

## Biochemical Effects of Mullicicides against the Land Snails, *Helicella vestalis* and *Theba pisana* Using Sublethal Doses

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### ABSTRACT

Land snails are serious pests in orchards, resulting in quantitative and qualitative losses in several plant species. The biochemical effects of two mullicicides; Agrinate and Biomagic were studied on two land snail species; *Helicella vestalis* and *Theba pisana*. The negative effects of sub-lethal concentration (1/4 LC<sub>50</sub>) of both mullicicides on aspartate aminotransferase, alanine aminotransferase, total lipids and total proteins were assessed. Chemical assessments were carried out at Precise Chemical Analysis Component, Plant Protection Research Institute. Results showed that the two tested compounds enhanced, in general, the activity of aspartate aminotransferase (AST) in most periods after treatment, except at the 3<sup>rd</sup> day after treatment, as both pesticides achieved a decrease in enzyme activity in *H. vestalis* and also at the 10<sup>th</sup> day in *T. pisana*. treatment as Agrinate reduced the activity of (AST). Activity of alanine aminotransferase (ALT) was reduced in both snails, except at the 3<sup>rd</sup> day of treatment with Agrinate, while Biomagic increased (ALT) activity at the 3<sup>rd</sup> and 7<sup>th</sup> days post-treatment, followed by a reduction towards the end of the test duration (two weeks). In addition, levels of total lipids (TL) and total proteins (TP) were reduced due to the application of Agrinate or Biomagic. Because enzymes, proteins and lipids are vital chemical compounds in all organisms, including land snails, it has become very necessary to assess their reactions towards mullicidal applications.

### INTRODUCTION

Land snails are considered serious pests attacking different types of crops, causing great damage to all plant parts. These animals attack plants at their different growth stages and reduce their yields (Khidr *et al.* 2011)

Many investigations were evaluated many insecticides and biocides against land snails under laboratory and field conditions to find out the suitable molluscicide for controlling these pests (Farg, 2012) and (Ismail *et al.*, 2015). The carbamate compounds appeared to have a high toxic effect on the land snails *Eobania vermiculata* and *Monacha cantiana* under laboratory and field conditions (Aioub *et al.* 2000). Among carbamates, methomyl had the highest molluscicidal activity (Radwan *et al.*, 2008, Hendawy *et al.*, 2015 and Khalil, 2016).

Biological insecticides are actual living organisms or the toxins produced by them; include viruses, bacteria, fungi, and non-target insects (Geasa *et al.*, 2013). Biomagic is a biological insecticide based on a selective strain of naturally occurring entomopathogenic fungus *Metarhizium anisopliae*. It contains spores and mycelial fragments. Fungal isolates of *M. anisopliae* are one of the most effective fungi against the two land snails; *E. Vermiculata* and *M. cantiana* (Gabal, 2013). At Kafr El-sheikh Governorate, the native isolates of fungi, *M. anisopliae* were compared to methomyl insecticide against *Monacha* spp. Methomyl exhibited the highest toxic effect against snails, while *M. anisopliae* had a moderate effect (Hendawy *et al.*, 2015). Transaminase enzymes and acetylcholine esterases as well as total proteins and total lipids are important in the biological processes in the land snails (Abd El-Aal, 2004).

This work aimed to determine the biochemical effect of two pesticides, Agrinate and Biomagic on the activities of two vital enzymes, aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) and also on total proteins (TP) and total lipids (TL) in the two land snails *Helicella vestalis* and *Theba pisana* which are considered the most common snails causing problems in orchards at Kafr El-Sheikh Governorate.

### MATERIALS AND METHODS

#### Tested compounds:

- **Agrinate:** Methomyl 24% WP. S-methyl-N-[(methyl carbamoyl) oxy] thioacetimidate.
- **Biomagic:** *Metarhizium anisopliae* 1.75% WP.;

The two compounds were obtained from Central Laboratory of Pesticides, Agric. Res. Center, Dokki, Giza.

#### Tested animals:

Adult individuals of the land snails; *H. vestalis* and *T. pisana* were collected from an ornamental orchard at Motobas district, Kafr El-sheikh Governorate during March and April, 2017 season. The snails were transferred to laboratory, kept in open-air cages and fed on fresh lettuce leaves.

#### Bait applications:

Sub-lethal concentration of each compound was obtained using the graphic method of the curve dose-effectes using the probit analysis, according to Finney (1971). The snails were treated with sub-lethal concentration (1/4 LC<sub>50</sub>) of each compound. For each treatment, 100 snails were allocated and kept in 10 plastic boxes (10 animals each). Baits of each compound was prepared by incorporating 40 ml. water containing 0.2 ml of Agrinate or 0.3 ml of Biomagic i.e. (1/4 LC<sub>50</sub>) with 10 g bran and provided to snails in each box. A parallel control test was conducted, using wheat bran bait with water only. The bait was renewed every 3 days till the end of the experiment. The snails were monitored 3, 7, 10, and 14 days after treatment, then subjected to biochemical analysis.

#### Biochemical studies:

**Sample preparation:** The soft parts of *H. vestalis* and *T. pisana* snails were excised from the shell, then the soft tissue was homogenized in 10 volumes (W/V) of 0.1 M phosphate buffer PH 7.4 using glass homogenizer for one minute. The homogenates were centrifuged at 1000xg for 20 minutes using a cooling centrifuge at 4°C. The supernatant was used as a substrate of enzymes (Mourad and Zedan, 1996).

#### Determination of biochemical activities:

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed according to Reitman and Frankel (1957). Total proteins

were assessed according to Bradford (1976), and total lipids were estimated according to Knight *et al.* (1972). Statistical analysis was done according to Duncan (1955).

## RESULTS AND DISCUSSION

### Effects of two pesticides on (AST), (ALT), (TP), and (TL) in *H. vestalis*:

The obtained data in Table (1) show the effect of Agrinate and Biomagic on (AST), (ALT), (TP), and (TL) in the land snail *H. vestalis* after different periods.

- **Effect on aspartate aminotransferase (AST):** Data showed that Agrinate significantly reduced the activity of AST after 3 days with 3.95%. On the contrary, this pesticide began to increase the level of enzyme from the 7<sup>th</sup> day of treatment with 16.83, 97.63, and 74.80% after 7, 10, and 14 days post treatment, respectively. The highest mean value of AST activity was noted after 10 days. Similar results were observed in Biomagic treatment, the pesticide reduced AST level only after 3 days with 5.33%. The ratio of AST activity achieved a slight increase with 0.45% beginning from the 7<sup>th</sup> day. This ratio gradually increased to 13.36% after 7 days, while the highest increase was observed two weeks post-treatment with 54.58%.
- **Effect on alanine aminotransferase (ALT):** Data showed that Agrinate reduced the activity of ALT after the four exposure periods but with different values. The reduction was 59.84% after 3 days, it reached to 1.5 and 9.23% after 7 and 10 days, respectively. The difference of ALT was achieved the highest value after 14 days with 70.98%. Biomagic

took another trend at the first two exposure periods, whereas it was significantly increased the level of ALT when applied against the snail but after 10 and 14 days, it caused a significant decrease in ALT level with 15.58% and 54.24%, respectively.

- **Effect on total proteins (TP):** Data revealed that the two mentioned pesticides, Agrinate and Biomagic reduced the levels of total proteins after all tested periods with significant different values. During the 3<sup>rd</sup> day of treatment, TP decreased with 78.41% and 62.02% difference of two pesticides, respectively. The 7<sup>th</sup> and 10<sup>th</sup> days showed significant decrease too, with 55.86% and 36.19% and 69.59% and 52.15% difference with control for Agrinate and Biomagic, respectively. The same trend was observed after two weeks with 46.27% and 19.98% difference comparative with control for the two previous pesticides.
- **Effect on total lipids (TL):** Data in Table (1) indicated that the two tested compounds reduced the levels of total lipids when applied against *H. vestalis*. There were significant differences between all days of treatment. The 14<sup>th</sup> day of exposure to Agrinate showed the highest decrease in TL with 72.23%. Biomagic was less effective than Agrinate in this concern, it also reduced lipids but with values lower than Agrinate. Biomagic gradually reduced mean of TL from 8.07±0.70 after 3 days to 6.44±0.49 and 6.20±0.44 after 7 and 10 days, respectively, then to 5.83±0.16 after 14 days.

**Table 1. Effect of ¼ LC<sub>50</sub> of Agrinate and Biomagic on the activity of (AST), (ALT), (TP) and (TL) in *Helicella vestalis* at different exposure periods.**

Pesticide	Exposure time (days)	AST(µ/L)		ALT (µ/L)		TP (g/100ml)		TL (g/100ml)	
		Mean ±SD	change % (±)	Mean ±SD	Change % (±)	Mean ±SD	Change % (±)	Mean ±SD	Change (±)%
Agrinate	3	51.03±1.00**	+3.95	6.96±0.57**	+59.84	6.98±0.85**	+78.41	6.96±0.33*	+29.20
	7	62.07±1.59**	-16.83	17.07±0.21**	+1.50	14.27±0.67**	+55.86	6.34±0.51*	+35.50
	10	105±4.36**	-97.63	15.73±0.55*	+9.23	9.83±0.21*	+69.59	8.17±0.91*	+16.89
	14	92.87±3.19**	-74.80	5.03±0.38	+70.98	17.37±1.27**	+46.27	2.73±1.16*	+72.23
Biomagic	3	50.3±1.04	+5.33	30.17±1.36**	-74.09	12.28±0.25**	+62.02	8.07±0.70*	+17.90
	7	53.37±0.81	-0.45	25.80±0.82**	-48.87	20.63±1.18**	+36.19	6.44±0.49*	+34.49
	10	60.23±1.16**	-13.36	14.63±0.64**	+15.58	15.47±1.36**	+52.15	6.20±0.44*	+36.93
	14	82.13±2.35**	-54.58	7.93±0.67**	+54.24	25.87±1.88**	+19.98	5.83±0.16*	+40.69
Control	--	53.13±2.35	-	17.33±1.27	-	32.33±1.99	-	9.83±0.76	-

\*Significant (P > 0.05)\*\* Highly significant (P > 0.01)

### Effects of two pesticides on (AST), (ALT), (TP), and (TL) in the land snail *T. pisana*:

Data in Table (2) show the effect of ¼ LC<sub>50</sub> of Agrinate and Biomagic on (AST), (ALT), (TP), and (TL) in the land snail *T. pisana*.

- **Effect on AST:** Data revealed that Agrinate increased the level of AST after detected days of treatment except after the 3<sup>rd</sup> day, it achieved % decrease with 1.17. The highest concentration of the enzyme was after 72 h with total mean of 75.97±3.62 while the lowest was after two weeks. On the other hand, Biomagic raised the activity of AST in *T. pisana* land snail all days post treatment with different values, these differences were high after 3 and 7 days with 35.09 and 38.33%, respectively. High significant

differences were observed between the total mean numbers of AST after 10 and 14 days.

- **Effect on ALT:** Similar results to those of *H. vestalis* were observed in ALT of *T. pisana* when treated with either Agrinate or Biomagic whereas a high significant increase was recorded after 3 days of Agrinate treatment where its change percentage was 85.43%
- **Effect on TP:** Results in Table (2) show decreasing the level of TP in *T. pisana* snails treated with both of Agrinate and Biomagic as reported in *H. vestalis*, where they reduced protein levels after all treatment periods. The highest decrease was after 10 days for Agrinate and 7 days for Biomagic with 64.33 and 66.60%, respectively.

• **Effect on TL:** Results revealed different values of TL levels among time periods for the two pesticides, whereas Agrinate achieved higher total lipids concentration after 3 days. The mean values of TL reduction were 21.29, 71.48, and 48.02% after 7, 10, and 14 days, respectively. Biomagic proved to be more effective on TL than Agrinate, since it reduced lipid levels with high ratio at all tested periods. On the other hand, the highest mean value of TL was after 3 days and the lowest was observed at the 10<sup>th</sup> day of treatment with 7.12±1.93 and 5.30±0.10, respectively.

Alternations in the activity of AST and ALT are known to be helpful in the diagnosis of hepatic disease and infarcts of the heart Abd EL-Wahed (2014). Plasma protein serves as a source for rapid displacement of tissue proteins during tissue depletions, as buffers in acid base balance and as transporters for the constituents of the blood such as lipids, vitamins, hormones and certain enzymes. Also, lipids play important roles in normal functions of the cell, not only lipids serve as highly reduced storage forms of energy, but also play an intimate role in the structure of cell membranes and the organelles found in the cell. (Wilson, 1986).

**Table 2. Effect of ¼ LC<sub>50</sub> of Agrinate and Biomagic on the activity of (AST), (ALT), (TP) and (TL) in *Theba pisana* at different exposure periods.**

Pesticide	Exposure time (days)	AST(µ/L)		ALT (µ/L)		TP (g/100ml)		TL (g/100ml)	
		Mean ±SD	Change % (±)	Mean ±SD	change % (±)	Mean ±SD	Change % (±)	Mean ±SD	change% (±)
Agrinate	3	75.97±3.62**	-67.33	37.03±2.68**	-85.43	13.47±0.67	+52.28	15.13±1.55**	-17.56
	7	56.50±1.80**	-24.45	17.50±1.00*	+12.37	12.57±0.38	+55.47	10.13±0.91**	+21.29
	10	44.87±0.95**	+1.17	19.53±0.68**	+2.20	10.07±0.87**	+64.33	3.67±0.38**	+71.48
	14	45.77±1.57**	-0.81	4.34±0.51**	+78.27	11.57±0.74*	+59.02	6.69±1.16**	+48.02
Biomagic	3	61.33±0.59	-35.09	20.03±0.15	-0.30	14.07±1.93**	+50.16	7.12±1.93**	+44.68
	7	62.80±1.47	-38.33	20.93±1.78	-4.81	9.43±1.07**	+66.60	6.22±0.51*	+51.67
	10	48.43±1.01**	-6.67	17.87±0.42	+10.52	14.77±0.86	+47.68	5.30±0.10**	+58.82
	14	54.80±1.60**	-20.70	18.47±0.57	+7.51	11.97±0.15	+57.60	5.83±0.76**	+54.70
Control	--	45.40±2.16	-	19.97±1.06	-	28.23±0.86	-	12.87±1.17	-

\*Significant (P > 0.05)\*\* Highly significant (P > 0.01)

A few investigations had been conducted on the effectiveness of pesticides; Agrinate and Biomagic on (AST), (ALT), (TP), and (TL) of land snails. Regarding the response of (AST) and (ALT) to mentioned pesticides, the two pesticides increased effects on (AST) while most treatments caused a decrease in the activity of (ALT) of *H. vestalis* and *T. pisana* snails. These results support the findings of Radwan *et al.* (1992), who found that carbamate compounds lead to elevations of the activity of (AST) and (ALT) when applied against the land snail *T. pisana*. They also reported that the deviation of both enzyme activities out of normal range could lead to biochemical impairment and lesions of the tissues and cellular function. The current data also agree with El-Gohary and Genena (2011) who reported that Gastrox, Molotov, and Mesurool increased the activity of (AST) and (ALT) when tested against the land snail, *M. cantiana*, but all treatments caused slight increase in the activity of (AST) whereas they caused a significant decrease in the level of (ALT) in the land snail *E. vermiculata*. Lannet like some other pesticides may cause alternation in the activities of (AST), (ALT), (ALP) and (LDH) (Celik *et al.*, 2009). Aminotransferases are very active in the liver and their activity can be detected in small amounts so, they are the most useful in monitoring people exposition to such pesticides (Lehninger *et al.*, 1992). The significant changes in (AST) and (ALT) activities in the land snails pointed out to functional disorder of the liver (Khalil, 2016).

In general, the current data indicated a reduction of total proteins (TP) and total lipids (TL) in the tissues of *H. vestalis* and *T. pisana* snails when treated with Agrinate and Biomagic. This agrees with El-Gohary and Genena (2011) and Khidr *et al.* (2011) who reported that the sub lethal concentration (¼ LC<sub>50</sub>) of the two compounds;

theophylline and furosemide significantly or insignificantly reduced total proteins and total lipids in the land snail *M. obstructa*. They also reported that the fluctuation in the level of total proteins and total lipids might be resulted from balance between the rate of synthesis and degradation. Also, the exposure to sub lethal doses of Lannet may influence total protein metabolism. The depletion of protein in snails may be due to the degradation of carbohydrates such cases are indicative of disorders in protein synthesis and metabolism (Khalil 2016). Gabr *et al.* (2007) reported that the depression in total lipids may be due to decline in lipid synthesis capacity and/or due to an increase in the hydrolysis of hepatic lipid to combat the stress conditions.

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### التأثير البيوكيميائي للجرعات تحت المميتة من مبيدات القواقع على قوقي هيلسيلافيستالس و ثيبا بيسانانا وفاء عبد المجيد شهاوي قسم بحوث الحيوانات الضارة - معهد بحوث وقاية النباتات - مركز البحوث الزراعية- الدقى - جيزة

تعتبر القواقع الأرضية من الآفات الخطيرة في البساتين، والتي ينتج عنها خسارة في أنواع كثيرة من النباتات سواء من حيث الكم أو الكيف. في هذه الدراسة تم تقدير التأثيرات الكيميائية الحيوية لنوعين من مبيدات القواقع هما الأجرينيت والبيوماجيك على نوعي القواقع هيلسيلافيستالس و ثيبا بيسانانا وذلك بدراسة تأثير التركيز تحت المميت ( $1/450 LC$ ) من كلا المبيدين على إنزيمات AST و ALT والبروتين والدهون الكليه وذلك بطريقة الطعوم السامة. وأخذت القياسات على فترات مختلفة من المعاملة. وأوضحت النتائج أن كلاً من الأجرينيت والبيوماجيك أحدثا زيادة في إنزيم ال AST في كلا القوقعين خلال أغلب فترات المعاملة في حين حدث نقص ملحوظ في نشاط الإنزيم في اليوم الثالث من معاملة القوقع هيلسيلافيستالس سواء بالأجرينيت والبيوماجيك، وكذلك في اليوم العاشر من معاملة القوقع ثيبا بيسانانا بمبيد الأجرينيت. أما بالنسبة لإنزيم ALT فقد أدى التعرض لمبيد الأجرينيت إلى انخفاض نشاطه في كلا القوقعين عدا في اليوم الثالث من المعاملة للقوقع ثيبا بيسانانا حيث زاد نشاطه مقارنة بالكنترول في حين أدى البيوماجيك لزيادة نشاط ال ALT خلال اليومين الثالث والسابع من المعاملة تبعها انخفاض لنشاط الإنزيم في باقي فترات المعاملة وذلك في كلا القوقعين. ومن ناحية أخرى أشارت النتائج إلى حدوث انخفاض في محتوى البروتين والدهون لكلا القوقعين بعد تعرضهما للمبيدين خلال فترة المعاملة.