

Original Article

Toxicity and Repellent Activity of Some Plant Essential Oils Against the Cotton Aphid, *Aphis gossypii*

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ABSTRACT

The fumigant, repellent, contact toxicity, and phytotoxicity of essential oils from *Mentha pulegium*, *M. piperita*, *Cuminum cyminum*, and *Myrtus communis* were investigated against the cotton aphid, *Aphis gossypii* (Glover). GC- MS analyzed the composition of essential oil. The primary constituents of the essential oils from *M. pulegium*, *C. cyminum*, *M. piperita*, and *M. communis* were pulegone (63.88%), cuminic aldehyde (28.20%), menthol (36.94%), and 1, 8-Cineole (36.13%), respectively. The LC₅₀ toxicity values showed that the essential oil from *M. pulegium* was the most toxic against *A. gossypii*. In the contact toxicity bioassay, the essential oil of *C. cyminum* was more harmful than the others. All formulations, except for *M. piperita* had a repellent effect on adult aphids. When used at the highest tested doses, all formulations of essential oils caused phytotoxicity to the cucumber leaves. Based on our results, the essential oils of *M. pulegium*, *C. cyminum*, *M. piperita*, and *M. communis* plants could be developed into effective fumigants.

INTRODUCTION

All around the world, the cotton aphid, *Aphis gossypii* (Glover), is a significant pest of various agriculturally important crops. High aphid populations can have a detrimental effect on crop yields and lead to financial losses. Direct damage to *A. gossypii* is caused by the removal of phloem. However, aphid feeding also damages the epidermis, fibrous sclerenchyma, rib parenchyma, and spongy mesophyll cells. Heavily-infested plants commonly show distorted and stunted leaves and reduced fruit sets, and sometimes individual plants may be killed by direct feeding. Indirect damage is caused either by honeydew or by the transmission of viruses. The cotton aphid is the vector of approximately 60 virus diseases in various plants [1, 2].

The major reasons for the resurgence in populations and pest status include the development of resistance to synthetic insecticides (O'Brien, P., and Graves 1992). Over the last few decades, *A. gossypii* has developed resistance to all the major synthetic insecticide groups, such as organophosphates, carbamates, organochlorines, and pyrethroids, in most cotton-growing areas worldwide [4].

Essential oils are aromatic compounds with a strong odor and are formed by aromatic plants as secondary metabolites [5]. They are composed of complex mixtures of terpenoids and phenylpropanoids, which are obtained from hydrodistillation, steam distillation, dry distillation, or mechanical cold pressing of plants [6]. Insecticides that contain essential oils are potential alternatives to synthetic insecticides for pest control [7]. They are eco-friendly, degrade quickly in the environment, and have minimal toxicity to non-target organisms. Essential oils possess contact and fumigant toxicity, repellent, and antifeedant activity. They also reduce fertility and modify behavior in many insect species [8, 9].

The objective of the present study was to assess the potential of utilizing essential oils as contact and fumigant insecticides and repellents against *A. gossypii*. Additionally, the study aimed to analyze the composition of the essential oils using GC-Mass analysis.

MATERIALS AND METHODS

Insects

Stock culture of the cotton aphid, *A. gossypii*, was obtained from the Entomological Laboratory cultures maintained on cucumber plants in the College of Agriculture and Natural Resources at the University of Tehran. The aphids were reared on cucumber plants (*Cucumis sativus*) without pesticide exposure, under standard conditions in a controlled environmental greenhouse at 25±1 °C with 70% relative humidity and a 16:8 h light-dark photoperiod. The plants were also grown in a greenhouse at a temperature of 25±1 °C and a photoperiod of 16 hours of light and 8 hours of darkness.

Essential Oil Extraction

The essential oils of pennyroyal (*M. pulegium*) aerial parts, cumin (*C. cyminum*) seeds, peppermint (*M. piperita*) aerial parts, and myrtle (*M. communis*) leaves were obtained from the plants. Essential oils were extracted from the plant samples using a Cleverer-type apparatus through hydrodistillation for 4 hours. Anhydrous sodium sulfate was used to remove water during the extraction process.

Gas Chromatography-mass Spectrometry Analysis

A GC-MS device with specific specifications and a temperature program were utilized to identify chemical compounds in essential oils. To identify chemical compounds in essential oils, a GC-MS device with specific specifications and a temperature program was utilized. Essential oil samples obtained from plants were analyzed using a chromatograph device combined with an Agilent HP-5973 mass spectrometer, equipped with a HP-5MS capillary column. The column had a stationary phase of methyl phenyl siloxane 5% (30 meters in length, 0.25 mm inner diameter, and 0.25-μL stationary layer thickness) and an ionization energy of 70 electron volts. The temperature program involved starting the oven at 60 °C, increasing to 246 °C at a rate of 3 °C/min, and then maintaining a temperature of 250 °C for 10 minutes to clean the column. The device included a divider at the column's end, simultaneously splitting the output into two parts for analysis in the mass spectrometer and FID detector. The injection chambers, FID detector inlet, and mass spectrometer inlet were set at 250 °C. The analysis utilized a split injection system with a 1 to 10 ratio, using 0.1 μL of pure sample and a constant flow of

helium carrier gas at 1.5 ml per minute. The carrier gas was helium at a 1.5 mL/min flow rate. The injector and detector temperatures were set at 250 °C. An injection volume of 1 μL was used. The compounds were identified by comparing their retention indices with those of known compounds. The relative percentage amounts were obtained directly from the peak areas of the GC.

Bioassays

Four essential oils (*C. cyminum*, *M. pulegium*, *M. piperita*, and *M. communis*) were used in bioassays. All bioassays were performed at 25±1 °C, 60±10% HR, and 16: 8 (L: D) photoperiod.

Fumigant Bioassay

To assess the fumigant toxicity of essential oils against *A. gossypii*, we utilized 700 ml glass jars. One cucumber leaf was placed on the surface of water agar (1.5%) at the bottom of a Petri dish with a diameter of 6.5 cm. Ten cotton aphids of the same age (adult or nymph) were carefully transferred onto the leaf using a fine brush. The Petri dishes were covered with fine mesh gauze. Each Petri dish was attached to the inner surface of the inverted lid of a jar. Essential oils were applied to a small piece of cotton using a micropipette, while cotton pieces without essential oils were used as the control treatment. The cotton was placed at the bottom of each jar, and the lid was sealed with parafilm to prevent any loss of essential oils. All concentrations and controls were replicated four times. Mortality was determined after 24 hours. The insects were considered dead when no leg or antennal movements were observed. Preliminary screening of the activity of the essential oil was carried out at three concentrations (1.4, 11.7, and 23.5 μl/l). Then, six essential oils with the highest activity at a concentration of 23.5 μl/l were chosen for adult fumigant bioassay, using five concentrations of each essential oil. Four essential oils with the lowest LC₅₀ values for adult *A. gossypii* were further tested on the first and third nymphal instar as fumigants and in contact toxicity tests against adults.

Contact Bioassay

To prepare formulations of essential oils, first dissolve polyvinyl-pyrrolidone (3% w/v) in 50 ml of 96% ethanol as an emulsifier. Then, essential oils (10% w/v) and an emulsifier (4% w/v) were added. Different concentrations were prepared in

water using these 10% formulations. Formulations of essential oils were used at 25,000, 12,500, and 6,250 mg/L (for *C. cyminum* and *M. pulegium*), at 25,000, 16,000, 12,500, and 8,000 mg/L (for *M. piperita*), and 50,000, 25,000, 16,000, and 12,500 mg/L (for *M. communis*). A cucumber leaf disc was placed in a Petri dish (6.5cm) filled with agar (1.5%) in contact toxicity tests. Ten adult cotton aphids of the same age were carefully transferred onto the leaf using a fine brush. Then, each Petri dish was sprayed with a hand sprayer containing 4.8 ± 0.003 mg/cm² of each concentration or control (formulation without essential oils). The Petri dishes were closed with a hole in the lid for ventilation. All treatments were replicated four times. Mortality was determined after 24 hours.

Repellent Bioassay

The repellency assay was done according to [10] with slight modifications. A choice bioassay system was used to evaluate the repellency of four essential oil formulations. Half of the leaf discs, each with a diameter of 9 cm, were treated with essential oil formulations at a concentration of 4.8 ± 0.003 mg/cm², which was equal to the LC₂₀ obtained in the contact toxicity bioassays. The remaining half of the leaf discs were treated with a control emulsion. After air-drying for one hour, the leaves were placed on 1.5% agar beds in 9 cm diameter Petri dishes. Ten adults were placed in the center of each Petri dish, and the lid was sealed with Parafilm. Four replicates were prepared for each treatment. After 24 hours, the number of aphids on each half of the leaf (treated and control) was recorded. The repellency index (% RI) was calculated using the following formula: $RI = (C - T) / (C + T) \times 100$, where C represents the number of insects in the untreated area, and T represents the number of insects in the treated area. "Significant positive values indicate repellency, while significant negative values indicate attraction."

Phytotoxicity Assay

Detached cucumber leaves were sprayed with 4.8 ± 0.003 mg/cm² of each concentration used in the contact toxicity bioassay. Each leaf was placed on the surface of the water agar (1.5%). All treatments were replicated four times. Leaves were scanned after 24 hours and the percentage of phytotoxicity was determined using a program written in the MATLAB® environment.

Statistical Analyses

Data from all bioassays were corrected for control mortality using Abbott's formula. LC₅₀ values and confidence limits for each essential oil were determined by the PROBIT procedure of the SAS system. Differences in toxicity were considered significant when 95% of fiducial limits did not overlap.

RESULTS

Essential Oil Extraction and Gas Chromatography-mass Spectrometry

The essential oil yields obtained by hydrodistillation for *C. cyminum*, *M. pulegium*, *M. piperita*, and *M. communis* were approximately 2.5%, 0.4%, 0.3%, and 0.2% of the fresh-dried plant material, respectively. Results presented in Tables 1-4 show the identified constituents and their percentage composition. Based on GC-MS analysis, the primary constituents in the *M. pulegium* oil were pulegone (63.88%) and menthofuran (11.65%) (Table 1). The primary constituents of *M. piperita* were menthol (36.94%) and L-menthone (23.37%) (Table 2), while the main components of *M. communis* were 1,8-cineole (36.13%) and α -pinene (22.47%) (Table 3). The main components of *C. cyminum* were cuminic aldehyde (28.20%) and *P*-cymene (14.62%) (Table 4).

Fumigant Bioassay

The activities of four essential oils against *A. gossypii* at the three concentrations assayed in the screening bioassay are shown in Table 5. The fumigant LC₅₀ values of the tested essential oils are shown in Table 6. Among the adult aphids, the essential oil of *M. pulegium* was the most toxic (LC₅₀ = 4.70 μ l/L), followed by oils from *C. cyminum* (LC₅₀ = 4.87 μ l/L), *M. piperita* (LC₅₀ = 5.08 μ l/L), and *M. communis* (LC₅₀ = 10.72 μ l/L). Based on the fiducial limits, there was no significant difference in the fumigant toxicity between the essential oils extracted from *M. pulegium*, *C. cyminum*, and *M. piperita*. For first-instar nymphs of *A. gossypii*, the essential oil of *M. pulegium* was the most toxic (LC₅₀ = 1.43 μ l/L), followed by oils from *M. piperita* (LC₅₀ = 1.65 μ l/L), *C. cyminum* (LC₅₀ = 1.82 μ l/L) and *M. communis* (LC₅₀ = 5.66 μ l/L). However, there was no significant difference in the fumigant toxicity between essential oils from *M. pulegium*, *M. piperita*, and *C. cyminum* (Table 6). Based on LC₅₀ values, third-instar nymphs of *A. gossypii* were susceptible to the essential oils

extracted from *M. pulegium* ($LC_{50} = 2.13\mu\text{l/L}$), *C. cyminum* ($LC_{50} = 3.20\mu\text{l/L}$), *M. piperita* ($LC_{50} = 3.41\mu\text{l/L}$), and *M. communis* ($LC_{50} = 7.05\mu\text{l/L}$), respectively. No significant difference was observed in fumigant toxicity between essential oils from *M. pulegium*, *M. piperita*, and *C. cyminum* (Table 6).

Contact Bioassay

The LC_{50} values of the tested essential oil formulations are shown in Table 7. The formulation of *M. communis* and *C. cyminum* essential oils showed the lowest and highest contact toxicity against adult aphids, respectively.

Repellent Bioassay

The choice-leaf assay showed that all the studied formulations, except for the formulation of *M. piperita*, had a repellent effect on aphids (Table 8). The essential oil

of *M. communis* exhibited a 20% repellency effect on adult aphids. *C. cyminum* and *M. pulegium* essential oils had a relatively low repellency effect (10%) on adult aphids. A negative repellency (-24.25 %) was observed on adult aphids when exposed to *M. piperita* oil.

Phytotoxicity Assay

It is evident from the results that the phytotoxicity increased in response to higher concentrations of essential oil formulations (Table 9). The percentage of phytotoxicity at a concentration of 25000 mg/L for *M. pulegium*, *C. cyminum*, and *M. piperita* oils was 71.55%, 92.62%, and 22.51%, respectively. *M. communis* oil caused very low phytotoxicity at this concentration but showed 59.92% phytotoxicity at a concentration of 50,000 mg/l.

Table 1 Identified chemical compounds of *M. pulegium* essential oil

Compound	R.I. (Source)	R.I. (Author)	Percent
α -pinene	926-1045	906	0.59
Camphene	946	922	0.15
Sabinene	973 - 1147	946	0.55
β -pinene	934 -1138	950	0.89
β -Myrcene	986 - 1187	960	0.44
3-Octanol	991 - 1400	964	0.17
Limonene	1031 - 1234	1001	0.48
1,8-Cineole	1026	1006	7.28
3,8- <i>p</i> -Menthadiene	1060 - 1072	1041	0.32
Isoamyl-2-methyl butyrate	1090	1067	0.14
Octanoic acid methyl ester	1124	1124	3.97
Menthone	1137 - 1478	1128	0.54
cis-Isopulegone	1159	1150	1.40
Menthofuran	1163 - 1464	1140	11.65
β -Fenchyl alcohol	1168	1169	0.31
delta-Terpineol	1174	1145	0.21
Pulegone	1209 - 1662	1223	63.88
Piperitone oxide	1251	1229	0.90
Borneol acetate	1147 - 1747	1256	0.11
Piperitenone	1342 - 1918	1312	1.52
Piperitenone oxide	1333	1336	1.32
(E)- Caryophyllene	1467	1396	0.49
Germacrene D	1480 - 1772	1456	0.27
Bicyclogermacrene	1475 - 1738	1471	0.27

Source: The Pherobase: Database of Pheromones and Semiochemicals

<https://pherobase.com/database/kovats/kovats-detail-borneol.php>

Table 2 Identified chemical compounds of *M. piperita* essential oil

Compound	R.I. (Source)	R. I. (Author)	Percent
Ethanol	485	427	0.14
α -Pinene	926 - 1045	855	0.54
Sabinene	973 - 1147	892	0.37

β -Pinene	934 - 1138	895	0.80
β -Myrcene	986 - 1187	912	0.31
3-Octanol	991 - 1400	913	0.29
α -Terpinene	1017 - 1315	934	0.19
o-Cymene	1011 - 1045	937	0.25
Limonene	1031 - 1234	948	3.29
1,8-Cineole	1026	945	3.74
γ -Terpinene	1055 - 1274	973	0.32
Linalool	1082 - 1570	1013	0.39
<i>trans</i> -Sabinene hydrate	1070 - 1459	976	0.51
Menthone	1137 - 1478	1057	23.37
Menthofuran	1163 - 1460	1065	1.90
neo-Menthol	1173	1069	3.78
Menthol	1173 - 2103	1090	36.94
β -fenchyl alcohol	986 - 1187	1094	0.62
Carvone	1242 - 1715	1127	3.81
Piperitone	1231 - 1739	1135	0.75
(E) Anethole	1283 - 1847	1165	1.26
Menthyl acetate	12881 - 1551	1183	4.53
β -Bourbonene	1380 - 1633	1294	0.33
(E) caryophyllene	1418 - 1657	1320	1.60
<i>trans</i> - β -Farnesene	1043 - 1270	1347	0.23
β -Cubebene	1389 - 1558	1359	1.30
Bicyclogermacrene	1475 - 1738	1369	0.27
Viridiflorol	1590 - 2112	11444	0.39

Source: The Pherobase: Database of Pheromones and Semiochemicals

<https://pherobase.com/database/kovats/kovats-detail-borneol.php>

Table 3 Identified chemical compounds of *M. communis* essential oil

Compound	R.I. (Source)	R.I. (Author)	Percent
α -Pinene	926-1045	863	22.47
Camphene	935	869	0.59
β -Pinene	934 - 1138	895	0.65
β -Myrcene	986 - 1187	912	0.27
Phellandrene	1002 - 1205	921	0.16
Δ -3-Carene	1004 - 1180	928	0.23
Limonene	1031 - 1134	956	3.84
1,8-Cineole	1026	954	36.13
γ -Terpinene	1055 - 1274	974	0.42
<i>trans</i> -Sabinene hydrate	1070 - 1459	977	0.42
Terpinolene	1084 - 1315	1001	0.56
Linalool	1082 - 1570	1016	8.38
Camphor	1120 - 1498	1034	0.37
Borneol	1147 - 1747	1061	0.35
Terpin-4-ol	1171 - 1732	1047	0.64
α -Terpineol	1171 - 1732	1089	4.38
γ -Terpineol	1183	1093	0.54
Carvone	1252 - 1255	1121	0.32
Linalyl acetate	1257 - 1569	1152	4.20
Bornyl acetate	1285 - 1580	1173	5.16
Piperitone	1231 - 1739	1202	0.30
α -Terpinyl acetate	1350 - 1700	1240	1.03
Eugenol	1035 - 2192	1236	0.37
Neryl acetate	1345 - 1742	1258	0.31
Geranyl acetate	1363	1284	1.87
Methyleugenol	1360 - 1410	1293	0.72
(E) Caryophyllene	1572 - 2068	1320	1.19
Caryophyllene oxide	1572 - 2068	1432	0.27

Source: The Pherobase: Database of Pheromones and Semiochemicals.

<https://pherobase.com/database/kovats/kovats-detail-borneol.php>

Table 4 Identified chemical compounds of *C. cyminum* essential oil

Compounds	R.I. (Source)	R.I. (Author)	Percent
α -Thujan	926 - 1045	927	0.12
α -Pinene	926 - 1045	935	0.35
Camphene	946	950	0.03
Sabinene	973 - 1147	976	0.07
β -Pinene	934 - 1138	977	4.08
β -Myrcene	986 - 1187	991	0.35
α -Phellandrene	1002 - 1205	1006	0.14
Carene (Delta 3-)	1004 - 1180	1013	0.03
p-Cymene	1016 - 1303	1027	14.62
Limonene	1031 - 1234	1031	1.36
1,8-Cineole	1026	1030	0.21
γ -Terpinene	1055 - 1274	1056	10.04
cis-Sabinenehydrate	1069 - 1465	1066	0.03
α -Terpinolene	1084 - 1315	1188	0.09
Linalool	1082 - 1570	1098	0.09
cis-thujone	1091 - 1419	1087	0.25
Methyl chavicol	1243 - 2340	1195	0.55
Cuminic aldehyde	1665 - 2349	1248	28.20
(E) Anethole	1283 - 1847	1288	9.41
Thymol	1282 - 1308	1291	12.49
Carvacrol	1298 - 1314	1296	0.54
(E) Caryophyllene	1572 - 2068	1412	0.07
Cumic acid	600 - 1477	1438	0.65
<i>trans</i> - β -Farnesene	1434 - 1650	1452	0.17
Caryophyllene oxide	1572 - 2068	1574	0.59

Source: The Pherobase: Database of Pheromones and Semiochemicals.

<https://pherobase.com/database/kovats/kovats-detail-borneol.php>

Table 5 Corrected mortality of ten essential oils against adults of *A.s gossypii* in the screening bioassay.

Essential oils	Concentration (μ l/l) and % mortality		
	1.4	11.7	23.5
<i>M. pulegium</i>	20 \pm 0.1	71.2 \pm 0.08	95 \pm 0.05
<i>C. cyminum</i>	20 \pm 0.05	70 \pm 0.05	80 \pm 0.05
<i>M. piperita</i>	23.6 \pm 0.05	61.05 \pm 0.05	100 \pm 0
<i>M. communis</i>	5 \pm 5.01	55 \pm 5.01	95 \pm 5.01

Table 6 Evaluation of essential oils applied as a fumigant against adult, first, and third instar nymphs of *A. gossypii*.

Developmental stage	Essential oils	Slope \pm SE	LC ₅₀ (μ l/l) (FL)	χ^2	df
Adult	<i>M. pulegium</i>	1.78 \pm 0.38	4.70 (2.69 - 7.05)	4.27	6
	<i>C. cyminum</i>	1.44 \pm 0.35	4.87 (2.64 - 7.44)	2.12	8
	<i>M. piperita</i>	1.55 \pm 0.37	5.08 (2.90 - 7.34)	8.24	12
	<i>M. communis</i>	2.47 \pm 0.36	10.72 (8.96 - 13.23)	8.45	18
First instar nymph	<i>M. pulegium</i>	2.97 \pm 0.47	1.43(1.23 - 1.67)	7.44	18
	<i>M. piperita</i>	1.83 \pm 0.30	1.65 (1.26 - 2.09)	5.14	18
	<i>C. cyminum</i>	1.93 \pm 0.31	1.82 (1.43 - 2.29)	3.84	18
	<i>M. communis</i>	3.23 \pm 0.58	5.66 (4.96 - 6.63)	4.98	18
Third instar nymph	<i>M. pulegium</i>	2.78 \pm 0.52	2.13 (1.80 - 2.49)	2.36	18
	<i>C. cyminum</i>	2.13 \pm 0.34	3.20 (2.57 - 3.93)	4.21	18
	<i>M. piperita</i>	1.94 \pm 0.34	3.78 (3.02 - 4.82)	4.48	18
	<i>M. communis</i>	3.15 \pm 0.58	7.05 (6.12 - 8.14)	4.71	18

χ^2 =chi-square; df= degree of freedom

Table 7 Evaluation of formulations of essential oils applied by contact method against adults of *A.s gossypii*.

Essential oils	Slope \pm SE	LC ₅₀ (mg/l) (FL)	χ^2	df
<i>C. cyminum</i>	3.31 \pm 0.59	9045 (7197 - 10821)	5.04	10
<i>M. pulegium</i>	3.58 \pm 0.59	10497 (8705 - 12421)	4.89	10
<i>M. piperita</i>	3.73 \pm 0.68	13620 (11696 - 15661)	4.74	14
<i>M. communis</i>	5.28 \pm 0.79	22310 (19765 - 25387)	6.88	14

χ^2 =chi-square; df= degree of freedom

Table 8 Repellent activity of essential oil formulations against adults of *A. gossypii*.

Essential oils	Concentration (mg/l)	Repellency index (%) \pm SE ^a
<i>M. pulegium</i>	6781	10 \pm a
<i>C. cyminum</i>	5795	10 a
<i>M. communis</i>	9125	20 a
<i>M. piperita</i>	8108	-24.25 b

Note: Different letters indicate significant differences ($P < 0.05$) between treatments according to ANOVA and LSD tests.

Table 9 Percentage of phytotoxicity of essential oils in different concentrations to cucumber leaves.

Essential oils	Concentrations (mg/L) a and % phytotoxicity					
	6250	8000	12500	16000	25000	50000
<i>M. pulegium</i>	4.70 \pm 0.02 b	-	5.45 \pm 0.01 b	-	71.55 \pm 0.08 a	-
<i>C. cyminum</i>	4.20 \pm 0.01 c	-	57.71 \pm 0.15 b	-	92.62 \pm 0.04 a	-
<i>M. piperita</i>	-	0.32 \pm 0.00 b	0.43 \pm 0.004 b	2.72 \pm 0.03 b	22.51 \pm 0.04 a	-
<i>M. communis</i>	-	-	0.63 \pm 0.50 a	0.35 \pm 0.15 b	1.99 \pm 0.69 b	50.92 \pm 20.53 b

Note: Different letters indicate significant differences ($P < 0.05$) between treatments according to ANOVA and LSD tests.

DISCUSSION

Substantial qualitative and quantitative differences were observed in the compositions of the oils extracted from various plants. GC-MS analysis of the essential oils revealed that out of 38 compounds, only three compounds, namely β -pinene, 1,8-cineole, and γ -terpinene, were consistently found in all the tested plants. These molecules are monoterpenes found in numerous aromatic plants and exhibit diverse insecticidal and pharmacological properties [11–13]. Pulegone and 1,8-cineole, which make up more than 75% of the total composition, are the two major components of *M. pulegium*. In another species of Mentha, *M. piperita*, menthol, and *L*-menthone are the predominant compounds, comprising more than 65% of the total composition. Menthol and menthone were already reported as the major components of *M. piperita* var. *citrata* [12]. Interestingly, there was a significant qualitative and quantitative difference found in the compositions of the essential oils of these closely related species. Analysis of the essential oil revealed that *P*-cymene, cuminic aldehyde, trans-anethole, and thymol are the four predominant molecules of *C. cyminum*, comprising more than 76% of the total components. In a recent study, cuminic aldehyde, α , β -dihydroxyethylbenzene, 2-carene-10-

al, γ -terpinene, and β -pinene were reported as the most abundant constituents of *C. cyminum* seeds [14]. In another study, cuminaldehyde, *p*-cymene, β -pinene, α -terpinen-7-al, γ -terpinene, *P*-cymen-7-ol, and thymol were found to be the major components of *C. cyminum* seeds essential oil [15]. α -pinene, limonene, 1,8-cineole, and linalool were reported as the major constituents of seeds of *C. cyminum* [16]. 1,8-cineole, α -pinene, and linalool L with more than 73% of total compositions are the main molecules in the essential oil of *M. communis*. In other studies, α -pinene, 1,8-cineole, and linalool were also reported as the major constituents of *M. communis* [17–19]. However, these data revealed that if the essential oils are composed of several components, a few of them serve as the major constituents of each essential oil. These molecules may be responsible for the biological activity of the oil. The qualitative and quantitative variations of the constituents of essential oil may be influenced by factors such as the variety of the plant, its growth stage and the part of the plant used, the geographical location and altitude at which it is distributed, the season in which it is sampled, and genetic variation [20–23].

In this study, the aphicidal activity of the essential oils varied depending on the life stage of the pest and

the method of application (fumigant or contact). In the fumigant toxicity bioassay, all oils exhibited strong insecticidal activity. The LC₅₀ values showed that the essential oil of *M. pulegium* was more potent than the other essential oils against adults and nymphs of *A. gossypii*. The essential oil of *M. pulegium* has also been reported to be highly active against adults of *Mayetiola destructor* [24], *Drosophila melanogaster*, *Bactrocera oleae* [25], *Dermatophagoides farinae*, and *D. pteronyssinus* [26]. In the contact toxicity bioassay, the formulation of essential oil from *C. cyminum* was more effective than the other formulations against adult *A. gossypii*. The essential oil of *C. cyminum* has also been reported to be highly effective against female *A. gossypii* and *Tetranychus cinnabarinus* [27], the eggs of *Tribolium confusum* and *Ephestia kuehniella* [28], and adults of *Callosobruchus chinensis* [29].

Pulegone, the primary compound found in *M. pulegium* oil, has been shown to increase the mortality rate of various pests, including *Lipaphis pseudobrassicae* Davis [30], *Bactrocera oleae* [25], *Tyrophagus putrescentiae* [10], and *D. melanogaster* [31]. Menthol, the main compound of the *M. piperita* essential oil, has shown high insecticidal activity against *Lipaphis pseudobrassicae* Davis [30], *T. castaneum*, *Musca domestica*, *Sitophilus oryzae*, *O. Surinamensi*, and *B. germanica* [32]. The primary compound found in the essential oil of *C. cyminum* exhibited significant insecticidal activity against *Acanthoscelides obtectus* [33] and *Lycoriella ingénue* [34]. 1,8-cineol, the main constituent of *M. communis*, showed high insecticidal activity against *T. confusum* [35]. These results suggest a correlation between the chemical composition of essential oils and their biological effects. The strong insecticidal activity of the essential oils presented here could be attributed to their main components.

The repellency of certain essential oils has been previously evaluated against aphids. In the repellent bioassay, the essential oils of *M. communis*, *M. pulegium*, and *C. cyminum* demonstrated a greater repellency effect on adults of *A. gossypii* compared to the essential oil of *M. piperita*. The essential oil of *M. pulegium* has shown moderate repellency in high concentrations against *Amblyomma cajennense* [36] and adults of *T. urticae* [37]. The essential oil of *M. piperita* has been reported to have a strong repellent action against adults of *Aedes aegypti*, *Anopheles*

stephensi, *Culex quinquefasciatus* [38] and *T. castaneum* adults [39].

Essential oils may be used as an insect control technology in organic farming systems, but it is necessary to have basic information on phytotoxicity before conducting field experiments. Plants exposed to oil formulations at a concentration of 25000 mg/L for 24 hours showed 92.62% leaf burn in *C. cyminum* and 71.55% leaf burn in *M. pulegium*. At a concentration of 50000 mg/L, the oil formulation showed 52.92% leaf burn in *M. communis*. Based on the present study, it can be concluded that the application of *C. cyminum* oil at high concentrations causes severe phytotoxicity when applied to the leaves.

CONCLUSION

Essential oils extracted from plants such as *M. pulegium*, *M. piperita*, *C. cyminum*, and *M. communis* have the potential to be developed into highly efficient insecticides. However, further research is needed in the areas of essential oil chemistry and formulation, entomology, and plant breeding to explore their effectiveness fully.

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