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

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Keywords: Calotropis procera, Pelargonium graveolens, Ethulia conyzoides Euphorbia milii, Lymnaea caillaudi, Biomphalaria alexandrina, Eobania vermiculata

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 (Miller), 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. mili (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-weighted 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 1 liter 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 (1988a). 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

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. mili . 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 differential susceptibility to the ethanol extract of E. miliii.

Keywords: Calotropis procera, Pelargonium graveolens, Ethulia conyzoides Euphorbia milii, Lymnaea caillaudi, Biomphalaria alexandrina, Eobania vermiculata
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).

<table>
<thead>
<tr>
<th>Plants</th>
<th>Used solvent</th>
<th>Molluscicidal Activity (ppm)</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Calotropis procera</em></td>
<td>Hexane</td>
<td>3412.995, 11561.991</td>
<td>2.419</td>
</tr>
<tr>
<td><em>Pelargonium graveolens</em></td>
<td>Hexane</td>
<td>5916.165, 24558.908</td>
<td>2.073</td>
</tr>
<tr>
<td><em>Ethulia conyzoides</em></td>
<td>Ethanol</td>
<td>8578.855, 24040.848</td>
<td>1.756</td>
</tr>
<tr>
<td><em>Euphorbia milii</em></td>
<td>Methanol</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

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.

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.
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.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Used solvent</th>
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<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calotropis procera</td>
<td>Hexane</td>
<td>56.608, 183.779 (3.28, 154.105)</td>
<td>2.778</td>
</tr>
<tr>
<td>Pelargonium graveolens</td>
<td>Hexane</td>
<td>25.41, 77.965 (3.149, 271.471)</td>
<td>2.632</td>
</tr>
<tr>
<td>Euphorbia conyzoides</td>
<td>Ethanol</td>
<td>17.443, 57.781 (3.906, 239.972)</td>
<td>2.464</td>
</tr>
<tr>
<td>Euphorbia milii</td>
<td>Methanol</td>
<td>9.304, 34.027 (3.859, 59.799)</td>
<td>2.264</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Used solvent</th>
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<th>Slope</th>
</tr>
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<tr>
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</tr>
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</table>

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.
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.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Used solvent</th>
<th>Molluscidal Activity (ppm)</th>
<th>Slope (Regression coefficient)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Calotropis procera</em></td>
<td>Hexane</td>
<td>33.12</td>
<td>78.64 (80.35, 78.95)</td>
</tr>
<tr>
<td><em>Pelargonium graveolens</em></td>
<td>Hexane</td>
<td>67.76</td>
<td>159.88 (143.67, 176.56)</td>
</tr>
<tr>
<td><em>Euphorbia conyzoides</em></td>
<td>Ethanol</td>
<td>29.92</td>
<td>52.09 (50.33, 53.73)</td>
</tr>
<tr>
<td><em>Euphorbia milii</em></td>
<td>Methanol</td>
<td>20.03</td>
<td>81.94 (71.75, 91.31)</td>
</tr>
</tbody>
</table>

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.
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 snakes pests such as (Hussein and El-Wakil, 1996; Ghamry, 1994, 1997). Zidan et al. (2001) used several plant extracts against Monacha obstruca, 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 obstruca and Eobania vermiculata. Afifi et al. (2007) studied the effect of some plant extracts on the glassy clover snail Monacha obstruca 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 wild plants against the two-land snails Monacha cartusiana and Theba pisana. These plants were Azadirachta indica, Nerium oleander, Calotropis procera and Urginea maritima (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 caullaudi 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 potnet 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 well 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).

**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 (Raphanus stivus), Oshar (Calotropis procera) and Datura (Datura stramonium). 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.

**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.
Shanta et al. (2008) examined the molluscicidal effects of some indigenous plants such as Dhol kalmi (Ipomoea fistulosa), lantana (Lantana camara) Rakta-Karabi (Nerium indicum), Polash (Butea frondosa), Mohavringoaj (Wedelia calandulacea), Nishinda (Vitex negundo), Bishkatali (Polygonum hydropiper), Kalmi (Ipomoea aquatica), Haicha (Alternanthera sessilis) and Shaora (Strebulus 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 acuminata. 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 carandas, 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 millii was the most effective one on B. alexandrina and L. calaaudi snails including the least concentrations.

References


