Molluscicidal potency of four plant extracts on three pulmonate snails (Gastropoda) in Egypt

By

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Abstract

In the present study four types of plant extracts were used to affect three gastropods in Egypt. These plants *were Calotropis procera, Pelargonium graveolens, Ethulia conyzoides* and *Euphorbia milii.*The treated snails were the land snail or garden snail *Eobania vermiculata* and the two freshwater ones *Lymnaea caillaudi* and *Biomphalaria alexandrina*.

Traditionally, the land snails demands very high concentrations in comparison with those for the freshwater snails. There was no effect to the extract of *E. milii* . The ethanol extract of *E. conyzoides* was highly more active than others on the snail *E. vermiculata.* While, the effect of *E. conyzoides* and *E. milii* on *L. caillaudi* and *B. alexandrina* were the most active according to the used concentrations. In comparison, the extract of E. milii exhibits the highest molluscicidal activity as it caused 50% or more mortality of *L. caillaudi* and *B. alexandrina* with the least concentrations. Moreover, the mortality rate of *L. caillaudi* was significantly higher than that of *B. alexandrina* , demonstrating a deffrential susceptibility to the ethanol extract of *E. milii*.

INTRODUCTION

In recent times, the use of plant products has gained unprecedented impetus all over the world. A large number of plant families have furnished many classes of products, which may vary in the degree of pesticidal activity. Several countries have promoted the use of plant products due to their wide range of ideal properties, such as high target toxicity, low mammalian toxicity, low cost, solubility in water, easy biodegradability, abundant growth in endemic areas and operator safety **(Singh *et al.*, 2000)**. Plants are the richest source of renewable bioactive organic chemicals. The total number of plant chemicals may exceed 400 000; of these, 10 000 are secondary metabolites whose major role in the plants is reportedly defensive **(Cooper and Johnson, 1984)**. Numerous defensive chemicals belonging to various categories (terpenoids, alkaloids, glycosides, phenols, tannins, etc.) that cause behavioural and physiological effects on pests have already been identified.

Materials and Methods

Collection of the Snails

Adult animals with a similar shell of the land brown garden snail, *Eobania vermiculata* (Müller), Adult freshwater harmful snails *Lymnaea caillaudi* and *Biomphalaria alexandrina* were collected from Dakahlia, Governorate, Egypt. Identification of the collected snails was according to **Genena (2003) and Ibrahim *et al.* (1999).** The snails were acclimatization under laboratory condition for two weeks before the treatments with the plant extracts.

Description of the tested plants

Euphorbia splendens var. milii (Crown of Thorns), *Calotropis procera* (osher), *Pelargonium graveolens* (Geranium), *Ethulia conyzoides* were collected from their natural habitat in winter season, and were identified in Botany Department Faculty of Science, El-Mansoura University, Egypt.

Preparation of the plant extracts

Known weights of fresh stem, bark and leaves were cut into small pieces, dried at 40°C over night and pulverized in a mortar and pestle. For partial purification, dried powder of the four plant species were extracted in Soxhlet apparatus, using 500 ml of the organic solvents (methyl alcohol, ethyl alcohol and hexane). The extracts concentrated under vacuum in a rotary evaporator, and weighed in pre-weighed beakers and stored in airtight desiccators at –20°C until.

Molluscicidal activity tests.

For molluscicidal activity testing, from the crude extracts, stock solutions were freshly prepared in distilled water and different dilutions ranging from 10 to 100 ppm as well as controls were prepared in 1liter beakers using dechlorinated tap water for the freshwater snails and up to 10000 ppm for the terrestrial ones for further use. Toxicity experiments were performed by the method of Singh and Agarwal (l988a). Ten of the freshwater snails were placed in each of different concentrations (5, 10, 20, 30, 40, 50, 60, 70, 80 ppm) of the plant extracts prepared by dilution with distilled water containing 0.05% of Triton-X 100, and kept in glass flasks containing 500 ml of the solution and covered with a plastic screen to allow the air in and keeps the snails from escaping. Control groups were kept in dechlorinated tap water/Triton-X 100 solutions without any treatment and the number of surviving after 24h exposure followed by 24h recovery period determined. For the terrestrial snail *Eobania vermiculata* Stock solutions of the tested plant extracts were prepared. Pieces of green lettuce leaves were dipped in glass jar containing 100 ml of the tested extract for 10 seconds, and then left until solution dropping stopped before being offered to the snails. After 72 h of exposure period, the treated leaves were placed daily with fresh untreated ones for 28 day. Mortality percentages were recorded after 1, 3, 5 and 7 days up to 28 day post treatment and corrected for natural mortality according to Abbott's formula (Abbott, 1925). Each set of experiments, were replicated three times at the room temperature. Experimental tests with more than 20% control mortality was discarded and then repeated. However, when the control mortality ranged from 5-20%, the percentage mortality (%M) was corrected by Abbott (1925).

$$\% M = \frac{\%\text{ Test mortality} - \%\text{ Control mortality} \times 100}{100 - \%\text{ Control mortality}}$$

Dead snails were detected by loss of response to a thin stainless steel needle according to the WHO (1965) procedure.

Method of statistical analysis

Probit regression analysis was carried out by a computerized log-probit analysis (Finney, 1971) for all the plants tested to determine the lethal concentration causing 50% mortality (LC50) and 90% mortality (LC90). The slope of the regression line was used to assess the effect of the extract, the steeper the slope, the more lethal the plant molluscicide effect.

RESULTS

Molluscicidal potency of the examined plant extracts

In the present study, four types of plant extracts were used to affect three gastropod species; they extracted from the plants *Calotropis procera*, *Pelargonium graveolens*, *Ethulia conyzoides* and *Euphorbia milii.* The gastropod species were the land snail *Eobania vermiculata* and two freshwater ones *Lymnaea caillaudi* and *Biomphalaria alexandrina.*

Traditionally, the treatment of the land snails with different plant extracts demands very high concentrations in comparison with those for the freshwater snails. This was obvious in the present study during the treatments of the terrestrial or the land snail *Eobania vermiculata*. Application of the treatment of the four extracts of the plants *Calotropis procera*, *Pelargonium graveolens*, *Ethulia conyzoides* and *Euphorbia milii* revealed that there is no effect to the extract of *E. milii*, table (1&2).

As illustrated in table (1) similar concentrations of the three effective plant extracts were used. These concentrations were 1000, 2000, 3000, 4000, 5000, 7000 and 9000 ppm for each.

Table (1): Mortality rate of *Eobania vermiculata* after the exposure to different concentrations of the plant extracts for 28 day.

Calotropis procera		*Pelargonium graveolens*		*Ethulia conyzoides*		*Euphorbia milii*	
Conc. (ppm)	Mortality rates %	Conc. (ppm)	Mortality rates%	Conc. (ppm)	Mortality rates%	Conc. (ppm)	Mortality rates%
Control	0	Control	0	Control	0	Control	0
1000	6.667	1000	6.667	1000	13.333	1000	0
2000	10	2000	13.333	2000	23.333	2000	0
3000	23.333	3000	23.333	3000	43.333	3000	0
4000	33.333	4000	40	4000	56.667	4000	0
5000	26.667	5000	50	5000	66.667	5000	0
7000	33.333	7000	53.333	7000	76.667	7000	0
9000	53.333	9000	63.333	9000	86.667	9000	0

Table (2): Molluscicidal activity (LC50 and LC90) of different plant extracts against the land snail *Eobania vermiculata* after 28 day of exposure under laboratory conditions.

Plants	Used solvent	Molluscicidal Activity (ppm)			Slope
		Lc50	Lc90	(95% CI)	(Regression coefficient)
Calotropis procera	Hexane	8578.855	46036.848	(31446.31:56054E+1)	1.756
Pelargonium graveolens	Hexane	5916.165	24558.908	(20518.34:11799E+1)	2.073
Ethulia conyzoides	Ethanol	3412.995	11561.991	(14833.228:59620.002)	2.419
Euphorbia milii	Methanol	—	—	—	—

— Not effective.

It was clear that *E. conyzoides* ethanol extract in general was highly more active than hexane extract of *P. graveolens* and that of *C. procera* on the common garden snail *Eobania vermiculata*, the LC50 values were 3412.995, 5916.165 and 8578.855 ppm respectively, where LC90 were 11561.991, 24558.908 and 46036.848 ppm respectively [table (2) and Figs. (1, 2&3)].

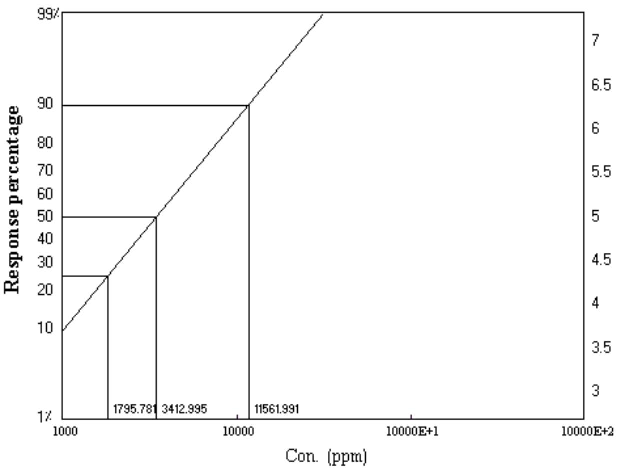

Fig. (1): Con/probit regression line of Ethulia conyzoides ethanol extract on Eobania vermiculata snail.

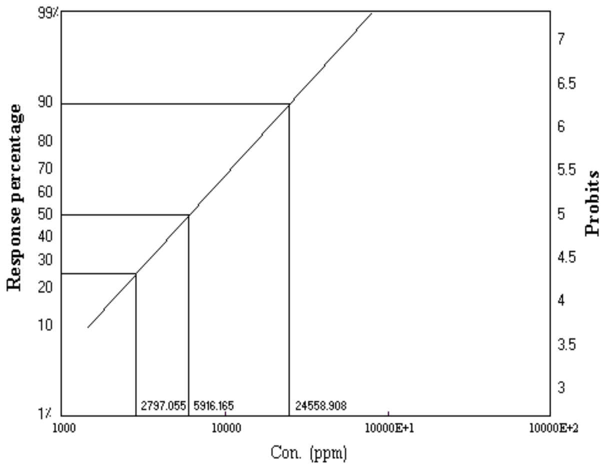

Fig. (2): Con/probit regression line of Pelargonium graveolens hexane extract on Eobania vermiculata snail.

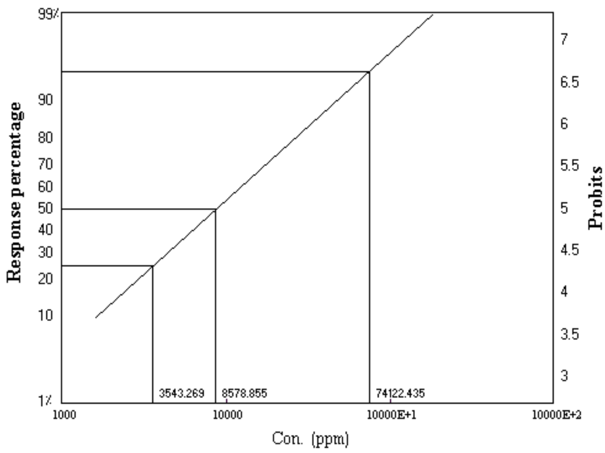

Fig. (3): Con/probit regression line of *Calotropis procera* hexane extract on *Eobania vermiculata* snail.

Table (3): Mortality rate of *Lymnaea caillaudi* after the exposure to different concentrations of the plant extracts for 24 hours.

Plants	Used solvent	Molluscicidal Activity (ppm)			Slope
		Lc50	Lc90	(95% CI)	(Regression coefficient)
Calotropis procera	Hexane	8578.855	46036.848	(31446.31:56054E+1)	1.756
Pelargonium graveolens	Hexane	5916.165	24558.908	(20518.34:11799E+1)	2.073
Ethulia conyzoides	Ethanol	3412.995	11561.991	(14833.228:59620.002)	2.419
Euphorbia milii	Methanol	—	—	—	—

— Concentration not used

Table (3) illustrated that besides using a control; four concentrations from two extract types (*P. graveolens* and *E. conyzoides*) and five concentrations from the others (*C. procera* and *E. milii*) were used to treat the freshwater snail *L. caillaudi*. These concentrations were different for each extract type as 20, 30, 40, 50 and 60 ppm for *C. procera*, 20,40,60 and 80 ppm for *P. graveolens*, 10, 20, 30 and 40 ppm for *E. conyzoides* and 5,10,20,30 and 40 ppm for *E. milii*.

It was clearly obvious that the effect of *E. conyzoides* and *E. milii* extracts were the most active according to the used concentrations. In comparison, the extract of *E. milii* exhibits the highest molluscicidal activity as it caused 50% mortality with the least concentrations.

Table (4): Molluscicidal activity (LC50 and LC90) of different plant extracts against the freshwater snail *Lymnaea caillaudi* after 24 hours of exposure under laboratory conditions.

Plants	Used solvent	Molluscicidal Activity (ppm)			Slope
		Lc50	Lc90	(95% CI)	(Regression coefficient)
Calotropis procera	Hexane	56.608	163.779	(135.28:714.105)	2.778
Pelargonium graveolens	Hexane	25.41	77.965	(73.192:271.471)	2.632
Ethulia conyzoides	Ethanol	17.443	57.781	(51.906:239.972)	2.464
Euphorbia milii	Methanol	9.304	34.027	(33.805:99.799)	2.264

From table (4) and figures (4, 5, 6&7) it was noticed that *E. milii*. Extract found to be the most active followed by *E. conyzoides* and *P. graveolens*, while *C. procera* was the least, LC50 values were 9.304, 17.443, 25.41 and 56.608 ppm respectively and LC90 values were 34.027, 57.781, 77.965 and 163.779 ppm respectively.

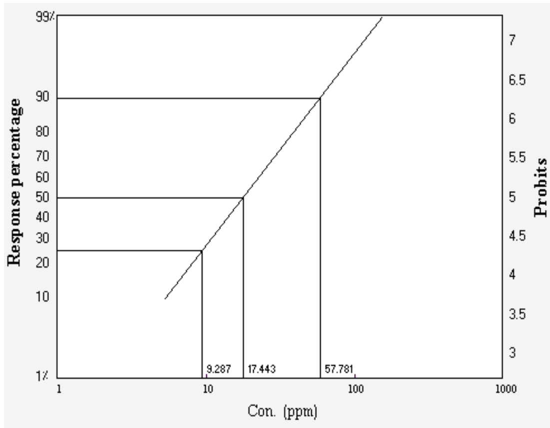

Fig. (4): Con/probit regression line of Ethulia conyzoides ethanol extract on *Lymnaea caillaudi* snail.

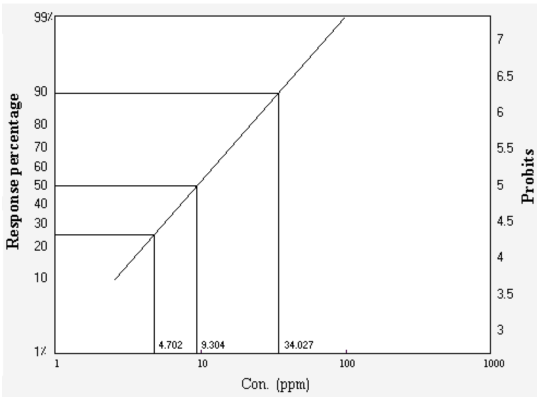

Fig. (5): Con/probit regression line of Euphorbia milii methanol extract on *Lymnaea caillaudi* snail.

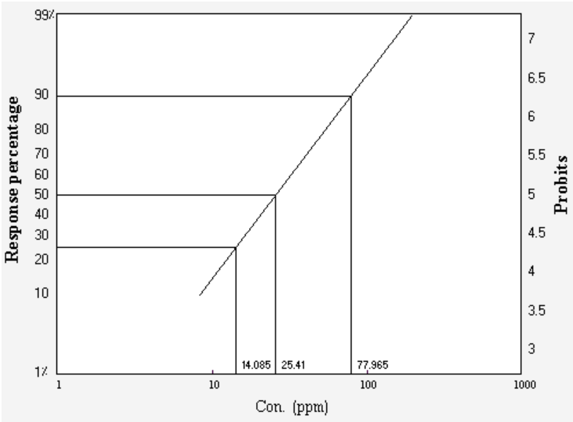

Fig. (6): Con/probit regression line of Pelargonium graveolens hexane extract on *Lymnaea caillaudi* snail.

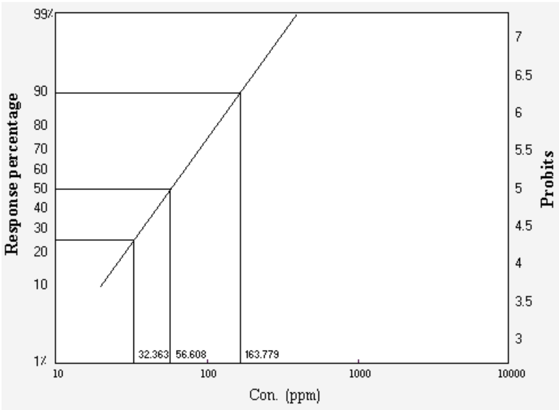

Fig. (7): Con/probit regression line of Calotropis procera hexane extract on *Lymnaea caillaudi* snail.

Table (5): Mortality rate of Biomphalaria alexandrina after the exposure to different concentrations of the plant extracts for 24 hours.

Calotropis procera		*Pelargonium graveolens*		*Ethulia conyzoides*		*Euphorbia milii*	
Conc. (ppm)	Mortality rates %	Conc. (ppm)	Mortality rates%	Conc. (ppm)	Mortality rates%	Conc. (ppm)	Mortality rates%
Control	0	Control	0	Control	0	Control	0
20	26.667	20	6.667	20	23.333	5	13.333
30	46.667	40	16.667	30	46.667	10	26.667
40	53.333	60	33.333	40	76.667	20	40
50	80	80	70	50	90	30	60
—	—	—	—	—	—	40	83.333

— Concentration not used

With respect to the treatment of *Biomphalaria alexandrina*, table (5) illustrated that four different concentrations of extracts types were used except in *E. milii* where five concentrations were applied. These concentrations were 20,30,40 and 50 ppm for both C. procera and E. conyzoides, while they were 20,40,60 and 80 ppm in P. graveolens and 5,10,20,30 and 40 ppm in E. milii. It was noticed that the most effective one was E. milii including the least concentrations. Moreover, from Tables (3&5) the mortality rate *of L. caillaudi* was significantly higher than that of the *B. alexandrina*, demonstrating a differential susceptibility to the Euphorbia milii methanol extract.

Table (6): Molluscicidal activity (LC50 and LC90) of different plant extracts against the freshwater snail *Biomphalaria alexandrina* after 24 hours of exposure under laboratory conditions.

Plants	Used solvent	Molluscicidal Activity (ppm)			Slope
		Lc50	Lc90	(95% Cl)	(Regression coefficient)
Calotropis procera	Hexane	32.12	78.651	(68.509:306.266)	3.259
Pelargonium graveolens	Hexane	67.706	159.883	(133.567:558.328)	3.434
Ethulia conyzoides	Ethanol	29.092	52.09	(50.522:89.79)	5.066
Euphorbia milii	Methanol	20.037	81.964	(71.752:351.176)	2.095

As provided in **table (6)** and figures (8,9,10&11) *Euphorbia milii* extract was found to be the most active followed by *E. conyzoides*, *C. procera* while *P. graveolens* was the least, LC 50 values were 20.037, 29.092, 32.12 and 67.706 ppm respectively, LC90 values were 81.964, 52.09, 78.651 and 159.883 ppm respectively.

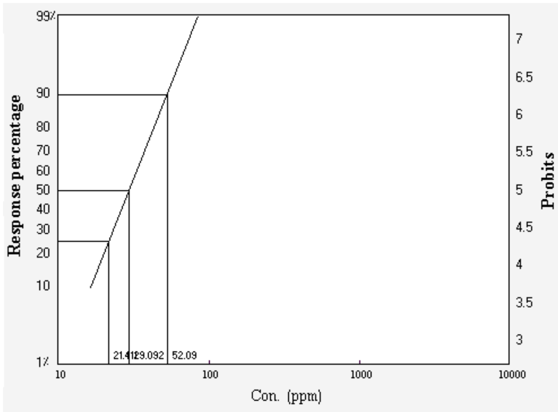

Fig. (8): Con/ probit regression line of *Ethulia conyzoides* ethanol extract on *Biomphalaria alexandrina* snail.

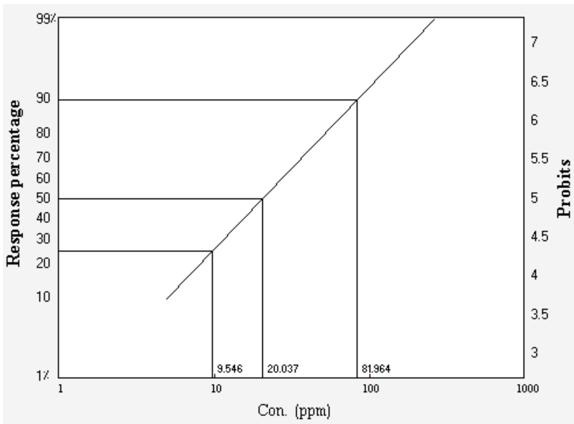

Fig. (9): Con/probit regression line of *Euphorbia milii* methanol extract on *Biomphalaria alexandrina* snail.

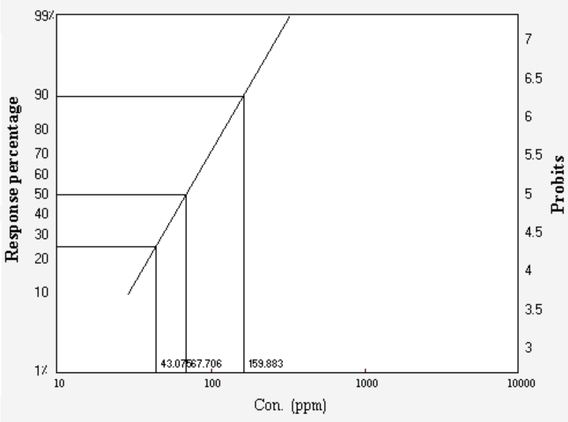

Fig. (10): Con/probit regression line of *Pelargonium graveolens* hexane extract on *Biomphalaria alexandrina* snail.

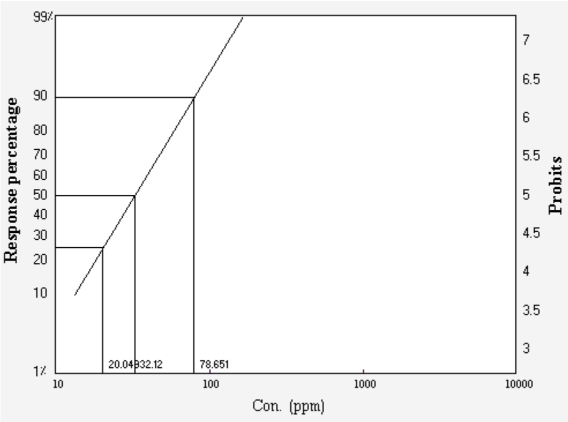

Fig. (11): Con/probit regression line of *Calotropis procera* hexane extract on *Biomphalaria alexandrina* snail.

Discussion

Effect of the plant extracts on the studied snail species

In the present study four plant extracts were used as molluscicides to show their effects on the mortality of three pulmonate species.

Many plants are known to be lethal to snails. Among these *Calotropis procera*, *Pelargonium graveolens*, *Ethulia conyzoides* and *Euphorbia milii* which are used in the present study. Many authors previously studied the effect of plant extracts on the land and freshwater snails, such as (Adewunmi and Marquis, 1980; Adewunmi *et al*., 1982; El-Hwashy *et al.,* 1996; Sermsart *et al*., 2005; Afifi *et al.,* 2007; Abdel-Kader *et al*., 2007; Shanta *et al*., 2008; Bakry, 2009a,b; Mello-Silva *et al*., 2006, 2007, 2010).

El-Hwashy *et al*., (1996) studied the toxicity effect of six plant extracts on the land snail *Eobania vermiculata* in Egypt. These plants were Cauliflower (*Brassica oleracea*), Atma (*Pergularia tomentosa*), Khilla (*Ammi visnaga*), Radish (*Rophanus stivus*), Oshar (*Calotropis procera*) and Datura (*Datura stramonuum*). The preliminary molluscicidal activity screening of these plant extracts showed that three of them were highly potent against snails when extracted with ethanol and tested as residue film technique. These extracts were of Cauliflower, Atma and Oshar, where Atma extract was found to be the most active followed by Oshar while Cauliflower was the least.

The results of the work described in this investigation showed that 4000 ppm of the effective extracts and above produced 33-100% mortality in the snail *E. vermiculata*, 1000 ppm and lower concentrations of the toxicant which did not appreciably affect most of the snails can be taken as sub-lethal according to (Ghandour and Webbe, 1975; Adewunmi *et al*., 1982). The prolonged exposure of *E. vermiculata* to sub-lethal concentrations of the plants is in agreement with similar studies of prolonged exposure to low concentrations of chemical molluscicides on oviposition and egg development of many snail species (Olivier and Haskins, 1960; Olivier *et al*., 1962; Cardarelli, 1974). Also, sub-lethal concentrations appear to slow down the growth rate of snails and could probably kill the snails when applied continuously for a period of a few months (Adewunmi *et al*., 1982).

Several authors used plant extracts to control land snails pests such as (Hussein and El-Wakil, 1996; Ghamry, 1994, 1997). Zidan *et al*. (2001) used several plant extracts against *Monacha obstructa*, *Eobania vermiculata* and *Theba pisana*. Ebenso (2004) used Neem extract and reported that there is no effect on the snails exposed to Neem seeds oil extract. Gabr *et al*. (2006) used Neem extract against land snails *Monacha obstructa* and *Eobania vermiculata*. Afifi *et al*. (2007) studied the effect of some plant extracts on the glassy clover snail *Monacha obstructa* and reported that plant extracts can be successfully used in controlling the injurious land snails, using the bait technique, specially extracts of Fennel and Pomegranate as they exhibit more than 90% mortality after seven days of treatment.

Abdel-Kader *et al*. (2007) used water extracts of some wiled plants against the two-land snails *Monacha cartusiana* and *Theba pisana*. These plants were *Azadirachta indica*, *Nerium oleander*, *Calotropis procera* and *Urginea maritime* (different parts: leaves, stems and flowers). The obtained results indicated that the using of some plant water extracts, as spraying technique was more efficient against land snails than in its addition to lettuce leaves as poisonous foods or using the grinded plant parts itself.

Concerning the effect of the plant extracts in the present study on the freshwater snails *Lymnaea caillaudi* and *Biomphalaria alexandrina*, it was noticed that they affect positively these snails mortality. Moreover, it was clearly obvious that the effect of *E. conyzoides* and *E. milii* extracts were the most active according to the used concentrations, but the extract of *E. milii* exhibited the highest molluscicidal activity including the least concentrations.

Sermsart *et al*. (2005) studied the effect of *Euphorbia milii* on the snail *Indoplanorbis exustus* and said that crude latex of *E. milii* is promising and very potent plant molluscicide for killing *I. Exustus* snails. One of the greatest advantages of *E. milii* is that it requires only a small volume of plant material during plant multiplication (as they used some *E. milii* hybrids) and extraction stages, as will as a small volume of extracted product needed for stack. They added also, handling the plant requires some care due to the numerous thorns along its stems, and with possible squirting of the crude latex into the eyes. Adoption of safely measures, such as wearing appropriate gloves and goggles during handling, is advised.

Mello-Silva *et al*. (2006, 2007, 2010) treated *Biomphalaria glabrata* by the latex of *Euphorbia splendens var. hislopii.* They concluded that molluscicides have been used as one of the strategies to control schistosomiasis. Many plant extracts with molluscicidal effects have been tested, but the action of the latex of *Euphorbia splendens var. hislopii* is considered the most promising because it meets the recommendations of the world health organization (WHO).

Shanta *et al*. (2008) examined the molluscicidal effects of some indigenous plants such as Dhol kalmi (*Ipomoea fistulosa*), lantana (*Lantana camara*) Rakta-Karabi (*Nerium indecum*), Polash (*Butea frondosa*), Mohavringoaj (*Wedelia calandulacea*), Nishinda (*Vitex negundo*), Bishkatali (*Polygonum hydropiper*), Kalmi (*Ipomoea aquatica*), Haicha (*Alternarnthera sessilis*) and Shaora (*Streblus asper*). The extracts of these plants were used against *Lymnaea auricularia*, *Lymnaea luteola* and *Indoplanorbis exustus*. Ethanol extracts were more toxic than other organic extracts.

Singh and Singh (2005) reported the mortality caused by the aqueous extract and latex of *Thevetia peruviana* against harmful freshwater snail *Lymnaea acuminate*. The observations in the present study are in accordance with many authors using different plant species as molluscicides.

In Egypt, **Bakry (2009b)** used the extracts of ten plant species. These plants were Guayacum officinalis, Euphorbia splendens, Chenopodium murale, Cestrum parqui, Calotropis procera, Carissa carandus, Conyza dioscoridis, Lantana camara, Atriplex stylosa and Calligonum comosum. He used these extracts to control Biomphalaria alexandrina snails. The author reported that among the ten tested plants E. splendens had the highest molluscicidal activity against B. alexandrina snails followed by A. stylosa then G. officinalis plants. In the present investigation a similar result was recorded, where the methanolic extract of Euphorbia milii was the most effective one on B. alexandrina and L. caillaudi snails including the least concentrations.

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