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EFFICIENCY OF ACETIC ACID AND METHOMYL ON TERRESTRIAL SNAILS EOBANIA VERMICULATA (MÜLLER) AND MONACHA OBSTRUCTA (FEROSSAC) UNDER LABORATORYAND FIELD CONDITION.

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ABSTRACT

Some laboratory experiments and field trials were conducted to study the effiecieney of acetic acid and the molluscicides methomyl against theterrestrial snails, *Eobania. Vermiculata* Müler and *Monacha. obstructa* Ferrosac were investigated under laboratory and field conditions. The results showed that, acetic acid had a highly significant effect on *Monacha. obstructa* than *Eobania. vermiculata*, whereas, the mortality percent was higher for *M. obstructa* (100%) at the concentration of 10% after 72 hrs. than *E. vermiculata*, which recorded (50%) mortality at the same concentration and the same time. The LC_{50} values recorded, 8.69 and 2.19% after 72 hrs of application for *E. vermiculata* and *M. obstructa* respectively. The effect of the applied compound (acetic acid) on certain biochemical parameters were recognized at the LC_{50} level, the total protein activities showed increasing for both snails species when compared with control .The same compound showed decrease in the level of total lipid from 4.96 *to* 2.78 for *E. vermiculata* while total lipid increased from 2.89 to 3.22 g/dl for *M. obstructa* respectively. Also decrease in the level of alkaline and acid phosphatase in both land snail species were recorded at Lc_{50} level .On the other hand, the results cleared that acetic acid achieved the decreased effect on the cholesterol for *E. vermiculata* while it increased the cholesterol for *M. obstructa* . On the other side, the obtained data of the field trials which carried out at Qalubyia Governorate Toukh district (shubra harris) in citrus orchards using acetic acid and methomyl proved that *M. obstructa* had more sensitivity to the tested compound than *E. vermiculata*.

Keywords: Acetic acid, Methomyl Eobania, vermiculata Müler, monacha obstructa ferrosac , Snails.

INTRODUCTION

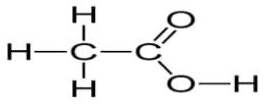
The terrestrial molluscs are considered a major pest of a wide range of agricultural crops in temperate and humid habitats worldwide (Speiser and Kistler [20]. They attack plants causing great damage to the cultivated plants. Economic damage caused by these molluscs is due to feeding and to contamination with their bodies, feces or slime leading to deterioration of the product quality, in addition the financial loss Iglesias *et al*., [11]. In Egypt terrestrial snails attack vegetables, field crops, orchard trees as well as ornamental and medical plants. *Monacha obstructa* (Ferussac) and *Eobania vermiculata* (Müler) are the important snail species in the Egyptian Governorates attacking various plantations Eshra [7], Khidr,E.k. [14]. Azzam and Abd El-Hady [3], recorded seven snail species and three slug species infesting about 47 species of plants in seven Governorates in Egypt including Cairo and Arish. Shell is very important for protecting snail from any risk. Therefore the main aim of this work is shedding some light on the efficiency of acetic acid on *E. vermiculata* and *M. obstructa* under laboratory and field conditions. Also its effect on some biochemical parameters affecting the synthesis of shell such as alkaline and acid phosphatase, total protein, total lipid and cholesterol contents had investigated.

MATERIALS AND METHODS

Tested Compounds .

a-Common name: Acetic Acid 96%

CH_3COOH

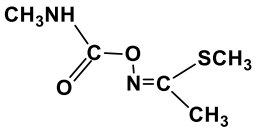


b- Common name: methomyl (90%)

Trade name: Newmyl (90% SP) Is a Carbamate compound.

Iupac Name S-methyl N-(methylcar-bamoyloxy) thioacetimidate. Chemical name**:**methyl N- [[(methyl amino)carbonyl] oxy] thioacetimidate. It was obtained from Kafr El-Zayat Company, Egypt. The Egyptian

Ministry of Agriculture and Land Reclamation (MALR) recommends this compound for use against land snail infestation in Egyptian fields, (Molluscicide)



Action: Insecticide**.**

1. Experimental Animals

Adult individuals of the two terrestrial snail species, *E. vermiculata* and *M. obstructa* were collected from the infested fields cultivated with Egyptian clover and ornamental plants at Qalubyia Governorate. Animals were transferred to laboratory, kept in glass boxes and fed on fresh lettuce leaves. For each treatment, 40 healthy animals were allocated and divided into four replicates (each of 10 individuals) and another for control.

2. Laboratory Contact Application

Animals were treated with different concentrations of the tested compound using thin layer film technique according to the method of **Ascher and Mirian** [2]**.** Two ml of the tested concentration of the tested compound were spread on inner surface of a Petri- dish using water moving the dish gently in circles. Water was evaporated under room conditions in a few minutes leaving a thin layer film of the tested compounds. Animals were exposed to different concentrations of tested compound for one week. A parallel control test was conducted using plain water. Mortality percentages were recorded and corrected according to **Abbott's formula** [1]**.**

$$\text{corrected\%} = 1 - \frac{\text{No.of snails in treatment after treatment}}{\text{No.of snails in control after treatment}} \text{ x } 100$$

3. Field Experiments:

Two compounds, acetic acid and methomyl (recommended compound) were tested against the two land snail species under field conditions. The performance of the tested compounds was studied under field conditions at Qalubyia Governorate Toukh district (shubra harris) on plantation of citrus nursery trees for *E. vermiculata* and *M. obstructa.* Four plots (each of 4 m^2) were chosen. Two plots were used for acetic acid, the third for methomyl and the four was left without treatment as control. Each plot was far from the other by at least 4m. The tested compounds were applied as a spray the snails infested plants were counted daily pre and post treatment during 15 days. The efficiency of compounds was based on the reduction of snails population after 15 days of treatment according to the formula of **Henderson and Tilton** [9] as follows :

$$\text{Reduction percentage} = 1 - \frac{(C_1 \text{ x } T_2)}{(C_2 \text{x } T_1)} \text{ x } 100$$

C_1= population of snails in control before application,

C_2= population of snails in control after application,

T_1= population of snails in treatment before application,

T_2= population of snails in treatment after application,

4. Biochemical Parameter Determination:

The activities of some enzymes and biochemical parameters were studied to clarify the physiological response of the terrestrial snail to the tested compounds i.e., alkaline and acid phosphatase, total protein, total lipid and cholesterol contents. The parameters were determined after 3 days of treatment. Samples were prepared according to the method of **Bergmeyer [5].**

4.1. Alkaline and acid Phosphatase (ALP and ACP): Both alkaline and acid phosphatase activity was determined according to method of **Kind and King [15]** and **Belfield and Goldberg [4].**

4.2. Total Protein: Soluble protein was determined using the method of **Henry** [10] **and Tietz** [21]**.**

4.3. Total lipid: Lipids are hydrolyzed by sulphoric acid, then treated with phosphovanilin mixture to produce sulphophosphovanilin complex of rose coloration which is measured photometrically according to **Zollner and Kirsch** [23]**.**

4.4. Cholesterol: Cholesterol concentration was determined according to method of **Ellefson and Caraway** [6]**.**

5. Statistical analysis:

The data was subjected to analysis of variance (ANOVA) and the means were compared by L.S.D test at 0.05 levels, using SAS program **(SAS Institute,)**[19].

RESULTS AND DISCUSSION

The gained results in table (1) and Fig.(1a&b) showed the effect of acetic acid on the adult age of the two snails species. The data revealed that, acetic acid caused 0.0, 25, 35, 40, 50, 65 and 75% mortality for *E. vermiculata* after 3 day of treatment when using concentrations of 2, 4, 6, 8, 10, 12 and 14 % respectively meanwhile for *M. obstructa* the concentrations 2, 4, 6, 8 and 10 % caused mortality 40, 90, 92.5, 100 and 100% respectively comparing with the untreated control. The LC_{50} values recorded, 8.69 and 2.19% after 72 hr. of application for *E. vermiculata* and *M. obstructa* respectively**. Mobarak** [16] reported that LC_{50} of abamectin biocide against the two snail species *E. vermiculata* and *M. obstructa* were 30.9 and 877.0 ppm consecutively. She also recorded that acetylsalicylic acid when used against the same two snail species had more effect against *M. obstructa* as its LC_{50} and LC_{90} reported 1354.6 and 38873.7 ppm than *E. vermiculata* since LC_{50} and LC_{90} were 14975.9 and 1156600 ppm respectively. **Youssef** [22] reported that abamectin was the most effective compound against *E. vermiculata* and *M. contiana* , where it achieved 90 and 100% mortality for two species, respectively.

Biochemical impacts of acetic acid on *E. vermiculata* and *M. obstructa*:

a. Total protein:

The response of total protein to the treatment as a contact against both snail species was shown in Table (2) .The results showed significant increase in total protein from 0.195 to 1.99 g/dL for *E. vermiculata* and from 0,168 to 2.10 g/dL for *M. obstructa* after 3 days of treatment with the tested compound.

Khater *et al*. [13] reported that the increase in total protein could be attributed to the increased biosynthesis process occurred by high enzyme stress.

b. Total Lipid:

The obtained results in table (2) showed also a different effect of the acetic acid on the total lipid on both snail species. whereas a decreased in the level of total lipid from 4.96 to 2.78 g/dL for *E. vermiculata* were recorded .In contrast the total lipid increased from 2.89 to 3.22 g/dL for *M. obstructa* after the 3 days of treatment.

Mobarak,. [14] reported that the acetylsalicylic acid affected on the total lipid and cholesterol which important for synthesis of shell.) Observed that acetylsalicylic acid when combined with methomyl was more effective against both alkaline and acid phosphatase enzymes than each compound alone in the two snail species, *E. vermiculata* and *M.obstructa*

c. Alkaline and acid phosphatase:

Data in the same table illustrated the effect of acetic acid on alkaline and acidic phosphatase in both land snails species after 3 days of treatment. Results revealed that acetic acid resulted in decreasing in the level of alkaline phosphatase from 666.58 to 86.9 U/L for *E. vermiculata* and from 333.92 to 212.41 U/L for *M. obstructa*. The acidic phosphatase level decreased from 2.39 to 1.91 U/L for *E. vermiculata* while it increased from 2.19 to 2.33 U/L for *M. obstructa*. **Kandil *et al.***[12] Observed that acetylsalicylic acid when combined with methomyl was more effective against both alkaline and acid phosphatase enzymes than each compound alone in the two snail species, *E. vermiculata* and *M.obstructa*.

d. Cholesterol:

The data in table (2) cleared the response of cholesterol for the investigated snails species to the previous tested compound. Results showed increasing of cholesterol level after 3 days of treatment with acetic acid from 222.3 to 374.6 for *E. vermiculata* meanwbile cholesterol decreased from 412.8 to 201.3 mg/dL for the *M. obstructa* **Mobarak,** [17]**.** Reported that acetylsalicylic had strong effect on ALP and ACP enzymes which are responsible to the mucus secretion of snail this effect lead to inhibit the production of the mucus and shell materials that is very important for snail life.

Also, the acetylsalicylic acid affected on the total lipid and cholesterol which important for synthesis of shell. In the present investigation, scratch and abrasion was observed on some treated snails (Fig.,2-4).

It has been reported that lipid content increased when the animals encountered stressful conditions **Nandurkar and Zambare** [18].This may explain the increasing in cholesterol and total lipids observed in the present investigation after treatment .

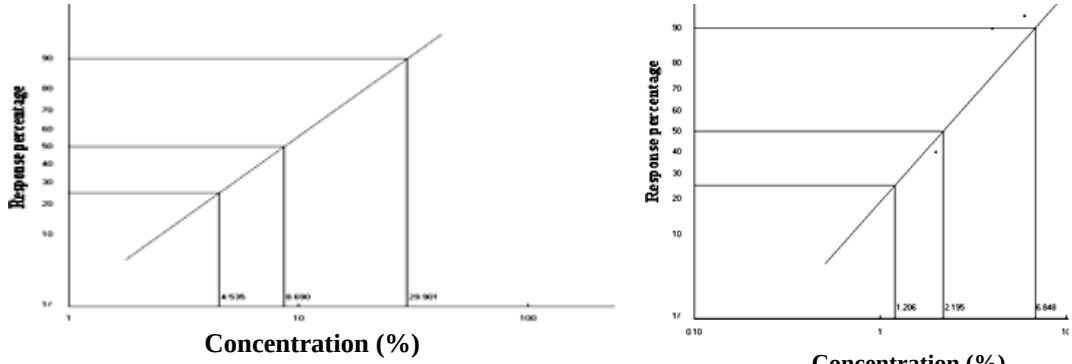
Farkas *et al.* [8] recorded that increase in ALP enzymes could be due to a variety of conditions, including muscle damage, intestinal and hepatopancreatic injury, and toxic hepatitis.

Field experiments:

The field performance of the tested compounds against *E. vermiculata* and *M. obstructa* was shown in Table (3&4). The results revealed that the **acetic acid** 20% achieved the highest population reduction of snail (63.72%) followed by **acetic acid** 16% (58.68%), then Methomyl (64.48%) for *E. vermiculata* snail. While in the case of *M. obstructa* the results indicated that, the Methomyl achieved the highest population reduction of snail (91.313%), followed by **acetic acid** 20% (72.45%) then **acetic acid** 16% (60.80%), Comparing the data in Tables (3&4) it is cleared that under field conditions *M. obstructa* was more sensitive than *E. vermiculata* to methomyl which caused 91.313% population reduction on the first species comparatively with 65.21% in case of second species. Field results are in contradicted with those obtained by laboratory trails. In spite of **acetic acid** (16& 20%), were the superior in the laboratory, it had the lowest effect under field condition. This may be attributed to the weather factors and natural conditions (temperature, humidity, and light ….etc.). These factors may be affected on this compound inducing degradation or decomposition led to reduce its toxic action against snails. In comparison, **Kandil *et al.*** [12] reported that the combination of methomyl with acetylsalicylic acid achieved the highest population reduction percentage of both snails *E. vermiculata* and *M. obstructa*., followed by methomyl alone.

Table (1): Toxicity of acetic acid on adult of *Eobania vermiculata* and *Monacha obstructa.*

Conc. (%)	*Eobania vermiculata*	*Monacha obstructa*
	Mortality % after 72hr	Mortality % after 72hr
Control	0.0	0.0
2	0.0	40
4	25	90
6	35	92.5
8	40	100
10	50	100
12	65	-
14	75	-
Slope	2.3880 ± 0.2987	2.5935 ± o.4573
LC_{50}	8.6897%	2.1949%
LC_{90}	29.9007%	6.8481%



Concentration (%)
a. *Eobania vermiculata*

Concentration (%)
b. *Monacha obstructa*

Fig (1,a&b).Ldp lines of acetic acid on laboratory of *E. vermiculata* and *M.obstructa.*

Table (2): Effect of LC_{50} of acetic acid on total protein, total lipid ,alkaline phosphatase acid phosphatase , cholesterol of *E. vermiculata* and *M. obstructa* terrestrial snails after three days.

Snail species	Treatment	Total protein (g/dl)	Total lipid (g/dl)	Alkaline phosphatase (U/L)	Acid phosphatase (U/L)	Cholesterol (mg/dl)
Eobania vermiculata	Control	0.195± 0.011	4.96 ± 0.05	666.58± 10.070	2.39± 0.131	222.3± 7.090
	LC_{50} of Acetic acid	1.99 ± 0.02	2.78± 0.061	86.867± 5.672	1.91± 0.0754	374.6± 14.674
	F Value	18341.7	2299.55	7547.44	30.18	261.99
	LSD	0.0368	0.1262	18.527	0.2426	26.124
Monacha obstructa	Control	0.168± 0.005	2.89± 0.061	333.92± 21.833	2.19 ± 0.03	412.8± 11.031
	LC_{50} of Acetic acid	2.1± 0.226	3.22± 0.267	212.41 ± 4.875	2.33 ± 0.078	201.3± 1.621
	F Value	219.02	4.37	88.51	8.40	1079.48
	LSD	0.363	0.438	35.86	0.134	17.87

Table (3): Field performance of certain compound against *Eobania vermiculata* snail

Treatment	Rate of application	No. of individual before treatment	No. of alive individual after treatment	% population reduction
Control	-	112	98	12.5
Acetic Acid	16%	130	47	58.68
Acetic Acid	20%	63	20	63.72
Methomyl (90%)	300g/fdean	74	23	64.48

Table (4): Field performance of certain compound against *Monacha obstructa* snail

Treatment	Rate of application	No. of individual before treatment	No. of alive individual after treatment	%population reduction
Control	-	45	28	37.7
Acetic Acid	16%	41	10	60.80
Acetic Acid	20%	35	6	72.45
Methomyl(90%)	300g/fdean	37	2	91.313

Fig. (2) scratch and abrasion of *Monacha* shell

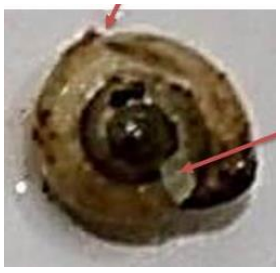


Fig.(3) scratch and abrasion of *Monacha* shell

Fig.(4) scratch and abrasion of *Eobania* shell

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الملخص العربي :

لدراسة كفاءة حمض الخليك والميثوميل على قوقع الحدائق البنى *E. vermiculata* وقوقع البرسيم الزجاجى *M. obstructa* اجريت بعض التجارب المعمليه والحقليه وقد أظهرت الدراسة المعمليه أن حمض الخليك له تأثير معنوى أكثر على قوقع البرسيم الزجاجى *M. obstructa* من قوقع قوقع الحدائق البنى *E. vermiculata* حيث ان معدل الموت كان (100%) بالنسبة لـقوقع البرسيم الزجاجى *M. obstructa* و (50%) بالنسبه لقوقع الحدائق البنى *E. vermiculata* عند تركيز10% بعد 72 ساعه من المعامله بالمقارنه بالكنترول .سجلت قيمة ال LC_{50} 8.69 % بالنسبه لقوقع *E. vermiculata* و 2.19% بالنسبه لقوقع البرسيم الزجاجى *M. obstructa* بعد 72 ساعه من المعامله .وبالنسبه للتاثيرات البيوكيميائيه باستخدام ال LC_{50} فقد كانت هناك زياده فى مستوى البروتين الكلى بالنسبه لنوعى القواقع مقارنة بالكنترول وانخفاض فى مستوى الدهون الكليه من 4,96 الى 2,78 g/dl بالنسبه لقوقع الحدائق البنى *E. vermiculata* بينما حدث زياده فى مستوى الدهون الكليه من 2.89 الى 3,22 g/dl بالنسبه لقوقع البرسيم الزجاجى *M. obstructa* . وايضا ادى استخدام قيمة ال LC_{50} انخفاض فى مستوى الفوسفاتيز القاعدى والحامضى لكلا نوعي القواقع ولكن حدث زياده طفيفه فى مستوى الفوسفاتيزالحامضى بالنسبه لقوقع البرسيم الزجاجى *M. obstructa* . واوضحت النتائج ان حامض الخليك سبب انخفاض فى مستوى الكوليستيرول بالنسبه لقوقع الحدائق البنى *E. vermiculata* وزياده بالنسبه لقوقع البرسيم الزجاجى *M. obstructa* ,اما تأثيره ضد نوعى القواقع السابق ذكرهم تحت الظروف الحقليه فقد كان قوقع البرسيم الزجاجى *M. obstructa* اكثر حساسيه من قوقع الحدائق البنى *E. vermiculata*