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Research Article
Toxicological Impacts of Some Compounds on *Massylaea vermiculata* (O.F. Müller 1774), (Gastropoda: Helicidae) Under Laboratory Conditions

[1]Soheir Mohamed Abd-Elhaleim, [2]Kadry Weshahy, [2]Heba Mohamed Emam and [3,4]Reham Fathey Ali

[1]Institute of Environmental Studies and Research, Ain Shams University, El-Khalyfa El-Mamoun Street Abbasya, Cairo, Egypt
[2]Faculty of Agriculture, Ain Shams University, Shubra Al Kheimah, Al Qalyubia Governorate, Egypt
[3]Department of Zoology and Agricultural Nematology, Faculty of Agriculture, Cairo University, El-Gammaa St., 12613, Giza, Egypt
[4]Faculty of Organic Agriculture, Heliopolis university for Sustainable Development, 3 Cairo-Belbeis Desert Rd., El-Nahda, Second Alsalam, 11785, P.O. Box, 3020 Cairo, Egypt

Abstract

Background and Objective: Effect of some compounds such as Plant extracts (Techno oil and Berna Star), natural origin compounds (Top-nine, Repcar and Chitosan 5%) and classical chemical pesticides (methomyl and lambda-cyhalothrin) were studied against the terrestrial snail *Massylaea vertmiculata* using the bait technique. **Material and Methods:** LC_{50} of the each tested compound of natural compounds were estimated after 14 days of treatment, while LC_{50} of pesticide were evaluated after 72 hrs of treatment. The impact of LC_{50} of each tested compound on some biochemical parameters, total protein content, alkaline phosphatase (ALP) and acid phosphatase (ACP) activity were determined 48 hrs post treatment. **Results:** The results revealed that the methomyl and lambda-cyhalothrin were the most effective compounds against test land snails, followed by Repcar, Top-nine and Techno oil, while Berna Star and Nema Ultra Chem come in the last rank. The pesticide compound methomyl was the most toxic one against the tested terrestrial snail species, while the Chitosan 5% was the least toxic one. The results showed that all tested compounds caused fluctuated effect whether increasing or decreasing on all the studies parameters such as total protein content, ALP and ACP activity as well. However, the Techno oil and the Berna Star caused sever decreasing on total protein content and ACP, followed by Top-nine, Repcar, Chitosan 5% (Nema Ultra Chem) and plant extracts. **Conclusion:** The both tested natural compounds and plant extracts recorded satisfying results compared with methomyl and lambda-cyhalothrin effect and that can be used in the pest controlling programs against terrestrial snails to reduce the environmental pollution.

Key words: Terrestrial snail, *Massylaea vermiculata*, control, techno oil, berna star, top-nine, repcar, chitosan 5% (nema ultra chem), methomyl, lambada-cyhalothrin, enzymes

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Corresponding Author: Soheir Mohamed Abd-Elhaleim, Institute of Environmental Studies and Research, Ain Shams University, El-Khalyfa El-Mamoun Street Abbasya, Cairo, Egypt

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Terrestrial snails were considered as one of the most serious and dangers agricultural pests to different varieties of commercial crops, fruits, vegetables and ornamental plants causing heavy damages to the plant leaves, fruits, tubers, buds and roots. The terrestrial snails occurred in high population number in Delta region and North Coast belt of Mediterranean Sea of Egypt[1-6]. The species *Massylaea vermiculata* has been reported at different governorates in Egypt such as El Sharkia[7,8], Beheira and Alexandria governorates[9], Sohag governorate[10], Gharbia, Dakahlia, Damietta, Ismailia governorate[4], Qalyubia, Monufia, Cairo and Giza governorate[5,6]. Certain pesticides were investigated as poisonous baits against this species, where this technique is the common chemical control method[11]. Many of previous studies focused on evaluating different insecticides and biocides against terrestrial snails whether under laboratory or field conditions to expose the suitable molluscicide for controlling these pests[12,13,14]. The species *Massylaea vermiculata* are previously known as *Eobania vermiculata* and re-identified to genus *Massylaea*[15]. The carbamate compounds such as methomyl and other substances i.e., urea and copper hydroxide seemed to have a toxicity on the terrestrial snail species *Massylaea vermiculata* under laboratory conditions[16] and field environments[17]. Among the carbamates compounds, methomyl had the highest molluscicidal activity[18-20]. Indoxacarb is a widely used and new insecticide that belongs to the oxadiazine pesticide group, which acts on target organism as a sodium channel blocker[21]. Lannate was the most toxic molluscicide against *Monacha cartusiana* and the highest mortality percentages followed by Ginger, Ice plant and Biozed, respectively, while Biogard caused the lowest lethal effect under laboratory conditions[21]. Though some chemical compounds had been proved molluscicidal effectiveness in the snails' mortality. However, the use of these chemicals is not recommended due to its harmful effect of environmental and chemical pollution. Therefore, more efforts should be made to control the pest through the use of natural products derived from plant origin[22]. The main objective of the current research was to study the toxic effect of certain naturally occurring compounds on some biochemical parameters of the terrestrial snail species *Massylaea vermiculata*.

MATERIAL AND METHODS

Experimental animals: The adult of *Massylaea vermiculata* species were collected at activity season during February and March, 2020 from the infested crop fields at Qalyubia governorate and then transferred the samples to the toxicity unit of Plant Protection department Ain Shams University for implementing the experiment. The snails were kept in a glass terrarium with dimensions (30 width×48 length×34.5 cm height) containing moist clay and sand soil by 1:1 of about 8-12 cm soil height. The soil was moistened with tap water twice a day in the summer and one every day in the winter to keep the humidity level reach to 80±5%, which is almost its optimal levels. The soil moisture level was measured by using light and moisture meter. Eggshells powder as a source of calcium were added to the soil surface and fresh leaves of lettuce were introduced daily as sources of food for the snails. The terrarium was covered with white muslin clothes secured with rubber band to prevent snails from escaping[23,24]. The walls of glass terrarium were cleaned each two weeks to remove the snail feces and prevent reproducing any other small insects. The land snails were kept in their glass terrarium for 2 weeks for adaption till to implement the experiment. The toxicological experiments and studying the impacts of the tested compounds and pesticides spent 2 months starting from April, till May, 2020 to implement and complete the experiments successfully, furthermore obtain the final results.

Tested compounds

Baiting method: This current experiment had tested two plant extracts, three natural compounds and two chemical pesticides against the adults of terrestrial snail *Massylaea vermiculata*, whereas all of the tested compounds were obtained commercially and had been purchased from a reliable source. Table 1 showed the tested pesticides whether naturally origin pesticides or synthetic formulations (common name, trade name, the producer and the treatment dose) that had been applied against this species in this study. Five concentrations of each tested compounds were used as a poison bait method and each concentration has four replicates, each replicate has five adults of *Massylaea vermiculata*. The poisonous baits of each compound prepared by mixing the five tested concentrations of each compounds with bran to complete 100 parts of poisonous baits with adding adequate water. About 25 g of poisonous baits were spread in to plastic boxes and five adult snails of *M. vermiculata* were used for control treatment that prepared by the same method without treatment. The tested boxes were covered with muslin clothes and closed with rubber band to prevent snails from escaping. The boxes were examined after 1, 3, 7, 14 and 21 days as post treatment. The dead snails were counted and removed from boxes calculating the Mortality percentages and corrected using Abbott's formula[25] as follows:

Table 1: Tested pesticides (naturally origin pesticides and synthetic formulations) that applied against terrestrial snail *Massylaea vermiculata*

Groups	Common name	Trade name	Producer	Dose of treatment
Plant extracts (naturally origin pesticides)	Techno oil	-	Star Chem. Company for Chemical Manufacturing, Plot 35, Sixth Industrial Zone, 6th of October City, Egypt	60-100 cm^3 L^{-1}
	Berna Star	Estate EL-gelisriin (32%)		100-150 cm^3 100 L^{-1}
Natural compounds (Naturally origin pesticides)	Top-nine	-	Star Chem. Company for Chemical Manufacturing, Plot 35, Sixth Industrial Zone, 6th of October City, Egypt	750 cm^3 L
	Repcar	-		350 mL
	Chitosan 5%	Nema Ultra Chem	Canadian Company for Humate Technologies and Agricultural Consulting, Egypt	2-3 L
Synthetic formulations	Methomyl	GOLDBEN (90% SP)	Star Chem. Company for Chemical Manufacturing, Plot 35, Sixth Industrial Zone, 6th of October City, Egypt	300 g
	Lambada-cyhalothrin	Lambada- cyhalothrin (5% EC)		375 mL

$$\text{Corrected mortality (\%)} = \frac{\text{Number of alive in Treatment after treatment}}{\text{Number of alive in untreated after treatment}} \times 100$$

LC_{50} and LC_{90} of natural compounds were determined after 14 days of treatment, while chemical compounds were determined after 72 hrs post treatment according to LdP line^R software[26]. Toxicity index of the tested compounds was determined according to the equation of LdP line^R software as well[26].

Biochemical studies: Some biochemical parameters were studied to clarify the toxicity response of the snail *M. vermiculata* to the tested compounds on total protein content, Acid phosphatase and Alkaline phosphatase activity after 48 hrs of the treatment. The samples were prepared[27] with LC_{50} of each compound for 48 hrs of treatment.

Determination of total protein content: Colorimetric method of soluble protein was done according to the method of Tietz, 1994[28] by using Biuret reagent. Using wavelength was measured at 540 nm spectrophotometric.

Determination of acid phosphatase activities (ACP): Acid phosphatase activity was measured using sodium phenyl phosphate as a substrate by the described method[29]. The wavelength was measured at 510 nm spectrophotometric.

Determination of alkaline phosphatase (ALP): Alkaline phosphatase (ALK) was determined and evaluated as the method described[30]. The wavelength was measured at 405 nm spectrophotometric.

Statistical analysis: The data of total protein and enzymes activity were statistically analyzed with (SAS) program[31]. On the other hand, the toxicity data were analyzed using LdP line^R software[26]. The level of significant of the means of the reveled data at the 0.05% level, while the highly significant where at 0.01 level.

RESULTS AND DISCUSSION

Toxicity of tested compounds against the adult stages of *Massylaea vermiculata*: Data in Table 2 showed the susceptibility of the land snail *Massylaea vermiculata* to the tested compounds. Methomyl was the most effective compound with Toxicity Index (T1) 100% followed by Lambda power, Repcar, Top-nine, Techno oil, Berna Star and Nema Ultra Chem with LC_{50} (1.9, 4.20, 11.1, 16.1, 17.3, 28.5 and 44.4%), respectively. Regarding the slope value of the tested compounds that registered different degree of steep where as Goldben recorded the lowest one, while Techno oil was the highest slope value recorded. These results mean that synthetic compounds were more toxic than Natural compounds or plant extract. The deltamethrin exceeded the other pesticides (chlorpyrifos-ethyl and methomyl) and revealed high initial toxicity of 70.0% and 93.3% against *Monacha cantiana* and *M. vermiculata*, respectively, after 3 days of exposure[32]. However, methomyl, belonging to carbamate group, surpassed chlorpyrifos-ethyl and gave 100% mortality after seven and 12 days for the two land snails, *Monacha cantiana* and *M. vermiculata*, respectively. On the other hand, chlorpyrifos-ethyl recorded the lowest mortality percentages against the tested land snails. Methomyl was the effective pesticides followed, in order, by deltamethrin, bensultap, lambda-cyhalothrin, diazonixy then chlorpyrifos-ethyl against the land snails *M. vermiculata* and *Monacha cantiana*. The species *M. vermiculata* exposed more sensitivity to the all tested pesticides remarkably such as methomyl and deltamethrin more than the other land snail species *Monacha cantiana*[32].

The terrestrial snail *Theba pisana* presented daily mortality against the insecticides cypermethrin, imidacloprid,

Table 2: Toxicity of the tested pesticides (naturally origin pesticides and synthetic formulations) against the adult terrestrial snail of *Massylaea vermiculata*

Type of compound	Tested compounds	LC_{50}	LC_{90}	Slope	Toxicity index
Plant extracts	Techno oil	17.30	59.90	26.06	10.98
	Berna star	28.50	59.90	17.61	6.70
Natural compounds	Repcar	11.10	34.90	171.81	17.10
	Top-nine	16.10	47.90	23.19	11.80
	Nema ultra chem	44.40	49.90	12.17	4.30
Synthetic formulations	Goldben	1.90	5.90	-28.39	100
	Lambda power	4.20	14.90	7.53	45.20
	Control	0.0	0.0	0.0	0.0

Table 3: Effect of LC_{50} of tested plant extracts on biochemical parameters of *Massylaea vermiculata* after 48 hrs of treatment

	Total protein content (g dL^{-1})			Alkaline phosphatase activity (µ L^{-1})			Acid phosphatase activity (µ L^{-1})		
	Control	Treatment		Control	Treatment		Control	Treatment	
Plant extracts	Mean±SE	Mean±SE	Diff. (%)	Mean±SE	Mean±SE	Diff. (%)	Mean±SE	Mean±SE	Diff. (%)
Techno oil	1.8 ± 0.8^{AB}	1.7 ± 0.4^{A}	-5.5	2727 ± 144.3^{AB}	2914.9 ± 152.5^{A}	6.9	49.5 ± 7.7^{AB}	37.0 ± 2.5^{B}	-25.3
Berna star		0.7 ± 0.1^{B}	-61.1		2548.2 ± 26.2^{B}	-6.6		65.8 ± 5.8^{A}	32.9
F-value	8.6**			27.3**			33.4**		
LSD	0.8			153.3			11.0		

Means with the same letter within each column were not significantly different from another at the 0.05% level by Duncan's multiple tests[43], SE: Standard error, **Highly significant at 0.01 level

methomyl and abamectin. The daily of mortality percentage averaged around 15.00 and 48.33% for imidacloprid, which is the less effective insecticide, 19.16 and 60.00% for cypermethrin, 22.50 and 68.33% for abamectin, as well as 22.50 and 70.00% for methomyl as the most effective insecticide[33]. The (LT_{50}) Half lethal time values were 2.58, 2.92, 4.21 and 5.0 days for the insecticide methomyl, abamectin, cypermethrin and imidacloprid, individually[33].

The averaged mortality percentages against the terrestrial snail *Monacha* sp. were 31.6% by Sulphonic acid, 36.6% by Ferrous sulphate, 72.5% by Potassium hydroxide and 87.5% by Methomyl, which was the most toxic insecticide[34].

The ethanol crude extract of Cumin *Cuminum* sp. had been recorded as the most toxic extract against the terrestrial snails *Monacha obstructa* and *M. vermiculata*, which applied as contact method.

The other plants extract such as Golden shower *Cassia* sp., Umbrella tree *Schefflera* sp., pomegranate *Punica granatum* extracts and Olive *Olea europaea* extract were the lowest effect in the same previous order[35].

M. vermiculata showed more resistance against the tested plant extracts than the land snail, *Monacha obstructa.* The LC_{50} values were 250 ppm of Cumin, 325 ppm of Golden shower, 635 ppm of Umbrella tree, 1500 ppm Olive and 910 ppm of Pomegranate extracts against *Monacha obstructa*, while the LC_{50} values were 288 ppm of Cumin, 380 ppm of Golden shower, 682 ppm of Umbrella tree, 1720ppm Olive and 965 ppm of Pomegranate extracts against the terrestrial snail *M. vermiculata*[35].

Ferrous sulphate was the most effective one against *Monacha cartusiana* followed by spicy and pepper, neem, black pepper, oshar fresh leaves, crude extracts and oshar leaves' powder extract, where mortality percentage of effected snails were 100, 70, 50, 45, 45 and 10%, respectively at highest concentration 7 days' post-treatment[36]. While, the Neomyl, natural compounds, was the most effective compound followed by Thymol and Kafrothrin then round up, which had the lowest affect against the adult snails of *M. cartusiana* with LC_{50} 0.79, 1.78, 3.62 and 0.49 ppm, respectively[37].

Biochemical impacts on *Massylaea vermiculata*: Effects of the concentration at the level of LC_{50} of tested compounds were investigated on the biochemical parameters of *Massylaea vermiculata* such as total protein content, Alkaline and Acid phosphatase activity and all of these parameters were determined after 48 hrs of treatment.

Effect of plant extract on some biochemical parameters in *Massylaea vermiculata*: Data in Table 3 and Fig. 1 indicated effect of the test plants and naturally origin pesticides on some biochemical parameters of *M. vermiculata* after 48 hrs of treatment. The results showed that treatment with Techno oil has no significant effect on total protein content, while, it increase ALP activity with difference percentage 6.9% (according to control). The adverse effect was observed in case of ACP whereas the Techno oil caused significant decreasing from in treatment with -25.3 difference

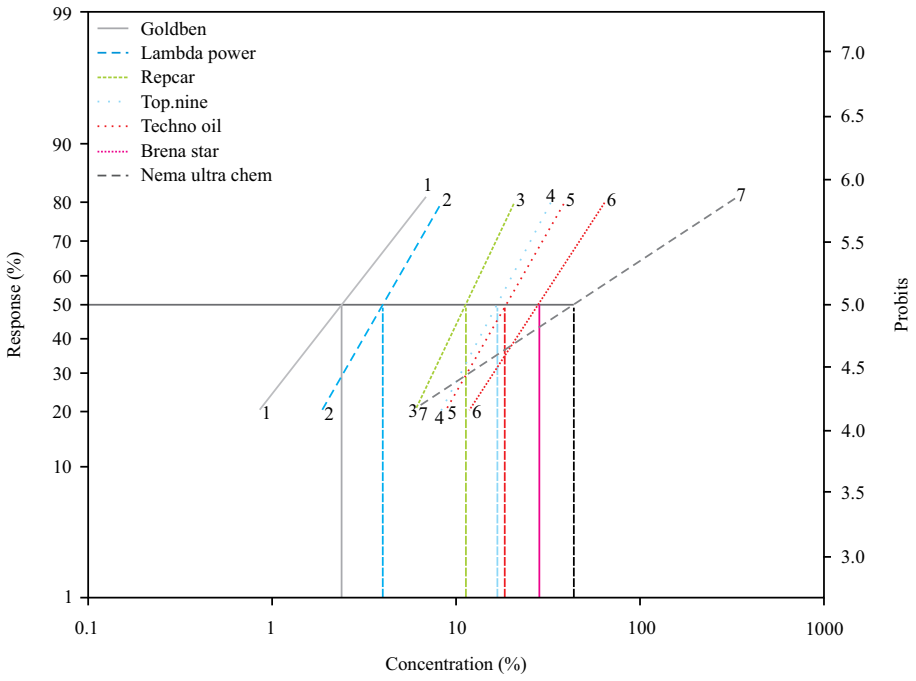

Fig. 1: Toxicity lines of the tested pesticides (naturally origin pesticides and synthetic formulations) under this study against the terrestrial snail *Massylaea vermiculata*

percentages (comparing with control). Regarding Berna Star, it decreased the total protein content with -61.1 difference percent (comparing with control). The same result occurred in ALP whereas it decreased from 2727.0 μ L^{-1} in control to 2548.2 μ L^{-1} in treatment with -6.6 difference percent, while it enhanced the activity of ACP from 49.5- 65.8 μ L^{-1} in treatment. The previous results, it proved that tested plant extracts can make fluctuation effect on the tested parameters and may be this could cause the death.

The least level of total protein was recorded among animals treated with 10% Oleander or Nerium plant *Nerium oleander* L. (Apocynaceae) as plant extract (6.30 mg mL^{-1}), while level of carbohydrate was the least by Yellow Ironbark plant *Eucalyptus melliodora* A. Cunn. Ex Schauer (Myrtaceae) as plant extract treatment at 10% concentration (1.41 mg mL^{-1})[38].

On the other hand, the highest reduction of total lipids and glucose levels were recorded after treatment by Neem tree *Azadirachta indica* A. Juss. (Meliaceae) as plant extract at 10% concentration. Also, the highest activity of peroxidase and catalase enzymes after *Eucalyptus melliodora* plant extract 20% treatment and the least activity for the same two enzymes occurred by *Nerium oleander* treatment. In addition, the highest reduction of (ALP) activity was registered after treatments by extracts of oleander *Nerium oleander* and Neem tree *Azadirachta indica* extracts.

Data in Table 4 and Fig. 1 showed effect of the tested natural compounds on some bio chemical parameters of *M. vermiculata* after 48 hrs of treatment. The results revealed that Top-nine as Natural Compound caused significant increasing with recorded to 38.8% in total protein content comparing with control and the same results occurred with ALP and ACP, whereas, it caused significant increasing in the activity of enzymes with difference percent 5.3 and 4.2%, respectively comparing with control. The opposite effect happened in Nema Ultra Chem, which caused significant decrease in the total protein content, ALP and ACP activity, where, it decreases with difference percent -47.2, -4.0 and -6.7%, respectively comparing with control.

Regarding to Repcare, it caused a fluctuated effect on the some parameters that it caused significant increase in total protein that enhanced from 18 g dL^{-1} in control to 3.1 g dL^{-1} in treatment, however it decreased the activity of ALP enzyme from 2727.0 μ L^{-1} in control to 2581.6 μ L^{-1} in treatment, while, it observed that the Repcar compound caused enhancing in activity of ACP enzyme with 33.9% comparing with control. From the previous results, it had been observed that Top-nine and Nema Ultra Chem were the most effective compounds comparing with Repcar whereas the first compound caused high level of increasing and the second one caused high level of decreasing in all test parameters.

Table 4: Effect of LC_{50} of the tested natural compounds on some biochemical parameters of *Massylaea vermiculata* after 48 hrs of treatment

	Total protein content (g dL^{-1})			Alkaline phosphatase activity (µ L^{-1})			Acid phosphatase activity (µ L^{-1})		
	Control	Treatment		Control	Treatment		Control	Treatment	
Natural Compounds	Mean±SE	Mean±SE	Diff. (%)	Mean±SE	Mean±SE	Diff. (%)	Mean±SE	Mean±SE	Diff. (%)
Top-nine	1.8 ± 0.8^{AB}	2.5 ± 0.9^{AB}	38.8	2727 ± 144.3^{AB}	2872.4 ± 83.3^{A}	5.3	49.5 ± 7.7^{AB}	51.6 ± 6.5^{AB}	4.2
Nema ultra chem		0.95 ± 0.6^{B}	-47.2		2617.9 ± 54.5^{B}	-4.0		46.2 ± 6.9^{B}	-6.7
Repcar		3.1 ± 0.7^{A}	72.2		2581.6 ± 83.3^{B}	-5.3		66.3 ± 7.98^{A}	33.9
F-value	4.8**			5.4**			4.5**		
LSD	1.9			253.8			19.1		

Means with the same letter within each column were not significantly different from another at the 0.05% level by Duncan's multiple tests[43], SE: Standard error, **Highly significant at 0.01 level

Table 5: Effect of LC_{50} of tested Synthetic formulations pesticides on some biochemical parameters of *Massylaea vermiculata* after 48 hrs of treatment

	Total protein content (g dL^{-1})			Alkaline phosphatase activity (µ L^{-1})			Acid phosphatase activity (µ L^{-1})		
Synthetic formulations	Control	Treatment		Control	Treatment		Control	Treatment	
Pesticides	Mean±SE	Mean±SE	Diff. (%)	Mean±SE	Mean±SE	Diff. (%)	Mean±SE	Mean±SE	Diff. (%)
Goldben 90% sp.	1.8 ± 0.8^{B}	7.5 ± 2.1^{A}	316.7	2727.0 ± 144.3^{B}	3654.2 ± 144.3^{A}	34.0	49.5 ± 7.7^{A}	0.2 ± 13.7^{B}	-99.6
Lambda power 5%		10.7 ± 1.6^{A}	494.4		2563.4 ± 237.7^{B}	-6		-33.2 ± 10.7^{C}	-167.1
F-value	25.6**			31.7**			42.9**		
LSD	3.9			453.2			27.6		

Means with the same letter within each column were not significantly different from another at the 0.05% level by Duncan's multiple tests[43], SE: Standard error, **Highly significant at 0.01 level

The activity of (ALT) recorded as reduction in the both land snails *Helicella vestalis* and *Theba pisana* except at the third of treatment with the insecticide Agrinate, while the biological insecticide Biomagic improved ALT activity at the third and seventh days at the post-treatment[39], however the reduction is gradually increased at the end of 2 weeks of the test duration. The same author recorded that the levels of Total Lipids (TL) and Total Proteins (TP) become lower due to the application of Agrinate or Biomagic. It becomes essential to evaluate the reactions of enzymes, proteins and lipids within the land snails' tissues against the molluscicidal applications[39].

The treatment with Sub-lethal doses of fenchone caused noticeable decrease in protein level of the two snail species. Aspartate transaminase (AST) and Alanine transaminase (ALT) activities were stimulated by the most tested doses of fenchone, responses of ALT toward fenchone treatments were more sensitive than AST. The two tested enzymes of *M. vermiculata* were more responsive than those of *Theba pisana* to fenchone treatments[40].

Data in the Table 5 and Fig. 1 indicated that the effect of LC_{50} of tested pesticides on biochemical parameters of *M. vermiculata* after 48 hrs of treatment.

The results indicated that Goldben 90% (Sp) compound caused significant increased on total protein content and ALP activity comparing with control with 316.7 and 34.0% difference, individually, while it affected significant decreased on ACP with -99.6% differences comparing with control treatment.

Lambda power 5%, cased significant increasing on total protein with 494.4% difference with control, while, it caused decreasing on ALP and ACP with -6 and -167.1% difference with control, consecutively. This means that two tested compounds had a strong effect on the tested parameters.

The effect of LC_{50} level of acetic acid had been evaluated on some biochemical parameters of the both land snails *M. vermiculata* and *Monacha obstructa* that showed increasing of total protein activities compared with control treatment[41].

The acetic acid presented a decrease in the total lipid level (4.96-2.78 g dL^{-1}) for *M. vermiculata,* while it presented an increasing level (2.89-3.22 g dL^{-1}) for *Monacha obstructa*[41].

Also, level of alkaline and acid phosphatase in both land snail species was recorded at LC_{50} level[41]. Methomyl, Lambda-cyhalothrin and biopesticide (Spinosad) had a potent affection total protein on *M. vermiculata*[42].

Methomyl 90%, Lambda-cyhalothrin10% Spinetoram 12% and emamectin benzoate (0.5, 1.92% EC) were augment the Alkaline phosphatase activity against the both field and laboratory individuals rearing of *M. vermiculata* and *Monacha obstructa*. While, all the tested compounds increased the Acid phosphatase activity when used against both laboratory individuals rearing and field. However,

Methomyl 90% and Lambda-cyhalothrin reduced the Acid phosphatase activity when used against collecting field samples of *M. obstructa* after 48 hrs and augment after 72 hrs, while Lambda-cyhalothrin10% reduced the Acid phosphatase activity when used against laboratory individuals rearing of *M. obstructa* after 48 hrs and augment after 72 hrs.

CONCLUSION

The results concluded that the tested chemicals had the most effective followed by the natural compounds and the plant extracts were ranked in the last level regarding to the three tested parameters. These results may be occurred due to the pesticides compounds and plant extracts having chronic and slow effects. On the other side, the determination of the activities of ALP and ACP with the previous tested compounds may be cause depletion of enzymes activities.

Finally, although the natural compounds and plant extracts were less toxic than the pesticide compound, the results are satisfied and can be listed in land snail control compounds and programs due to their environmentally friendly effect.

SIGNIFICANCE STATEMENT

Studying the toxic effect of Plant extracts, natural origin compounds and classical chemical pesticides are essential purpose to determine the effect on some biochemical parameters of the terrestrial snail species *Massylaea vermiculata.* These tested compounds could be used as part of successful controlling program to reduce land snail species numbers in agricultural fields or nurseries and eliminate them effectively.

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