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Some laboratory studies on the efficacy of some plant extracts on biochemical and biological aspects of *Monacha cartusiana* land snail (Stylommatophora: Helicidae)

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ABSTRACT

Three plant extracts of *Nerium oleander, Eucalyplus melliodora* and *Azadirachta indica* at 10 and 20% concentrations were tested against adult individuals of *Monacha cartusiana* land snail under laboratory conditions to show their toxicity effects on the activity levels of GOT, GPT, AchE, GST, Alpha Estrase, Beta Esterase and phenolase enzymes. The results indicated that *N. oleander* extract had the highest effect on six enzymes expect AchE which was more affected with *E. melliodora* extract. Different five concentrations of *N. oleander* leaves extract were used against adult stage of the tested animal, mortality percentages and LC_{25}, LC_{50} and LC_{90} were recorded. Based on LC_{50} of the tested extract caused high effectness on some aspects of *M. cartusiana* life cycle (number of eggs, oviposition inhibition, depth of eggs, weight of eggs, incubation period and hatchability percentage) under laboratory conditions.

Keywords: Terrestrial snails, *Monacha cartusiana*, plant extracts, biochemical, biological aspects.

Introduction

Land molluscs are belonging to phylum: Mollusca. This phylum is one of the largest phyla in the animal kingdom. Moreover these animals are very successful group that have invaded most of environments and have a wide range of feeding habits (Barker, 2002). Many of them are play an important role in the general economy of man. They may be beneficial to man or harmful, causing a lot of problems to different cultivated plants (Speiser and Kistler, 2002). In some localities of Egypt, land snails have been increased as agricultural pests, where they attack numerous parts of plants feeding on it (Al-Akraa and Mohammed, 2015). Furthermore these animals leave mucus and faces on the invaded plants which leading to appearance and spreading some plant diseases (Iglesia, *et al.,* 2003). On the other hands, some of them are known as intermediate hosts for some parasites (Barker, 2002).

Some plant extracts are used as useful bioactive compounds recently (Abdel-Khalek, *et al.,* 2010 and El-Khayat, *et al*., 2014). Because that the aim of these laboratory experiments is determine some biochemical and life cycle aspects changes of *M. cartusiana* land snail treated by some plant extracts.

Materials and Methods

Tested animals:

The individuals of *Monacha cartusiana* land snail were collected from different plants and herbs of the fields, in Moshtohor village at Kalubia Governorate, and then transported to the laboratory. Adults of healthy snails were chosen and put in plastic cags with 65-70% moistened soil, under laboratory conditions (25°C ± 2 and 70 ± 5% R.H), for 15 days, the snails were feeding daily on fresh leaves of lettuce.

Plant material and extraction:

Leaves parts of *Eucalyplus melliodora* and *Nerium oleander* and fruits parts of *Azadirachta indica* were choiced and cut into very small pieces, then placed inside the oven for dry at 50°C. The parts of tested plants were floured into the miller to make plant powder. Powder of different plants were weighed and soaked in ethylacohol, hexan aceton and water. The extracts of the different plants

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were filtered and evaporated at 50°C by vacuum rotary evaporator separately. The extracts were saved in refrigerator at 18°C until used, (Su and Horvat, 1981).

Biochemical methods:

10 and 20% concentrations of plant extracts were prepared to biochemical experiments against *M. cartusiana* land snail. Snails were fed on fresh leaves of *Lactuca sativa* treated with the tow concentrations of plant extracts beside the control. The confiding snails after 1, 3, 5 and 7 days were collected for biochemical measurements. The tissues of surviving snails were dissected and homogenized in 0.1m phosphate buffer PH 7.4 using a polytron homogenizer directly. The homogenates were centrifuged at 5000 rpm for 20 min at 4°C (Bergmenyer, 1963). Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were determined colorimetrically according to the method of (Reitman and Frankle, 1957). While AchE (acetylcholine esterase) activity was measured according to the method described by Simpson *et al.,* (1964), using acetylcholine bromide (AchBr) as substrate. As well as Glutothione S-transferase (GST) was determined by (Habig *et al.,* 1974). On the other hand Alpha esterases (a-esterases) and Beta esterases (β-esterases) were determined according to(Van Asperen , 1962) using a-naphyl acetate and B-naphthyl acetate as substrates, respectively.And Phenoloxidase activity was determined by (Ishaaya, 1971).

Effect of *N. oleander* extract on the land snail *M. cartusiana*:

Five concentrations of *N. oleander* extract 2.5, 5, 5.5, 10 and 10.5% were prepared and used against healthy adults of *M. cartusiana*. Animals were fed on fresh leaves of *Lactuca sativa* sprayed with different concentrations of plant extract. Three replicates beside the control were used for each concentration; each replicate consisted of five individuals. The mortality percentages were counted according to (Abbott, 1925) after 1, 2, 3, 4, 5 and 6 days. The lethal concentration (LC_{25}, LC_{50} and LC_{90}) values were calculated by probit analysis in the end of experiment.

$$\text{Mortality \%} = (a - b / a) \times 100$$

Where: -

a = The total initial numbers of animals.

b = Mean numbers of animals still alive.

The effect of LC_{50} on some aspects of *M. cartusiana* life cycle was stated by using six replicates of a live adult snails treated with LC_{50} of *N. oleander* leaves extract. Each replicate was consisted of a pair of animals for mating. Number of lied eggs, oviposition inhibition, depth of lied eggs, weight of 10 eggs, incubation period and hatchability percentage were determined when the experiment was finished comparative with control. The oviposition inhibition and hatchability percentages were calculated as following.

$$\textit{Oviposition inhibition \%} = \frac{\textit{No. of eggs in control- No. of eggs in treatment}}{\textit{No. of eggs in control}} \times 100$$

$$\textit{Hatchability \%} = \frac{\textit{No. of hatching eggs}}{\textit{The total No. of lied eggs}} \times 100$$

Results and Discussion

As shown in tables (1, 2, 3 and 4) results indicated that plant extracts were very effective on the activities of enzymes and some life cycle aspects of *Monacha cartusiana* land snail.

Toxicity of some plant extracts against adult of *Monacha cartusiana*:

Data in table (1) showed the effect of three plant extracts *N. oleander, E. melliodora* and *A. indica* on the activity of GOT, GPT, GST and AchE enzymes in *M. cartusiana* snail. The tabulated data indicated that *A. indica* was the highest effect on GOT activity where increased from 56.00 in control to 132.17 *M/L* comparison with *E. melliodoroa* with mean of the two tested concentrations 109.83 *M/L*, while *N. oleander* extract gave the lowest effect with 87.00 *M/L*. It is clear also that 20% concentration of all tested plants was the most impression on GOT activity with 155.33, 183.33 and

138.67 *M/L* for *N. oleander, E. melliodora* and *A. indica* respectively. On the other side GPT enzyme activity was decreased to 7.28, 8.28 and 11.02 *M/L* when the snail treated with *N. oleander, E. melliodora* and *A. indica* extracts respectively and 10% concentration was the most effectiveness on lowering of enzyme level with 3.17, 5.43 and 9.17 *M/L* for all tested plants respectively. While data observed that the activity of GST enzyme had little decreasing by three plant extracts with means 2.04, 3.18 and 2.50 *M/L* regularity.

But GST level had little increasing with 4.24 *M/L* under 20% concentration of *E. melliodora* extract and decreased to 1.20 *M/L* with 10% concentration of *N. oleander.* The results in the same table cleared that the highest level of AchE 1788 nol/min/mg was recorded when the animal treated with *A. indica* extract comparison with control 864 nol/min/mg, while the lowest level was recorded for *E. melliodora* where the level decreased to 278 nol/min/mg, in addition, 20% concentration of all tested plants was the most effect on this enzyme level especially 20% *A. indica* where the level increased to 2176 nol/min/mg.

Generally, 10% concentration of *N. oleander* extract had the most effect on GOT, GPT and GST *M. cartusiana* enzymes where the activities of them decreased to the lowest levels comparison with control followed by 10% concentration of *E. melliodora* extract. While *E. melliodora* had the most effect on AchE enzyme especially when the snail treated with 10% concentration of this extract, (Mohamed and Elshwey, 2016) indicated that *N.oleander* extract had the most effect on total protein of *E. vermiculata* land snail.

Data in table (2) can showed that, *M. cartusiana* enzymes as Alpha and Beta esterase were more affected with *N.oleander* extract 469 and 193 ug/min/ml comparison with control respectively. And 10% concentration of this plant extract was the most effective on these enzymes when the activity was decreasing more and more recording 293 and 152 ug/min/ml for two enzymes regularity. Besides that the activity of phenolse enzyme had a little decreasing when *M.cartusiana* treated with *N. oleander* and *A. indica* extracts with means 752 and 775 O.D/min/ml. On the other hand phenolse was increased from 785 O.D/min/ml in control case to 1170 O.D/min/ml by *E. melliodora* extract. In addition the research can clear that 20% concentration of *N. oleander* and *E.melliodora* extracts due to increase the level of phenolase enzyme activity reaching to 1048 and 1897 O.D/min/ml respectively, when compared with concentration 10% of the same two plant extracts.

Generally results can investigate that 10% concentration of *N. oleander* and *E. melliodora* was caused inhabiting the levels of Alpha esterase, Beta esterase and phenolase activities.

Effect of *N. oleander* leaves extract on mortality and life cycle of *M. cartusiana*:

Data in tables (3 and 4) showed the effect of *N. oleander* leaves extract on the percent of mortality and some aspects of life cycle for *M. cartusiana*.

As shown in table (3) results indicated that the mortality percentage after days was 20% for 10 and 10.5 concentrations in the first day, while this percent increased to 86.7 and 93.3% with the end of experiment. On the other hand the lowest effeteness on the animal mortality recorded by 2.5 concentration when the percent was 6.7% in third day and reached to 20% with the end of days. LC_{25}, LC_{50} and LC_{90} values for the tested plant extract were 2.466, 3.881 and 9.186% respectively.

Results in table (4) showed that, the lethal concentration LC_{50} of *N. oleander* leaves extract decreased the number of lied eggs by treated snails with mean 30.3 eggs comparative with 104.8 eggs for untreated snails, and the ovipositor inhibition percentage recorded 71.1%. On the other hand the depth of lied eggs shorted to 0.53 cm compared with control 2.18 cm under the soil. The mean weights of eggs were 0.021 and 0.073 gm. for 10 eggs for *N. oleander* extract and control respectively. But the incubation period was increased recorded 22.3 days for *N. oleander* when was 11.4 days only for control. In the end the percent of hatchability were 17.2 and 83.4% for treated and untreated cases respectively.

Bruna *et al.* (2013) showed that the effeteness of *Bidens pilosa* extract on some life cycle aspects of *Subulina octona* land snail, at LC_{50} and LC_{90} of plant extract was affected on reduction snail's growth, hatchability and fecundity. The same outhers (2014) studied the effect of *Mikania glomerata* plant extract on the same species of land snails, and showed that, this plant extract was very effective on the snails' fecundity, growth of different stages, hatchability and the offspring produced after exposure. Amal (2017) studied the efficiency of *Azadirachta indica* (neem) leaves

Table 1: Effect of some plant extracts on the activities of GOT, GPT, GST and AchE enzymes of *M. cartusiana* land snail, under laboratory conditions.

Plant extract	(Daflaa) *N.oleander*			(Cafour) *E.melliodora*			(Neem) *A.indica*			Control	L.S.D. 0.05
Enzymes	**10%**	**20%**	**Mean**	**10%**	**20%**	**Mean**	**10%**	**20%**	**Mean**		
GOT	18.67 ± 1.86[a]	155.33 ± 7.54[e]	87.00 ± 30.76	36.33 ± 1.86[b]	183.33 ± 8.82[f]	109.83 ± 33.12	125.67 ± 3.76[d]	138.67 ± 1.45[d]	132.17 ± 3.42	56.00 ± 4.93[e]	15.47
GPT	3.17 ± 0.27[a]	11.40 ± 0.32[d]	7.28 ± 1.85	5.43 ± 0.34[b]	11.13 ± 0.18[d]	8.28 ± 1.29	9.17 ± 0.44[c]	12.87 ± 0.67[e]	11.02 ± 0.90	11.53 ± 0.65[de]	1.35
AchE	903 ± 11.85[b]	920 ± 22.88[b]	912 ± 12.18	258 ± 7.36[a]	298 ± 6.01[a]	278 ± 9.9	1401 ± 35.51[e]	2176 ± 95.27[d]	1788 ± 179.09	864 ± 15.95[b]	122.12
GST	1.20 ± 0.06[a]	2.89 ± 0.11[c]	2.04 ± 0.38	2.11 ± 0.14[b]	4.24 ± 0.20[d]	3.18 ± 0.49	2.50 ± 0.12[bc]	2.51 ± 0.10[bc]	2.5 ± 0.07	3.96 ± 0.14[d]	0.39

Table 2: Effect of some plant extracts on the activities of Alpha-estrase, Beta-esterase and Phenolase enzymes of *M. cartusiana* land snail, under laboratory conditions.

Plant extract	(Daflaa) *N.oleander*			(Cafour) *E.melliodora*			(Neem) *A.indica*			Control	L.S.D. 0.05
Enzymes	**10%**	**20%**	**Mean**	**10%**	**20%**	**Mean**	**10%**	**20%**	**Mean**		
Alpha- esterase	293 ± 6.51[a]	644 ± 20.33[c]	469 ± 79.14	403 ± 6.57[b]	823 ± 17.58[d]	613 ± 94.21	637 ± 7.57[c]	782 ± 8.88[d]	710 ± 32.91	1746 ± 30.33[e]	49.50
Beta-esterase	152 ± 4.73[a]	234 ± 7.97[b]	193 ± 18.87	205 ± 3.06[b]	298 ± 4.36[c]	252 ± 20.93	794 ± 8.62[e]	743 ± 13.65[d]	769 ± 13.50	1156 ± 29.61[f]	40.56
Phenolase	427 ± 6.77[a]	1048 ± 32.20[e]	752 ± 132.97	443 ± 8.54[a]	1897 ± 53.56[d]	1170 ± 326.03	803 ± 8.41[b]	747 ± 10.82[b]	775 ± 13.87	785 ± 7.42[b]	74.90

Table 3: Toxicity effect of *Nerium oleander* leaves extract on *Monacha cartusiana* under laboratory conditions.

Mortality %	Mortality % Days after							LC_{25} %		LC_{50} %		LC_{90} %		Slope ±	R.
Concentration	Frist day	Second day	Third day	Fourth day	Fifth day	Sixth day	mean							SD	
2.5	-	-	6.70	13.3	13.3	20.0	13.33	2.466		3.881		9.186			
5	-	-	13.3	20.0	26.7	33.3	24.00	Upper	Lower	Upper	Lower	Upper	Lower	3.425	
5.5	-	6.70	20.0	33.3	40.0	73.7	34.74							±	0.969
10	20.0	20.0	33.3	40.0	73.7	86.7	45.62	1.234	3.335	2.655	4.867	7.055	16.109	6.796	
10.5	20.0	26.7	40.0	53.3	86.7	93.3	53.33								
Control	-	-	-	-	-	-	-								

R = Correlation coefficient of the regression line.
SD = Standard deviation of the mortality regression line.

Table 4: Effect of LC_{50} of *Nerium oleander* leaves extract on some of *Monacha cartusiana* life cycle aspects under laboratory conditions.

Life cycle aspects / Plant extract	Av. No. of eggs	Oviposition inhibition %	Mean depth of eggs (cm)	Mean weight of 10 eggs (g)	Incubation period (days)	Hatchability %
N. oleander	30.3	71.1	0.53	0.021	22.3	17.2
Control	104.8	-	2.18	0.073	11.4	83.4

powder in controlling the land snail *Monacha cartusiana* under laboratory and field conditions, and indicated that this plant was very effective on this snail.

Conclusion

N. oleander plant extract was the highest successfully in decreasing the activity levels of GOT, GPT, GST, Alpha esterase, Beta esterase and phenolase enzymes of *M. cartusiana* land snail under laboratory conditions especially where the snail was under treating with 10% concentration. But AchE enzyme level was decreased by each of 10 and 20% concentrations of *E. melliodara* extract. And the activities levels for all seven tested enzymes were unregular from increasing or decreasing where the animal treated by *A. indica* extracts. LC_{50} of *N. oleander* extract was very effective on life cycle aspects of *M. cartusiana* land snail.

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