

## EFFECT OF METHOMYLON CERTAIN ENZYME SYSTEMS IN SOME LAND SNAIL AND SLUGS.

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The effect of methomyl compound in vivo on three vital enzymes namely Glutamic Pyruvic Transaminase (GPT), Glutamic Oxaloacetic Transaminase (GOT) and Acetylcholinestrase (AchE) activities of *succinea putrus*, *chocelicella acuta* and *Deroceras reticulatum* tissues were investigated after 1,3 and 7 days of exposure under laboratory conditions.

The results revealed that the biochemical response of (GPT) and (GOT) enzymes to methomyl compound were significant increase differences in activity during 1,3 and 7 days post-treatment for all the previous tested gastropods. Also, there were slight significant differences in (GPT) and (GOT) enzymes activities among either all treatments or time intervals. On the other hand, (AchE) activity was induced a significant decrease. Also, methomyl compound has inhibitory effect on this enzyme, the values were significantly for the first day of exposure till the end of the experimental period.

تأثير الميثوميل على بعض النظم الإنزيمية على بعض القواقع والبرازقات

الأرضية

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أجريت دراسة معملية على بعض القواقع والبرازقات الأرضية لدراسة تأثير مركب الميثوميل (نيوميل ٩٠%) على بعض النظم الإنزيمية مثل إنزيم (GPT)، (GOT) و (AchE). ووضحت النتائج أن مركب الميثوميل ٩٠% يسبب زيادة تصل إلى ٣٠% عن المقارنة في الإنزيم الأول وكذلك زيادة تصل إلى ٢٨% في الإنزيم الثاني كما أدى إلى تثبيط بنسبة تصل إلى ٧٥% للإنزيم الثالث.

ويمكن تفسير ذلك بأن زيادة إفراز إنزيمي (GPT) و (GOT) أو انحرافهم عن النسبة الطبيعية تؤدي إلى الموت حيث يحدث تغيرات بيوكيميائية وتتضخم الأنسجة ولا تستطيع جدر الخلايا القيام بوظيفتها ويحدث الموت. وعلى العكس من ذلك فإن تأثير الميثوميل على إنزيم (AchE) بالنقص حيث يتم تثبيط الإنزيم بنسبة كبيرة مما يؤدي إلى حدوث شلل في العضو مكان ملامسة المبيد ثم للجهاز العصبي للحيوان كليا.

## INTRODUCTION

Investigation on the effect of carbamate compounds on gastropods were first made in the USA and Germany , These have shown that these compounds are just as toxic to molluscs as metaldehyde . (Godan , 1983).

The molluscicidal activity of carbamate compounds against terrestrial gastropods was studied by several authors ( El-Okda, 1989; Radwan *et al.*,1992 ; Zedan *et al.*,2006 and Martada, 2006 ).

Methomyl and metaldehyde are two of the main molluscicides that been in continuous for snail control in Egypt more than ten years (as recommended) .

Numerous authors were also performed experiments on the effects of oxime carbamates on the enzyme activities of vertebrates ( El- Toweissy , 1990). While few studies were carried out on the action of carbamates upon the enzyme activity of land snails. ( Radwan *et al.*, 1992 ) on *Theba psiana* land snails and on *Eobania vermiculata* land snails. (Mourad and Zedan 1996.)

Biochemical studies of snails are very important to see whether there any pathways , enzyme systems peculiar to snails that open them up to specific chemical control ( Duncan , 1983 ).

The present study aim to throw light on the effect of methomyl compound in some vital enzymes systems in the terrestrial gastropods *succinea putrus* , *chocelicella acuta* land snails and *Deroceras reticulatum* in vivo. As well as more information about the biochemical processes of relevance for understanding the mode of methomyl action on the terrestrial snail and slugs .

## MATERIALS AND METHODS

### Animals :

Snails and slugs were collected from fields and orchards at Aga and El-Mansoura districts Dakahlia Governorate, they were transferred in cloth sacs to the laboratory. Snails and slugs were kept – separately each species – at room temperature in small plastic boxes (32 × 21 × 25 cm) and supplied with fresh green lettuce leaves for two weeks before treatment .

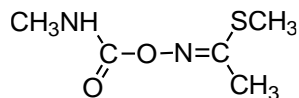
Ten health individual snails and slugs were selected for each replicate and starved for 24 hours before starting the tests.

### Tested pesticides :

Methomyl compound belonging to chemical group of (carbamate pesticides) were tested. Common name , trade name , chemical structure and chemical name of this compound were as follows:

- Methomy ( Neomyl 90% WP ) insecticide / molluscicide .

- structural formula.



- chemical name .

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

**Sample preparation**

The soft part of the snails or slugs was removed 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 10000 xg for 20 minutes using a cooling centrifuge at 4°C. The supernatant was used as a source of the enzymes . ( Radwan et al., 1992 and Mourad and Zedan 1996 )

**Determination of Glutamic Pyruvic Transaminase activity (G P T) .**

The (GPT) of the treated and untreated snails and slugs were determined according to Reitman and Frankel ( 1957 ) , using (GmbH) Diagnostics kits, with simple modification, it is the volume of the sample ( 0.1 ml ) was used .

**Principle :**



photometric determination of the concentration of the pyruvic hydrazine formed for 2,4 dinitrophenyl hydrazine was done at 505 nm by using spectrophotometric method.

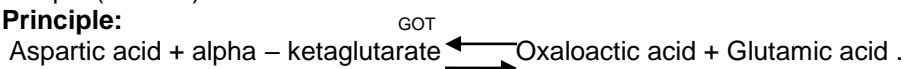
**Calculation :**

The number of (GPT) units / ml of sample was calculated using the standard curve for alanine as the substrate for GPT, the curve shows a relationship between of GPT units/ml and optical density.

**Determination of Glutamic Oxaloacetic Transaminase activity (GOT) :**

Determination of Got activity of the treated and untreated snails and slugs were determined according to Reitman and Frankel(1957), using (GmbH ) diagnostics kits, with simple modification, it is The volume of the sample ( 0.1 ml ) was used.

**Principle:**



measured by using colorimeter at wave length of 505nm.

**Calculation :**

The number of GOT units / ml of samples was calculated using the standard curve for aspartate as the substrate for GOT, the curve shows a relationship between number of GOT units / ml and optical density .

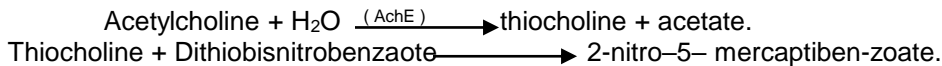
**Assay of Acetylcholinestrse ( AchE ) activity :**

The method of Ellman et al., (1961) was used for measuring the AchE activity using kits purchased from ( GmbH) company , Germany.

**Principle:**

The principle of the tested depend on the following :

**Reaction:**



The colored solution formed is measured colorimetircally at a wave length 405 nm.

**Calculation:**

Calculate the activity of ( AchE ) in the sample as follows :  
 $U/L (25^{\circ}C) = 23460 \times \Delta$ .

**RESULTS AND DISCUSSION**

**Biochemical studies :**

In vivo effects of the Neomyl 90 % pesticide (methomyl) carbamate group which it has the great effective toxic compound for snails and slugs on the activities of the Glutamic – pyruvic transaminase (GPT), Glutamic – Oxaloacetic transaminase (GOT) and Acetylcholinestrerase (AchE) enzymes of *succinea putrus* , *chocelicella acuta* land snails and *Deroceras reticulatum* land slugs were investigated after 1,3 and 7 days of exposure in the basis of their  $1/4$  LC<sub>50</sub> and  $1/8$  LC<sub>50</sub> 's values and recorded in Tables (1 to 5) .

**The effect on Glutamic – pyruvic transaminase ( GPT )**

The biochemical response of GPT enzyme to selected pesticide compounds on *S.putrus* , *C. acuta* land snails and *D. reticulatum* land slug are presented in Tables (1&2) for the two sub-lethal concentrations, respectively.

Results in Table (1) the effect of  $1/4$  LC<sub>50</sub> of the tested compound on GPT enzyme, revealed that methomyl increased the enzyme activity during 1<sup>st</sup> day post-treatment with (23.71, 8.80 and 30.01) % than control treatment, while during 3<sup>rd</sup> and 7<sup>th</sup> days, its activity significantly increased too, with ( 20.78 , 20.46 ) ( 6.05 , 7.89 ) and ( 10.13 , 9.83 ) for *S.putrus* , *C.acuta* land snails and *D.reticulatum* land slugs , respectively.

Data in Table (2) showed that the effect of  $1/8$  LC<sub>50</sub> of the tested compounds in GPT enzyme in *S.putrus* , *C.acuta* and *D.reticulatum* . Results revealed that there were slight significant differences in GPT activity among either all treatments or time intervals. On the other hand, the highest mean value of GPT activity was noted after first day the lowest one was observed after 7 days following treatment.

**Table (1): Effect of Methomyl (Neomyl 90%) pesticide (  $1/4$  LC<sub>50</sub> PPM) on the activity of Glutamic-Pyruvic Transaminase (GPT) in some terrestrial gastropods at different exposure periods.**

Gastropod species	Exposure periods (days)	Control mean ± S.E	Methomyl mean ± S.E	% difference
<i>S.putrits</i>	1	15.73 ± 0.51	14.46 ± 1.73	+ 23.71
	3	13.33 ± 1.49	16.10 ± 0.64	+ 20.78
	7	19.01 ± 1.52	22.90 ± 1.02	+ 20.46
<i>C.acuta</i>	1	7.04 ± 1.04	7.66 ± 0.96	+ 8.80
	3	6.61 ± 0.75	7.01 ± 0.45	+ 6.05
	7	8.11 ± 0.46	8.75 ± 1.20	+ 7.89
<i>D.reticulatum</i>	1	17.03 ± 0.92	22.16 ± 1.73	+ 30.01
	3	21.71 ± 1.07	23.91 ± 0.97	+ 10.13
	7	19.23 ± 1.12	21.12 ± 1.03	+ 9.83

Means significant at P < 0.05

**Table (2): Effect of Methomyl (Neomyl 90%) pesticide (  $1/8$  LC<sub>50</sub> PPM) on the activity of Glutamic-Pyruvic Transaminase (GPT) in some terrestrial gastropods at different exposure periods.**

Gastropod species	Exposure periods (days)	Control mean $\pm$ S.E	Methomyl mean $\pm$ S.E	% difference
<i>S.putrits</i>	1	15.73 $\pm$ 0.51	17.63 $\pm$ 0.75	+ 12.07
	3	13.33 $\pm$ 1.49	15.31 $\pm$ 0.65	+ 14.77
	7	19.01 $\pm$ 1.52	20.41 $\pm$ 0.91	+ 7.36
<i>C.acuta</i>	1	7.04 $\pm$ 1.04	8.61 $\pm$ 0.72	+ 22.30
	3	6.61 $\pm$ 0.75	7.88 $\pm$ 0.73	+ 19.21
	7	8.11 $\pm$ 0.46	9.02 $\pm$ 1.01	+ 11.22
<i>D.reticulatum</i>	1	17.03 $\pm$ 0.92	18.17 $\pm$ 1.31	+ 6.69
	3	21.71 $\pm$ 1.07	23.26 $\pm$ 0.63	+ 7.13
	7	19.23 $\pm$ 1.12	13.22 $\pm$ 0.43	+ 7.17

Means significant at  $P < 0.05$

#### Effect on Glutamic Oxaloacetic Transaminase ( GOT )

The effect of the tested compound on (GOT) enzyme in *S.putrus* , *C.acuta* and *D.reticulatum* presented in Tables (2 and 4) for the previous two sub-lethal concentrations. The same trend was observed as GPT. Data revealed that methomyl compound was the higher increasing the activity of GOT enzyme activity, when they were tested for ( $1/4$  LC<sub>50</sub> and  $1/8$  LC<sub>50</sub>) after 1<sup>st</sup> , 3<sup>rd</sup> and 7<sup>th</sup> days post treatment. The difference between than control treatment were [(8.93, 1.46 and 4.02) and (17.57, 4.39 and 18.64] for *S.putrits*: [(9.64 , 5.70 and 11.78) and ( 9.94 , 12.68 and 13.89 ) ] for *C.acuta* and [(15.18, 7.38 and 28.09) and (15.18, 13.68 and 0.37) % , for *D.reticulatum* , respectively.

A few investigation had been conducted on the effect of Methomly on the activity of GPT , GOT and AchE enzymes in land snails and slugs . Regarding the response of GPT and GOT to the previous pesticide. Results cleared that the methomyl had an increasing effect against GPT and GOT activity in *S.putrus* , *C.acuta* land snails and *D. reticulatum* land slug.

The possible mechanism involved in the elevation of GPT and GOT levels observed in the present study may be due to tissue damage or due to increasing synthesis or decreasing catabolism of both enzymes. On the other hand, the decreasing activity of GOT and GPT may be due to either to leakage of the enzyme into extra-cellular compartments or actual enzyme inhibition by these pesticides, thus, the deviation of both enzymes activities out of the normal range could be lead to biochemical impairment and lesions of the tissues and cellular functions.

**Table (3): Effect of Methomyl (Neomyl 90%) pesticide (  $1/4$  LC<sub>50</sub> PPM) on the activity of Glutamic-Oxaloacetic Transaminase (GOT) in some terrestrial gastropods at different exposure periods.**

Gastropod species	Exposure periods (days)	Control mean $\pm$ S.E	Methomyl mean $\pm$ S.E	% difference
<i>S.putrits</i>	1	10.30 $\pm$ 0.42	11.22 $\pm$ 0.81	+ 8.93
	3	8.88 $\pm$ 0.93	9.01 $\pm$ 0.90	+ 1.46
	7	9.44 $\pm$ 1.30	9.82 $\pm$ 0.74	+ 4.02
<i>C.acuta</i>	1	10.16 $\pm$ 0.96	11.14 $\pm$ 1.63	+ 9.64
	3	9.46 $\pm$ 1.17	10.00 $\pm$ 0.87	+ 5.70
	7	8.06 $\pm$ 0.73	9.01 $\pm$ 1.45	+ 11.78
<i>D.reticulatum</i>	1	12.25 $\pm$ 0.55	14.11 $\pm$ 1.31	+ 15.18
	3	11.11 $\pm$ 0.97	11.93 $\pm$ 2.13	+ 7.38
	7	13.17 $\pm$ 1.61	16.87 $\pm$ 0.90	+ 28.09

Means significant at P &lt; 0.05

**Table (4): Effect of Methomyl (Neomyl 90%) pesticide ( $1/8$  LC<sub>50</sub> PPM) on the activity of Glutamic-Oxaloacetic Transaminase (GOT) in some terrestrial gastropods at different exposure periods.**

Gastropod species	Exposure periods (days)	Control mean $\pm$ S.E	Methomyl mean $\pm$ S.E	% difference
<i>S.putrits</i>	1	10.30 $\pm$ 0.42	12.11 $\pm$ 0.35	+ 17.57
	3	8.88 $\pm$ 0.93	9.27 $\pm$ 0.73	+ 4.39
	7	9.44 $\pm$ 1.30	11.20 $\pm$ 0.45	+ 18.64
<i>C.acuta</i>	1	10.16 $\pm$ 0.96	11.17 $\pm$ 0.35	+ 9.94
	3	9.46 $\pm$ 1.17	10.66 $\pm$ 0.73	+ 12.68
	7	8.06 $\pm$ 0.73	9.18 $\pm$ 0.45	+ 13.89
<i>D.reticulatum</i>	1	12.25 $\pm$ 0.55	14.11 $\pm$ 0.71	+ 15.18
	3	11.11 $\pm$ 0.97	12.63 $\pm$ 0.63	+ 13.68
	7	13.17 $\pm$ 1.61	13.22 $\pm$ 0.42	+ 0.37

Means significant at P &lt; 0.05

Our results are in agreement with those reported by Lebsack *et al.*, (1980) mentioned that the possible mechanism involved in the elevation of GPT and GOT levels may be due to tissue damage or due to increase of it . El-wakil (1990) found that GPT and Got activities were not significant affected by the Bayluscide, Dursban and Gramaxon however a significant damages in activities of these enzymes were noticed among the different periods after treatment. Radwan *et al.*, (1993) reported that Chlorfluazuron (1k 17899) and Cascade increased the activities of GPT and GOT in *Helix aspersa* snail tissue.

**The effect on Acetylcholinesterase activity (AchE):**

Acetyl cholinesterase (AchE) activity of the tested compound presented in Table (5), it is obvious induced a significant decrease in AchE activity. Also, the tested compound has inhibitory effect on this enzyme, the values were significantly from the first day of exposure till the end of the experimental period.

The results showed that a considerable variation in their effects on AchE, Methomyl compound the maximum inhibition of this enzyme was noted among snails treated with values (-55.83,- 58.46 and -61.77) (-69.13,- 67.70 and -75.57 ) and ( -64.82 , - 67.82 and -63.11 ) % inhibition of this enzyme than control , for *S.putrus* , *C.acuta* and *D.reticulatum* during 1<sup>st</sup> , 3<sup>rd</sup> and 7<sup>th</sup> days post- treatment, respectively.

The present findings are in agreement with those reported by Banne (1980) who reported that Frescon induced the release of cholinesterase and nonspecific Estrase in some tissue of *Bulinus truncates* snail. Radwan, (1993) found that (AchE) activity in *Theba pisana* snail tissue inhibited after exposure for 1 and 5 days with certain oxime carbamate pesticides.

**Table (5): Effect of Methomyl (Neomyl 90%) pesticide ( $1/8$  LC<sub>50</sub> PPM ) on the activity of Acetylcholinesterase ( AchE ) in some terrestrial gastropods at different exposure periods.**

Gastropod species	Exposure periods (days)	Control mean $\pm$ S.E	Methomyl mean $\pm$ S.E	% difference
<i>S.putrits</i>	1	4.03 $\pm$ 0.41	1.78 $\pm$ 0.17	- 55.83

	3	5.61 ± 0.71	2.33 ± 0.23	- 58.46
	7	5.18 ± 0.93	1.98 ± 0.33	- 61.77
<b>C.acuta</b>	1	3.37 ± 0.63	1.04 ± 0.19	- 69.13
	3	4.18 ± 0.17	1.35 ± 0.13	- 67.70
	7	3.89 ± 0.41	0.95 ± 0.40	- 75.57
<b>D.reticulatum</b>	1	6.14 ± 0.33	2.16 ± 0.16	-64.82
	3	5.14 ± 0.55	1.67 ± 0.40	- 67.82
	7	7.13 ± 0.45	2.63 ± 0.46	- 63.11

AchE activity is Expressed a  $\mu$  m/mg protein / min

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**تأثير الميثوميل على بعض النظم الإنزيمية على بعض القواقع والبزاقات الأرضية**  
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أجريت دراسة معملية على بعض القواقع والبزاقات الأرضية لدراسة تأثير مركب الميثوميل (نيوميل ٩٠ %) على بعض النظم الإنزيمية مثل إنزيم ( GPT ) ، ( GOT ) و ( AchE ) .  
واوضحت النتائج أن مركب الميثوميل ٩٠ % يسبب زيادة تصل الى ٣٠ % عن المقارنة في الانزيم الأول وكذلك زيادة تصل الى ٢٨ % في الانزيم الثاني كما أدى إلى تثبيط بنسبة تصل الى ٧٥ % للإنزيم الثالث.  
ويمكن تفسير ذلك بأن زيادة إفراز إنزيمي ( GPT ) و ( GOT ) أو انحرافهم عن النسبة الطبيعية تؤدي إلى الموت حيث يحدث تغيرات بيوكيميائية وتتضخم الأنسجة ولا تستطيع جدر الخلايا القيام بوظيفتها ويحدث الموت. وعلى العكس من ذلك فإن تأثير الميثوميل على إنزيم ( AchE ) بالانقص حيث يتم تثبيط الإنزيم بنسبة كبيرة مما يؤدي إلى حدوث شلل في العضو مكان ملامسة المبيد ثم للجهاز العصبي للحيوان كليا.