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Biochemical Effect of Three Molluscicide Baits Against the Two Land Snails, *Monacha cantiana* and *Eobania vermiculata* (Gastropoda: Helicidae)

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ABSTRACT

The present study was investigated the biochemical effect of three molluscicides in the tissues of the two land snails, *Monacha cantiana* (Montagu) and *Eobania vermiculata* (Muller). The field recommended rate of three commercial molluscicide baits namely: Gastrotov, mlotov and mesurol were evaluated. The activities of five vital enzymes, total protein (TP) and total lipid (TL) were laboratory tested. The five vital enzymes were acetylcholine-esterase (AChE), alanine amino transaminase (ALT), aspartate amino transaminase (AST), lactate dehydrogenase (LDH) and mixed function oxidase (MFO). Results showed that all tested molluscicides lead to increase the activity of AST, LDH and MFO in the tested land snails, *E. vermiculata* and *M. cantiana*, except mesurol treatment showed a decrease in MFO when applied against the two land snails. All of the tested molluscicides had inhibitory effect on the activity of AChE in both tested land snails. The results also revealed that all tested molluscicides increased the activity of ALT in the tissue homogenate of the land snail, *M. cantiana*. Obviously, all of the tested molluscicides decreased the activity of ALT in the land snail, *E. vermiculata*. On the other hand, the levels of total protein and total lipid were increased after treatment with all tested molluscicides when applied against *E. vermiculata*. Moreover, the levels of total protein and total lipid decreased after treatment in the tissue homogenate of the land snail, *M. cantiana*. In general, the three molluscicides were significantly affected on the activities of enzymes, total lipid and total protein compared with control treatment when applied against the tested snails.

Key words: Land gastropods, chemical compounds, biochemical analysis, enzymes, protein, lipid

INTRODUCTION

The garden brown snail, *Eobania vermiculata* (Muller) and the clover land snail, *Monacha cantiana* (Montagu) are particularly the most common and serious pests in Egypt. They caused a substantial damage to different agricultural crops in various governorates. *E. vermiculata* and *M. cantiana* were recorded with a relatively high population density on the major economic crops at Dakahlia governorate (Awad, 2000; Genena, 2003).

Control of land snails on different crops is heavily dependent on the use of molluscicides that limit the effect of these pests below damaging level. Hence, the synthetic molluscicides are the most effective measures available at present for the control of terrestrial gastropods (Heiba *et al.*, 2002;

Genena, 2003; Abd-El-Ail, 2004; Ismail *et al.*, 2005; Zedan *et al.*, 2006; Genena *et al.*, 2008). Bait formulations of molluscicides was the most effective application method in the field for controlling terrestrial gastropods rather any other technique (Kassem, 2004). Carbamate molluscicides are known to act as nerve toxins by inhibition of cholinesterase. Metaldehyde molluscicides caused an excessive increase of fluid excretion in the soft snail body, so leading to snail death (Kassem *et al.*, 1993). Both carbamate and metaldehyde are successfully used in Egypt as well as in many other countries to control land snails (Heiba *et al.*, 2002). Transaminases enzymes and acetylcholine esterase as well as total proteins and total lipids are important in the biological processes in the land snails (Abd-El-Ail, 2004).

The aim of this work was to determine the biochemical effect of three molluscicides namely, mesurol (carbamate), gastrotax and mlotov (metaldehyde) on the activities of five vital enzymes, Total Protein (TP) and Total Lipid (TL) to throw a light on the toxicity and mode of action of these molluscicides in the two land snails, *M. cantiana* and *E. vermiculata*. The enzymes selected for this study were; acetylcholine-esterase (AChE), alanine amino transaminase (ALT), aspartate amino transaminase (AST), lactate dehydrogenase (LDH) and mixed function oxidase (MFO).

MATERIALS AND METHODS

Tested snails: Adult snails of *M. cantiana* and *E. vermiculata* were collected from infested nurseries and field crops in Mansoura city, Dakahlia Governorate, Egypt, during the spring of 2011. The obtained snails were transferred in plastic bags to the laboratory, then kept in plastic cages (40×30×30 cm, with 100 individuals per cage) filled with moist sterilized sandy loamy soil 1:1 (v:v) and fed on fresh leaves of lettuce (*Lactuca sativa* L.) for 14 days to be laboratory acclimatized.

Tested molluscicides: Three molluscicides belonging to two different chemical groups were tested. The trade name, Common name, chemical name and field recommended rates are shown in Table 1.

Experimental design: The experiment took place under laboratory condition at 22±1°C and 60±2% R.H. Field recommended rate for each molluscicide was introduced to each land snail species. Ten adult snail individuals with approximately similar size were then transferred from stock culture to plastic cups 10 cm-diameter filled with 100 g moist sterilized sandy soil: loamy soil 1:1 (v:v). Each cup was then covered with muslin cloth held by rubber bands. Each of the above mentioned molluscicide and the control were replicated five times. Biochemical studies were made after three days of treatment.

Biochemical studies

Sample preparation: After three days of treatment, shells of tested snails were removed by making a cut around the whorls in a continuous manner starting at the aperture opening using bone scissors and the broken fragments of the shell were carefully removed. Snail tissues were dissected out and all tissues of each treatment were homogenized in distilled water (50 mg mL⁻¹). The homogenates were centrifuged at 8000 rpm for 15 min at 5°C in refrigerated centrifuge. The deposits were discarded and the supernatants were kept in a deep freezer till use to determine the activities of acetylcholine-esterase (AChE), alanine amino transaminase (ALT), aspartate amino transaminase (AST), lactate dehydrogenase (LDH), mixed function oxidase (MFO), total protein (TP) and total lipid (TL).

Table 1: List of molluscicides, their trade name, common name, chemical name and field recommended rate

Trade name	Common name	Chemical name	Rate/Fed (kg)
Gastrotox (5% RB)	Metaldehyde	2,4,6,8-tetramethyl-1.3.5.7 tetraoxacyclooctane, acetaldehyde tetramer	2
Mlotov (3% RB)	Metaldehyde	2,4,6,8-tetramethyl-1.3.5.7 tetraoxacyclooctane, acetaldehyde tetramer	5
Mesurool (2% RB)	Methiocarb	3,5-dimethyl-4-(methylthio) phenyl methylcarbamate	4

Biochemical measurements: AchE activity was assayed by the method of Symphon *et al.* (1964). The activity of AST and ALT were determined according to the method of Reitman and Frankel (1957) using commercial reagents. Lactate dehydrogenase (LDH) was assayed using the colorimetric method described by Cabaud and Wroblewski (1958). Total proteins were calorimetrically determined according to Bradford (1976) while total lipids were assayed by the method of Knight *et al.* (1972). Mixed function oxidase was measured according to the method of Hansen and Hodgson (1971).

All biochemical measurements were conducted in the micro-chemical analysis unit at Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt.

Data analysis: Data were calculated as Mean±SD and analyzed using analysis of variance technique (ANOVA) followed by Least Significant Difference (LSD). Probability of 0.05 or less was considered significant. All statistical analysis was done with CoHort Software 2004.

RESULTS

Activity of acetylcholine-esterase (AChE): Results in Table 2 showed that mesurool was found to be most inhibitory compound to AChE in the tissue homogenate of the two land snails, *E. vermiculata* and *M. cantiana* followed by mlotov and gastrotox, respectively. The percentages activities of the enzyme in the two land snails, *E. vermiculata* and *M. cantiana* were decrease than control with mean values, 0.80 ± 0.06 and 2.30 ± 0.36 after 72 h of application with mesurool, respectively. Data in Table 2 also indicated that both of gastrotox and mlotov were decreased the activity of AChE than control in the land snail, *E. vermiculata* with mean values 1.06 ± 0.05 and 0.85 ± 0.05 , respectively. On the other hand, no significant inhibitors between gastrotox and mlotov when applied against the land snail, *M. cantiana* with mean values 2.88 ± 0.08 and 2.84 ± 0.13 , respectively compared with control.

Activity of alanine amino transaminase (ALT): Data in Table 3 indicated that, ALT activity was decreased in the land snail, *E. vermiculata* after treated with all tested molluscicides. Mlotov gave the highest decrease in the activity of enzyme with mean value 22.17 ± 2.31 less than control and no significant differences between gastrotox and mesurool with mean values, 32.70 ± 3.20 and 29.17 ± 1.13 less than control, respectively. The level of this enzyme was increased in the land snail, *M. cantiana* after treated with all tested molluscicides. There were significant differences between all treatments and control. Mlotov and mesurool gave the highest increase in ALT activity with mean values, 272.00 ± 9.17 and 269.00 ± 2.00 more than control, respectively. Gastrotox gave the lowest increase in the level of this enzyme with mean value, 242.30 ± 6.11 more than control.

Activity of aspartate amino transaminase (AST): Data in Table 4 showed that all tested molluscicides were increase the level of (AST) when applied against the two tested land snails, *E. vermiculata* and *M. cantiana*. Data also showed that there were not significant increase between

Table 2: Acetylcholine-esterase (AchE) activity in the two land snails, *E. vermiculata* and *M. cantiana* after 72 h of molluscicides treatment

Land snail species	Treatment	kg/Fed	Activity of AchE (Mean±SD)
<i>E. vermiculata</i>	Gastrotox	2	1.06±0.05b
	Mlotov	5	0.85±0.05c
	Mesurool	4	0.80±0.06c
	Control	--	1.26±0.14a
LSD (0.05) = 0.15			
<i>M. cantiana</i>	Gastrotox	2	2.88±0.08a
	Mlotov	5	2.84±0.13a
	Mesurool	4	2.30±0.36b
	Control	--	2.92±0.08a
LSD (0.05) = 0.38			

Specific activity of AchE: (ug Ach Br/ min/ mg protein). Values followed by the same letter (s) are not significantly different at the 0.05 level according to Duncan's test

Table 3: Alanine amino transaminase (ALT) activity in the two land snails, *E. vermiculata* and *M. cantiana* after 72 h of molluscicides treatment

Land snail species	Treatment	kg/Fed	Activity of ALT (Mean±SD)
<i>E. vermiculata</i>	Gastrotox	2	32.70±3.20b
	Mlotov	5	22.17±2.31c
	Mesurool	4	29.17±1.13b
	Control	--	45.97±4.32a
LSD (0.05) = 6.53			
<i>M. cantiana</i>	Gastrotox	2	242.30±6.11b
	Mlotov	5	272.00±9.17a
	Mesurool	4	269.00±2.00a
	Control	--	221.70±3.06c
LSD (0.05) = 12.72			

Specific activity of ALT: (U x 10³/ mg protein). Values followed by the same letter (s) are not significantly different at the 0.05 level according to Duncan's test

Table 4: Aspartate amino transaminase (AST) activity in the two land snails, *E. vermiculata* and *M. cantiana* after 72 h of molluscicides treatment

Land snail species	Treatment	kg/Fed	Activity of AST (Mean±SD)
<i>E. vermiculata</i>	Gastrotox	2	673.33±23.50a
	Mlotov	5	594.00±12.80b
	Mesurool	4	614.33±1.80b
	Control	--	586.33±16.50b
LSD (0.05) = 42.97			
<i>M. cantiana</i>	Gastrotox	2	952.70±8.39b
	Mlotov	5	1102.00±39.40a
	Mesurool	4	1088.30±43.70a
	Control	--	923.70±25.11b
LSD (0.05) = 30.85			

Specific activity of AST: (U x 10³/ mg protein). Values followed by the same letter (s) are not significantly different at the 0.05 level according to Duncan's test

mesurool and mlotov in the activity of enzyme when applied against land snail, *E. vermiculata* with mean values, 614.33±1.80 and 594.00±12.80 more than control, respectively. Gastrotox caused

the highest increase in the activity of (AST) with mean value, 673.33 ± 23.50 . Regarding the effects of the tested molluscicides on the activity of (AST) against the tested snail, *M. cantiana* the same trend was observed that no significant increase between mesurol and mlotov in the activity of enzyme with mean values, 1088.30 ± 43.70 and 1102.00 ± 39.40 more than control, respectively. On the other hand gastrotox caused the lowest increase in the activity of (AST) with mean value, 952.70 ± 8.39 .

Activity of lactate dehydrogenase (LDH): The activity of Lactate dehydrogenase (LDH) in the tissue homogenate of the two land snails was represented in Table 5. Data indicated that all tested molluscicides were significantly increased the level of this enzyme in both tested land snails compares with control treatment. Mesurol gave the highest increase in the level of (LDH) in the tested land snails, *E. vermiculata* and *M. cantiana* with mean values, 7.63 ± 0.88 and 16.14 ± 1.80 more than control, respectively followed by mlotov and gastrotox.

Activity of mixed function oxidase (MFO): As demonstrated in Table 6 gastrotox and mlotov caused increase in the level of MFO in both tested land snails. They were gave (5.84 ± 0.11 and 8.65 ± 1.08) in *E. vermiculata* and (17.06 ± 2.33 and 14.16 ± 0.86) in *M. cantiana* more than control,

Table 5: Lactate dehydrogenase (LDH) activity in the two land snails, *E. vermiculata* and *M. cantiana* after 72 h of molluscicides treatment

Land snail species	Treatment	kg/Fed	Activity of LDH (Mean±SD)
<i>E. vermiculata</i>	Gastrotox	2	$4.56 \pm 0.25c$
	Mlotov	5	$5.88 \pm 0.12b$
	Mesurol	4	$7.63 \pm 0.88a$
	Control	--	$3.59 \pm 0.37c$
LSD (0.05) = 1.11			
<i>M. cantiana</i>	Gastrotox	2	$11.19 \pm 1.05b$
	Mlotov	5	$14.26 \pm 1.77a$
	Mesurol	4	$16.14 \pm 1.80a$
	Control	--	$8.17 \pm 0.92c$
LSD (0.05) = 2.57			

Specific activity of LDH: (U x 103/ mg protein). Values followed by the same letter (s) are not significantly different at the 0.05 level according to Duncan's test

Table 6: Mixed function oxidase (MFO) activity in the two land snails, *E. vermiculata* and *M. cantiana* after 72 h of molluscicides treatment

Land snail species	Treatment	kg/Fed	Activity of MFO (Mean±SD)
<i>E. vermiculata</i>	Gastrotox	2	$5.84 \pm 0.11b$
	Mlotov	5	$8.65 \pm 1.08a$
	Mesurol	4	$2.79 \pm 0.18d$
	Control	--	$4.71 \pm 0.42c$
LSD (0.05) = 1.11			
<i>M. cantiana</i>	Gastrotox	2	$17.06 \pm 2.33a$
	Mlotov	5	$14.16 \pm 0.86b$
	Mesurol	4	$1.71 \pm 0.21c$
	Control	--	$3.76 \pm 0.41c$
LSD (0.05) = 2.52			

Specific activity of MFO: (n mole sub. oxidized/ min/ mg protein). Values followed by the same letter (s) are not significantly different at the 0.05 level according to Duncan's test

respectively. On the other hand, the level of this enzyme was decreased in the two treated land snails, *E. vermiculata* and *M. cantiana* when applied with mesurol with mean values, 2.79 ± 0.18 and 1.71 ± 0.21 less than control treatment, respectively.

Total lipids level: Data in Table 7 indicated that all tested compounds were increase the level of total lipids when applied against *E. vermiculata* and there were no significant differences between all treatments and control except mesurol. Mesurol gave the highest increase in total lipids followed by mlotov and gastrotox with mean values, 1.41 ± 0.11 , 1.21 ± 0.09 and 1.16 ± 0.11 more than control, respectively. Data also showed that all tested compounds were decrease the level of total lipids when applied against, *M. cantiana*. Mesurol gave the highest decrease in total lipids compare with control with mean value, 2.12 ± 0.25 followed by mlotov and gastrotox with mean values, 2.72 ± 0.24 and 3.13 ± 0.24 more than control, respectively.

Total proteins level: Data in Table 8 indicated that all tested compounds were increase the level of total proteins when applied against *E. vermiculata*. Mlotov gave the highest increase in total proteins followed by mesurol and gastrotox with mean values, 17.42 ± 1.21 , 14.23 ± 0.55 and 13.60 ± 0.50 more than control, respectively. While, in the land snail, *M. cantiana* all tested molluscicides caused decreased in the level of total proteins. Mlotov gave the highest decrease followed by mesurol and gastrotox with mean values, 4.34 ± 0.11 , 4.57 ± 0.22 and 5.29 ± 0.20 less than control, respectively.

Table 7: Total lipids level in the two land snails, *E. vermiculata* and *M. cantiana* after 72 h of molluscicides treatment

Land snail species	Treatment	kg/Fed	Total lipids (TL) (Mean±SD)
<i>E. vermiculata</i>	Gastrotox	2	1.16±0.11b
	Mlotov	5	1.21±0.09b
	Mesurol	4	1.41±0.11a
	Control	--	1.13±0.18b
LSD (0.05) = 0.15			
<i>M. cantiana</i>	Gastrotox	2	3.13±0.24a
	Mlotov	5	2.72±0.24ab
	Mesurol	4	2.12±0.25b
	Control	--	3.22±0.47a
LSD (0.05) = 0.71			

Total lipids: (mg/ snail). Values followed by the same letter (s) are not significantly different at the 0.05 level according to Duncan's test

Table 8: Total proteins level in the two land snails, *E. vermiculata* and *M. cantiana* after 72 h of molluscicides treatment

Land snail species	Treatment	kg/Fed	Total proteins (TP) (Mean±SD)
<i>E. vermiculata</i>	Gastrotox	2	13.60±0.50bc
	Mlotov	5	17.42±1.21a
	Mesurol	4	14.23±0.55b
	Control	--	12.90±0.22c
LSD (0.05) = 1.16			
<i>M. cantiana</i>	Gastrotox	2	5.29±0.20b
	Mlotov	5	4.34±0.11c
	Mesurol	4	4.57±0.22c
	Control	--	5.79±0.26a
LSD (0.05) = 0.45			

Total proteins (T.P): (mg/ snail). Values followed by the same letter (s) are not significantly different at the 0.05 level according to Duncan's test

DISCUSSION

It is well known that the most significant biochemical effects of carbamate compounds are the ability to reversibly inhibit AchE. Also, they bind to the enzyme that is normally responsible for breaking down Ach after it has carried its message across the synapse. The present study showed that all tested molluscicides inhibited the activity of AchE. Mesurol (carbamate compound) was found to be the most inhibitor compound to AchE when applied against the two land snails, *E. vermiculata* and *M. cantiana*. These results support the finding of Essawy *et al.* (2009) found that some carbamate compounds such as methiocarb induced reductions in the activity of AchE in the land snail, *E. vermiculata*. Also, Daoud (2010) indicated that carbamates gave a significant decrease in AchE activity in the two land snails, *Cochlicella acuta* and *Succinea putris*.

The present study revealed that all tested molluscicides increased the activities of AST and ALT when tested against the land snail, *M. cantiana*. On the other hand, all treatments caused slightly increase in the activity of AST whereas, they were caused significant decrease in the level of ALT in the tissue homogenate of the land snail, *E. vermiculata*. The transaminases enzymes; AST and ALT are not solely located in hepatocytes but rather are also in many body organs. Also, they elevation in their activities could be due to a variety of conditions including muscle damage, intestinal and hepatic injury and toxic hepatitis (Farkas *et al.*, 2004). On the other hand, the decrease activities of AST and ALT may be due to either to leakage of the enzymes into extracellular compartments or to actual enzymes inhibition by these molluscicides. Thus, the deviation of both enzymes activities out of the normal range could lead to biochemical impairment and lesions of the tissues and cellular functions (Radwan *et al.*, 1992). Accordingly, the present elevations or reductions in the activities of AST and ALT enzymes in tissues of the two land snails, *E. vermiculata* and *M. cantiana* treated with tested molluscicides could be partially due to cell injury of their different organs and this may be led to disturbances in their enzymatic systems (Mahmoud, 2006). These results support the findings of Radwan *et al.* (1992) they found that carbamate compounds lead to significant elevation of the activity of AST and ALT when applied against the land snail, *Theba pisana*.

In general, the present data indicated that all tested compounds increased the level of LDH in land snail species, *E. vermiculata* and *M. cantiana*. The enhancement of release of LDH is indicative of cellular and / or membrane damage. These results agree with the finding of Abd-El-Ail (2004), who found that niclosamid pesticides were increased the level of LDH in *E. vermiculata* from the first day of exposure till the end of the experiment. Also the current data agree with the finding of Salama *et al.* (2005) reported that carbamates enhanced the release of LDH in the land snail, *H. aspersa*.

MFO an enzyme complex found in animals that oxidize toxic compounds to render them more susceptible to metabolism and excretion. The enzyme is localized in hepatopancreas in invertebrates. The present study clearly indicates that Metaldehyde compounds (gastrotox and mlotov) caused increase in the level of MFO in both tested land snails, while the level of this enzyme was decreased in the two treated land snails, *E. vermiculata* and *M. cantiana* when applied with carbamate compound (mesurol). Both NADPH-neotetrazolium reductase (NTR) and arylhydrocarbon hydroxylase (AHH) belong to the MFO system, which is normally involved in the metabolism of steroid hormones. NTR was activated after metaldehyde application because metaldehyde induced less degenerative effect in the cells of the hepatopancreas (Henderson and Tribskorn, 2002). Also, NTR was activated after metaldehyde application because ultra structural alternations in the endoplasmic reticulum in the crypt cells may possibly be related to the activation

of the NTR which know to be localized on the smooth endoplasmic reticulum (Henderson and Triebkorn, 2002). Mesurool leads to heavy ultra structure damage dose not know.

In general the current results indicated that a reduction of total lipids (TL) and total proteins (TP) in the tissues of land snail, *M. cantiana*. On the other hand, there were significant increase in (TL) and (TP) in the tissue of land snail, *E. vermiculata* when exposed to the tested molluscicides. The present study also disagrees with Radwan *et al.* (2008) found that methiocarb caused significant decrease in total proteins and total lipids in the tissues of *E. vermiculata*. Also, the current results are agreement with the findings of Abd-El-Ail (2004) found that niclosamide molluscicide were increased the level of total lipids and total proteins more than control after 24, 48, 72, 96 h of treatments when applied against the land snail, *E. vermiculata*.

The decrease in the level of both total protein and total lipids may be partly resulted from imbalance between the rate of synthesis and the rate of degradation. Gabr *et al.* (2007) reported that the depression in total lipids may be due to decline in lipid synthesizing capacity and/or due to an increase in the hydrolysis of hepatic lipids to combat the stress conditions. The harmful effect of chemical compounds could be attributed to enhancement of energy utilization and/or destruction of cells organelles of treated snails that may be led to inhabitation of protein synthesis (Eissa *et al.*, 2002).

The data presented in this study, provide that these chemical compounds caused an alternation in some biochemical targets which could lead to serious metabolic and cellular damage. In general, the three molluscicides were affected in the activities of five vital enzymes, total lipid and total protein when applied against the tested land snails. Further studies are needed to clearly the most probable mode of action of these chemical compounds on the terrestrial snails.

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