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Efficacy of Sodium Hypochlorite as a biocontrol agent for the land snail: *Eobania vermiculata*

Ismail GH[1], Kandil RS[2*]
[1] Department of Harmful Animal Research, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt
[2] Department of Field Crop Pests Research, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

Abstract
The use of Sodium hypochlorite (NaOCl) as a molluscicide for controlling land snail populations was evaluated in this study. Sodium hypochlorite, commonly used for its disinfectant properties, was tested for its efficacy in reducing the population of land snails, *Eobania vermiculata*. Laboratory assays were conducted to determine the lethal dose (LD_{50}) of Sodium hypochlorite that causes 50% mortality in snails after exposure. The snails were subjected to varying doses of NaOCl (***0***.5, 1, 1.5 and 2 ppm) over different exposure periods 12 to 72 hours. Results demonstrated a dose-dependent relationship, with higher doses and prolonged exposure times leading to increased mortality rates. The lowest dose showed no mortality, but increased to 6.67% at 48 hours and 23.33% at 72 hours. The maximum dose of 2 ppm caused a 13.33% death rate. Sodium hypochlorite increases AST and ALT activity, while decreasing total protein and albumin dose in land snail; *E. vermiculata.* In addition to its lethal effects, Sodium hypochlorite (NaOCl) caused sub-lethal effects, including reduced movement and feeding, at lower doses (0.5–1 ppm). The study suggests Sodium hypochlorite can effectively control land snail populations, but its application must be managed to prevent environmental impacts. It may serve as an alternative to traditional molluscicides in agricultural settings.

Keywords: Land snails, molluscicides, chemical control

Introduction

Land snails, particularly species as *Eobania vermiculata*, are considered significant agricultural pests due to their destructive feeding habits, which damage crops and affect plant health. Traditional methods for controlling land snail populations have predominantly involved the use of metaldehyde (Abobakr *et al.*, 2021 [1]) and iron phosphate-based molluscicides (Bashandy and Raddy, 2021) [2]. Calcium Hypochlorite gave a high mortality rate in Potadoma snails, with 100% mortality observed at higher doses within 32 hours (Oniya *et al.*, 2023) [3]. Sodium hypochlorite could offer a viable alternative to traditional molluscicides due to its broad availability, relatively low cost, and potential effectiveness against snails. The most practical form of active oxygen is Sodium hypochlorite (NaOCl), which is made chemically from aqueous Sodium chloride solutions. It is safe to use in drinking water (Taha *et al*., 2014) [4]. Because of its small size and low molecular mass, it can readily cross cellular membranes and be rapidly evacuated (Eventov *et al*., 1998) [5]. Sodium hypochlorite has effective antimicrobial mechanisms, tissue dissolution capacity and showed a biological compatibility in less concentrated solutions (Gerba and Denise, 2007 [6] and Abadias *et al.*, 2008 [7]). Sodium hypochlorite (NaOCl) acts as a molluscicide by interacting biochemically with target species, particularly freshwater snails like *Biomphalaria alexandrina*. It induces significant physiological changes, including immune system disruption and direct poisoning. The lethal dose (LC50) for *B. alexandrina* is 1.25 ppm, indicating its potency. At sublethal levels, NaOCl alters metabolism by increasing transaminase activity and decreasing steroid hormone and total protein levels. Additionally, NaOCl causes morphological changes in hemocytes, such as ruptured plasma membranes and cytoplasmic vacuolation, which impair the snails' immune response (Ibrahim *et al.*, 2023) [8]. So, the purpose of this present experiment was to evaluate the effectiveness of Sodium hypochlorite at varied doses on the land snail, *Eobania vermiculata*.

Material and methods

Tested snails

Individuals of the brown garden snail, *E. vermiculata* were collected from ornamental plants in Zagazig city Sharika Governorate, Egypt and were transported to the standard laboratory of Plant Protection Research Institute, Agricultural Research Center, Giza Governorate, Egypt, in March 2024. The snails were placed in small glass boxes containing moist soil (1:1 mixture of clay and sand, 10cm high). Each box included fresh green lettuce leaves and was kept in the lab for two weeks to allow for acclimatization. It was covered with a muslin fabric that was fastened with a rubber band to keep snails from escaping. Ten animals in total were utilized as the control group and for each treatment dose.

Tested compound

Sodium hypochlorite; a commercial bleaching solution, Clorox® (5.0% NaOCl solution), was used in this study. The stock solution was prepared using 1000 ppm distilled water based on a volume/volume (V/V) ratio.

Experimental design

In this study, four serial dilutions (0.5, 1, 1.5, and 2 ppm) of the NaOCl stock solution were prepared with dechlorinated tap water to determine the LD_{50} and LD_{95}. Each treatment included three replicates and each replicate contained ten adult *E. vermiculata* snails. The tested snail fed on lettuce leaves treated with NaOCl using baits prepared by the same method reported by (Shahawy, 2019) [9] as follows: (95 gm. Of wheat bran mixed with an appropriate amount of water containing the tested above doses of chlorine plus 3g of sugar cane honey to attract snails. Untreated control was contained bait without pesticides. Mortality percentages were monitored and calculated after 12 hours up to 72 hours from exposure using Probit analysis (Finney, 1971) [10].

Biochemical examinations

Tissue Preparation was made by dissecting the snails from the control and exposed groups to sublethal doses of NaOCl, the soft tissues from the hermaphroditic and digestive glands were extracted from the shells using forceps. The soft tissues were homogenized using a glass Dounce homogenizer after being weighed (1 g tissue/10 ml phosphate buffer). The supernatants obtained after centrifuging the homogenates at 3000 rpm for 10 minutes were used for the analysis.

The activity of Aspartate and alanine aminotransferase (AST and ALT) was measured using the method of (Reitman and Frankel, 1957) [11]. Protein content was determined using the method of Lowry *et al.*, (1951) [12]. Albumin levels were measured according to (Gustafsson, 1976) [13]. Acid and alkaline phosphatase (ACP/ALP) activities were measured using the method adapted by (Bergmeyer, 1985) [14] and (Singh and Agarwal, 1991) [15].

Statistical analysis

‘’F’’ test and analysis of variance (ANOVA) were applied to the data. A computer software is used to compare the means of the various treatments (Costat Software, 1988) [16] was used to calculate the least significant differences (L.S.D.) at the 0.05 level, and means were also compared using Duncan’s Multiple Range Test (Duncan, 1955 [17]).in addition to calculation of standard error.

Results

The mortality percentages of *E. vermiculata* land snails exposed to different Sodium hypochlorite doses (ppm) from 12 hours to 72 hours are shown in Table 1. The results showed that the mortality percentages increased with increasing doses and longer exposure times. After 12 hours, no mortalities were reported at low doses (0.5 and 1 ppm). However, after 24 hours, mortality percentages rose to 10% and 13.33% for 1.5 and 2 ppm doses, respectively without significant differences. Mortality percentages increased significantly over time, reaching 23.33% at 0.5 ppm and 33.33% at 1 ppm after 72 hours, compared to 56.67% and 76.67% at 1.5 and 2 ppm doses, respectively with significant differences. This supports a strong dose-response relationship, with toxicity increasing with higher doses and longer exposure times.

Table 1: The mortality percentages of *E. vermiculata* land snail exposed to different Sodium hypochlorite doses

Dose of NaOCl (ppm)	Mortality percentage after exposure time ± Standard error			
	12 hrs	24 hrs	48 hrs	72 hrs
0.5	$0.00\pm0.00^{a*}$	0.00 ± 0.00^{b}	6.67 ± 3.33^{d}	23.33 ± 3.33^{c}
1	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	23.33 ± 3.33^{c}	33.33 ± 3.33^{c}
1.5	0.00 ± 0.00^{a}	10.00 ± 0.00^{a}	36.67 ± 3.33^{b}	56.67 ± 6.66^{b}
2	0.00 ± 0.00^{a}	13.33 ± 3.33^{a}	50.00 ± 5.77^{a}	76.67 ± 3.33^{a}
Untreated control	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
$LSD_{0.05}$	NS	4.70	11.51	12.43

* Means in each column followed by the same letter (s) are not statistically different at the probability of 0.05

Data in Table (2) indicated the toxic effects of Sodium hypoclorite over two time periods: 24 hours and 72 hours. The LD_{50} showed a decrease from 2.017 to 1.195, suggesting that the dose required to achieve the same level of lethality decreases with prolonged exposure, indicating that extended exposure increases the sensitivity of living organisms to toxicity. Similarly, the LD_{95} decreased from 9.400 to 5.940, reflecting the need for lower doses to achieve a 95% lethality rate over time. The confidence intervals indicated greater uncertainty in the data for the 24-hour period compared to the 72-hour period, although the relationship between dose and effect remains strong, with statistically significant p-values. These results highlight the importance of monitoring exposure levels to toxic substances over extended periods, as lower doses can lead to lethal outcomes over time, necessitating careful risk assessment and the establishment of safety protocols.

Table 2: Lethal Sodium hypoclorite dosage for *E. vermiculata* land snail exposed to 24–72 hours

Dose	LD_{50}	Confidence limits		LD_{95}	Confidence limits		Slope ± SEM	Regression equation	Probability
		Lower	Upper		Lower	Upper			
24 hrs	2.017	1.717	2.519	9.400	5.773	19.975	2.461 ± 0.128	Y = -0.750 + 2.46X	0.966
72 hrs	1.195	1.051	1.364	5.940	4.148	10.498	2.362 ± 9.453E-02	Y = -0.183 + 2.362X	0.024

Results of Figure (1) showed that Sodium hypoclorite with LD_{50} increased the activity of AST with value 121 U/I and ALT with value 135 U/I, however LD_{95} maximized AST with value 143 U/I and ALT with value 148 compared to the control (58 and 63, respectively). At the same time, the LD_{50} and LD_{95} of Sodium hypochlorite decreased the total protein of the land snail; *E. vermiculata* with values (2.80 and 1.40 g/100 ml), respectively, and it also showed a decrease in albumin dose at LD_{50} and LD_{95} with values (1.20 and 0.80) compared to the control, which recorded total protein and albumin with values (7.00 and 4.30, respectively).

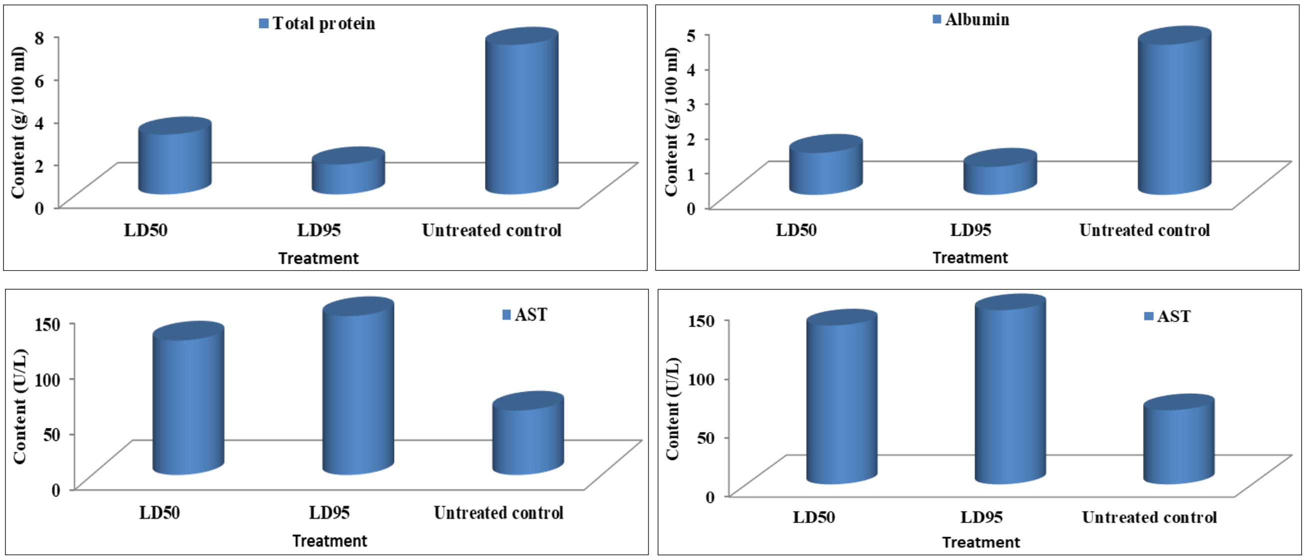

Fig 1: Effect of LD_{50} and LD_{95} of Sodium hypoclorite on total protein content, albumin, Aspartate aminotransferase, and Alanine Transaminase activity in the land snail, *E. vermiculata* after 72 hrs of treatment

Data in Table (3) demonstrated how Sodium hypochlorite causes clinical symptoms in *E. vermiculata* during treatment. The weight of the treated snails (1 and 2 g) decreased as a result of the compound as compared to the untreated snails (4 and 3 g). The loss of a portion of the body's water content could have caused this. Mucus colors in treated animals altered from creamy in control animals to brown in treated animals, which could be because of the compound's impact on mucus cells and structure. Additionally, following treatment, the snails died as a result of the mucus secretion's decision and bubbles.

Table 3: Clinical symptoms on the land snail; *E. vermiculata* during seven days of treatment with the Sodium hypochlorite

Parameters	Un treated snails	Treated snails
Body weight	3 - 4 g	1 - 2 g
Color of mucus	Creamy	Brown
Viscosity of mucus	High viscosity	Low viscosity (liquid).
Mucus status l	Normal	Bubbles and then dehydration
Animal movement	Normal	Slower

Discussion

The use of sodium hypochlorite for controlling land snails presents a promising approach, particularly due to its effective molluscicidal properties. Sodium hypochlorite solutions have demonstrated high biocidal power, particularly when combined with agents that generate hypochlorous acid upon application (Fukuzak, 2006) [18]. Ibrahim *et al.*, (2023 [8]) Illustrated the toxic effect of NaOCl on *B. alexandrina* snails at LC_{50} (1.25 ppm) and agree with the results of current study the maximum dose of 2 ppm caused a 13.33% death rate, the results of the study illustrated the activity of the liver enzymes AST and ALT and that matched with by El-Deeb *et al.*, (2017) [19].

The snails that received sub lethal doses of NaOCl (LC_{10} and LC_{25}) had hemolymph with lower levels of albumin and total protein than the control group. This study was comparable to one by (Mohammed 2012) [20], who reported that following four weeks of exposure to sub lethal doses of the insecticide's regent, hemolymph total protein and albumin doses decreased in *B. alexandrina* snails. The damage and cellular degeneration of the treated snails' digestive glands and ovotestis caused this decline. Exposure of the land snail; *E. vermiculata* to NaOCl, decrease the body weight of snails, change the color of mucus and increase the secretion of it causing dehydration and this in agreement of (Guglielmi, *et al.*, 2024) [21]. Sodium hypochlorite, a biocidal compound, can cause cellular damage and oxidative damage in snails, impacting their metabolic processes and broader ecological impact in water bodies (Ebenezer and Ki 2014) [22]. Sodium hypochlorite, a biocidal compound, can cause cellular damage and oxidative damage in snails, impacting their metabolic processes and broader ecological impact in water bodies (Ebenezer and Ki 2014) [22].

Conclusion

The study investigated the effects of sodium hypochlorite on mortality rates of *E. vermiculata* land snails at different doses and exposure times. Mortality rates rose at higher doses and longer exposure, with no deaths at low doses after 12 hours but considerable increases after 72 hours. The LD_{50} and LD_{95} values indicated that prolonged exposure made snails more sensitive to toxicity, requiring lower doses for lethality. Additionally, sodium hypochlorite elevated the activity of key enzymes (AST and ALT) while decreasing total protein and albumin levels in the snails. Clinical symptoms included weight loss and changes in mucus color, suggesting damage to mucus-producing cells.

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