**How does Acetylcysteine Compound affect the Shell of Land Snails?**

**Heba Y. Ahmed, Randa A. Kandil, Soha A. Mobarak**

**Harmful Animals Research Department, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza.**

**Ministry of Agriculture and Land Reclamation (MALR), Giza, Egypt.**

**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 agriculture fields. The present study aims to test the effect of acetylcysteine on the shells of two land snail species, *Monacha cartusiana and Eobania vermiculata*, 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 medium lethal concentration (LC50) was calculated. Toxic actions of quarter lethal concentration (LC25) on carbonic anhydrase activity and some elements level of shell; including calcium, phosphorus, magnesium and potassium; were estimated. The efficiency of acetylcysteine was tested against the two land snail species, *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 LC50 were 0.6 and 1.5 % both species*,* consecutively. Moreover, the compound induced remarkable decrease in carbonic anhydrase activities and caused depression in calcium levels in the shell of both species. While treatment caused rising in the content of other elements. In addition, it caused the shell of *M. cartusiana* to be weaker*.* Concerning the field results, acetylcysteine compound achieved 94.7 and 90.1 % reduction in the population of snails comparing with methomyl (MALR recommended compound) which gave 76.4 and 74.9 % reduction in snail population for *M. cartusiana* and *E. vermiculata*, respectively. Results revealed that *M. cartusiana* were more susceptible to the tested compound than *E. vermiculata*. Finally, it can be concluded that acetylcysteine achieved satisfied results, under laboratory and field conditions, against the two species of land snails by reduced the number of snails through damaging their protective shells, so it can be used as an effective molluscicide.

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

**Introduction**

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

**Material and Methods**

**Experimental Compound**

**Acetylcysteine** (600mg powder), the medium lethal dose (LD50) for rats was 5050 mg/ kg (Golden 1971). It was supplied by South Egypt drug Industries Company (Sedico), 6 October City, Egypt.

**Methomyl.** Lannate (90% Powder) 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 LD50 value for rats is 17- 24 mg/ kg. It was produced by Kafer El-Zayat Company, Egypt.

**Tested Animals**

Adult animals of the two species of land snails; 1- clover land snail, *Monacha cartusiana* (Müller 1774), were obtained from clover field in Sumasta area, Beni- Suef Governorate, Egypt, coordinate (N28°54’13 E30°54’36) and 2- chocolate band snail, *Eobania vermiculata* (Müller 1774), were collected from citrus trees at the nursery of Abu- Rawash district, Giza Governorate, Egypt, coordinate (N30°”8” E 31°. 5” 26”). Snails were transported to the laboratory of the Harmful Animals Research Department, Sids Research Station, Agriculture Research Center, coordinate (N28°54”21” E 30°57”12”). Snails of each species were put in plastic boxes consist of 8-10 cm moist soil, offered with fresh leaves of lettuce and covered with muslin secured with rubber band to impede snail from escaping. Snails were acclimated for two weeks under 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). Consecutive concentrations (0.15, 0.3, 0.6, 1.2, 1.8, 2.4, and 3.6) of acetylcysteine compound were applied in Petri-dishes, for each of *Monacha cartusiana* and *Eobania vermiculata* individually. Two ml of each concentration of the compound spread on the inner surface of each Petri-dish by moving the dish in circles. Water was evaporated in a few minutes under room conditions leaving a thin film layer of the tested compound. A parallel control test was conducted using tap water without treatment. The dead animals were daily counted and removed. The mortality percentages were calculated and LC50 value was determined after seven days of treatment according to (Finney 1971).

**Biochemical studies**

Each of tested land snail species were treated individually with LC25 of acetylcysteine for seven days to estimate the effect of the tested compound on carbonic anhydrase activity and the shell content of calcium, phosphorus, magnesium and potassium.

**Sample preparation**

After seven days of treatment, the shell was removed from treated and untreated animals of each 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 for three minutes, by homogenizer under cooling with 10 ml of sodium chloride 0.9 N, and then centrifuged (5000 round per one minute for 30 minute) resulting supernatant to determine carbonic anhydrase activity.

**Determination of carbonic anhydrase activity**

Elisa kit allies to determine the carbonic anhydrase activity according to Barman (1974) using Novus Biologicals kits (USA). The developed color was measured at 450 nm, spectrophotometrically.

**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. The method is according to (Gindler and King 1972) using kits purchased from Biodiagnostic Company, Egypt.

**Phosphorus (P) level determination**

Inorganic phosphorus 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 colorimetrically at 640 nm. This method is according to (El- Merzabani *et al*. 1977) using kits obtained from Biodiagnostic Company, Egypt.

**Magnesium (Mg) level determination**

Magnesium ions react in an alkaline medium with the metallochromic dye calmagite to form a chromophore which absorbs at 520 nm. This procedure is according to (Teitz 1983) using 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 colorimetrically at 420 nm. This method is according to (Sunderman and Sunderman 1958) using kits purchased from Biodiagnostic Company, Egypt.

**Field Experiments**

Four plots (20 m2 each) planted with clover and infected with *Monacha cartusiana* were chosen at Quftan village, Sumsta district, Beni- Suef Governorate, Egypt, coordinate (N 28ᵒ54’13 E30ᵒ54’36). Another four plots planted with young citrus trees, and infested with *Eobania vermiculata* were chosen at Abu-Rawash, Giza Governorate, Egypt, coordinate (N 30ᵒ”8” E 31.5ᵒ “26”). Other plots were left without any treatment as a 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 for each treatment and others for control with ten meters between each plot. Survival 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 of treatment. The reduction in population of snails was calculated after 21 days post treatment according to (Henderson and Tilton 1952).

**Statically analysis**

Experimental design was completely randomized with different replicates**.** The obtained results were statically 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**

Data in Table (1) describe the efficacy of acetylcysteine against the two tested land snail species, *Monacha cartusiana* and *Eobania vermiculata*, after seven days of treatment. The results cleared that mortality percent increased gradually with increasing compound concentrations. Whereas the concentrations of 0.15, 0.3, 0.6, 1.2, 1.8, 2.4, and 3.6% gave 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 LC50 values were 0.6 and 1.5 % for *M. cartusiana* and *E. vermiculata*, consecutively, after seven days of treatment.

**Biochemical studies**

**Carbonic anhydrase activity**

As shown in Table (2), the results revealed the effect of LC25 of acetylcysteine on the two tested land snail species, *M. cartusiana,* and *E. vermiculata,* after seven days of treatment. The results depicted that the activity of carbonic anhydrase decreased from 4.2 in control to 1.5 ng/mg in *M. cartusiana*. Also, it showed the same trend in case of *E. vermiculata,* whereas it decreased from 5.10 in control to 2.47 ng/mg in the treated snails. There were significant decreases in the enzyme activities between control and treated snails.

**Effect of acetylcysteine on shell elements levels**

The impacts of LC25 of acetylcysteine on shell levels of Ca, P, Mg and K were reported in Table (3) and Fig (1-4). There were significant differences between treated and untreated snails. In treated snails*,* Ca level decreased to 19.5 mg/g comparing with 24.1 in 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 comparing to 13.7 mg/g, 0.67 mg/ g and 25.5 m/ mol in control, consecutively. Concerning *E. vermiculata,* the Ca level decreased from 21.5 in control to 18 mg/g in treated snails and K level reduced from 25.1 to 23.5 m mol / L. While P level enhanced 14.9 in treated snails compared with 13.4 mg/g in control. However, Mg level value still as it is 0.55 mg/g in both treated and untreated snails´ shells.

**The field performance of acetylcysteine**

Table (4) tabulated the efficiency of acetylcysteine against land snail, *M. cartusiana* compared with methomyl after three weeks of application using spray technique. 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 < 0.5) in *M. cartusiana* snail numbers. Regarding the same application against *Eobania vermiculata* in Table (5), results referred that the acetylcysteine achieved 90.1% reduction in snails´ population compared with methomyl which caused 74.9% reduction only. These results achieved significant reduction at (p < 0.5) in snail numbers after treatment.

**Discussion**

The present study revealed the efficacy of acetylcysteine against *Monacha cartusiana* and *Eobania vermiculata*. The mortality percentages of both snail species were increased with increasing the compound concentrations. These results may be due to that the higher concentrations reach the point of action rapidly and make the snails ability to repel the compound from their bodies by the mucus weaker. Also, results showed that the species *M. cartusiana* was more susceptible than *E. vermiculata*. These results may be attributed to the size of land snail as *M. cartusiana* is smaller than *E. vermiculata*. Moreover, *M. cartusiana* secrete mucus less than *E. vermiculata*. Therefore, *M. cartusiana* individuals become unable to expel the compound from their bodies by mucus like *E. vermiculata.* Mucus is very important to snails, so as a result of the treatment; mucus viscosity altered as well as its production and secretion (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 Soha *et al*. 2021).

In our study, treatment of both snail species with LC25 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 speed up the formation of biocarbonate and the production of calcium carbonate to form the shell (Wilbur and Jodrey 1955). Also, the results attributed to reduce the rate of calcium in the shell, so the shell became weaker and breakable and this perception agreed with (Wilbur and Jodrey 1955). Also, the mucus of animals is very thick and has high concentration of calcium contents (South 1992). Acetylcysteine changed the mucus viscosity from thick to liquid in the treated animals resulting in decreasing the calcium content in the mucus. This may be due to carbonic anhydrase inhibition and these data are in agreement with (Mobarak Soha *et al.* 2021). The carbonic anhydrase has an important role in the precipitation of calcium in the land snail mantle. The tested compound may be inhibiting the activity of the enzyme leading to prevent calcium depositions. Previous study recorded that shell thinning due to decrease carbonic anhydrase activity by abamectin and thiamethoxam led to prevent calcium carbonate production in land snail, *Theba pisana* post treatment and death (El-Gendy Kawther *et al*. 2019). Other study showed a significant inhibition in the activity of carbonic anhydrase of the mussel of *M. galloprovincialis* post treatment with cadmium (Lionetto *et al*. 2016).

Our results cleared that the *M. cartusiana* species was more susceptible to acetylcysteine than the *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 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 Soha and Kandil 2014), tannic acid reduced alkaline and acid phosphatase activities responsible for calcium participation in the shell of land snail, *E. vermiculata* and *M. cartusiana*. The results cleared that P level increased in the shell of each tested land snail species, when the level of P exceeds Ca in the body, Ca is reabsorbed from the shell leading to pathological changes. As mentioned in previous study (Taylor and Bushinsky 2009), that disturbances in the body level of phosphorus and calcium can lead to pathological changes. In addition, the calcium and magnesium ions are known to have specific and opposite effect at the prejunctional nerve terminals of several cholinergic synapses (Jenkinson 1957). This study supports our results regarding *M. cartusiana*, whereas the Mg level increased, compared to control, led to decrease the Ca level causing weakness and breaking in the shell*.* The opposite relationship also seems to be between Ca and Mg levels in relation to eggshell quality 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 decreasing in Ca level led to weak in shell of *M. cartusiana*, this may be causing breakable in the shell. It was reported by (Leach 1974) that K content reduction in the hen causes shell thickness in the egg of poultry. While K level reduced in *E. vermiculata* compared to 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 easy breaking. It was investigated that acetylcysteine caused remarkable increasing in alkaline phosphatase activity in *M. cartusiana* (Mobarak Soha *et al*. 2021). This result confirms that the compound influences shell negatively, whereas alkaline phosphatase is the responsible enzyme for producing the shell in the snails.

Concerning the field application, the results agreed with those obtained in the laboratory and took the same trends, whereas it is proved that acetylcysteine and methomyl were 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 highly reduction percent in snail population in case of the two species of snails, when compared with methomyl. These results may be due to the ability of acetylcysteine to weaken snail shells which enhanced penetration of the compound rapidly to arrive easily 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 baiting method (Mobarak Soha *et al*. 2021). The opposite results occurred with (Mobarak Soha 2008), who reported that acetylsalicylic acid failed to give satisfied result as it only gave 33.4% reduction with *M. cartusiana*, while it caused 86.0% reduction in *E. vermiculata*. It was mentioned that chitosan achieved 74.3% reduction in *E. vermiculata* numbers, after 21 days of treatment, using the spray method (Abbass Nada 2020).

**Conclusion**

From the previous findings, acetylcysteine proved to have strong effect on the shells of land snails by playing the role as a carbonic anhydrase inhibitor, which is responsible for shell formation leading to shell weakness and breakability. This effect supports the penetration of the compound into the site of action and causing snail death. Therefore, it can be concluded that the compound could be used as an effective and safe molluscicide molluscicide under Egyptian agricultural field conditions.

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**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.

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**Table 1: LC50 determination of acetycysteine against land snails, *Monacha cartusiana* and *Eobania vermiculata*, after one week of treatment using thin film layer**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Concentration%** | ***Monacha cartusiana*** | | ***Eobania vermiculata*** | |
| **Mortality %** | **LC50 %** | **Mortality %** | **LC50 %** |
| 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 LC25 of acetylcysteine on carbonic anhydrase (ng/mg) activity of land snails, *Monacha cartusiana* and *Eobania vermiculata,* after one week of treatment**

|  |  |  |
| --- | --- | --- |
| **Carbonic anhydrase activity (ng/ mg)** | | |
| **Group** | ***Monacha cartusiana*** | ***Eobania 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 LC25 on shell elements content of two land snails, *Monacha cartusiana and Eobania vermiculata,* after one week of treatment**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Shell**  **component** | **Species** | | | | | |
| ***Monacha cartusiana*** | | | ***Eobanaia 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.

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

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Rate of application**  **(g/l)** | **No. of survival snail pre-treatment** | | **No. of survival snail 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, *Eobania vermiculata* comparing with methomyl after three weeks of application as a spray technique**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Rate of application**  **(g/l)** | **No. of survival snail pre-treatment** | | **No. of survival snail 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.

|  |  |
| --- | --- |
|  |  |
| Fig. (1) untreated adult *M. cartusiana* | Fig. (2) Adult *M. cartusiana* treated with acetylcysteine shows broken shell. |
| C:\Users\hp\Documents\Bluetooth\Share\IMG_20220918_143812.jpg | C:\Users\hp\Documents\Bluetooth\Share\IMG-20220906-WA0015.jpg |
| Fig. (3) Untreated adult *E. vermiculata.* | Fig. (4) Adult *E. vermiculata* treated with acetylcysteine shows color change of the shell. |