ESSENTIAL OILS FUMIGANT, CONTACT AND REPELLENT POTENTIALS AGAINST *OLIGONYCHUS AFRASIATICUS* MCGREGOR (ACARI: TETRANYCHIDAE)

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Abstract. The efficacy of essential oils (EOs) extracted from *Mentha spicata, Mentha puleguim, Ocimum basilicum,* and *Ruta graveolens* was examined against *Oligonychus afrasiaticus* in a controlled laboratory conditions. The EOs were tested to assess fumigant toxicity, contact impact, and repellency. The essential oil of *R. graveolens* was characterized by the high concentration of 2-nonanone (11.9%), 2-undecanone (16.22%) and 1-nonene (19.4%). The composition of *O. basilicum* EO was characterized by linalool (29.23%), whereas 1,8-cineole (14.60%) was the major constituent in *M. puleguim* EO. The principal component of *M. spicata* was carvone (50.6%). The laboratory bioassay results demonstrated that the essential oils derived from *M. pulegium*, *O. basilicum* and *M. spicata* caused a mortality rate of 50% among females when administered at a concentration of 1 μL/L. With this concentration, *R. graveolens* EO caused significantly higher acaricidal effects (98%) with LC_{50} = 0.11 μL/L air. All oils showed a significant level of pest-repellent activity, with their repellency effects reaching 95% after 1 h of exposure against female adults of *O. afrasiaticus* at doses of 0.318 and 1.9 μL/cm^2. The *R. graveolens* EO exhibited higher toxicity through residual contact compared to other oils, with LC_{50} values of 0.18 μL/L air. Toxicity of the highly effective *R. graveolens* plant can occur via residual contact, repulsive and fumigant action.
Keywords: *date palm, old world date mite, essential oil, toxicity, botanical*

Introduction

The tetranychid mite *Oligonychus afrasiaticus* (McGregor) (Acari: Tetranychidae), is an important pest affecting date palm fruits on global scale (Hussain, 1969; Saleh et al., 1979; Talhouk, 1991; Elwan, 2000; Ben Chaaban et al., 2011). The high-value Deglet Nour cultivar in Tunisia experienced the most significant damage by this species (Ben Chaaban et al., 2011). Traditionally, the mitigation of this mite depend on the application of chemical sprays for several years. The utilization of pesticides leading to the development of pest resistance, presence

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of product residues, significant risks to non-target organisms, and unwanted persistence in the environment (George, 2022). Synthetic acaricides are expensive and unsafe for pest control and more effective alternatives are needed to establish. There are a lot of integrated pest management methods have been studied in recent years, with a focus of using chemicals derived from plants as insecticides. Protecting agricultural commodities against various insect pests with chemicals derived from plants has been a common practice worldwide (Nikpay, 2006). In this context, essential oils made from plants are being considered as potential alternatives (Isman et al., 2011). Tunisian plant heritage is renowned for its richness and diversity, especially medicinal plants (Akrout, 2004), which have therapeutic properties, possess bactericidal, fungicidal, insecticidal and acaricidal effects (Chiasson et al., 2001). Different studies on the insecticidal qualities of plant-based essential oils have been conducted in Tunisia (Jemâa et al., 2012; Hamdi et al., 2015; Ben Chaaban et al., 2019; Amari et al., 2022) and no particular study has inquired how effective essential oils are against *O. afrasiaticus*.

The purpose of this research is to examine the chemical components of *Ruta graveolens*, *Ocimum basilicum*, *Mentha spicata* and *Mentha pulegium* EOs and to their toxicity against the date spider mite *O. afrasiaticus*.

Materials and methods

Mite culture

Mites (*O. afrasiaticus*) were collected from severely infested fruit variety Deglet Noor, from pesticide free oases in Tozeur, South Tunisia (8° 08′E; 33° 55′N). A stock colony was established on *Vigna unguiculata* plants using hundreds of various stages spider mites. Cultures were kept under laboratory conditions (25 ± 1°C temperature, 50 ± 10% relative humidity and a photoperiod of 16/8) in the laboratory of Entomology, Regional Research Centre of Oasis Agriculture, Degache, Tunisia.

Plant materials

In May 2018, fresh foliage specimens of *Mentha spicata*, *Ocimum basilicum*, *Ruta graveolens*, and *Mentha pulegium* were procured from the Tozeur oases. The samples underwent a three-week air-drying period at ambient temperature (20-25°C) before the essential oil extraction process.

Extraction of essential oil

The essential oils were extracted through hydro-distillation for 4 h, using a Clevenger-type apparatus and 100 g of desiccated plant leaves per sample in 500 ml of distilled water. Following this, the essential oils obtained were placed in tinted glass containers and chilled to a temperature of 4°C until requirement. The oil yields were quantified in g/100 g of dried plant material, expressed as a percentage.

Analysis of essential oil

Utilizing gas chromatography with a 6980 N Network GC system and an HP-5MS capillary column (30 m 0.25 mm ID, 0.25 m film thickness; Agilent Technologies, Little Falls, CA, USA), the volatile hydrocarbons were analyzed. The carrier gas

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utilised was helium gas with a consistent flow rate of 1 mL/min. The column's temperature was intentionally programmed to rise linearly, at a rate of 4°C/min, from 80°C to 220°C. Three minutes were spent maintaining the lower temperature, whereas 10 min were spent maintaining the upper temperature. Utilizing split mode, a split/splitless injector was affixed to the chromatograph. A 1.0 L sample was administered at a 1:100 ratio using the split mode. To identify the compounds, their retention indices (RI) were compared to values reported in the literature and authentic standards that were available in our laboratory. To determine the retention indices, they were compared to a succession of n-alkanes (C9–C24) in identical experimental conditions. Through a comparison of their mass spectra with those contained in the NIST08 and W8N08 libraries, the volatile components were identified.

Fumigant toxicity

To assess the toxicity of the fumigant, test chambers made of glass with a capacity of 1 L were utilized. Using a fine brush, twenty newly emerged female adults (<24 h old) were transferred to cut-out bean leaves of *V. unguiculata* (3 cm diameter). The specimen was placed with its upper side facing up on moist cotton soaked with water in Petri dishes to preserve the sturdiness of the disks and prevent mites from escaping. Each Petri dish was enclosed within a glass container, and a filter paper (Whatman No.1) disc (Ø 3.0 cm), soaked with the essential oil being tested and affixed to inner surface of the glass container before sealing it tightly. Different concentrations of the tested essential oils 0.02, 0.2, 1, 2, and 12 µL/L air were prepared by dissolving in ethanol. The control group that did not receive treatment were kept in glass containers containing filter paper soaked in ethanol alone. A single replication involved placing 20 mites on each disk within a Petri dish. The experiments were replicated five times. After 24 h of exposure, the Petri dishes containing spider mites were removed from their containers. A brush was then delicately employed to evaluate mortality by making contact with the mites. Mites that exhibited no movement were categorized as deceased. The insecticidal activity assays were carried out in a controlled environment maintaining a relative humidity of 50% ± 5, a temperature of 25 ± 1°C, and a photoperiod of 16 h of light and 8 h of darkness.

Contact assay

We employ a leaf disc painting technique, modified by Miresmailli et al. (2006), to assess the impacts of essential oil. The experiments were carried out in Petri dishes with a diameter of 10 cm. The concentrations evaluated were achieved by diluting the essential oils with ethanol. These concentrations ranged from 0.0159 $\mu L/cm^2$ to 0.95 $\mu L/cm^2$. A 20 µL portion of each concentration was applied to the underside of the Leaf discs obtained from bean leaves (4 cm diameter) using a micropipette. Following 20 min of air drying, each disc was positioned with its upper side facing down on moist cotton located at the base of a Petri dish. Using a fine brush and a binocular loupe, 25 adult females were placed on the treated disks. The studies were conducted using Petri plates that were not covered to avoid any potential interference caused by the volatile chemicals found in the oils. Using ethanol, control mites were maintained on leaf discs under identical conditions. Each treatment was repeated four times. Binary magnification was utilized to ascertain mortality 24 h after the treatment. Mites that exhibited no signs of life were considered dead.

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Repellency bioassay

The assessment of the repellent effects of essential oils on adult females of *O. afrasiaticus* was estimed using the method of Nerio et al. (2009). Watman filter papers (diameter 9 cm) were cut in half. The test solutions were prepared by diluting the essential oil in ethanol to obtain concentrations of the order of: 0.003, 0.03, 0.318 and 1.9 μL/cm². Each concentration was uniformly applied to a half-leaf disc using a micropipette. For comparison, the other half of the leaf was subjected just to ethanol treatment. To remove the solvent, the treated half discs were allowed to undergo evaporation by air drying for 5 min. The two parts, whether treated or untreated, were joined together using adhesive tape and then placed inside glass Petri dishes with a diameter of 10 cm.

In the central region of the petri dish, ten adult females that had emerged within the previous 24 h and were treated with half discs were positioned. After 1, 4, and 24 h, the number of mites on the treated and untreated portions was determined using a binocular loupe. Calculating percentage repellency (PR) was performed as follows:

$$PR = [(NC - NT)/(NC + NT) \times 100$$

NC represented the mite population in the untreated area after the exposure interval, while NT denoted the mite population in the treatment area after the exposure interval.

Statistical analysis

The statistical analyses were performed using SPSS statistical software version 20.0. Statistical analyses in the experiments of the fumigant, contact and repellent activities were compared using analysis of variance (ANOVA) followed by LSD test at the 0.05 significance level. For the insecticidal activity, bioassay data were performed using Probit analysis to determine the lethal concentrations (LC_{50} and LC_{90}) (Finney, 1971).

Results and discussion

Chemical composition of essential oils

Dried leaves of *M. pulegium*, *O. basilicum*, *R. graveolens*, and *M. spicata* produced essential oils at respective yields of 1.56, 0.23, 0.21 and 0.33%. According to the findings of Salem et al. (2017), the essential oil yield of *M. pulegium* leaves sourced from Tunisia was 1.5%. Moreover, studies has documented reduced yields of 0.65% (Kamkar et al., 2010) and 0.27% (Mahboubi and Haghi, 2008). According to Ouakouak et al. (2015) study, the harvests of *M. pulegium* plants collected in Algeria and Morocco were 2.33% greater. For the *O. basilicum*, the obtained yield (0.23%) is lower than that reported in previous works 0.7% (Brada et al., 2011) and 1.56% (Dris et al., 2017). Regarding *M. spicata* (0.33%), it is lower than that found by Mkaddem (2009) (0.8%). The *R. graveolens* EO extraction yields achieved are similar in magnitude to those documented in the scientific literature. For example, the percentage was calculated 0.18% in Algeria (Haddouchi et al., 2013) and 0.3% in Tunisia (Fredj et al., 2007). In comparison, the yield achieved in Saudi Arabia was 1.29% and in Egypt 0.6% (Kalmosh et al., 2019; Reddy and Al-Rajab, 2016).

The findings from the chromatographic studies of *M. pulegium*, *R. garaveolens*, *O. basilium*, and *M. spicata* the essential oils (EOs) are presented in *Table 1*.

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Table 1. *Essential oil composition (%) of M. pulegium, R. graveolens, O. basilicum and M. spicata leaves*

N°	Compounds	*M. pelegium*	*R. graveolens*	*O. basilicum*	*M. spicata*	RT (min)
	Monoterpene hydrocarbons					
1	α-Pinene	2.43	0.53	0.37	0.12	7.64
2	Camphene	3.68	-	0.16		8.04
3	Sabinene	-	1.13	-	0.18	8.77
4	β-Pinene	4.4	-	1.08	0.29	8.85
5	β-Myrcene	1.34	0.47	1.56	0.24	9.29
6	2-Methylanisole	-	0.15	-		9.84
7	β-Phellandrene	0.02	-	-		9.99
8	α–Terpinene	-	-	0.17		10.00
9	Limonene	-	7.25	-	5.10	10.56
10	γ-Terpinene	-	0.03	0.17	0.25	11.50
11	α-Terpinolene	0.09	-	0.27	0.10	12.10
12	Carene	10.19	-	-		15.69
	Oxygenated monoterpenes					
13	1,8-Cineole	14.6	-	5.74	0.94	9.188
	cis-Sabinene hydrate				1.32	10.221
14	β-Ocimene	-	-	0.65	0.36	10.99
16	Geyrene	-	3.77	-		13.54
	Linalool L	-	-	29.23		13.73
17	Camphor	-	-	0.42		14.31
18	Borneol	-	-	1.18		14.86
19	Menthol	2.62	-	2.01		15.80
	Terpinene-4-ol					13.25
20	2-Decanone	-	2.16	-		15.44
21	Estragol	-	-	4.72		15.63
22	Fenchyl acetate	-	-	0.96		16.15
23	Linalylpropanoate	-	0.13	0.71		16.26
	Carvone				50.6	16.671
24	Menthyl alcohol	6.14	-	-		16.86
					0.82	16.97
25	Geraniol	-	-	0.91		17.16
26	Bornyl acetate	-	-	2.33		17.94
27	α-Bornyl acetate	0.19	-	-	0.55	18.42
	trans-Carvylacetate					18.532
28	Methyl cinnamate	-	-	18.97		18.57
29	Thymol	0.06	-	-		19.02
30	2-Methoxy-4-vinylphenol	-	0.09	-		19.09
31	Dodecyl acetate	-	0.17	-		20.24
32	Piperitenone oxide	0.16	-	-		20.25
33	Eugenol	-	-	5.84		20.04
34	Methyl eugenol	-	-	0.29		21.29
	Sesquiterpene hydrocarbons					
	β-Bourbonene				2.75	19.082
35	α-Copaene	0.02	-	0.54		20.39
36	β-Elemene	0.30	-	-	3.57	20.70
	α –Humulene				0.47	20.78
37	trans-Caryophyllene	6.64	-	-	**5.55**	21.61
38	β-Cubebene	0.69	-	0.64	2.01	21.69
39	Germacrene-D	1.93	-	1.09	**7.67**	21.77
40	1-Pentadecene	-	12.62	-		22.24
41	Caryophyllene	0.91	-	0.43		22.26
43	Aromadendrene	0.62	-	0.09	0.29	23.05
44	δ-Cadinene	0.37	0.06	0.66	0.78	23.16
45	α-Amorphene	0.13	-	0.08	0.19	23.27
46	Germacrene-B	-	-	0.45		23.25

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47	γ-Cadinene	-	-	2.48	0.16	23.76
48	Calamenene	0.25	-	-		23.84
49	α-Muurolene	0.13	-	-		24.17
50	α-Guaiene	0.03	-	-		24.52
51	Eremophilene	0.04	-	-		25.49
52	α-Selinene	0.16	-	-		26.20
53	1,3-Cyclooctadiene	-	0.05	-		27.04
54	Neophytadiene	-	0.27	-		30.67
55	1,3-Cyclooctadiene	-	0.1	-		31.79
	Oxygenated sesquiterpenes					
	Spathulenol				0.63	23.701
56	Elemol	-	0.26	-		24.55
57	Caryophyllene oxide	0.38	-	- 0.47		25.25
58	α-Cadinol	4.58	-	5.69 0.89		26.72
59	β –Eudesmol	-	-	0.15		26.93
60	Levomenol	-	-	0.18		27.54
	Ketones					
61	2-Nonanone	-	11.92	-		12.74
62	L-Menthone	3.25	-	0.80	0.33	14.24
63	p-Menthan-3-one	14.9	-	-		14.83
64	Pulegone	0.49	-	3.32	0.35	17.10
65	1-Nonene	-	19.37	-		17.31
66	2-Undecanone	-	16.22	-	1	17.45
67	Isophorone	0.52	-	-		18.29
68	2-Dodecanone	-	2.86	-		19.84
69	Pulespenone	11.41	-	-		19.93
70	Jasmone	0.6	-	-	1.36	21.06
71	β-Damascenone	-	0.15	-		20.54
72	2-Tridecanone	-	1.77	-		23.32
73	3,4-Methylenedioxybenzylacetone	-	0.20	-		25.70
	Others compounds					
74	Octyl acetate	-	-	0.31		15.92
75	Trans-carane	4.75	-	-		16.07
76	Acetic acid, decyl ester	0.11	-	-		18.79
77	Cyclododecane	0.06	-	-		19.22
78	Lepidozene	-	-	0.25	2.34	19.15
79	Exo-2-hydroxycineole acetate	-	-	0.21		19.30
80	α-Bergamotene	-	-	4.24		21.91
81	Mint furanone	0.07	-	-		23.69
82	Zingiberene	0.17	-	-		27.56
83	2-Acetoxytridecane	-	0.28	-		26.24
84	Myristinic acid	-	0.05	-		29.40
85	(Z)-8-(3,5-dimethyl-4-hydroxyphenyl)-2-octene	-	4.13	-		30.38
86	Hexahydrofarnesyl acetone	-	0.11	-		30.81
87	Hexadecanoic acid	-	0.46	-		33.45
88	3-ethoxy-4-hydroxy-4-(4-methoxyphenyl)cyclopent-2-enone	-	1.39	-		34.67
89	Phytol	-	1.65	-	0.45	36.03
90	Linolenic acid, methyl ester	-	0.14	-		36.62
91	Isomaturinin	-	2.61	-		37.28
92	Pentacosane	-	0.09	-		42.29
93	Heptacosane	-	0.08	-		45.56
TOTAL		99.45	93.08		99.74	92.22

The chromatographic examination of the *M. pelegium* EO detected 99.45%. The primary constituents consisted of 1,8-cineole (14.60%), p-menthan-3-one (14.9%), piperitenone (11.4%), and carene (10.19%). Marzouk et al. (2008) conducted a study in Tunisia and found that the primary constituents of *M. pelegium* leaves were menthol

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(46.60-49.86%), 1.8 cineole (13.53-17.31%), menthone (11.13-12.34%), and pulegone (3.76-4.78%). Our findings contradict many published studies that identify pulegone as the primary component of the essential oil (Hajlaoui et al., 2009). Nonetheless, other studies showed that the rate of pulegone did not exceed 2.3, 3.76 and 6.42%, respectively in Morocco and Tunisia (Zekri et al., 2013; Marzouk et al., 2008).

The essential oil of *R. graveolens* has 25 primary components, distinguished by a significant prevalence of ketones, particularly 1-nonene (19.4%), 2-undecanone (16.22%), and 2-nonanone (11.9%). Previous studies have found the most common essential oil found in the above-ground sections of *R. graveolens* which is composed mainly of 2-undecanone and 2-nonanone (De Feo et al., 2002; Fredj et al., 2007; Reddy and Al-Rajab, 2016). The gas chromatography-mass spectrometry (GC-MS) study of the essential oil (EO) extracted from *O. basilicum* confirmed the high concentration of linalool (29.23%), methyl cinnamate (18.97%), eugenol (5.84%), 1,8-cineole (5.74%), and α-cadinol (5.69%). The findings of our study align with prior research conducted in Tunisia on the *O. basilicum* essential oil (Snoussi et al., 2016; Bouzouita et al., 2012). These investigations also identified linalool, E-methyl cinnamate, and 1,8-cineole as the primary components. A total of 32 components were discovered from the *M. spicata* EO. The primary constituents consisted of carvone (50.6%), limonene (5.10%), Germacrene-D (7.67%), and trans-Caryophyllene (5.55%). The prevalence of carvone aligns with the findings of Ait-Ouazzou et al. (2012). The differences observed in the amount and chemical makeup of essential oils, both in terms of quality and quantity which can be attributed to various ecological factors, the specific plant part used, the age of the plant, and the stage of its growth cycle.

Fumigant toxicity

The outcomes of fumigating adult female *O. afrasiaticus* with essential oils from *R. graveolens*, *O. basilicum*, *M. spicata*, and *M. pulegium* are displayed in *Table 2*.

Table 2. *Mortality rate of Oligonychus afrasiaticus adult females after fumigation with different concentrations of Mentha pulegium, Ruta graveolens, Ocimum basilicum and Mentha spicata essential oils*

(EOs) concentration µL L^{-1}	*R. graveolens*	*O. basilicum*	*M. spicata*	*M. pulegium*
0.02	17.71 ± 5.7a	0 ± 0a	0 ± 0a	0 ± 0a
0.2	98.13 ± 2.5b	6.89 ± 5.9b	5.51 ± 1.8a	4.9 ± 3.5b
1	100 ± 0b	53.92 ± 7.7c	49 ± 13.8b	45.09 ± 6.7c
2	100 ± 0b	100 ± 0d	93.13 ± 8.3c	99.06 ± 2d
12	100 ± 0b	100 ± 0d	100 ± 0c	100 ± 0d

Means ± standard deviation (SD) within a column (between concentration within oil type) followed by the same letter are not significantly different according to LSD test at $p < 0.05$

The effect of fumigants differed depending on the type of plant and concentration of essential oils used. All the essential oils that were tested exhibited significant fumigant toxicity, resulting in a mortality rate of over 90% when applied at a concentration exceeding 2 µL/L. When exposed to the vapors of essential oils (EOs) derived from *O. basilicum*, *M. spicata*, and *M. pulegium* at a concentration of 1 µL/L air, roughly 50% of female subjects were fatally affected. With this concentration, *R. graveolens* EO

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caused significantly higher acaricidal effects (98%). Consequently, *R. graveolens* exhibited lowest LC_{50} (0.11 µL/L) and LC_{90} (0.23 µL/L) values, which resulted in higher toxicities achieved at lower concentrations (*Table 3*).

Table 3. *LC_{50} and LC_{90} values calculated for mortality of Oligonychus afrasiaticus adult females fumigated with different concentrations of Mentha pulegium, Ruta graveolens, Ocimum basilicum and Mentha spicata essential oils*

Essential oils	CL_{50}	CL_{90}	χ2	Slope ± SEM
M. spicata	1.1 (0.1-1.9)	1.77 (1.6-1.9)	6.15	1.89 ± 0.13
O. basilicum	0.92 (0.8-1)	1.46 (1.3-1.6)	5.17	2.3 ± 0.2
M. puleguim	1.03 (0.9-1.1)	1.57 (1.4-1.7)	3.3	2.3 ± 0.18
R. graveolens	0.11 (0.04-0.27)	0.23 (0.15-0.73)	30.7	10.36 ± 1

Units LC_{50} and LC_{90} = µL/L air, applied for 24 h at 25°C; 95% lower and upper confidence limits are shown in parenthesis

Kalmosh et al. (2019) determined that the LC_{50} and LC_{90} of *R. graveolens* EO for *T. urticae* adults were 0.018 and 0.075 µL/mL air, respectively. They attribute the fumigation toxicity of this oil to its high concentration of methyl ketones, particularly 2-Undecanone (60.54%). This also aligns with our findings, as *R. graveolens* essential oil is notably abundant in ketones. Our findings about *M. spicata* concur with those of other studies which estimated the fumigant's activity on adult *T. urticae* (LC_{50} = 7.53 µL/L air, 25.11 mg/L air,1.3 µL/L air), respectively (Kheradmand et al., 2018; Reddy and Dolma, 2018; Pavela, 2016). In another study, Sertkaya et al. (2010) investigated the insecticidal impact of *M. spicata* against *T. Cinnabarinus* and reported that the value of LC_{50} was 1.83 µg/mL. Previouss studies revealed that *M. pulegium* EO has a high fumigant activity against adults of *T. urticae* with LC_{50} values of 0.49 (Topuz et al., 2018) and 2.75 µL/L air (Mozaffari et al., 2013) after 24 h of exposition. The presence of some chemicals including monoterpene are known for their strong activity. *Ocimum basilicum* EO has been highlighted for its fumigant characteristics. When it was used on adult female *T. urticae*, the LC_{50} was determined to be 0.6 µL/L and the LC_{90} to be 5.9 µL/L (Pavela et al., 2016). Previous investigations reported that pesticidal properties of the genus *Ocimum* were due to various groups of compounds in its essential oil including linalool which exhibits toxic effects (Regnault-Roger et al., 2002; Santoro et al., 2007; Chang et al., 2009).

Repellency assay

The results of the repellency tests conducted on the essential oil against *O. afrasiaticus* are detailed in *Table 4*.

Data showed the highest repellent potentials (more than 95%) at concentrations of 0.318 and 1.9 µL/cm^2 following 1 h of exposure for all tested oils (class V). Following 24-h exposure to *M. spicata* EO, the repellent efficacy did not exceed 50% at low doses (0.003 and 0.03 µL/cm^2). The essential oil of *O. basilicum* exhibited significant repellent properties, with an efficacy of 81%, even when applied at low quantities

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(0.003 µL/cm^2) after 24 h of exposure. *M. pulegium* EO showed excellent results against *O. afrasiaticus* and after 1 h of exposure the repulsion rates exceeded 50% and 80%, respectively, with 0.003 and 0.03 µL/cm^2. *R. graveolens* EO had a very high repellent power. In fact, at doses of 0.003 µL/cm^2, pulsivity of 66% and 75% were noted, respectively, during one and 4 h of exposure. The tested dose of 0.03 µL/cm^2 had a strong repulsive activity (class V) after 1 h of exposure. Although there is no research proving the essential oil of *R. graveolens* repellent properties against Tetranychidae. Several studies have proven the repulsive efficacy of *R. graveolens* EO against insects like *Sitophilus oryzae* (Perera and Karunaratne, 2016), *Sitophilus zeamais* (Perera et al., 2017), *Rhynchophorus ferrugineus* (Perera and Karunaratne, 2016). Witting-Bissinger et al. (2008) found that the insect repellent activities of *R. graveolens* essential oil can be attributed to the major component 2-undecanone. Our research indicates that *M. spicata* EO has a repellent effect on *O. afrasiaticus*. Other researchers' findings confirmed its excellent repellent activity against *T. urticae* adults (Mozaffari et al., 2013; Kheradmand et al., 2018). *M. spicata* EO demonstrated 97% repellent activity on *T. urticae* adults after 1 h of exposure at 10 mg/L (Reddy and Dolma, 2018).

Table 4. *Percentage repellency of M. pulegium, R. graveolens, O. basilicum and M. spicata essential oils against O. afrasiaticus adult females after various periods of exposure and concentrations*

Period of exposure (h)	Concentration (µL/cm^2)				Response class			
	0.003	0.03	0.318	1.9				
M. spicata								
1	27.52 ± 19.3a	40.5 ± 30.19a	98.18 ± 4.06b	100 ± 0b	I	II	V	V
4	40.4 ± 28.45a	35.16 ± 28.79a	100 ± 0b	100 ± 0b	III	II	V	V
24	48.89 ± 27.93a	54.14 ± 29.7a	96 ± 8.9b	100 ± 0b	III	III	V	V
M. puleguim								
1	57.79 ± 25.44a	82.34 ± 21.75b	100 ± 0c	100 ± 0c	III	V	V	V
4	57.33 ± 30.06a	82.33 ± 20.76b	100 ± 0c	100 ± 0c	III	V	V	V
24	50.44 ± 30.16a	98 ± 6.32b	96.44 ± 4.87b	100 ± 0b	III	V	V	V
R. graveolens								
1	66.45 ± 42.64a	98 ± 0.6b	100 ± 0b	100 ± 0b	IV	V	V	V
4	75.27 ± 27.9a	83.34 ± 20.7a	100 ± 0b	100 ± 0b	IV	V	V	V
24	85.34 ± 21.38a	100 ± 0b	100 ± 0b	100 ± 0b	IV	V	V	V
O. basilicum								
1	44.06 ± 27.6a	80.43 ± 12.87a	100 ± 0b	100 ± 0b	III	V	V	V
4	64.7 ± 22.07a	68.26 ± 26.25a	100 ± 0b	100 ± 0b	IV	IV	V	V
24	81.78 ± 21.53a	87.78 ± 21.6a	100 ± 0b	100 ± 0b	V	V	V	V

Means ± standard deviation (SD) within a column (between exposure time within oil concentration) followed by the same letter are not significantly different according to the LSD test at $p < 0.05$
Repellency classes: O = < 0.1: is not repulsive; class I = 0.1–20: Very weak repulsive; class II = 20.1–40: weakly repulsive; class III = 40.1–60: moderately repulsive; class IV = 60.1–80: repulsive; class V = 80.1–100: very repulsive (based on Juliana and Su, 1983)

In a prior investigation, *M. pulegium* EO performed well in repellency bioassays. Mozaffari et al. (2013) found that *M. pulegium* at 1000 mg/L air exhibited 100% repellency in adults of *T. urticae*. The insecticidal action ascribed to this phenomenon is

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typically linked to the existence of monoterpenoids and sesquiterpenes, which have a neurotoxic impact on these insects (Nerio et al., 2009). Concerning *O. basilicum* EO, it is remarkable for its significant repellent action. According to the studies carried out by Mansour et al. (1986), at doses of 0.1 to 2% produced a remarkable repellent effect against *Tetranychus cinnabarinus* within 48 h. Additionally, the essential oil of *Ocimum basilicum* has been documented to act as a repellent against various insect species, ranging from *Nephotettix virescens* (Chittihunsa and Samngamnim, 1999) to the tick *Rhipicephalus* (Martinez-Velazquez et al., 2011). Martinez-Velazquez et al. (2011) proposed the significant repellent effect of *O. basilicum* might be attributed to its primary components, namely 1,8-cineole (6.2%), eugenol (6.61%), α-farnesene (6.96%) linalool (30.61%) and estragole (20.04%) which have repellent potential.

Contact essay

The mortality of *O. afrasiaticus* increased significantly after being exposed to different concentrations of the tested essential oils for 24 h (*Fig. 1*).

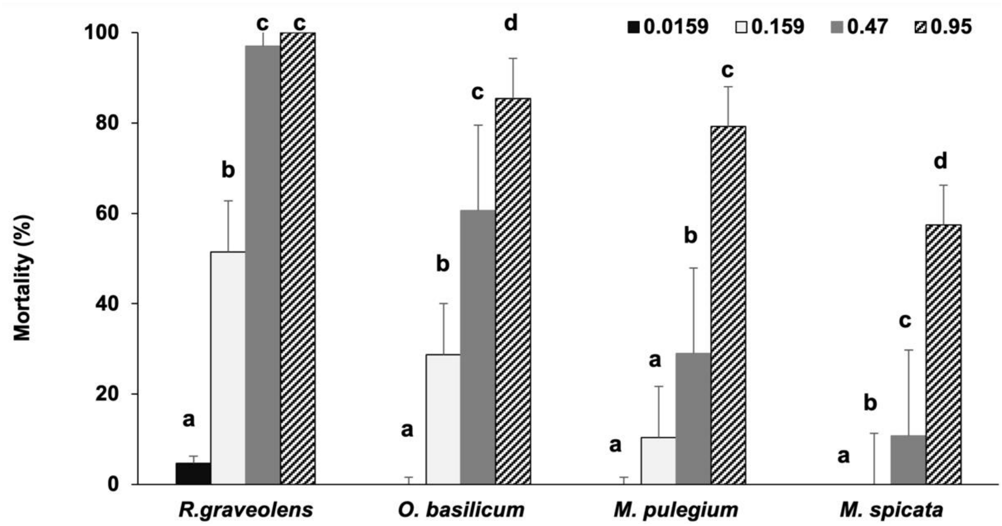

Figure 1. *The mortality rate of Oligonychus afrasiaticus adult females exposed to residues of various concentrations of Mentha pulegium, Ruta graveolens, Ocimum basilicum and Mentha spicata essential oils. Means ± standard deviation (SD) between concentration within oil type followed by the same lowercase letter are not significantly different according to LSD test at $p < 0.05$*

The mortality rates varied depending on the concentration of the essential oil used. Essential oil of *R. graveolens* had more acaricidal effects on *O. afrasiaticus* female adults. At the lowest tested concentration (0.0159 µl/cm^2) it proved to be the only active oil. At 0.47 µl/cm^2 *R. graveolens* caused high adult mite mortalities (55.5%) compared to the other tested oils. For the highest concentration (0.95 µl/cm^2), mortality was attained, respectively, 57.2, 79.2, 85.4, and 100% mortality with *M. spicata*, *M. pulegium*, *O. basilicum* and *R. graveolens*.

Table 5 shows the estimated lethal concentration values (LC_{50} and LC_{90}) for each essential oil. The lowest LC_{50} and LC_{90} values for the mite were obtained for *R. graveolens* EO (0.18 and 0.34 µl/cm^2), followed by *O. basilicum* (0.31 and 1.2 µl/cm^2), *M. puleguim* (0.66 and 1.1 µl/cm^2) and *M. spicata* (0.88 and 1.2 µl/cm^2).

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Table 5. *LC_{50} and LC_{90} values calculated for mortality of O. afrasiaticus adult females exposed to residues of various concentrations of M. pulegium, R. graveolens, O. basilicum and M. spicata essential oils*

Essential oils	LC_{50}	LC_{90}	χ^2	Slope ± SEM
M. spicata	0.88 (0.82-0.95)	1.2 (1.1-1.4)	1.6	3.3 ± 0.35
O. basilicum	0.31 (0.26-0.37)	1.2 (0.99-1.8)	1.1	2.1 ± 0.23
R. graveolens	0.18 (0.23-0.54)	0.34 (0.23-1.8)	7.6	7.6 ± 0.8
M. puleguim	0.66 (0.6-0.72)	1.1 (1-1.2)	4.6	2.8 ± 0.24

Units LC_{50} and LC_{90} = µL/L air, applied for 24 h at 25°C. 95% lower and upper confidence limits are shown in parenthesis

Regarding *M. pulegium*, studies published by Aissaoui et al. (2018) showed that at a concentration of 1%, it killed all adults of *T. urticae* and 89.47% of the larvae. Additionally, other research findings revealed a contact toxicity potential of *M. pulegium* EO against the beetle *Rhyzopertha dominica* (Benayad et al., 2012), against *Mayetiola destructor* (Lamiri et al., 2001). *O. afrasiaticus* adults appeared to be vulnerable to the effects of *O. basilicum* EO upon residual contact. According to Aslan et al. (2004), *O. basilicum* EO was a contact toxic to nymphs and adults of *T. urticae* and resulted in 60% mortality at the highest dose. Refaat et al. (2002) discovered that basilica oil exhibited miticidal properties against tetranychid mites, specifically *T. urticae* and *Eutetranychus orientalis* pests. While our results have shown the effectiveness of *M. spicata* EO is limited. Biological tests executed by Sertkaya et al. (2010) demonstrated that the essential oil of *M. spicata* led to the complete death of adults *T. cinnabarinus* at concentrations in the air of 5.0 and 7.5 µg/ml, respectively, whose mean lethal concentration (LC_{50}) was 1.83 µg/ml of air. *R. graveolens* essential oil was very toxic and caused 100% contact mortality within the initial 24 h in a contact toxicity test conducted against weevils *R. ferrugineus* (Perera et al., 2017).

Conclusion

Our findings confirm that essential oils from *M. spicata*, *M. puleguim*, *O. basilicum* and *R. graveolens* plants possess acaricidal activity against *O. afrasiaticus* female adults. The laboratory bioassay results demonstrated that the essential oils derived from *M. pulegium*, *O. basilicum* and *M. spicata* caused a mortality rate of 50% among females when administered at a concentration of 1 µL/L. The highly effective plant was *R. graveolens* and its toxicity can occur via residual contact, repulsive and fumigant action. Given the large number of studies on EO there is a strong interest in extending Eos research work. It can help in making competitive and environmentally friendly products.

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