INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
22–0421/2023/29–2–127–132
DOI: 10.17957/IJAB/15.2011
http://www.fspublishers.org

Full Length Article

How does Acetylcysteine Compound Affect the Shell of Land Snails?

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Received 26 October 2022; Accepted 12 December 2022; Published 27 January 2023

Abstract

Shell is the first line of defense in land snails protecting them from any risk; however, it is considered one of the impediments when controlling this pest in agricultural fields. The present study was aimed at to test the effect of acetylcysteine on the shells of two land snail species, *Monacha cartusiana* (Muller, 1774) and *Eobania vermiculata* (Muller, 1774) under laboratory and field conditions. Both species were treated with consecutive concentrations of the tested compound for one week using thin film layer technique, and the median lethal concentration (LC_{50}) was calculated. Toxic actions of sub lethal concentration (LC_{25}) on carbonic anhydrase activity and some elements level of shell; including calcium, phosphorus, magnesium and potassium; were estimated. The efficiency of acetylcysteine was also tested *via* spray technique for three weeks in the field. The laboratory results indicated that the most effective concentrations, achieving 100% mortality, were 1.8 and 3.6% for *M. cartusiana* and *E. vermiculata*, respectively, while the LC_{50} were 0.6 and 1.5% for both species, respectively. Moreover, the chemical compound induced remarkable decrease in carbonic anhydrase activities and caused reduction in calcium levels in the shell of both species. Treatment caused rising the contents of other elements. In addition, it caused the shell of *M. cartusiana* to be weaker. Concerning the field results, acetylcysteine achieved 94.7 and 90.1% reduction in the population of snails comparing with methomyl (MALR recommended compound) which showed 76.4 and 74.9% reduction of *M. cartusiana* and *E. vermiculata* population, respectively. Results revealed that *M. cartusiana* were more susceptible to acetylcysteine than *E. vermiculata*. Finally, it can be concluded that acetylcysteine achieved significant results, under laboratory and field conditions, against the two species of land snails by reducing the number of snails through damaging their protective shells. So, acetylcysteine can be used as an effective molluscicide *via* spray technique under Egyptian agricultural field conditions.

Keywords: Acetylcysteine; Shell elements; Carbonic anhydrase; Molluscicides; Land snails

Introduction

Terrestrial molluscs, snails and slugs are very important group that normally spread to different areas through human activities. Snails are regarded as pests due to their damage to cultivated crops as well as their role in carrying parasitic diseases affecting humans (Barker 2002; Hajian-Forooshani *et al*. 2020). Land snail, *Monacha cartusiana* and *Eobania vermiculata* (Muller 1774) are the most prevalent species in Egypt. They are recorded on clover, wheat, mango, orange, grapes and wood trees (Reham and Ramadane 2020). Land snails have shell which cover their soft body and allows the snails to survive under severe conditions of drought and heat (Crowell 1977). It is established that carbonic anhydrase speeds up the formation of biocarbonate, production of calcium carbonate and development of the shell (Wilbur and Jodrey 1955; Muller *et al*. 2013). The enzymes involved in the formation of the shell are phosphatase, phosphorylase and carbonic anhydrase (Digby 1968; Mobarak and Kandil 2014). It is difficult to control land snails because of their shells that protect them from any foreign compounds. Chemical molluscicide such as metaldehyde has strong effect on land snails. However, it cannot be used in moist places because treated snails quickly regain its moisture loss from their bodies and recover. Likewise, methomyl compound has negatively affected non-target species and increases environmental pollution (Mobarak and Kandil 2021; Mobarak *et al*. 2021). Therefore, alternative effective safe products should be tested against land snails. N-acetylcysteine (drug) is a sulfhydryl consisting of – compound and derived from amino acid L-cysteine. It is usually used to reduce the viscosity of mucus secretions and increase the ciliary clearance rate (Blackwell *et al*. 1996; Overveld *et al*. 2005; Tardiolo *et al*. 2018; Mobarak *et al.* 2021). Keeping in mind the above discussions, this study was designed to estimate the effect of acetylcysteine on the shells of two species of land snail; clover land snail, *M. cartusiana* and chocolate band snail, *E. vermiculata*, under laboratory and field conditions.

To cite this paper: Ahmed HY, RA Kandil, SA Mobarak (2023). How does acetylcysteine compound affect the shell of land snails? *Intl J Agric Biol* 29:127–132

Materials and Methods

Experimental compound

Acetylcysteine (600 mg powder) was purchased from South Egypt drug Industries Company (Sedico) Egypt. The median lethal dose (LD_{50}) of acetylcysteine for rats is 5050 mg/kg (Golden 1971). Methomyl Lannate (90% Powder, Kafer El-Zayat Company, Egypt), is a carbamate insecticide compound recommended by Ministry of Agriculture and Land Reclamation (MALR) against land snail infestation in agriculture crops, at the rate of 8–10 kg/feddan. The LD_{50} value for rats is 17–24 mg/kg.

Tested animals

Adult animals of the two species of land snails; *M. cartusiana* were obtained from clover field of Sumasta, Beni-Suef Governorate, Egypt, (N28°54'13 E30°54'36) and *E. vermiculata*, were collected from citrus trees at the nursery of Abu-Rawash district, Giza Governorate, Egypt, (N30°"8" E 31°. 5" 26"). Snails were transported to the Laboratory of the Harmful Animals Research Department, Sids Agriculture Research Station, Agriculture Research Center, (N28°54"21" E 30°57"12"). Snails of each species were put in plastic boxes having 8–10 cm moist soil, offered with fresh leaves of lettuce and covered with muslin cloth secured with rubber band to impede snail from escaping. Snails were acclimated for two weeks at 20 ± 2°C in the laboratory before beginning of the experiments.

Laboratory experiments

Thin film layer technique: The method of thin film layer was used according to Asher and Mirian (1981). Serial concentrations (0.15, 0.3, 0.6, 1.2, 1.8, 2.4 and 3.6%) of acetylcysteine were applied in Petri-dishes, for each of *M. cartusiana* and *E. vermiculata* individually. Two mL of each concentration of the compound was spread on the inner surface of each Petri-dish by moving the dish in circles. Water was evaporated in few minutes under room temperature leaving a thin film layer of the tested compound. A parallel control test was conducted using tap water only. The dead animals were daily counted and removed. Mortality percentages were calculated and LC_{50} value was determined after seven days of treatment according to Finney (1971).

Biochemical studies: Each of the tested land snail species were treated individually with LC_{25} of acetylcysteine for seven days to estimate the effect of acetylcysteine on carbonic anhydrase activity and the shell contents of calcium, phosphorus, magnesium and potassium.

Sample preparation

After seven days of treatment, the shell was removed from treated and untreated snail species. Then, the shell was grinded to determine the elements content of the shell. On the other hand, one gram of the snail soft tissue was homogenized under cooling for three minutes with 10 mL of sodium chloride 0.9 N, and then centrifuged (5000 rpm for 30 min). The resulting supernatant was used to determine the carbonic anhydrase activity.

Determination of carbonic anhydrase activity

The carbonic anhydrase activity was determined according to Barman (1974) using Novus Biologicals kits (USA). The developed color was measured at 450 nm using JENWAY 6305 UV/Vis Spectrophotometer.

Shell element content determination

Calcium (Ca) level determination: Calcium ion produces a blue color with methylthymol blue in an alkaline medium. The intensity of color is in proportion to the calcium concentration. The presence of hydroxyl 8-quinoline eliminates the interference due to the magnesium ions. The developed color was measured at 585 nm according to Gindler and King (1972) using Biodiagnostic (diagnostic and research reagents) kits purchased from Biodiagnostic Company, Egypt.

Phosphorus (P) level determination: Inorganic P present in shell solution as phosphate forms a phosphomolybdate complex with molybdic acid. The complex is reduced by stannous chloride to a blue color which can be measured calorimetrically at 640 nm according to El-Merzabani *et al*. (1977) using Biodiagnostic (diagnostic and research reagents) kits obtained from Biodiagnostic Company, Egypt.

Magnesium (Mg) level determination: Mg^{2+} react in an alkaline medium with the metallochromic dye calmagite to form a chromophore which absorbs at 520 nm according to Teitz (1983) using Biodiagnostic (Diagnostic and Research Reagents) kits purchased from Biodiagnostic Company, Egypt.

Potassium (K) level determination: Potassium ions in protein-free filtrate react with sodium tetraphenyl boron forming colloidal solution which can be measured calorimetrically at 420 nm according to Sunderman and Sunderman (1958) using Biodiagnostic (Diagnostic and Research Reagents) kits purchased from Biodiagnostic Company, Egypt.

Field experiments

Four plots (20 m^2 each) planted with clover and infected with *M. cartusiana* were chosen at Quftan, Sumsta district, Beni-Suef Governorate, Egypt, (N 28°54'13 E30°54'36). Another four plots planted with young citrus trees and infested with *E. vermiculata* were chosen at Abu-Rawash, Giza Governorate, Egypt, (N 30°"8" E 31.5° "26"). Other plots left without any treatment were taken as control. The most effective concentrations of acetylcysteine in the laboratory

tests were 1.8 and 3.6% for *M. cartusiana* and *E. vermiculata*, respectively. These concentrations were evaluated, in the field, against both land snail species and compared with methomyl 2% (MALR recommended compound) using spray method. Two replicates were applied for each treatment and others for control. A distance of ten meters was left between the plots. Survived snails were counted in each plot (in four corners and in center of each plot) pre and post treatment at 1, 3, 7, 15 and 21 days. The reduction in population of snails was calculated 21 days' post treatment according to Henderson and Tilton (1952).

Statistical Analysis

Experimental design was completely randomized with different replicates**.** The results were statistically analyzed by one-way analysis of variance (ANOVA) and least significant difference (LSD) at ($P < 0.05$) using the COSTAT program (Glenn 2005).

Results

Laboratory studies: The efficacy of acetylcysteine

The results depicted that mortality percentage increased gradually with increasing acetylcysteine concentrations. However, the concentrations of 0.15, 0.3, 0.6, 1.2, 1.8, 2.4 and 3.6% presented 0.0, 10, 40, 80, 100, 100, and 100% mortality for *M. cartusiana* and 0.0, 0.0, 10.0, 30, 60, 90 and 100% for *E. vermiculata*, respectively. The LC_{50} values were 0.6 and 1.5% for *M. cartusiana* and *E. vermiculata*, respectively after seven days of treatment (Table 1).

Biochemical studies: Carbonic anhydrase activity

The results revealed the effect of LC_{25} of acetylcysteine on *M. cartusiana*, and *E. vermiculata*, after seven days of treatment. The results depicted that the activity of carbonic anhydrase decreased from 4.2 ng/mg in control to 1.5 ng/mg in treated *M. cartusiana*. It also showed the same trend in case of *E. vermiculata*, whereas it decreased from 5.10 ng/mg in control to 2.47 ng/mg in the treated snails. There were significant decreases in the enzymatic activities between control and treated snails (Table 2).

Effect of acetylcysteine on shell elements levels

The impacts of LC_{25} of acetylcysteine on shell levels of Ca, P, Mg and K are reported in Table 3 and Fig. 1–4. There were significant differences between treated and untreated snails. In the treated snails, Ca level decreased to 19.5 mg/g comparing with 24.1 mg/g in the control. Regarding P, Mg and K levels in *M. cartusiana* shell, values elevated to 15.1 mg/g, 0.9 mg/g and 27.5 mmol/L in treated snails compared to 13.7 mg/g, 0.67 mg/g and 25.5 mmol/L in the control, respectively. Concerning *E. vermiculata*, the Ca level decreased from 21.5 mg/g in control to 18 mg/g in treated snails and K level reduced from 25.1 mmol/L to 23.5 mmol/L. While P level was enhanced 14.9 mg/g in treated snails compared with 13.4 mg/g in the control. The level of Mg remained unchanged (0.55 mg/g) in both treated and untreated shells.

Table 1: LC_{50} determination of acetycysteine against land snails, *M. cartusiana* and *E. vermiculata*, after one week of treatment using thin film layer technique

Concentration (%)	*M. cartusiana*		*E. vermiculata*	
	Mortality (%)	LC_{50} (%)	Mortality (%)	LC_{50} (%)
0.15	0.0	0.6	0.0	1.5
0.3	10.0		0.0	
0.6	40.0		10.0	
1.2	80.0		30.0	
1.8	100.0		60.0	
2.4	100.0		90.0	
3.6	100.0		100.0	

Table 2: Effect of LC_{25} of acetylcysteine on carbonic anhydrase (ng/mg) activity of land snails, *M. cartusiana* and *E. vermiculata*, after one week of treatment

Group	Carbonic anhydrase activity (ng/mg)	
	M. cartusiana	*E. vermiculata*
Control	4.2 ± 0.23^{a}	5.10 ± 0.23^{a}
Treated	1.5 ± 0.12^{b}	2.47 ± 0.09^{b}
LSD	0.72	0.69

P < 0.05

* Data are expressed as mean ± SE

* Means, which share the same superscript symbol(s), are not significantly different

Table 3: Effect of LC_{25} on shell elements content of two land snails, *M. cartusiana and E. vermiculata*, after one week of treatment

Shell component	Species					
	M. cartusiana			*E. vermiculata*		
	Control	Treated	LSD	Control	Treated	LSD
Ca mg/g	24.1 ± 0.25^{a}	19.5 ± 0.28^{b}	1.1	21.5 ± 0.60^{a}	18.0 ± 0.46^{b}	2.1
P mg/ g	13.7 ± 0.08^{b}	15.1 ± 0.15^{a}	0.5	13.4 ± 0.25^{b}	14.9 ± 0.07^{a}	0.7
Mg mg/ g	0.67 ± 0.01^{b}	0.9 ± 0.01^{a}	0.02	0.55 ± 0.01^{a}	0.55 ± 0.01^{a}	-
K mmol /L	26.5 ± 0.28^{a}	27.5 ± 0.26^{a}	-	25.1 ± 0.10^{a}	23.5 ± 0.48^{b}	1.35

P <0.05

* Data are expressed as mean ± SE

* Means, which share the same superscript symbol(s), are not significantly different

The field performance of acetylcysteine

The efficiency of acetylcysteine on *M. cartusiana* compared with methomyl after three weeks of application using spray technique was evaluated. The results indicated that the tested compound caused 94.7% reduction in snails' population compared with 76.4% for methomyl. These results achieved significant reduction ($P < 0.05$) in *M. cartusiana* numbers (Table 4). Similar application against *E. vermiculata*, depicted that acetylcysteine achieved 90.1% reduction in snails´ population compared with methomyl which caused 74.9% reduction only. These results displayed significant reduction ($P < 0.05$) in snail numbers after treatment (Table 5).

Fig. 1: Untreated adult *M. cartusiana*

Fig. 2: Adult *M. cartusiana* treated with acetylcysteine showing broken shell

Fig. 3: Untreated adult *E. vermiculata*

Fig 4: Adult *E. vermiculata* treated with acetylcysteine showing color change of the shell

Discussion

The present study revealed the efficacy of acetylcysteine against *M. cartusiana* and *E. vermiculata*. The mortality percentages of both species were increased with increasing the compound concentrations. It may be due to the higher concentrations that reduced the ability of snails to repel the compound from their bodies by the mucus. The results also showed that *M. cartusiana* was more susceptible than *E. vermiculata*. These results may be attributed to the smaller size of *M. cartusiana* than *E. vermiculata*. Moreover, *M. cartusiana* secretes less mucus than *E. vermiculata*. Therefore, *M. cartusiana* is unable to excrete the compound from their bodies by mucus like *E. vermiculata*. Mucus is very important to snails and the mucus viscosity and production are changed post treatment (Livingstone *et al*. 1990; King and Rubin 2002). It was mentioned that 3.6% of acetylcysteine gave 100% mortality against *M. cartusiana* after seven days of treatment using bait technique (Mobarak *et al*. 2021).

In our study, treatment of both snail species with LC_{25} of acetylcysteine produced noticeable decrease in carbonic anhydrase activity. These results may be attributed to the impact of acetylcysteine on hepatopancrease which inhibits the activity of carbonic anhydrase. It was clarified that carbonic anhydrase speeds up the formation of bicarbonate and the production of calcium carbonate to form the shell (Wilbur and Jodrey 1955; Muller *et al*. 2013). Also, the results may be attributed to reduction of shell calcium rate of treated animals that led to the shell became weaker and breakable. This perception agreed with Wilbur and Jodrey (1955). The mucus of animals is very thick and has high concentration of calcium contents (South 1992). Acetylcysteine decreased the snail mucus calcium rate and consequently, leading to reduction in mucus viscosity. This effect may be due to carbonic anhydrase inhibition post treatment. This finding is agreed with (Mobarak *et al.* 2021). The tested compound may inhibit the activity of the enzyme leading to prevention of calcium depositions. Previous study recorded that shell thinning due to decreasing the carbonic anhydrase activity by abamectin and thiamethoxam led to prevent calcium carbonate production and mortality of land snail, *Theba pisana* post treatment (El-Gendy *et al*. 2019). Other study showed a significant inhibition in the activity of carbonic anhydrase of the mussel of *Mytilus galloprovincialis* post treatment with cadmium (Lionetto *et al*. 2016).

The findings of the current study showed that *M. cartusiana* was more susceptible to acetylcysteine than *E. vermiculata*. This may be due to the smaller size and weaker shells of *M. cartusiana* compared to *E. vermiculata*. Moreover, the results revealed that Ca level was significantly decreased in the shell of both species after treatment. These results may be attributed to carbonic anhydrase inhibition which is responsible for deposition of calcium in shells. As reported by Mobarak and Kandil

Table 4: Field application of acetylcysteine against land snail, *M. cartusiana* comparing with methomyl after three weeks of application as a spray technique

Treatment	Rate of application (g/L)	No. of snails survived pre-treatment		No. of snails survived post treatment		LSD	Reduction population (%)
		No.	Mean ± SE	No.	Mean ± SE		
Acetylcysteine	18	248	24.8 ± 1.0 [b]	12	1.2 ± 0.5 [d]		94.7
Methomyl	20	388	38.8 ±1.2 [a]	84	8.4 ± 0.5 [c]		76.4
Control	-	314	31.4 ± 4.0 [a]	288	22.8 ± 2.9 [b]	6.9	

P < 0.05.
* Data are expressed as mean ± SE
* Means, which share the same superscript symbol(s), are not significantly different

Table 5: Field application of acetylcysteine against land snail, *E. vermiculata* comparing with methomyl after three weeks of application as a spray technique

Treatment	Rate of application (g/L)	No. of snails survived pre-treatment		No. of snails survived post treatment		LSD	Reduction population (%)
		No.	Mean ± SE	No.	Mean ± SE		
Acetylcysteine	36	706	70.6 ± 8.9[a]	64	6.4 ± 0.8[c]		90.1
Methomyl	20	314	31.4 ± 1.9[b]	72	7.2 ± 0.9[c]		74.9
Control	-	704	70.4 ± 8.6[a]	644	64.4 ± 7.7[a]	17.6	

P < 0.05
* Data are expressed as mean ± SE
* Means, which share the same superscript symbol(s), are not significantly different

(2014), tannic acid reduced alkaline and acid phosphatase activities responsible for calcium participation in the shell of *E. vermiculata* and *M. cartusiana*. The results showed that P level increased in the shell of each tested snail species leading to reduction in the Ca level and reabsorbed Ca from the shell resulting in pathological changes. As mentioned in the previous study Taylor and Bushinsky (2009) that disturbances in the body level of P and Ca can lead to pathological changes. In addition, the Ca and Mg ions are known to have specific and opposite effect at the prejunctional nerve terminals of several cholinergic synapses (Jenkinson 1957). This finding supports our results, whereas the increased Mg level led to decreased Ca level causing weakness and breaking of shell of treated *M. cartusiana.* Similar results were reported in the eggshell in hens (Shastak and Rodehutscord 2015; Skrivan *et al.* 2016). The same trend occurred in case of K level, whereas its increase in the shell of *M. cartusiana* caused decreased Ca level which led to weakening of the shell of *M. cartusiana*. It was reported by Leach (1974) that K contents reduction in the hen causes egg shell thickness. While K level reduced in *E. vermiculata* compared to the control causing disturbance in shell component. Finally, treatment with acetylcysteine caused inhibition in the carbonic anhydrase responsible for the formation of the shell, consequently led to a decrease in the Ca level which led to shell weakness and making it fragile. It was investigated that acetylcysteine caused remarkable increase in alkaline phosphatase activity in *M. cartusiana* (Mobarak *et al*. 2021).

On the other side, the field results were in harmony with laboratory results, whereas it is proved that acetylcysteine was more effective against *M. cartusiana* than *E. vermiculata*. Moreover, the results confirmed that acetycysteine was more effective than methomyl against the two tested land snail species, as it caused high reduction percent in snail population. It may be due to the ability of acetylcysteine to weaken snail shells which enhanced penetration of the compound rapidly at the site of action. As recorded in our previous study, that acetylcysteine caused 95.0% reduction in snail numbers after three weeks of application using the bait method (Mobarak *et al.* 2021). Acetylsalicylic acid showed 33.4% reduction in *M. obstructa* population, while it caused 86.0% reduction in *E.* vermiculata population (Mobarak 2008). Chitosan achieved 74.3% population reduction of *E. vermiculata* after 21 days of treatment using the spray method under field conditions (Nada 2020).

Conclusion

From the previous findings, acetylcysteine proved to have strong effect on the shells of land snails leading to shell weakness and breakable, causing snail death. Therefore, it can be concluded that the compound could be used as an effective and safe molluscicide under Egyptian agricultural field conditions.

Acknowledgment

I wish to express my deepest gratitude to Dr. Aly El-Sherbiny Professor of Vertebrate Ecology, at the Harmful Animal Research Department, Plant Protection Research Institute, ARC, for correcting the manuscript's language, and for scientific reviewing. Great and special thanks to Dr. Waheed Gabr, Professor at the Harmful Animal Research Department, Plant Protection Research Institute, ARC, for correcting the manuscript scientifically. We thank Dr. Ehab A. Abdallah, Senior Researcher at the Poultry Physiology and Director of the Physiology Lab, Poultry Department Unit, Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Egypt, for helping in evaluation of the elemental contents in the snail's shell.

Author Contributions

Heba Y. Ahmed, Randa A. Kandil and Soha A. Mobarak proposed the research plan, processed the laboratory and field experiments and shared in writing the manuscript. All authors read and approved the final manuscript.

Consent for Publication

The authors´ consent for publication.

Funding Source

No fund, it is the personal effort of the author.

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