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Anti-fertility effect of three inorganic salts against land snail, *Massylaea vermiculata*, (O.F. Müller1774) and their field efficiency



Abstract

Background: The ovotestis gland (the hermaphrodite gland) is a reproductive system organ that affects fertility of land snails. Thus, the present study was conducted to evaluate the effect of three inorganic salts (sodium carbonate, sodium benzoate, and sodium nitrate) against the land snail, *Massylaea vermiculata* under laboratory and field conditions. Snails were treated with serial concentrations of the tested salts for seven days using thin-layer film technique. The LC_{50} of each salt was determined, and the effect of $\frac{1}{2}LC_{50}$ of each was tested on egg-laying. In addition, LPO enzyme was determined, and the histological effect of the ovotestis gland was inspected. The field efficiency of sodium carbonate and sodium nitrate were evaluated as a spray for 21 days on pomegranate nursery trees at Giza Governorate. The effects of inorganic salts were compared with methomyl (the compound recommended by the Egyptian Ministry of Agriculture against land snails in the field).

Results: The laboratory results revealed that sodium carbonate and sodium nitrate were more toxic than sodium benzoate, whereas the LC_{50} were 2.4, 9.6, and 11.8% for the three inorganic salts, respectively. Sodium carbonate and sodium nitrate achieved complete inhibition for egg production, while sodium benzoate gave 37.7% compared to 96.8% for hatchability control. Sodium carbonate and sodium benzoate exhibited significant effect on LPO enzyme, while a non-significant effect was recorded by sodium nitrite compared with control. Regarding the histological effect, sodium carbonate and sodium nitrate had the same effect on ovotestis, as a necrobiotic change was observed in the ova, and diminishing in spermatozoal concentration, while sodium benzoate caused decreasing in spermatozoal concentration without any alteration in the ovarian compared with control. Regarding the field results, sodium carbonate and sodium nitrate achieved 78.4 and 79.0% reduction in land snail populations, respectively, compared with 75% for methomyl compound.

Conclusions: The tested inorganic salts have highly toxic effect against the land snail, *M. vermiculata*, and they can be used in field control programs, after conducting more studies on their effect on the soil and other environmental components.

Keywords: Massylaea vermiculata, Inorganic salts, Toxicology, Histology, LPO enzyme, Egg lying, Pest control

Background

Land snails cause damage to different kinds of plants causing great economic loss in nurseries, agricultural field, orchards, and greenhouses, in many parts of the world (Bonnelly, 1965; Calabrese et al., 1977). In Egypt, the chocolate band snail, *Massylaea vermiculata* (O.F. Müller 1774) (*Eobania vermiculata*, The name was modified by Bouaziz-Yahiatene et al., 2017) is one of the most known pest species, widespread in several areas in ornamental, horticulture, and field crops (Shehata et al., 2009). Therefore, continued efforts are conducted to identify and test more effective molluscicides against this

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snail species. Many publications, about molluscicides' effect, are found in the literature. Most researches are restricted to investigate the toxicological effects against target snails. However, information about the biological effects, which helps in more understanding of their mode of action are still lacking (El-Bolkiny et al., 2000). Methomyl is usually recommended to be used as a molluscicide against land snails (Abo-Bakr, 1997). It is still the common compound usually used to protect crops from land snail's attack. For controlling land snails in agricultural fields, there should be more studies on new effective and safe materials for the environment (EL-Shahat et al., 2009; Abbas Nada, 2020).

Sodium benzoate is a widely used preservative in the food industry. It is used as an antifungal agent in juices and sweet products (Salkowski, 1975; Stanojevic et al., 2009). Sodium nitrate is also used to conserve fish and meat products, with an effective concentration of 0.02% (Noel et al., 1990; Stanojevic, et al., 2009). European commission limits for sodium benzoate is 0.5% (EC, 1995).

Inhibition of growth and activity of microorganisms can be achieved by lowering pH of medium, or by increasing the concentration of sodium nitrate or sodium benzoate (Restaino et al., 1981; Stanojevic et al., 2009). Sodium benzoate has decreased the cell line viability and induced both cell cycle arrest and toxic events indicating their high potential of being cytotoxic and genotoxic material (Aldwany Amal et al., 2018). LPO product accumulation in human tissues is a major cause of tissue and cellular dysfunction that plays a major role in aging and most age-related and oxidative stress-related diseases (Negere Anne et al., 2010).

The present work aims to study the biological and toxicological effect of sodium carbonate, sodium benzoate, and sodium nitrite against the chocolate band snails, *Massylaea vermiculata*, to expand the possibility for more effective damage control programs of this pest species in Egyptian agricultural fields.

Methods

Tested compounds

- a. **Inorganic salts.** Three tested inorganic salts were used in this study i.e., sodium carbonate (NaCO₃), sodium benzoate (C₇H₅NaO₃), and sodium nitrate (NaNO₃). Each salt is a white powder 99%, produced by Biochem for library chemicals.
- b. Methomyl compound (Common name).

Trade name. Newmyl (20% SL) is a carbamate insecticide recommended to be used The EMALR, as bait against land snail infestation in Egyptian Agricultural

fields, at the rate of $8-10~\rm kg/Fed$. The LD $_{50}$ value for rats is $17-24~\rm mg/kg$. It was obtained from Kafr El-Zayat Company, Egypt.

Experimental animals

Adult individuals of chocolate band snails, *Massylaea vermiculata* were collected from citrus nursery at Abu-Rawash, Giza Governorate. Animals were transferred to the laboratory of the Plant Protection Research Institute, Agricultural Research Center. Animals were kept in small glass boxes $(55 \times 65 \times 40 \text{ cm})$ containing moist soil (8-10-cm-high) and covered with a rubber band to prevent snails from escaping. Boxes were provided with fresh green lettuce and carrot slices. Calcium powder was spread on soil surface as a source of calcium, three times per week. Dead animals and detritus of rearing were daily removed.

Laboratory experiments

Contact (thin layer film) technique

The thin layer film technique was used according to Asher and Mirian (1981). Different concentrations of each tested inorganic salt were applied using water in a Petri-dish, (9-cm-diameter). Two ml of each concentration was spread on the inner surface of each Petri-dish, by moving the dish gently in circles. Water was evaporated under room conditions in a few minutes, leaving a thin layer film of each compound on the surface of the Petri-dish. Snails were exposed to the candidate concentration for one week (two snails/one Petri dish, and five replicates for each concentration). A parallel control test was conducted using plain water. Dead snails were daily counted and removed. Mortality percentages and LC_{50} values were determined according to Finney (1971).

Effect of inorganic salts on egg-laying

Snails were treated with ½ LC $_{50}$ of each tested inorganic salt *i.e.*, 1.2, 5.9, and 4.8% for sodium carbonate, sodium benzoate, and sodium nitrate, respectively, using the thin layer film technique for one week. Three replicates were used (each of 20 animals) for each treatment. Animals were transferred in small glass boxes containing a mixture of sandy soil, clay soil, and patmos (1:1:1 ratio), and moistened with water three times a week, and kept in 20 ± 2 °C and 80 ± 5.0 R.H%. The cultural boxes were checked daily searching for clutches of eggs. Newly deposits clutches were removed with soil, placed in another box and observed daily until hatching to calculate the hatchability percentages (Hussein et al., 2005).

Effect of lipid peroxidase (LPO) activity

LPO activity was measured, after seven days of treatment with $\frac{1}{2}$ LC₅₀ of each tested compound compared with control using contact technique.

· Sample preparation.

After removing of snail shells, 1 g of snail tissue was homogenized for 3 min under cooling in a homogenizer with 10 ml of sodium chloride 0.9 N per 1 g tissue and then centrifuged (4000 r.p.m. for 15 min.). The enzyme activity was determined according to Ohkawa et al. (1979).

The obtained data were statistically analyzed by ANOVA and LSD at (p < 0.05) using the Costat program (COHORT, 2005).

Histological studies

The effect of ½ LC₅₀ of the three tested inorganic salts on the ovotestis tissues of treated M. vermiculata was studied after seven days of treatment comparing with untreated snails. Autopsy samples were taken from the ovotestis of treated and untreated snails, then fixed in 10% formol saline for 24 h. Washing was done in tap water, and then serial dilutions of alcohol (methanol, ethyl, and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degrees in a hot air oven for twenty-four hours. Paraffin bee wax tissue blocks were prepared for sectioning at 4-microns-thickness by slide microtone. The obtained tissue sections were collected on glass slides, deparaffinized, and stained by hematoxylin and eosin stain for routine examination by light electric microscope (Banchroft et al., 1996).

Field application

Two inorganic salts, sodium carbonate, and sodium nitrite were tested as a spray compared with methomyl compound against *Massylaea vermiculata* on pomegranate nursery trees which planted in Nursery of the Agricultural Ministry, EL-Dokki district Giza Governorat. Eight plots were chosen (each of 50 m²), two replicates were used for each compound and others for control. At least two meters distance was kept between each plot and the other. Live snails were counted on random trees in each plot, pre- and post-treatment at 1, 3, 7, 15, and 21 days of treatment. The reduction in the snail population was calculated after 21 days of treatment according to the formula described by Henderson and Tilton (1952).

$$Population \, Reduction \, \% \, = 1 - \frac{C_1 \times T_2}{C_2 \times T_1} \times 100$$

 C_1 =Number of snails in control before application. C_2 =Number of snails in control after application. T_1 =Number of snails in treatment before application. T_2 =Number of snails in treatment after application.

· Statistical analysis

Data Analysis, obtained data were analyzed as one way ANOVA, using Proc ANOVA in SAS software (Version 9.1; SAS Institute, Cary, NC, USA) (SAS Institute, 2008).

Results

Laboratory studies

Toxicity tests

Data in Table 1 show the efficacy of three tested inorganic salts on land snails, *Massylaea vermiculata* using contact technique after one week of treatment. The concentrations of sodium carbonate at 1, 2, 4, 6, and 8% caused 20, 40, 50, 80, and 90% mortality, respectively, while sodium benzoate achieved 10, 30, 50, and 70% mortality with 6, 8, 13, and 15% concentrations, respectively. Farther more, sodium nitrite gave 10, 20, 40, 50, and 70% mortality at the rate of 0.5, 2, 8, 13, and 15%, respectively. The LC_{50} values were 2.4, 11.8, and 9.6% for sodium carbonate, sodium benzoate, and sodium nitrite, respectively.

Effect of the tested inorganic salts on egg production and hatching percentage

The effect of the tested inorganic salts on the egg production of land snail, *Massylaea vermiculata*, shown in Table 2. Results indicated that sodium carbonate and sodium nitrite exhibited complete inhibition for egg

Table 1 Effect of three inorganic salts against land snail, *Massylaea vermiculata*, after seven days of treatment

Salt	Concentration (%)	Mortality (%)	LC ₅₀ (%)
Sodium carbamate	1	20	
	2	40	2.4
	4	50	
	6	80	
	8	90	
Sodium benzoate	6	10	11.8
	8	30	
	13	50	
	15	70	
Sodium nitrite	0.5	10	9.6
	2	20	
	8	40	
	13	50	
	15	70	

Table 2 Effect of ½ LC₅₀ of three inorganic salts on egg product of land snail, *Massylaea vermiculata*

Treatment	Concentration %	No. of egg masses Mean \pm SE	No. of egg in one mass Mean \pm SE	No. of hatching in one mass Mean ± SE	Hatchability %	
Control	_	16.7 ± 1.5 ^b	96.5 ± 0.4 ^b	93.6 ± 1.7 ^b	96.9	
Sodium carbamate	1.2	0.0	0.0	0.0	0.0	
Sodium benzoate	5.9	3.1 ± 0.0^{a}	16.6 ± 2.1^{a}	13.6 ± 1.1^{a}	81.9	
Sodium nitrite	4.8	0.0	0.0	0.0	0.0	

Hatchability $\% = \frac{\text{No. of hatching in one mass}}{\text{No. of egg in one mass}} \times 100$

Table 3 Effect of $\frac{1}{2}$ LC₅₀ of three inorganic salts on lipid proxidase (LPO) activity (nmol/g) tissues in land snail, *Massylaea vermiculata*

Control	Treatment					
	Sodium carbonate	Sodium benzoate	Sodium nitrite	LSD		
Mean ± SE 2.5 ± 0.6 ^b	Mean±SE 11.9±2.3 ^a	Mean±SE 15.3±1.9 ^a	Mean ± SE 4.2 ± 1.6 ^b	5.6		

^a Significant, ^bnon significant at p < 0.05

production in treated snails while sodium benzoate recorded 81.9% hatchability comparing with 96.9% for untreated snails. There was a significant difference in the fecundity of treated snails with sodium benzoate compared with control.

Effect of tested inorganic salts on lipid peroxidase

Data in Table 3 show the effect of the three tested inorganic salts on lipid peroxidase enzyme in *M. vermiculata* after 7 days of treatment. The results revealed that sodium carbonate and sodium benzoate achieved significant effects compared with control. Both compound caused a severe increase in the secretion of the enzyme as the level of the enzyme was increased from 2.5 to 11.9 and 15.3 nmol/g tissue for the two salts, respectively. While it slightly increased to 4.2 nmol/g tissue in case of sodium nitrite.

Effect of three inorganic salts on ovotestis tissues of Massylaea vermiculata

The histological effect on ovotestis of M. vermiculata treated with ½ LC_{50} of the three tested inorganic salts (sodium carbonate, sodium benzoate, and sodium nitrite) is shown in Figs. 1, 2, 3, 4, and 5 after 7 days of treatment. Figure 1 showed the normal tissues of the ovarian structure with different follicular and ova in the untreated snails. Figure 2 showed the normal spermatozoal in the sac. Figure 3 showed necrobiotic changes in the ova, with the reduction in spermatozoal concentration in

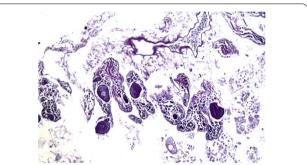


Fig. 1 Showed ovotestis of untreated *M. vermiculata,* different follicular and ova

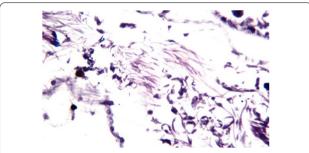


Fig. 2 Showed the normal spermatozoa in the sac. of untreated *M. vermiculata*

the ovotestis treated with sodium carbonate. Figure 4 showed no histopathological alteration in the ovarian while the spermatozoal showed diminished concentration after treatment with sodium benzoate, while Fig. 5 showed the effect of sodium nitrite which caused the same effect of sodium carbonate, whereas, it induced necrobiotic change in the ova with a reduction in spermatozoal concentration.

Field studies

The field performance of the two tested inorganic salts (sodium carbonate and sodium nitrate) compared with

^a Significant, ^bnon significant at p < 0.05

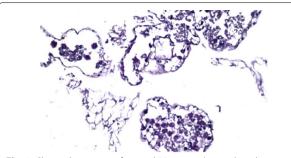


Fig. 3 Showed ovotestis of treated *M. vermiculata* with sodium carbonate. Necrobiotic change was detected in the ova and some follicles, associated with diminished in the spermatozoal concentration

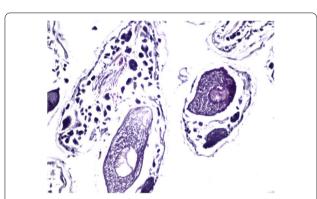


Fig. 4 Showed ovotestis of treated *M. vermiculata* with sodium benzoate. There was no histopathological alteration in the ovarian structure while the spermatozoa showed diminished concentration

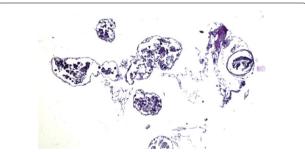


Fig. 5 Showed ovotestis treated *M. vermiculata* with sodium nitrate, Necrobiotic change was detected in the ova and some follicles, associated with diminished in the spermatozoal concentration

methomyl (recommended compound) against *Massylaea vermiculata* population is shown in Table 4. The two tested inorganic salts achieved good result against land snail, *M. verniculata*, compared with the recommended compound. Results revealed that sodium carbonate and sodium nitrite caused 84.6 and 80.8% population

reduction in snails, respectively, compared with 73.7% by methomyl compound.

Discussion

Laboratory studies

A gradual increase in the mortality percentage was observed with the increase in increasing concentration of the tested salts. Sodium carbonate was the most toxic salt against land snail, Massylaea vermiculata, followed by sodium nitrite and sodium benzoate. It was noticeable that inorganic salts extended a highly toxic effect after a long time of treatment. This result may be due to that these compounds take a long time arriving to the site of action in the treated snails' body. Corrao Norah et al. (2006) reported that 500 ppm of nitrite gave low mortality against adult and juvenile snails till 96 h after treatment. The influence of adding chloride on nitrite enhanced toxicity against Cray fish, Orconectes limosus after 96 h (Dickinson, 2016; Kozak et al., 2005). Hegab et al. (2013) studied the effect of copper sulphate against Eobania vermiculata and found that 1, 3, 5, and 7% concentrations caused 65, 70, 80, and 85% mortality, respectively, after seven days of treatment. The juveniles of *E*. vermiculata were more sensitive to inorganic salts than adults (Hegab et al., 2013; Ismail & Hegab, 2006). While Ismail et al. (2010) reported that copper hydroxide had a low effect against Monacha cartusiana under laboratory and field conditions.

The effect of the tested inorganic salts on land snail, M. vermiculata, egg production may be due to deformation in embryogenesis and losing embryos. Goddard and Martin (1966) recorded that hatching new snails were reduced after treatment with embactin benzoate. Moreover, Adewunmi et al. (1987) observed that the reduction in the glycogen and protein content leads to decreased egg production in Biomophalaria glabrata and Lymnaea columella. El-Bolkiny et al. (2000) found that 0.3 ppm of Diethyldithiocarbamate caused inhibition in egg hatchability of Biomophlaria alexandrina after five days of treatment. Chauhan et al. (2011) reported that 20 and 40% Plant extract, Lantana indica against water snail, Lymnaea acuminate, reduced the fecundity and hatchability of snails. After 15 days of treatment with nitrite, the snail egg production was reduced compared with untreated snails (Cofone et al., 2020). Sharaby et al. (2008) found that inorganic salts as ZnO₄ KBr, CuCl₂ KOH, and KI gave 100% sterility against red palm weevil, Rhynchophprus ferrugineus. El-Sabagh Marwa et al. (2015) showed that inorganic salts NaOH, Ca (OH)₂, and ZuSO₄ achieved 100% sterility against cotton leaf worm, Spedopatera littoralis, causing several malformations in the pupal stage. The effect of the three tested inorganic salts on lipid peroxidase (LPO) enzyme in M. vermiculata

Table 4 Field Performance of two inorganic salts against land snail, *Massylaea vermiculata*, comparing with methomyl compound after 21 days of treatment

Tested compound	Concentration	No. of snails before treatment		No. of live snails after treatment		Population
		Total	$Mean \pm SD$	Total	Mean \pm SD	reduction%
Control	_	247	24.7 ± 9.3 ^b	192	19.2 ± 7.3 ^b	_
Sodium carbonate	80 g/L	338	33.8 ± 11.1 ^a	40	$4.4 \pm 1.6^{\circ}$	84.6
Sodium nitrite	150 g/L	394	39.4 ± 11.3 ^a	59	5.9 ± 3.3 ^c	80.8
Methomyl	20 mL/L	234	23.4 ± 2.2^{b}	48	$4.8 \pm 5.0^{\circ}$	73.7
LSD					7.36	

^a Significant, ^bnon significant at p < 0.05

after seven days of treatment affected membrane fluidity association of biomolecules (membrane-bound proteins or cholesterol) with membrane and cell health (Halliwell & Gutteridge, 2015). Lipid peroxidase is wildly used as a biomarker of oxidative stress as raising the level of oxidation causing severe damage to the enzyme in snail species (Barky et al., 2012; El-Shenawy et al., 2012; Al-Daihan et al., 2010; Ali et al., 2015; Bhagat et al., 2016). Wang et al. (2014) reported that LPO enzyme increased in the digestive gland of Achtina fulica after treatment with broad-spectrum antimicrobial agent. The increase in LPO enzyme activity at high temperature is due to an increase in polyunsaturation in mitochondrial membrane leading to high mitochondrial respiration, with increase in the information of ROS (Bhagat et al., 2016). The same results of the histological effect on ovotestis of M. vermiculata treated with ½ LC₅₀ of three tested inorganic salts (sodium carbonate, sodium benzoate, and sodium nitrite) were obtained by Abbas Nada (2020) on ovotestis of E. vermiculata after being treated with an emamectin benzoate. The necrobiotic changes and reduction in spermatozoal concentration may be attributed to the tested inorganic salt, which causes mortality for snail sperms, and induced problems to the transport of sperms to the ova that prevent the production of new hatching. This result may be due to that the inorganic salts were absorbed by the reproductive system tissues. Xiao et al. (2000) reported that the lipophilic of botanical extracts affected membrane lipid, and changed permeability leading to water loss cause dehydration, and lead to abnormalities of egg laying. This may be due to the molluscicidal effect that destroyed the ova, by preventing formation and development. These results agreed with Zhou et al. (1993) clarifying that the niclosamide caused a reduction in the number of spermatozoal of Biomophalaria glabrata. Also, Mohamed et al. (2004) registered that Mepiquat chloride caused abnormal changes in the tissues of ovotestis glands in Biomophlaraia alexandrina. The same results were observed by Amaeze et al. (2011) they found that high concentrations of NPK fertilizers caused necrosis in the tissues of the genital organs of the land snail, *Archachatina marginata*. Dethiert (1955) and Frings (1946), indicated that inorganic salts are transported across the cell membrane, and their penetration was according to the order of ionic mobility which will determine the order of effectiveness. Moreover, the effect of inorganic salts may be due to salts ion accumulation in the snails' tissue after treatment, causing an impending effect on the viability of sperm and ovaries of snails.

Field studies

The two tested inorganic salts achieved good results against land snail, M. verniculata compared with methomyl. Abbas Nada (2020) reported that embactin benzoate and chitosan achieved 66.6% and 74.3% population reduction of snails, respectively, under field conditions. Mobarak Soha et al. (2021) indicated that clodinafoppropargyl caused 90.7% population reduction in the land snail, Helix aspersa, compared with 75.0% for methomyl, after 21 days of treatment. Ali (2017) reported that Round Up caused 17.2% population reduction of the land snail, Monacha cartusiana. Also, NPK fertilizer was examined by Eshra et al. (2016) under field conditions against Land snails, Monacha obstructa, it gave 66.8% population reduction after seven days of treatment. Samy et al. (2015) reported that methomyl reduced land snail, Monacha sp., population in cabbage and lettuce fields. Chen et al. (2019) evaluated the novel molluscicide PPUO7 (the mixture of Kaolin and black carbon 25% PPUO7 WP) in the field against land snail, Oncomelania hupensis. They revealing that the molluscicidal effect at 2.0 g/m² provided 95% reduction in snails population.

Conclusions

From previous results, it is cleared that the three tested inorganic salts have a high toxic effect on the tested land snail, *Massylaea vermiculata*. They caused mortality and fertility reduction of live treated snails under laboratory

conditions by inhibiting the egg production. Therefore, they can recommend to be used in land snail control programs in Egyptian agricultural fields. However, more future studies should be conducted on the effect of this inorganic salts on soil and other environmental components.

Abbreviations

 LC_{50} : Half Lethal Concentrations; LPO: Lipid Peroxidase Enzyme; SL: Soluble Liquid; EMARL: Egyptian Ministry of Agriculture and Land Reclamation; LD_{50} : Half Lethal Dose; Kg/Fed: Kilogram per Feddan; rpm: Round per minutes; ANOVA: Analysis of Variance; LSD: Least significant difference; SAS: Statistical Analysis System.

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Authors' contributions

Not applicable. One author is executing the search. The author read and approved the final manuscript.

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Availability of data and materials

The data sets used and analyzed during the current study are available in this article, contain studies on animals (snails) performed by the author.

Declarations

Ethics approval and consent to participate

Regarding animals ethics approval by Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt. This article does not contain any studies with human participants performed by the author.

Consent for publication

The author consent for publication.

Competing interests

The authors declare that they have no competing interests.

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