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Molluscicidal toxicity of abamectin against *Eobania vermiculata* and *Theba pisana in vivo* and the estimation of GABA-Transaminase activity by HPLC

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ABSTRACT

The molluscicidal toxicity of abamectin against two economic snails namely, the brawn garden snail (*Eobania vermiculata*) and the white garden snail (*Theba pisana*), was established under *in vivo* conditions. Abamectin was used by the topical application technique. The mortality of snails was recorded after 24 and 72 hrs of exposure. The obtained results revealed that *E. vermiculata* was more resistant to abamectin than *T. pisana*. The LD_{50} for *E. vermiculata* after 24 and 72 hrs of exposure were 0.379 & 0.250 µg/ gm b.w, respectively. Whereas the LD_{50} for *T. pisana* were 0.199 and 0.125 µg/ gm b.w after 24 and 72 hrs of exposure, consecutively. On the other hand, the impact of abamectin upon GABA- Transaminase concentration was estimated in both snails. For the first time, the activity of GABA- T was estimated by HPLC. Abamectin has been assessed with 1/2, 1/5 and 1/10 of LD_{50} for 24 and 72 hrs of exposure. The most effective dose on GABA- T activity was 1/2 of LD_{50} after 24 and 72hrs of application for both snails.

Keywords: Abamectin, *Eobania vermiculata*, *Theba pisana*, GABA- T, HPLC.

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INTRODUCTION

Terrestrial gastropods are from the most significant threats to sustainable agriculture around the world (Barker, 2002). The land snails (subclass: Pulmonata) are one of the most numerous with almost 35,000 described species of the world which belong to the terrestrial gastropods. The losses which caused by land snails depends not only on their activity and population density, but also on their feeding habits, which differ from one species to another. Different parts of plant such as leaves, flowers, roots, buds, stems and even the trunk of trees are attacked by land snails causing great damages.

In Egypt, land snails are known as dangerous pests to field crops, vegetables, orchards and ornamental plants (Abdallah *et al*., 1998; Ibrahim 1995; Mohamed 1995). Damage caused by snails is mainly attributed to feeding and to contamination with their bodies, faces or slime, leading to deterioration of the product quality besides, the financial loss (Lglesias *et al*., 2003). The importance of land snails as pest organisms has drastically increased in the past few decades (Gathwaite and Thomas, 1996).

Growers and farmers often experience difficulty in controlling land gastropods with conventional bait pellets containing molluscicides as in wet conditions. The efficacy of these pellets can be very low leading to unsatisfactory control levels (Schuder *et al*., 2003). In addition, the toxic baits have effects on other non-targeted life farms (Martin, 1993; Purvis, 1996).

Avermectins, is a new generation of macrocyclic lactones group produced by a fermentation process of soil bacterium; *Streptomyces avermitilis* (Omura and Shiomi, 2007; Pitterna *et al*., 2009). Abamectin or avermectin B1 is a combination among avermectin B1a (>80%) and B1b (<20%) (Pitterna *et al*., 2009), and they have very similar biological and toxicological properties (Khalil, 2013). Abamectin is currently used as a miticide, insecticide, and nematicide in several crops (Khalil, 2012; Khalil *et al*., 2012).

Therefore, the main target of this work is to assess the molluscicidal activity of the non-conventional pesticide, abamectin against the most two abundant species of the terrestrial snails brown garden snail (BGS) *Eobania vermiculata* and white garden snail (WGS) *Theba pisana* using topical application technique under laboratory conditions, as well as determination the activity of GABA-Transaminase in both snails by HPLC as new method.

METRIALS AND METHODS

Common name of biopesticide: Abamectin (97% a. i)

IUPAC name : 10*E*,14*E*,16*E*,22*Z*)-(1*R*,4*S*,5'*S*,6*S*,6'*R*,8*R*,12*S*, 13*S*,20*R*, 21*R*,24*S*)-6'-[(*S*)-*sec*-butyl]-21,24-dihydroxy-5',11,13,22-tetramethyl-2-oxo-3,7,19-trioxatetracyclo [15.6.1.$1^{4,8}$.$0^{20,24}$]pentacosa-10,14,16,22-tetraene-6-spiro-2'-(5',6'-dihydro-2'*H*-pyran)-12-yl 2,6-dideoxy-4-*O*-(2,6-dideoxy-3-*O*-methyl-∝-L-*arabino*-hexopyranosyl)-3-*O*-methyl-∝-L-*arabino*-hexopyranoside (i) mixture with (10*E*,14*E*,16*E*,22*Z*)-(1*R*,4*S*,5'*S*, 6*S*,6'*R*,8*R*,12*S*, 13*S*,20*R*,21*R*,24*S*)-21,24-dihydroxy-6'-isopropyl-5',11,13,22-tetramethyl-2-oxo-3,7,19-trioxatetracyclo[15.6.1.$1^{4,8}$.$0^{20,24}$] pentacosa-10,14,16,22-tetraene-6-spiro-2'-(5',6'-dihydro-2'*H*-pyran)-12-yl2,6-dideoxy -4-*O*-(2,6-dideoxy-3-*O*-methyl-∝-L-*arabino*-hexopyranosyl)-3-*O*-methyl-∝-L-*arabino*-hexopyranoside (ii).

Topical application

Topical application technique was used according to Abdallah *et al*. (1992). In this method technical grades of abamectin was dissolved in dimethyl sulfoxide (DMSO). DMSO has been shown to be the most appropriate solvent for topical application as it causes little distress to snails (Young and Wilkins, 1989). The stock solution of abamectin was serially diluted with the same solvent to achieve the wanted doses. The tested doses of abamectin for *E. vermiculata* were 0.077, 0.155, 0.232, 0.310, 0.388, 0.775 and 1.55 µg/gm b.w., whereas the doses in case of *T. pisana* were 0.085, 0.170, 0.255, 0.340, 0.425 and 0.890 µg/gm b.w. Each treatment (dose) was replicated three times and each replicate included ten animals. The replicate was a cup (diameter 10 x height 7 cm) that was covered with cloth netting secured with rubber bands to prevent snails from escaping. The tested doses were gently applied once on the surface of the snail body inside the shell using a micropipette. The positive control was exposed to DMSO. Mortality counts were recorded after 24 and 72 hrs of treatment and mortality percentages were calculated. The mortality in untreated check was corrected according to Abbott formula (Abbott, 1925) and then subjected to probit analysis (Finney, 1971). The dead animals were detected by probing the snails with a needle to elicit typical withdrawal movement according to WHO (1965).

Abamectin on the GABA-Transaminase (GABA-T) of *E. vermiculata* and *T. pisana*

E. vermiculata and *T. pisana* snails were topically treated with abamectin at 1/10, 1/5 and 1/2 of calculated LD_{50}. Treated snails were collected after 24 and 72 hrs of treatment. Snail shells were removed and the soft tissue was homogenized in 1:10 (w/v) 200 m*M* potassium phosphate buffer, pH 6.8 using Polytron Kinemetica homogenizer. The homogenate was centrifuged at 5000 rpm for 30 min at 4ºC using IEC-CRU 5000 cooling centrifuge. Supernatant was used as the enzyme source for the determination of enzyme activity.

Enzyme assay was carried out according to the method of Allen and Griffiths (1984) with some modifications. The modification was as

follows: To 100 µl of enzyme source, 500 µl (200 m*M* potassium phosphate buffer, pH 6.8), 20 µl (50 m*M* GABA) and 55 µl (0.2 m*M* pyridoxal 5'-phosphate (PLP) were added. After incubation for 20 min at 37°C, 675 µl absolute methanol was added at room temperature to terminate the reaction. The suspension was centrifuged at 1500 rpm (10 min, 0°C). GABA present in the supernatant was derivatization, following the indications of (Hamza *et al.*, unpublished data) as follows: GABA present in the supernatant and or in a standard solution of GABA at 1:1 (v/v) OPA derivatization reagent and leave about 30 min at room temperature to achieve derivatization well be done.

The *O*- phthaldialdehyde (OPA) derivatization reagent was prepared according to Liu and Worthen (1992) by dissolving 3 mg of OPA in 50 µl of methanol, adding 450 ml of sodium borate buffer (0.5 mol/l, pH 10.2) and 5 µl of 3-mercaptoprobionic acid (3-MPA). Borate buffer was prepared from 0.5 M boric acid solution adjusted to pH 10.2 with 5 M sodium hydroxide solution. This OPA solution was placed in an amber crimp top vial with a silicone rubber PTFE-coated cap and kept in the dark at –20°C. Fresh solution was prepared each week.

HPLC separation and evaluation of GABA after derivatization:

Mobile phase solution

The sodium acetate buffer (0.015 M) in mobile phase was prepared by dissolving sodium acetate in HPLC-grade water and titrating to pH (6.8 for solvent A) with glacial acetic acid and methanol (solvent B). The mobile phases were filtered by passing through a 0.45-µm Durapore membrane filter (Millipore Inc., Milford, MA). The mobile phase program illustrated in Table (1).

Table 1. Mobile phase programme:

Time/min.	0.05	15.00	18.50	22.00	25.0	40.00
A	100.00	60	57.5	45.0	100	100
B	0.0	40	42.5	55.0	0.0	0.0

Agilent Hewlett-Packard 1200 series HPLC system with solvent degasser system, quaternary pump, and autosampler fitted with a diode array and fluorescence detector was used. The system was controlled by a Hewlett-Packard Vectra Xm series 4 data analysis work station. The used column was stainless steel Zorbax SB C18 with 250 mm. × 4.6 mm. I.D. The mobile phase for isocratic elution was pumped at 1 ml/min, at 40°C; detection was at excitation 230 and emission 450 nm. Standard curve for GABA were carried out.

The standard curve of GABA

The standard curve for GABA potted as shown in Figure (1), was determined by applying the same derivatization procedure to five standard solutions of GABA (0.15, 0.3, 0.6, 1.2 and 2.4 *n* mole). The corresponding optical density was recorded as previously mentioned. GABA was calculated as n*M*/ mg protein.

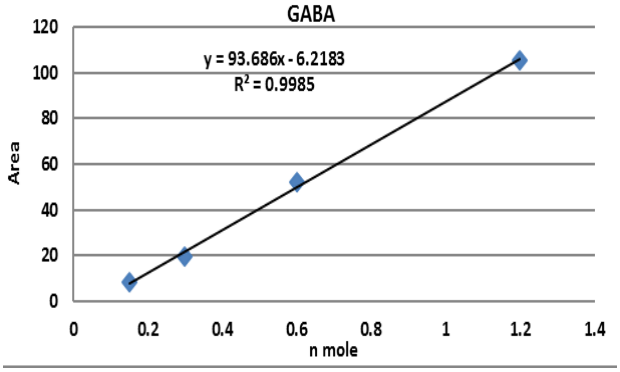

Fig. 1. Standard calibration curve for GABA linear response over the concentration range of 0.15-2.4 *n* mole.

Protein measurements

Protein estimation was done according to Lowry *et al*. (1951).

Statistical analysis

The LD_{50} value is expressed as microgram of pesticide per gram body weight of snail with confidence limit and slope were computed using the Probit analysis program based on Finney (1971). All statistics were performed using the SAS software programme.

RESULTS AND DISCUSSION

Data presented in Table 2 and Fig. 2 indicated the toxicity impact of abamectin on both snails, *E. vermiculata* and *T. pisana* by using the topical application technique. The mortality of snails was recorded after 24 and 72 hrs of application. Abamectin was evaluated at seven doses for *E. vermiculata*.

While it was tested at six doses for *T. pisana*. The tested doses were chosen depending on preliminary tests to specify the range of doses. The obtained results showed that the highest doses of abamectin recorded 86 and 100% mortality after 24 and 72 hrs of exposure for *E. vermiculata*, respectively. Otherwise, the LD_{50} of abamectin after 24 and 72 hrs for *E. vermiculata* were 0.379 and 0.250 µg/gm b.w, consecutively. Abamectin achieved mortality by 100% for *T. pisana* with the highest dose for both tested periods of exposure. The values of LD_{50} were 0.199 and 0.125 µg/gm b.w after 24 and 72 hrs of exposure, respectively.

The findings of the present study are in agreement with Mortada *et al.* (2012) who reported that molluscicide compounds were the most effective to population density reduction of land snails *M. contiana* compared with biocides (Agrien, Diple 2x, Protecto and Vertimec) on sugar beet and pea plantation. Also, Mortada *et al.* (2013) found that Agriflex® (abamectin + thiamethoxam) has molluscicidal activity against adults of *Monacha obstructa* land snails using leaf dipping and bait technique methods. The recorded LC_{50} of Agriflex® was 1430.8 mg/L for leaf dipping technique, while LC_{50} was 12800 mg/L for bait technique. Daoud (2004) tested abamectin against *Eobania vermiculata* and *Monacha contiana* on Egyptian clover crop. Results revealed that Neomyl exhibited the highest toxic action followed by abamectin under field conditions. Abdelgalil (2011; 2016) and Abdallah *et al.* (2015) proved that abamectin recorded significant mortalities against *Eobania vermiculata* (BGS) and *Theba pisana* (WGS) snails by using wheat bran bait and topical application techniques.

Table (2): Toxicity of abamectin against *Eobania vermiculata* (BGS) and *Theba pisana* (WGS) snails using topical application technique, shown as mortality percentage and LD_{50} values after 24 and 72 hrs of exposure.

Hours	The tested doses (µg/ gm b.w.)							LD_{50} (µg/ gm b. w)	Conf. Limits		Slope
	0.077	0.155	0.232	0.310	0.388	0.775	1.55		Lower	Upper	
Mortality % of *Eobania vermiculata* (BGS)											
24	0	20	33	45	55	73	86	0.379	0.33	0.42	1.94
72	19	33	46	57	68	86	100	0.250	0.21	0.27	2.01
Hours	The tested doses (µg/ gm b.w.)							LD_{50} (µg/ gm b. w)	Conf. Limits		Slope
	0.085	0.170	0.255	0.340	0.425	0.890			Lowe r	Upper	
Mortality % of *Theba pisana* (WGS)											
24	25	40	58	67	79	100		0.199	0. 17	0.22	2.06
72	35	60	80	86	93	100		0.125	0.10	0.14	2.52

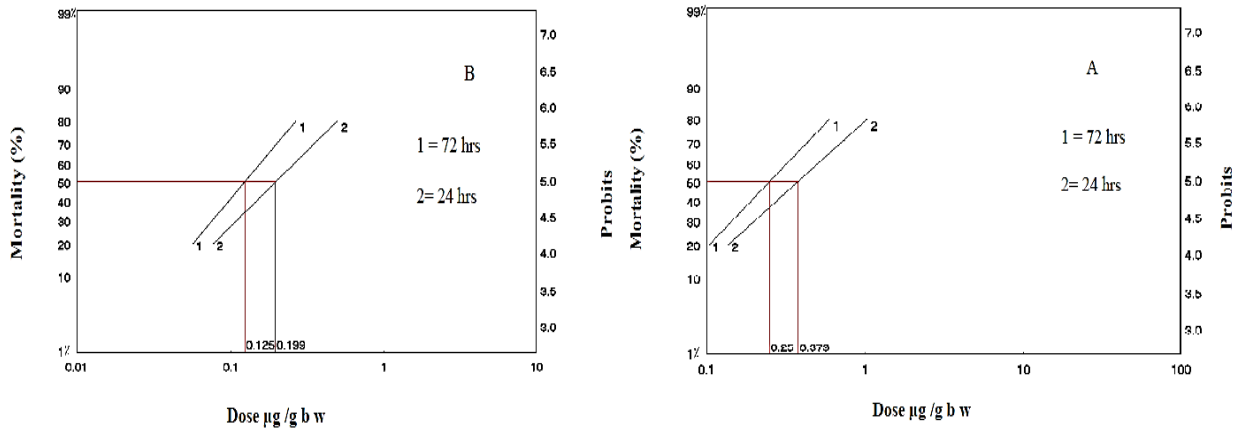

Fig. 2. Probit regression lines representing the effect of abamectin using topical application against terrestrial snails: A- *Eobania vermiculata* (BGS) and B- *Theba pisana* (WGS).

The impact of abamectin on GABA-Transaminase activity was determined in both *E. vermiculata* and *T. pisana* Table (3)**.** Abamectin at 1/2, 1/5 and 1/10 of LD_{50} were evaluated after 24 and 72 hrs of exposure. The gained results after 24 of exposure at 1/2 of LD_{50} recorded the least activity by 35.3% followed by 1/5 and 1/10 of LD_{50} values with 62.5 and 74.59%, respectively. Moreover, the 1/2 of LD_{50} after 72 hrs of exposure was the superior treatment which recorded 59.46% activity. The LD_{50} at 1/5 and 1/10 after 72 hrs of exposure gave 76.55 and 94.46% activity, respectively.

Table 3. Effects of *in vivo* abamectin on *Eobania vermiculata* (BGS) and *Theba pisana* (WGS) GABA-Transaminase (GABA-T) activities.

Dose		Abamectin			
		Eobania vermiculata		*Theba pisana*	
		S.A (n*M*GABA)/mg protein/min ± SD	Activity %	S.A (n*M*GABA)/mg protein/min ± SD	Activity %
LD_{50} at 24 hr	½	10.476±0.104	35.3	6.794 ±0.116	26.74
	1/5	18.552±0.101	62.51	10.597 ±0.072	41.71
	1/10	22.138±0.091	74.59	18.966 ±0.11	74.65
$LSD_{0.05}$		0.271		0.183	
LD_{50} at 72 hrs.	½	17.647±0.116	59.46	7.866 ±0.0587	30.96
	1/5	22.719±0.084	76.55	14.306 ± 0.109	56.31
	1/10	28.036 ±0.042	94.46	22.361 ± 0.097	88.01
$LSD_{0.05}$		0.259		0.167	

Specific activity of untreated ***E. vermiculata*** snail (GABA-T) is 29.679± 0.231(S.A n*M* GABA)/ mg protein/min) ± SD.
Specific activity of untreated ***T. pisana*** snail (GABA-T) is 25.406± 0.081 (S.A n*M* GABA)/ mg protein/min) ± SD.

In respect to *T. pisana* GABA-T was also determined with 1/2, 1/5 and 1/10 of LD_{50} after both 24 and 72 hrs of exposure. The highest activity after 24 hrs was recorded with 1/10 LD_{50} by 74.65% followed by 1/5 and 1/2 of LD_{50} by 41.71 and 26.74%, respectively. Whereas after 72 hrs of exposure, 1/2 of LD_{50} gave 30.96% activity of GABA-T, followed by 1/5 and 1/10 of LD_{50} values with 56.31 and 88.01% activity, respectively.

The mechanism by which avermectins produce the pesticide is the release of γ-aminobutyric acid (GABA) and the enhancement of its inhibitory action (Cambell, 1989). Also, Abdel baky (2004) observed that GABA concentrations were increased in earthworm, (*Allolobophora caliginosa*) as result to treat by ivermectin.

Yamazaki *et al*. (1989) reported that ivermectin is an agonist for the GABA neurotransmitter. Thus, the binding of ivermectin to a neuronal membrane increases the release of GABA which binds to the GABA-receptor-chloride channel complex of postsynaptic neuronal membranes causing an influx of chloride ions. The influx of chloride ions causes hyperpolarization of neuronal membrane. The hyperpolarization of neuronal membrane mediates aflaccid paralysis in arthropods and nematodes.

Furthermore, Kass *et al*. (1980; 1984) used *Ascaris lumbricoides* as a model system. Their results demonstrated avermectun's function as a GABA agonist that stimulates GABA release from pre-synaptic inhibitory membranes. Abou-Taleb *et al*. (2009) reported that emamectin benzoate increased the GABA neurotransmitter and glutamic acid concentrations in the field strain of cotton leaf worm to lesser extent than in the laboratory strain. GABA and glutamic acid augmentation are depending on the emamectin benzoate concentration and the time of exposure. The results come in complete agreement with what obtained by Abdelgalil (2011; 2016) and Bedair (2010).

The chromatogram profile of HPLC for standard GABA after derivatization was

appeared with retention time at 17.05 min (Fig.3). While in Fig. (4) panels A and B represents the chromatogram profile of the sample after derivatization in case BGS and WGS snails after 24 hrs at 1/2 LD_{50} with retention time at 16.95 and 17.00 min, respectively.

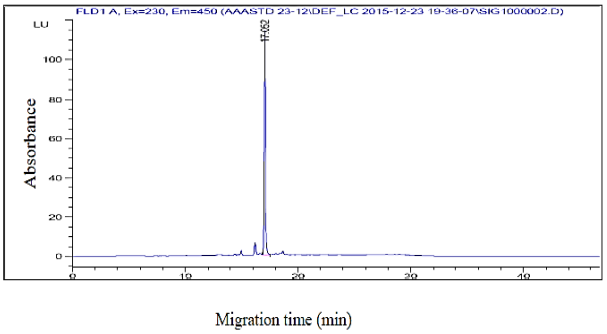

Fig. 3. HPLC chromatogram of standard γ-aminobutyric acid (GABA).

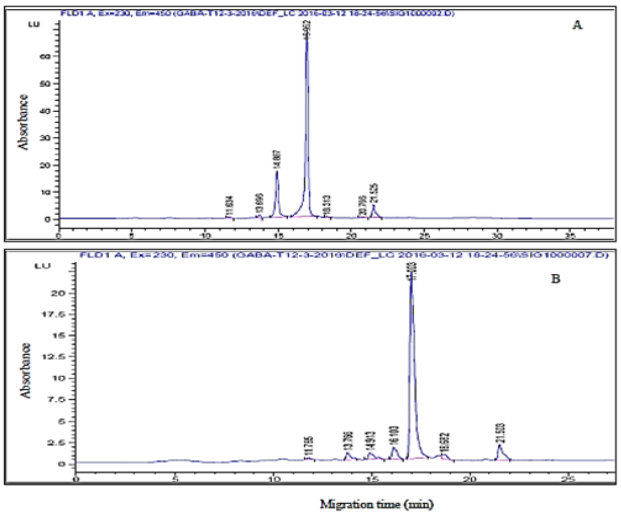

Fig. 4. Chromatogram of GABA derivatives HPLC separation due to abamectin treatments of: (A) *Eobania vermiculata* (BGS) and (B) *Theba pisana* (WGS) after 24 hr at ½ LD_{50}.

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