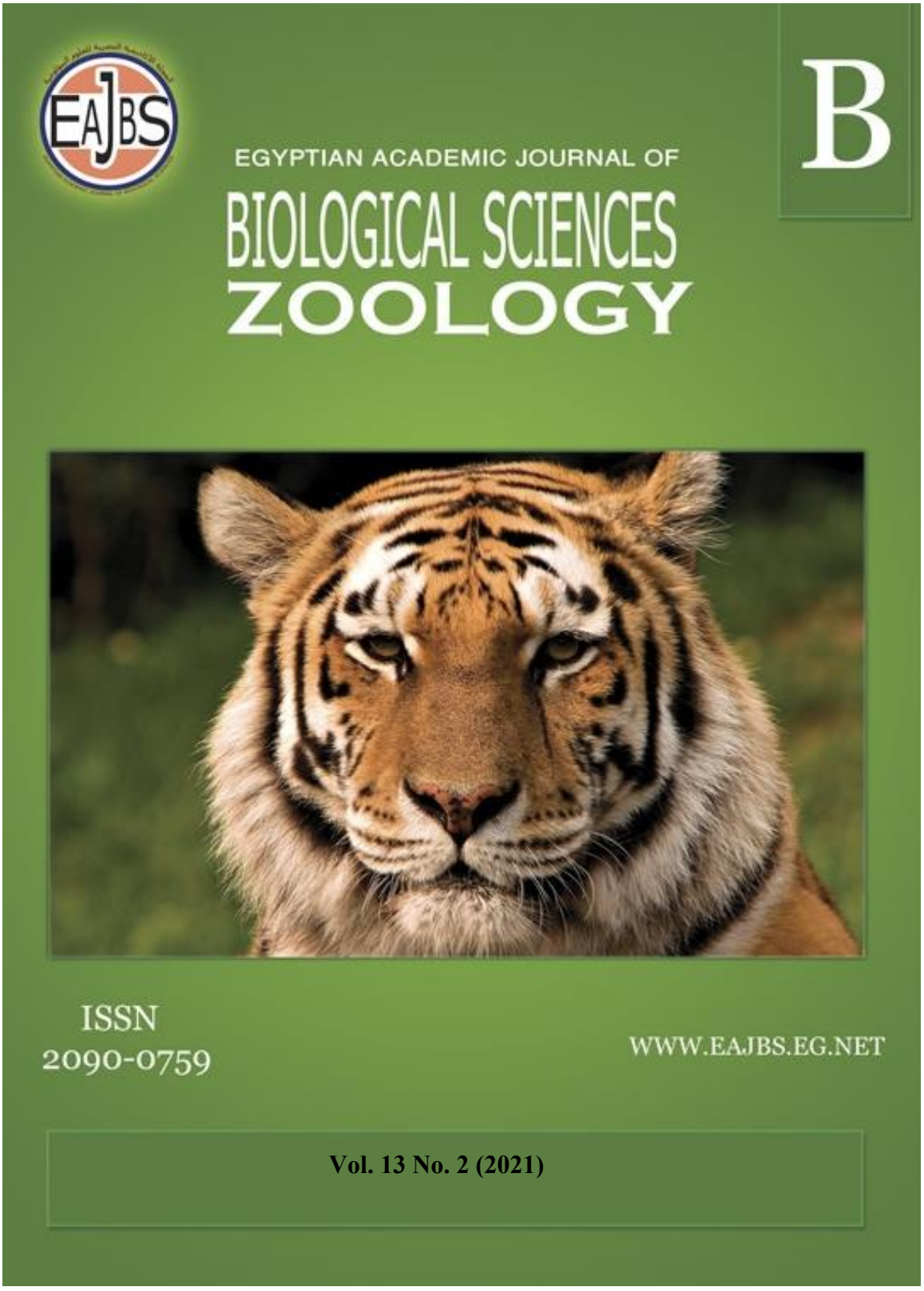

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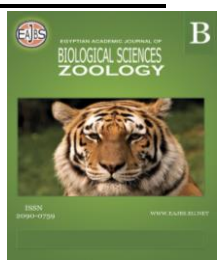

The Efficiency of Acetylcysteine (Drug) As A Molluscicide

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ABSTRACT

Mucus is very important for the protection of land snails. It is the second defense line after the shell. This study was conducted to test the effect of acetylcysteine (drug) against the mucus in the foot of a clover land snail, *Monacha cartusiana* (O.F. Müller 1774), under laboratory and field conditions. Snails were exposed to serial concentrations of the tested compound for seven days as bait. LC_{50} of the compound was calculated and the effect of ¼ LC_{50} was tested on Total Protein (TP) and Alkaline phosphatase (ALK). The histopathology of land snail foot was studied after treatment with ¼ LC_{50}. The field performance of the compound was evaluated against *M. cartusiana* as poison bait 3.6% for 21 days in a clover field at Sumsta district, Beni- Suef Governorate. The laboratory results revealed that the LC_{50} was 2%, and the most effective concentration was 3.6% which gave 90 % mortality. Moreover, the compound caused high significant effect on TP and ALK with a severe increase in their biochemical parameters. Based on the histopathological effect on the foot, the compound caused focal necrosis in the epithelium and degeneration in the connective tissue. The field results indicated that the compound achieved a 95% reduction in the population of *M. cartusiana* compared with methomyl (the compound recommended by the Ministry of Agriculture), which gave an 88.0**%** population reduction in the snails' population. Therefore, acetylcysteine compound can be used as a safe molluscicide in agriculture fields, to reduce the number of pest snails to avoid environmental pollution and the long-term pest resistance to methomyl compound.

INTRODUCTION

Terrestrial gastropod molluscs (snails and slugs) are herbivorous pests that cause significant crop damage around the world (Feldkamp, 2002). Land snails can cause damage directly by feeding on several plant species in the field, and indirectly through infection by bacteria, fungi and viruses *via* scratching the plants during feeding (Barker, 2002). Terrestrial snails are considered important economic pests infesting and causing intense damages to ornamental plants, orchard trees, vegetables and field crops (Desoky, 2018).

Clover land snail, *Monacha cartusiana* (O.F. Müller 1774) is one of the most well-known species widespread in the most governorates in Egypt e.g., Beni- Suef, Fayoum,

Giza, Alexandria, Behira, Monufeia, Qulyubeia, Gharbeia, Kafr El- Shikh and Sharkeia. It was recorded on orange, mango, wheat, grapes and wood trees (Ali Reham, 2020).

Snails secret the mucus to remove any foreign substances exposed to their body, therefore, it is difficult to control them. Chemical control is beneficial, but it could pollute the environment and harm other organisms. Chemical molluscicides may negatively affect non-target species, organisms and increase environmental pollution (Gabr *et al*., 2006). For this reason, efforts should be increased towards the use of non-traditional safe substances to control this pest (Massoud and Habib, 2003).

N-Acetylcysteine (NAC- drug) is a sulfhydryl-containing compound, a precursor of amino acid L-cysteine. It has been used for decades as a mucolytic agent in the treatment of different respiratory diseases reducing the viscosity of airway secretions and increasing the ciliary clearance rate (Blackwell *et al*., 1996; van Overveld *et al*., 2005; Tardiolo *et al*., 2018). The present study aimed to assess the efficacy and efficiency of N-Acetylcysteine as a non-traditional compound for land snail control under laboratory and field conditions.

MATERIALS AND METHODS

Tested Compounds:

1.Acetylcistein 600 drug (Acetylcysteine 600 mg powder), The LD_{50} for rats was 5050 mg/ kg (Golden, 1971). Produced by South Egypt Drug Industries Company (SEDICO), 6 October City- Egypt.

2.Methomyl.

Trade Name: Newmyl (20% SL) is an insecticide carbamate compound recommended by the MALR against land snail infestation agricultural crops as poison bait, at the rate of 8 - 10 kg/ fed. The LD_{50} value for rats is 17- 24 mg/ kg. It was obtained from KZ. CO., Egypt.

Experimental Animals.

The adult individuals of clover land snail, *Monacha cartusiana*, were collected from clover field in Sumasta area, Beni-Suef Governorate, and transported to the laboratory of Sids Research Station, Plant Protection Research Institute, Agriculture Research Center, coordinate (N28°54’21 E30°57’12). Animals were kept in small plastic boxes containing 8-10 cm moist soil provided with fresh green lettuce leaves, covered with muslin secured with a rubber band to prevent snails from escaping, and kept under 20±2°c in the laboratory for two weeks for acclimatization.

Laboratory Experiments:

1.Baiting Technique:

Serial concentrations of Acetylcysteine compound (0.6, 1.2, 1.8, 2.4, and 3.6 %) were evaluated against *M. cartusiana,* used as poison baits were prepared by mixing each concentration with 5% molasses + 93% bran. Five grams of the poison bait were put on a plastic sheet and placed on the surface of the soil in each glass box. Ten animals were exposed to the candidate concentration, and three replicates for each concentration were used. A control test was conducted with plain carriers. Mortality percentages were calculated after seven days of treatment and LC_{50} value was determined according to Finney (1971).

2.Biochemical Studies:

Animals were treated with ¼ LC_{50} of acetylcysteine for seven days using the poison bait method to determine the effect on Alkaline Phosphatase Activity (ALK) and Total Protein content (TP) to clarify the physiological effect of the compound compared with untreated animals.

Sample Preparation:

After removing the shell of treated snails, 1g of them was homogenized for three min. under cooling in a homogenizer with 10 ml of sodium chloride 0.9N and then centrifuged (3000 rpm., for 15 min) to determine the (TP) content according to Bergmeyer (1967) and modified according to Singh and Agarwal (1987). Another 1g was centrifuged (5000 rpm., for 20 min to determine (ALK) activity as described by Moss (2016). The extraction process takes not more than 24h under cooling conditions in the refrigerator. A parallel control test was also conducted.

Determination of (TP) Content:

Soluble protein was determined spectrophotometrically as described by Tietz (1994), using Buiret reagent. The developed color was measured at 546 nm.

Determination of (ALK) Activity:

ALK activity was determined spectrophotometrically according to Moss (2016). The development of color was done according to the following reaction:

Phenylphosphate $\xrightarrow[\text{pH 10}]{\text{Alkaline phosphatase}}$ phenol + phosphate

The liberated phenol was measured in the presence of amino -4- antipyrine and potassium ferricyanide.

The obtained results were statistically analyzed by one-way ANOVA and LSD at ($P < 0.05$) using the Costat program (COHORT, 2005).

Histopathological Studies:

The histopathological effect of acetylcysteine on the foot of *Monacha cartusiana* was checked. Snails were treated with ¼ LC_{50} for seven days using the baiting technique. After anesthetized, the foot of treated and untreated snails was separated, then fixed in 10% formol saline for 24 hours. 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 bees 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 Experiment:

Six plots (4 m^2each) cultivated with clover and infested with clover land snail, *M. cartusiana* were chosen at Quftan village, Sumsta district, Beni- Suef Governorate, coordinate (N28°54’13 E30°54’36). The most effective concentration of acetylcysteine bait 3.6% (36g/kg) in the laboratory was evaluated under field conditions. The compound was compared with the recommended compound methomyl 2% (20g/kg). 100 gm of poison bait was put on a blue plastic sheet (to attract the snails). Two replicates for each treatment and the other for control with two meters between each plot. The bait was renewed every four days. Live snails were counted in each plot (in four corners and in the center of each plot) pre and post-treatment at 1, 3, 7, 15, and 21 days of treatment. The reduction in snails’ population was calculated after 21 days of treatment according to Henderson and Tilton, (1952).

Statical Analysis.

Obtained data were analyzed using one-way ANOVA, in SAS software Version 9.1; SAS Institute, Cary, NC, USA (SAS Institute, 2008), and means were compared by Tukey’s HSD (P= 0.05 level) using the same software.

RESULTS AND DISCUSSION

Laboratory Studies:

1-LC_{50} of Acetylcysteine Determination:

Table (1**)** shows the efficacy of acetylcysteine on a land snail, *Monacha cartusiana,* after seven days of treatment using the baiting method. The results manifested that mortality percent increased progressively with the increase of the acetylcysteine concentrations. The concentrations of 0.6, 1.2, 1.8, 2.4, and 3.6 induced 0.0, 15, 45, 60, and 90 % mortality percentage, respectively. The calculated LC_{50} was 2% with a 4.9 slope after seven days of treatment. This result may be attributed to the palatability and taste of the baits. Classical mucolytics, like acetylcysteine and other thiol reducing agents, degrade the three-dimensional network that forms the mucus by reducing the disulphide bonds (S-S) to a sulfhydryl (SH) bond (-SH) that no longer participates in the cross-linking. They may act on the mucus elasticity and viscosity as well as modulate its production and secretion (Livingstone *et al.*, 1990; King and Rubin, 2002). In humans, acetylcysteine has been reported to reduce the viscosity of sputum in both cystic fibrosis and COPD, facilitating the removal of pulmonary secretions (Ventresca *et al.,* 1989). Moreover, maintaining the airway clearance, it prevents bacterial stimulation of mucin production and hence mucus hypersecretion (Adler *et al.*, 1986). Based on the previous function of acetylcysteine, it exerts its toxicity on snails *via* its mucolytic activity. Mobarak, Soha *et al.,* (2017) indicated that the LC_{50} of acetylsalictlic acid was 210.6 ppm, after7 days of treatment using the contact method.

Table 1: Effect of acetylcysteine (Drug) against Land snail, *Monacha cartusiana*, after seven days of treatment as a bait**.**

Concentration %	Mortality %	LC_{50} %	Slope
0.6	0	2	4.9
1.2	15		
1.8	45		
2.4	60		
3.6	90		

2.Effect of ¼ LC_{50} of Acetylcystein on TP and ALK:

Data in **Table (2)** represented the effect of ¼ LC_{50} of acetylcysteine on Total Protein (TP) and Alkaline phosphatase (ALK) in land snail, *Monacha cartusiana*, after seven days of treatment. The results cleared that the compound induced a marked increase in TP level and ALK activity compared to control. It caused a significant severe increase in TP level from 2.4 u/l in control to 28.4 u/l in treated snails. Also, the same result occurred in ALK enzyme whereas, a significant increase was occurred from 7.5 g/l in control to 16.6 g/l in treated snails. These data revealed that the increase in TP content may be due to the damage caused *via* acetylcysteine treatment in the protein synthesis, whereas it caused a mucolytic activity, which acts directly by splitting the disulfide bonds of mucoproteins and reducing the mucus viscosity making the snails lose a big of the amount of mucus which led to death. Moreover, the increase in ALK activity affected mucus production in the mucus gland, which resulted in stress to the gland ultimately leading to the death of the treated snails. The mucous cells had acid and ALK activities and the mucous released by the mucus cells showed activity in both enzymes (Ning *et al*., 2005). Acetylsalicylic acid increased TP content and ALK activity to reach 2.3 g/dl and 264.2 U/L after 7 days, consecutively, but still less than normal activity of 0.71 g/dl

and 437.5 U/L for control, respectively (Kandil *et al*., 2014). Opposite results occurred with Mobarak, Soha *et al*. (2015) they reported that LC_{50} of clove extract decreased ALK activity in the land slug, *Limax flavus,* after treatment. Khater *et al*., (1990) pointed that the increase in total protein could be attributed to the increase in the biosynthesis process occurred by high enzyme stress.

Table 2: Effect of ¼ LC_{50} of acetylcysteine on two biochemical parameters in land snail, *Monacha cartusiana,* after seven days of treatment.

Parameter	Control Mean ± SE	Treatment Mean ± SE	LSD
Total Protein (u/l)	2.4 ± 0.4^{b}	28.4 ± 2.3^{a}	1.2
Alkaline phosphatase (g/l)	7.5 ± 0.4^{b}	16.6 ± 0.2^{a}	6.4

P <0.05.

* Data are expressed as mean ± SE.

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

3.Histological studies:

Figures (1&2) showed the histopathological findings of the mucous glands of the foot of *Monacha cartusiana* treated with ¼ LC_{50} of acetylcysteine compound compared with the untreated foot.

In Figure (1) there was the normal histological structure of the sole aspect showed and underlying sub epithelium, connective tissue and glandular structure in the untreated foot. While Figure (2) cleared the treated foot whereas, the ventral and dorsal aspects showed focal necrosis in the epithelium cells and glands and also degeneration in the connective tissue. This result may be due to the compound causing damage and breakdown of the protein cells of the mucus gland in the foot, which led to induce necrosis. Acetylsalycilic acid caused increase in the volume of the mucus gland, swelling cells, and necrosis (Kandil *et al*., 2014, and Mobarak, Soha *et al*., 2017). Gland mucus cells showed histological alterations after being treated with boric acid and necrosis was observed in the cells with alum compound (Mahmoud, Maha *et al.*, 2012).

Field Studies:

The field performance of acetylcysteine against land snail, *Monacha cartusiana* compared with methomyl (The recommended compound) was shown in Table (3). Results showed that acetylcysteine achieved a 95.0% reduction in the snail population compared with 88.0 % with methomyl after 21 days of treatment using the baiting application. Application of acetycysteine achieved significant reduction (P< 0.5) in snail numbers compared with pre-treatment. The compound activity may be due to its mucolytic effect on snails. Also, the taste of the bait may be attractive to snails. Acetylsalicylic acid was low effective whereas it failed to achieve a good result as it gave only 33.4 % population reduction against *M. obstructa* under field conditions (Mobarak, Soha, 2008). Ali (2017) investigated the effect of the roundup compound against *M. cartusiana* and reported a 17.20% population reduction. Mobarak Soha, (2016) also recorded that 94% and 78.7% population reduction in *Eobania vermiculata* treated with methomyl and chlorfluazuron, respectively.

Table 3: Field efficiency of acetylcystiene against land snail, *Monacha cartusiana*,compared with methomyl compound after 21 days of treatment as a bait.

Treatment	Bait concentration %	No. of live snails before treatment	No. of live snails after treatment	Population Reduction %
Control	-	31.4 ± 9.86^{a}	24.8 ± 7.09^{ab}	-
Acetylcysteine	3.6	28.6 ± 7.20^{a}	1.2 ± 1.30^{c}	95.0
Methomyl	2.0	19.4 ± 7.64^{b}	2.6 ± 1.67^{c}	88.0

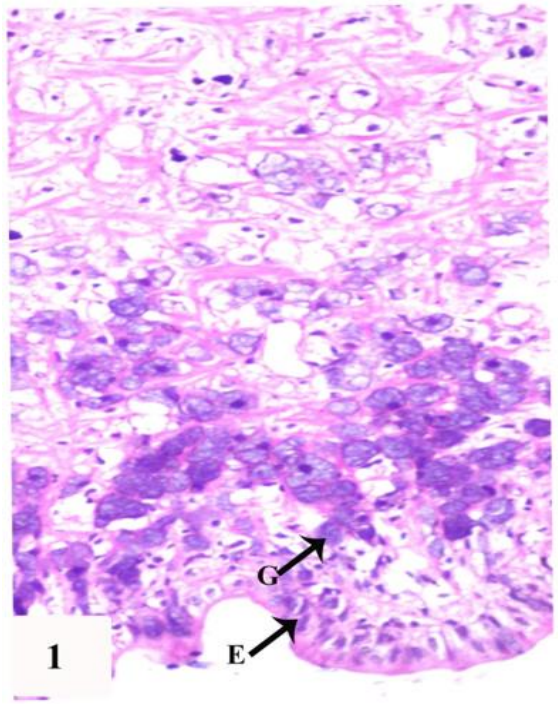

Fig.1. Photomicrograph of foot of *Monacha cartusiana*, from the control group and stained with hematoxyline and eosin showing normal epithelial cells (E) and normal glands (G). X 400

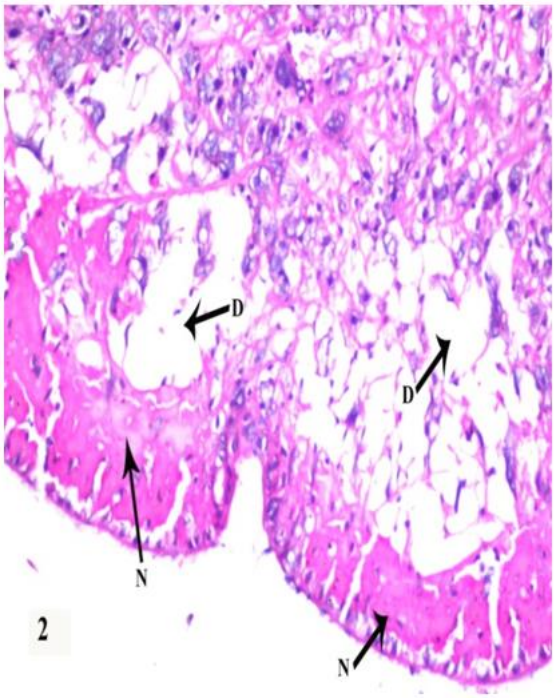

Fig.2. Photomicrograph of foot of *Monacha cartusiana*, treated with acetylcysteine and stained with hematoxyline and eosin showing focal necrosis of epithelial cells and glands (N), as well as degeneration of connective tissue. X 400

Conclusion

The acetylcysteine compound achieved satisfactory results against clover land snail, *Monacha cartusiana*, under laboratory and field conditions. Therefore, it can be used as a molluscicide against land snails in the form of poison bait affecting the mucus, leading to the reduction number in snail's, while avoiding pollution of the environment and long-term resistance to methomyl (the recommended compound) for snails control by the Egyptian Ministry of Agriculture.

List of Abbreviations:

LC50, Half Lethal Concentrations.
SL. Soluble Liquid.
EMARL., Egyptian Ministry of Agriculture and Land Reclamation.
LD50, Half Lethal Dose.
KZ., Co. Kafr El-Zayat Companey.
Kg/Fed, Kilogram per Feddan.

r.p.m. Round per minute.
ANOVA. Analysis of Variance.
LSD. Least significant difference
SAS. Statistical Analysis System.

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