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Molluscicidal effect of some pesticides on *Monacha cantiana* (Gastropoda: Hygromiidae) and its injury on sugar beet plants in vitro and in vivo

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Abstract

This investigation was carried out to examine the influence of four pesticides namely, Acrobat® Copper 73.2% WP, Lannate® 90% SP, Delta Star® 10% EC and Mesurol® 2% bait on the mortality of the land snail *Monacha cantiana* (Montagu) (Gastropoda: Hygromiidae) and their effects on the characteristics of sugar beet plants in both *in vitro* and *in vivo* during the fall season of 2022/2023. Under the laboratory conditions (*in vitro*), the results revealed that Mesurol® induced the highest mortality percentage among the tested pesticides after a 72-hour exposure period, followed by Acrobat Copper®. Mesurol® also demonstrated the highest toxic effect, as indicated by its LD_{50} and LD_{95} values after 72 hours of exposure, along with the most favorable LT (Lethal time) values. Acrobat Copper® and Lannate® showed a moderate effect with LD_{50} and LT values (LT_{50} and LT_{95}). Concerning damage criteria, Mesurol® resulted in the lowest number of infested sugar beet leaves and the least leaf-eating area, leading to the lowest injury, while Acrobat Copper® and Lannate® showed a moderate effect on the number of infested leaves and leaf-eating area. In the field experiment (*in vivo*), Mesurol® produced the highest foliage, and roots, as well as total fresh and dry weight compared to other treatments. This trend was also observed with Acrobat Copper®, which had a similar pattern of Mesurol, but with lower values. Furthermore, using Mesurol® resulted in the highest quantities of top, root, and sugar yields, followed by Acrobat Copper®.

Introduction

Sugar beet is considered the first source of sugar production in Egypt, with a crop yield of 1,708,400 tons of roots, producing 61.2 % of Egypt's total sugar production (Annual Report of Sugar Crops Council, 2022). Sugar beet yields are affected by insects, diseases, weeds, and snails; therefore, control of such pests is necessary.

Land snails are dangerous agricultural pests, classified under the phylum Mollusca, Class Gastropoda. These snails have increased rapidly causing economic damage to the field crops,

vegetables as well as horticultural crops; some of them have been identified as disseminators of the spores of phytopathogenic fungi (Puslednik, 2002). Recently, the snails have invaded sugar beet fields at Nubaria (Abo El-Ftooh, 2012), Kafr El-Sheikh (Shalaby *et al.* 2007), and Sharkia (Ibraheem *et al.*, 2008).

The land snail *Monacha cantiana* (Montagu) (Gastropoda: Hygromiidae) severely attacks sugar beet fields in Kafr El-Sheikh and Dakahlia Governorates. No one estimated damage and losses, or reduction of sugar content caused by land snails eating on foliage or root yield. Therefore, increasing invasions of this pest drew attention to its control. To assess the molluscicidal activity of several pesticides against *M. cantiana* land snails, laboratory and field studies were carried out.

Different pesticides demonstrated different degrees of molluscicidal action among the chemicals studied (Al-Sarar *et al.*, 2012; Mortada *et al.,* 2013 and Maaroof *et al.,* 2023). Controlling snails on different crops mainly depends on the use of pesticides that limit the effect of these pests below damaging levels. Hence, synthetic molluscicides or pesticides are the most effective measures available at present for the control of terrestrial gastropods (Heikal, 2015).

The present study was conducted to examine the effect of some pesticides on *M. cantiana* and its effect on sugar beet plant characteristics in vitro and in vivo.

Materials and methods

1. Laboratory experiment:

Adults of *M. cantiana* land snail was collected from sugar beet fields infested with that pest at Nubaria region during the 2022/2023 season. The collected land snails were kept in laboratory conditions at 27ºC ± 2ºC and 65% ± 5% RH. in metal containers (60 × 60 cm, with a 5 cm layer of moistened soil at the bottom: 10 land snails in keeping with glass terrarium) designed to keep land snails from escaping. These containers had ventilation holes, a big hole for adding fresh sugar beet leaves (15 leaves per replicate) as a food source, and moistened soil that had been sterilized and mixed with loamy sand. The molluscicidal effect of the tested compounds was carried out against the adult snails for 72 hrs. The tested compounds were prepared and diluted for application in three concentrations as follows; Delta Star®10 % [Deltamethrin 10% EC (0.35, 0.40 and 0.45 ml/l), Lannate®90% (Methomyl 90% SP) and Acrobat® Copper 73.2% WP (Dimethomorph 6 % + Copper Oxychloride 67.2 %) at 0.50, 0.75 and 1.0 g/l, with three replicates by dipping sugar beet leaves for two minutes and drying them for one hour in the laboratory to be introduced to the targeted snails for feeding. The recommended molluscicides; Mesurol® (Methiocarb 2 % bait) were applied at the rate of 0.25, 0.50, and 0.75 g/m^2. The untreated sugar beet leaves were sprayed with distilled water. After 6, 12, 24, 48, and 72 hrs. of application, the tested snails were examined to count the dead ones. Moreover, untreated sugar beet leaves were introduced for snails as feed-in check treatment. The corrected mortality percentage, infestation percentage, and injury reduction percentage were calculated as follows (Bakr, 2005):

$$\textbf{Corrected mortality (\%)} = \frac{\textbf{Mean number of land snails after treatment}}{\textbf{Mean number of land snails in Control (10 land snails)}} \times 100$$

$$\textbf{Infestation (\%)} = \frac{\textbf{Mean number of infested leaves}}{\textbf{Total number of leaves (15 leaves/replicate)}} \times 100$$

$$\textbf{Injury Reduction (\%)} = \frac{\textbf{Leaf eating area in control – Leaf eating area in treatment}}{\textbf{Leaf eating area in control}} \times 100$$

2. Field experiment:

The field experiment was conducted at a private farm during the 2022/2023 season in a completely randomized block design with three replicates under the flooding irrigation system. Seeds of the polygerm Gloria sugar beet variety were sown in hills spaced at 15 cm, on the 15th of October. The plot area was 10.5 m^2 (3 x 3.5 m). Each plot had 6 rows of 50 cm apart. All agricultural operations were carried out as recommended by Sugar Crops Research Institute. The tested compounds were prepared for application as follows: Acrobat® Copper 73.2% WP (2.5g/l), Lannate® 90% SP (0.75g/l) and Delta Star® 10% EC (0.5 ml/l). The recommended molluscicide, Mesurol® 2% bait was applied at the rate (0.5g/m^2).

3. Statistical analysis:

Statistical analysis was performed using a program of Costat software (1988) with the least significant difference (LSD) at the 0.05 level of probability. Probit analysis was done to estimate LD, LT values, and confidence limits as well as the slope value of LCP lines for the tested pesticides using SPSS V. 10 system software (SPSS, 1999). The corrected mortalities (M %) of snails were calculated.

Results and discussion

1. Laboratory experiment:

Data in Table (1) cleared that the mortality % of snails by the tested pesticides was raised significantly and gradually as the period after treatments was prolonged from 6 to 12, 24, 48, and 72 hrs. Moreover, the mortality % increased by increasing the concentration of the applied pesticides. Under this study, it is important to be clear that the highest mortality percentages were recorded at the highest concentrations of all treatments. Mesurol® achieved the highest mortality percentage after 72 hrs. and showed superiority over the other tested pesticides with an average of 71.2 %, followed by Acrobat® Copper, which achieved an average of mortality%. However, Delta star ranked the lowest pesticide, recording an average of 25% mortality.

Table (1): Corrected mortality percentage of the tested pesticides on the land snail *Monacha contiana* under laboratory conditions.

Pesticide	Dose/liter	Mortality of adults/hours					
		6 hrs.	12 hrs.	24 hrs.	48 hrs.	72 hrs.	Mean
Acrobat copper®	0.50 g	20.0	23.3	33.3	43.3	43.3	32.6
	0.75 g	30.0	33.3	43.3	43.3	53.3	40.6
	1.00 g	40.0	46.7	60.0	63.3	73.3	56.7
Mean		30.0	34.4	45.5	50.0	56.6	43.3
Lannate®	0.50 g	20.0	23.3	33.3	36.7	36.7	30.0
	0.75 g	23.3	30.0	33.3	43.3	53.3	36.6
	1.00 g	40.0	43.3	53.3	53.3	63.3	50.6
Mean		27.8	32.2	40.0	44.4	51.1	39.1
Delta star®	0.35 ml	10.0	13.3	16.7	20.0	23.3	16.7
	0.40 ml	13.3	20.0	26.7	33.3	36.7	26.0
	0.45 ml	10.0	30.0	33.3	43.3	53.3	34.0
Mean		11.1	21.1	25.6	32.2	37.8	25.5
Mesurol®	0.25 g	33.3	46.7	70.0	86.7	91.3	65.6
	0.50 g	33.3	60.0	83.3	96.7	96.7	74.0
	0.75 g	40.0	60.0	76.7	96.7	96.7	74.0
Mean		35.5	55.6	76.7	93.4	94.9	71.2
Untreated	-	0.00	0.00	0.00	0.00	0.00	0.00

1.1. Lethality effects of the tested pesticides:

The present study aimed to determine the lethal exposure concentrations, LD_{50} and LD_{95} at exposure periods from 6 to 72 hrs. after exposing *M. cantiana* to the tested pesticides. Data in Table (2) showed that all of the tested pesticides were lethal to *M. cantiana* adults with different percentages of mortality according to the concentration of the tested molluscicides and time of exposure. The results also showed that the tested sub-lethal concentrations of Mesurol® (0.015 and 0.427 g/m^2) exhibited the highest lethal effect with LD_{50} and LD_{95} with 72 hrs. of exposure, respectively. Acrobat copper® and Lannate® had a moderate effect, with LD_{50} values of 0.614 and 0.957 g/l, while Delta star® had a slight effect, with LD_{50} and LD_{95} values of 0.406 and 0.996 ml/l. According to the LD values, there was a negative correlation between the time and dose-mortality values (Table 2). This means that the longer the time of treatment the greater toxicity. Data in Table (3) showed that the best LT values (LT_{50} and LT_{95}) were 8.647 and 56.22 hrs. respectively, with Mesurol® being the most effective followed by Acrobat copper®, Lannate® and Delta star® pesticides. Likewise, a negative correlation was found between the dose of pesticide and the LT values.

Table (2): Dose-mortality values of the tested pesticides against the land snail, *Monacha contiana* under laboratory conditions after different exposure times using the topical application bioassay method.

Pesticide	LD_{50}	Confidence limits		LD_{95}	Confidence limits		Slope ± SE**	Probability
		Lower	Upper		Lower	Upper		
Acrobat copper®	0.614^{b*}	0.515	0.709	2.764^{ab}	1.553	6.453	2.52 ± 0.36	0.211
Lannate®	0.957^{a}	0.816	1.184	3.929^{a}	2.001	10.943	2.68 ± 0.39	0.507
Delta star®	0.406^{c}	0.375	0.439	0.996^{b}	0.617	1.628	4.22 ± 1.34	0.270
Mesurol®	0.015^{d}	5x10^{-5}	0.597	0.427^{c}	0.246	0.777	1.12 ± 0.39	0.732

***Values with different letters in each column are significantly different**
**** Standard Error**

Table (3): Time-mortality values of the tested pesticide against the land snail, *Monacha contiana* under laboratory conditions at different doses using the topical application bioassay method.

Pesticide	LT_{50}	Confidence limits		LT_{95}	Confidence limits		Slope ± SE**	Probability
		Lower	Upper		Lower	Upper		
Acrobat copper®	13.49b*	9.157	19.66	1772a	345.2	10009	0.78 ± 2.17	0.849
Lannate®	60.37^{a}	42.42	86.75	2145^{a}	583.2	8302	1.06 ± 2.52	0.222
Delta star®	21.02^{b}	12.63	34.85	35281^{a}	622.4	3x10^{6}	0.51 ± 0.02	0.710
Mesurol®	8.647^{c}	7.143	10.43	56.22^{b}	42.42	75.06	2.02 ± 3.76	0.627

***Values with different letters in each column are significantly different**
**** Standard Error**

1.2. Investigation and injury levels:

Concerning the effect of the four pesticides on *M. cantiana* infestation and damage to sugar beet leaves under laboratory conditions, data in Table (4) indicated that Mesurol® gave the lowest number of infested leaves (2.33) with an infestation of 15.56 % at the concentration of 0.75 g/l. Also achieved edging with all treatments the lowest leaf eating area (9.00 and 11.00 cm^2/15 leaf) with injury reduction of (75.68 and 70.27 %) at the concentration of 0.75 and 0.5 g/l, respectively, without variances. Acrobat copper® and Lannate® had a moderate effect, with a number of infested leaves (4.00 and 4.67) at the concentration of 1.0 and 0.5 g/l with infestation of 26.67 and 31.11%, respectively. It also gave a moderate effect of leaf eating area (10.00 and 14.00 cm^2) at the concentration of 1.0 g/l with injury reduction of 72.97 and 62.16% at the same concentration, respectively. However, Delta star® had a slight effect with all readings.

Table (4): Effect of tested pesticides against the land snail, *Monacha contiana* infestation and damage on sugar beet leaves under laboratory condition.

Pesticide	Dose	No. of infested leaves/15 leaves	Infestation %	Leaf eating area cm^2/15 leaves	Injury reduction %
Acrobat copper®	0.50	5.33	35.56	17.00	54.05
	0.75	4.33	28.89	15.00	59.46
	1.00	4.00	26.67	10.00	72.97
	Mean	4.56	30.37	7.67	79.27
Lannate®	0.50	5.33	35.56	20.00	45.95
	0.75	5.00	33.33	17.00	54.05
	1.00	4.67	31.11	14.00	62.16
	Mean	5.00	33.33	10.67	71.16
Delta star®	0.35	8.33	55.56	26.00	29.73
	0.40	7.33	48.89	23.00	37.84
	0.45	6.33	42.22	19.00	48.65
	Mean	7.33	48.89	17.33	53.16
Mesurol®	0.25	4.00	26.67	14.00	62.16
	0.50	3.00	20.00	11.00	70.27
	0.75	2.33	15.56	9.00	75.68
	Mean	3.11	20.74	3.67	90.08
Untreated	-	9.33	62.22	37.00	0.00
$LSD_{0.05}$	-	0.46	4.22	2.3	1.75

2. Field experiment:

2.1. Impact of the tested pesticides on sugar beet fresh and dry weight of plant parts:

The results in Table (5) indicated that applying Mesurol® pesticide, to get rid of snails, resulted in higher fresh weight of leaves, roots, and the total plant compared to the other tested pesticides with 621.44, 1216.89 and 1838.33 g/plant, respectively. A similar trend was recorded with the same order on the dry weight of the concerned plant portions (78.40, 330.50, and 408.90 g/plant, successively), with a general mean of 749.08 g/plant, followed by Acrobat copper® with the same trend with a mean of 581.11 g/plant. On the contrary, Delta star®showed the lowest influence, regarding all studied measurements, with a mean value of 416.97 g/plant.

Table (5): Influence of the tested pesticides on the fresh and dry weight of sugar beet during 2022/2023 season.

Pesticide	Leaves fresh weight (g/plant)	Root fresh weight (g/plant)	Total fresh weight/ plant (g/plant)	Leaves dry weight (g/plant)	Root dry weight (g/plant)	Total dry weight/ plant (g/plant)	Mean
Acrobat copper®	495.65	945.44	1441.09	69.54	232.70	302.24	581.11
Lannate®	454.33	870.44	1324.77	57.30	190.30	247.6	524.12
Delta star®	340.00	712.34	1052.34	48.22	150.34	198.56	416.97
Mesurol®	621.44	1216.89	1838.33	78.40	330.50	408.90	749.08
Untreated	205.24	287.33	492.57	13.42	94.87	108.29	200.29
$LSD_{0.05}$	73.00	121.34	292.63	10.80	53.92	97.61	48.34

2.2. Impact of the tested pesticides on sugar beet yield:

Data in Table (6) revealed that all tested pesticides were highly efficient and significantly increased the sugar beet yield components. The highest top, root, and sugar yield was recorded for Mesurol® by 26.78, 34.24, and 5.76 ton/feddan respectively, with a mean of 22.26 ton/feddan, followed by Acrobat copper®with a mean of 16.08 tons/feddan followed by Lannate®14.61 tons/feddan. While the treatment of Delta star® gave the lowest action with all studied characteristics with a mean of 12.53 ton/feddan.

Table (6): Effect of the tested pesticides on sugar beet yields during 2022/2023 season.

Pesticide	Top yield/fed (ton)	Root yield/fed (ton)	Sugar yield/fed (ton)	Mean
Acrobat copper®	18.80	28.40	4.94	17.38
Lannate®	15.33	24.00	4.50	14.61
Delta star®	11.74	22.32	3.54	12.53
Mesurol®	26.78	34.24	5.76	22.26
Untreated	9.95	19.20	2.20	10.45
$LSD_{0.05}$	5.23	2.83	0.75	0.96

The effect of the tested pesticides on a land snail, *M. cantiana* was estimated, concerning the mode of action of each pesticide, which can be summarized as follows:

The toxicity of Acrobat copper®fungicide may be attributed to the explanation reported by Schiide *et al.* (2003), who concluded that the feet of terrestrial gastropods are the sites of copper uptake. The foot is an important penetration route for highly soluble copper salt, which can be expected to cause some internal damage and irritant effects. Also, the effect of copper salts can cause significant dehydrogenation of snails and slugs. Lannate®90 % is a carbamate pesticide, which is widely used and has known toxic effects on non-target organisms including land snails. Lannate has toxic effects in cellular damage of the digestive glands of the snails, which could be correlated with disturbed enzyme activities (Heiba *et al*., 2002). Delta star® (Deltamethrin 5 %) is a synthetic version of pyrethrins, that acts on tiny channels, through which sodium is pumped to cause excitation of neurons, prevent the sodium channels from closing, resulting in continual nerve impulse transmission, tremors, and eventually death (Brown, 2006). Mesurol® makes its toxic effect by inhibiting the site action of post-synaptic nicotinic acetylcholine receptors (nAChRs), which weakens nerve signals and prevents the entry of ions into neurons. Das and Kaviraj (1994) pointed out that pesticides such as copper sulfate, including Mesurol, are highly toxic to freshwater biota such as snails through absorption in the internal organs to store in different tissues of the snail's body. These effects, caused by the tested pesticides, on land snail, *M. cantiana* led to enhancing growth and performance of sugar beets, as well as improving its characteristics indirectly by reducing or/and preventing the damage caused by the snails to sugar beet green leaves, which convert carbon dioxide in the presence of air and water from the soil into sucrose, translocated and stored in its root.

In conclusion, the study's findings demonstrated that all tested pesticides had a lethal effect on *M. cantiana*, with different levels of mortality depending on the concentration and duration of exposure. Mesurol® was found to be the most effective pesticide in terms of its toxic effect on the

snails, followed by Acrobat Copper® and Lannate® but Delta Star® had the lowest mortality rate. In the field experiment, also all tested pesticides were effective in reducing the number of infested sugar beet leaves, with Mesurol® being the most effective. Overall, this study provides valuable information on the molluscicidal effect of pesticides on land snails and their impact on sugar beet plants, which can be useful for developing effective pest management strategies.

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