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EFFICACY OF SOME PLANT ACTIVE INGREDIENTS AS MOLLUSCICIDES AGAINST THE GLASSY CLOVER LAND SNAIL, *MONACHA OBSTRUCTA*

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ABSTRACT: *This study aimed to evaluate the bioactivity of some active ingredients of plant extracts , menthol, camphor and their combination, on the adults of clover land snail, Monacha obstructa (Montagu) by two methods of application, leaf dipping method and contact method (thin layer film). Also, sub-lethal concentration (¼ LC_{50}) of the combination using the contact method (thin layer film) was used to measure some biochemical parameters as alanine amino transaminase (ALT), aspartate amino transaminase (AST), alkaline phosphatase (ALP) and acid phosphatase (ACP). The results revealed that, the combination of camphor and menthol was more effective than each ingredient alone, by the two application methods, with LC_{50} 11757.11 and 8907.73, respectively. However, the results of all biochemical parameters, (ALT, AST, ALP & ACP) for the tested samples were significantly different from control.*

Key words: *Monacha obstructa*, *camphor, menthol, biochemical effects.*

INTRODUCTION

Land snails are serious pests attacking the vegetation including vegetables, horticultural plants and field crops in most area of Egypt. *Monacha obstructa* (Montagu) is the most common and serious pests in Egypt. It caused a substantial damage to different agricultural crops in various governorates. Many environmental problems such as the harmful effects against non-target organisms including mammals, poultry and wildlife result from using synthetic compounds. Some attempts were carried out to evaluate alternative, effective natural pesticides to replace the conventional synthetic pesticides (Abdelgaleil, 2005; Khidr *et al.*, 2006; El-Zemity and Radwan, 2001; Hussein *et al.* 1994, 1999, 2007a, b). Natural products are an excellent alternative to synthetic pesticides as a means to reduce negative impacts towards to human health and the environment (Opender *et al*., 2008). Transaminases enzymes are important in the biological processes in the land snails (Abd El-Ail, 2004).

The aim of this work was to determine the effect of some active ingredients and their combination on mortality of the land snail, *M. obstructa* and on the activities of vital enzymes. The enzymes selected for this study were, alanine amino transaminase (ALT), aspartate amino transaminase (AST), alkaline phosphatase (ALP) and acid phosphatase (ACP).

MATERIALS AND METHODS

Tested snails:

Adult individuals of the glassy clover snail, *Monacha obstructa* were collected from untreated clover field at Dakahlia Governorate. The snails were transferred directly in muslin bags to the laboratory and were kept in glass boxes and fed on fresh lettuce leaves (El-Deeb *et al*. 2003) for two weeks for acclimatization. Healthy adult snails with the same shell diameter were selected for each treatment.

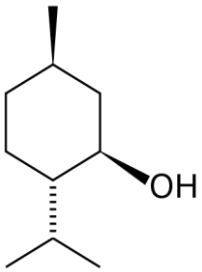
Tested plant active ingredients:

Camphor is crystalline material, ($C_{10}H_{16}O$) and bought from (El- Gomhoria company-Mansoura).

Menthol is crystalline material ($C_{10}H_{20}O$) and was bought from (El- Gomhoria company-Mansoura).

Mixture of the two materials made by mix camphor and menthol 1:1 proportion (Amal, 2014; Salvador *et al*., 2014; Ghada and Amal, 2015).

Camphor formula (Opender *et al.*, 2008)



Menthol formula (Opender *et al.*, 2008)

Methods of application:

1-Leaf dipping method:

This method was used to detect the toxicity action of the snail. The snails were fed on treated lettuce which dipped into the treatments for 20 seconds, then left for air dryness under laboratory conditions (Ghamry, 1994), 10 snails for each replicate were released to each leaf disc placed. Four concentrations and three replicated were used to estimate each concentration-mortality line. The same number of leaf discs per treatment was dipped into distilled water as an untreated check. The concentrations were 10000, 15000, 17500 and 20000 ppm for each ingredient. The mortality was recorded after one, three, five and seven days and the data were corrected relatively to control mortality (Abbott, 1925). LC_{50} values were determined using probit analysis statistical method of Finney, 1971. Toxicity index of LC_{50} was computed according to the Equation of Sun, 1950 :

Toxicity index of LC_{50} =

$$\frac{LC_{50} \text{ of the most effective compound}}{LC_{50} \text{ of the least effective compound}} \times 100$$

2- Contact method:

This method was used to detect the toxicity action on the body enzymes of the snail. The snails were treated with sub-lethal concentration (1/4 LC_{50}) of each ingredient using thin layer film technique according to Ascher and Mirian (1981). Four concentrations and five replicates were used and 5 snails for each replicate. Two ml of each ingredient concentration were spread on the inner surface of a petri-dish by moving the dish gently in circles. Water was evaporated under room conditions in a few minutes leaving a thin layer film of the applied concentration. The snails were exposed to the concentrations of each ingredient and the results were taken after one, three, five and seven days. A parallel control test was conducted using water only.

Biochemical studies:

Preparation of samples:

Samples were prepared according to El-Gohary (2011). After each days (one, three, five & seven) shells of tested snails were removed by making a cut around the whorls in a continuous manner starting at the aperture opening using bone scissors and the broken fragments of the shell were carefully removed. Snails tissues were dissected out and all tissues of each treatment were homogenized in distilled water. The homogenates were centrifuged at 3000 rpm for 15 min. at 5^{o}C in refrigerated centrifuge. The deposits were discarded and the supernatants were kept in a deep freezer till use to determine the activities of alanine amino transaminase (ALT), aspartate amino transaminase (AST), alkaline phosphatase (ALP) and acid phosphatase (ACP).

Determination of ALT and AST:

The activity of alanine amino transaminase (ALT) and aspartate amino transaminase (AST) was determined according to the method of Reitman and Frankel (1957) using commercial reagents.

Determination of ALP and ACP:

Alkaline phosphatase (ALP) activity in haemolymph was estimated according to the method of DGKC (1972). Acid phosphatase (ACP) activity in haemolymph was measured following the method of Kind and King (1954).

RESULTS AND DISCUSSION

1- Toxicity Effect:

Efficiency of the active ingredients on *Monacha obstructa* by leaf dipping method*:*

The data in Table (1) indicated that, the mixture of camphor and menthol caused high mortality proportion on the adults of *M. obstructa* than menthol or camphor alone.

However, Table (2) and Fig. (1) Demonstrated that, the mixture of camphor and menthol was more effective than each one alone with LC_{50}: 11757.11ppm and the toxicity index was 100% for it. Camphor was following the mixture in the effectiveness on the snail with LC_{50}: 17555.65. The slope values indicated that, the mixture of both ingredients had the lowest value was 2.08 followed by 2.51 and 2.87 for camphor and menthol, respectively. Hanan *et al.* (2012) proved that camphor extract has a significant effect on larval mortality of cotton leaf worm.

Efficiency of the active ingredients on *Monacha obstructa* by contact method*:*

Data shown through (Table 3) indicated that, the mixture of camphor and menthol caused high mortality proportion on the adults of *M. obstructa* than menthol or camphor alone. Ghada and Amal (2015) revealed that, the mixture of camphor and menthol had significant effect on 2nd instar larvae of *Spodoptera littoralis* than everyone alone.

Table (1): Corrected mortality *Monacha obstructa* under laboratory conditions by leaf dipping method 25±2 °C and 75±5% RH.

Treatments	Conc. (ppm)	Mortality after treatments %				Total Mortality %
		One day	Three days	Five days	Seven days	
Camphor	10000	-	3.33	10	6.67	20.0
	15000	10	10	6.67	6.67	33.33
	17500	13.33	23.33	6.67	3.33	46.66
	20000	16.67	30	13.33	10	70.0
Menthol	10000	-	3.33	6.67	10	20.0
	15000	16.67	10	-	3.33	30.0
	17500	3.33	16.67	16.67	10	46.0
	20000	26.67	16.67	13.33	3.33	60.0
Mixture of camphor + menthol	10000	6.67	20	6.67	6.67	40.01
	15000	33.33	6.67	3.33	-	43.33
	17500	10	43.33	13.33	-	66.66
	20000	26.67	30	16.67	6.67	80.01

Table (2): Efficiency of some plant active ingredients against *Monacha obstructa* by leaf dipping method.

Conc. (ppm)	corrected mortality %	LC_{50}	LC_{90}	Slope± S.D.	Toxicity index LC_{50}	LC_{90}/LC_{50}	R	P
Camphor								
10000	20	17555.65	56911.66	2.51± 0.59	77.36	3.24	0.894	0.142
15000	33.33							
17500	46.66							
20000	70							
Menthol								
10000	20	18904.69	52846.34	2.87± 0.66	71.54	4.14	0.97	0.609
15000	30							
17500	46							
20000	60							
Mixture of camphor + menthol								
10000	40.01	11757.11	48652.35	2.08± 0.51	100	4.14	0.876	0.140
15000	43.33							
17500	66.66							
20000	80.01							

R: Regression **P: Probability**

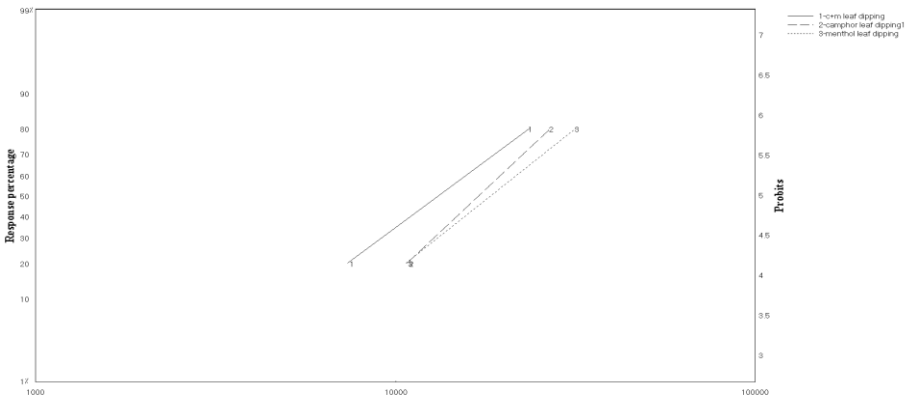


Fig. (1): LD-P lines for some plant active ingredients against the clover land snail, *M. obstructa.*

Table (3): Corrected mortality by contact method of *M. obstructa* under laboratory conditions 25±2 ◦C and 75±5% RH.

Treatments	Conc. (ppm)	Mortality after treatments %				Total Mortality %
		One day	Three days	Five days	Seven days	
Camphor	10000	12	8	8	-	28
	15000	16	16	8	-	40
	17500	28	16	12	-	56
	20000	40	16	4	8	68
Menthol	10000	16	8	8	-	32
	15000	16	20	8	4	48
	17500	32	20	12	-	64
	20000	40	32	8	-	80
Mixture of camphor + menthol	10000	16	20	12	-	48
	15000	28	12	20	4	64
	17500	28	24	24	-	76
	20000	48	40	-	-	88

The mixture of active ingredients was more effective than each ingredient alone with LC_{50}: 8907.73 ppm and with toxicity index 100% , while LC_{50} for camphor & menthol was 15691.57& 12958.78, respectively. These results showed through the Table (4) and Fig. (2). The obtained results were in agreement with Hussein *et al.* (2016) who proved that, the clover snail, *M. obstructa* was affected by the plant active ingredients. These results proved that, the effect of every ingredient increased when each one was added to each other. Salvador *et al*., 2014 was also proved that, mixtures of ethanolic plant extracts were more effective against food pathogen bacteria than each material alone.

2- Biochemical Experiments:

Effect of ¼ LC_{50} of the plant active ingredients on the snail biochemical activity :

2-1- The effect of the mixture on AST and ALT enzymes:

The results in Table (5) indicated that, there was highly significant difference in AST results in 1, 4 and 7 days and the control. The mixture of camphor and menthol increased ALT enzyme than control. This means that, diffusion of this enzyme from intracellular sites; this is may be due to the damage caused by the mixture on the sub- cellular level. These results were in agreement with Amer *et al.* (1994). While the results of AST enzyme were decreased than control. This decrease of the enzyme level may be due to the diffusion of this enzyme from the liver to the blood and through the kidney to outside with the urea or/ and due to the decrease in its synthetic due to liver tissue disorders. Also, these results were in agreement with Amer *et al.* (1994).

Table (4): Efficiency of some plant active ingredients against *Monacha obstructa* by contact method.

Conc. (ppm)	corrected non emergence %	LC_{50}	LC_{90}	Slope ± S.D.	toxicity index LC_{50}	LC_{90}/LC_{50}	R	P
Camphor								
10000	28	15691.57	42057.2	2.993	67.39	2.68	0.984	0.806
15000	40							
17500	56							
20000	68							
Menthol								
10000	32	12958.78	36941.85	2.82	76.80	2.85	0.949	0.447
15000	40							
17500	64							
20000	80							
Mixture of camphor + menthol								
10000	48	8907.73	30537.02	2.4	100	3.43	0.941	0.519
15000	64							
17500	76							
20000	88							

R: Regression P: Probability

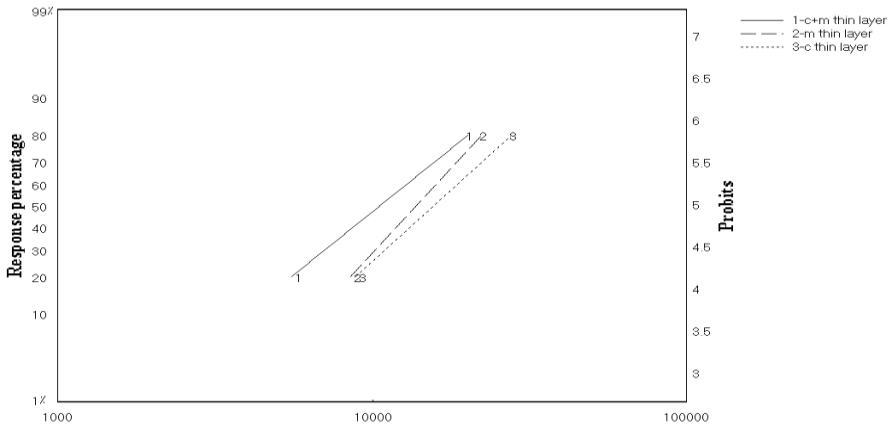


Fig. (2): LD-P lines for plant active ingredients against the clover land snail, *M. obstructa.*

2-2- The effect of the mixture on ALP and ACP:

The results in Table (6) indicated that, there was highly significant difference in ALP results in 1, 4 and 7 days and the control. There was high decrease between the obtained results and the control. Alkaline Phosphatase has critical roles on protein synthesis (Pilo *et al.*, 1972) and shell formation (Timmermans, 1969). It plays an important role in spermatogenesis (Pavlikova and Repas, 1975). In the current study, exposure of *M. obstructa* snails to both sub lethal dose of the camphor and menthol mixture showed a marked inhibition in the activity of ALP enzyme. Reduction of ALP activity may be related to the cessation of protein synthesis due to the effect of the toxin on the general metabolism of the animal (Henderson and Triebskorn, 2002). However, the result of ACP enzyme activity was decreased than control. Acid phosphatase is a lysosomal enzyme and plays an important role in catabolism, pathological necrosis, autolysis and phagocytosis (Abu-Donia, 1978). In the present work, the mixture of the compounds cause the decreases of enzyme level may be due to disease or damage in the major organ of enzymes synthesis. Similar results have been obtained by Soha and Randa (2014).

Table (5): ¼ LC_{50} effect of camphor+menthol mixture on aspartate transaminase (AST) and alanine transaminase (ALT) activities in *M. obstructa* at different periods of treatment

Days after treatment	Parameters mean of 5 snail ± SE.	
	AST (U/ml)	**ALT (U/ml)**
1	3.52 ^{c} ± 0.32	8.154 ^{a} ± 0.66
4	5.53 ^{b} ± 0.52	6.9 ^{b} ± 0.32
7	0.99 ^{d} ± 0.06	2.26 ^{d} ± 0.05
Control	12.5 ^{a} ± 0.88	4.81 ^{c} ± 0.29
LSD 0.05	**1.62**	**1.19**

Values followed by the same letter (s) in each column are not significantly different

Table (6): ¼ LC_{50} effect of camphor+menthol mixture on alkaline phosphatase and acid phosphatase activities in *M. obstructa* at different periods of treatment

Days after treatment	Parameters (mean of 5 snail ± SE)	
	Alkaline phosphatase (ALP) activity (U/L)	**Acid phosphatase (ACP) activity (U/L)**
1	132.93 ^{d} ± 2.06	2.48 ^{b} ± 0.03
4	276.06 ^{b} ± 4.4	2.38 ^{b} ± 0.06
7	165.45 ^{c} ± 2.92	2.09 ^{b} ± 0.09
Control	362.84 ^{a} ± 2.48	3.46 ^{a} ± 0.33
LSD 0.05	**9.32**	**0.52**

Values followed by the same letter (s) in each column are not significantly different

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كفاءة بعض المواد الفعالة النباتية كمبيدات للرخويات ضد قوقع البرسيم الزجاجى

منى عبد الحميد على ، غادة السيد عبد الله ، أمل السيد معروف

معهد بحوث وقاية النباتات – الدقى – الجيزة – مصر

الملخص العربى

استهدفت الدراسة معرفة مدى تأثير المواد الفعالة لبعض المستخلصات النباتية وخليط هذه المواد على قوقع البرسيم الزجاجى وهذه المواد الفعالة هى الكامفور، المنثول وخليطهما. وقد تم تطبيقهما بطريقتين مختلفتين وهما غمر أوراق التغذية فى المواد الفعالة والأخرى بطريقة الملامسة ، وقد أظهرت النتائج ان خليط المادتين له تأثير أعلى من كل مادة على حدة ، وذلك فى كلتا الطريقتين ، وتم حساب التركيز النصف مميت وكان ٣١٨٩,٤٤ ، ١٥٣١٨,٠٣ ، ٥٣١٤,٧٨ جزء فى المليون لكل من خليط المادتين، الكامفور والمنثول ، على التوالى. كما تم عمل تحليل لأربع أنواع من الانزيمات وذلك بتطبيق ربع التركيز نصف المميت لخليط المادتين لأنها الأكثر تأثيرا وقد أظهرت النتائج أن هناك تأثيرا سلبيا على انزيمات القوقع مقارنة بالكنترول.