Governorate, in April 2023, a field experiment was conducted in a sugar beet field heavily infested with this land snail at Abofreen village, Sidi Salem district, Kafr El-Sheikh Governorate. We applied the laboratory-tested materials to the infested area, dividing it into plots. Each treatment, including the control, was divided into three plots, and each plot was divided into five subplots. We used concentrations of 0.2%, 0.3%, and 1% for emamectin benzoate, indoxacarb, and tolfenpyrad, respectively. We prepared these concentrations as poison baits by combining the appropriate amount of each compound with 5 parts of black sugar cane syrup and then mixing them with wheat bran to produce 100 grams of bran bait. We designed the control treatment in the same manner, excluding any compounds. Baits were offered on plastic pieces. We recorded the live snail numbers within each plot before the treatment and at 1, 7, 14, and 21 days after the application. The percentages of reduction were determined based on the statistical formula outlined by Henderson and Tillton (1955).

5. Data analysis:

Mortality percentage was determined and corrected using the formula of Abbott (Abbott, 1925). The slope, the LC_{50} values were determined using the "LdP Line"® software, following the method outlined by Finney (1971). The toxicity index of the examined compounds was calculated using the methodology outlined by Sun (1950) as follows: The toxicity index is calculated by dividing the LC_{50} of the most effective compounds by the LC_{50} of the tested compounds and multiplying the result by 100.

Results and discussion

1. Toxicity tests under laboratory conditions:

1.1. Toxicity of certain compounds against the land snail, *Monacha cantiana* using two techniques in laboratory conditions:

Molluscicidal activity for indoxacarb, emamectin benzoate, and tolfenpyrad compounds on the terrestrial snail *M. cantiana*, using two techniques in laboratory conditions, is portrayed in Table (1). Regarding the poison bait technique, it was found that indoxacarb exhibited the most toxic effects, followed by emamectin benzoate, with LC_{50} values of 2.70 ppm and 8.29 ppm and toxicity index of 100% and 32.57%, respectively, while Tolfenpyrad showed the lowest toxicity, with LC_{50} and toxicity index values of 87.92 ppm and 3.07. The same materials, when applied using the leaf-dipping method, resulted in LC_{50} values of 1.28 ppm, 12.03 ppm, and 95.31 ppm with a toxicity index of 100%, 10.64%, and 1.34% for indoxacarb, emamectin benzoate, and tolfenpyrad, respectively.

Table (1): Comparative toxicity of certain compounds against the terrestrial snail, *Monacha cantiana* using two techniques under laboratory conditions.

Application technique	Compounds	LC_{50} (ppm)	Slope &Variance	T. I	R. P
Poison baits	Agrovent (Indoxacarb)	2.70	0.89 ± 0.36	100	32.56
	Core (Emamectin benzoate)	8.29	1.56 ± 0.43	32.57	10.60
	Tolficide (Tolfenpyrad)	87.92	3.46 ± 1.45	3.07	1
Leaf dipping	Agrovent (Indoxacarb)	1.62	0.35 ± 0.34	100	58.83
	Core (Emamectin benzoate)	12.03	1.56 ± 0.43	13.46	7.92
	Tolficide (Tolfenpyrad)	95.31	4.68 ± 1.46	1.69	1

TI= Toxicity index; RP = Relative potency

1.2. Toxicity of certain compounds against the land snail, *Succinea putris* using two techniques under laboratory conditions:

The toxicity of indoxacarb, tolfenpyrad and emamectin benzoate against *S. putris* using leaf dipping and poison bait techniques was illustrated in Table (2). Data demonstrates that emamectin benzoate shows the highest mortality rate, followed by indoxacarb and tolfenpyrad. The estimated LC_{50} values were 2.79 ppm, 3.23 ppm and 62.69 ppm with a Toxicity index of 100%, 86.37%, and 4.45% when tested by bait technique, respectively. The mortality rate of snails rose progressively with higher concentrations following each treatment, while LC_{50} values by leaf dipping technique were 3.33 ppm, 4.19 ppm, and 67.14 ppm with a Toxicity index of 100%, 79.47% and, 4.96% for emamectin benzoate, indoxacarb and tolfenpyrad, respectively. It was also observed that the poison bait technique was more effective than the leaf dipping treatment.

Table (2): Comparative toxicity of certain compounds on the terrestrial snail, *Succinea putris* using two techniques under laboratory conditions.

Application technique	Compounds	LC_{50} (ppm)	Slope &Variance	T. I	R. P
Poison baits	Agrovent (Indoxacarb)	3.23	2.32 ± 0.47	86.37	19.40
	Core (Emamectin benzoate)	2.79	1.75 ± 0.52	100	22.46
	Tolficide (Tolfenpyrad)	62.69	7.55 ± 1.46	4.45	1
Leaf dipping	Agrovent (Indoxacarb)	4.19	2.10 ± 0.42	79.47	16.02
	Core (Emamectin benzoate)	3.33	1.18 ± 0.45	100	20.16
	Tolficide (Tolfenpyrad)	67.14	6.17 ± 1.78	4.96	1

TI= Toxicity index
RP = Relative potency

2. Toxicity tests under field conditions:

Data in Table (3) and Figure (1) revealed that the effect of the tested pesticides in reducing populations of *M. cantiana* snail exhibited varying degrees of percent reduction compared to the control. Where the population density reduction after 7 days of these materials was (81.86%, 52.99% and 49.41%) reduction for indoxacarb, emamectin benzoate and tolfenpyrad, respectively. The same trend was observed after 21 days since indoxacarb gave the highest reduction percentage of 99.5% followed by emamectin benzoate and tolfenpyrad with reduction percentages (85.83% and 83.07%) respectively. Regarding the general mean reduction percentages of these materials, they were (80.26%, 58.68% and 55.42%) for indoxacarb, emamectin benzoate, and tolfenpyrad, respectively.

Table (3): Reduction ratios of *Monacha cantiana* snail after applying with the toxicants in the field conditions at Sidi Salem district, Kafr El-Sheikh Governorate.

Treatments	(%) Reduction after treatment / Days				General Mean
	1 day	7 days	14 days	21 days	
Agrovent (Indoxacarb)	46.68	81.86	92.99	99.5	80.26
Core (Emamectin benzoate)	19.57	52.99	76.33	85.83	58.68
Tolficide (Tolfenpyrad)	23.48	49.41	65.73	83.07	55.42
Mean	29.91	61.42	78.35	89.47	

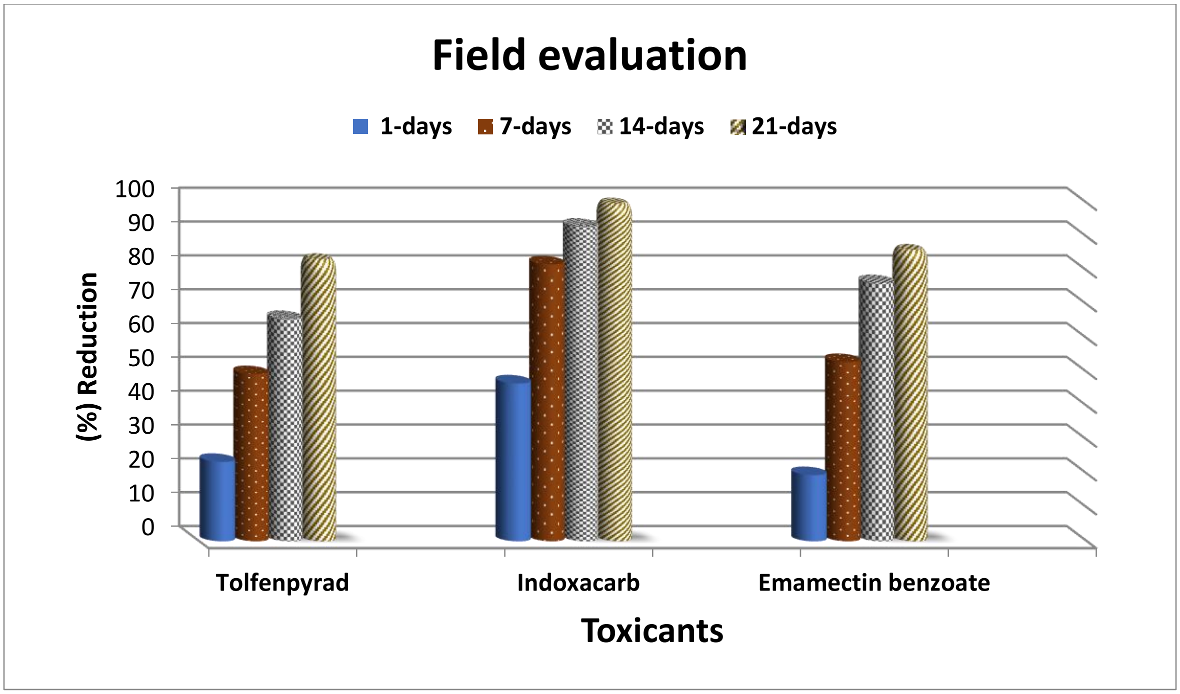

Figure (1): Reduction ratios of *Monacha cantiana* snail after applying the toxicants in the field conditions at Sidi Salem district, Kafr El-Sheikh Governorate.

The findings of the present study indicate that the examined compounds have potential for use in integrated pest management strategies targeting land snails. The data demonstrates that indoxacarb and emamectin benzoate had significant efficacy against *M. cantiana* and *S. putris* snails, followed by the tolfenpyrad compound. Some researchers have investigated the effectiveness of indoxacarb against land snails, and their results were consistent with the results of the current study. Hussein and Sabry (2019) demonstrated that indoxacarb showed the highest efficacy against *E. vermiculata* adults, outperforming abamectin and spiromesifen. The LC50 values were reported as 58.6, 83.3, and 280.9 ppm, respectively. Shams El Din *et al.* (2015) highlighted that a key advantage of indoxacarb is its brief pre-harvest interval (PHI), which ranges from 1.4 to 2.1 days during summer and 2.8 to 4.8 days in winter.

Gad (2022) indicated the effectiveness of emamectin benzoate (EMB) against the white garden snail, *T. pisana*, the median lethal dose (LD_{50}) at 48 hrs. of EMB treatment was 5.34 µg g−1 body weight (b.w.). According to Kandil *et al.* (2020), when the land snail *E. vermiculata* was treated with a thin-film layer technique, the weight of its reproductive organs changed and the two biopesticides, chitosan (Ch) and emamectin benzoate (EMB), demonstrated mollusccidal activity. Their respective LC_{50} values were 68.8 and 120.12 ppm. In a study conducted by Abd-El-Haleem *et al.* (2022), it was shown that methomyl had the greatest impact on the glassy clover snail, *M. cartusiana*, followed by indoxacarb, chlorpyrifos, and glyphosate pesticides. The study used the toxic baits technique in a laboratory setting to assess the effects on adult snails.

The study concludes that the efficacy of pesticides against the land snail species *M. cantiana* and *S. putris* varies based on the method of application, pesticide type, and environmental conditions. Under laboratory conditions, the poison bait method outperformed the leaf-dipping method in delivering toxicity. Indoxacarb was identified as the most effective compound for *M. cantiana*, outperforming emamectin benzoate and tolfenpyrad, while emamectin benzoate proved most effective against *S. putris*. Field tests confirmed the high effectiveness of indoxacarb, achieving near-complete mortality (99.5%) for *M. cantiana* after 21 days, with emamectin benzoate and tolfenpyrad also demonstrating significant mortality rates. These findings highlight the potential of indoxacarb and other tested compounds as effective molluscicides for integrated pest management programs targeting these snail species.

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