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# Apoptotic and histopathological impacts of *Moringa oleifera* seed oil on the land snail *Cornu aspersum* (O. F. Müller, 1774)

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## Abstract

*Cornu aspersum* (O. F. Müller, 1774) is among the most harmful pests for many crops. Using natural molluscicides is essential to reduce the adverse effects of chemical ones on the biosystem. The toxicity of *Moringa oleifera* seed oil was determined, and $LC_{50}$ and $LC_{90}$ were 20.06% and 29.5%, respectively, after 72 hr of exposure. *Moringa* oil diminished the survival rate of *Cornu aspersum* (O. F. Müller, 1774) by 50% compared to the control (98.6%). Moreover, *Moringa* oil significantly increased the apoptosis and necrosis of digestive and ovotestis gland cells ($P \leq 0.02$). By the end of the experiment, the proportion of apoptotic cells rose dramatically to 62.6% and 50.3% in the digestive and ovotestis glands, respectively, compared to the control (10.8% and 12.3%). In addition, the percentage of necrotic cells significantly increased to 21.5% and 24.7% ($P \leq 0.05$), while the control values were 5.6% and 4.5%, respectively, at the 8% concentration. Regarding the digestive gland, *Moringa* oil caused vacuolation, nuclear pyknosis, and haemocyte infiltration. Deficiency of mature ova and spermatozoa, fibrosis, degeneration, and necrosis were recorded in the ovotestis. *Moringa* oil has proved its effectiveness as a natural molluscicide.

**Keywords:** *Cornu aspersum* (O. F. Müller, 1774), *Moringa oleifera* Seed Oil, survival rate, Apoptosis, Histology.

## Introduction

*Mollusca* are characterized as the second-largest phylum in the animal kingdom due to their great reproductive capacity-especially slugs and snails-which enables them to spread quickly throughout agricultural fields, making population control extremely challenging [1; 2]. The damage caused by these snails is attributed to their rasping feeding habits and the deposition of waste products such as slime and feces. Crop losses of 50–90% in soybean and corn have been reported in India [3; 4]. Land snails are notorious pests that cause significant damage to various crops and vegetation throughout Egypt [5; 6; 7]. These Land snails (gastropods) can damage multiple plant species, leading to major economic losses in agricultural fields, gardens, orchards, and greenhouses [8; 9; 10; 11]. Several authors have recorded different snail species and investigated their harmful effects: *Cornu aspersum* (O. F. Müller, 1774), *Achatina fulica*, *Helix vestalis*, and *Theba pisana* and *Monacha* sp. and *Oxychilus* sp. [12; 13; 14; 15]. These pests have caused serious damage to vegetables, banana crops, tomatoes, ornamental plants, mulberries, grapevines, and germinated seeds [16]. Land mollusks also harm potatoes, grains, lettuce, cabbage, carrots, maize, clover, and other horticultural and field crops. They feed on roots, seedlings, seeds, and tubers of nearly every fruit, vegetable, oil plant, and ornamental species in gardens, greenhouses, and fields. Damage includes killing seedlings (leading to poor stands) and destroying the leaves of young plants. Molluscs' detrimental effects, however, differ depending on the crop. For example, some consume freshly planted wheat seeds, scrape strips of leaves from maize and many small grain plants, and make craters in the cotyledons and ragged holes in the leaves of soybean crops [17; 1; 18].

Pest control for snails requires a continuous and integrated approach. Physical methods such as hand collection and device innovation, the use of simple chemicals like sodium chloride, and the application of registered molluscicides such as methomyl are commonly employed, often in combination with biological control methods [18; 19; 20]. However, the extensive use of pesticides

by agricultural workers in many countries has raised serious health concerns due to the toxicity of these chemicals [21]. Moreover, they often affect non-target organisms and disrupt the ecosystem [22]. Given the side effects associated with each method, there is an urgent need for safe and cost-effective alternatives. As a result, ongoing efforts are focused on discovering and evaluating promising, effective, and eco-friendly molluscicides against pest snail species. Natural plant derivatives, also known as botanical pesticides, have gained attention for their environmental safety. Oil extracts from these plants are excellent natural products-they are biodegradable into non-toxic compounds, easy to access and dispose of, and economically affordable [23].

**Table 1.** Physiochemical properties of *Moringa* seed oil

| **Physical properties** | |
|---|---|
| Refractive index | 1.471 ±0.00 |
| Specific gravity | 0.903 ±0.2 |
| Acid value (mg/g) | 0.58 ±0.21 |
| Peroxide value (Meq/kg) | 2.37 ±0.06 |
| Saponification value (mg KOH/g) | 160.32 ±0.4 |
| Iodine value (g/100g) | 65.27 ±0.21 |
| **Chemical properties (Fatty acids composition)** | |
| **Name** | **Concentration(%)** |
| Palimitic acid C 18:0 | 6.61 ±0.5 |
| Stearic acid C18:0 | 5.30 ±0.5 |
| Oleic acid C 18:1 | 66.12 ±0.04 |
| Linolinic acid C 18:3 | 1.03 ±0.4 |
| Palmitoleic acid C 16:1 | 1.78 ±0.02 |
| Vaccinic acid C16:1 | 5.86 ±1 |
| Linoleic acid C18:2 | 1.47 ±0.1 |
| Arachidic acid C 20:0 | 3.46 ±0.04 |
| Gadolic acidic C20:1 n9 | 2.75 ±0.1 |
| Behenic acid 22:0 | 6.36 ±0.03 |
| Total unsaturated fatty acids | 79.01 ±1.2 |
| Total saturated fatty acids | 21.73 ±1 |

(n=3 replicates, data are mean ±SD)

One of the most well-known and widely distributed species is *Moringa oleifera* [24]. The seed oil extract contains fatty acids similar to olive oil, with linoleic acid serving as a substitute [25]. It is rich in protein, and its leaves contain high levels of minerals such as iron, vitamins, and calcium, making it useful for treating malnutrition [26]. Different parts of the tree including the root, leaf, fruit, and seedalong with their extracts or oils, possess various medicinal properties and have been used in both non-food products and traditional medicine [27]. It possesses antioxidant, anti-inflammatory, antianemic, and antidiabetic properties, supports the immune system, and combats neurological, reproductive, cardiovascular, and bone illnesses. [28; 29]. Furthermore, the bioactive constituents of botanical molluscicides, such as flavonoids, saponins, and tannins found in *Moringa* spp., have been studied for their effects on snails [30; 31; 32]. Thus, the aim of this study is to evaluate the molluscicidal efficacy of *Moringa oleifera* seed oil against *Cornu aspersum* (O. F. Müller, 1774).

## Materials and Methods

### Experimental materials

*Moringa oleifera* seed oil was purchased from the Moringa Unit at the National Research Center, Dokki, Egypt. The analysis of the oil, including its components and physicochemical properties, was conducted at the same unit **(Table 1)**. Methomyl (Copter 90% SP), a carbamate compound (S-methyl N-[(methylcarbamoyl) oxy]thioacetimidate), with the molecular formula C5H10N2O2S, was used as a standard pesticide. It was obtained from Egyptchem International for Agrochemicals (Cairo, Egypt). The required concentrations of *Moringa* oil were prepared by dissolving the oil in a 1% Tween 80 solution (v/v) [33; 34]. The chemical structure of Tween 80 is 2-[2-[3,4-bis(2-hydroxyethoxy) oxolan-2-yl]-2-(2-hydroxyethoxy)ethoxy]ethyl octadec-9-enoate.

### Determination of $LC_{50}$ and $LC_{90}$

To determine the $LC_{50}$ and $LC_{90}$ of *Moringa oleifera* seed oil, acclimatized snails (mean weight: 4.4 ± 0.6 g) were divided into three replicates, with 10 snails in each group. Each group of 10 individuals was sprayed with 10 ml of its specific concentration. A range of concentrations (1%, 3%, 6%, 9%, 15%, 21%, 27%, and 30%) of *Moringa* oil was freshly prepared using a 1% Tween 80 solution (v/v) [35]. Snail mortality was observed and recorded daily for up to 72 hours. After counting and removing the dead individuals, the lethal concentrations ($LC_{50}$ and $LC_{90}$) were calculated using Probit analysis in the SPSS statistical software package (IBM Corp., Armonk, NY, USA). Two sub-lethal concentrations, 4% and 8%, were selected as $LC_{10}$ and $LC_{20}$, respectively.

### Collection of snails and experimental design

*Cornu aspersum* (O. F. Müller, 1774) individuals were collected from garden plants and decorative trees, then transported in plastic boxes to the laboratory. The snails were maintained at a temperature of 23 ± 2°C, under a 12 h photoperiod and relative humidity ranging from 75% to 85%. Each box contained a layer of soil at the bottom and was covered with muslin cloth. The snails were fed fresh lettuce leaves throughout the acclimatization and experimental periods. Snails were randomly divided into six groups, with three replicates per group, and each replicate consisting of 15 snails. The experimental groups were as follows: (a) Control group (unexposed). (b) Two groups exposed to 4% and 8% *Moringa oleifera* seed oil. (c) Two groups exposed to 4% and 8% methomyl. (d) One group exposed to 1% Tween 80 solution. Snails were sprayed daily with 1 ml of the respective treatment solution per snail (15 ml per replicate) over the course of three weeks. Survival rate was recorded as the percentage

**Table 2.** The effect of *M. oleifera* seed oil on the survival rate of *Cornu aspersum* (O. F. Müller, 1774).

| Exposure period | Survival rate | | | | | |
|---|---|---|---|---|---|---|
| | Control | Methomyl | | *Moringa* | | Tween 80 |
| | | 4 | 8 | 4 | 8 | 1 |
| Zero time | 100 ±0 | 100 ±0 | 100 ±0 | 100 ±0 | 100 ±0 | 100 ±0 |
| 1st week | 100 ±0 | 67.6 ±0.5 | 64.3 ±0.5 | 82 ±0.4 | 75.3 ±0.3 | 100 ±0 |
| 2nd week | 100 ±0 | 54.3 ±1.5 | 47.6 ±1.1 | 72 ±0.5 | 63.3 ±1 | 98.9 ±0.5 |
| 3rd week | 98.6 ±0.5 | 34.3 ±1.5$^{a}$ | 30 ±1$^{b}$ | 63.3 ±1 | 50 ±1$^{ab}$ | 97.6 ±0.5 |

n = 3 replicates; data were reported as mean ±SD; $^{a}$ denotes a significant difference between the exposed and control groups and $^{b}$ vs methomyl-exposed group when $P \leq 0.05$.

of snails remaining alive at each observation point during the exposure period. At the end of the experiment, the digestive and ovotestis glands were dissected for apoptosis and necrosis assessment using cell cycle analysis and AnnexinV/PI staining, in addition to histological investigations.

### Cell cycle analysis

Digestive and ovotestis glands (0.08 g) were homogenized in 1500 $\mu$l phosphate-buffered saline (cold) (PBS) and centrifuged at 1,000 rpm for 1 min at 4°C. The supernatant was collected for the assay. 200 $\mu$l of cell suspension in citrate buffer was added, along with propidium iodide (PI). There were 10,000 assessed nuclei on average for each specimen, and 120 nuclei were scanned every second. Accuri™ C6 flow cytometer analysis (Becton Dickinson, United States) was used to assess apoptotic cells utilizing sub-$G_1$ peak labeling with PI [36; 37].

- **$G_0/G_1$ peak**: Normal diploid cells.
- **S phase (Synthesis)**: Cells synthesizing DNA.
- **$G_2$/M peak (Gap 2 / Mitosis)**: Cells ready to divide.
- **Sub-$G_1$**: Apoptotic cells with fragmented DNA.

### Assay of PI/annexin-V dual staining

Discrimination of the apoptotic profile was determined using the Apoptosis Detection Kit I (Cat. No. 556547BD, Pharmingen™, Bioscience, New Zealand) according to the manufacturer's instructions. The principle of this procedure relies on the interaction between the membrane phospholipid phosphatidylserine of apoptotic cells and Annexin V, a phospholipid-binding protein that requires calcium. To differentiate between viable and non-viable cells, propidium iodide (PI) was employed as a conventional flow cytometric viability probe. Non-viable or damaged cells with permeable membranes allow PI to enter, while viable cells with intact membranes exclude PI. The BD Accuri™ C6 flow cytometer was used to analyze the cells immediately. Four distinct cell populations were identified: (1) Early apoptotic cells (bound to Annexin V only), (2) Late apoptotic/necrotic cells (bound to both Annexin V and PI), (3) Necrotic cells (stained with PI only), (4) Viable cells (unstained). The percentage of fluorescent cells in each quadrant was calculated after the fluorescence distribution was presented in a two-color dot plot analysis [38].

### Histological investigation

After three weeks, the digestive and ovotestis glands were dissected and fixed in Bouin's fluid. After 24 hr of fixation, dehydration was performed through a series of alcohols and cleared in xylene. Paraffin blocks were sectioned at 5 $\mu$m thickness on glass slides for hematoxylin and eosin staining [39; 40]. A good wash with tap water was performed after staining. A photo-automated camera (Optika, Italy) was used to capture images of the histological sections, which were then analyzed for histopathological syndromes.

### Statistical analysis

The Statistical Package for the Social Sciences (SPSS; IBM Corp., Armonk, NY, USA) was used to analyze the data, which were presented as mean ± standard deviation. One-way ANOVA was performed, followed by the Least Significant Difference (LSD) post-hoc test to determine the significance between the control and different concentrations of the tested materials. A significance level of $P < 0.05$ was considered statistically significant.

## Results

### Toxical effect of *Moringa oleifera* seed oil

The values (%) of $LC_{50}$ and $LC_{90}$ were 20.06 (95% confidence limit for log = 1.3) and 29.5 (95% confidence limit for log = 1.4), respectively, after 72 hr of exposure. The slopes of $LC_{50}$ and $LC_{90}$ were 2.5 and 3, respectively. The sublethal concentrations used were 4% *Moringa* oil (40 mL/L of 1% Tween 80) and 8% *Moringa* oil (80 mL/L of 1% Tween 80) (v/v) solutions. Additionally, 4% methomyl (4 g / 100 mL) and 8% methomyl (4 g / 100 mL) (w/v) solutions were used.

### The survival rate of *Cornu aspersum* (O. F. Müller, 1774) under the effect of *Moringa* oil

The survival rate of *Cornu aspersum* decreased gradually according to the concentration and exposure duration. The reduction was more significant in methomyl-exposed snails, followed

**Table 3.** Percentage of cells in different cell cycle phases after treatment

| Concentration | Exposure Period | %Sub G1 | % Cells/Cell Cycle | G0/G1 | S | G2/M |
|---|---|---|---|---|---|---|
| **Control** | 1st week | 12.8 ±0.1 | 91.9 ±0.1 | 51.03 ±1 | 4.4 ±0.1 | 24.5 ±0.8 |
| | 2nd week | 11.8 ±0.2 | 95.8 ±0.3 | 52 ±1 | 4.1 ±0.3 | 24.8 ±1 |
| | 3rd week | 11.7 ±0.4 | 96 ±0.4 | 52.1 ±1.2 | 4 ±0.2 | 24.3 ±0.5 |
| **4% *Moringa* oil** | 1st week | 14.9 ±0.6 | 97.2 ±2.9 | 70.1 ±3.3 | 2.1 ±0.2 | 10.4 ±1.9 |
| | 2nd week | 15.02 ±0.1[ab] | 83.2 ±1.4 | 40.1 ±0.7 | 15.03 ±0.05 | 15.5 ±0.7 |
| | 3rd week | 12.4 ±1.2 | 90.9 ±2.9 | 60.1 ±2.07 | 4.1 ±0 | 15.3 ±1.4 |
| **8% *Moringa* oil** | 1st week | 18.2 ±0.3[ab] | 99.2 ±1.3 | 78.3 ±1.4 | 1.8 ±0.2 | 0.9 ±0.1 |
| | 2nd week | 10.4 ±0.05 | 91.03 ±1.1 | 57.1 ±0.6 | 3.7 ±0.05 | 20.6 ±0.8 |
| | 3rd week | 13.3 ±0.9 | 90.2 ±2.03 | 59.5 ±2.4 | 4.03 ±0.1 | 14.5 ±3.2 |
| **4% Methomyl** | 1st week | 21.3 ±0.3[ab] | 96.3 ±1.1 | 64.3 ±1.06 | 1.3 ±0.05 | 9.93 ±0.1 |
| | 2nd week | 14.1 ±0.5[b] | 92.1 ±0.8 | 51.4 ±1.1 | 6.5 ±0.05 | 21.1 ±1.6 |
| | 3rd week | 14.7 ±0.6 | 93.8 ±1.05 | 52.06 ±2.2 | 1.8 ±0.05 | 26.1 ±2.8 |
| **8% Methomyl** | 1st week | 22.1 ±0.1[ab] | 96.9 ±0.6 | 63.6 ±1.5 | 1.6 ±0.05 | 10.1 ±1.7 |
| | 2nd week | 12.8 ±0.6 | 90.7 ±3.03 | 41.9 ±2.4 | 13.4 ±0.3 | 23.6 ±3.9 |
| | 3rd week | 14.1 ±0.5[b] | 92.6 ±1.5 | 45.4 ±1.2 | 5.2 ±0.1 | 28.9 ±2.4 |
| **1% Tween** | 1st week | 12.8 ±2.7 | 94.03 ±1.7 | 76.1 ±1.2 | 2.23 ±0.2 | 3.5 ±0.1 |
| | 2nd week | 9.2 ±0.7 | 87.8 ±4.9 | 65.6 ±4.2 | 13.3 ±0.1 | 0.8 ±0.1 |
| | 3rd week | 14.02 ±1 | 86.3 ±4.4 | 54.3 ±3.9 | 3.7 ±0.4 | 16.1 ±0.2 |

Data are stated as mean ± SD, $n$ = 3 replicates; significant differences are represented as [a] when compared with the control and [b] when compared with methomyl at $P \leq 0.05$.

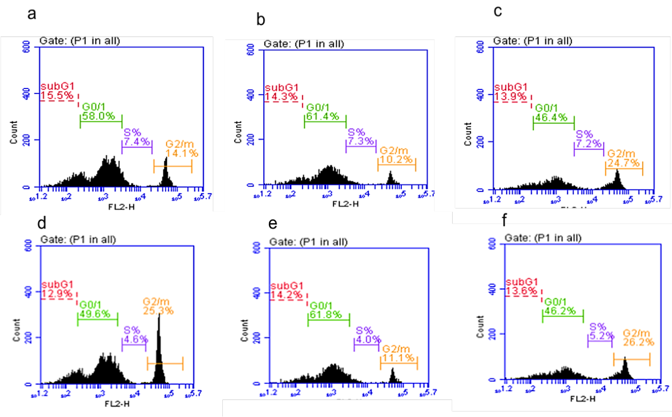


**Figure 1.** Representative flow cytometry histogram showing *Moringa* oil's effect on cell cycle distribution of *Cornu aspersum* (O. F. Müller, 1774) after the third week of exposure. The digestive gland is represented by panels (a), (b), and (c): (a) control; (b) 8% *Moringa* oil; (c) 8% methomyl. The ovotestis gland is represented by panels (d), (e), and (f): (d) control; (e) 8% *Moringa* oil; (f) 8% methomyl.

by those exposed to *M. oleifera* seed oil. A substantial decrease ($P$ = 0.01) was observed in the third week of exposure, with survival rates of 50% for *M. oleifera* oil and 30% for methomyl, compared to 98.6% in the control. Methomyl exposure significantly reduced the survival rate compared to *M. oleifera* seed oil (**Table 2**).

### Cell cycle distribution after *Moringa* oil exposure

The proportion of cells in the $G_0/G_1$, S, and $G_2/M$ phases of the cell cycle was determined in both digestive and ovotestis glands after 1, 2, and 3 weeks. Exposure to 4% and 8% *M. oleifera* seed oil resulted in a significant increase in fragmented cells (sub-$G_1$) compared to the control ($P \leq 0.003$) and methomyl ($P \leq 0.01$). Notably, the number of $G_0/G_1$ phase cells increased to 78.3 ± 1.4% in the 8% *Moringa* oil group, compared to 51.03 ± 1% in the control and 63.6 ± 1.5% in the methomyl group. Consequently, the proportions of S and $G_2/M$ phase cells decreased at the same concentration **(Table 3, Figure 1)**.

In the digestive gland, 8% *Moringa* oil significantly increased sub-$G_1$ phase cells compared to the control ($P \leq 0.001$) and

**Table 4.** Percentage of cells in different cell cycle phases after treatment

| Concentration | Exposure Period | %Sub G1 | % Cells/Cell Cycle | G0/G1 | S | G2/M |
|---|---|---|---|---|---|---|
| **Control** | 1st week | 9.3 ±1 | 96.5 ±1.3 | 52.2 ±0.1 | 3.1 ±0.2 | 11.4 ±1 |
| | 2nd week | 10.4 ±0.3[a] | 95.4 ±0.8 | 52.2 ±0.1 | 3.5 ±0.3 | 10.9 ±1 |
| | 3rd week | 10.4 ±0.5 | 95.3 ±0.4 | 52.5 ±0.2 | 3 ±0.2 | 10.8 |
| **4% *Moringa* oil** | 1st week | 15.4 ±0.4 | 93.6 ±0.5 | 74.1 ±1.1 | 10.5 ±0.1 | 1.1 ±0.1 |
| | 2nd week | 17.06 ±0.5[a] | 88.5 ±3.2 | 74.1 ±2.4 | 10.5 ±0.2 | 0.9 ±0.1 |
| | 3rd week | 15.2 ±0.05 | 93.6 ±0.2 | 75.1 ±0.5 | 10.3 ±0.05 | 1.2 ±0.3 |
| **8% *Moringa* oil** | 1st week | 16.2 ±0.04 | 92 ±0 | 67.1 ±0.2 | 9.1 ±0.05 | 2.9 ±0.2 |
| | 2nd week | 30.2 ±0.1[a] | 82.2 ±0.5 | 67.1 ±0.4 | 14.1 ±0.1 | 3 ±0.1 |
| | 3rd week | 15.7 ±0.7 | 96.1 ±1.4 | 67.4 ±1.5 | 13.1 ±0.2 | 2.8 ±2.4 |
| **4% Methomyl** | 1st week | 18.1 ±0.1[a] | 91.5 ±0.5 | 63.6 ±0.7 | 17.9 ±0.05 | 14 ±0.8 |
| | 2nd week | 12.9 ±0.1 | 86.2 ±0.2 | 63.7 ±0.5 | 17.6 ±0.1 | 14.1 ±0.9 |
| | 3rd week | 15.1 ±0.1 | 90.4 ±0.8 | 63.6 ±0.2 | 12 ±0.05 | 14.2 ±1.05 |
| **8% Methomyl** | 1st week | 18.07 ±0.8[a] | 91.3 ±1.4 | 68.6 ±3 | 12 ±0.05 | 13.4 ±1.8 |
| | 2nd week | 14.6 ±0.05 | 93.3 ±2.8 | 68.9 ±2.7 | 12.1 ±0.05 | 13.3 ±0.1 |
| | 3rd week | 17.9 ±0.4[a] | 94.7 ±1.4 | 68.8 ±2.3 | 11.9 ±0.1 | 13.4 ±0.7 |
| **1% Tween** | 1st week | 11.2 ±0.1 | 96.3 ±0.4 | 56 ±0.2 | 25.3 ±0.3 | 0.6 ±0.05 |
| | 2nd week | 11.5 ±0.2 | 96.6 ±0.7 | 59 ±0.2 | 25.7 ±0.7 | 0.7 ±0.1 |
| | 3rd week | 14.6 ±0.2 | 87.3 ±0.8 | 52.5 ±0.5 | 5.3 ±0.1 | 16.5 ±0.8 |

Data are expressed as mean ± SD, $n$ = 3 replicates; significant differences are represented by [a] vs. control when $P \leq 0.05$.

methomyl ($P \leq 0.005$). The $G_0/G_1$ phase cell counts increased to 75.1 ± 0.5% (4% *Moringa*) and 67.4 ± 1.5% (8% *Moringa*) versus 52.5 ± 0.2% in the control. Furthermore, $G_2/M$ phase percentages decreased with both *Moringa* oil concentrations **(Table 4**, **Figure 1)**.

### Detection of apoptosis by annexin-V/PI

Using flow cytometry, apoptotic cells were identified by incubating them with FITC-labeled annexin V and PI. To differentiate between necrotic and apoptotic cells, PI was utilized. The exposure of snails to *Moringa* oil caused a significant increase in apoptotic cells (early and late apoptosis) in digestive gland cells at both concentrations ($P \leq 0.001$) and in the two concentrations of methomyl ($P \leq 0.01$) compared with the control group. At the end of the experiment, the percentages of apoptotic cells were 50.3% and 63.9% at 8% *Moringa* oil and methomyl, respectively, while that of the control was 12.3%. Depending on the material, the increase in apoptotic cells in both concentrations of *Moringa* oil was significant compared with methomyl ($P \leq 0.001$).

In ovotestis gland cells, a significant increase in apoptotic cells was observed at both concentrations of *Moringa* oil ($P \leq$ 0.003) and at the two concentrations of methomyl ($P \leq 0.02$). At the third week of exposure, the percentages of apoptotic cells were 62.6% and 50.8% at 8% *Moringa* oil and methomyl, respectively, while the control was 10.8%. Depending on the time of exposure, the increase in apoptotic cells in the third week was significant when compared with that in the first week ($P = 0.01$). Cells that were stained with PI were identified as necrotic, and they significantly increased in the digestive gland cells after exposure to both *Moringa* oil concentrations ($P \leq 0.005$) and methomyl ($P \leq 0.02$) compared with the control. The percentages were 21.5% and 14.9% at 8% *Moringa* oil and methomyl, respectively, while that of the control was 5.6%. In the ovotestis gland, a significant increase was noticed at both *Moringa* oil concentrations and methomyl ($P \leq 0.03$) compared with the control. Regarding exposure time, the increase in the third week was 24.7% and 31.1% ($P = 0.02$) at the 8% concentration of *Moringa* oil and methomyl, respectively, compared with their values in the first week (5.5% and 3.7%), when the control was 4.5%. As a result, the number of viable cells was significantly decreased in *Moringa* oil and methomyl (70.03% and 60.2%, respectively) compared with the control (84.6%) in the third week of exposure

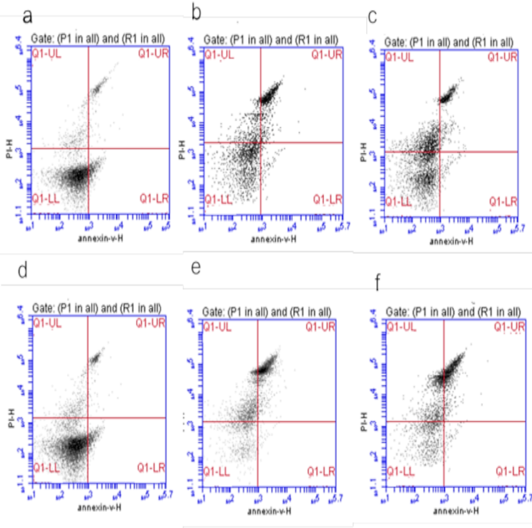


**Figure 2.** Fluorocytograms of one representative experiment from three separate trials following a three-week exposure to *M. oleifera* seed oil.

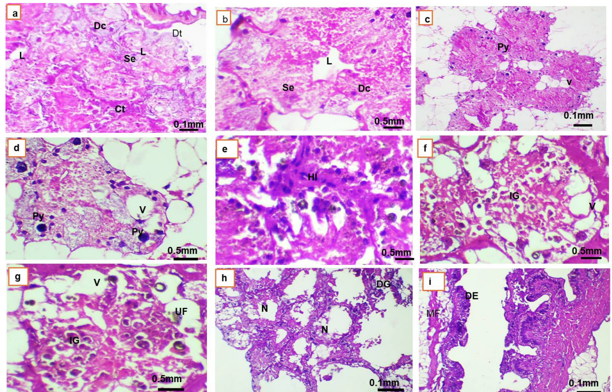


**Figure 3.** Light photomicrographs of sections through the digestive gland of *Cornu aspersum* (O. F. Müller, 1774) after three weeks of exposure. (a & b) Control, (c, d, e, & i) exposed snails to 4% and 8% *M. oleifera* seed oil, and (f, g, h, & j) exposed snails to 4% and 8% methomyl. Dt, digestive tubule; Dc, digestive cell; Se, secretory cell; Ct, connective tissue; L, lumen; Py, pyknosis; V, vacuolization; Dg, degeneration; Uf, undigested food; IG, increasing the granules in digestive cells; N, necrosis; Hi, hemocytic infiltration; Mf, muscle fibers.

**(Figure S1, Figure S2, Figure 2)**.

### Histopathological signs after exposure to *Moringa* oil

Histological sections of the normal (unexposed) digestive gland of *Cornu aspersum* (O. F. Müller, 1774) revealed that it is mainly composed of digestive tubules, each lined with columnar epithelial cells of various types, including digestive and excretory cells. The digestive cells are typically characterized by numerous green and yellow cytoplasmic granules. These tubules enclose a distinct central lumen and are separated by interlobular connective tissue containing hemolymphatic sinuses rich in actively circulating hemocytes. Externally, the tubules are encased by a distinct circular muscle layer.

Exposure to 8% *M. oleifera* oil caused pronounced pathological alterations in the digestive gland, including marked vacuolization and pyknosis of nuclei. Hemocytic infiltration, a clear sign of inflammation, was also observed. Severe epithelial damage, including extensive vacuolization, degeneration, and necrosis, was evident following exposure to both 4% and 8% concentrations of methomyl. Additionally, *Moringa* oil induced progressive degeneration of muscle fibers **(Figure 3)**. The ovotestis of unexposed snails consists of numerous small follicles (acini) lined with germinal epithelium, which is clearly differentiated into primary and secondary spermatogonia/oogonia and mature spermatozoa/ova. After three weeks of exposure, the high concentration (8%) of *Moringa* oil caused notable alterations in the histological architecture of the ovotestis. Histopathological signs included degeneration of ova and spermatozoa, inhibition of ova maturation, appearance of dense fibrous tissue, and necrosis. Methomyl (8%) exposure led to a substantial reduction or complete absence of mature ova and degeneration of spermatozoa **(Figure 4)**.

## Discussions

### Effect of *Moringa oleifera* seed oil on the survival rate of *Cornu aspersum* (O. F. Müller, 1774)

The present study recorded the influential role of *Moringa* oil in reducing the survival rate of *Cornu aspersum* snails. As primary consumers, land snails serve as models for rapid physiological responses to dietary habits and breeding conditions. Previous research [41; 42] confirmed the sensitivity of Mollusca to minimal dietary changes due to their rapid metabolism. *Moringa* oil is composed of unsaturated and saturated fatty acids. Exposure of snails to this oil may alter their fatty acid profile, which is sufficient to change the physiological state of gastropods [43]. A previous study also indicated that fluctuations in polyunsaturated fatty acids can serve as a valuable ecotoxicological test in snails [44]. Benzylamine extracted from *M. oleifera* has demonstrated molluscicidal potency by reducing survival and reproductive rates in *Biomphalaria alexandrina* snails [45]. Plant extracts containing essential oils, flavonoids, terpenes, saponins, and tannins have been reported as effective agents in snail control [46; 47]. The negative impact of such extracts on the land snail *Monacha obstructa* has also been documented [48].

### Apoptotic effect of *M. oleifera* seed oil on *Cornu aspersum*

The findings indicated the apoptotic potential of *M. oleifera* oil on both digestive and ovotestis gland tissues. This can be explained by the fact that mollusks possess a unique composition of fatty acids. Given this, previous studies have shown that unsaturated and saturated fatty acids exert different effects on steatosis and cell death, influencing apoptosis in a variety of experimental systems and being identified as the most harmful lipid types [49; 50; 51]. The presence of polyunsaturated fatty acids in cellular membranes influenced membrane permeability. Previous research showed that apoptosis was caused by palmitic acid

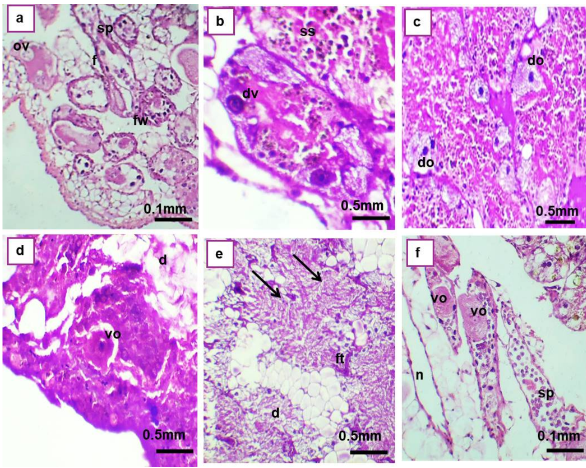


**Figure 4.** Light photomicrographs of sections through the ovotestis gland of Cornu aspersum (O. F. Müller, 1774) after three weeks of exposure. (a) Control; (b, c, & f) exposed snails to 8% *M. oleifera* oil; (d & e) exposed snails to 8% methomyl. f, follicle; fw, follicular wall; ov, ovum; sp, spermatozoa; dv, developing oocyte; ss, stages of spermatogonia; d, degeneration; ft, fibrous tissue; n, necrosis; vo, vitellogenic oocyte. Reduction/lack of mature ova and spermatozoa (black arrows).

through caspase-dependent Beclin 1 cleavage. The researchers concluded that essential fatty acids induce apoptosis of tumor cells by overexpressing cytochrome P450 [52]. Mitochondria play a major role in apoptosis by releasing cytochrome c and caspase 3, which activate apoptotic enzymes [53]. Fatty acids that stimulate lipotoxicity were also reported to play a crucial role in pathogenesis [54]. Moreover, the toxicity of metals has been linked to disturbances in lipid membranes [55]. The observed changes in fatty acid levels represent metabolic adjustments initiated by external stimuli [56]. A recent study explored the ability of *Moringa oleifera* leaf extract to promote apoptosis in adipocyte cells (3T3-L1 cells) by increasing caspase 3 activity and regulating the expression of *BAX* and *BCL2* genes [57]. *Moringa* seed extract has been shown to induce apoptosis and cell cycle arrest in cancer cells, with fatty acids like caprylic acid, oleic acid, and stearic acid contributing to apoptosis in lung, leukemia, and ovarian cancer cells [58]. Autophagy and hepatic steatosis were reported in the mouse liver after high-fat diets [59]. Another study demonstrated the harmful effects of *Moringa* extract (containing saponins, flavones, and flavonols) on *Biomphalaria glabrata* embryos, adults, and *Schistosoma mansoni* adult worms [60].

#### Histopathological effect of *M. oleifera* seed oil on *Cornu aspersum*

Exposure to seed oil induced histopathological changes in both digestive and ovotestis glands. This observation aligns with previous findings [61], where damage such as fragmentation and vacuolization was reported in the digestive and excretory cells of *B. alexandrina* and *B. truncatus* snails following treatment with Egyptian wild plant extracts. Exposure to aqueous seed extract of *M. oleifera* caused severe damage in digestive cells, including tip loss, degeneration, and increased numbers of secretory cells [62]. Additional histopathological features included vacuolation of digestive and secretory cells. Degeneration and rupture of sperm and ova in *B. truncatus* were observed following exposure to cerium oxide nanoparticles synthesized using *Moringa* seeds [63]. Chlorophyllin from deep-frozen *M. oleifera* leaves caused deformation of secretory cells and rupture of connective tissue between tubules, leading to degeneration of digestive cells in *B. truncatus*. When combined with magnesium or copper, this compound also caused histological malformations due to photosensitization [64; 65]. In *Cornu aspersum*, degeneration of digestive tubules and damage to the basement membrane and hepatopancreas were reported following exposure to thiamethoxam (200 mg/L) [66].

## Conclusions

*Moringa oleifera* seed oil has significant molluscicidal potency, exhibiting both apoptotic and histopathological effects on the ovotestis and digestive glands of *Cornu aspersum* (O. F. Müller, 1774). This study investigated the effects of *Moringa* oil as a whole against the snail *Cornu aspersum*. Further studies will aim to isolate the main active constituents of the oil and assess their effects on non-target organisms to ensure greater eco-friendliness compared with conventional chemical molluscicides.

## List of Abbreviations

- **PBS**: Phosphate-buffered saline
- **PI**: Propidium iodide
- **COI**: Cytochrome oxidase subunit I
- **ND1**: NADH dehydrogenase subunit 1
- **DNA**: Deoxyribonucleic acid

## Supplementary

**Figure S1**: Exposure to *M. oleifera* seed oil increased cell apoptosis and necrosis of the digestive gland of *Cornu aspersum* (O. F. Müller, 1774) after exposure for3 weeks: (a) first week, (b) second week, and (c) third week. Values expressed as mean ±SD. [c] The significant difference in necrosis between control and exposed snails when $P \leq 0.02$. The significant difference in apoptosis was indicated as follows: [a,b], between *Moringa* exposed groups vs. control and methomyl exposed groups when $P \leq 0.05$, ANOVA.

**Figure S2**: Exposure to *M. oleifera* seed oil increased cell apoptosis and necrosis of the ovotestis gland of *Cornu aspersum* (O. F. Müller, 1774) after exposure 3 weeks: (a) first week; (b) second week; and (c) third week. Values expressed as mean ±SD. [c] The significant difference in necrosis between control and exposed snails when $P \leq 0.03$. The significant difference in apoptosis was indicated as follows: [a,b] between the *Moringa* exposed group vs. control and methomyl exposed groups when $P \leq 0.05$, ANOVA.

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