



Article

The effect of four botanical volatile oil against aphid (*Aphis gossypii*) and mealybug pests (*Phenacoccus solenopsis*). A toxicological and chemical composition study

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Abstract: Given the economic damage caused by cotton mealybugs *Phenacoccus solenopsis*, (*P. solenopsis*) and aphids *Aphis gossypii* (*A. gossypii*), it was a need to look for more acceptable and safe materials. Four local plant extracts were assessed *Ocimum basilicum*, *Origanum majorana*, *Piper nigrum*, and *Schinus terebinthifolius* represented by their volatile oils, against these two pests in the laboratory, with the following LC50 values for each applied volatile oil extract against aphid and mealybug 1938.05, 7391.33, 674.81, 5548.65, 644.45, 4383.52, 727.88, and 6735.62 ppm using the four previous extracts respectively. The highest mortality percentage was 50.0 and 49.3% for *piper nigrum*, while the lowest percentage was for *O. basilicum* with 36.2 and 31.2% against *P. solenopsis* and *A. gossypii*, respectively. The oils that gave the highest mortality percentages *piper nigrum* and *Origanum majorana* were analyzed using GCMS technique revealed the existence of major compounds belonging to the terpenoids chemical group, which have a noticeable role in the process of controlling these pests.

Key words: Cotton, *Phenacoccus solenopsis*, *Aphis gossypii*, plant extracts, mealybugs pests.

1. Introduction

Natural compounds and plant-based extracts are frequently utilized in pest control operations, whether insect or animal; these compounds have features that can be applied to control some types of hazardous animals such as land snails and mice, and they are also used in combatting insects. (Ali and Ayyad 2022, Ali 2023). Natural extracts of plant origin are considered promising materials that are of interest to researchers, whether those working in the natural products chemistry or those interested in studying and control agricultural pests in general, and aphids (*Aphis gossypii*) and Egyptian mealybugs (*Phenacoccus solenopsis*) in particular. The whole plant extract obtained from the tea leaves was tested for insecticidal efficacy against green aphid. The efficiency of various concentrations of tea extract formulation on green peach aphid was assessed using two traditional methods: leaf dipping

and leaf spraying. The results have demonstrated that tea leaf extract could be considered as a possible source for the development of a pesticide for aphid control (**Khoshraftar, Shamel *et al.*, 2019**). The efficacy of nine botanical extracts (neem, neem oil, neem leaf extract, lantana leaf extract, tobacco decoction, garlic bulb extract, karanj oil, and ginger rhizome) against cabbage aphid, *Lipaphis erysimi*, was compared to a control group. The tobacco decoction and ginger rhizome extract were shown to be considerably superior to neem oil, garlic bulb extract, and neem seed kernel extract (**Chavada *et al.*, 2020**).

The insecticidal activity of alcoholic plant extracts on *Aphis craccivora* Koch in cowpea under greenhouse conditions was evaluated. Hydrated ethanol was used as a solvent, the botanical extracts with more than 50% efficiency were: *Caesalpinia ferrea*, *Allium tuberosum*, *Piper aduncum*, *Dieffenbachia picta*, *Carica papaya*, *Cucurbita moschata* and the control treatment, *Annona squamosa*. Some of the botanical extracts did not have an effect of more than 50% (**Dutra *et al.*, 2020**).

Botanical insecticides are environmentally friendly, cost-effective, and unlikely to lead to pest resistance property. The effect of five different botanical extracts on the bean aphid, *Aphis craccivora*, and the second larval instar of the green lacewing, *Chrysoperla carnea* was assessed in a laboratory setting. Furthermore, HPLC analysis revealed the existence of many flavonoid molecules; the flavonoids in the methanolic extracts of these plants were identified by HPLC analysis. This investigation reveals that *O. baccatus* could be used as an effective natural extract-based pesticide utilized in pest management operations against *A. craccivora* (**Sayed *et al.*, 2020**).

Among the most harmful aphids in the Brassicaceae family is *Brevicoryne brassicae*, which can reach exceptionally serious densities if left uncontrolled. The relationship between two well-known systemic pesticides, acetamiprid and pirimicarb, and extracts of *Peganum harmala*, *Melia azedarach*, *Calendula officinalis*, and *Otostegia persica* that are methanolic, ethanolic, and aqueous. By using leaf spraying in a greenhouse, the population percentages of *B. brassicae* were determined. One of the greenhouses housing the leaves was sprayed with chemicals. According to **Shafiei *et al.* (2018)**, the study stated that different plant extracts have distinct systemic effects. For example, the methanolic extract of *P. harmala*, the ethanolic extract of *O. persica*, and the aqueous extract of *M. azedarach* all decreased the population of *B. brassicae*.

In a laboratory, numerous essential oils were examined for their toxicity against *H. armigera* larvae. After 24 and 48 hours of application, respectively, *Ocimum basilicum* essential oil showed strong larvicidal activity at 2.5%, with 88% and 92% larval mortality. When compared to the control, the field data showed that *O. basilicum* oil at 2.5% was very effective in lowering pod borer larvae and pod damage six days after the first and second sprays. According to **Boulamtat *et al.*, 2021**, the findings indicate that *O. basilicum* essential oils may be used in the creation of biological pesticides that are safe, effective, and acceptable for the environment in order to control the chickpea pod borer.

In a laboratory, **(El-Sherbini and colleagues, 2014)** evaluated marjoram extracts' insecticidal efficacy against lice caused by *Pediculus humans capitis* was conducted. A comparison was made between the contact insecticidal activity of a 0.25 mg/cm² dosage of marjoram oil and the commonly used insecticides, pyrethrum and phenothrin, against adult *P. humanus capitis*. In terms of LT50 values, pyrethrum or &-phenothrin were less hazardous than marjoram oils. Compared to phenothrin, it was 2.0 times more harmful. The hazardous concentration was 0.0625 mg/cm. When using 0.25 mg/cm² of *P. humanus capitis* for fumigation, marjoram oils worked better in enclosed spaces than in open ones.

Aqueous and methanolic extracts of *Curcuma longa* (turmeric) and *Piper nigrum* (black pepper) were tested on *Anopheles gambiae* larvae, pupae, and adults. In laboratory conditions, individuals were tested for repellence experimentation using moulded insecticidal coils and plant essential oils. The essential oils were particularly effective against *A. gambiae* larvae, with 100% mortality for both *P. nigrum* and *C. longa* at LC50 values of 15 and 149 ppm, respectively. The methanolic extracts revealed LC50 values of 27 and 214 ppm for *P. nigrum* and *C. longa*, correspondingly. The essential oils of *C. longa* and *P. nigrum* oils were concentrated (100%) and provided above 95% protection. The

concentration (100%) of *P. nigrum* and *C. longa* reduced adult mosquitos by 76.7% and 70%, respectively. As a result, *P. nigrum* and *C. longa* can be regarded repellents against *A. gambiae* and can be utilized in chemical control methods (Kemabonta *et al.*, 2018).

Aphis craccivora Koch is one of the most damaging pests bean crop *Vigna unguiculata* (L.). The ability of essential oils from *Hymenaea courbaril*, *Copaifera langsdorffii* and *Schinus terebinthifolius* to kill nymphs and adults of *A. craccivora*. The biotests were carried out in two stages: the first was conducted in the laboratory, under controlled conditions of temperature, relative humidity and photophase, and the following stage was carried out in the greenhouse, with only the treatment that gave the greatest results in the laboratory tests. After exposure 24, 48, 72, 96 and 120 hours, the insect mortalities were determined.

The aroeira oil indicated an 83.33% and 75.75% efficiency of mortality in nymphs and adults, respectively, in the experiment's first phase. Regarding its safe usage as a natural pesticide for the control of the black aphid, the same oil demonstrated 73.52% in nymphs and 62.85% in adults in the greenhouse trial (Bezerra *et al.*, 2023).

The current study investigates the toxicological influence of four plant-based essential oils that can be used in chemical control operations for mealybugs (*Phenacoccus solenopsis*) and aphids (*Aphis gossypii*), two of the most common pests. The experiment is conducted in a laboratory conditions.

2. Materials and Methods

2.1. Plant Collected Materials: *Origanum majorana* L. (Lamiaceae), *Piper nigrum* L. (Piperaceae), *Ocimum basilicum* L. (Lamiaceae), *Schinus terebinthifolius* R. (Anacardiaceae) were collected from many farms' localities in Damietta governorate.

2.2. Essential Oils Processing:

Hydrodistillation of the four essential oils operating a Clevenger apparatus type were performed for 8 h and stored in the dark at -20°C, until analysis.

2.3. Insecticidal application techniques

2.3 a. Insects

2.3 a1. On cotton seedlings (*Gossypium hirsutum* L.), *Aphis gossypii* were collected from a commercial nursery of several ornamental plants and was placed for experimentation. Aphid populations were maintained in tiny mesh cages measuring 47.5 × 47.5 × 47.5 cm. Plants were changed each week and fresh plants infested with aphids taken from the discarded plants. Insect petri dishes were placed in controlled environment rooms set to 25 °C, 70% relative humidity and 12:12 (light-dark) hours.

This work was carried out from Feb to May 2024. *A. gossypii* rearing was started with aphids collected from a cotton field located at Damietta University, Egypt. The aphids were then transferred to cotton plants maintained plastic cups, containing a sandy soil. *A. gossypii* individuals were selected at random, transferred to cotton leaf discs of 5.0 cm in diameter Petri dishes. In order to evaluate the effect, the applied four volatile oils on *A. gossypii*, the extracts were prepared, 5 hours prior to the experiment starts. The treatments consisted of four concentrations of 625, 312.5, 156.25 and 78.12 ppm water and control contained of distilled water. Each leaf disc was sprayed by the applied extracts.

2.3 a2. Mealybug cultures

The host plant was *Ficus benjamina*. Individuals collected from an infested *Ficus benjamina* street trees in the region of Mansoura city, Egypt. The culture was maintained on sprouted potatoes in sandwich boxes. The boxes were kept at 26° C and constant dark.

2.3 b. Applied concentrations

For the preparation of the target extract, one gram of the volatile oil was weighed and diluted in (100 mL of distilled H₂O containing 1% Triton X as an emulsifier), giving a solution of 1% concentration (g mL⁻¹) as a stock solution of each volatile extract.

Four volatile oils concentrations, (5000, 2500, 15000 and 1000 ppm) four replicates, 40 individuals each replicate for *Phenacoccus solenopsis* and (625, 312.5, 156.2 and 78.1 ppm), four replicates, 40 individuals each replicate for *aphis gossepii* were prepared.

2.3 c. Spray technique

The activity of four volatile oils of varying concentrations (5000, 2500, 15000 and 1000 ppm) for *Phenacoccus solenopsis* and (625, 312.5, 156.2 and 78.1 ppm) were tested. Four replicates corresponding to each concentration were performed, the control treatment was presented with untreated discs. The mortality rate was measured after one, three, five, and seven days.

2.4. Gas chromatography–mass spectrometry (GC-MS) analysis

The samples were adjusted using GC-TSQ spectrometer mass device (Thermo Scientific, Austin, TX, USA), with a straight direct column TG–5MS (30-meter x 0.25-millimeter x 0.25-micrometer). The temperature was originally set at 60°C and then gradually increased by 6°C /min to 250°C, held for 1 min and then increased to 300 at 30 C/min. The injector temperature was maintained at 250°C. Helium was employed as a carrier gas with a flow rate of 1 ml/min. Using Autosampler AS3000 coupled with GC, diluted samples of 1 µl were injected automatically after a 4-minute solvent delay. EI mass spectra were taken at 70 eV. The components were identified by comparing their corresponding mass to those of WILEY 09 and NIST14 mass (Ali and Ayyad, 2022).

2.5. Analytical statistics

LDP line software program was used to calculate the LC50 values, were expressed in ppm unit, the slope for the target plant extracts, were plotted using the Probit analysis (Helmy *et al.*, 2022).

3. Results and Discussion

The mortality percentage recorded in Table 1 showed that the volatile extract of *Piper nigrum* through the applied four concentrations was the highest percentage with 50.0 and 49.3%, while the lowest percentage was for *O. basilicum* with 36.2 and 31.2% against *P. solenopsis* and *A. gossypii*, respectively while *Origanum majorana* and *Schinus terebinthifolius* were 48.7, 44.3% and 43.1, 45.6% against *P. solenopsis* and *A. gossypii*, respectively.

3.1. Toxicity tests under laboratory conditions

We elucidated the insecticidal activity of *Ocimum basilicum*, *Origanum majorana*, *Piper nigrum*, and *Schinus terebinthifolius* against the two target pests (Aphid and *Phenacoccus solenopsis*) using leaf dipping, respectively. The results are summarized in Table 2 demonstrate that *Piper nigrum* shows the highest mortality rate for Aphid and *Phenacoccus solenopsis* with LC50 values 644.45 and 4383.52 ppm followed by *Origanum majorana* with LC50 values 674.81 and 5548.65ppm. The impact of these extracts could be attributed to the presence of the major identified terpene (mono and sesquiterpenes) chemicals. *Schinus terebinthifolius* showed LC50 of 727.88 and 6735.62 ppm, while *Ocimum basilicum* was the highest LC50 values with 1938.05 and 7391.33 ppm for Aphid and *Phenacoccus solenopsis*, respectively.

Table (1). The applied concentrations in ppm for *Ocimum basilicum*, *Origanum majorana*, *Piper nigrum* and *Schinus terebinthifolius* extracts against Aphid and *Phenacoccus solenopsis* and mortality percentage

No	Essential Oil Extract	Con, ppm	Pest	Mortality %
1	<i>Ocimum basilicum</i>	5000	<i>Phenacoccus solenopsis</i>	36.2
		2500		18.7
		1250		6.8
		1000		3.7
		625	<i>Aphid</i>	31.2
		312.5		28.1
		156.25		22.5
		78.12		9.3
2	<i>Origanum majorana</i>	5000	<i>Phenacoccus solenopsis</i>	48.7
		2500		36.8
		1250		30.0
		1000		24.3
		625	<i>Aphid</i>	44.3
		312.5		40.0
		156.25		24.3
		78.12		11.2
3	<i>Piper nigrum</i>	5000	<i>Phenacoccus solenopsis</i>	50.0
		2500		39.3
		1250		24.3
		1000		19.3
		625	<i>Aphid</i>	49.3
		312.5		40.0
		156.25		30.0
		78.12		21.2
4	<i>Schinus terebinthifolius</i>	5000	<i>Phenacoccus solenopsis</i>	43.1
		2500		33.7
		1250		23.1
		1000		16.2
		625	<i>Aphid</i>	45.6
		312.5		41.2
		156.25		28.7
		78.12		20.0

Table (2). The toxicity of *Ocimum basilicum*, *Origanum majorana*, *Piper nigrum* and *Schinus terebinthifolius* extracts against Aphid and *Phenacoccus solenopsis*.

Plant extract	Pest	LC50, ppm	Lower and upper values, ppm	LC90, ppm	Slope ± S.E
<i>Ocimum basilicum</i>	Aphid	1938.05	1017.11 and 8193.97	70746.39	0.82±0.16
	<i>Phenacoccus solenopsis</i>	7391.33	5808.93 and 10649.43	33261.51	1.96±0.23
<i>Origanum majorana</i>	Aphid	674.81	507.48 and 1056.36	8441.80	1.16±0.16
	<i>Phenacoccus solenopsis</i>	5548.65	3846.36 and 12015.19	154188.68	0.88±0.18
<i>Piper nigrum</i>	Aphid	644.45	453.11 and 1213.37	19693.29	0.86±0.15
	<i>Phenacoccus solenopsis</i>	4383.52	3542.58 and 5909	34871.91	1.42±0.15
<i>Schinus terebinthifolius</i>	Aphid	727.88	482.99 and 1525.72	25101.09	0.83±0.15
	<i>Phenacoccus solenopsis</i>	6735.62	4723.99 and 13203.04	102205.42	1.08±0.19

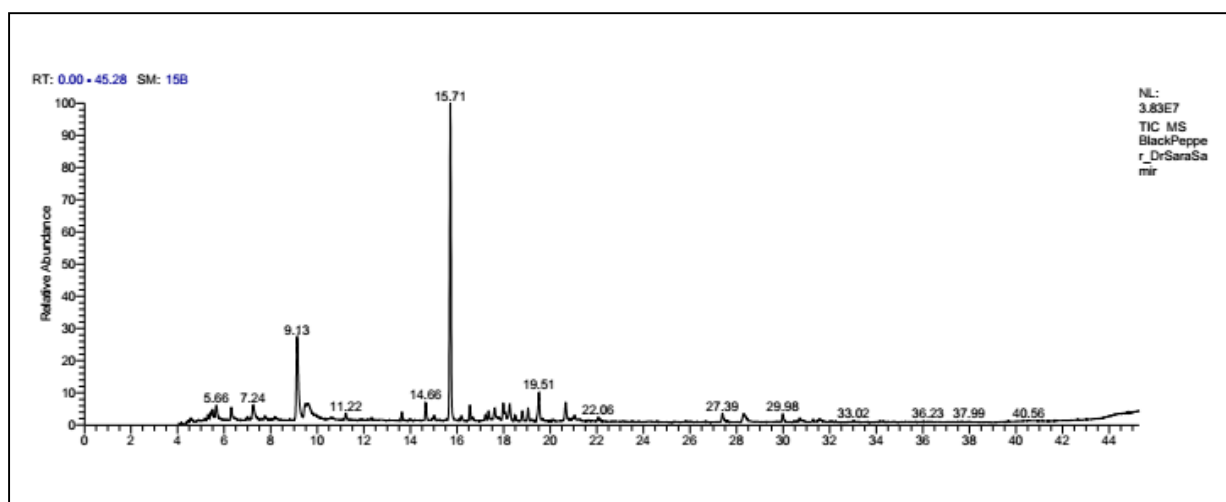


Figure (1). GC/MS analysis chart of *pepper nigrum*

Natural products and essential oil components such as terpenes have been shown to have a significant potential for insect control. The toxicity of terpene compounds against three insects: lice, cockroaches, and Triatominae bugs has been assessed to evaluate the importance of bioactive terpenes (Dambolena et al., 2016).

Three terpenes based biopesticides were employed to reduce the population of two aphid species, *Myzus persicae* and *Aphis gossypii*, in ornamental crops. The three products tested were neem oil, orange oil, and the essential oil from *Chenopodium ambrosioides*. The biopesticides were applied as foliar sprays, where, all the applied essential oils reduced the numbers of aphids by 85% at least during the experimentation period (Smith et al., 2018).

Table (3). Main chemical components of *Pepper nigrum* identified by GC/MS technique

No	Compound Name	R.T	Molecular Formula	Area %
1	6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane	4.56	C ₁₀ H ₁₆	0.52
2	2,6,6-trimethylbicyclo[3.1.1]hept-2-ene	5.31	C ₁₀ H ₁₆	0.54
3	1-methyl-4-(propan-2-ylidene)cyclohex-1-ene	5.41	C ₁₀ H ₁₆	0.69
4	m-cymene	5.49	C ₁₀ H ₁₄	1.11
5	1-methyl-4-(prop-1-en-2-yl)cyclohex-1-ene	5.66	C ₁₀ H ₁₆	1.93
6	1-isopropyl-4-methylenebicyclo[3.1.0]hexane	6.30	C ₁₀ H ₁₆	1.99
7	3,7-dimethylocta-1,6-dien-3-ol	7.24	C ₁₀ H ₁₈ O	2.16
8	1-isopropyl-4-methylcyclohex-3-enol	9.13	C ₁₀ H ₁₈ O	12.8
9	2-(4-methylcyclohex-3-enyl)propan-2-ol	9.47	C ₁₀ H ₁₈ O	1.97
10	1-allyl-4-methoxycyclohexane	9.58	C ₁₀ H ₁₂ O	2.64
11	1-isopropyl-4-methyl-3-(prop-1-en-2-yl)-4-vinylcyclohex-1-ene	13.63	C ₁₅ H ₂₄	1.09
12	1,2,3,4,4a,5,8,8a-octahydro-4-isopropyl-1,6-dimethylnaphthalene	14.65	C ₁₅ H ₂₄	2.32
13	(3Z,6E)-3,7,11-trimethyldodeca-1,3,6,10-tetraene	16.19	C ₁₅ H ₂₄	0.69
14	(1E,5E)-1,5,8,8-tetramethylcycloundeca-1,5-diene	16.55	C ₁₅ H ₂₄	2.0
15	1,2,3,4,4a,5,6,8a-octahydro-2-isopropyl-4a,7-dimethylnaphthalene	17.35	C ₁₅ H ₂₄	1.13
16	1-methyl-4-(4-methylpenta-1,3-dien-2-yl)cyclohex-1-ene	17.98	C ₁₅ H ₂₄	2.84
17	1,2,4a,5,8,8a-hexahydro-1-isopropyl-4,7-dimethylnaphthalene	18.26	C ₁₅ H ₂₄	2.30
18	(1S, 2R, 7S, 8S)-2,6,6-trimethyl-9-methelenetricyclo[5.4.0.0 ^{2,8}]undecane	18.49	C ₁₅ H ₂₄	0.82
19	(1Z,5E)-1,5-dimethyl-8-(propan-2-ylidene)cyclodeca-1,5-diene	19.05	C ₁₅ H ₂₄	1.54
20	decahydro-1,1,7-trimethyl-4-methylene-1H-cyclopropa[e]azulen-7-ol	20.66	C ₁₅ H ₂₄ O	2.54
21	1,2,3,4,4a,7,8,8a-octahydro-4-isopropyl-1,6-dimethylnaphthalen-1-ol	21.05	C ₁₅ H ₂₆ O	0.87
22	2-methyl-5-(6-methylhept-5-en-2-yl)bicyclo[3.1.0]hexan-2-ol	22.06	C ₁₅ H ₂₆ O	0.87

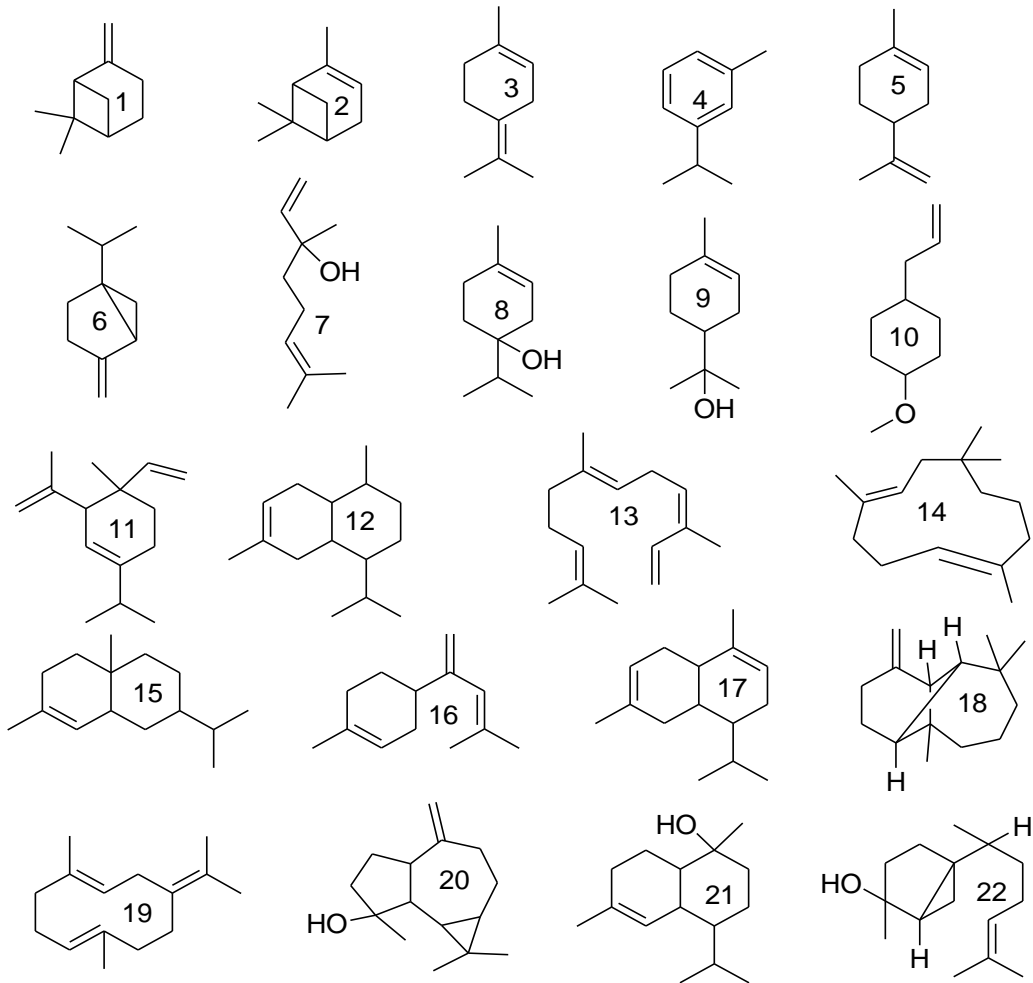


Fig (2). The chemical structures of *Pepper nigrum* compounds identified by GC/MS technique

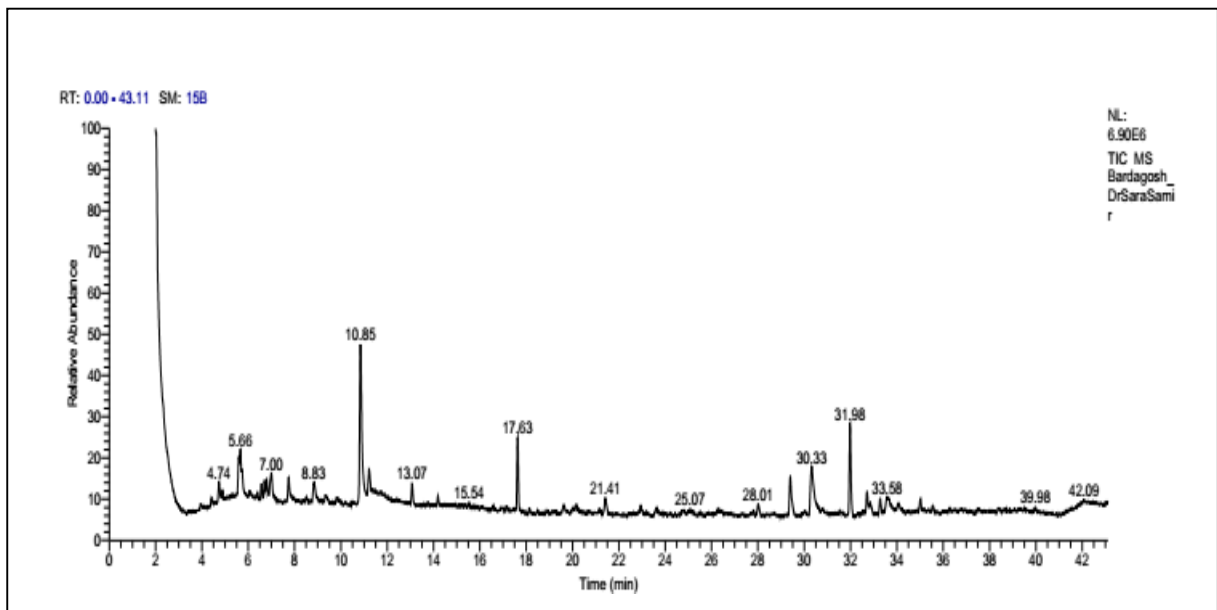
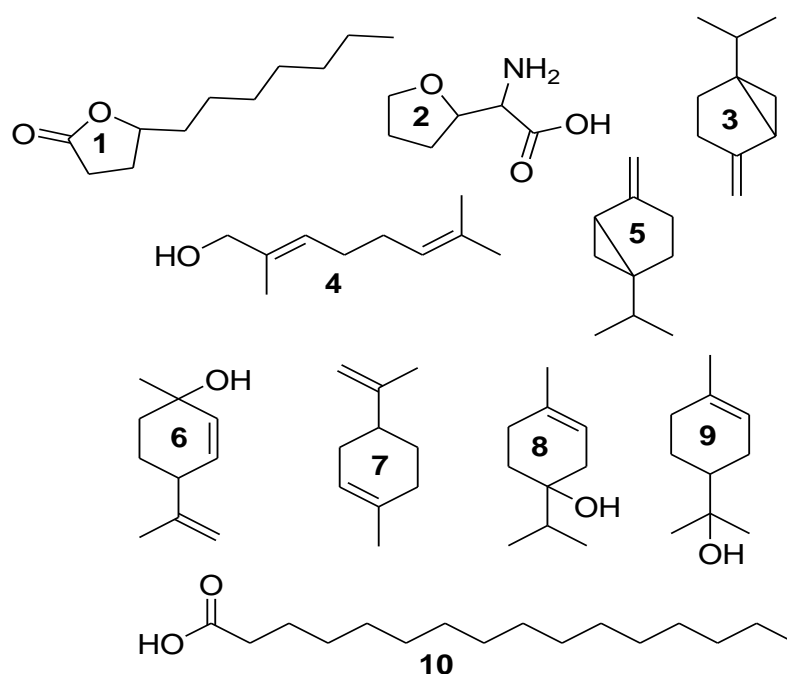


Figure (3). GC/MS analysis chart of *Origanum majorana*

Table (4). Main chemical components of *Origanum majorana* identified by GC/MS technique

No	Compound Name	R.T	Molecular Formula	Area %
1	5-heptyl-dihydrofuran-2(3H)-one	4.73	C ₁₁ H ₂₀ O ₂	2.25
2	2-amino-2-(tetrahydrofuran-2-yl)acetic acid	5.59	C ₇ H ₁₁ NO ₃	3.67
3	1-isopropyl-4-methylenebicyclo[3.1.0]hexane	5.66	C ₁₀ H ₁₆	4.37
4	(E)-2,7-dimethylocta-2,6-dien-1-ol	5.74	C ₁₀ H ₁₈ O	1.78
5	1-isopropyl-4-methylenebicyclo[3.1.0]hexane	6.70	C ₁₀ H ₁₆	1.57
6	1-methyl-4-(prop-1-en-2-yl)cyclohex-2-enol	6.79	C ₁₀ H ₁₆ O	2.09
7	1-methyl-4-(prop-1-en-2-yl)cyclohex-1-ene	7.0	C ₁₀ H ₁₆	3.54
8	1-isopropyl-4-methylcyclohex-3-enol	10.85	C ₁₀ H ₁₈ O	21.07
9	2-(4-methylcyclohex-3-enyl)propan-2-ol	11.22	C ₁₀ H ₁₈ O	3.62
10	Hexadecanoic acid	30.32	C ₁₆ H ₃₂ O ₂	8.89

**Fig (4). The chemical structures of *Origanum majorana* separated compounds identified by GC/MS technique**

Mealybugs are a spreading worldwide pest in many vineyards due to their direct damage, natural and botanical products valuable as an acceptable alternative to synthetic insecticides for an effective control process.

To evaluate the insecticidal action of kaolin and citrus essential oil laboratory tests and field experiments were conducted in several locations including *Planococcus ficus* (Signoret), *Pseudococcus calceolariae* (Maskell) and *Pseudococcus longispinus*. Although kaolin increased *Ps. calceolariae* mortality in the lab, it was unproductive at controlling the three mealybug species in the vineyards. On the other hand, citrus essential oil increased *Ps. calceolariae* and *Pl. ficus* mortality in the lab and reduced leaf infestations of the latter in the vineyards. Citrus essential oil may be regarded as a good alternative to synthetic insecticides against mealybugs (Tacoli et al., 2018).

4. Conclusion

Natural materials are a safe source for many agricultural operations, especially pest control, such as mealybugs and aphids, due to the environmentally friendly properties of these materials, ease of application, and acceptable results through laboratory or field application. The results obtained were so good that they reduced the population for black pepper, 50% and 49.3% and an acceptable LC50 values of 4383.52 and 644.45 ppm against *Phenacoccus solenopsis* and *Aphis gossypii*, respectively. The existing analyzed compounds, the majority of which are traced to the group of terpenes through GCMS technique. Therefore, it may be possible to use these materials as safe alternatives within ongoing and evolving control operations day after day.

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