



Full Length Article

Molluscicidal Activity of *Artemisia herba-alba* and *Zingiber officinale* Extracts on Some Biological Parameters of *Monacha cartusiana* Snail

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Abstract

This study investigated the molluscicidal activity of aqueous extract of *Artemisia herba-alba* and *Zingiber officinale* on the incubation and hatching periods, hatchability and the newly hatched offspring from the treated eggs of *Monacha cartusiana* snail at different periods of laying. Eggs of 1 day, 7 days and 10 days–old were dipped in the concentrations of 1, 2, 5 and 10% of each extract individually with the dipping times of 10, 20, 30 and 50 s for each concentration. The results demonstrated that both extracts significantly affected on all the tested parameters. The one-day old eggs were the most affected by these extracts compared to 7 and 10-days old eggs, respectively. The effect of *A. herba-alba* was higher than that of *Z. officinale* at all ages of the eggs. Increasing the concentration of both extracts and the dipping time increased the incubation and hatching periods and decreased the hatchability and the survival rate of newly hatched snails. The highest impact on hatchability was achieved by 5% *A. herba-alba* extract which completely prevented the hatching of one-day old eggs when dipped for 50 s. *A. herba-alba* extract also revealed the similar effect against one-day old eggs when dipped in a concentration of 10% for 30 and 50 s, respectively. Treating snail's eggs with plant extracts is one of the effective methods due to its effectiveness, safety to environment, and small amount of extracts which makes it an economical and useful strategy. © 2023 Friends Science Publishers

Keywords: Snail; Eggs; Biological parameters; Dipping times; Plant extracts

Introduction

The glassy clover snail, *Monacha cartusiana* (Muller) is the most predominant species in the Egyptian fields (Mahrous *et al.* 2002; Ramzy 2009; Abdel-Kader *et al.* 2016). It causes serious damage to vegetables and field crops (El-Deeb *et al.* 2003), It chews the vegetative growth, roots, flower and tubers of plants. Additionally, it left unpleasant slimy secretions on the injured parts that has unfavorable smell which making humans and animals to refuse the eating of these plants (El-Massry 1997). This species also acts as an intermediate host to human and livestock parasites, and vector of many plant pathogens (Godan 1983; Grewal *et al.* 2003). Thus, it is necessary to control this pest. Moreover, *M. cartusiana* represents an ideal biological model for laboratory studies due to some characteristics of its biological behavior such as fast sexual maturity, high rates of reproduction, egg hatchability, and short incubation period (Mohamed and Ali 2009) which make easy to investigate the efficiency of extracts as potential molluscicides. The excessive use of synthetic molluscicides is highly toxic to environment and human health (Joshi *et al.* 2008). Therefore, the plant molluscicide was recommended

by the World Health Organization due to its lower toxicity to the environment (WHO 1983). In addition, botanical molluscicides are easily biodegradable, less expensive in their crude form, easily obtainable and more associated with indigenous self-sufficient strategies of snail control than imported synthetic pesticides (Guruswamy *et al.* 2017; Abdel-Rahman 2020).

Artemisia herba-alba is a medicinal plant used to treat Diabetes mellitus and digestive system disorders in human (Haghighian *et al.* 2008). It has also strong effect against many parasites causing dangerous diseases to domestic animals and showed observed toxic effects against many pests (Asta 2016). *Zingiber officinale* another plant is known to have antioxidants, anti-inflammatory, analgesic and antimicrobial activities (Nikoli *et al.* 2014; Amri and Touil-Boukoffa 2016). In addition, it has a strong molluscicidal activity against *M. cartusiana* snail, as it causes severe disturbances in the enzymatic activities of this snail (El-Atti *et al.* 2019). It also causes an inhibition in the reproductive capacity of *Biomphalaria alexandrina* snail and rapid decline in its survival of its individuals (Bakry *et al.* 2013). The treatment of the golden apple snail eggs with metaldehyde

and niclosamide after short period of laying makes the effect of these molluscicides on the hatching rate of these eggs is stronger (Sisa *et al.* 2016). *A. herba-alba* and *Z. officinale* plants contains chemical compounds such as tannins, flavonoids and saponins (Kahlouche-Riachi *et al.* 2015; Sharma *et al.* 2016) all of these compounds have proven biocidal activities, including against snails (Filho 2010; Lopes *et al.* 2011). If these extracts have the ability to inhibit the hatching of snail eggs, the glassy clover snail, *M. cartusiana* can be controlled in the fields to minimize its damage.

The aim of this study is to evaluate the molluscicidal efficacy of *A. herba-alba* and *Z. officinale* ethanol-based extracts on the eggs of *M. cartusiana* snail after different periods of egg laying.

Materials and Methods

Collection and rearing of snails

Adult snails, *M. cartusiana* were collected from a snail – infested navel orange field located in Tal – Haween village, Zagazig district, Sharkia Governorate, Egypt. The snails were acclimated in plastic boxes (3/4 kg capacity) containing moistened clay soil. The rearing boxes were provided daily with fresh cabbage leaves and closed with muslin cloth and rubber band to prevent snails from crawling out (Clemente *et al.* 2008). The boxes were examined daily to observe the laying of eggs, and all egg clutches deposited in the boxes soil were carefully collected for later experiments.

Preparation of plant extracts

The leaves and stem of *A. herba-alba* were collected from Sinai desert, Egypt and were further taxonomically identified for correct classification in the Botany Department, Faculty of Science, Zagazig University, Egypt. Fresh rhizomes of *Z. officinale* were purchased from the International Company, Cairo, Egypt. The plant parts of *A. herba-alba* were washed with tap water and air – dried at the room temperature. The *Z. officinale* rhizomes were chopped into small pieces and then shade dried for three days. The dried materials of each plant were ground up into a fine powder by using a domestic blender. The 250 g of these powders was macerated in 95% ethanol separately according to the method of Mau *et al.* (2004). The obtained extract was filtered using a Whatman filter paper and concentrated under vacuum in a rotary evaporator at 30°C. The crude ethanol-based extract of each plant was stored at – 20°C until using in the later experiments in the study by dissolving in water (Kamel *et al.* 2015).

Ovicidal activity assays

The toxic effect of *A. herba-alba* and *Z. officinale* extracts

was tested against *M. cartusiana* eggs by dipping technique at different laying periods (1, 7 and 10 days) to determine the period during at which the eggs are most affected by these extracts. Four concentrations (1, 2, 5 and 10%) from each extract were prepared by dissolving the amount of each tested extract in water to prepare the respective concentrations (Mahmoud 1994). Suitable number of eggs laid at different laying periods (1, 7 and 10 days) were collected carefully by a fine hair brush and dipped in each extract concentration at different dipping times (10, 20, 30 and 50 s) using a piece of white cloth. The treated eggs were later transferred to the boxes at the same depth of laid eggs. Three replications were prepared for each extract concentration, and additionally three boxes containing eggs were dipped in distilled water as control. All boxes were covered with muslin cloth and secured with rubber band. The eggs were observed daily to determine the incubation period, hatching period and hatching percentage of eggs (Shokry 2013).

Survival of offspring hatched from the treated eggs

The juvenile's snails hatched from the treated eggs were kept in the same boxes. Snails were fed on fresh cabbage leaves and the soil remoistened with water were replaced every three days. The mortality was assessed every three days for 28 days, and the final survival percent of snails was calculated (Hegab and Sh Hend 2016).

Statistical Analysis

Data were statistically analyzed using Costat (2005) statistical program analysis, computer software (COHORT, Monterey, California).

Results

Influence of the tested plant extracts on the one-day old eggs

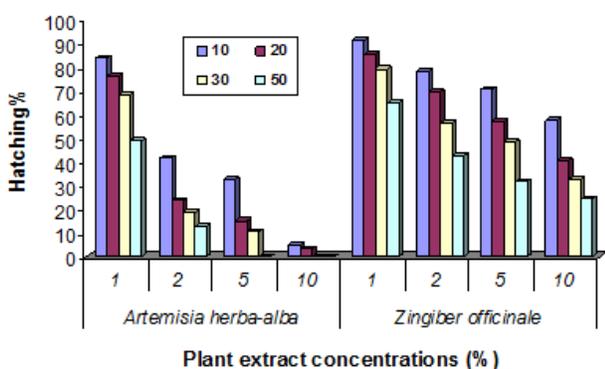
Effect on the incubation and hatching periods of one-day old eggs: The impact of *A. herba-alba* and *Z. officinale* extracts at different tested concentrations (1, 2, 5 and 10%) and dipping times (10, 20, 30 and 50 s) was evaluated on the incubation and hatching periods of one-day old treated *M. cartusiana* eggs. The results in Table 1. showed the effect of each extract on incubation and hatching periods. The periods of incubation and hatching increased significantly with the increase in concentrations of each extract and dipping times of eggs in these tested concentrations. It was observed that *A. herba-alba* extract had a stronger effect than *Z. officinale* on incubation and hatching periods. *A. herba-alba* extract achieved the highest elongation of the incubation and hatching periods at the highest tested concentration of 10% with value of 28.66 days (incubation) and 7 days (hatching) at a 20 s (dipping time) compared to

Table 1: Effect of plant extracts on the incubation and hatching periods of one-day old eggs

Tested plant extracts	Conc. (%)	Incubation period (days) at dipping times (s)				Hatching period (days) at dipping times (s)			
		10	20	30	50	10	20	30	50
<i>Artemisia herba-alba</i>	1	22.00 ^d	22.33 ^{de}	23.00 ^d	24.33 ^a	4.33 ^{bcd}	4.33 ^d	4.66 ^b	5.00 ^{bc}
	2	24.00 ^{bc}	24.66 ^b	25.00 ^b	25.00 ^a	4.33 ^{bcd}	4.66 ^{cd}	5.00 ^{ab}	5.66 ^{abc}
	5	25.66 ^{ab}	28.00 ^a	28.00 ^a	0.00 ^d	5.00 ^{bc}	5.33 ^{bc}	6.00 ^a	0.00 ^e
	10	26.33 ^a	28.66 ^a	0.00 ^e	0.00 ^d	6.66 ^a	7.00 ^a	0.00 ^d	0.00 ^e
<i>Zingiber officinale</i>	1	21.00 ^d	21.00 ^e	21.66 ^e	22.00 ^b	3.66 ^{de}	4.00 ^d	4.33 ^b	4.66 ^c
	2	21.66 ^d	22.33 ^{de}	23.00 ^d	23.66 ^a	4.00 ^{de}	4.66 ^{cd}	5.00 ^{ab}	5.66 ^{abc}
	5	22.66 ^{cd}	22.66 ^{cd}	23.66 ^{cd}	24.00 ^a	4.66 ^{bcd}	5.33 ^{bc}	5.33 ^{ab}	6.00 ^{ab}
	10	24.00 ^{bc}	24.00 ^{bc}	24.33 ^{bc}	25.00 ^a	5.33 ^b	5.66 ^b	6.00 ^a	6.33 ^a
Control		18.00 ^e	18.00 ^f	18.00 ^f	18.00 ^e	3.00 ^e	3.00 ^e	3.00 ^e	3.00 ^d
P		0.000 ^{***}	0.000 ^{***}	0.000 ^{***}	0.000 ^{***}	0.000 ^{***}	0.000 ^{***}	0.000 ^{***}	0.000 ^{***}
LSD _{0.05}		1.83	1.47	1.27	1.47	1.27	0.99	1.14	1.32

Table 2: Effect of plant extracts on the hatching percentage of one-day old eggs

Tested plant extracts	Conc. (%)	Hatching % at dipping times (s)			
		10	20	30	50
<i>Artemisia herba-alba</i>	1	83.78 ^c	76.08 ^c	68.18 ^c	48.88 ^c
	2	41.46 ^e	23.80 ^e	18.64 ^e	12.50 ^e
	5	32.60 ^b	15.00 ^b	10.64 ^b	0.00 ^b
	10	5.00 ^j	3.12 ^j	0.00 ^j	0.00 ^b
<i>Zingiber officinale</i>	1	91.30 ^b	85.29 ^b	79.16 ^b	64.66 ^b
	2	78.12 ^d	69.44 ^d	56.25 ^d	42.42 ^d
	5	70.58 ^e	57.14 ^e	48.27 ^e	31.48 ^e
	10	57.69 ^f	40.54 ^f	32.50 ^f	24.13 ^f
Control		100.00 ^a	100.00 ^a	100.00 ^a	100.00 ^a
P		0.000 ^{***}	0.000 ^{***}	0.000 ^{***}	0.000 ^{***}
LSD _{0.05}		1.77	2.53	1.40	2.16

**Fig. 1:** Effect of plant extracts on the hatching % of one-day old eggs at different dipping times (s)

control (18 and 3 days for both periods) sequentially. The lowest concentration 1% of *A. herba-alba* extract had the least effect on the two periods (incubation: 22 days and hatching: 4.33 days) compared to the other tested concentrations of the same extract at the lowest dipping time 10 s, respectively. The highest effect of this concentration was achieved at the highest dipping time 50 s, recording 24.33 days and 5 days for both periods sequentially. It is worth noting that the concentrations 5 and 10% of *A. herba-alba* extract did not show any effect on incubation and hatching periods because they completely prevented eggs from hatching at the dipping time 50 s.

Z. officinale extract achieved its highest impact or

elongation of the incubation and hatching periods at the highest tested concentration 10% and the highest dipping time 50 s, by recording 25 and 6.33 days for both periods, respectively. Whereas, the lowest concentration 1% of this extract gave the least effect on both periods, with a record of 21 and 3.66 days at the lowest dipping time 10 s consecutively. There is a high significant difference between the means of incubation and hatching periods compared to the control.

Effect on the hatchability of one-day old eggs

The effect of *A. herba-alba* and *Z. officinale* extracts on the hatchability (hatching percentage) of one-day old *M. cartusiana* eggs was studied. As indicated in Table 2 and Fig. 1, both extracts influenced negatively on the hatchability of eggs. The tested concentration and dipping time also influenced the hatching rates. By increasing the concentration of both extracts and dipping time, the hatchability of eggs was decreased. The efficiency of *A. herba-alba* extract on the hatching rate of eggs was higher than that of *Z. officinale*. The *A. herba-alba* extract completely prevented the egg hatching at the highest concentration 10% at the dipping times 30 and 50 s. The 5% concentration of *A. herba-alba* extract also showed the same effect and prevented the hatching of eggs at the highest dipping time 50 s. At the same trend, the highest tested concentration 10% recorded the lowest hatching (3.12%) only at the dipping time 20 s. While, the lowest effect of *A. herba-alba* extract on the hatching rate was recorded at the lowest concentration 1% with 83.78% hatching of eggs at the lowest dipping time 10 s, compared to 100% hatching of the untreated eggs in the control. The extract of *Z. officinale* achieved its highest efficacy on egg hatching at the highest concentration 10%, with values of 24.13% hatchability of eggs at the highest dipping time 50 s. While, the lowest efficiency on the reduction of egg hatching was recorded at the lowest concentration 1% by achieving the highest hatchability 91.30% at the lowest dipping time 10 s.

In general, both of *A. herba-alba* and *Z. officinale* extract significantly affect the hatchability, as they decreased it at all dipping times compared to the hatchability of untreated eggs in the control.

Table 3: Survival of the offspring hatched from treated one-day old eggs

Tested plant extracts	Conc. (%)	Survival (%) of juveniles at dipping times (s)							
		10		20		30		50	
		2 w	4 w	2 w	4 w	2 w	4 w	2 w	4 w
<i>Artemisia herba-alba</i>	1	87.09	80.64 ^d	80.00	72.50 ^d	73.33	66.66 ^d	68.18	63.63 ^d
	2	72.50	68.18 ^f	69.09	56.75 ^f	54.05	45.45 ^f	42.85	34.21 ^e
	5	63.79	56.41 ^e	58.18	47.91 ^e	48.07	32.69 ^e	0.00	0.00 ^b
	10	47.61	31.57 ^h	36.66	24.44 ^h	0.00	0.00 ^b	0.00	0.00 ^b
<i>Zingiber officinale</i>	1	95.74	91.48 ^b	92.10	87.50 ^b	84.21	81.48 ^b	83.33	78.94 ^b
	2	93.61	89.63 ^c	88.88	83.33 ^c	86.48	70.19 ^c	73.68	69.56 ^c
	5	84.09	81.57 ^d	79.24	71.69 ^d	77.14	65.71 ^d	63.63	56.81 ^c
	10	77.55	71.79 ^e	69.44	62.16 ^e	58.33	52.77 ^e	49.18	40.98 ^f
Control		100.00	100.00 ^a	100.00	100.00 ^a	100.00	100.00 ^a	100.00	100.00 ^a
<i>P</i>			0.000 ^{***}		0.000 ^{***}		0.000 ^{***}		0.000 ^{***}
LSD _{0.05}			1.87		1.75		1.63		1.38

w = week

Survival of the offspring hatched from treated one-day old eggs

The survival of snails hatched from the one-day old, treated eggs with *A. herba-alba* and *Z. officinale* extracts was investigated for 4 weeks after eggs hatching. As demonstrated in Table 3, the survival of the offspring hatched from eggs treated with both extracts was significantly lower compared to the control. But the impact of *A. herba-alba* extract on the survival of these hatched juveniles was higher than that of *Z. officinale*.

The lowest survival of juveniles was achieved by the highest concentration 10% of *A. herba-alba* extract which recorded 36.66 and 24.44% survival at the dipping time 20 s after two and four weeks, respectively. *A. herba-alba* extract showed its lowest effect on the survival of hatched juveniles at the lowest tested concentration 1% which achieved 87.09 and 80.64% survival at the dipping time 10 s after two and four weeks, respectively.

Z. officinale extract gave its highest impact on the survival of hatched juveniles at the highest concentration 10% by achieving 49.18 and 40.98% survival at the highest dipping time 50 s after two and four weeks, respectively. It showed the lowest effect at the lowest concentration 1% by recording 95.74 and 91.48% survival of hatched juveniles at the lowest dipping time 10 s after two and four weeks, respectively. The two extracts showed a high significant difference in the survival of new hatched juveniles in comparison with the control.

Influence of the tested plant extracts on the seven-days old eggs

Effect on the incubation and hatching periods: The influence of *A. herba-alba* and *Z. officinale* extracts on the incubation and hatching periods of eggs that were treated with each extract after seven days of laying was illustrated in Table 4. The obtained results showed that both extracts elongated the incubation and hatching periods compared to the control. The effect or elongation which caused by *A. herba-alba* extract for both periods was greater than the *Z.*

officinale extract at all dipping times.

The extract of *A. herba-alba* gave the highest elongation of the incubation period at the highest concentration 10% and the highest dipping time 50 s by record of 25.66 days. Moreover, this concentration achieved the highest impact or elongation of the hatching period at the dipping time 30 s by record of 6.33 days compared to 18 and 3 days for both periods at all times of dipping in the control, respectively. *A. herba-alba* extract showed its lowest effect on both periods at the lowest tested concentration 1% and the lowest dipping time 10 s by achieving 20.66 and 3 days for the incubation and hatching periods, respectively.

Z. officinale extract showed its highest impact on the incubation and hatching periods at the highest concentration 10%, with a record of 24 and 5.66 days for both periods at the dipping time 50 s, consecutively. It achieved the least effect on the two periods at the lowest concentration 1% by record of 20 and 3 days for both periods at the lowest dipping time 10 s, respectively. High significant difference was recorded between both of incubation and hatching periods of treated eggs with tested extracts and the same periods of the untreated eggs in the control.

Effect on the hatchability of seven-days old eggs

The hatching rate of seven-days old eggs that were treated with each of *A. herba-alba* and *Z. officinale* extracts was investigated. As shown in Table 5 and Fig. 2, *A. herba-alba* extract achieved the highest reduction in the hatching percentage by recording only 3.03% hatching at the highest concentration 10% and the highest dipping time 50 s. The lowest impact of *A. herba-alba* on the hatchability of eggs was recorded at the lowest tested concentration 1% which gave 92.33% hatching at the lowest dipping time 10 s. The hatchability in the control replicates was 100% at all the dipping times. The efficiency of *Z. officinale* extract in reducing the rate of egg hatching was lower than that of *A. herba-alba*. It was achieved 58.18% hatching at the highest concentration 10% and the highest dipping time 50 s. Moreover, the lowest reduction in hatchability 97.24% was

Table 4: Effect of plant extracts on the incubation and hatching periods of seven-days old eggs

Tested plant extracts	Conc. (%)	Incubation period (days) at dipping times (s)				Hatching period (days) at dipping times (s)			
		10	20	30	50	10	20	30	50
<i>Artemisia herba-alba</i>	1	20.66 ^d	21.00 ^{ef}	21.66 ^e	22.00 ^d	3.00 ^b	3.00 ^c	3.66 ^{cde}	4.00 ^{bc}
	2	22.00 ^e	22.66 ^{cd}	23.00 ^{cd}	23.33 ^{bc}	3.00 ^b	3.33 ^c	4.00 ^{bcde}	4.00 ^{bc}
	5	24.00 ^{ab}	24.00 ^{ab}	24.33 ^{ab}	25.00 ^a	4.00 ^{ab}	4.66 ^b	4.66 ^{bc}	6.00 ^a
	10	24.66 ^a	25.00 ^a	25.00 ^a	25.66 ^a	4.66 ^a	6.00 ^a	6.33 ^a	0.00 ^d
<i>Zingiber officinale</i>	1	20.00 ^d	20.00 ^f	20.33 ^f	21.00 ^e	3.00 ^b	3.00 ^c	3.33 ^{de}	3.66 ^c
	2	20.66 ^d	21.33 ^e	22.00 ^{de}	22.66 ^{cd}	3.00 ^b	3.33 ^c	3.66 ^{cde}	4.00 ^{bc}
	5	22.00 ^e	22.00 ^{de}	22.66 ^{cde}	23.00 ^e	3.66 ^{ab}	4.00 ^{bc}	4.33 ^{bcd}	5.00 ^{ab}
	10	23.00 ^{bc}	23.33 ^{bc}	23.33 ^{bc}	24.00 ^b	4.00 ^{ab}	4.66 ^{bc}	5.00 ^b	5.66 ^a
Control		18.00 ^e	18.00 ^e	18.00 ^e	18.00 ^f	3.00 ^b	3.00 ^c	3.00 ^e	3.00 ^c
P		0.000 ^{***}	0.000 ^{***}	0.000 ^{***}	0.000 ^{***}	0.003 ^{***}	0.003 ^{***}	0.003 ^{***}	0.000 ^{***}
LSD _{0.05}		1.14	1.27	1.09	0.99	1.23	1.14	1.14	1.09

Table 5: Effect of plant extracts on the hatching % of seven-days old eggs

Tested plant extracts	Conc. (%)	Hatching % at dipping times (s)			
		10	20	30	50
<i>Artemisia herba-alba</i>	1	92.33 ^c	84.49 ^c	77.50 ^d	63.22 ^d
	2	85.28 ^d	72.11 ^f	65.39 ^f	50.68 ^f
	5	80.00 ^f	68.42 ^g	51.16 ^g	32.43 ^g
	10	75.75 ^g	39.50 ^h	18.64 ^h	3.03 ^h
<i>Zingiber officinale</i>	1	97.24 ^b	88.09 ^b	85.33 ^b	79.15 ^b
	2	91.76 ^c	85.54 ^c	80.37 ^c	72.88 ^c
	5	86.66 ^d	81.13 ^d	77.29 ^d	65.40 ^d
	10	82.28 ^e	79.60 ^e	71.03 ^e	58.18 ^e
Control		100.00 ^a	100.00 ^a	100.00 ^a	100.00 ^a
P		0.000 ^{***}	0.000 ^{***}	0.000 ^{***}	0.000 ^{***}
LSD _{0.05}		1.48	1.32	1.71	2.22

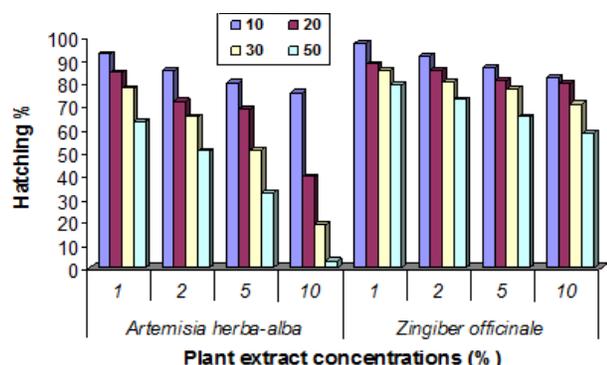


Fig. 2: Effect of plant extracts on the hatching % of seven-days old eggs at different dipping times (s)

recorded at the lowest concentration 1% and the lowest dipping time 10 s. In general, by increasing the tested concentrations of both extracts and the dipping times, the hatching rate decreases. Moreover, a high significant difference was recorded between the hatchability of treated eggs with both extracts and the hatchability of untreated eggs in the control.

Survival of the offspring hatched from treated seven-days old eggs

The survival of the offspring hatched from eggs that treated with each of *A. herba-alba* and *Z. officinale* extract after

seven days of its laying was explained in Table 6. The obtained results revealed that by increasing the concentration of each extract and the dipping times, hatching survival decreases. The extract of *A. herba-alba* recorded the highest reduction of juvenile’s survival to 51.93% after four weeks of hatching at the highest concentration 10% and dipping time 50 s. While, the lowest impact of this extract was recorded at the lowest concentration 1% and the lowest dipping time 10 s, achieving 91.06% survival after four weeks of hatching. The effect of *Z. officinale* extract on the survival of juveniles was less than that of *A. herba-alba*. The highest effect of *Z. officinale* extract was achieved with values of 68.03% at the highest concentration 10% and dipping time 50 s after four weeks of hatching. After the same period, *Z. officinale* extract recorded the lowest reduction of juvenile’s survival at the lowest concentration 1% and the lowest dipping time 10 s, with the highest juvenile’s survival rate of 96.25%. The obtained results also indicated that the differences in the survival of new hatched juveniles from the treated eggs with extracts were highly significantly in comparing with the control.

Influence of the tested plant extracts on ten-days old eggs

Effect on the incubation and hatching periods: The effect of *A. herba-alba* and *Z. officinale* extracts on the incubation and hatching periods of eggs that were treated with these extracts after ten days of its laying was shown in Table 7. The obtained data indicated that the influence of the two extracts increased on both periods by increasing the tested concentrations and times of dipping. The extract of *A. herba-alba* achieved the highest elongation of incubation and hatching periods at the highest concentration 10% and the highest dipping time 50 s with record of 23.66 and 5 days compared to 18 and 3 days in the control for each period, respectively. The concentration 1% of *A. herba-alba* not record any effect on the incubation and hatching periods at the dipping times 10 and 20 s.

The *Z. officinale* extract achieved its highest impact on the incubation and hatching periods at the highest concentration 10% and the highest dipping time 50 s, with

Table 6: Survival of the offspring hatched from treated seven-days old eggs

Tested plant extracts	Conc. (%)	Survival (%) of juveniles at dipping times (s)							
		10		20		30		50	
		2 w	4 w	2 w	4 w	2 w	4 w	2 w	4 w
<i>Artemisia herba-alba</i>	1	94.35	91.06 ^d	86.33	81.15 ^e	82.27	78.14 ^e	80.29	75.84 ^d
	2	89.66	85.40 ^f	80.11	75.38 ^e	77.17	72.40 ^f	73.55	68.12 ^e
	5	84.93	81.85 ^g	78.22	73.63 ^h	74.30	69.44 ^h	66.68	62.50 ^f
	10	76.49	72.18 ^h	73.56	68.12 ⁱ	69.85	63.17 ⁱ	59.36	51.93 ^g
<i>Zingiber officinale</i>	1	98.16	96.25 ^b	95.82	92.03 ^b	94.47	90.88 ^b	87.34	84.56 ^b
	2	96.66	93.50 ^c	92.18	89.22 ^c	90.39	87.51 ^c	85.20	81.41 ^c
	5	92.48	89.17 ^c	88.60	84.27 ^d	85.63	82.79 ^d	81.77	75.90 ^d
	10	88.39	84.62 ^f	82.44	77.96 ^f	79.12	75.38 ^f	72.54	68.03 ^e
Control		100.00	100.00 ^a	100.00	100.00 ^a	100.00	100.00 ^a	100.00	100.00 ^a
<i>P</i>			0.000 ^{***}		0.000 ^{***}		0.000 ^{***}		0.000 ^{***}
LSD _{0.05}			1.71		1.57		1.32		2.54

w = week

Table 7: Effect of plant extracts on the incubation and hatching periods of ten-days old eggs

Tested plant extracts	Conc. (%)	Incubation period (days) at dipping times (s)				Hatching period (days) at dipping times (s)			
		10	20	30	50	10	20	30	50
<i>Artemisia herba-alba</i>	1	18.00 ^d	18.00 ^f	18.33 ^f	19.00 ^{ef}	3.00 ^b	3.00 ^b	3.00 ^e	3.33 ^{cd}
	2	19.66 ^e	20.33 ^{cd}	21.00 ^{cd}	22.00 ^{bc}	3.00 ^b	3.00 ^b	3.33 ^{bc}	3.66 ^{bcd}
	5	22.00 ^{ab}	22.00 ^{ab}	22.33 ^{ab}	23.00 ^{ab}	3.33 ^b	3.33 ^b	4.00 ^{ab}	4.33 ^{abc}
	10	22.66 ^a	23.00 ^a	23.00 ^a	23.66 ^a	4.00 ^a	4.33 ^a	4.33 ^a	5.00 ^a
<i>Zingiber officinale</i>	1	18.00 ^d	18.00 ^f	18.00 ^f	18.66 ^f	3.00 ^b	3.00 ^b	3.00 ^e	3.33 ^{cd}
	2	18.66 ^{cd}	19.00 ^{ef}	19.66 ^e	20.33 ^{de}	3.00 ^b	3.00 ^b	3.33 ^{bc}	3.66 ^{bcd}
	5	19.33 ^c	19.66 ^{de}	20.00 ^{de}	21.00 ^{cd}	3.00 ^b	3.33 ^b	3.33 ^{bc}	4.00 ^{abcd}
	10	21.00 ^b	21.00 ^{bc}	21.33 ^{bc}	22.00 ^{bc}	3.33 ^b	3.33 ^b	4.00 ^{ab}	4.66 ^{ab}
Control		18.00 ^d	18.00 ^f	18.00 ^f	18.00 ^f	3.00 ^b	3.00 ^b	3.00 ^e	3.00 ^d
<i>P</i>		0.000 ^{***}	0.000 ^{***}	0.000 ^{***}	0.000 ^{***}	0.003 ^{**}	0.003 ^{**}	.0003 ^{**}	0.003 ^{**}
LSD _{0.05}		1.19	1.09	1.04	1.40	0.46	0.66	0.87	1.14

Table 8: Effect of plant extracts on the hatching % of ten-days old eggs

Tested plant extracts	Conc. (%)	Hatching % at dipping times (s)			
		10	20	30	50
<i>Artemisia herba-alba</i>	1	100.00	100.00 ^a	95.44 ^b	89.37 ^d
	2	100.00	100.00	97.04 ^b	77.83 ^f
	5	100.00	100.00	94.66 ^c	58.29 ^g
	10	100.00	100.00	65.47 ^d	30.82 ^h
<i>Zingiber officinale</i>	1	100.00	100.00 ^a	100.00 ^a	97.34 ^b
	2	100.00	100.00 ^a	100.00 ^a	95.18 ^c
	5	100.00	100.00 ^a	98.53 ^a	90.26 ^d
	10	100.00	100.00 ^a	92.08 ^c	81.75 ^e
Control		100.00	100.00 ^a	100.00 ^a	100.00 ^a
<i>P</i>		-	0.000 ^{***}	0.000 ^{***}	0.000 ^{***}
LSD _{0.05}		-	0.49	2.00	2.07

record of 22 and 4.66 days for each period, respectively. The lowest concentration 1% of this extract had no effect on the two periods and dipping times of 10, 20 and 30 s, as it was recorded 18 and 3 days, respectively for each period. That is a high significant difference in the incubation and hatching periods of treated eggs with both extracts in comparison with the control.

Effect on the hatchability of ten-days old eggs

The efficacy of both *A. herba-alba* and *Z. officinale* extract was evaluated on the hatching rate of eggs treated with each extract after ten days of laying. The results in Table 8. and Fig. 3. showed that all concentrations of the two extracts did not achieve any reduction in egg hatching at the lowest

dipping time 10 s. The *Z. officinale* extract also did not give any reduction in egg hatching at the all tested concentrations at the dipping time 20 s. Although both extracts did not record significant results in reducing egg hatching, but *A. herba-alba* extract gave a stronger effect than *Z. officinale* in decreasing hatchability. *A. herba-alba* achieved the highest effect by recording the lowest hatching percentage 30.82% at the highest concentration 10% and the highest dipping time 50 s. The lowest concentration 1% of *A. herba-alba* extract did not record any reduction in hatching at the lowest times of dipping 10 and 20 s. While, the same concentration recorded 95.44 and 89.37% hatching at the other dipping times 30 and 50 s, respectively.

The *Z. officinale* extract showed its highest impact on egg hatching at the highest concentration 10% by recording

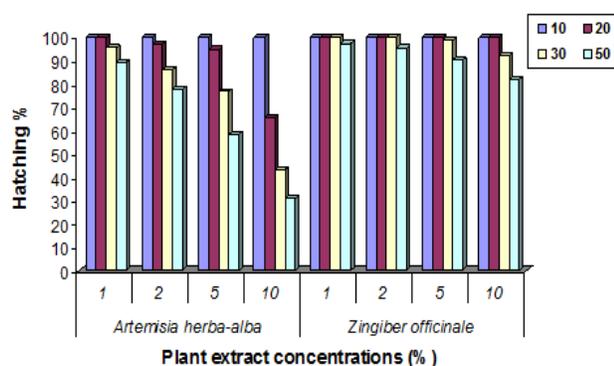


Fig. 3: Effect of plant extracts on the hatching % of ten-days old eggs at different dipping times (s)

81.75% hatching at the highest dipping time 50 s. Eggs treated with the lowest concentration 1% of this extract were fully hatched at all dipping times except the highest dipping time 50 s, in which a slight reduction in hatching rate was recorded by recording 97.34% hatching. A high significant difference was recorded between the hatchability values of treated eggs with extracts and the hatchability of eggs in the control.

Survival of the offspring hatched from treated ten-days old eggs

The impact of *A. herba-alba* and *Z. officinale* extracts on the survival of offspring hatched from eggs that were treated after ten days of laying is demonstrated in Table 9. The survival rate of hatching from eggs treated with both extracts was high but the effect of *A. herba-alba* extract was slightly greater than that of *Z. officinale*. *A. herba-alba* extract gave highest effect on hatching snails survival at the highest concentration 10% and the highest dipping time 50 s, with the lowest survival rate of 66.42% after four weeks of hatching eggs. While at the lowest concentration 1% and the lowest dipping time 10 s of *A. herba-alba* extract, no snails died after two weeks of hatching. After four weeks, the same concentration gave the lowest effect as well by record of 97.54% survival.

The *Z. officinale* extract, did not give any reduction in the number of juveniles at the lowest concentrations 1 and 2% and the lowest dipping time 10 s after four weeks of hatching. After the same period of hatching, *Z. officinale* extract showed its highest effect on the juveniles survival at the highest concentration 10% and the highest dipping time 50 s by record of 82.22% survival. The obtained data also showed a high significant difference in the survival rate of the hatched juveniles from treated eggs with extracts in comparing with the control.

Discussion

The effect of *A. herba-alba* and *Z. officinale* extracts on the incubation and hatching periods, hatchability and survival of

the newly hatched offspring of *M. cartusiana* eggs was investigated in this study. Both extracts significantly affected on all these biological parameters, but the effect of *A. herba-alba* extract is higher than *Z. officinale* especially against the hatchability of the *M. cartusiana* snail eggs. The efficiency of both extracts against all these biological parameters are related to their dipping time in these extracts. Our data showed that the higher dipping time was related to long incubation and hatching periods and the lowest hatching rate of eggs. These results were in agreement with the findings of Ezzat (2021), who reported that plants have an effective impact on the incubation and hatching periods of snail eggs, as linseed oil elongated both periods of *M. cartusiana* eggs to 20.33 and 10.67 days at the highest tested concentration 16% compared to 16.33 and 5.67 days in the control for each period, respectively. In addition, this oil at the same concentration has been reduced the eggs hatchability to 54.53% in comparing with 94.43% of the untreated eggs in the control.

The low rates of hatching are important for the snail's control because if the hatchability is low, the adult population would be low (Bessa and Araujo 1995). Hmamouchiu *et al.* (2000) showed that the plant species *A. herba-alba* has a significant molluscicidal influence on *Bulinus truncatus* snail and reported the presence of flavonoids and saponins in this plant. Other studies have recorded the molluscicidal activity of these compounds against other snail species (Bezerra *et al.* 2002; Cantanhede *et al.* 2010; Lopes *et al.* 2011). In the same trend, Silva (2007) confirmed that the plants with higher content of saponins, tannins and flavonoids were more effective in the control of land snails. The molluscicidal activity of saponins is due to its ability to form complexes with proteins, steroids and phospholipids is responsible for the action on cell membranes causing their destruction (Schenkel *et al.* 2004).

Other study reported by Souza *et al.* (2013) demonstrated that the exposure of *Subulina octona* eggs to the sublethal concentration of *Bidens pilosa* extract was significantly reduced the hatchability of these eggs. The presence of saponins, flavonoids and tannins in this extract can explain the molluscicidal and ovicidal effects of it against this snail. The molecular structure of these components has a very simplified structure that make easy the penetration into the membrane pores that involves the embryo. Moreover, the exposure time has a highly influence on the egg hatching rate. Our study showed that by increasing the tested concentrations of *A. herba-alba* and *Z. officinale* extracts and increasing the dipping period, the hatching rate of *M. cartusiana* eggs decreased. Moreover, the one day old eggs were more affected by both extracts than the seven days and ten days old eggs. *A. herba-alba* extract at its highest concentration of 10% completely prevented the hatching of one day old eggs at dipping times of 30 and 50 s. These findings were supported by the previous study where another extract, *Mikania glomerata* is negatively affected on the hatchability of *S. octona* snail

Table 9: Survival of the offspring hatched from treated ten-days old eggs

Tested plant extracts	Conc. (%)	Survival (%) of juveniles at dipping times (s)							
		10		20		30		50	
		2 w	4 w	2 w	4 w	2 w	4 w	2 w	4 w
<i>Artemisia herba-alba</i>	1	100.00	97.54 ^b	95.30	92.14 ^c	93.62	90.18 ^d	91.33	87.95 ^d
	2	96.73	94.08 ^c	92.17	89.46 ^d	90.58	86.20 ^f	84.39	79.16 ^f
	5	94.65	91.44 ^d	88.93	85.45 ^e	86.28	82.70 ^g	80.25	73.38 ^g
	10	91.30	87.75 ^e	84.93	78.11 ^f	81.25	74.57 ^h	71.68	66.42 ^h
<i>Zingiber officinale</i>	1	100.00	100.00 ^a	100.00	97.64 ^b	99.23	96.04 ^b	97.88	94.31 ^b
	2	100.00	100.00 ^a	98.12	95.70 ^b	96.11	93.55 ^c	93.36	91.28 ^c
	5	99.34	96.59 ^b	96.42	92.15 ^c	92.86	89.23 ^{de}	90.47	88.53 ^d
	10	95.18	92.45 ^{cd}	93.78	91.30 ^{cd}	90.26	87.52 ^{ef}	86.14	82.22 ^e
Control		100.00	100.00 ^a	100.00	100.00 ^a	100.00	100.00 ^a	100.00	100.00 ^a
P			0.000 ^{***}		0.000 ^{***}		0.000 ^{***}		0.000 ^{***}
LSD _{0.05}			1.87		2.05		2.09		2.24

w = week

eggs and the efficiency of this extract was related to the exposure time. So the higher exposure period was related to a successfully control of snails (Souza *et al.* 2014).

Simoes *et al.* (2010) added that *M. glomerata* extract reduced the hatchability of snail eggs due to its saponins and tannins content, which easily dissolved in the water and can penetrate through the pores in the thin calcite layer that envelops the embryo. El-Atti *et al.* (2019) revealed that the ethanolic extract of *Z. officinale* caused deformation of *M. cartusiana* eggs. The inhibition of eggs hatchability may be due to a defect that occurred in the embryonic development (Aioub *et al.* 2000).

In the current study, the negative effect of the *A. herba-alba* and *Z. officinale* extracts on the survival of newly hatched snails is an important result for the control of *M. cartusiana* snail. These results suggest that both extracts reduced the survival rate of newly hatched snails compared to the control and the *A. herba-alba* extract had the strongest effect against *M. cartusiana* eggs. These findings are in accordance with Davila and Bessa (2005) who recorded the negative impact of different extract *B. pilosa* on the growth of the newly hatched juveniles of *S. octona* snail species. In the same direction, Souza *et al.* (1992) reported that the newly hatched *Biomphalaria glabrata* snail were more sensitive to the *Anacardium occidentale* extract. This effect may be related to the energy content of the newly hatched individuals. Due to the stress caused by the exposure to molluscicides, the stored energy of the snails can be reduced (Mello-Silva *et al.* 2006).

The exposure time of snails to the extract also affected on the snails survival, the higher exposure period is related to a decrease in the survival rate of snails (Souza *et al.* 2014).

In present study, the effect of *A. herba-alba* and *Z. officinale* extracts on incubation and hatching periods, hatchability and survival of the offspring hatched juveniles was associated with the age of the eggs being treated after laying with each extract. The results strongly indicated that both extracts achieved the highest effect on these parameters in the case of eggs that were treated after one-day of laying compared to the other which treated after seven and ten days

of laying. These results are confirmed by Sisa *et al.* (2016) who showed that the one-day old eggs of the golden apple snail were more sensitive to the earlier treatment with metaldehyde and niclosamide.

In our study, the negative effect of *A. herba-alba* and *Z. officinale* extracts on the hatchability of one-day old *M. cartusiana* eggs was more than that against 7- and 10-days old eggs. These results were in agreement with the findings of Omobhude *et al.* (2017) who determined the molluscicidal effectiveness of curcumin-nisin poly lactic acid against 1 day and 7 day – old eggs of *Biomphalaria pfeifferi* snail. The results demonstrated that the one-day old eggs age was more susceptible to the sub-lethal concentration of CurNisNp molluscicide than the 7-day old eggs by cause significant reduction in the hatchability of these eggs. Thus, the treatment of the eggs after short period of laying is the best way to achieve a strong effect on them.

Conclusion

Artemisia herba-alba and *Zingiber officinale* extracts have a significant effect on the incubation and hatching periods, hatchability and survival of the offspring hatched juveniles of *Monacha cartusiana* snail eggs. *A. herba-alba* is more effective against the hatchability of snail eggs compared to *Z. officinale*. Increasing the concentration of both extracts and increasing the dipping times of eggs gives the highest reduction of the eggs hatchability. Both extracts had the highest effect on one-day old eggs compared to the 7- and 10-days old eggs. This observation has suggested that the earlier treatment within a day of egg laying could be more effective on the hatching process. Based on these results and the easy preparation and application of these extracts, and cheap cost, it can be considered an alternative molluscicides to control this snail species. Moreover, *A. herba-alba* and *Z. officinale* are promising plants for other studies that aims to control other snail species.

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Author Contributions

Hend Sh. Ghareeb is a single author and responsible for everything in the research.

Conflict of interest

The author declares that she has no competing interests.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approvals

None

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