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# Molluscicidal efficacy of ethanol extracts of cumin, moringa and golden shower against glassy clover land snail, *Monacha Cartusiana* (Gastropoda: Hygromiidae) under laboratory conditions

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**Abstract**

The molluscicidal effects of three ethanolic crude extracts, cumin (*Cuminum cyminum*), golden shower (*Cassia fistula*) and moringa (*Moringa oleifera*) were evaluated against *Monacha cartisiana* (O.F.Müller) (Gastropoda: Hygromiidae) land snail under laboratory conditions. Three methods of bioassay were used, *i.e.* contact, leaf-dipping and bait techniques. The results indicated that the ethanol crude extract of cumin was the most toxic extract for the *M. cartisiana* land snail followed by moringa extracts while golden shower extract had the lowest effect. Results showed that the contact technique of the tested plant extracts was the most effective method of application. The $LC_{50}$ values of cumin, moringa and golden shower extracts when applied as contact were 235.9, 266.0 and 292.8 ppm, respectively. The plant constituents present in the three extracts that may be responsible for their molluscicidal activity are sterols and triterpenes, alkaloids, carbohydrates and glycosides, tannins, saponins, cardiac glycosides, and flavonoids.

## Introduction

Terrestrial snails were considered as one of the most serious and dangerous agricultural pests to different varieties of commercial crops, fruits, vegetables, and ornamental plants causing heavy damage to the plant leaves, fruits, tubers, buds and roots. Globally, terrestrial gastropods pose the greatest threat to sustainable agriculture, and they are crucial in the transmission and spread of diseases to domestic plants (De Ley *et al*., 2020; Gaber *et al*., 2022; Kandil *et al*., 2020 and Shahawy, 2019). In Egypt, terrestrial snails occur in high population number in the Delta region and North Coast belt of the Mediterranean Sea of Egypt, (Kassab and Daoud, 1964; El-Okda, 1979, 1980, 1981, 1984; Godan, 1983; Mohammed, 2015 and Ali and Robinson, 2020) causes serious economic damage especially in horticulture and ornamental plants (Godan, 1983).

Terrestrial snails are usually controlled chemically using traditional

pesticides or molluscicides, these molluscicides were applied as dust, baits, or sprays (Mortada *et al*., 2013; Shahawy, 2019 and De Ley *et al*., 2020). The application of low doses of such molluscicides was effective, more practical, and less harmful to non-target species, however, high doses cause environmental contamination and has a negative impact on non-target species like humans and vertebrate animals (Hamed *et al*., 2007 and Radwan *et al*., 2008).

Although the number and types of specific molluscicides used for controlling the snails are limited, they cause different environmental problems in addition to the toxic effects on non-target organisms (Buchs *et al*., 1989). Scientists' attention has been directed toward monitoring the molluscicidal activity of different plants (Hamdy *et al*., 1994; El-Hawashy *et al*., 2001; Truiti *et al*., 2005 and Mortada *et al*., 2012).

The present study was carried out to evaluate the molluscicidal efficacy of three ethanolic crude plant extracts, cumin (*C. cyminum)*, moringa (*Moringa oleifera*), and golden shower (*C. fistula*) against *Monacha cartisiana* (O.F.Müller) (Gastropoda: Hygromiidae) land snail.

## Materials and methods

### 1. Tested plants:

The experimental plants selected for this study are listed in Table (1).

**Table (1): Names, used parts and location of the different plant species studied.**

| English Name | Latin Name | Used part | Location |
|---|---|---|---|
| **Cumin** | *Cuminum cyminum* | Flowers | Giza |
| **Moringa** | *Moringa oleifera* | Leaves | Sohag |
| **Golden shower** | *Cassia fistula* | Seeds | Giza |

### 2. Extraction procedure:

The extraction of the tested samples was conducted according to Freedman *et al*. (1979) with minor modification (where tested samples were soaked in the chosen solvent instead of using the soxhlet procedure). Plant material was ground into fine powder, then 150 gm of the dried powder was extracted with about 750 mL of ethyl alcohol 95%. The produce extracts were concentrated using a rotary evaporator and kept in the refrigerator until testing. The crude concentrates of extracts were diluted with distilled water. Five concentrations for each plant extract were used.

### 3. Preliminary screening of the phytochemical constituents in plant extracts:

**3.1. Sterol and triterpenes test:** Sterol and triterpenes were determined according to the method adapted by Wall *et al*. (1964).

**3.2. Tanins:** Tanins were determined by method described by Claus (1961).

**3.3. Phenolic glycosides:** Balbaa (1981) determined. Phenolic glycosides by the following procedure, some drops of sulfuric acid were added to 1ml plant extract red color is produced and disappears with addition of water Phenolic glycoside is present.

**3.4. Cardic glycosides:** Cardic glycosides were determined according to Balbaa (1981).

**3.5. Anthraquinone glycosides:** Anthraquinone was calculated according to Balbaa (1981).

**3.6. Alkaloids:** Alkaloids were estimated by the method described by Romo (1966).

**3.7. Saponins glycosides:** Saponins glycosides were calculated according to the method mentioned by Wall *et al*. (1964).

**3.8. Carbohydrates and glycosides:** Carbohydrates and glycosides were determined using the method Adopted by Karawya and Abd El-Wahab (1975).

**3.9. Flavonoids:** Flavonoids were determined according to the method adopted by Claus (1961).

**4. Tested snails:**

Adults of the land snail *M. cartusiana* were handily collected from infested fields at Bahada Locality, EL-Qanter ELkhaereia, district, Kalubia Governorate. The obtained snails were transferred in plastic bags to the laboratory, then kept in plastic containers filled with (5 - 7 cm) moist sterilized sand soil loamy 1:1 (v:v) and fed on fresh lettuce leaves for 14 days to be laboratory acclimatized. Dead snails were removed, and only healthy ones were used in the experiments. Laboratory conditions at 25 °C ± 2 °C and 75 % RH. ± 5 % Soil moisture (Mortada, 2002; Khidr, 2010 and Asran *et al*., 2011).

**5. Bioassay test:**

**5.1. Contact method:**

The thin layer film technique was used according to Ascher and Mirian (1981). Five concentrations of the tested plant extract were prepared using distilled water. Two ml of each concentration were deposited and distributed on the bottom of a petri dish by moving the dish gently in circles. Water evaporated under room conditions in a few minutes leaving a thin layer film of the applied concentration of the tested plant extracts. Five healthy adults snails of the tested species were placed and exposed to the candidate concentration of the tested extract for 72 hrs., then transferred to another plastic boxes (24× 10 ×12 cm), closed with muslin cloth containing optimal soil (3-5 cm) and provided with fresh lettuce leaves. A parallel control test was carried out using water only.

**5.2. Leaf-dipping method:**

Serial concentration of each plant extract, fresh lettuce leaves (10 × 15 cm) were dipped for three minutes and were left to dry under laboratory conditions (Ghamry, 1994). The treated leaves were placed inside plastic boxes (24 × 10 × 12 cm) containing optimal soil and a piece of filter paper to adsorb the moisture. Five healthy adult snails of each tested species were used for each replicate. The snails were supplied with treated leaves for 72 hours, then supplied with untreated leaves for 4 successive days. Untreated control snails were fed on water treated leaves.

**5.3. Poisonous baits method:**

The tested extracts were evaluated as poisonous baits. The poison bait was prepared by mixing each concentration with 93% bran and 5% molasses (Aly, 1994). Five grams of each bait were spread on the bottom of a cylindrical glass vessel (9 cm diameter × 7 cm height) and five individuals of the adult snails of the tested species were confined in each vessel and the vessel was covered with muslin cloth and fastened with a rubber band to prevent snails from escaping. Animals were exposed to candidate concentrations of the tested compounds for 72 hrs., then transferred to another plastic boxes (24 × 10 × 12 cm) containing optimal soil (3 – 5 cm) supplied with fresh lettuce leaves. In all bioassay methods and in the control group, five replicates of five individual adult snails for each concentration were used. Dead snails were counted daily for up to 7 days and mortality percentages were estimated and corrected according to Abbott`s formula (Abbott, 1925). The lethal concentration that killed 50% of the snails ($LC_{50}$) values, and slopes were calculated as described by Finney (1971).

**Results and discussion**

This study evaluated the molluscicidal effects of ethanolic extracts of three plants, cumin, moringa, and golden shower, against the terrestrial snail (*M. cartusiana)*, using three different application techniques (Contact, dipping, and bait). The $LC_{50}$ values were determined for each plant extract and application technique.

Table (2) shows the results of a study that tested the efficacy of three ethanolic plant extracts against *M. cartusiana* using a contact technique. Results showed that on the basis of $LC_{50}$ values, Cumin and moringa

extracts proved to be more toxic than golden shower extract against the *M. cartusiana.* The corresponding $LC_{50}$ values were 235.9, 266.0, and 292.8 ppm, respectively. The LSD value is a measure of the smallest difference between the means that is statistically significant. In this case, the LSD value of 11.5 ppm is significant at the $p < 0.05$ level, which means that there is a 95% probability that the difference between the means is due to a real difference between the groups, rather than chance.

**Table (2): Effect of some ethanolic plant extracts against *Monacha cartusiana* using contact technique.**

| Plant extract | $LC_{50}$ ppm | 95% Confidence limits | | P value |
|---|---|---|---|---|
| | | Lower | Upper | |
| **Cumin** | 235.9 | 208.28 | 265.36 | 0.670 |
| **Moringa** | 266.0 | 247.34 | 290.47 | 0.704 |
| **Golden shower** | 292.8 | 250.40 | 344.20 | 0.078 |
| **LSD** | 11.5 | | | |

As shown in Table (3) similar results were obtained when the tested plant extracts were applied using leaf-dipping technique. The corresponding $LC_{50}$ values of cumin, moringa and golden shower extracts were 266.8, 319.0 and 345.5, ppm, respectively. The results of this experiment suggest that cumin extract may be a potential alternative to synthetic insecticides for the control of *M. cartusiana*. The data were analyzed using a one-way analysis of variance (ANOVA) to compare the $LC_{50}$ values of the three plant extracts. The ANOVA results showed that the F-value was significant at the $p < 0.05$ level, indicating that there was a significant difference between the three plant extracts. The LSD value for the ANOVA was 20.9.

**Table (3): Effect of some ethanolic plant extracts against *Monacha cartusiana* using dipping technique.**

| Plant extract | $LC_{50}$ ppm | 95% Confidence limits | | P value |
|---|---|---|---|---|
| | | Lower | Upper | |
| **Cumin** | 266.79 | 247.3 | 290.4 | 0.7044 |
| **Moringa** | 319.0 | 300.7 | 337.5 | 0.9705 |
| **Golden shower** | 345.5 | 291.8 | 415.8 | 0. 746 |
| **LSD** | **20.9** | | | |

Table (4) shows that the same trend of susceptibility to the tested plant extracts among the *M. cartusiana* land snail was observed when the tested plant extracts were used as baits. The $LC_{50}$ values of cumin, moringa, and golden shower extracts were 316, 370 and 374 ppm, respectively. The results of this study showed that the ethanolic plant extracts of cumin, moringa, and golden shower were all effective in controlling *M. cartusiana*. The $LC_{50}$ values for cumin were the lowest, indicating that it was the most effective extract. The $LC_{50}$ values for moringa and golden shower were higher, indicating that they were less effective than cumin.

**Table (4): Effect of some ethanolic plant extracts against *Monacha cartusiana* using bait technique.**

| Plant extract | $LC_{50}$ ppm | 95% Confidence limits | | P value |
|---|---|---|---|---|
| | | Lower | Upper | |
| **Cumin** | 316 | 249.8 | 338.9 | 0.745 |
| **Moringa** | 370 | 348.36 | 394.5 | 0.556 |
| **Golden shower** | 374.8 | 342.17 | 405.15 | 0.1095 |
| **LSD** | **15.24** | | | |

The obtained results showed that there are different susceptibility levels *M. cartusiana* snail according to type of plant extracts and method of application (Contact or leaf dipping or bait). Godan (1983) stated that the phases of greater or lesser sensitivity differ from one species to another with shorter or longer life spans, but the general pattern of changing susceptibility with physiological conditions remains.

The molluscicidal activity of different plants was previously studied proving the molluscicidal activity of powder and crude extract of some cruciferous seeds on the three land snail species (Ghamry, 1994). El-Deeb *et al*. (1999) recorded that ethanol extract of khella fruits was effective against *M. contiana* land snail. El- Sebaii *et al*. (2000) indicated that the Calotropis procera plant was found to have molluscicidal activity against the two terrestrial snail species. El-Hawashy *et al*. (2001) reported that the extracts of cauliflower, oshar and pergulania were effective against *E. vermiculata*. Ebenso (2004) found that the crude extracts of bark, root and leaf of neem produced mortality for land snails. Truiti *et al*. (2005) proved acute molluscicidal activity of the ethanolic extract of *Melochia arenosa* and *Nectandra falcifolia* on the snail, *Biomphalaria globrata*. Also, Mahmoud and Bakr (2008) found that Hellebore plant extract suppresses the reproductive rate of the land snails, *E. vermiculata* and *M. obstructa*. Also, Mourad (2014) found that the ethanol crude extract of cumin was the most toxic extract for the two tested land snail species followed by golden shower, Umbrella tree and pomegranate extracts while olive extract had the lowest effect.

The plant constituents present in the three extracts that may be responsible for their molluscicidal activity are sterols and triterpenes, alkaloids, carbohydrates and glycosides, tannins, saponins, cardiac glycosides, and flavonoids. Cumin and golden shower extracts both contain high levels of sterols and triterpenes, alkaloids, and flavonoids, all of which are known to have molluscicidal activity (Table 5). Moringa extract contains high levels of carbohydrates and glycosides, as well as moderate levels of tannins, saponins, and cardiac glycosides (Table 5).

**Table (5): Preliminary photochemical screening of seeds cumin, golden shower and moringa plant extract with ethanol.**

| No. | Pant extraction constituents | Plant used | | |
|---|---|---|---|---|
| | | Cumin | Golden shower | Moringa |
| 1 | Sterols and triterpenes | ++ | + | + |
| 2 | Tannins | ± | ++ | ++ |
| 3 | Phenolic glycosides | + | + | + |
| 4 | Cardiac glycosides | + | + | ++ |
| 5 | Anthraquinone | ++ | + | ± |
| 6 | Alkaloid | ++ | + | + |
| 7 | Saponins | ± | + | + |
| 8 | Flavonoids | ++ | + | ++ |
| 9 | Carbohydrate | +++ | +++ | + |

**+++ high amount ± Trace amount ++ Moderate amount + slight amount**

The obtained results confirm that sterols and triterpenes, anthraquinone, flavonoids, alkaloids and carbohydrates and glycoside were found in varying amounts in all tested plants, these compounds mostly act as pesticide agents (Su, 1984 and 1990). Such results agree with many investigators. Edward *et al*., (1993) studied the terpenoid

composition of 6 species of Eucalyptus, all 6 Eucalyptus species showed that cineole (eucalyptol) content ranged from 13% to 78% of the total oil. Ali (1999) separated nine phytochemical components in leaves and seeds of both red and spotted gum. Such components were found in different amounts according to plant species, plant part and solvent used.

The partial chemical analysis of methanol extract of *C. coggygria*, showed flavonoids, tannins and phenolic compounds (Stanic *et al.*, 2009). Muley *et al*. (2009) found the presence of active substances in the *C. officinalis* flowers by phytochemical study. The results obtained were terpenoids, flavonoids, quinones, volatile oil, carotenoids, amino acids and carbohydrates.

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