Archives of Agriculture Sciences Journal
Print ISSN: 2535-1680
Online ISSN: 2535-1699

ARCHIVES OF AGRICULTURE SCIENCES JOURNAL

Volume 7, Issue 1, 2024, Pages 73–84

Available online at https://aasj.journals.ekb.eg

DOI: https://dx.doi.org/10.21608/aasj.2024.255815.1158

In vitro assessment of plant extracts for controlling land snail (*Monacha obstraucta*)

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Abstract

Land snails (*Monacha obstructa*) are commonly found in a variety of plant species, making them an important target for this study. Therefore, the opjective of this study is to compare eleven plant extracts and select the most efficient one to evaluate the acute toxicity of their extracts using the contact method against *Monacha obstructa*. The findings revealed that the plant extracts with the highest effectiveness against land snails were chinaberry (*Melia azedarach*), apple of Sodom (*Calotropis procera*), Nerium (*Nerium oleander*), and Basil (*Ocimum basilicum*) leaf extract. Consequently, the results indicated that the aqueous extracts from the leaves of *M. azedarach* exhibited high toxicity, with an LC_{50} value of 11.88 mg/L and a 100% mortality rate. Following that, *C. procera* leaves had an LC_{50} value of 16.82 mg/L and a mortality rate of 70.61%. The aqueous extracts from *N. oleander* (flowers and leaves) displayed a moderate mortality rate of 58.06% and 56.96% with LC_{50} values of 20.46 mg/L and 20.85 mg/L, respectively. The decoction of the leaves of the plants with *O. basilicum* leaf extract had the lowest death rate for individuals after 24 h which was 45.90%, and the toxicity rate for land snails was recorded at 25.88 mg/L. However, the rate of death of treated snails were decreased compared to that of control ones. These findings suggest the potential recommendation of utilizing a decoction of the extracted plants as an alternative to expensive and environmentally harmful pesticides for controlling clover snails.

Keywords: plant extracts, *Monacha obstructa*, *Melia azedarach*, *Calotropis procera*, *Nerium oleander*, *Ocimum basilicum*.

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1. Introduction

Gastropods, including terrestrial snails, are a significant and widespread pest problem for many plants globally. In Egypt, terrestrial gastropods have emerged as the most crucial agricultural pests, leading to substantial crop damage across different regions (Ali-Asmaa, 2014; El-Deeb *et al*., 1999; Ibrahim, 2017; Mahrous *et al*., 2002). Furthermore, Ali and Ramadane (2020) reported that clover land snails (*Monacha* sp.), are a widely recognized species in Egypt, found in all governorates and observed on various crops and trees. According to Gabr *et al*. (2006), chemical control techniques are still often used to manage land snails, (*Monacha* sp.), even though there may be unexpected consequences for humans and other creatures. The employment of pest control methods obtained from natural sources is encouraged by the high cost of synthetic pesticides and the development of resistance to them (Massoud and Habib, 2003). Due to their low cost, quick biodegradability, and minimal mammalian toxicity, natural products are being used in pest control in a growing number of nations (Abd El-Atti, *et al*., 2019; Singh *et al*., 2000). Synthetic molluscicides are expensive, environmentally damaging, and toxicity-prone, while plant molluscicides offer powerful activity, low resistance, and a high biodegradation rate (Abd El-Atti *et al*., 2019; Nikoli *et al*., 2014; Thakur *et al*., 2019). The effect of several plant extracts was evaluated on the glassy clover snail, *M. obstructa* using the baiting approach, and it found that plant extracts caused more than 90% mortality after seven days of treatment (Afifi *et al*., 2007). Other studies investigated the efficacy of eleven different water extracts on *Eobania vermiculata*. After 24 h, aqueous extracts of wild beets and oleander exhibited the most significant percentage mortality of 90 - 100%, whereas Jimpson weed showed 70% mortality (Kassem *et al*., 1993), as well as Olofintiye *et al*. (2011) found larvae mosquito mortality rate under 50%, but at 100% concentration, it reached 100%. Additionally, Baleta *et al*. (2015) observed it was toxicity more pronounced in small golden apple snails than large snails. Also, the boiling water extract for the leaf of *Datura stramonium* has phenolic compounds that cause 57.5% repulsion for insects (Amira *et al*., 2020). Using the baiting method, Afifi *et al*. (2007) assessed the effects of different plant extracts on the glassy clover snail, *M. obstructa*, which caused more than 90% of deaths after seven days of exposure. The effectiveness of eleven water extracts on *Eobania vermiculata* was examined by Kassem *et al*. (1993). Aqueous extracts of oleander and wild beets demonstrated the highest rate of mortality after 24 hours, with a moderate 95%, while Jimpson weed only showed 70% death. Furthermore, Amira *et al*. (2020) found that phenolic chemicals in the boiling water extract of *Datura stramonium* leaves generated 57.5% repelling of insects. The objectives of this study are to assess the effectiveness of

eleven plant extracts for anti-land snail (*Monacha obstructa*) activity, using the contact poison approach to select eleven plant extracts that are effective against snails. Also, the LC_{50} for the finest plant extracts were measured by making many concentrations.

2. Materials and methods

2.1 Tested snails

Mid-May 2022 saw the manual collection of adult *M. obstructa* snails from infesting nurseries inside the Giza governorate, Egypt. The animals were placed in clear bags and brought to Agricultural Zoology and Nematology Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. The animals were housed in plastic dishes that were filled with a mixture of soil enough moisture and covered to keep the snails from fleeing. *In vitro*, they were fed leaf lettuce for two weeks at 27±2 ºC and RH 65%. Each replication consisted of three sets of ten mature snails. Before the investigation started, the animals were deprived for 48 h (Shetaia, 2005).

2.2 Tested plants

Test seven families containing ten plant species. The families were Apocynaceae (Nerium, *Nerium oleander*; Apple of Sodom, *Calotropis procera*); Euphorbiaceae (Fireplant, *Euphorbia heterophylla*; Jatropha, *Jatropha curcas*); Lamiaceae (Basil, *Ocimum basilicum*); Lauraceae (Camphora, *Cinnamomum camphora*); Meliaceae (Neem, *Azadirachta indica*; chinaberry, *Melia azedarach*); Rosaceae (Bitter Orange, *Citrus aurantium*); and Solanaceae (Jimsonweed, *Datura stramonium*). Their leaves were used to make a plant extract. In addition to using Nerium flowers next to the leaves.

2.3 Procedure for extraction

With certain adjustments, aqueous plant extraction was performed similar to the technique reported by Mwonga *et al*. (2015), Ahmed (2020) and Al-Jebory *et al.* (2023). 250 g of the green leaf plant was cooked in half a liter of distilled water for five minutes. After boiling, the solution was allowed to cool to room temperature. Following that, the extract was transferred to a 500 mL glass cup and filtered under lab conditions with filter paper (18.5 cm) before being transferred to a 500-mL clean, dry conical flask. The combination was filtered until the sample became transparent. The aqueous extracts were refrigerated until they were employed in the bioassay.

2.4 Contact toxicity assay

The contact approach was employed with various changes, according to Asher and Mirian (1981). Only crude extract was employed in the first trial to differentiate between the crud-boiled water extracts of ten plants. The following study used different concentrations (10, 20, 30, 40, 50, and 100% v/v) of the most effective

crude extracts (basil, chinaberry, Apple of Sodom, and "flowers and leaves" for Nerium). Five mL of each concentration was deposited on the inner surface of a petri dish measuring 15 cm by gently moving the dish in circles. At normal temperatures, the water evaporated after a few minutes, leaving a thin layer of film of the tested compounds on the top of the Petri plates. In the three repetitions, ten snails were treated for 24 h to assess the varying doses of the five tested extracts. As a control, plain water was utilized. In the first study, mortality was tallied and documented over 3 days, while in the second trial, it was reported after 24 h. Snails that stayed fully dry within their shells and did not respond to mechanical probing were considered dead. All extracts' activity against adults of *M. obstructa* was represented as a percentage of mortality.

2.5 Data analysis

After the period, mortality percentages were computed and adjusted for natural mortality using LdP line oftware Bakr (2005) to calculate probit analyses using Finney's (1971) and Abbott's (1925) formulas.

3. Results and Discussion

Figure (1) showed that on the first day, Nerium flower extract provided 100% mortality from the molluscicide action of crud-boiled water plant extracts, and Apple of Sodom extract caused 100% fatality on the second day. On the other hand, over the two days, varying mortality rates of snails appeared in the aqueous extracts of (chinaberry tree, basil, and Nerium leaves), and bitter orange was (60 and 90%) and (20 and 60%) and (10 and 60%) and (10 and 40%), respectively. While individuals' mortality rate reached 100% on the third day for chinaberry tree, basil, and Nerium leaf extracts. Besides, the mortality rate for bitter orange extract was 80% on the third day. The main components of sweet and bitter orange essential oils are linalyl acetate and linalool, which are effective on biomarkers of land snail species (Haj Ammar *et al*., 2012; Fagodia *et al*., 2017; Gonzalez-Mas *et al*., 2019). Moreover, the rest of the aqueous extracts of other plants did not affect the snails on the first day and showed their effect on the second and third days with mortality rates ranging from 20 to 40% for plant leaf extracts such as jatropha, fireplant, jimsonweed, and neem which were (40, 30, 20, and 20%), respectively. These findings are consistent with Maruni, *et al*. (2022) discovered that an extract from *Jatropha curcas* leaves were harmful to an immature golden snail and caused death within 24 h. Thapa Magar (2021) discovered that plant extracts of plant parts of neem and tirupati were more efficient in repelling and eradicating Giant African Land Snails at 5% concentration. While Sarma *et al*., (2019) found the Aqueous extract of the medicinal plant, *Azadirachta indica* (Neem) caused (98.00 ± 0.29) mortality at 6% concentration after 72 h of an adult

insect, banana beetle with an LC_{50} rate was 3.95 mg/L, by direct spray way under the laboratory. Nonetheless, the toxicity of aqueous neem leaf crude extract was lower than that of aqueous neem seed crude extract for both small and large golden apple snails (Massaguni and Latip, 2011). But Hasballah (2015) observed that the petroleum ether extract of *Azadirachta indica* was shown to be the most effective plant extract, then *Ocimum basilicum*, which showed moderate effectiveness against mosquitos. Also, According to Mesbah *et al*. (2022), it was discovered that the essential oil derived from *Azadirachta indica* exhibited higher toxicity toward *Theba pisana* than *E. vermiculata* after 72 hours, with an LC50 of 0.87 and 2.6%, respectively. Additionally, our aqueous extract of camphor leaves did not show an effect on animals during the three days of the experiment. On the contrary, Yang *et al*. (2014) found that leaf extracts from *Cinnamomum camphora* have a linalool component which was effective as a molluscicide and larvicidal for *Schistosoma japonicum* and the snail intermediate host. Also, combining camphor with menthol and its components was more effective than chemical alone on land snails (*M. obstructa*) over seven days utilizing the contact approach (thin-layer film) (Mona *et al*., 2017).

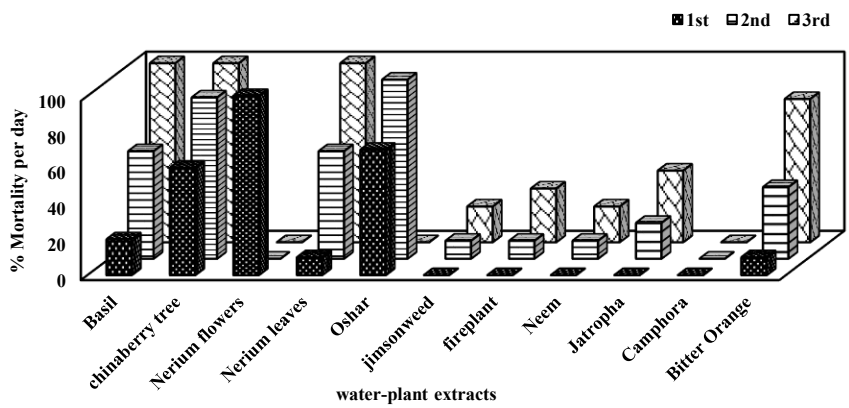

Figure (1): Screening between eleven water-plant extracts of plant´s leaves against land snail (*M. obstructa*) under lab conditions for three days.

The findings reported in Figure (2) from the second experiment showed that five extracts were the most efficient natural products for killing snails after 24 h of application. The study analyzed data (Tabel 1 and Figure 3) on toxicity levels after one-day exposure, using Finney (1971) Abbott's formula (1925), and Ldp line software by Baker (2005). Hussein (2007) indicated that *Nerium oleander*, a

natural molecule extract, is more toxic to *Theba pisana* snails than two chemicals, with an LD_{50} value of 12.039 µg/gm of body weight. Basil leaf extract was the lowest death rate for individuals after 24 h which was 45.90%, and the toxicity rate for land snails was recorded at 25.88 mg/L. As Youssef (2006) stated, the ethyl alcohol crude extract of sweet basil was weakly affected by the land snail *M. obstructa.* Nevertheless, according to Yousif and Satti (2012), aqueous extracts of *Azadirachta indica* were the most effective plant, followed by *Ocimum basilicum*, with 100% mortality for Khapra beetles within 18 days of treatment.

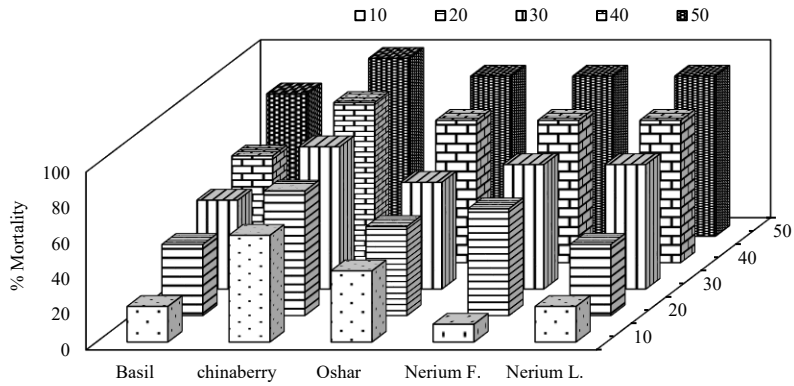

Figure (2): Evaluation mortality for five water-plant extracts against land snail (*M. obstructa*) during 24 h.

The regeneration lines of tested aqueous extracts of plant leaves or flowers against tested animals were high mortality and LC_{50} values which were recorded (100% and 11.88 mg/L) and (70.61% and 16.82 mg/L), for chinaberry and Apple of Sodom leaves, respectively. Chinaberry leaf extract contains potent triterpenoid saponins that block eating and induce 100% mortality and molting failure in the following generations of insects (Bullangpoti *et al*., 2012; Chen *et al*., 1996; Vergara *et al*., 1997).

Table (1): LC_{50} of certain plant extracts after five days on adult snails, using contact technique under laboratory conditions.

Plants	Corrected response (%)	LC_{50} (mg/L)	95% fiducial limits		Slope ±SE
			Lower limit	Upper limit	
Chinaberry	100	11.881	3.152	18.309	2.298±0.731
Apple of Sodom	70.611	16.826	7.363	24.416	2.296±0.670
Nerium flowers	58.064	20.462	13.535	26.796	3.363±0.814
Nerium leaves	56.967	20.856	13.612	27.538	3.204±0.783
Basil	45.901	25.884	15.567	37.333	2.369±0.676

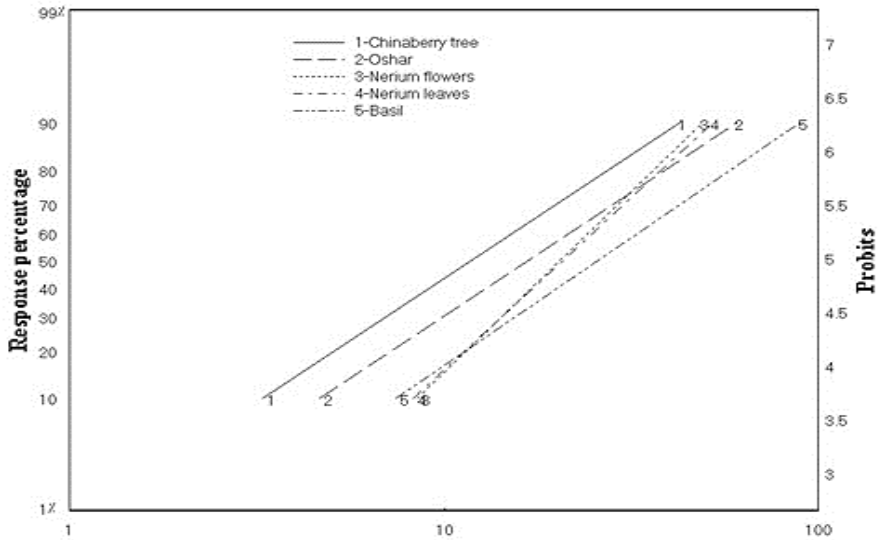

Figure (3): Under laboratory circumstances, the Ldp line was tested against the land snail (*M. obstructa*), for 24 h using a contact approach.

Besides, after 72 h of exposure, the LC_{50} value was 161.4 ppm of *Melia azedarach* fruit water extract for adult freshwater snails according to Mohamed *et al*. (2006). Also, Apple of Sodom had an active compound that was more toxic than Lannate for the land snails (*T. pisana*), (Hamdy *et al*., 1994; Hussein *et al.,* 1994). In addition, using water extracts of Neem, Nerium*,* Apple of Sodom, and Red Squill as a spraying technique was more efficient against Carthusian and white garden snails than lettuce leaves as toxic meals or utilizing ground plant components directly (Abdel Kader *et al*., 2007). Our study indicated that aqueous extracts for Nerium (flowers and leaves) were enrolled with moderate percentage mortality which was (58.06 and 56.96%) respectively. Furthermore, LC_{50} values for Nerium extracts of flowers and leaves were 20.46 mg/L and 20.85 mg/L respectively. On the contrary, leaf extract *Nerium oleander* has cardenolide which is more toxic to white garden snails (Hussein, 2007).

4. Conclusion

The current study found that *Melia azedarach* has the most efficient molluscicide ability to diminish (*M. obstructa*), followed by *C. procera* and *Nerium oleander* (flowers and leaves). As a result, there is the prospect of generating a source of substitute molluscicide chemicals for the long-term control of terrestrial mollusk pests. Consequently, integrating plant extracts, which have been determined to be less dangerous and are an essential component of today's eco-friendly environment, is perhaps a method to reduce the hazardous impact of a chemical.

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