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Molluscicidal effects of acetone and ethanol extracts of clove (*Syzygium aromaticum*) against *Monacha cartusiana* (Gastropoda: Hygromiidae) snails under laboratory and field conditions at Sharkia Governorate

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Abstract:

The present study aimed to investigate the effects of ethanol and acetone extracts of clove (*Syzygium aromaticum)* flower bud against the adult snails of *Monacha cartusiana* (Müller) (Gastropoda: Hygromiidae), at concentrations of 1, 2, 4 and 6% w/v, through using spray, contact and bait techniques under laboratory conditions, and at concentration of 6% under field conditions through bait technique only. The results showed that the mortality percentage of *M. cartusiana* was increased with increasing concentration of both crude extracts and time elapsed under the used methods. The mortality and the assessment of the lethal concentrations for 50% (LC_{50}) were determined at 3, 5 and 7 days of treatment. The bioassay of *S. aromaticum* against the land snail *M. cartusiana,* indicated that ethanol extract had a more potent effect than acetone extract as regard spray and bait methods all over the experiment, however, it had a less potent effect during 5^{th} and 7^{th} day**s** with contact method**.** The lowest lethal concentration LC_{50} values of ethanol extract were 0.94, 1.14 and 3.47% for spray, contact and bait techniques for 7 days exposure period and for acetone extract were 1.06 and 0.52% at 5^{th} and 7^{th} days exposure period, respectively. Field application by using a higher concentration (6%) as toxic baits, 60.28 and 49.28 % of reduction of *M. cartusiana* snails were recorded for both clove ethanol and acetone extract, respectively within 21 days. Snails display hyperactivity, a massive of air bubbles around the shell a perature, the body water lost as a result of excessive production of mucous secretion, complete withdrawal into their shell and died by the end of the experiment.

Introduction

The land snail *Monacha cartusiana* (Müller) (Gastropoda: Hygromiidae) is an economically important terrestrial hygromnidae widely distributed all over the world (Feldkamp, 2002) especially in the Mediterranean region, causing a severe damage in cultivated and newly reclaimed agroecosystems in Egypt. *M. cartusiana* is not only one of the worst pests of agricultural crops but also acts as a mechanical carrier and /or as a reservoir of several pathogens which infects human, animals and birds

(Cossignani and Cossignani, 1995 and Hausdorf, 2000). The last studies indicated that this snail was the most abundant snail in all localities of Sharkia Governorate (Mahrous *et al*., 2002; Abou Senna *et al*., 2016 and Ismail *et al.*, 2017).

During the last decade the use of natural biochemical products for pest control has been greatly increased. Plant-derived extracts and photochemical have received much effort as a potentially useful bio-active compounds to alternate the other synthetic counterparts (Isman and Grieneisen, 2014), in a trial to avoid recurrent resistance of snails (Rapado *et al*., 2011). Clove is the dried flower bud of *Syzygium aromaticum* (L.) Merr. and Perry (Family : Myrtaceae), cultivated in many parts of the world is a well-known as food additive and it has a broad spectrum medical effects including anticarcinogenic, antibacterial, antiviral, antifungal, anti-helminthic, anti-oxidant and anti-spasmodic (Burt and Reinders, 2003; Hirotaka *et al.,* 2003 ; Dorai and Aggarwal, 2004; Fatehi *et al.,* 2004 and Chaieb *et al*., 2007). The essential oil of *S. aromaticum* mainly act as a local anesthetic (Park *et al*., 2009) and are known to have strong antifeedant, growth regulatory, insecticidal, acaricidal, termicidal, metamorphosis, repellency and sterility effects on insects (Fichi *et al.*, 2007; Akhtar and Yeoung , 2008; Knio and Usta (2008) and Oyeniyi *et al*., 2015).

Most of our knowledge of a molluscicidal activity of *S. aromaticum* against snails is restricted either to aquatic snails, which are vectors for many diseases (El-Din, 2006 and Kumar and Singh, 2006) and or to terrestrial slug (Mobarak *et al*., 2015) and there is little information, on terrestrial gastropods (Ismail and Abdel-Kader, 2011).

The current study aimed to investigate the toxicity of acetone and ethanol 70% extracts of *S. aromaticum* (clove) against the harmful snail *M. cartusiana* using spray, contact and bait technique under laboratory and field conditions at Sharkia Governorate.

Materials and methods

1.Tested animals:

Adult snails of *M. cartusiana* were collected locally in April 2015 from heavily infested fields cultivated with Egyptian clover at Sharkia Governorate, then transferred in plastic bag to the laboratory, healthy individuals snails were kept in glass boxes containing moistened soil, fed on cabbage leaves for 10 days to acclimatization.

2. Plant material and extraction:

The buds of clove were procured from local market in Zagazig, Sharkia Governorate. The flower buds were roasted at 35 °C until weight stabilized then grounded in grinder to obtain a fine dry powder and the clove extract was obtained by maceration process. For defatting and remove fat soluble ingredients, the ground plant material (250gm.) was extracted by soaking for a weak in one liter of n-hexane at room temperature then filtered and washed with hexane three times 500 ml each. The defatted plant biomass was re-extracted successively with acetone and ethanol 70% by same way of defatting. The filtrates were separately evaporated to dryness under reduced pressure in a rotary evaporator until viscous matter as pal green and dark brown was obtained for both acetone and ethanol 70% extracts, respectively. Extracts were stored at 4°C in an airtight container until use **(Freedman *et al.*,1979).**

3.Toxicity studies:

3.1. Under laboratory conditions:

3.1.1. Spray toxicity assay:

For spraying technique, ten adult snails were placed in a covered plastic boxes and exposed to three concentrations of each acetone and ethanol extracts of clove, 2,4 and 6% by spraying it with the corresponding solution of the tested extract (3ml/10 snails) using a hand sprayer atomizer. Three replicates (ten snails each) of each

concentration were used. A parallel standard test was conducted using ethanol and acetone (3ml/10 snails) and in the control using plain water as the basic test. After 1,3,5,7 and 21 days of post treatment, plastic boxes were checked, and number of dead snails were recorded and removed. The concentrations - mortality percentages were calculated after 3, 5 and 7 days of treatment.

3.1.2. Contact toxicity assay:

Contact technique (thin layer film) was used according to Asher and Marian (1981). Different concentrations (1, 2, 4 and 6% w/v) of crude extracts, two ml of each concentration were deposited on inner surface of a petri-dish by moving the dish gently in circles. Solvents were evaporated under room conditions in a few minutes leaving a thin layer film of the tested compounds on the surface of petri-dishes. Ten snails in three replicates were exposed to the candidate concentration of both tested extracts for 24h. A parallel standard test was conducted using ethanol and acetone and, in the control, using plain water as the basic test. Mortality were counted and recorded after 1,3,5,7 and 21 days. All the snails remained completely dried within their shells without response to mechanical prodding were considered dead. The activity of both extracts against adults of *M. cartusiana* was expressed as mortality percentages.

3.1.3. Bait toxicity assay:

For preparation of baits, three concentrations of each acetone and ethanol extracts of clove, 2, 4 and 6% of clove were used as poisonous baits by incorporating it with wheat bran to give 100 parts of poisonous baits for each concentration ten snails were used in each box. About 10 gms of each poison bait were spread into each box that was covered with cloth netting secured with rubber bands to prevent snails from escaping. Four replicated were run in each case. Another two control groups were concurrently run containing wheat bran, one with ethanol and acetone extract as standard test and the second with plain water test but without treatment. Few milliliters of water were added daily into each box to provide suitable humidity for snail activity. The tested boxes were checked after 1,3,7,14 and 21 days to recorded and removed dead animals. Dead snails were counted and reported the results as percent mortality.

3.1.4. Data analysis:

Mortality percentages were corrected for natural mortality according to Abbott's formula (1925), then subjected to statistical analysis using Finney's method (Finney, 1971) to determine the lethal concentration causing 50% mortality(LC_{50}) and 95% mortality (LC_{95}) with 50 % fiducial limits of the tested extracts for *M. cartusiana* adults and slope in each case was calculated.

3.2. Under field conditions:

To evaluate reduction percentages of *M. cartusiana* snails after 1, 7, 14 and 21days post treatment, the experiment was carried out in heavy infested field cultivated with Egyptian clover at Hehia district, Sharkia Governorate during April 2016. The toxicity of one concentration (6%) In the laboratory by maxing 6 parts of each concentration with 89 parts of wheat bran and mixed with black sugarcane syrup 5% as attractant substances. About 100 grams of bait was offered on plastic pieces and spread along the edge of fields after the dawn. Four replicates were used for each clove extract (ethanol and acetone), plus control without treatment. Number of dead and alive snails were counted in check and treatment area before application and after 1,3,5,7,14 and 21 days. Reduction percentages were calculated according to formula of Henderson and Tiliton (1955).

Results and discussion

The results obtained on the molluscicidal activities as well as LC_{50} and LC_{95}values of *S. aromaticum* ethanol and acetone extracts at 2,4,6% concentration, against adult *M. cartusiana* after 3, 5 and7 days exposure are represented in Tables (1, 2 and 3). Data

indicated that the toxicity of organic solvent extracted (ethanol and acetone) were dependent in time and concentration when applied as contact, spray and bait technique. Through direct spray technique, it is cleared from data shown in the Table (1), that *S. aromaticum* ethanolic extract was highly active than acetone extract, indicated by the lethal concentration that killed 50% of adult snails *M. cartusiana* were being 1.67, 0.94, 0.94% for ethanol and, 5.10, 1.82 and 1.40% for acetone extract after 3, 5 and 7 days exposure, whereas, the lethal concentrations that killed 95% of adult snails *M. cartusiana* were 13.34, 14.56, 14.56% for ethanol extract and were 1717.6, 50.65and 15.90% for acetone extract of *S. aromaticum* after 3,5 and 7 days respectively .

Regarding the clove extracts effectiveness against adult *M. cartusiana* when applied by contact method, it is cleared from mortality data (3rd day) that ethanolic extract was more potent for adult *M. cartusiana* (LC_{50}=3.63 and LC_{95}=23.83) than acetone extract (LC_{50}=4.43 and LC_{95}=1429.8), while mortality data in days 5 and 7 showed that acetone extract was the most toxic botanical for adult *M. cartusiana* whereas, LC 50 =1.06 and 0.52 LC_{95}=26.83 and 11.40% compared to ethanol extract (LC_{50}=2.18 and 1.14, LC_{95}=46.29 and 17.45%) at the same time (Table, 2).

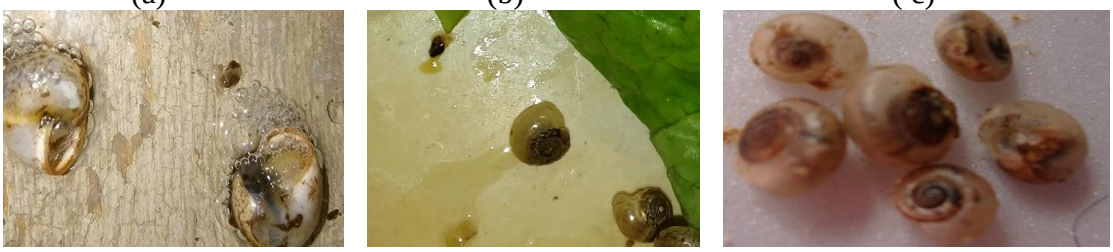
As for baits techniques, the adult *M. cartusiana* was not affected by both clove extracts at the first 3 days (no mortality was recorded), so it was difficult to determine LC_{50} and LC_{95} values (Table,3). The lethal effect recorded at 5 and 7 days post treatment, as showed in Table (3) LC_{50} and LC_{95} values of ethanol extract were relatively higher in molluscicidal activity (LC_{50}, 4.24, 3.47% and LC_{95}7.52 and 7.32%) than that obtained by acetone extract (LC_{50}, 5.67 and4.53% and LC_{95} 49.92 and 28.93%), at 5 and 7 days, respectively.

Considering the toxicity regression analysis, it was appeared from our data that fiducial limits (lower and upper limits) were positively correlated with LC_{50} values which ranged between 0.48, 12.92, and 0.56, 1.99% for acetone and ethanol extract respectively (spay method) and between 3.84 and 6.67 % for acetone extract (Bait method). The slope values indicated a homogeneity of the adults *M. cartusiana* snails in their response to both clove extracts toxicants as recorded in Tables (1,2 and 3).

In all techniques, no mortality was observed in the control groups either exposed to plain water or standard solvent. Our results showed that ethanol extract was more active in spray and bait form than acetone, however, acetone was relatively more active than ethanol when applied by contact technique. Generally, as can be seen from the above-mentioned result, that *S. aromaticum* ethanol and acetone extracts showed more molluscicidal activity against adult *M. cartusiana* when applied by contact and spray forms, than bait technique. After exposure to clove extracts examined in the present study, the snails showed the following symptoms for death including, hyperactivity, a massive of air bubbles, continuous excessive mucous secretion, and complete withdrawal inside the shell just in contact of the extract with their soft bodies followed by dehydration and finally death (Photos a, b and c).

Regarding, field application, the baits of both clove ethanol and acetone extract were applied at 6%concentration. Results in Table (4) indicated that ethanol extract was more active than acetone extract against adult *M. cartusiana* and their activity found to be the durations of exposure and concentration dependent. The population reduction percentages of these extracts after the first three days (initial effect) were to 7.93 and 7.25 % for ethanol and acetone extract respectively. While the reduction percentages after 21 days post-treatment were 60.28 and 49.28% for ethanol and acetone extract respectively.

(a) (b) (c)



Photos (a, b and c): Morphological feature of snail death after the treatment with plant extracts.

Table (1): Susceptibility of adult snails *Monacha cartusiana* towards the effect of clove extracts (acetone and ethanol) using spray technique under laboratory conditions.

Treatments		LC_{50} % (w/v)	Fiducial limits		LC_{95}% (w/v)	Slope (b)
			Lower	Upper		
Ethanol extract	3	1.67	1.33	1.99	13.34	1.82±0.23
	5	0.94	0.56	1.27	14.56	1.38±0.23
	7	0.94	0.56	1.27	14.56	1.38±0.23
Acetone extract	3	5.10	3.23	12.92	1717.6	0.65±0.14
	5	1.82	1.43	2.31	50.65	1.14±0.15
	7	1.40	0.48	2.62	15.90	1.56±0.16

N. D. = Not detected (Contact)

Table (2): Susceptibility of adult snails *Monacha cartusiana* towards the effect of clove extracts (acetone and ethanol) using contact technique under laboratory conditions.

Treatments		LC_{50} % (w/v)	Fiducial limits		LC_{95}% (w/v)	Slope (b)
			Lower	Upper		
Ethanol extract	3	3.63	3.07	5.42	23.83	2.01±0.60
	5	2.18	1.76	2.74	46.29	1.24±0.15
	7	1.14	0.35	1.87	17.45	1.38±0.15
Acetone extract	3	4.43	---------	-------	1429.8	0.65±0.11
	5	1.06	-------	-------	26.83	1.17±0.11
	7	0.52	0.39	0.65	11.40	1.22±0.14

Table (3): Susceptibility of adult snails *Monacha cartusiana* towards the effect of clove extracts (acetone and ethanol) using baits technique under laboratory conditions.

Treatments		LC_{50} % (w/v)	Fiducial limits		LC_{95}% (w/v)	Slope (b)
			Lower	Upper		
Ethanol extract	3	-	-	-	-	-
	5	4.24	-	-	7.52	0.65±0.65
	7	3. 47	-	-	7.32	1.16±0.63
Acetone extract	3	-	-	-	-	-
	5	5.67	4.9221	6.6723	49 .92	1.923±0.13
	7	4.53	3.8445	5.6952	28.93	1.22±0.14

Table (4): Susceptibility of adult snails *Monacha cartusiana* towards the effect of clove extracts (acetone and ethanol) using baits technique under field conditions.

Treatment	%Reduction during indicated days						
	Initial effect		Mean	Residual effect			Mean
	1	3		7	14	21	
Ethanol extract	6.05	9.81	7.93	28.72	48.04	60.28	45.68
Acetone extract	2.41	12.09	7.25	20.12	31.04	49.28	33.48

The present results clearly indicated that the clove ethanol extract and acetone extract can be used as an effective molluscicide in the control of land snail *M. cartusiana*. Their toxic effects were mainly dependent on time, concentration and the way in which the

extract was applied, as was evident from the negative correlations between LC_{50} and the exposure period. Adult *M. cartusiana* was susceptible to both clove ethanol and acetone extracts at different concentration whereas the ethanolic extract of clove showed higher potency in molluscicidal activity than acetonic extract along the whole time of experiment for the spray, baits and contact techniques (in 3rd day), while the acetonic extract was more potent than ethanolic extract at 5th and 7th days only for contact technique. Result revealed that ethanol extract was more toxic than acetone extract. The variation in the toxicity may be due to the fact that moluscicidal component present in clove was more soluble in ethanol than acetone solvent (Kumar and Singh, 2006), susceptibility-tolerance of the tested animals (Akhtar and Isman, 2004), or and phytochemical constituents of the plants (Olofintoye, 2010). A few studies were focused on the aquatic and terrestrial gastropods for the molluscicidal activity of *S. aromaticum*. Among the former studies, those carried out by Kumar and Singh (2006) recorded that the LC_{50} of *S. aromaticum* was 51.98 mg/l against the snail *Lymnaea acuminata* Lamarck (Gastropoda : Lymnaeidae) after 96hr and ethanol extract was more toxic than other organic extract. Also, Mobarak *et al.* (2015) they reported that clove extracted by ethanol was more toxic to the land slug, *Limax flavus* (L.) (Gastropoda : Limacidae) with an LC_{50} 0.6% than LC_{50} 0.36% of clove acetone extract.

Other researchers, Al-Zanbagi, 2005; Chauhan and Singh (2011); El-Tantawy *et al.* (2012) and Otarigho and Morenikeji (2012), they showed similar higher potency of ethanol extract in extracting the secondary products which exhibited molluscicidal activity against different species of aquatic and terrestrial snails. Furthermore, the toxicity of any molluscicide depends on the point of entry of the toxins (Franz *et al*., 2011). The potency of clove ethanol and acetone extract as observed in our study was higher in death rate when used as spray and contact methods than the bait method. This partially in agreement with Abdel-Kader *et al*. (2007) who found that using of some plant water extracts, as spraying technique was more efficient against land snails [*M. cartusiana* and *Theba pisana* (Müller) (Gastropoda : Helicidae)] than a poisonous foods or using the grinded plant parts itself. Similarly, a high effective contact technique was reported by Mourad (2014), for other tested plants extracts against *Monacha obstructa* (Pfeiffer) (Gastropoda : Hygromiidae) and *Eobania vermiculata* (Müller) (Gastropoda : Helicidae) . The effect of ethanol and acetone extract in bait technique is less harmful than spray and contact techniques. This is mainly due to the way of its application, since spray and contact technique causing rapid death of snail soft tissue before digestive tract is affected (in bait technique) which needs a considerable length of time for death or may be due to other factors such as temperature, light and the presence of impurities in the compound structures that govern stability of clove application (Turek and Stintzing, 2013).

The death of the snails in the present study is shown to be primarily correlated with the foot tissue destruction due to release of excessive mucous secretion leading to the body water lost, disrupting the cell membrane of the snail and changing its permeability (Juven *et al*., 1994 and Devi *et al*., 2010). These toxicity symptoms and signs could be linked to the presence of several secondary metabolites such as saponins, anthraquinones, tannins, cardiac glycosides and flavonoids present in *S. aromaticum* (Agbaje, 2008) which may exert molluscicidal effect and subsequently leading to the death of the *M. cartusiana* snails in this experiment. It is believed that the action of these naturally compounds causes over excitation of the

cholinergic nervous system (Young *et al*., 2010) and later brings a low paralysis and loss of muscular tone (Srivastava *et al*., 2013) or causes a metabolic disturbance by inhabiting the enzyme system primarily those of respiratory chain (Sokker *et al*., 2012 and Gonzales Correa *et al*., 2015). However, further research is necessary to isolate and identify the molluscicidal compounds and to elucidate the mechanism of its action upon snail body.

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