Inhibition of Terrestrial Snails Glutamate decaboxylase (GAD) by Abamectin and Emamectin benzoate

Presented by

Elsayed A.M. Abdallah

Prof. of Pesticide Chemistry & Toxicology

Chemistry & Technology of Pesticides Dept.

Fac. Agric., Alexandria Univ.

EGYPT

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INTRODUCTION

Introduction

The aim of this research is to assess the molluscicidal activity as well as biochemical impact of non conventional pesticides as Abamectin and its deoxy-4'–epi- methylamine derivative (Emamectin benzoate) as well as standard conventional molluscicide methomyl against two species of the terrestrial snails brown garden snail (BGS) *Eobania vermiculata* and white garden snail (WGS) *Theba pisana*.

Introduction

In Egypt, land snails are known as dangerous pests to field crops, vegetables, orchards and ornamental plants. Damage caused by snails is mainly due to feeding and contamination with their bodies, feces or the exudated slime material, leading to deterioration of the product quality besides the financial loss.

Introduction

Land snails attack leaves, flowers, roots, buds, and even the trunk of trees causing great damage to the several types of cultivated plants.

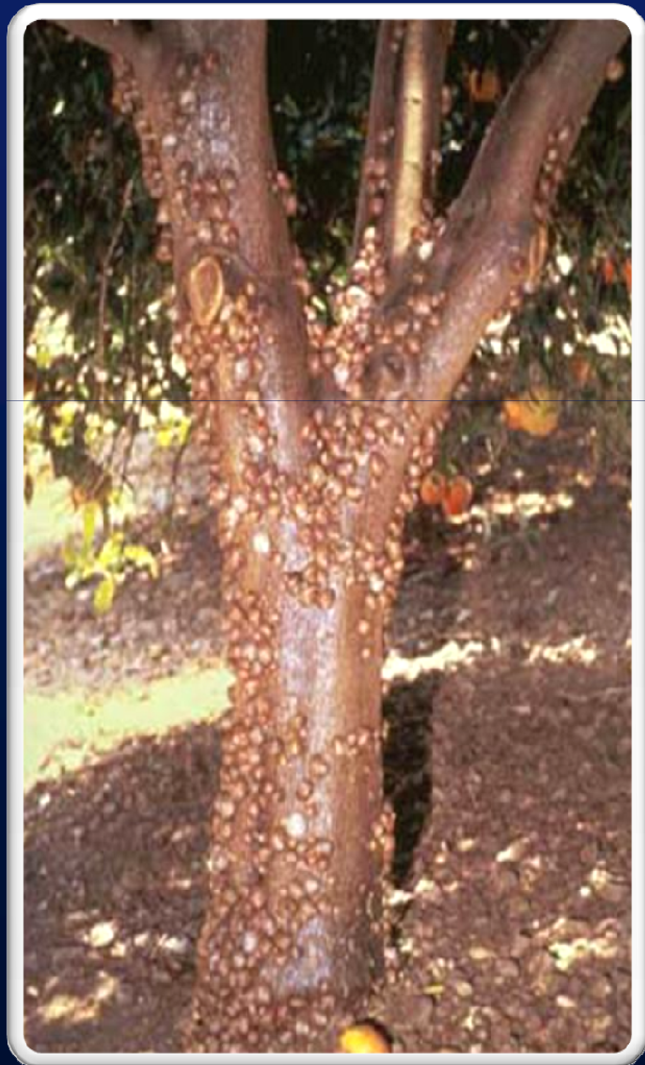
Glutamate decarboxylase (GAD, EC 4.1.2.5) has been shown to play an important role in the regulation of brain excitability through the synthesis of γ-aminobuteric acid (GABA) the major inhibitory neurotransmitter in the central nervous system and is considered a specific marker for GABAergic neurons and their processes

In Mollusca, GABA has been shown to elicit both inhibitory and excitatory actions in the central neurons.

In vivo effects of abamectin, emamectin benzoate and methomyl were investigated against the activities of *Eobania vermiculata* (BGS) and *Theba pisana* (WGS) glutamate decaboxylase (GAD).

GAD activity was evaluated by measuring the formed GABA after derivatization to phenylthio carbamoyl GABA (PTC-GABA) using HPLC with UV absorbance detection at 245 nm.

Introduction



Introduction

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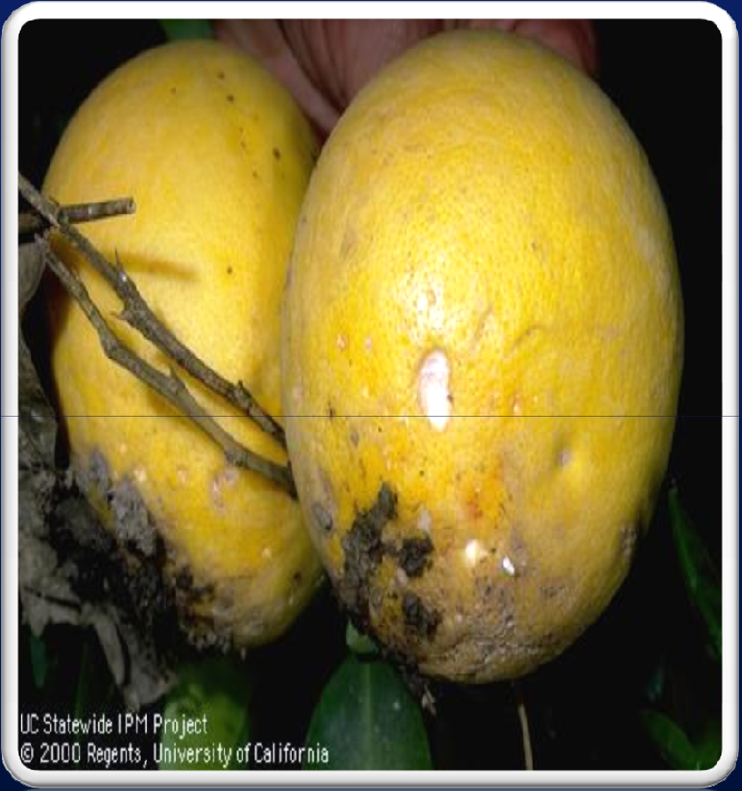


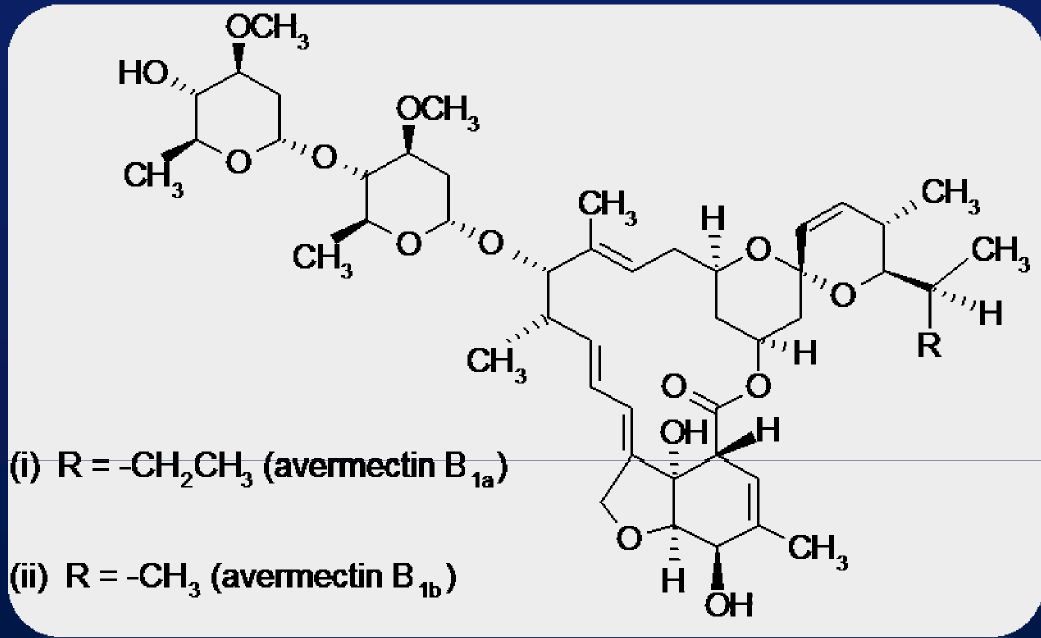
Introduction

Materials & Methods

Materials & Methods

- **I- Tested Pesticides**
  - **Abamectin**
  - **Emamectin benzoate**
  - **Methomyl**
- **II- Tested Snails**
  - ***Eobania vermiculata***
  - ***Theba pisana***
- **III- Biochemical Studies**
  - **GABAergic**
    - GAD

Chemical structure of Abamectin



Chemical name

5-*O*-demethylavermectin A1a (i) mixture with 5-*O*-demethyl-25-de(1-methylpropyl)-25-(1-methylethyl)avermectin A1a (ii)

Biochemistry

Acts by stimulating the release of γ-aminobutyric acid, an inhibitory neurotransmitter, thus causing paralysis. See M. J. Turner & J. M. Schaeffer in Ivermectin and Abamectin, W. C. Cambell ed., Springer-Verlag, New York (1989) p. 73.

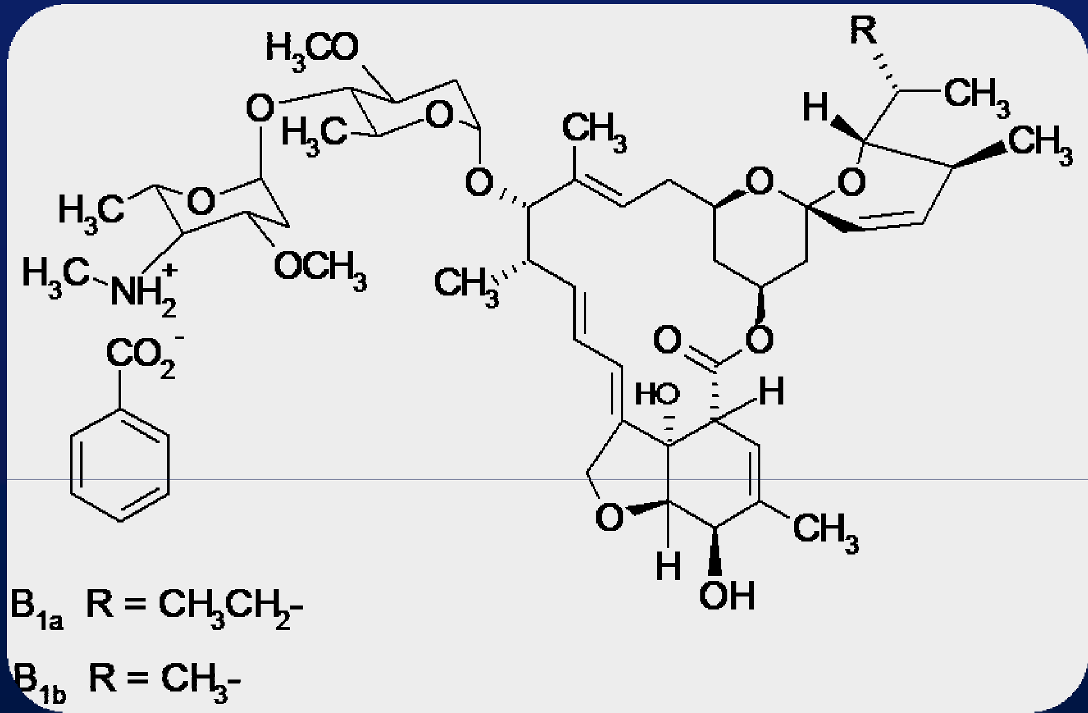
Mode of action

Insecticide and acaricide with contact and stomach action. Has limited plant systemic activity, but exhibits translaminar movement.

Uses

Control of motile stages of mites, leaf miners, suckers, Colorado beetles, etc. on ornamentals, cotton, citrus fruit, pome fruit, nut crops, vegetables, potatoes, and other crops. Application rates are 5.6 to 28 g/ha for mite control, 11 to 22 g/ha for control of leaf miners. Also used for control of fire ants.

Chemical structure of Emamectin benzoate



Chemical name

(4"*R*)-5-*O*-demethyl-4"-deoxy-4"-(methylamino)avermectin A1a + (4"*R*)-5-*O*-demethyl-25-de(1-methylpropyl)-4"-deoxy-4"-(methylamino)-25-(1-methylethyl)avermectin A1a (9:1)

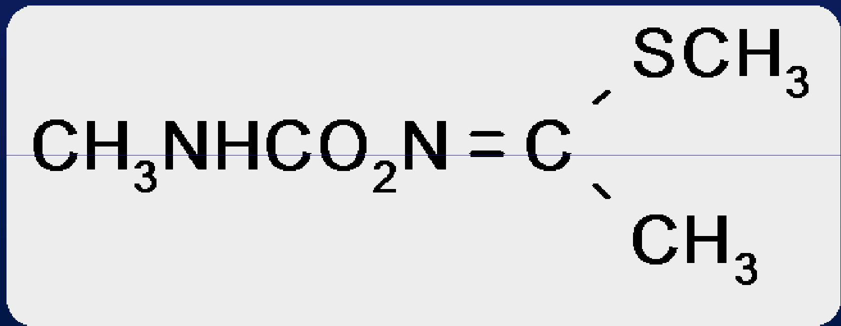
Mode of action

Non-systemic insecticide which penetrates leaf tissues by translaminar movement.

Uses

For control of lepidoptera on vegetables, brassicas and cotton, at up to 16 g/ha, and in pine trees at 5-25 g/ha.

Chemical structure of Methomyl



Chemical name

methyl *N*-[[(methylamino)carbonyl]oxy]ethanimidothioate

Biochemistry

Cholinesterase inhibitor.

Mode of action

Systemic insecticide and acaricide with contact and stomach action.

Uses

Control of a wide range of insects (particularly Lepidoptera, Hemiptera, Homoptera, Diptera and Coleoptera) and spider mites in fruit, vines, olives, hops, vegetables, ornamentals, field crops, cucurbits, flax, cotton, tobacco, soya beans, etc. Also used for control of flies in animal and poultry houses and dairies.

Tested mollusca

Order : Stylommatophora

Super family : Helicoidea

Family : Helicidae

Sub family : Helicinae

Genus : Eobania

Species

Eobania vermiculata

Tested mollusca

Order : Stylommatophora

Super family : Helicoidea

Family : Helicidae

Sub family : Helicinae

Genus : Thepa

Species

Theba pisana

Bioassy

Topical Application technique

- The collected snails were adapted to the laboratory conditions for two weeks before they were treated.
- Dimethyl sulfoxide (DMSO) solutions of Abamectine, Emamectin benzoate and Methomyl were applied topically on the terrestrial snails *Theba pisana* and *Eobania vermiculata* and the mortality percentages were recorded after 24, 48 and 72 hours.

Biochemical Studies

- Snails were initially treated with $^{1}/_{10}$ LD_{50}, $^{1}/_{5}$ LD_{50} and $^{1}/_{2}$ LD_{50} of tested pesticides
- After 24, 48 and 72 h Snails were collected.

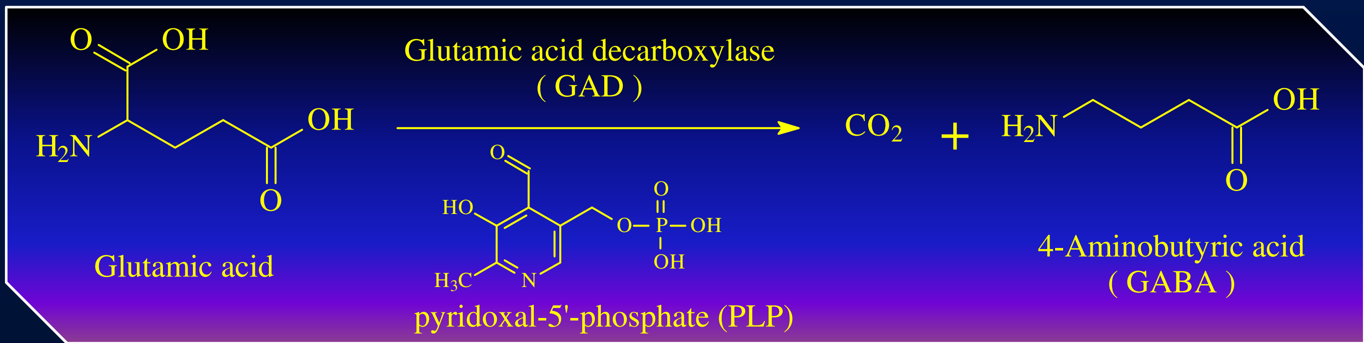
Materials & Methods

III- Biochemical Studies

Glutamic acid decarboxylase (GAD)

Assay principle

(According Allen and Griffiths (1984). Glutamate decarboxylase or glutamic acid decarboxylase (GAD) is an enzyme that catalyzes the decarboxylation of glutamate to GABA and CO_2. GAD uses PLP as a cofactor. The reaction proceeds as follows:



Materials & Methods

Glutamic acid Decarboxylase (GAD)

Allen and Griffiths (1984).

snails were homogenized in 200 m*M* potassium phosphate buffer, pH 6.8

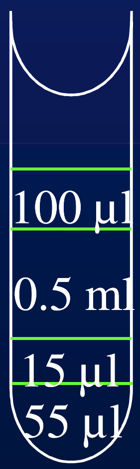
↓

Centrifuged at 5000 rpm for 30 min at 4 ^{O}C

↓

Supernatant served as enzyme source

↓



Enzyme source

200 m*M* potassium phosphate buffer, pH 6.8

50 m*M* L-Glu

0.2 m*M* pyridoxal-5'-phosphate (PLP)

↓

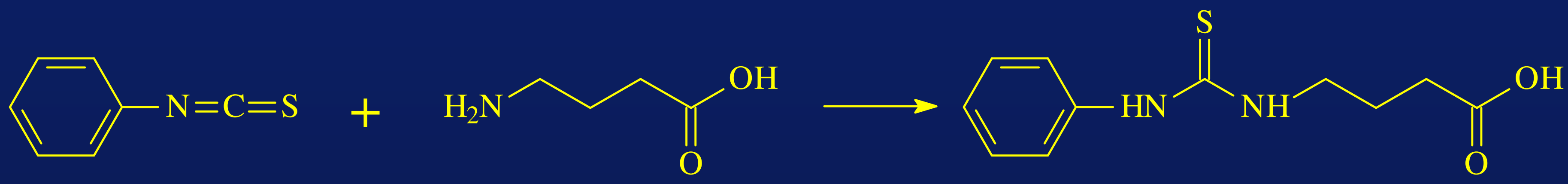
incubation for 20 min at 37°C

↓

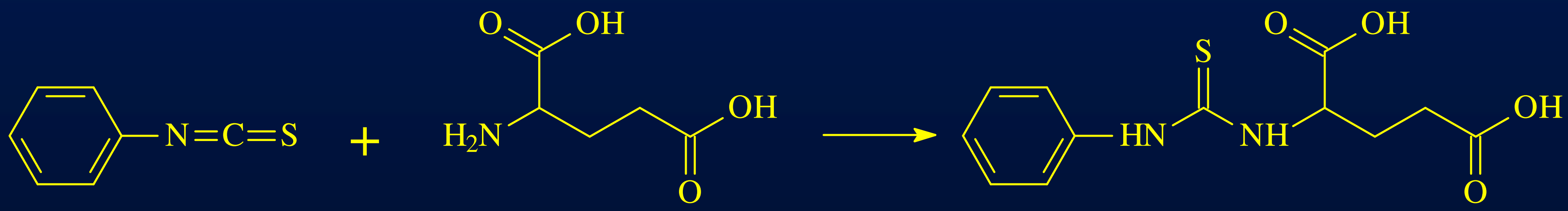
3 ml absolute ethanol was added to terminate the reaction.

The suspension was centrifuged at 1500 rpm (10 min, 0°C).

Derivatization of GABA and Glutamic acid to Phenylthiocarbamyl-GABA (PTC-GABA) and Phenylthiocarbamyl-Glutamic (PTC- GLU) According to Gunawan *et al*. (1990).



Phenylisothiocyanate | 4-Aminobutyric acid (GABA) | Phenylthiocarbamyl-GABA (PTC- GABA)



Phenylisothiocyanate | Glutamic acid | Phenylthiocarbamyl-Glutamic (PTC- Glu)

Derivatization of GABA to phenylthiocarbamyl-GABA (PTC-GABA) Gunawan *et al*. (1990).

A 100 µl aliquot of supernatant (or of standard solution of GABA)

Standard solutions of GABA & Glu. (0.125, 0.25, 0.50 and 0.75 m*M* GABA & Glu.).

↓

dried under vacuum

↓

The residue was dissolved in 20 µl of ethanol-water- triethylamine (2:2:1) and evaporated to dryness under vacuum

↓

Add 30 µl volume of ethanol-water- triethylamine-PITC (7:1 : 1 : 1)

↓

React for 20 min at room temperature to form PTC-GABA or Glu. and the excess reagent was then removed under vacuum

↓

the dry residue dissolved in 100 µl of mobile phase

HPLC operating conditions.

Type	: HPLC system (Agilent 1100)
Column.	: 250 mm. × 4.6 mm. I.D. stainless steel Zorbax SB C18
Temperature	: 30 ^{0}C
Detector	: HP 1100 UV variable wave length detector.
Wave length	: 245 nm
Injector volume	: Injection loop -20 µl
Mobile phase	:80% solution A (aqueous solution of 8.205 g sodium acetate, 0.5 ml triethylamine, 0.7 ml acetic acid and 5.0 ml acetonitrile in 1000 ml) :20% solution B [Acetonitrile-water (60:40)] :Adjusted to pH 5.8.
Flow rate	: 0.6 ml/min

Results

Table (1): Effect of Abamectin against *Eobania vermiculata* (BGS) and *Theba pisana* (WGS) snails using topical application technique, shown as mortality percentage % and LD_{50} values, at 24, 48 and 72 hr.

Hours	Dose µg/ Snail							LD_{50} µg/ Snail	Conf. Limits 95%		Slope
	2.5	5	10	15	20	25	50		Upper	Lower	
Eobania vermiculata											
24	37.78	51.11	55.56	60.00	68.89	71.11	77.78	5.738	7.742	3.706	0.813
48	53.33	68.89	71.11	75.56	77.78	80.00	86.67	1.552	2.777	0.504	0.716
72	66.67	71.11	75.56	80.00	84.44	88.89	93.33	0.927	1.816	0.251	0.782

Hours	Dose µg/ Snail					LD_{50} µg/ Snail	Conf. Limits 95%		Slope
	0.125	0.25	0.625	1.25	2.5		Upper	Lower	
Theba pisana									
24	15.56	33.33	62.22	82.22	100	0.421	0.498	0.358	1.923
48	42.22	62.22	86.67	95.56	100	0.163	0.199	0.125	1.804
72	42.22	66.67	86.67	95.56	100	0.155	0.189	0.119	1.881

Table (2): Effect of Emamectin benzoate against *Eobania vermiculata* (BGS) and *Theba pisana* (WGS) snails using topical application technique, shown as mortality percentage % and LD_{50} values, at 24, 48 and72hr.

Hours	Dose µg/ Snail							LD_{50} µg/ Snail	Conf. Limits 95%		Slope
	2.5	5	10	15	20	25	50		Upper	Lower	
Eobania vermiculata											
24	15.56	22.22	26.67	28.89	37.78	40.00	57.78	43.013	73.866	30.636	0.888
48	33.33	33.33	44.44	44.44	55.56	60.00	77.78	12.615	16.296	9.76	0.887
72	44.44	51.11	60.00	64.44	66.67	71.11	93.33	4.808	6.944	1.82	0.962

Hours	Dose µg/ Snail							LD_{50} µg/ Snail	Conf. Limits 95%		Slope
	1.25	2.5	5	7.5	10	12.5	25		Upper	Lower	
Theba pisana											
24	35.56	46.67	55.56	66.67	71.11	77.78	100	2.997	3.771	2.204	1.088
48	55.56	77.78	82.22	84.44	88.89	93.33	100	0.799	1.212	0.394	1.165
72	66.67	77.78	88.89	93.33	100	100	100	0.63	0.991	0.249	1.358

Table (3): Effect of Methomyl against *Eobania vermiculata* (BGS) and *Theba pisana* (WGS) snails using topical application technique, shown as mortality percentage % and LD_{50} values, at 24, 48 and 72 hr.

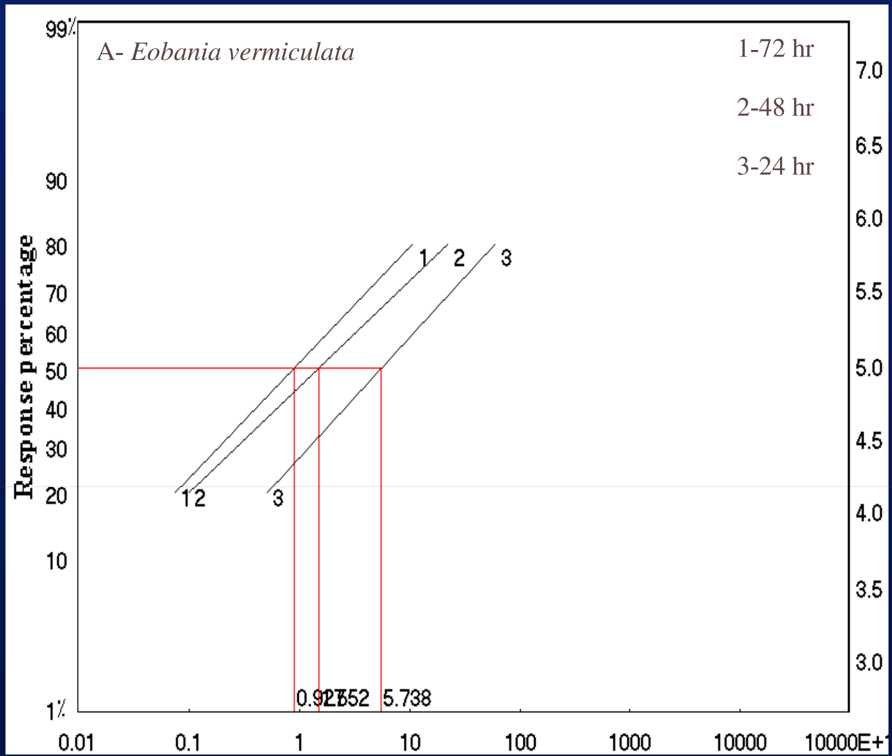
Hours	Dose µg/ Snail							LD_{50} µg/ Snail	Conf. Limits 95%		Slope
	2.5	5	10	15	20	25	50		Upper	Lower	
Eobania vermiculata											
24	4.44	11.11	11.11	22.22	26.67	28.89	37.78	89.507	186.2	58.037	1.059
48	11.11	15.56	22.22	33.33	33.33	44.44	44.44	53.772	97.787	37.265	0.926
72	15.56	33.33	33.33	44.44	51.11	51.11	53.33	26.148	40.096	19.48	0.812

Hours	Dose µg/ Snail							LD_{50} µg/ Snail	Conf. Limits 95%		Slope
	1.25	2.5	5	7.5	10	12.5	25		Upper	Lower	
Theba pisana											
24	4.44	8.89	17.78	22.22	24.44	24.44	31.11	70.39	221.87	38.55	0.886
48	22.22	33.33	33.33	37.78	44.44	44.44	51.11	21.839	59.069	13.431	0.572
72	22.22	51.11	55.56	55.56	60.00	62.22	66.67	4.853	6.426	3.487	0.777

Table (4): Comparative toxicity of tested pesticides against *Eobania vermiculata* (BGS) and *Theba pisana* (WGS) snails using topical application technique under laboratory conditions.

Hours		Abamectin				Emamectin benzoate				Methomyl			
		LD_{50} µg/snail	Conf. Limits 95%		Slope	LD_{50} µg/snail	Conf. Limits 95%		Slope	LD_{50} µg/snail	Conf. Limits 95%		Slope
			Upper	Lower			Upper	Lower			Upper	Lower	
Eobania vermiculata													
24		5.738	7.742	3.706	0.813	43.013	73.866	30.636	0.888	89.507	186.2	58.037	1.059
	RTI	1559.8				208.1				100			
48		1.552	2.777	0.504	0.716	12.615	16.296	9.76	0.887	53.772	97.787	37.265	0.926
	RTI	3464.6				426.2				100			
72		0.927	1.816	0.251	0.782	4.808	6.944	1.82	0.962	26.148	40.096	19.48	0.812
	RTI	2820.7				543.8				100			
Theba pisana													
24		0.421	0.498	0.358	1.923	2.997	3.771	2.204	1.088	70.39	221.87	38.55	0.886
	RTI	16719.7				2348.6				100			
48		0.163	0.199	0.125	1.804	0.799	1.212	0.394	1.165	21.839	59.069	13.431	0.572
	RTI	13398.1				2733.2				100			
72		0.155	0.189	0.119	1.881	0.63	0.991	0.249	1.358	4.853	6.426	3.487	0.777
	RTI	3130.9				770.3				100			

Relative Toxicity index (RTI): (LD_{50} of the Methomyl / LD_{50} of tested compounds * 100) at each time interval.



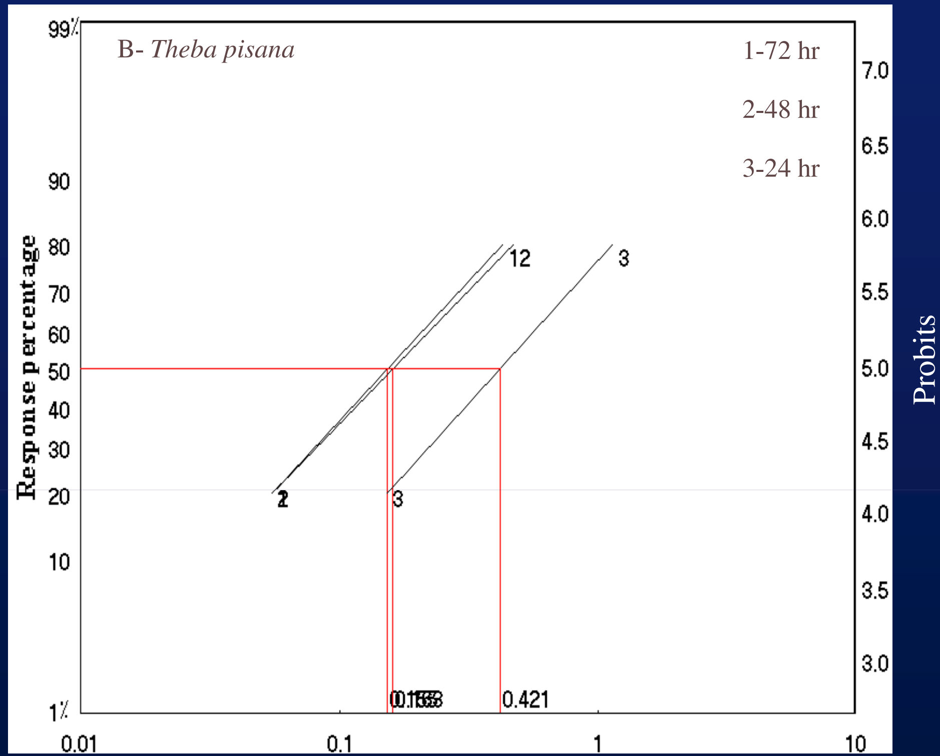


Fig. (1): Probit regression lines representing the effect of Abamectin topical application against terrestrial snails: A- *Eobania vermiculata* (BGS) and B- *Theba pisana* (WGS).

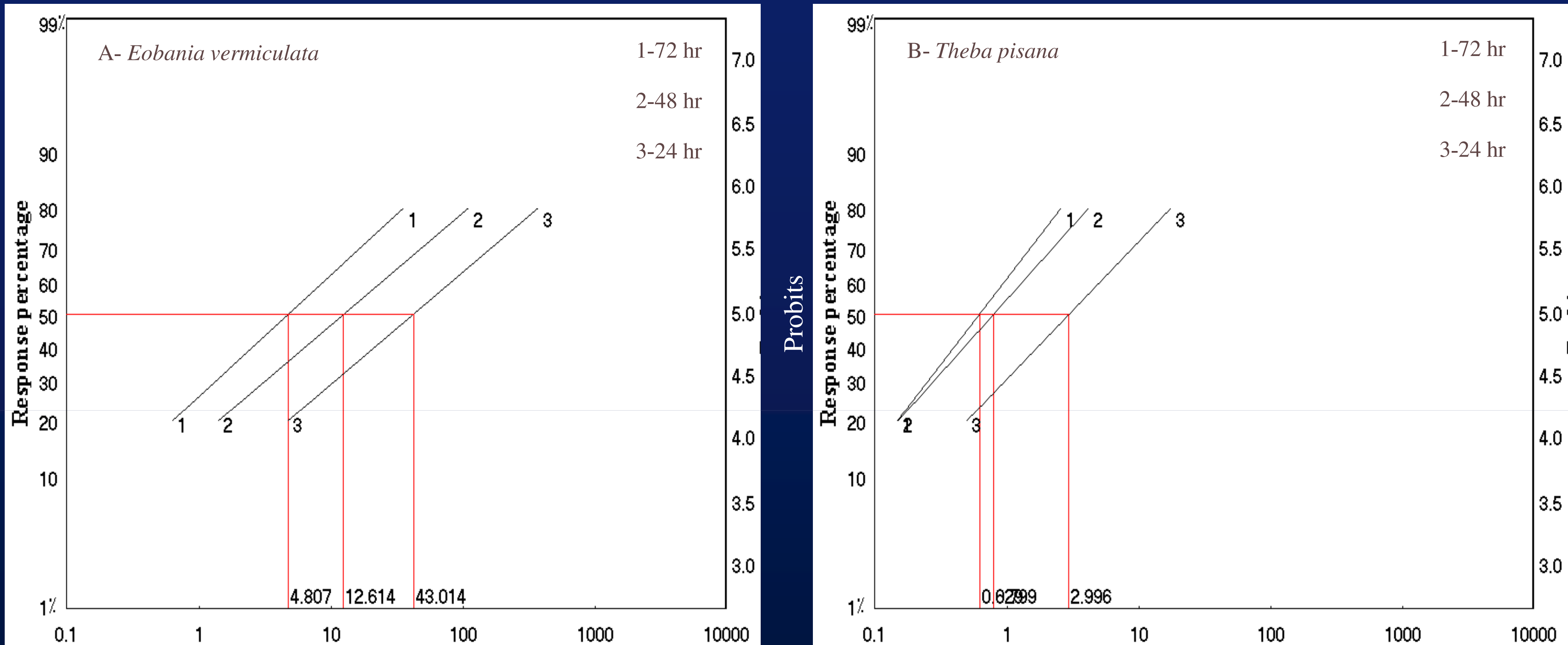


Fig. (2): Probit regression lines representing the effect of Emamectin benzoate topical application against terrestrial snails: A- *Eobania vermiculata* (BGS) and B- *Theba pisana* (WGS).

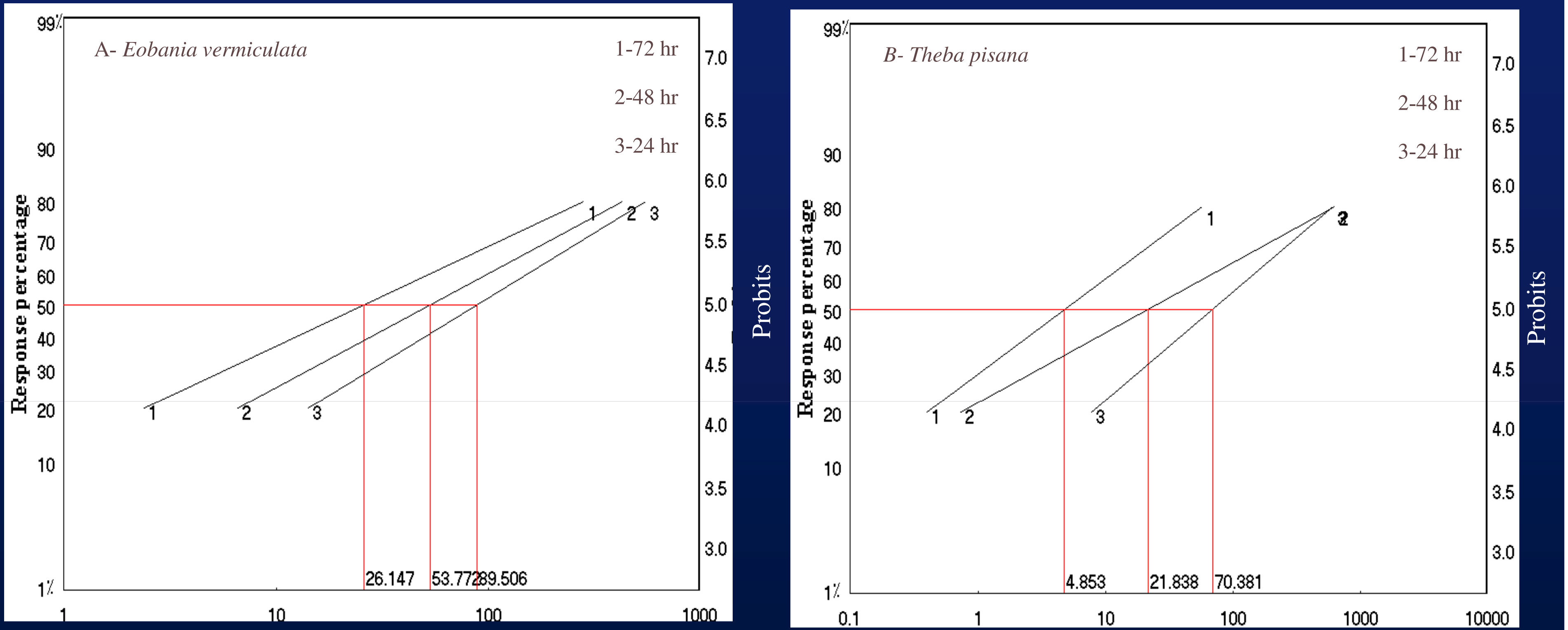


Fig. (3): Probit regression lines representing the effect of Methomyl topical application against terrestrial snails: A- *Eobania vermiculata* (BGS) and B- *Theba pisana* (WGS).

Results of

Biochemical studies

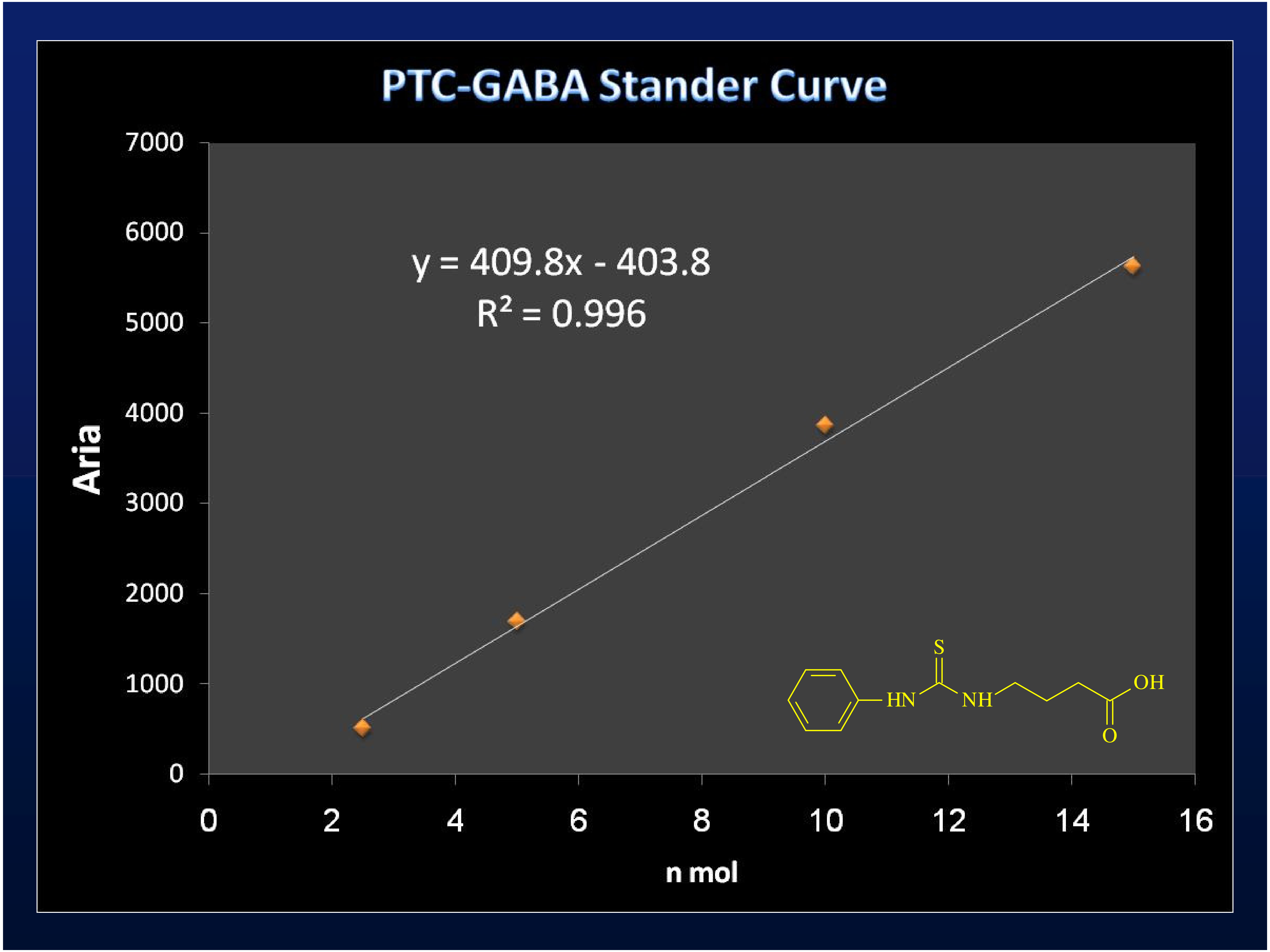
Results of the effects of abamectin, emamectin benzoate and methomyl on γ-aminobuteric acid (GABA) formation that considered an endogenous neurotransmitter to the central nervous system of both vertebrate and invertebrates will be introduced.

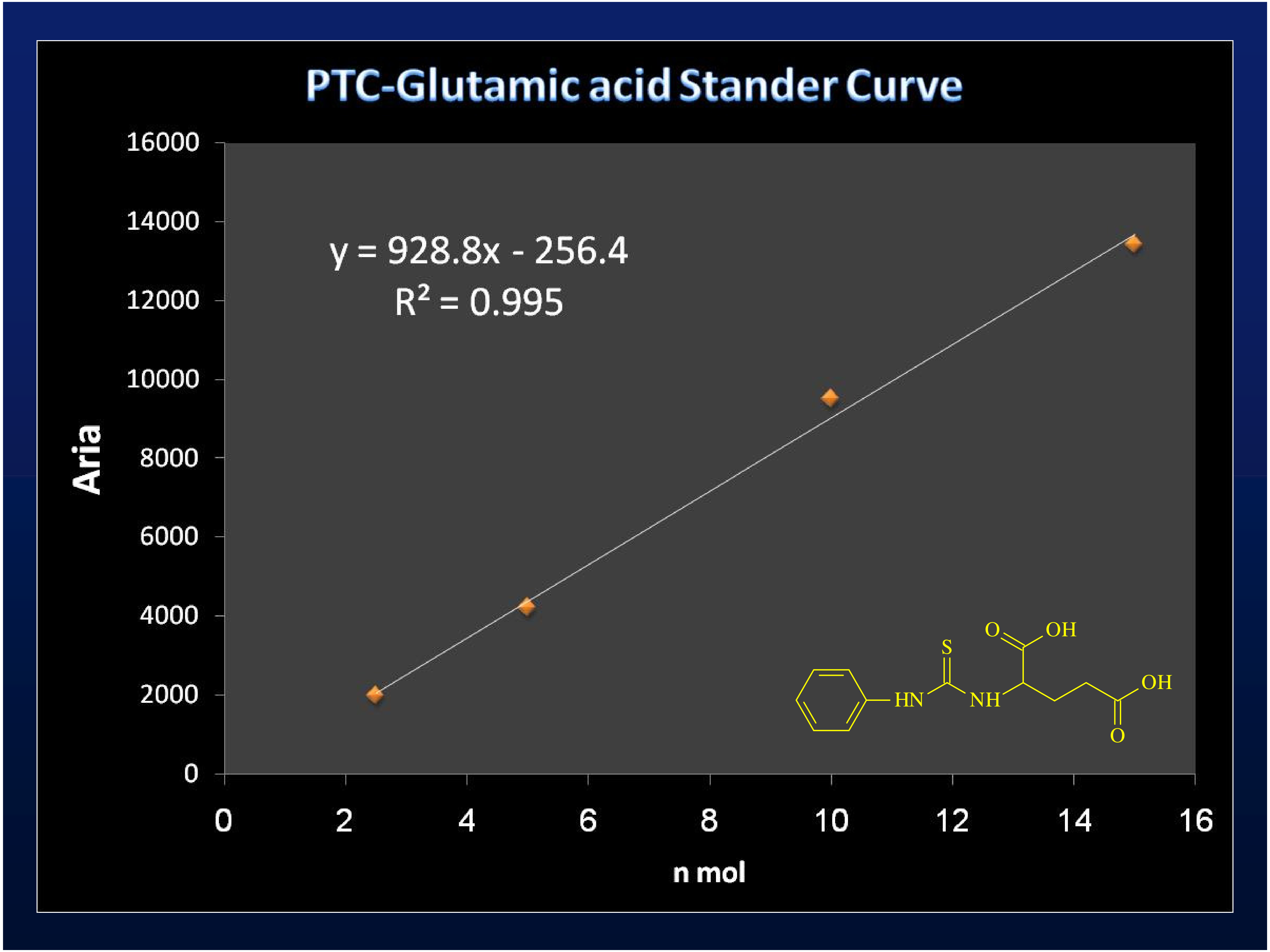
Results

GABAergic

Glutamic Acid Decarboxylase Activity

GAD


PTC-GABA Stander Curve
y = 409.8x - 403.8
R² = 0.996
Aria
n mol
0
1000
2000
3000
4000
5000
6000
7000
0
2
4
6
8
10
12
14
16
S
HN
NH
OH
O


PTC-Glutamic acid Stander Curve
y = 928.8x - 256.4
R² = 0.995
Aria
n mol
16000
14000
12000
10000
8000
6000
4000
2000
0
0
2
4
6
8
10
12
14
16
O
OH
S
HN
NH
OH
O

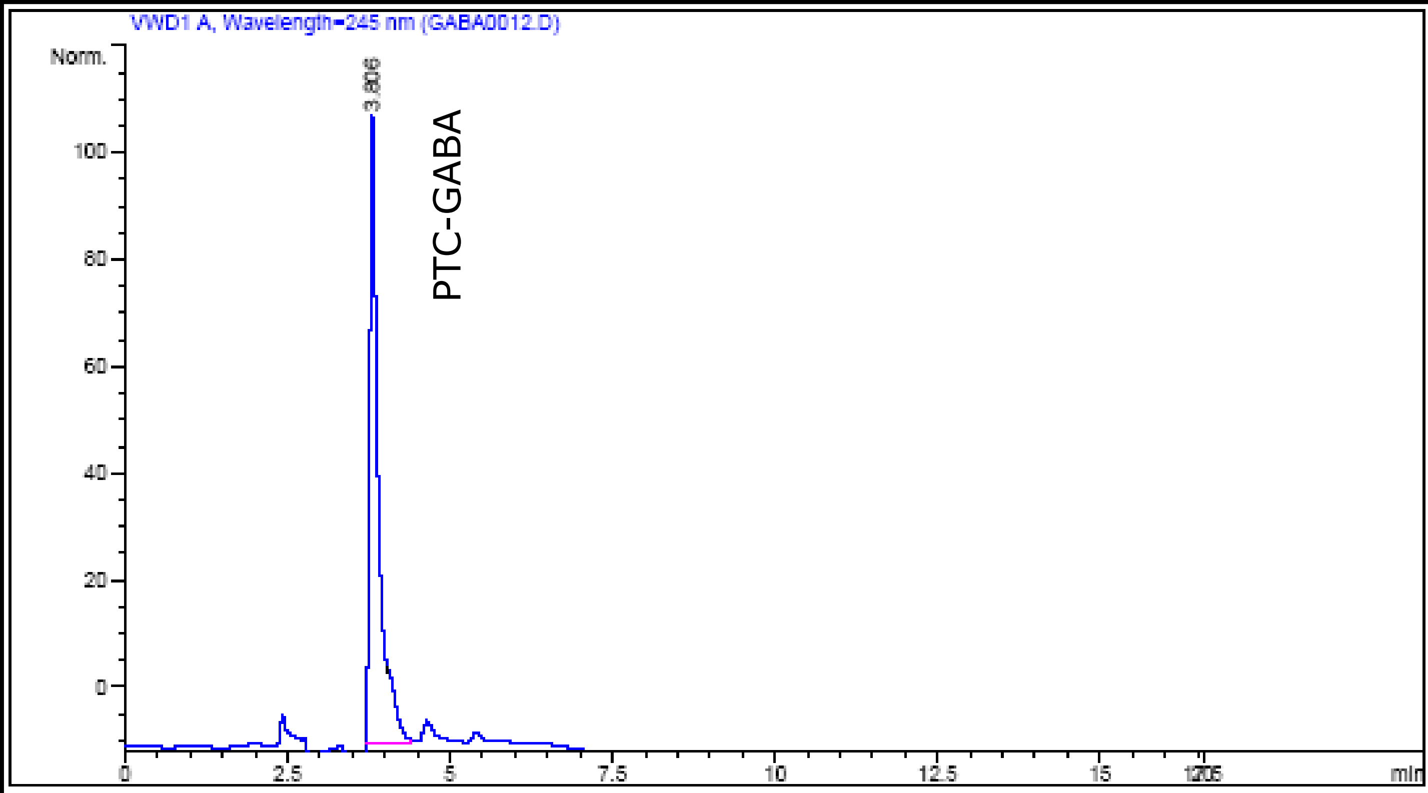


Fig. (4) HPLC chromatogram of PTC-GABA standard

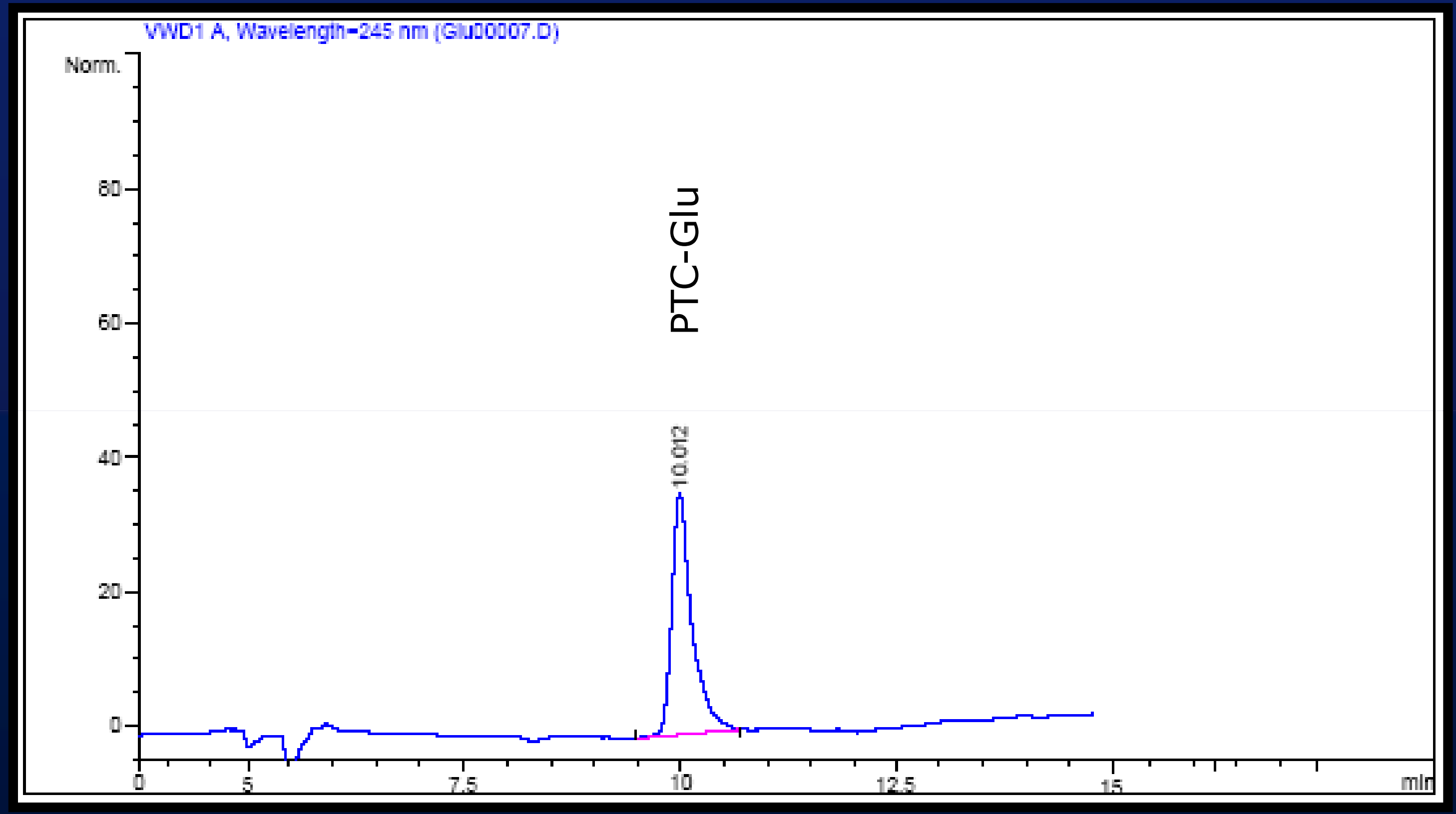


Fig. (5): HPLC chromatogram of PTC-Glutamic acid standard

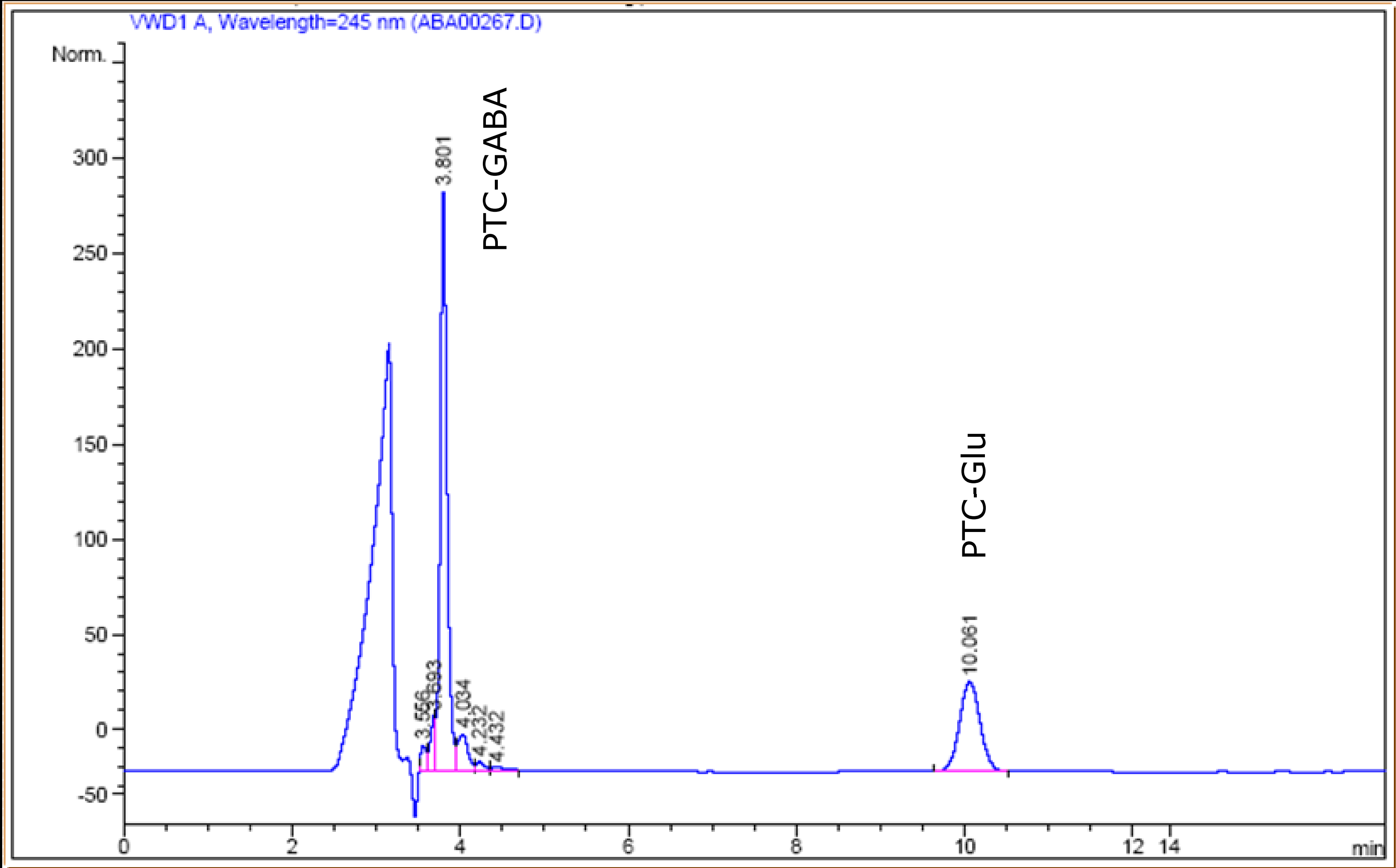


Fig. (6): Spectrum of PTC-GABA and PTC-Glutamic acid derivatives HPLC separation due to Abamectin treatments of *Eobania vermiculata* after 24 hours.

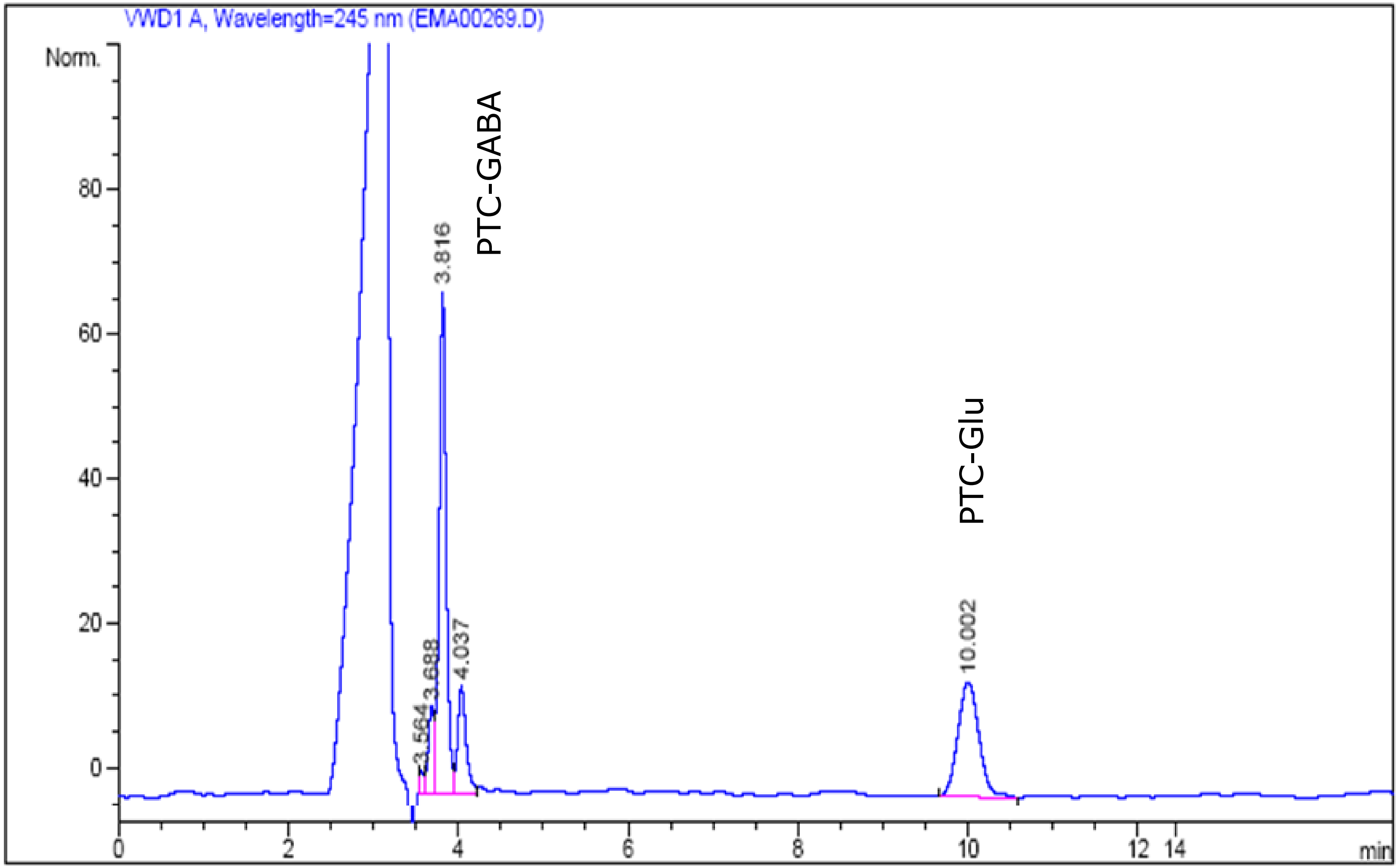


Fig. (7): Spectrum of PTC-GABA and PTC-Glutamic acid derivatives HPLC separation due to Emamectin benzoate treatments of *Eobania vermiculata* after 24 hours.

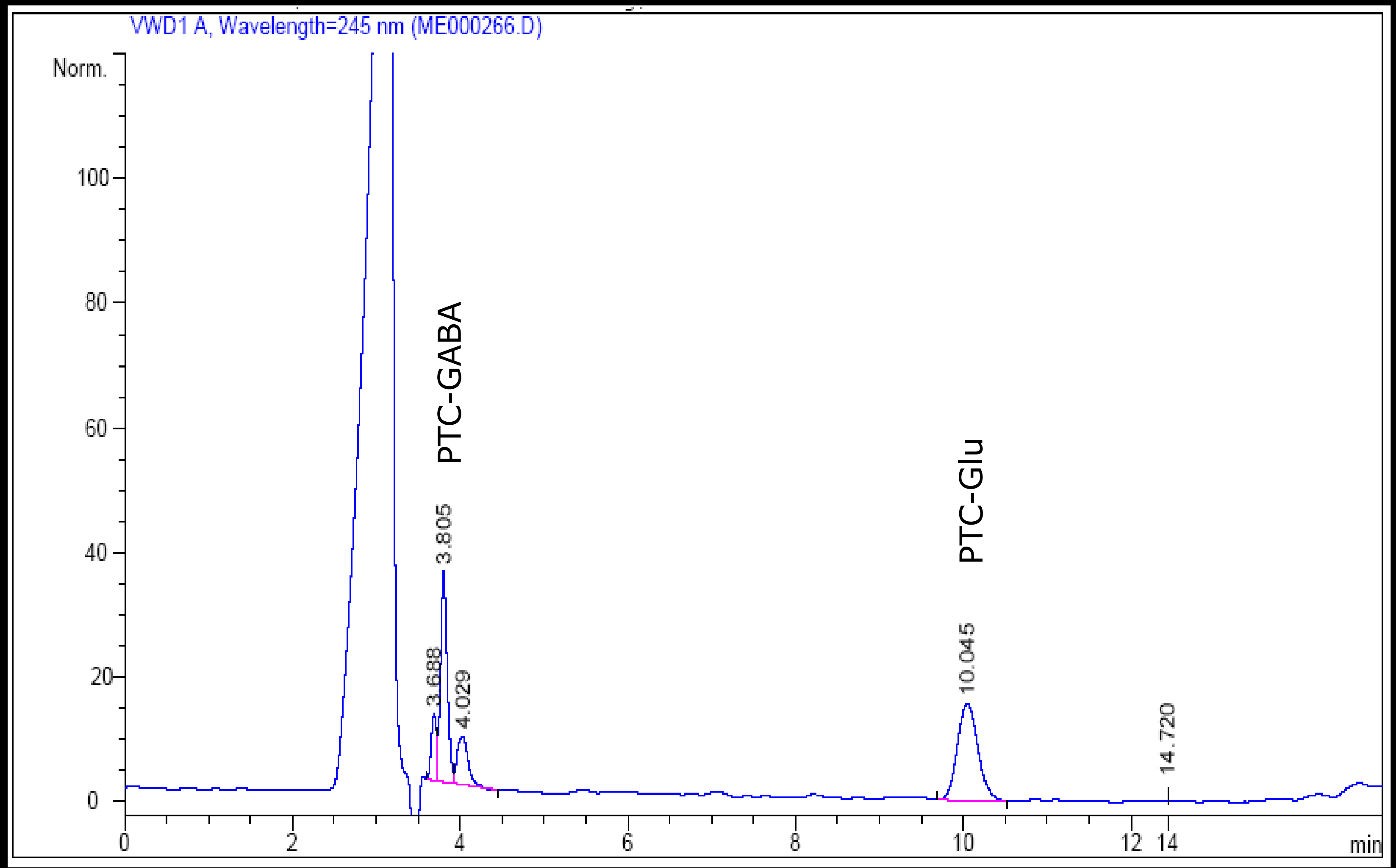


Fig. (8): Spectrum of PTC-GABA and PTC-Glutamic acid derivatives HPLC separation due to Methomyl treatments of *Eobania vermiculata* after 24 hours.

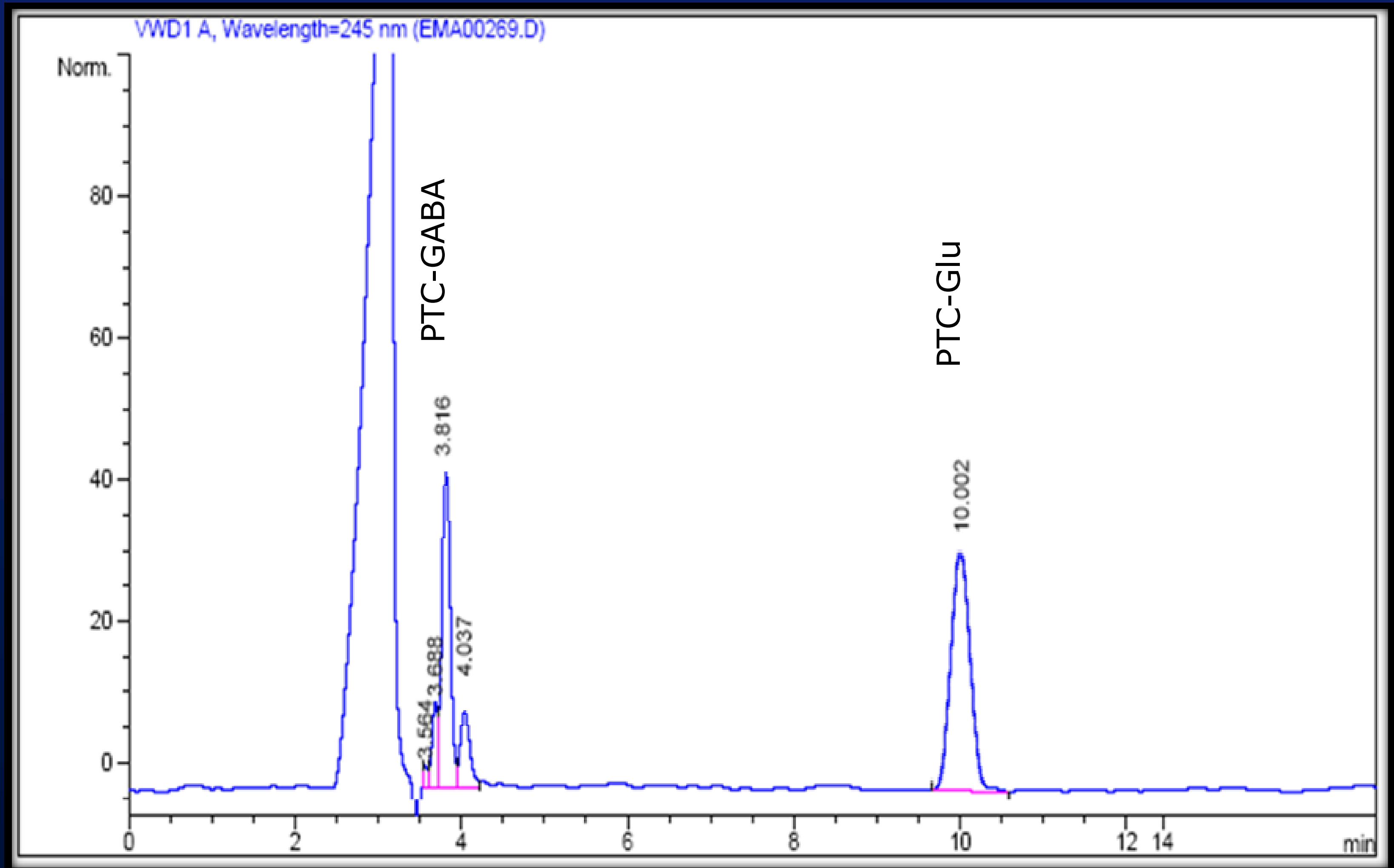


Fig. (9): Spectrum of PTC-GABA and PTC-Glutamic acid derivatives HPLC separation due to Control treatments of *Eobania vermiculata* after 24 hours.

Table (5): Effect of *in vivo* Abamectin, Emamectin benzoate and Methomyl interactions on *Eobania vermiculata* (BGS) glutamic acid decarboxylase (GAD) activities.

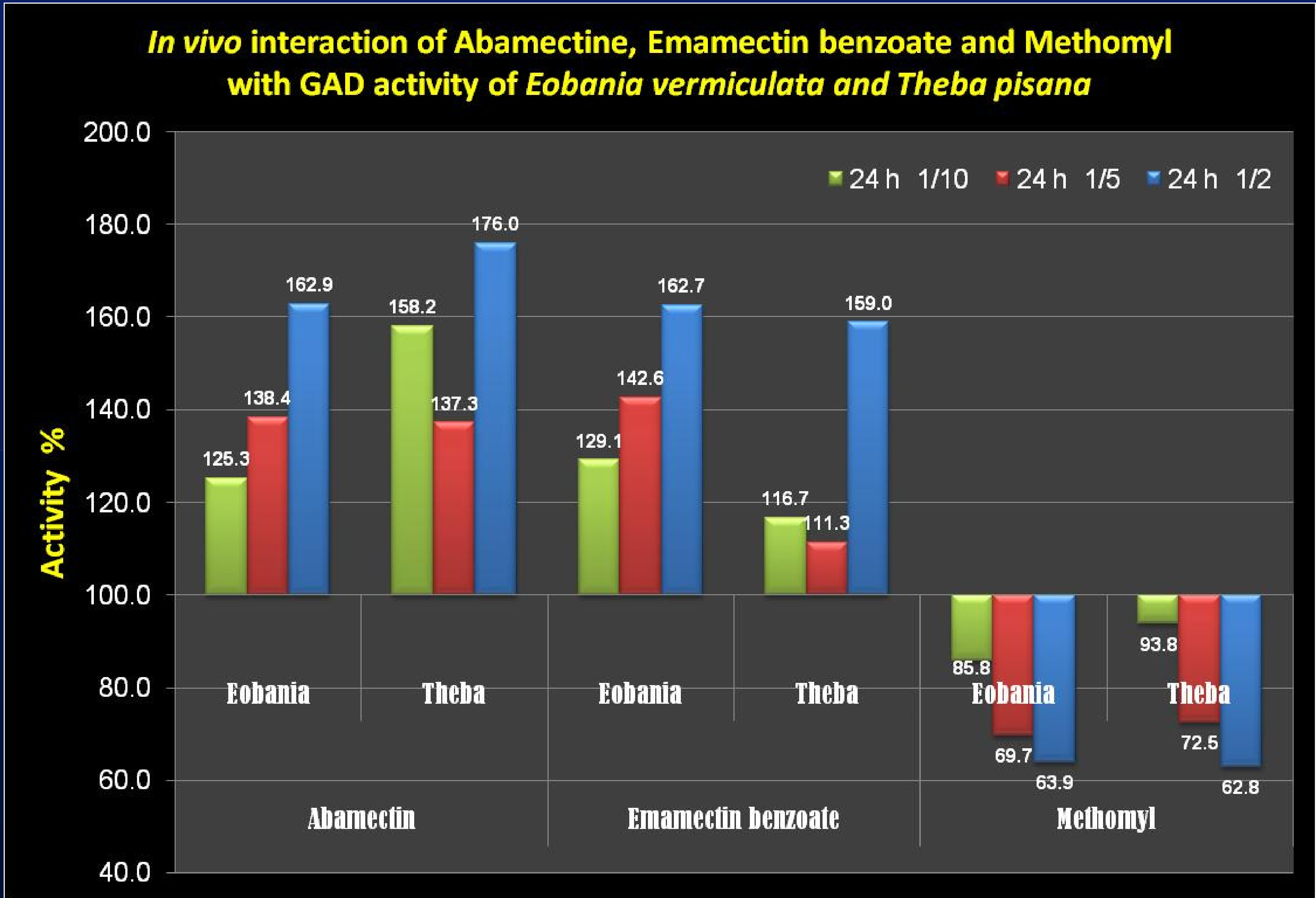
Dose		Abamectin S.A (n*M* glu./mg protein/min) ± SD	Abamectin Activity %	Emamectin benzoate S.A (n*M* glu./mg protein/min) ± SD	Emamectin benzoate Activity %	Methomyl S.A (n*M* glu./mg protein/min) ± SD	Methomyl Activity %
LD_{50} at 24 hr	1/2	19.243±1.02*	162.88	19.222±0.79*	162.71	7.544±0.74*	63.86
	1/5	16.356±1.04*	138.45	16.852±0.99*	142.65	8.229±0.92*	69.65
	1/10	14.804±0.49*	125.31	15.257±0.38*	129.14	10.137±0.99*	85.80
$LSD_{0.05}$		1.165		1.058		0.911	
LD_{50} at 48 hr	1/2	22.766±1.10*	192.71	22.173±1.07*	187.69	6.144±0.64*	52.01
	1/5	20.945±0.94*	177.30	17.964±0.66*	152.06	6.643±0.38*	56.23
	1/10	17.938±0.67*	151.84	17.157±0.51*	145.23	8.321±0.71*	70.43
$LSD_{0.05}$		1.206		1.106		1.026	
LD_{50} at 72 hr	1/2	16.989±0.49*	143.81	16.829±0.92*	142.45	7.672±0.82*	64.95
	1/5	14.724±0.72*	124.64	15.463±0.33*	130.89	11.323±0.74	95.85
	1/10	14.839±0.38*	125.61	13.836±0.63*	117.12	11.483±0.55	97.20
$LSD_{0.05}$		1.188		1.014		0.815	

* Control specific activity of untreated snail (GAD) is 11.814 ± 0.62(n*M*glu/mg protein/min) ± SD.

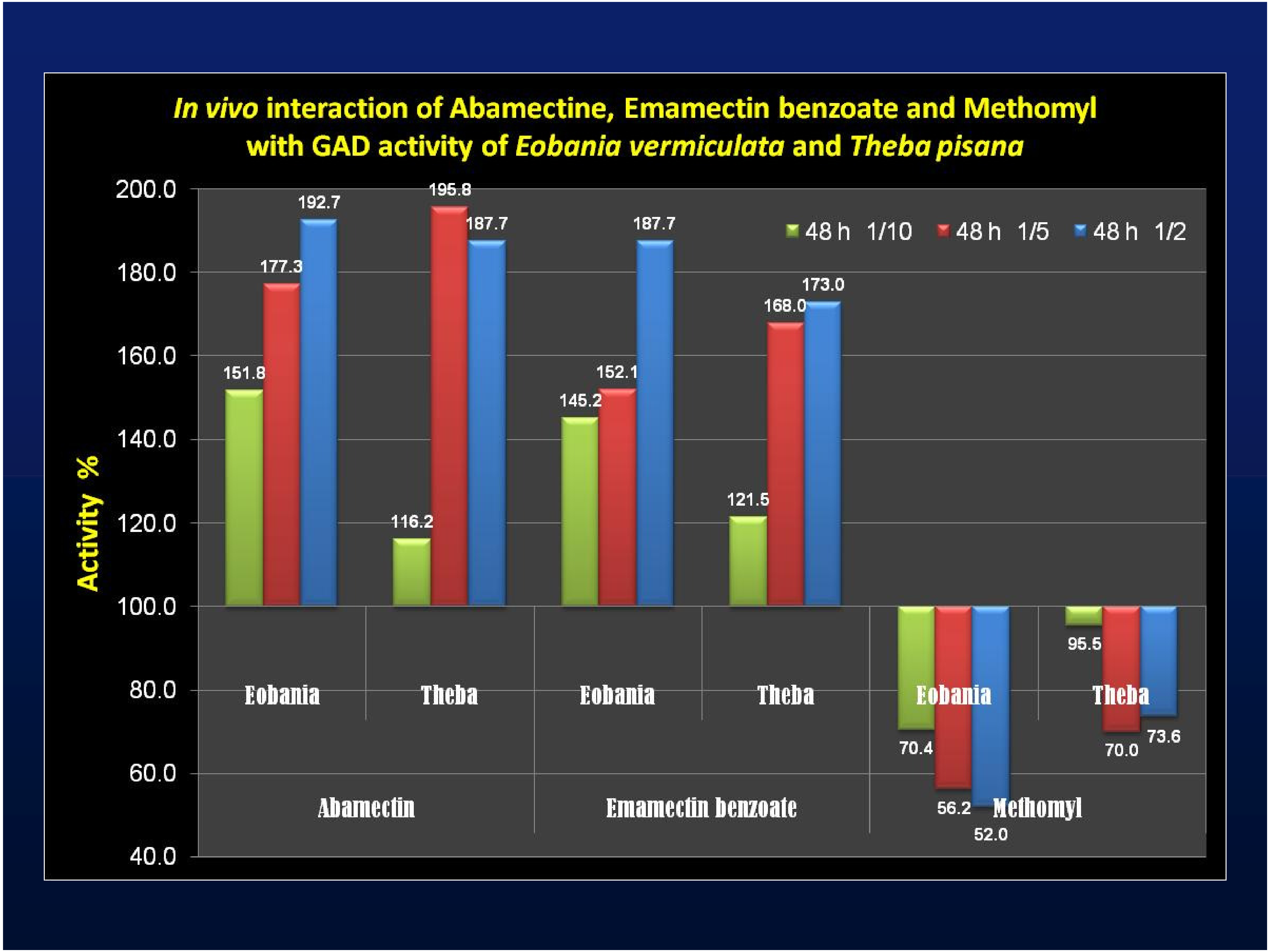
Table (6): Effect of *in vivo* Abamectin, Emamectin benzoate and Methomyl interactions on *Theba pisana* (WGS) glutamic acid decarboxylase (GAD) activities

Dose		Abamectin		Emamectin benzoate		Methomyl	
		S.A (n*M* glu./mg protein/min) ± SD	Activity %	S.A (n*M* glu./mg protein/min) ± SD	Activity %	S.A (n*M* glu./mg protein/min) ± SD	Activity %
LD_{50} at 24 hr	1/2	10.824±0.91*	176.04	9.774±0.42*	158.95	3.864±0.39*	62.84
	1/5	8.440±0.83*	137.26	6.843±0.18*	111.28	4.457±0.51*	72.48
	1/10	9.725±0.74*	158.17	7.178±0.22*	116.73	5.768±0.63	93.81
$LSD_{0.05}$		0.759		0.792		0.702	
LD_{50} at 48 hr	1/2	11.539±0.86*	187.66	10.636±0.97*	172.97	4.525±0.72*	73.59
	1/5	12.038±0.88*	195.79	10.328±0.62*	167.96	4.304±0.57*	70.00
	1/10	7.142±0.43*	116.15	7.470±0.49*	121.48	5.871±0.26	95.49
$LSD_{0.05}$		0.991		0.841		0.616	
LD_{50} at 72 hr	1/2	9.901±0.86*	161.03	7.846±0.28*	127.59	4.578±0.28*	74.45
	1/5	10.479±1.07*	170.43	7.767±0.19*	126.31	5.832±0.56	94.84
	1/10	8.863±0.73*	144.14	6.611±0.84	107.51	5.112±0.33*	83.14
$LSD_{0.05}$		1.159		0.757		0.984	

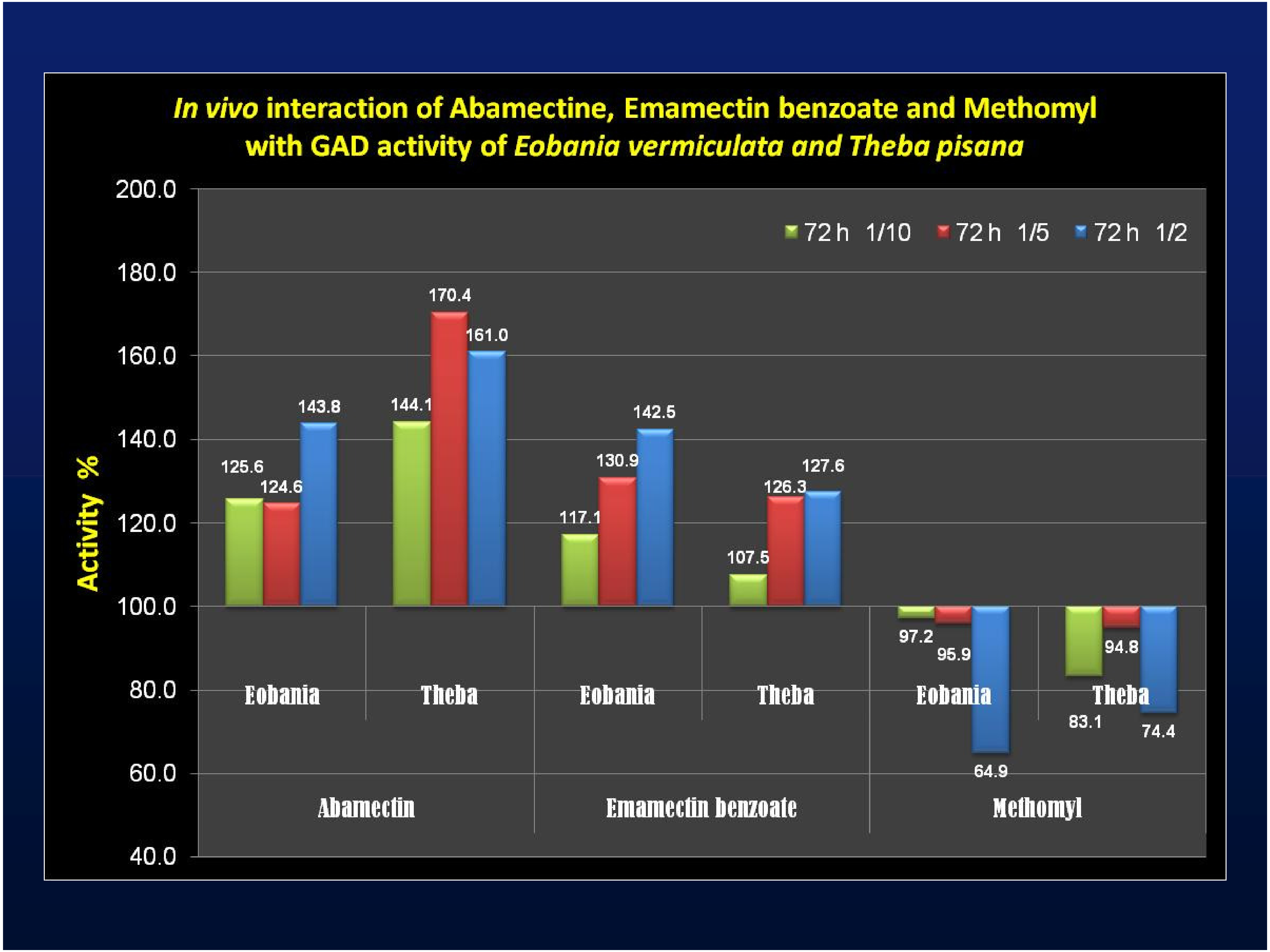
* Control specific activity of untreated snail (GAD) is 6.149±0.78 (n*M*glu/mg protein/min) ± SD.



In vivo interaction of Abamectine, Emamectin benzoate and Methomyl with GAD activity of Eobania vermiculata and Theba pisana
Activity %
24 h 1/10
24 h 1/5
24 h 1/2
200.0
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
125.3
138.4
162.9
158.2
137.3
176.0
129.1
142.6
162.7
116.7
111.3
159.0
85.8
69.7
63.9
93.8
72.5
62.8
Eobania
Theba
Eobania
Theba
Eobania
Theba
Abamectin
Emamectin benzoate
Methomyl



In vivo interaction of Abamectine, Emamectin benzoate and Methomyl with GAD activity of Eobania vermiculata and Theba pisana
Activity %
200.0
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
48 h 1/10
48 h 1/5
48 h 1/2
151.8
177.3
192.7
116.2
195.8
187.7
145.2
152.1
187.7
121.5
168.0
173.0
70.4
56.2
52.0
95.5
70.0
73.6
Eobania
Theba
Eobania
Theba
Eobania
Theba
Abamectin
Emamectin benzoate
Methomyl


In vivo interaction of Abamectine, Emamectin benzoate and Methomyl with GAD activity of Eobania vermiculata and Theba pisana
Activity %
72 h 1/10
72 h 1/5
72 h 1/2
200.0
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
125.6
124.6
143.8
144.1
170.4
161.0
117.1
130.9
142.5
107.5
126.3
127.6
97.2
95.9
64.9
83.1
94.8
74.4
Eobania
Theba
Eobania
Theba
Eobania
Theba
Abamectin
Emamectin benzoate
Methomyl

1-Same type of response was noticed between both types of the snails BGS and WGS. While methomyl clearly inhibited GAD activity, abamectin and emamectin benzoate stimulated markedly the GAD activity in both types of the used land snails.

2- The inhibitory effect of methomyl was dose dependent manner. That the activity of GAD enzyme increased by decreasing the dose treatments in both types of snails. However, the inhibition of GAD activity was more pronounced with BGS than WGS.

3- Natural fermentation products of *Streptomyces avermitilis*, avermectin B1 (abamectin) and its 4-deoxy-4-epi-methylamine derivative (emamectin benzoate) induced a significant GAD stimulatory effect for both type of snails BGS and WGS as indicated in Tables (9 and 10).

4- Abamectin interaction with GAD activity was higher than emamectin benzoate especially in the case of WGS when the stimulatory effect on GAD activity was less than BGS.

5- Both compounds abamectin and emamectin benzoate caused similar degree of GAD stimulation of BGS. The stimulation was dependent, however the stimulatory effect decreased by time, the lowest stimulation obtained for BGS was at 72 hr with the least concentration used 1/10 of LD_{50}.

6- While abamectin and emamectin benzoate caused equal degree of GAD activation with BGS, Abamectin effect was significantly higher in its stimulatory effect on GAD-WGS than GAD-BGS as shown in (Table 10).

7- Specific activity value of GAD-BGS was higher than the value of GAD-WGS indicating more participation of GABAergic system of *Eobania vermiculata* compared with *Theba pisana* in this respect.

8- These findings could illustrate how abamectin and emamectin benzoate induces the level of GABA neurotransmitter in *E. vermiculata* and *T. pisana* land snails, as it activates the biosynthesis of GABA and inhibit its degradation.

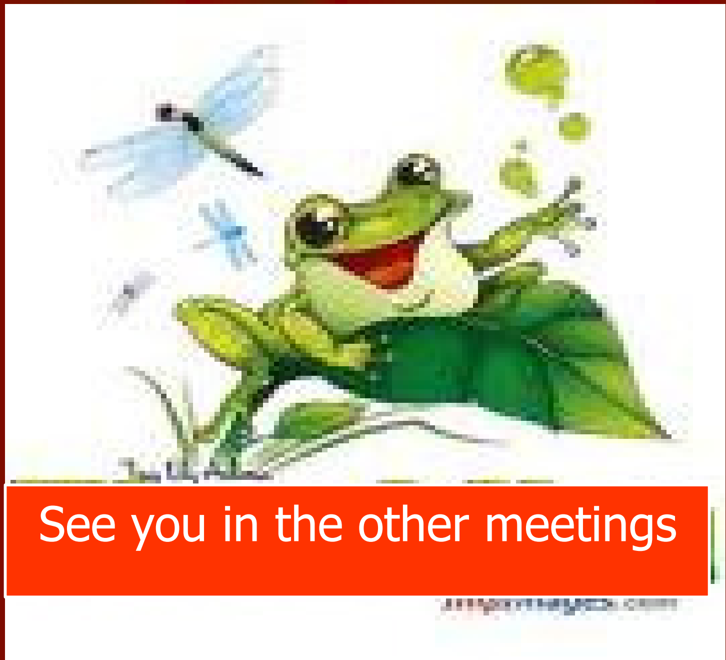
Conclusion

- Same type of response was noticed between both types of the snails BGS and WGS. While methomyl clearly inhibited GAD activity, abamectin and emamectin benzoate stimulated markedly the GAD activity in both types of the used land snails

- Abamectin and emamectin benzoate induced a significant GAD stimulatory effect for both type of snails BGS and WGS.

- Abamectin interaction with GAD activity was higher than emamectin benzoate especially in the case of WGS when the stimulatory effect on GAD activity was less than BGS.

ACKNOLEDGMENT



elsayedabdallah@yahoo.com

THANKS


Thanks
L.O.G.O