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Molluscicidal activity of some plant constituents

Ahmed S. Abdel-Aty
Department of Pesticide Chemistry and Technology, Faculty of Agriculture, 21545-El-Shatby, Alexandria University; Alexandria, Egypt

ABSTRACT

Several plant samples were tested for their lethal effects against white garden snail, *Theba pisana* (Muller), Heliecidae. These samples are *Aloe vera*, Liliaceae (dried leaves); *Allium sativum* L., Alliaceae (dried leaves); *Chenopodium ambrisoides*, Chenopodiaceae (leaves and seeds); *Ipomea carnea*, Convolvulaceae (leaves, seeds and seed coat); *Mangifera indica* L, Anacardiaceae (seed kernels); *Prunus armeniaca* L., Rosaceae (seed kernels); *Punica granatum*, Punicaceae (fruit peel) and *Zygophyllum album*, Zygophyllaceae (areal parts). The tested plant samples were mixed with wheat bran at ratios; 1:2, 1:1 and 2:1 (w/w). Mortality was differed among the tested plant parts as well as their ratios. *P. armeniaca* and *I. carnea* (leaves), followed by *C. ambrisoides* (leaves), *I. carnea* (seed-coat) and *P. granatum* were active with 84.4, 72.2, 65.6, 62.3 and 61.2% mortality, respectively after 14 days at (2:1) ratio. So, seed kernels of *P. armeniaca* were separated, extracted with different solvents. The obtained extracts were further fractionated and the produced constituents were checked for their mortal effect. The activity was differed among the separated constituents depending on both concentration and exposure time. The most active constituent caused 100% mortality after 72 hours at a concentration of 10% with LT_{50} equaled 25.7 (23.7-27.9) hours. GC-MS identification of the active fraction proved that it is enrich in oleic acid, flavanol derivatives, isoquercetrin and cyanogenic glycosides (amygdalin and prunasin). This study exhibited a positive relation between the molluscicidal activity and the cyanogenic glycosides content.

Key words: *Prunus armeniaca*, *Theba pisana*, isolation, cyanogenic glycosides

INTRODUCTION

White garden snail, *Theba pisana* (Muller) is a serious agricultural pest fed on leaves, flowers and soft apical parts of plants causing economic problems to fruits, vegetables, ornamentals and field crops (El-Okda, 1980; Miller *et*

al., 1988). Till now, synthetic molluscicides and insecticides, which are not environmentally safe, are still being applied for controlling snails (Lokma and Al-herby, 1999). Intensive studies have been carried out to find safe alternatives as toxicants present in different plant families (Okka, 1997). Plan *et al*. (2008) proved that the molluscicidal activity of cyclized peptides extracts from *Oldenlandia affinis* and *Viola odorata* plants is comparable to metaldehyde against *Pomacea canaliculata*, serious pest of rice. Extracts of *Punica granatum* Linn. (Punicaceae) bark and *Canna indica* Linn. (Cannaceae) root showed molluscicidal activity against the snail *Lymnaea acuminata* with toxic effect for the fish *Colisa fasciatus*, which shares the same habitat with the snail (Tripathi and Singh, 2000). Li *et al*. (2005) referred the excellent killing effect on *Oncomelania* snails by *Solanum xanthocarpum* to the alkaloid α-solamarrgine. Singh and Singh (2005) showed that the aqueous extracts of *Thevetia peruviana*, *Alstonia scholaris* and *Euphorbia pulcherrima* latex may eventually be of great value for control both *Lymnaea acuminata* and *Indoplanorbis exustus* and other molluscan pests. Crude extracts of bark, root and leaf of neem at 500 and 700 mg/kg produced mortality after exposure for 48 and 72 hours for *Limicolaria aurora* and *Archachatina marginata*, respectively (Ebenso, 2004). Chen *et al*. (2001) proved intense antifeeding activity of *Cryptomeria japonica* methanol extract against *Acusta despesta* snail as a pest of many vegetables and crops.

The purpose of this study is to evaluate the lethal effect of some plant species meals against the land snail, *Theba pisana* (Muller), extract the active plant origin as well as identification of the molluscicidally active constituents.

MATERIALS AND METHODS

Tested plant species: Eleven samples of eight plant species from different families were collected and studied for their lethal effects against the tested snail. Ripened fruits of *Mangifera indica* L and *Prunus armeniaca* L were purchased from the local market and the fleshly cover was removed to obtain seeds. The upper cover of seeds was cracked with hammer to give soft kernels inside, which were dried and blended before introducing to the baits. *Punica granatum* fruits were locally collected and the peel was dried and powdered. Samples *of Zygophyllum album*, *Chenopodium ambrisoides*, *Ipomea carnea* and *Allium sativum* L. were collected locally from Behira

governorate, Egypt, washed from soil, air dried in shadow and crushed to powders.

The tested samples:

Plant species	Family	Part(s) used
Aloe vera	Liliaceae	Leaves
Allium sativum L.	Alliaceae	Leaves
Chenopodium ambrisoides	Chenopodiaceae	Leaves, Seeds
Ipomea carnea	Convolvulaceae	Leaves, Seeds , Seed coat
Mangifera indica L	Anacardiaceae	Seed kernels
Prunus armeniaca L.	Rosaceae	Seed kernels
Punica granatum	Punicaceae	Fruit peel
Zygophyllum album	Zygophyllaceae	Areal parts

Tested animal: The white garden snail, *Theba pisana* (Mueller), family Heliecidae was collected from the research station of Faculty of Agriculture, Alexandria University, Abbis, Alexandria, Egypt and kept for adaptation under laboratory conditions for two weeks.

The bioassay test: The used bait was prepared on the wheat bran according to Miller *et al*. (1988). Water was added at 20% of the prepared bait. Three grams of the tested bait were introduced in a Petri dish (9 cm) to ten snails in each replicate. The Petri dish was placed in a plastic pot covered with a piece of fixed cloth. The cloth pieces were daily sprayed with water to keep the moisture. Three replicates were used for each treatment and control was concurrently carried out. The preliminary tests of the collected plant species were carried out at 1:2, 1:1 and 2:1 ratios in weight. Separated fractions from the active plant, *P. armeniaca* L extract and its separated fractions were dissolved in dimethylsulfoxide (DMSO) and tested at 0, 5, 10, 15, 20, 30 and 50% concentrations of the used bait. Number of dead snails was recorded at different times. LC_{50} and LT_{50} for each concentration were calculated. Feeding inhibition percentages of the bran poison baits were measured by comparing the average consumed food of each snail in control and treatment based on Abivandl and Benz (1984).

Extraction of *Prunus armeniaca* seed kernels: Fruits of *P. armeniaca* L, which showed a persuasive molluscicidal activity, were extracted at the prevailed conditions. They were collected from the market and the flesh was removed. Collected seed kernels (165 gm) were blended and extracted by Soxhlet successively with methanol and acetone for 10 and 14 hours,

respectively to give the methanol and acetone extracts. Successive extraction of the plant residue with hot water was not quantitatively usable. The yellow methanol extract (450 ml) was concentrated under vacuum at < 70^{O} C to 74.7 gm of yellow oily material. Partitioning of 48 gm with chloroform (100 ml × 2) gave the chloroform layer that was concentrated under reduced pressure to fraction I (brown oily materials, 7.5 gm, 4.54 % of the un-extracted seed kernels). The chloroform insoluble fraction was washed with 100 ml of petroleum ether (60-80) and the remained organic solvents were evaporated at < 80^{O} C to produce brownish yellow oil (fraction II). Freezing of this produced oil at -20^{O} C for 48 hours gave a precipitate that was filtered, air dried and completely dried over sodium hydroxide to fraction II-A (off white powder, 5.17 gm, 3.13 % of the un-extracted seed kernels). The remained filtrate was considered as fraction II-B (9.55 gm of brownish yellow). The original acetone extract was concentrated to 45.8 gm of turbid yellow oil. Removal of the organic solvent at 70^{O} C cleared the extract to fraction III (38.6 gm, clear yellow oil, 0.9327 gm ml^{-1}, 23.4 % of the un-extracted kernels). Through the evaporation process, some resinous drops were separated, recovered with chloroform: methanol (1:3 in volume) and concentrated to fraction IV (brown oily resin, 0.9 gm, 0.55 % of the un-extracted sample). The extraction process is briefed in Figure (1).

GC-MS identification of the active extract constituents: GC-EIMS analysis was performed under the prevailed conditions on a Shimadzu GC-MS 2010 QP spectrometer, Column HP5 (coated with 5% diphenyl/95% dimethyl polysiloxane (30 m × 0.25 µm film thickness × 0.32mm ID) was used as the stationary phase. Helium served as mobile phase with a constant pressure of 187.1 kPai. Direct injection of the sample in acetone (1µl of the standard solution, concentration 0.5 mg/ml) was performed in a splitless mode with an inlet temperature of 220^{O} C. Column oven temperature: 80.0^{O} C. Column Flow: 3.2 ml/min with linear velocity: 63.8 cm/sec. The applied oven temperature program included an initial step for 1 min at 80^{O} C, temperature shifted up to 250^{O} C with 10^{O} C /min, followed by 40 min at 250^{O} C. Mass conditions continued to 60 min with m/z range of 45.00-800.00.

Statistical analysis: Mortality percents were analyzed using the analysis of variance (ANOVA). Both LC_{50} and LT_{50} values with 95% confidence limits were determined using probit analysis (Finney 1971).

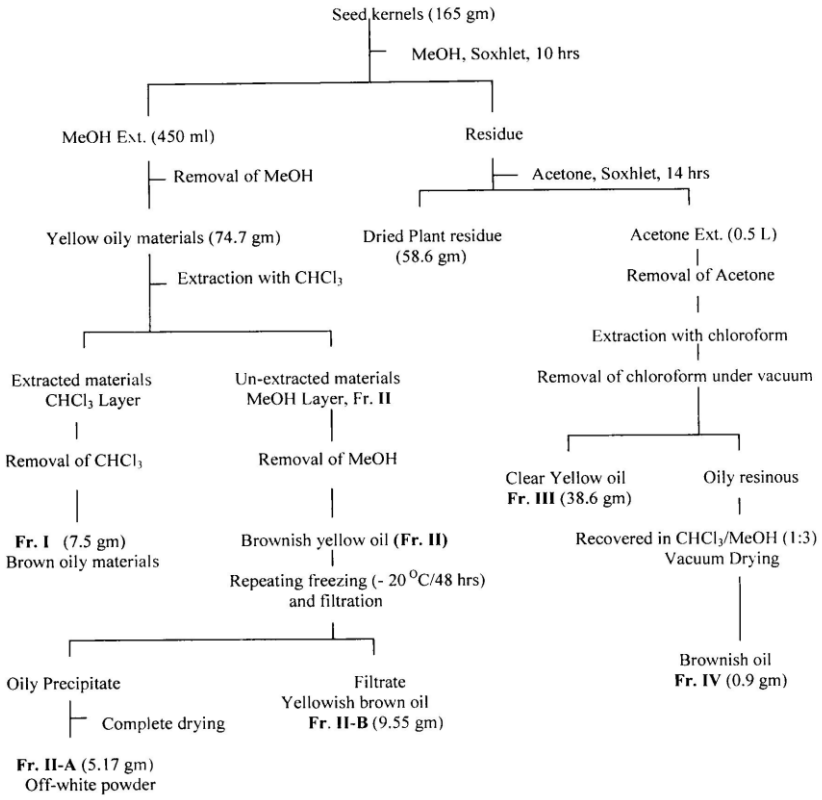

Figure 1: Extraction and separation of *Prunus armeniaca* L seed kernels constituents**.**

RESULTS AND DISCUSSION

Preliminary screening test: The tested plant species caused different mortality percents against the tested snail, *Theba pisana* (Table 1). Comparing with control, the effect of all tested species systemically increased with increasing their concentration and feeding periods. Baits of *Zygophyllum album* (aerial parts), *Chenopodium ambrisoides* (seeds), *Aloe vera* (leaves) and *Ipomea carnea* (seeds) caused weak lethal effect with mortality percentages as high as < 50% at the highest ratio (2:1) after 14 days feeding period. This effect was increased on population treated with *Prunus armeniaca* (seed kernels), *C. ambrisoides* (leaves), *Punica granatum* (fruits peel), *I. carnea* (leaves and seed-coat), they caused 55.5, 54.4, 55.6, 64.4 and 56.7% mortality of the treated population, respectively at a ratio of (1:1) with wheat bran after 14 days feeding. Both *Mangifera indica* (seed

kernels) and *Allium sativum* (leaves) caused 47.8 and 44.4% mortality, dramatically increased to 54.4 and 51.2 % mortality, at 1:1 and 2:1 ratios, respectively. From the previous mentioned data, it is clear that lethality on the tested snail differed among the tested plant species and even origins of the same plant. High mortality was achieved with treatment of *P. armeniaca* (seed kernels) and *I. carnea* (leaves), followed by *C. ambrisoides* (leaves), *I. carnea* (seed-coat) and *P. granatum* (fruit peel). They respectively exhibited 84.4, 72.2, 65.6, 62.3 and 61.2% mortality after 14 days at (2:1) ratio. So, seed kerneles of *P. armeniaca* were extracted, separated and checked for its molluscicidally active constituents.

Table 1: Preliminary mortality effects of the tested samples on *Theba pisana.*

Plant species	Origin	Ratio	Mortality % ± SD at different time (Days)				
			0	4	7	10	14
Mangifera indica L.	Seed-kernels	1:2	0.0	7.8±1.92	15.6±1.95	23.4±0.22	26.6±3.35
		1:1	0.0	25.4±1.91	32.2±1.90	41.2±1.89	47.8±1.90
		2:1	0.0	36.5±1.85	37.7±2.04	50.1±3.33	54.4±1.95
Prunus armeniaca L.	Seed kernels	1:2	0.0	24.4±1.17	25.6±1.93	41.3±1.76	45.6±1.96
		1:1	0.0	31.1±1.92	43.3±1.71	52.2±1.91	55.5±1.93
		2:1	0.0	54.4±1.14	62.2±1.91	72.2±1.91	84.4±1.96
Zygophyllum album L.	Areal parts	1:2	0.0	7.7±1.81	14.4±1.95	17.7±2.03	24.4±1.96
		1:1	0.0	14.4±1.17	21.2±1.82	26.1±3.53	30.0±3.3
		2:1	0.0	18.2±1.35	27.7±2.03	35.6±1.96	44.6±1.83
Chenopodium ambrisoides	Leaves	1:2	0.0	15.1±1.68	22.2±1.91	34.3±1.73	45.6±1.95
		1:1	0.0	22.3±1.75	24.3±1.73	45.6±1.92	54.4±1.97
		2:1	0.0	26.7±0.02	34.4±1.96	53.3±3.34	65.6±1.96
	Seeds	1:2	0.0	4.4±1.17	5.6±1.96	15.6±1.95	22.2±1.91
		1:1	0.0	11.1±1.95	16.7±3.35	25.6±1.94	33.3±3.34
		2:1	0.0	21.1±0.19	24.4±1.96	35.6±1.95	38.9±1.91
Aloe vera	Leaves	1:2	0.0	3.0±0.54	4.4±1.96	11.1±1.91	15.6±1.97
		1:1	0.0	4.4 ±1.93	7.8±1.93	13.4±3.20	15.6±1.96
		2:1	0.0	14.4±1.17	17.8±1.91	25.6±1.95	31.1±1.91
Punica granatum	Fruit peel	1:2	0.0	24.8±1.71	34.4±1.96	42.2±1.91	47.8±1.91
		1:1	0.0	34.6±1.83	44.4±1.96	47.7±2.03	55.6±1.96
		2:1	0.0	40.1±2.54	47.9±1.85	55.6±1.96	61.2±1.82
Ipomea carnea	Leaves	1:2	0.0	14.4±1.93	18.8±2.14	47.8±1.91	54.4±1.96
		1:1	0.0	24.1±1.35	32.2±1.92	56.7±3.34	64.4±1.96
		2:1	0.0	34.1±1.4	45.6±1.95	62.5±2.12	72.2±1.91
	Seeds	1:2	0.0	5.6 ±1.93	12.2±1.91	15.6±1.94	23.3±0.02
		1:1	0.0	11.8±1.68	22.5±1.33	27.8±1.92	34.6±1.83
		2:1	0.0	17.3±2.40	27.9±2.08	35.6±1.95	43.3±3.35
	Seed coat	1:2	0.0	5.6 ± 1.95	24.4±1.96	35.5±1.95	45.6±1.94
		1:1	0.0	23.4±0.20	38.9±1.91	50.1±3.33	56.7±0.02
		2:1	0.0	33.5±0.21	47.8 ± 1.9	55.5±1.91	62.3±2.03
Allium sativum L.	Leaves	1:2	0.0	13.9±1.15	17.9±2.09	24.4±1.95	32.2±1.91
		1:1	0.0	22.2±1.92	28.9±1.92	32.2±1.91	44.4±1.95
		2:1	0.0	27.9±2.10	37.8±1.91	47.8±1.90	51.2±1.82

Mortality effects of *P. armeniaca* constituents: As recorded in (Table 2), the separated fractions behaved differently in their lethal effects. Fraction I showed no mortality at 5%. Its lethal effect started after 6 days feeding on 10 % poisoned bait with mortality percent increased to 48.9 with LT_{50} (lethal time of 50% population) was higher than 14 days at 30%.

It caused mortality increased systematically to 60.2% at 50% after 14 days with 10.9 days LT_{50} value. Fraction II appeared the most active fraction. It was lethal at 5% and its mortality was systematically increased to kill all treated population at 30% after 11 days of treatment. Its LT_{50} values were 10.6, 7.01, 5.02 and 4.36 days at 15, 20, 30 and 50% poisoned baits, respectively.

Table 2: Mortality effects of *P. armeniaca* L seed kernels extracts against *Thepa pisana.*

Fraction	Conc. %	Mortality % ± SD at different time (Days)							LT_{50} (95% CL) Days	Slope ±SE
		0	3	5	6	8	11	14		
Control	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	> 14	
	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	> 14	
Fr. I	10	0.0	0.0	0.0	4.5 ±1.02	8.9 ±1.02	12.2 ±1.17	15.2 ±1.19	> 14	
	15	0.0	0.0	0.0	15.6 ±1.2	17.8 ±1.9	21.1 ±1.93	22.2 ±1.12	> 14	
	20	0.0	11.1 ±1.9	15.6 ±1.2	18.9 ±1.9	21.1 ±1.92	28.1 ±1.6	32.2 ±1.2	> 14	
	30	0.0	12.2 ±1.92	15.6 ±1.95	25.6 ±1.92	31.1 ±1.93	37.8 ±1.92	48.9 ±1.92	> 14	
	50	0.0	18.9 ±1.17	24.1 ±1.7	31.1 ±1.93	43.1 ±0.48	47.8 ±1.92	60.2 ±3.34	10.9 (9.13-13.06)	1.75 ±0.064
Fr. II	5	0.0	0.0	0.0	0.0	7.78 ±1.92	13 ±0.58	14.1 ±1.4	> 14	
	10	0.0	10.8 ±1.3	22.6 ±1.3	31.1 ±3.8	37.8 ±1.9	43.3 ±3.3	46.7 ±3.3	> 14	
	15	0.0	20.4 ±0.81	33.3 ±3.34	42.2 ±1.93	45.6 ±1.87	48.9 ±1.91	54.5 ±2.03	10.6 (8.44-13.35)	1.33 ±0.059
	20	0.0	30.0 ±3.42	40 ±3.33	45.2 ±1.72	51.9 ±1.71	61.2 ±2.1	70.3 ±3.42	7.01 (6.02-8.16)	1.56 ±0.058
	30	0.0	31.6 ±1.72	48.6 ±1.71	57.7 ±1.78	76.7 ±3.32	100	100	5.02 (4.65-5.41)	3.91 ±0.096
	50	0.0	32.2 ±1.92	56.7 ±3.33	65.2 ±1.67	86.7 ±3.33	100	100	4.36 (4.02-5.73)	4.14 ±0.114

Table 2: cont.

Fraction	Conc. %	Mortality % ± SD at different time (Days)							LT_{50} (95% CL) Days	Slope± SE
		0	3	5	6	8	11	14		
Fr. III	15	0.0	0.0	0.0	8.5 ±0.7	14.5 ±0.9	17.1 ±0.8	21.6 ±1.6	> 14	
	20	0.0	5.9 ±1.33	11.9 ±1.23	17.1 ±0.82	21.4 ±1.73	28.6 ±1.71	34.5 ±1.92	> 14	
	30	0.0	7.1 ±0.67	17.5 ±1.33	21.1 ±1.91	25.9 ±1.42	34.5 ±1.92	41.7 ±2.41	> 14	
	50	0.0	10.8 ±1.34	22.9 ±0.64	30.4 ±0.77	40.0 ±3.34	43.3 ±3.34	48.9 ±1.92	> 14	
Fr. II/Fr. III (1:1)	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	> 14	
	10	0.0	0.0	8.2 ±1.68	15.2 ±1.73	20.4 ±0.77	25.2 ±1.71	31.6 ±1.68	> 14	
	15	0.0	0.0	11.4 ±1.71	21.4 ±1.70	31.1 ±1.92	36.7 ±3.33	42.3 ±1.95	> 14	
	20	0.0	5.89 ±1.35	17.8 ±1.91	25.2 ±1.71	41.8 ±1.35	47.3 ±1.15	53.0 ±0.59	11.7 (10.2-13.46)	2.04 ±0.077
	30	0.0	7.78 ±1.92	22.2 ±1.93	35.6 ±1.92	45.6 ±1.93	52.2 ±1.93	58.5 ±1.92	10.51 (9.16-12.07)	2.20 ±0.069
	50	0.0	10.8 ±2.27	28.5 ±1.69	42.9 ±0.77	56.0 ±1.15	60.4 ±0.77	79.1 ±2.28	7.85 (7.15-8.62)	2.67 ±0.07
Un-extracted residue	15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	> 14	
	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	> 14	
	30	0.0	0.0	0.0	0.0	0.0	0.0	8.6 ±1.71	> 14	
	50	0.0	0.0	0.0	0.0	8.5 ±0.77	15.2 ±1.73	21.6 ±1.68	> 14	

However, Fraction III was less effective. Its lethal effect was increased with LT_{50} values equaled >14 days in all cases. Mixing of fraction III with fraction II reduced its toxic effect to 11.7, 10.51 and 7.85 days LT_{50} values at 20, 30 and 50%, respectively. This lowered effect reflects that there is no synergistic effect between these two fractions, which confirm that the most molluscicidally active constituents are contained in fraction II indicating good separation method. The remained un-extracted plant meal was poorly effective at the highest tested concentration.

So fraction II may contains molluscicidally active constituents. Further separation of fraction II into subfractions II-A and II-B was carried out. Their toxic effects differed as subfraction II-A exhibited its mortality with LT_{50} values equaled 77.1, 32.06, 25.7, 15.4, 13.5 and 10.8 hours in comparison to >144, 144, 88.3, 45.4, 27.5 and 25.3 hours in case of

subfraction II- B, respectively at 1, 5, 10, 15, 20 and 30% concentration indicating that the former is more active at least two times of the last one (Table 3).

Table 3: Mortality effects of fractions II-A and II-B against *T. pisana.*

Fraction	Conc %	Mortality % ± SD at different time (Hours)							LT_{50} (95% CL) Hours	Slope± SE
		0	12	24	36	48	72	144		
Control	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Fr. II-A	1	0.0	0.0	15.2 ±1.71	24.4 ±1.92	38.8 ±1.91	49.2 ±1.92	63.3 ±2.98	77.1 (67.0-88.8)	2.19 ±0.039
	5	0.0	5.3 ±1.02	35.6 ±2.01	61.1 ±1.94	72.2 ±1.91	84.4 ±1.96	95.6 ±1.98	32.06 (29.5-34.85)	3.49 ±0.067
	10	0.0	15.8 ±3.11	35.6 ±1.96	68.9 ±1.93	86.6 ±1.96	100	100	25.7 (23.7-27.9)	4.02 ±0.091
	15	0.0	36.7 ±3.42	72.2 ±1.89	91.1 ±1.94	100	100	100	15.4 (13.9-17.2)	3.98 ±0.12
	20	0.0	45.3 ±3.34	76.7 ±3.44	100	100	100	100	13.5 (11.9-15.2)	4.07 ±0.23
	30	0.0	60.0 ±3.33	85.6 ±1.98	100	100	100	100	(10.8) (9.2-12.6)	3.94 ±0.22
Fr. II-B	1	0.0	0.0	0.0	0.0	2.5 ±1.42	8.4 ±1.37	15.2 ±1.7	> 144	
	5	0.0	0.0	8.9 ±1.92	15.5 ±1.95	18.9 ±1.91	24.4 ±1.94	31.1 ±1.96.	> 144	
	10	0.0	14.4 ±1.95	21.1 ±1.87	27.8 ±1.89	31.1 ±1.92	47.8 ±1.93	63.3 ±2.98	88.3 (69.7-112.3)	1.37 ±0.03
	15	0.0	26.7 ±3.33	36.7 ±3.42	44.4 ±1.94	51.1 ±1.91	56.7 ±3.34	74.4 ±1.95	45.4 (36.9-55.7)	1.16 ±0.025
	20	0.0	30.8 ±3.91	44.4 ±1.97	52.2 ±1.91	57.8 ±1.95	71.1 ±1.93	85.5 ±1.95	27.5 (22.3-33.9)	1.26 ±0.026
	30	0.0	33.7 ±3.42	44.7 ±3.44	54.4 ±1.94	65.6 ±1.95	85.5 ±1.92	100	25.3 (22.1-28.9)	2.14 ±0.036

It is a point of importance that consumption of the tested poisoned baits was differed among the *Prunus armeniaca* extracted fractions. Comparing with 72.7% of non-poisoned bait consumption by control, baits of fractions I, II and II-B were less consumed at all the tested concentrations.

While that of fraction III was consumed around the control value except at the highest concentration, its mixture with fraction II reduced the consumption percents at the high concentrations especially in comparison with control. Fraction II-A was the lowest fraction in its consumption with

the highest mortality percentages indicating also good separation method (Table 4).

Table 4: Comparison between the consumption percents of fractions poisoned bait

Extract	Consumed poisoned bait % at different concentrations						
	0	5	10	15	20	30	50
Fr. I	72.7 ± 1.2	58.5 ± 1.0	45.7 ± 1.5	43.6 ± 2.1	41.5± 0.8	41.1 ± 2.0	37.5 ± 1.9
Fr. II	72.7 ± 1.2	69.2 ± 0.7	60.1± 0.8	57.1± 1.4	54.3 ± 2.3	45.4 ± 2.6	37.5 ± 1.7
Fr. II-A	72.7 ± 1.2	27.9 ± 2.6	23.2 ± 1.6	18.9 ± 0.5	17.4 ± 0.9	16.9 ± 0.8	16.0 ± 1.0
Fr. II-B	72.7 ± 1.2	70.8 ±1.6	68.1± 1.5	44.6 ± 1.9	39.1 ± 0.4	36.3 ± 2.3	32.8 ± 2.4
Fr. III	72.7 ± 1.2	76.0 ± 1.7	70.5 ± 1.6	75.4 ± 0.8	72.7 ± 1.7	77.8 ± 1.2	43.7 ± 1.3
Fr. II / III (1:1)	72.7 ± 1.2	77.1 ± 1.7	72.4 ± 1.1	67.1± 1.4	56.2 ± 1.7	47.5 ± 0.8	37.5 ± 1.0

GC-MS identification of the active fraction constituents: The identified compounds, their retention times and their fragment ions are recorded in (Table 5). Compound eluted at 13.7 min. was identified as oleic acid. Fragment ions at m/z 227, 213, 199, 185, 171 and 157 are produced through chain breaking with successive loss of CH_2 groups, from fragment at m/z 239 that was produced by loss of CO_2 from the parent molecule.

However Gumus and Kasifoglu (2010) proved that oleic acid ($C_{18}H_{34}O_2$) and linoleic acid ($C_{18}H_{32}O_2$) are the highest fatty acids contained in apricot, *P. armeniaca* oil, with 67.31% and 24.68%, respectively accompanying with others as 8.01% in total. Absence of linoleic acid descriptive fragment at m/z 236 and its successive produced fragments at m/z 236, 212, 208, 194, 180, 166 and 152 confirmed the contained fatty acid as oleic acid ($C_{18}H_{34}O_2$), octadec-9-enoic acid.

Table 5: Total identified compounds in fractions II-A and II-B

No	Compound	Rt min.	GC-MS identification m/z (abundance)
1	A cyanogenic derivative	12.0	219 (1.34); 187 (89); 169 (2.7); 116 (100.0); 115 (97.3); 99 (22.2); 89 (15.1); 77 (7.6); 63 (10.5); 57 (14.5)
2	Oleic acid	13.7	270 (10.6); 239 (4.4); 227 (9.2); 157 (3.1); 143 (18.3); 129 (8.4); 101 (6.9); 99 (8.5); 98 (8.7); 87 (64.6); 74 (100.0); 69 (13.6); 57 (15.5); 55 (23.9)
3	Prunasin	15.3	295 (M^+) (2.1); 294 (9.6); 220 (3.0); 190 (2.7); 180 (1.4); 150 (12.1); 149 (9.3); 137 (7.6); 136 (12.0); 135 (11.8); 133 (5.1); 120 (3.3); 116 (1.0); 113 (1.3); 77 (10.5); 69 (34.2); 68 (49.1); 67 (100.0); 55 (68.6); 54 (39.6)
4	Amygdalin	15.4	297 (1.02); 296 (4.0); 281 (0.6); 278 (M-Glu) (0.62); 264 (24.1); 222 (13.3); 211 (0.3); 180 (12.1); 165 (4.4); 163 (1.5); 162 (1.2); 146 (0.3); 144 (0.6); 133 (4.2); 116 (1.4); 107 (4.8); 106 (1.3); 83 (53.8); 81 (33.6); 74 (56.3); 69 (69.0); 68 (26.1); 67 (36.3); 57 (32.2); 55 (100.0); 54 (32.7)
5	Quercetin-3-glucopyranoside	19.3	405 (0.6); 277 (16.1); 167 (42.1); 150 (12.5); 149 (100.0); 123 (1.0); 113 (13.9); 94 (16.2); 71 (27.2); 57 (35.4)
6	2,3-Dihydroisoquercetrin	19.8	288 (19.0); 287 (100.0); 286 (95.7); 270 (16.4); 258 (9.0); 207 (7.7); 228 (1.7); 168 (7.3); 164 (1.2); 152 (1.4); 147 (0.7);143 (7.3); 140 (9.1); 139 (9.7); 138 (1.0); 137 (0.6); 129 (4.1); 128 (1.5); 118 (0.6); 115 (7.7); 111 (0.6); 109 (0.8); 94 (14.8); 87 (0.7); 77 (19.8)
7	Flavan-3-ol-(4β-2) - phloglucinol	21.0	290 (12.1); 289 (62.7); 288 (39.0); 273 (0.4); 272 (1.6); 154 (2.4); 153 (1.2); 152 (1.5); 139 (2.7); 123 (0.2); 121 (8.1); 120 (100.0); 109 (0.4); 93 (8.9); 77 (11.5); 65 (15.5)

Amygdalin and prunasin (cyanogenic glycosides) are confirmed at retention time of 15.4 and 15.3 min, respectively. As Amygdalin is larger than prunasin with a glucose unit, it was eluted later than it with 0.1 min. through the obtained spectra, discrimination between amygdalin and prunasin was carried out by fragments at m/z 264 with 24.1% and 1.6% in

case of amygdalin and prunasin, respectively and at m/z 222 with 13.3% in case of amygdalin indicating separation of two glucose unit. Amygdalin and prunasin are found in more than 2500 different species including *P. armeniaca* and many important crop plants (Bak *et. al.*, 2006).

GC-MS spectrum showed the general structure of flavanol units at retention time of 19.3, 19.8 and 21.0 minutes. Compound eluted at retention time of 19.3 min. showed the general spectrum of flavanol structure. Fragment at m/z 149 reflects a substituted sugar unit on the flavanol compound. Fragments at m/z 207, 167, 152, 124, 123 and 99 are produced from the heteroaromatic ring (ring C) cleavage, while fragment at 277 is referred to loss CO of the aglycone moiety. These data go with that of quercetin-3-glucopyranoside.

The compound eluted at 19.8 minutes retention time is identified as isoquercetrin as glucosidic bond cleavage gave the sugar moiety at m/z 147 that was fragmented at m/z 129, 116, 111 and 87 due to loss of H_2O, CHO, $2H_2O$ and 2CHOH groups, respectively. The aglycone moiety through retro-diels alder (RDA) fission gave fragment, at 138 and 137. It losses CO group to m/z 227. Its fragments appeared at m/z 286 and 287 indicate saturation at positions 2 and 3. So this compound is identified as 2,3-dihydroisoquercetrin. Absence of fragment at m/z 180 confirmed the sugar moiety is a 6-deoxyhexose. Fragment at m/z 270 is referred to loss of H_2O molecule from the aglycone.

Compound at retention time 21.0 seams to be a flavanol molecule as fragment at m/z 289, 288 and 272 revealed $(M-1)^+$, $(M-2)^+$ and $(M-H_2O)^+$ ions of flavan-3-ol unit are referred to de-protonation and forming double bond between C_3 and C_4 carbon atoms. Retro-diels alder fission of the heteroaromatic ring (ring C) gave fragments at m/z 139 and 152, that gave fragments at m/z 125 and 124 by loss CH_2 and CO group, respectively. Cleavage of the heteroaromatic ring gave fragment at m/z 138, which losses H_2O to fragment at m/z 120 that produces fragment at m/z 93 by loss CO group. Relative abundance of fragment at m/z 272 (1.6) appeared > 273 (0.4) indicating the substitution on carbon atom (C_4) and not on the 3-OH position. These data identified the compound as epicatechin-(4β-2)-phloroglucinol (Figure 2). These data go with literature as from the studied plant, Isoquercetrin (Silverstein *et al*. 1991); gallocatechin (Lakhnapal and Deepak, 2007); epicatechin-(4β-2)-phloroglucinol (Kimura *et al.*, 2008) were isolated.

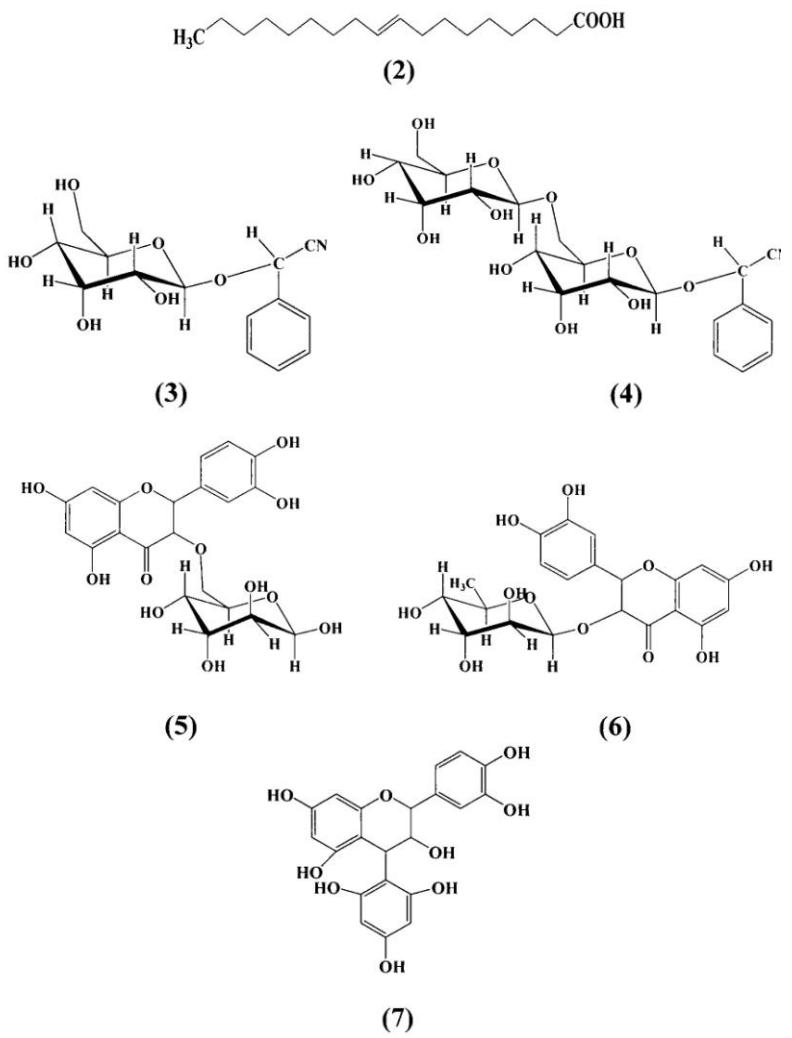

Figure (2): Chemical structures of the active fraction constituents

(**2**) Oleic acid (**3**) Prunasin (**4**) Amygdalin (**5**) Quercetin-3-glucopyranoside
(**6**) Isoquercetrin (7) Flavan-3-ol-(4β-2)-phloglucinol

Prunus armeniaca, a cyanogenic plant contains amygdalin and other cyanogenic glycosides, which have antimicrobial activities against *Helicobacter pylori*, both Gram positive and Gram negative bacteria and fungi (Myiazawa *et al*., 2006). They protect their contained plants from animals (Stowe and Kil, 1983).

The effect of cyanogenic glycosides is referred to hydrolysis liberating cyanide expressed as hydrocyanic acid (HCN), which has acute toxicity as it is well known and other corresponding carbonyl components (Bhunya *et al*, 2009). The effect of cyanide might be owed to coordination with the active site of peroxidases at which H_2O_2 reduction is catalyzed (Tatsuma *et al*., 1998). Cyanide also inhibits antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase. Cyanide can be transformed to thiocyanate which has chronic toxicity or its accumulation leads to ataxic neuropathy (Poulton, 1983).

Flavanol or flavanolone structures affect the snail through hydrogen bonding of their phenolic hydroxyl group with protein to the cross-linked insoluble and indigestible protein complex depending on number of hydroxyl groups (Nakamura *et al*., 1990). Hydrophobicity is also an utmost important factor for reaching its receptor in reasonable time and concentration giving the maximum activity (Kubinyi, 1976).

They affect as oxidative phosphorylation uncouplers through the acidity of hydroxyl groups destroying the proton differential produced by electron transport that is required for ATP formation (Laks and Pruner, 1989). Quercetin antagonists calmodulin, a calcium regulatory protein transports calcium ion across cellular membranes, initiating numerous cellular process. Through this mechanism, quercetin functions at cell membrane level with a membrane stabilizing action inhibiting calmodulin dependent enzyme present at cell membrane such as ATPases and phospholipases thereby influencing membrane permeability **(**Buss *et al*., 1984).

So, the difference between lethal effects of the active fractions II-A and II-B may be due to their contents of both amygdalin and prunasin. They are contained with 59.96 and 22.93%, respectively in fraction II-A, and with 34.71 and 12.42%, respectively in fraction II-B, respectively (Table 6).

Table 6: Differences in fractions II-A and II-B contents; shown as compound percents

Compound	Rt min.	Fraction II-A	Fraction II-B
A cyanogenic derivative	12.0	5.88	--
Oleic acid	13.7	6.64	10.70
Prunasin	15.3	22.93	12.42
Amygdalin	15.4	59.96	34.71
Quercetin-3-glucopyranoside	19.3	4.59	--
Isoquercetrin	19.8	--	10.09
Flavan-3-ol-(4β-2) -phloglucinol	21.0	--	32.08
Total		100.0	100.0

Amygdalin is hydrolyzed to glucose and prunasin, which is subsequently hydrolyzed to a glucose and mandelonitrile and finally mandelonitrile is cleaved to benzaldhyde and hydrogen cyanide. This work helped for discovering simple chemical molecules can be easily prepared with good molluscicidal activity.

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التأثير الإبادى لبعض المكونات النباتـية على القواقع

أحمد صبرى عبد العاطى

الاسكندرية- مصر – الشاطبى – جامعة الإسكندرية – كلية الزراعة –قسم كيمياء و تقنـية المبيدات

تم دراسة التأثيرات المميتة لمجموعة من العينات النباتية على قوقع الحدائق الأبيض (تيـبا بيـسانا). العينات التى تم دراستها هى الصبار (الورق)، الزربيح (الأوراق و البذور)، إيبـوميا كارنيا (الأوراق، البذور، غطاء البذور)، المانجو و المشمش (لب البذرة)، الرمان (قشرة الثمرة) بالإضافة إلى الأجزاء الهوائية للنبات الصحراوى "العرفص".

تم تجميع بذور كل من المانجو و المشمش من الأسواق المحلية بينما تم تجميع الأجزاء الهوائية للنبات الصحراوى من قرية الجعار- مركز وادى النطرون- محافظة البحيرة , فى حين تم تجميع باقى العينات من الحقل و تنظيفها من الأتربة ثم تجفيفهاو طحنها لصورة المسحوق. تم التقييم بطريقة الطعوم السامة خلطا مع نخالة القمح.

أحدثت هذه العينات النباتية موتا إختلفت نسبته بإختلاف العينة المختبرة و كذلك نسبتها فى الطعم المستخدم. ظهر طعم كل من عينة لب بذرة المشمش و أوراق الإيبوميا كارنيا أكثر هذه الطعوم نشاطا من حيث تأثيرها المميت على القوقع المدروس , يليها طعم عينة أوراق الزربيح و طعم غطاء بذرة الإيبوميا كارنيا و كذلك عينة طعم قشرة ثمار الرمان حيث أحدثت هذه الطعوم % موت قدرها 84.4، 72.2، 65.6، 62.3، 61.2 % على الترتيب بعد 14 يوم معاملة بنسبة 1:2 (المادة المختبرة: نخالة القمح).

تم إستخلاص لب ثمار المشمش بمذيبات مختلفة و تقييم التأثير المميت للمستخلصات الناتجة على نفس القوقع محل الدراسة. إختلف نشاط هذه المستخلصات من حيث تأثيراتها الإبادية معتمدا على كل من نوع المستخلص و تركيزه بالإضافة إلى زمن تعرض القوقع محل الدراسة له. وجد مستخلص الميثانول أكثر هذه المستخلصات فعالية و تم فصله إلى جزئين سبب أكثرهم فعالية 100% موت بعد 72 ساعة على تركيز 10% بزمن لازم لقتل 50% من العشرة المعاملة قدره 25.7 ساعة. التعرف على مكوناتهما بواسطة جهاز التحليل الكروماتوجرافى- مطياف الكتلة GCMS. ظهرت هذه الـ fractions غنية بمركبات الفلافانول و الأيزوكويرسيتين بالإضافة إلى جليكوسيدات السيانوجين (الأميجدالين، البروناسين). تم ربط العلاقة بين التأثير الإبادى و محتوى هذه الأجزاء من المركبات المتعرف عليها.