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Use of botanical extracts to control the land snail *Eobania vermiculata* (Gastropoda: Helicidae) under laboratory conditions

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Abstract

The laboratory experiment was conducted at Plant Protection Research Institute, Agricultural Research Center, Egypt, in March 2024 to study the efficacy of three plant extracts (*Allium sativum*, *Croton tiglium*, and *Senecio cineraria*) with three concentrations (20, 25, and 30 mg/ml) for each on the land snail, *Eobania vermiculata* (Müller) (Gastropoda: Helicidae). Results showed that *C. tiglium* had the highest mortality rate (21.48%), followed closely by *A. sativum* mortality rate (20.20%), while *S. cineraria* had the lowest mortality rate 18.24%. Mortality rates increased with higher concentrations of the extracts, with *A. sativum* achieving the highest mean mortality rate of 30.68% at 30 g/l. The study evaluated the lethal effects of various plant extracts on the land snail species; *E. vermiculata* over exposure durations from 1^{st} to 7^{th} days, focusing on lethal concentrations (LC_{50} and LC_{95}). *S. cineraria* demonstrated the highest effectiveness (LC_{50}: 34.95 mg/ml, LC_{95}: 89.43 mg/ml), while *C. tiglium* and *A. sativum* exhibited moderate effectiveness (LC_{50}: 32.28 mg/ml and 29.89 mg/ml, respectively). A positive correlation was observed between extract concentration and mortality rates. The phytochemical analysis of all three extracts revealed flavonoids and alkaloids, which indicate possible molluscicidal activities.

Introduction

The use of botanical extracts for the control of land snails has been an area of growing interest in integrated pest management (IPM) and sustainable agriculture. Botanical extracts are considered environmentally friendly alternatives to synthetic molluscicides, and they can offer selective control against land snails while minimizing harm to beneficial organisms. Garlic is one of the most widespread botanical extracts used to control land snails. Allicin is the active ingredient in garlic bulbs that kill snails within 24 hrs. of treatment, according to research on the molluscicidal qualities of garlic (Picardal *et al.*, 2018).

Snails fed either *Allium cepa* or *A. sativum* showed significant changes in their reproductive activity. Furthermore, the growth rate of freshly born snails fed either *A. sativum* or *A. cepa* was impacted. Snail poisoning was created by exposing snails to water containing either *A. cepa* or *A. sativum*;

this could have been caused by changes in the snails' habitat (Mantawy, 2001). Garlic contains a lot of bioactive compounds that are mostly toxic to snails, such as tannins, alkaloids, triterpenes, steroids, saponins, and cardiac glycosides (Batiha *et al.,* 2020). According to Pascual *et al.* (2024), fermented organic insecticides made from garlic (*A. sativum*) and Makabuhay (*Tinospora crispa*) were efficient against golden apple snails, *Pomacea canaliculata* (Lamarck) (Gastropoda: Ampullariidae).

Croton tiglium L. is a member of the Euphorbiaceae family, which is found in both tropical and temperate climates worldwide. This family comprises over 280 genera and 8000 species. Crude extract of croton seeds gave promising results as a molluscicide (Ibrahim *et al.,* 2022a) A preliminary analysis indicates that *Croton tiglium* is harmful due to its irritating oils and poisonous proteins (Liu *et al*., 2017). Aqueous seed extract of *C. tiglium* has the potential to cause pesticidal activity as well as genotoxic activity (Yumnamcha, 2014). Crotocaudin, an extracted substance, has the potential to be an effective molluscicide; plant products are less costly, more readily accessible, more soluble in water, and safer for non-target species than synthetic molluscicides. *C. tiglium* is used for the treatment of various ailments in developing countries, but the plant contains some poisonous compounds (Dey *et al*., 2018).

Senecio cineraria, commonly known as silver ragwort or dusty miller, is a plant that has been studied for various bioactive compounds. *S. cineraria* is known to contain compounds such as alkaloids, flavonoids, and terpenoids, which can have pesticidal, antifungal, and antibacterial properties. Their use specifically for snail control hasn't been extensively documented. *Senecio jacobaea* contains pyrrolizidine alkaloids, which are known to be toxic to pests and herbivores (Ababsa *et al*., 2018).

So, the present study aims to study the efficacy of three plant extracts (*A. sativum, C. tiglium*, and *S. cineraria*) as natural molluscicides for controlling the land snail *Eobania vermiculata* (Müller) (Gastropoda: Helicidae).

Materials and methods

1. Laboratory experiment:

The laboratory experiment was conducted at the Harmful Animals Research Department, Plant Protection Research Institute, Agricultural Research Center, Egypt, during March 2024. Adults of the land snail *E. vermiculata* were collected from vegetable fields infested with land snails in Sharkia Governorate. The collected snails were washed with tap water (Godan, 1983 and Badawey, 2002). The land snails were kept in a glass cage (50 × 50 cm) filled up with mixed sterilized soil (clay: sand) roughly (1:1) with reasonable dampness and shut with a muslin material to avoid snails escaping. They fed on lettuce leaves for 7 days under lab conditions at RH 60%±5 and 25±3 ºC (Shetaia, 2005). Each cage contained 25 adults of the land snail, *E. vermiculata.*

2. Experimental design:

The experiment included three plant extracts (*Allium sativum, Croton tiglium*, and *Senecio cineraria*) with three concentrations for each plant extract. The cage featured ventilation holes and a large opening for adding fresh lettuce leaves (500 g per replicate) as a food source. The molluscicidal effects of the tested plant extracts were evaluated against the adult snails over 7 days. Three concentrations (20, 25, and 30 mg/ml) for each plant extract were tested, with three replicates. Lettuce leaves were dipped in the extracts for two minutes and then dried for ten minutes in the laboratory before being offered to the targeted snails as feed. At the same time, untreated lettuce leaves were simply sprayed with distilled water for control treatment.

3.1. Preparation of the three plant extracts under study:

3.1.1. Aqueous *Allium sativum* extract preparation:

The *Allium sativum* (garlic) bulbs (1 kilogram) were obtained from the market with high quality. The garlic extraction procedures were adapted from research by Soteyome and Theeramongkol (2023), in which garlic bulbs were unhusked and put in a blender with 100 ml of distilled water. It was mixed for 3-5 minutes (On high speed) until a sticky paste was formed. The sticky substance was then strained and sifted through cheesecloth. The filtrate acts as a pure aqueous garlic extract.

3.1.2. Preparation of *Croton tiglium* seeds organic-soluble extracts:

The seed is refined, sun-dried, and ground to powder. To get the hexane-soluble extract, about 200 g of powdered seeds were continuously extracted by maceration with hexane solvent (3x1 L) and evaporated at 35°C. The residues (5 g) were macerated in 50 ml of ethanol for 24 hours, repeated three times, and then filtered. The filtrate was evaporated, and a sticky ethanol-soluble extract was produced. The sticky extract was dried using a vacuum oven at 35°C.

3.1.3. Preparation of the methanol extract of *Senecio cineraria*:

The plant's aerial parts (leaves, blooms, and stems) have been properly cleaned and air-dried at room temperature, away from moisture and sunlight. To make the dried plant usable, it was eventually processed into a fine powder with an electric grinder. The methanolic extract was made following the Markham technique (Markham, 1983), which entailed adding 950 g of vegetable powder to a beaker holding the hydroalcoholic mixture (methanol/H_2O 7:3) for an overnight duration (Alcoholic maceration). With the solvent changed, this maceration was repeated three times, each lasting twenty-four hours. Following that, methanol was extracted. The National Research Center is studying three plants to discover their active components using gas chromatography (GC).

4. Data collection:

The mortality values were recorded daily up to seven days post-application for each concentration. Additionally, untreated lettuce leaves were introduced to the untreated control group. The mortality percentage was calculated using the following formula:

Mortality (%) = (Mean No. of mortality land snails / Total No. of tested land snails) × 100

5. Statistical analysis:

Statistical analysis was carried out using Costat software (1988), with the least significant difference (LSD) applied at the 0.05 level of probability. Using SPSS version 10 software (SPSS, 1999), probit analysis was used to estimate the LC value, as well as their confidence limits and the slope value of LCP lines for the investigated plant extracts.

Results and discussion

1. Impact of plant extract concentrations on mortality rates:

The average daily mortality values for 25 land snails, *E. vermiculata*, with three concentrations for each plant extract (*A. sativum*, *C. tiglium*, and *S. cineraria*) throughout the intervals from the first to the seventh day are shown in Table (1). The data revealed that *C. tiglium* had the highest average mortality value of 5.38/25 land snails with a mortality percentage of 21.48%, followed by *A. sativum* with an average mortality value of 5.05/25 land snails and a mortality percentage of 20.20%, with significant differences. *S. cineraria* had the lowest average mortality value of 4.56/25 land snails with a mortality percentage of 18.24%, compared to the untreated control, which had an average mortality value of 0.28/25 land snails and a mortality percentage of 1.12%, with significant differences. The mean mortality rate per 25 land snails increased at defined intervals for each plant extract concentration (from the first to the seventh day). It is important to point out that as concentration increased, it also led to the highest mortality values, so *A. sativum* achieved reaching the highest mean mortality rate (30.68%) at 30 g/l. The average general mortality rate across all treatments was 3.75%, demonstrating the efficacy of plant extracts in decreasing snail populations under laboratory conditions. In conclusion, this table shows how varied concentrations of plant extracts may significantly increase *E. vermiculata* death rates, which has implications for pest management strategies in agricultural environments.

Table (1): Average mortality per 25 land snails, *Eobania vermiculata* under laboratory conditions during March 2024.

Plant extract (A)	Concentration (B)	Average mortality per 25 land snails after (B) Intervals (C)							Mean (A)	Mortality (%)
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day		
Allium sativum	20 g/l	1.00	1.33	2.33	3.00	3.33	4.33	5.00	2.90	11.60
	25 g/l	0.67	2.00	3.33	4.67	6.33	7.33	8.33	4.57	18.28
	30 g/l	1.67	4.00	6.00	8.00	9.33	11.67	13.00	7.67	30.68
Mean		1.11	2.44	3.89	5.22	6.33	7.78	8.78	5.05[b]	20.20
Croton tiglium	20 g/l	1.33	2.33	2.33	3.33	4.33	4.67	5.33	3.38	13.52
	25 g/l	0.67	2.00	3.67	5.67	6.67	7.33	8.67	4.91	19.64
	30 g/l	2.00	4.67	6.00	8.00	10.67	12.00	11.33	7.81	31.24
Mean		1.33	3.00	4.00	5.67	7.22	8.00	8.44	5.38[a]	21.48
Senecio cineraria	20 g/l	0.67	2.00	2.67	3.33	3.33	3.67	4.33	2.86	11.44
	25 g/l	1.33	2.00	3.00	4.33	5.33	6.00	7.33	4.16	16.64
	30 g/l	2.67	5.00	5.67	6.67	7.67	9.00	10.00	6.67	26.68
Mean		1.56	3.00	3.78	4.78	5.44	6.22	7.22	4.56[c]	18.24
Untreated Control	-	0.00	0.33	0.33	0.33	0.33	0.33	0.33	0.28[d]	1.12
General Mean (C)		1.00	2.11	2.92	3.92	4.75	5.50	6.11	3.75	
$LSD_{0.05}$	A =0.29 B = 0.25 C= 0.39									

2. Lethal concentrations of the tested plant extracts:

The results in Table (2) summarize a study investigating the lethal effects of various plant extracts on the land snail species *E. vermiculata* over exposure durations ranging from 1st to 7th days. The primary metrics evaluated were the lethal concentrations, specifically LC_{50} (the concentration required to kill 50% of the test population) and LC_{95} (The concentrations required to kill 95% of the test population) for each extract. The results indicated that all tested extracts effectively caused mortality in adult snails, with varying levels of potency. *S. cineraria* was identified as the most effective extract, with an LC_{50} of 34.95 mg/ml and an LC_{95} of 89.43 mg/ml, suggesting that lower concentrations are sufficient for significant lethality. In comparison, *C. tiglium* and *A. sativum* exhibited moderate effectiveness, with LC_{50} values of 32.28 mg/ml and 29.89 mg/ml, respectively. According to the LC values, there was a positive correlation between plant extract concentrations and mortality values, indicating that increasing the plant extract concentrations led to increased mortality. The probability values indicated the statistical significance of the results, with higher values reflecting greater confidence in the findings. All extracts showed high probabilities (0.54 for *A. sativum*, 0.81 for *C. tiglium*, and 0.85 for *S. cineraria*), indicating that their effects were statistically significant. The regression equations provided a mathematical model to predict the relationship between concentration and mortality for each extract. For example, for *A. sativum*, the equation (Ý = -7.70 + 5.22 X) suggested that as the concentration (X) increases, the predicted mortality rate (Ý) also increases significantly.

Table (2): Dose-mortality values of the tested plant extracts against the land snail, *Eobania vermiculata* under laboratory conditions during March 2024.

Plant extract	LC_{50}	Confidence limits		LC_{95}	Confidence limits		Slope ± SE**	Probability	Regression Equation
		Lower	Upper		Lower	Upper			
Allium sativum	29.89	27.09	32.98	61.79	42.13	90.70	5.22 ± 1.26	0.54	Ý = -7.70 + 5.22 X
Croton tiglium	32.28	27.41	38.03	84.25	43.19	164.64	3.95 ± 1.22	0.81	Ý = -5.96 + 3.95 X
Senico cineraria	34.95	28.56	42.77	89.43	43.96	182.27	4.04 ± 1.32	0.85	Ý = -6.22 + 4.03 X

3. Phytochemical screening of the plant extracts:

Results in Table (3) showed that all three plant extracts contain flavonoids and alkaloids, while tannins are present in *A. sativum* and *S. cineraria*. Saponins are absent in *C. tiglium* and *S. cineraria* but found in *A. sativum*, while steroids are present in *A. sativum* and *C. tiglium* but absent in *S. cineraria*. This screening highlights the diverse phytochemical profiles of these plants, which may contribute to their potential molluscicidal properties.

Table (3): Phytochemical screening of plant extracts under study.

Active ingredient	Plant extracts		
	Allium sativum	***Croton tiglium***	***Senecio cineraria***
Flavonoids	+	+	+
Alkaloids	+	+	+
Tannin	+	-	+
Saponin	+	-	-
Steroids	+	+	-

Present + Absent –

The use of plant extracts as alternative pesticides for controlling land snails has gained attention in recent years, particularly as concerns grow over the environmental and health risks posed by synthetic chemicals (Ibrahim *et al.*, 2022b and Abo-Elwfa *et al.*, 2024). Also, plant extracts tend to be less harmful to beneficial organisms compared to chemical pesticides, preserving biodiversity (Mesbah *et al.*, 2022). The molluscicidal properties of plant extracts are attributed to various chemical compounds, primarily alkaloids, flavonoids, and other phytochemicals. Plant families such as Solanaceae and Papaveraceae contain alkaloids with notable molluscicidal activity. The hydroalcoholic extract of Ricinus communis revealed the presence of flavonoids and tannins, although it showed limited molluscicidal activity (Mendes, 2018). Flavonoids may disrupt cellular processes in snails, leading to lethargy and behavioral changes, as observed in studies with extracts from *Persea americana* (Silva *et al.*, 2020). The effectiveness of extracts varies with concentration, as *Calotropis procera* was more potent against adult snails and agrees with our results (Oyeyemi *et al.*, 2022).

According to the results, *S. cineraria* had the lowest death rate at 18.24%, while *C. tiglium* had the highest at 21.48%, followed by *A. sativum* at 20.20%. Higher concentrations resulted in higher mortality rates. All extracts included flavonoids and alkaloids, according to a phytochemical study, which may indicate molluscicidal effects.

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