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Molluscicidal and antifeedant effects of some botanical oils against the land snail *Monacha obstructa* snail (Gastrda: Hygrmiidae)



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التأثيرات الإبادية والمضادة للتغذية لبعض الزيوت النباتية على قوقع البرسيم الزجاجي

ABSTRACT

The molluscicidal and antifeedant activities of six botanical oils namely sweet basil (Ocimum basilicum), clove (Eugenia aromaticum), peppermint Mentha piperita, garlic (Allium sativum), chamomile (Artemisia herba-alba) and geranium (Pelargonium graveolens) were tested against the adult stage of Monacha obstructa (Gastropoda: Hygromiidae) under laboratory conditions. The results indicated that using contact and spraying methods, sweet basil oil was the most toxic based on IC50 values. Chamomile and clove oils were the least efficient using the same methods. Also, sweet basil oil caused 82.4% inhibition in feeding activity to the land snail *M. obstructa* at 4 mg/ml. This inhibition was 74.3% by garlic oil and 57.1% by chamomile oil at the same concentration. Therefore, garlic oil at the IC₅₀ level and sweet basil at the IC₉₀ level appeared the more potent as feeding deterrents while chamomile oil was the lower effective of both levels. Conversely, botanical oils of clove, geranium and peppermint exerted negative antifeeding effects. Moreover, twenty essential oils were identified by GC/MS analysis in sweet basil. These oils were divided into twelve monoterpene hydrocarbons (70.83%) and eight sesquiterpene hydrocarbons (19.17%). It is evident that linalool (35.90%) was the most abundant compound followed by eucalyptol (12.73%) and α -farnesene (8.68%). While, among the identified compounds were eugenol (7.64%), γ -muurolene (5.42%), α -humulene (2.39%) and β pinene (1.03%). It is worth noting that sweet basil exhibited strong molluscicidal and antifeedant activities against the adults of *M. obstructa* and may be that the detected oils are responsible for causing this activity. The current study showed safe alternatives from plant origin such as sweet basil oil which may be used as a botanical molluscicide to control land snails or as antifeedant to protect the crops from them.

Keywords: Botanical oils, Antifeeding effect, *Monacha obstructa*, Chemical constituent, GC/MS analysis

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1. INTRODUCTION

Terrestrial snails are considered very serious pests resulting in significant loss of several agricultural crops causing a severe. damage to fruit trees as well as field crops, vegetables and ornamental plants in many countries (Nakhla et al., 2002; Abdel-Rahman, 2017; Idris et al., 2020). The harmful land snail Monacha obstructa, (Gastropoda: Hygromiidae) is one of the most prevalent snails in Egypt (Ali et al., 2020; Mesbah et al., 2022). Botanical oils can be successfully used in controlling land snails. Dill and fennel oils exhibited strong molluscicidal and antifeedant activities against Theba pisana land snails (El-Zemity and Radwan, 2001). Also, the essential oils of Origanum vulgare and Artmisia judaica appeared fumigant toxicity on T. pisana (Saad and Abou-Taleb, 2015). Therefore, the essential oils extracted from plants act as safe alternatives replace synthetic pesticides in pest control regardless of whether they are toxic or repellent. It is known that synthetic pesticides lead to environmental pollution and pest resistance with their effects on beneficial living organisms and phytotoxicity. So the search was for new, influential and natural materials represented in the plant kingdom which is a rich source for finding this and among them was botanical oils. The toxic impact of botanical oils against the land snail Monacha obstructa was reported by other researchers (Shoaib et al., 2010; Mourad, 2014; Farag and Sabry, 2017; Abdel-Rahman, 2020; Abobakr et al., 2022). The objective of the present investigation is to study the molluscicidal and antifeedant effects of six botanical oils namely: sweet basil, clove, peppermint, geranium, garlic and chamomile against the adult stage of land snail M. obstructa beside identification of chemical

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components of the more active oil using GC/MS analysis.

2. MATERIALS AND METHODS 2.1. Tested land snails

Adults of land snail Monacha obstructa were collected from infested alfalfa (Medicago sativa L.) field at Khaldia village, Abshwai district, Fayoum governorate. The obtained snails transferred to the laboratory in polyethylene bags. Healthy individuals and approximately the same shells diameter about (11-13 mm) for all experiments were selected and caged in plastic boxes (15×12 \times 4 cm) and supplied with fresh leaves of lettuce. The snails were acclimatized for two weeks before bioassay and starvation for 24 h. Dead snails were removed immediately and discarded (Eshra, 2014).

2.2. Tested botanical oils

Six botanical oils were selected for this study namely: sweet basil (Ocimum *basillicum* L.), geranium (Pelargonium graveolens L.), clove (Eugenia aromaticum L.), garlic (Allium sativum L.), peppermint (Mentha piperita L.) and chamomile (Artemisia herba-alba L.) extract steam distillation method (Gunther. 1948). plant essential oils Commercial were obtained from The National Research Center. These botanical oils were kept in a refrigerator until used. A series of aqueous concentrations of each essential oil was prepared with 0.5 ml tween 80 as an emulsifier.

2.3. Laboratory tests

Three different tests were carried out in the laboratory using six botanical oils assayed on the land snail *M. obstructa* adult.

2.3.1. Spraying test

The tested essential oils were applied as spray solution by atomizer on the adults of *M. obstructa* (3 ml/10 snails) in plastic boxes with green lettuce. Ten adults were placed in

each box and four replicates were used after that treated by being sprayed with different concentrations (40, 20, 10, 5 and 2.5 mg/ml) of essential oil prepared with 0.5 ml tween 80 as a surfactant. Untreated control was sprayed only with distilled water with tween 80. Mortality was estimated for snails after 24 hours of spraying and corrected by using **Abbott (1925)** formula as follows:

	,		
Corrected	mortality	(%)	=
Observed mortali	ty-Control mortality	100	
100 – Con	trol mortality ×	100	

2.3.2. Residual film (contact) test

film technique was Residual used according to Ascher and Marian (1981). The test involves five concentrations of the treated plant essential oils being applied using 1ml of each concentration deposited on the surface of petri dish 9 cm dia., by moving the box gently in circles and the solution is allowed for uniform spreading and then leaving it to dry up at room concentration temperature. Each was replicated four times and each replicate contained ten adult snails per dish which were exposed to the concentrations as a thin film. Mortality percentages were calculated after 24 h of treatment and the obtained results were corrected for the natural mortality using Abbott' (1925) formula as mentioned before. The untreated check was done by adding 40 adult snails to the empty dish

2.3.3. Antifeedant test

Homogenous discs (5 g) of fresh lettuce leaves were treated with the tested concentrations (4, 2, 1, 0.5 and 0.25 mg/ml) of each plant oil using 1 ml pipette. Plant discs were placed in a plastic box in three replications for every concentration. Ten adults were introduced into each box, 12cm dia. Control discs were treated with distilled water and 0.5 ml tween 80 alone. The consumption of leaf material was recorded

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by reweighed after 24 hours of feeding. The antifeeding effect was measured as an inhibition percentage in feeding activity and was corrected according to the following formula (**Barratt** *et al.*, **1993**)

Inhibition (%) = $(C-T)/C \times 100$, where: C = mean weight gain of control discs within 24 h and T= mean weight gain of treated discs within 24 h.

2.4. Identificationbygaschromatography-massspectrometry(GC/MS) analysisspectrometry

The GC column was 30 m (025 mm i.d., film thickness 0.25 Mm) HP-SMS (5% diphenyl) dimethylopolysiloxane capillary column. The GC conditions were as follows; injector temperature, 240 °C; column temperature, isothermal at 50 °C for 2 min, then at this temperature for programmed to 280" C at 6 °C/min and held at this for 2min; temperature ion source temperature, 200° C; detector temperature, 300C. Helium was used as the carrier gas at the rate of 1 ml/min. The effluent of the GC column was introduced directly into the ion source of the MS. Spectra were obtained in the EI mode with 70 eV ionization energy. The sector mass analyzer was set to scan from 40 to 400 amu for 5 s Gas chromatography-mass spectrometry analysis (GC-MS) was carried out using agilent auto system 7890B GC-MS equipped with HB-5MS capillary column (5% phenyl-95% dimethyl polysiloxane, 30 m \times 0.25 mm \times 0.25 µm). These data were obtained from environmental and food pollutants laboratory Faculty of Agriculture, Fayoum at University.

2.5. Statistical analysis

Toxicological data obtained were subject to statistical analysis to evaluate the relative efficiency of the tested plant oils. Mortality percentages were adopted using probit analysis program according to the

method developed by **Finney** (1971) to estimate the concentration required to kill 50 and 90% (LC $_{50}$ and LC $_{90}$) as well as the concentrations which caused 50 and 90% inhibition (IC $_{50}$ and IC $_{90}$). The values of slope \pm SE and chi-square (χ^2) were also determined. Withal, the data were statistically analyzed according to **Snedecor and Cochran** (1980). Means were compared using the least significant difference (LSD) test at 0.05 level of probability.

3. RESULTS AND DISCUSSION 3.1. Toxicity of some botanical oils against the adult stage of *M. obstructa*

The effect of six botanical oils namely: clove, garlic, peppermint, sweet basil, chamomile and geranium as spraying and contact were tested

3.2. Botanical oils as spray solutions

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Data in Table 1 indicate that sweet basil oil was the most effective with LC₅₀ of 5.23 mg/ml followed by peppermint and garlic oils with LC₅₀ values of 19.7 and 22.2 mg/ml respectively. Clove oil was the least toxic with LC_{50} of 51.4 mg/ml, the LC_{90} values followed the same order as that of LC_{50} . Therefore, sweet basil was the most toxic with LC_{90} of 24.3 mg/ml while clove was the least toxic with LC₉₀ value of 465 mg/ml. The relative potency at the LC_{50} and LC₉₀values elucidated that sweet basil oil had 9.83 and 19.1 folds as effective as clove oil. Likewise, garlic oil at the same values were 2.31 and 5.67 folds more toxic than clove oil. Thus, toxicity index illustrated that clove at the values of LC₅₀ and LC₉₀ were 10.2 and 5.22% as toxic as sweet basil. Withal, garlic oil at the same values were 23.6 and 29.6% lower potent than sweet basil.

Oil	LC ₅₀ (mg/ml)		nfidence nits	LC ₉₀ (mg/ml)		nfidence nits	Slope±SE	Toxicit (%)		potenc	ative cy (fold) at
	(IIIg/IIII)	Lower	upper	(IIIg/IIII)	Lower	upper		LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀
Sweet basil	5.23	3.849	6.678	24.3	17.42	41.16	1.92 ± 0.27	100	100	9.83	19.1
Peppermint	19.7	44.35	131.6	89.2	56.09	194.7	1.95 ± 0.28	26.5	27.2	2.60	5.21
Garlic	22.2	17.79	29.47	82.0	54.46	161.7	2.25 ± 0.31	23.6	29.6	2.31	5.67
Chamomile	39.3	27.67	71.52	219	106.1	930.9	1.72 ± 0.30	13.3	11.1	1.30	2.12
Geranium	48.4	35.34	88.21	170	92.09	661.8	2.35 ± 0.46	10.8	14.3	1.06	2.74
Clove	51.4	31.74	138.2	465	162.9	5239	$1.34{\pm}0.27$	10.2	5.22	1.0	1.00

Table 1. LC₅₀, LC₉₀, slope, toxicity index and relative potency values of some botanical oils of *M. obstructa* adults using spraying technique

 LC_{50} : Concentration which is lethal to 50% of a population of test animals. LC_{90} : Concentration which is lethal to 90% of a population of test animals.

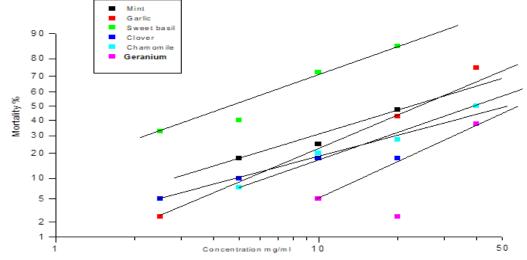


Fig. 1. Toxicity lines of essential oils by spraying technique.

3.3. Botanical oils as contact poisons

Data presented in Table 2 revealed that sweet basil oil as a residual film proved to be the highly toxic with LC_{50} values of 7.70 mg/ml followed by clove and peppermint oils with LC₅₀ of 8.18 and 8.39 mg/ml respectively. Based on LC₉₀ level, only peppermint and clove oils were more toxic than sweet basil, this level for peppermint and clove oils were 34.9 and 36.0 mg/ml while, sweet basil was 54.2 mg/ml. Chamomile oil was the lower efficient with LC₅₀ and LC₉₀ were 26.9 and 240 mg/ml respectively. The relative potencies, at the LC50 and LC90 values exhibited that sweet basil oil had 3.49 and 4.42 times more potent than chamomile oil. Therefore, the toxicity index showed that chamomile had 28.6 and

14.5% as effective as sweet basil at LC₅₀ and peppermint at LC₉₀ respectively. Toxic action of botanical oils as molluscicides was also reported by El-zemity and Radwan (2001)using garlic. geranium, and peppermint oils on Theba pisana, Ismail et al. (2011) using clove oil on Monacha cartusiana, Farag and Sabry (2017) using sunflower and soybean oils on М. cartusiana, Idris et al. (2020) using citronella and clove oils on Pomaceoa canaliculate, Mesbah et al. (2022) using neem and peppermint oils on T. pisana, and Eobania vermiculata and Abobakr et al. (2022) using lavender, Juniper and wild mint oils on *M. obstructa*, whom showed that these botanical oils have molluscicidal activity.

Table 2. Toxic effect of some tested botanical oils of *M. obstructa* adults using residual film technique

()1]	LC_{50}	95% confidence limits		LC_{00} (mg/ml)			Slope±SE	Toxicity index (%) at		Relative potency (fold) at	
	(mg/ml)	Lower	upper		Lower	upper	•	LC50	LC ₉₀	LC ₅₀	LC ₉₀
Sweet basil	7.70	5.558	10.27	54.2	33.32	127.7	1.51 ± 0.24	100	64.3	3.49	4.42
Clove	8.18	6.397	10.29	36.0	25.43	62.05	1.99 ± 0.26	94.1	96.9	3.29	6.67
Peppermint	8.39	6.624	10.49	34.9	25.01	58.64	$2.07{\pm}0.27$	91.8	100	3.21	6.88
Geranium	13.9	9.829	21.54	160	72.72	828.5	1.21 ± 0.23	55.4	21.8	1.94	1.50
Garlic	15.6	12.63	19.99	60.2	41.59	108.1	$2.18{\pm}0.28$	49.3	57.9	1.72	3.99
Chamomile	26.9	18.79	48.49	240	104.5	1312	$1.35{\pm}0.25$	28.6	14.5	1.0	1.00

 LC_{50} : Concentration which is lethal to 50% of a population of test animals. LC_{90} : Concentration which is lethal to 90% of a population of test animals.

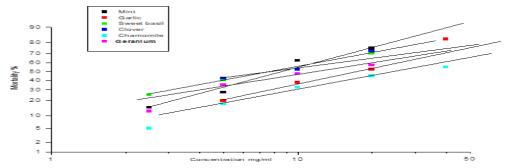


Fig. 2. Toxicity lines of essential oils by film method.

3.4. Antifeedant activity of the tested oils against *M. obstructa*

3.3.1. The inhibition rate

It is clear from Table 3 that the oil of sweet basil caused 82.4% inhibition in feeding activity at 4 mg/ml to the adult stage of *M. obstructa*. while the inhibition was 74.3% by garlic oil and 57.1%. by chamomile oil at the same concentration. The food consumption of the snail adult was 0.37 g at 4 mg/ml by sweet basil oil, thereby the feeding on lettuce leaves was less after 24 h of treatment and antifeeding effect was

observed. Likewise, the food consumption was relatively greater by garlic and chamomile oils were 0.54 and 0.90 g at 4 mg/ml compared to 2.10 g in control. Apparently, chamomile oil was not given a positive percentage of inhibition in feeding activity for the snails at 0.25 mg/ml because the food consumed was more than control. Only 9.52 and 6.19% inhibition resulted from the feeding on lettuce leaves treated with garlic and sweet basil oils at 0.25 mg/ml.

Table 3. Effect of three botanical oils on inhibition rate as a percentage (%) in feeding activity to of *M. obstructa* adults

Concentration (mg/ml)	Mean weight after 24 h	Food consumption (g)	Inhibition (%)
	Sweet I	oasil oil	
Control	2.90a	2.10	-
0.25	3.03a	1.97	6.19
0.50	3.27b	1.73	17.6
1.0	3.63c	1.37	34.8
2.0	4.20d	0.80	61.9
4.0	4.63e	0.37	82.4
LSD at 0.05	0.139	-	-
	Chamo	mile oil	
Control	2.90a	2.10	-
0.25	2.73b	2.27	-8.09
0.50	3.13c	1.87	10.9
1.0	3.46d	1.54	26.7
2.0	3.54 d	1.46	30.5
4.0	4.10 e	0.90	57.1
LSD at 0.05	0.131	-	-
	Garl	ic oil	
Control	2.90a	2.10	-
0.25	3.10 b	1.90	9.52
0.50	3.53c	1.47	30.0
1.0	3.80d	1.20	42.9
2.0	4.13e	0.87	58.6
4.0	4.46f	0.54	74.3
LSD at 0.05	0.135	-	-

The weigh before treatment was 5 g/disc of fresh lettuce leaves.

3.3.2. Inhibitory concentrations (IC₅₀ and IC₉₀)

The concentrations which cause 50 and 90% inhibition (IC₅₀ and IC₉₀) in the feeding activity by snails are shown in Table 4. It was explained that of garlic oil was the most active as antifeedant with IC₅₀ of 1.40 mg/ml followed by sweet basil with IC₅₀ was 1.45 mg/ml. Based on IC₉₀ value, sweet basil was more active on *M. obstructa* adults than garlic oil as feeding deterrent, the IC₉₀ for sweet basil and garlic oils were 6.05 and 10.0 mg/ml respectively. The antifeedant activity decreased with chamomile oil with IC₅₀ and IC₉₀ of 2.78 and 14.6 mg/ml at IC₅₀ level, chamomile was half the activity of garlic, and the relative potency was almost

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two folds as effective as chamomile. At IC₉₀ level, sweet basil was 2.4 times more active than chamomile oil and the last one was 41.4 activity compared to sweet basil (100%). In contrast, clove, geranium, and peppermint oils failed to show any antifeeding action. botanical oils that acted as antifeedants against land snails was recorded on Allium sativum by El-Zemity and Radwan (2001) on Artemisia herba-alba and Azadirachta indica by Afifi et al. (2007), on Lupinus termis by Azzam et al. (2014), on Anagallis arvensis by Ali et al. (2020) and on Mentha longifolia by Abobakr et al. (2022) who found that these natural plants contain oils which have powerful antifeedant properties.

Table 4. Antifeeding activity of three botanical oils against *M. obstructa* adults

Oil	IC ₅₀ (mg/ml)	95% cor lim		Fold	Activity (%)	IC ₉₀ (mg/ml)	95% cor lim		Fold	Activity (%)	Slope±SE	X ²
	(mg/nn)	Lower	upper		(/0)	(ing/iii)	Lower	upper		(70)		
Sweet basil	1.45	1.258	1.686	1.92	96.6	6.05	4.658	8.598	2.41	100	$0.293 {\pm} 0.066$	2.062
Garlic	1.40	1.686	1.717	1.99	100	10.0	6.720	17.97	1.46	60.5	1.499 ± 0.061	2.511
Chamomile	2.78	2.294	3.549	1.00	50.4	14.6	9.649	26.79	1.00	41.4	1.779 ± 0.073	5.812

3.3.3. Identification of the chemical constituents in the essential oil of sweet basil using GC/MS analysis

Twenty essential oils were identified in sweet basil as listed in Table 5 and their chemical structures in Fig. 3. These oils were divided into twelve monoterpene hydrocarbons (70.83%)and eight sesquiterpene hydrocarbons (29.17%). It is evident that linalool (35.90%) was the most abundant compound followed by eucalyptol (12.73%) and α -farmesene (8.68%). Also, among the identified compounds were β pinene (1.03%), estragole (7.59%), bornyl acetate (1.81%), eugenol (7.64%) and ocymene (0.53%) that tracking monoterpene oils. Besides the identified essential oils which belong to sesquiterpenes such as γ muurolene (5.42%), tau-candiol (4.50%), α - humulene (2.39%) and germacrene D (3.95%). It is worth noting in the current study that sweet basil oil exhibited strong molluscicidal and antifeedant activities against the adults of *M. obstructa*. This may be that the terpene hydrocarbons which included monoterpenes and sesquiterpenes found in the essential oil of sweet basil are responsible for causing this activity. These results are in agreement to the findings of Salama et al. (2012) reported that the major sesquiterpene hydrocarbon in essential oil of vulgare Marrubium was γ-cadinene (17.68%) while Z-caryophyllene was the major sesquiterpene hydrocarbon in essential oil of Thymus capitatus (6.15%). The two

plant oils appeared dominated by the oxygenated constituents, these were *mainly* composed of phenols among which carvacrol (32.98%) in T. capitatus and thymol (34.55%) in M. vulgare. Both oils showed promising molluscicidal activity against Biomphalaria alexandrina snails concluding that this activity may be attributed to thymol and carvacrol. Gomes et al. (2019) found that the major constituent is limonene (58.81%) as monoterpene and the minority component is α -mulene (0.11%) when extracted the oils from Citrus limon peels. Also, among the identified oils using GC/MS analysis were γ -terpinene (9.01%), sabinene (5.08%), β -pinene (4.91%) and α citral (1.61%). The essential oil is highly toxicity to the adults of Biomphalaria glabrata snails than Aedes aegypti and not toxic to the larvae of Artemid salina. Abobakr et al. (2022) identified the

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chemical constituents from the essential oil (EO) of Juniperus procera and Lavandula dentata. Both tested plants exerted antifeedant and molluscicidal effects on M. obstructa. In the J. procera, five compounds were specified which represented 97.87% of the EO composition. The oil components were α -pinene (53.70%), P- cymene (24.83%), β -ocimene (10.78%), γ -elemene (5.25%) and thymol (3.31%). Camphor (45.74%) was the most abundant compound in L. dentata followed by eucalyptol (18.63%) and β -myrcene (9.02%). However, additional research is needed to evaluate the tested botanical oils as molluscicides against the terrestrial snails under field conditions and testing pure separated oils such as monoterpenes which represent 70.83% of the total oils in sweet basil to know it's combat efficiency on snails.

NO	Essential oil	Molecular formula	Molecular weight	RT (min)	Area (%)
		Monoterr	benes		
1	α-pinene	C10H16	136.23	6.30	1.07
2	Camphene	$C_{10}H_{16}$	136.23	6.66	0.26
3	β -pinene	$C_{10}H_{16}$	136.23	7.18	1.03
4	o-cymene	$C_{10}H_{14}$	134.22	7.35	0.53
5	Eucalyptol	$C_{10}H_{18}O$	154.25	7.44	12.73
6	β -ocimene	$C_{10}H_{16}$	136.23	8.25	0.62
7	Linalool	$C_{10}H_{18}O$	154.25	8.74	35.90
8	Estragole	$C_{10}H_{12}O$	148.20	10.10	7.59
9	a-terpineol	$C_{10}H_{18}O$	154.25	10.44	1.33
10	Sabinene	$C_{10}H_{16}$	136.23	11.43	0.32
11	Bornyl acetate	$C_{12}H_{20}O_2$	196.28	11.79	1.81
12	Eugenol	$C_{10}H_{12}O_2$	164.20	12.65	7.64
		Sesquiter	penes		
13	γ-Elemene	C15H24	204.35	12.81	0.40
14	α-cubebene	C15H24	204.35	13.12	1.01
15	α-farnesene	C15H24	204.35	13.58	8.68
16	α-humulene	C15H24	204.35	14.02	2.39
17	Germacrene D	C15H24	204.35	14.20	3.95
18	γ-muurolene	C15H24	204.35	14.53	5.42
19	β -guaiene	C15H24	204.35	16.41	2.82
20	Tau-cadinol	C15H260	222.37	16.17	4.50
Fotal ident	ified essential oils				100
Fotal mono	oterpene hydrocarbons				70.83
	iterpene hydrocarbons				29.17

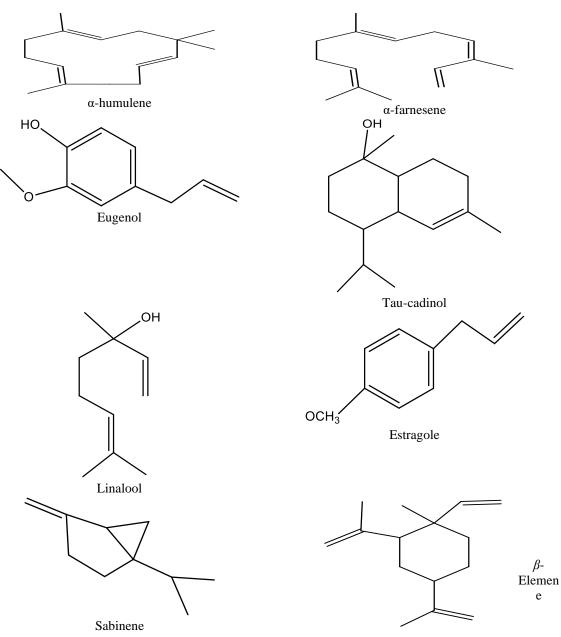


Fig. 3. The chemical structures of terpenes from sweet basil.

4. CONCLUSION

These results indicated that sweet basil oil was the most toxic using contact and spray methods. Also, the same oil caused highly inhibition infeeding activity to the land snail M. *obstruct*. Moreover, 20 essential oils were identified in sweet basil, linalool was the most

abundant oil (35.9%). Therefore, sweet basil oil can be exploited as molluscicide or as antifeedant

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