Acaricidal Activity of Essential Oils from *Lantana camara* (Verbenaceae) and *Ruta chalepensis* (Rutaceae) against *Oligonychus afrasiaticus* (Acari: Tetranychidae)[1]

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Abstract *Oligonychus afrasiaticus* (McGregor) (Acari: Tetranychidae) is a major pest that causes significant economic losses to date palm, *Phoenix dactylifera* L. (Arecales: Arecaceae), fruit in southern Tunisia and Algeria, where climatic conditions are highly conducive to development of this insect. In our efforts to develop sustainable management alternatives for *O. afrasiaticus,* essential oils extracted from *Lantana camara* L. and *Ruta chalepensis* L. were tested against *O. afrasiaticus* adult females. Both essential oils exhibited acaricidal activity against *O. afrasiaticus* in laboratory bioassays of concentration–mortality responses. At 96 h after exposure, the median lethal concentrations were 5,259 μl/ml for the *L. camara* extract and 3,329 μl/ml for the *R. chalepensis* extract. Based on median lethal times, the acute toxicity of the extracts against *O. afrasiaticus* was higher with *L. camara* than with *R. chalepensis* at a concentration of 50 μl/ml. Gas chromatography–mass spectrometry analysis revealed that the primary constituents of the essential oil extracted from *L. camara* were humulene (26.65%), caryophyllene (26.33%), and γ-muurolene (14.22%). The predominant compounds in the essential oil obtained from *R. chalepensis* were 2-undecanone (50.52%), 2-nonanone (11.27%), and 2-octanol, acetate (9.17%). These two essential oils have potential for development as botanical acaricides for the management of *O. afrasiaticus* in date palm production in this region.

Key Words Tetranychidae, acaricidal activity, biological control, botanical acaricides, alternative control

The date palm, *Phoenix dactylifera* L. (Arecales: Arecaceae), is an economically important crop in arid subtropical and tropical regions. According to the Food and Agriculture Organization of the United Nations (FAO), Algeria ranks fourth and Tunisia tenth globally in terms of date production and is expected to reach 1.247 and 0.369 million ton of dates in those respective countries by 2022 (Food and Agriculture Organization of the United Nations Statistics 2023). However, this production might decline because of attacks by four phytophagous pests: *Ectomyeloïs*

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ceratoniae (Zeller) (Lepidoptera: Pyralidae), *Parlatoria blanchardi* (Targioni Tozzetti) (Hemiptera: Diaspididae), *Oryctes agamemnon* Burmeister (Coleoptera: Scarabaeidae), and *Oligonychus afrasiaticus* (McGregor) (Acari: Tetranychidae) (Dhouibi 1991).

Oligonychus afrasiaticus is an economically important pest of date palm fruit in the Middle East (Arbabi et al. 2017, Negm et al. 2014, Palevsky et al. 2005) and in North Africa in Tunisia (Ben Chaaban et al. 2018) and Algeria (Lakhdari et al. 2015). Prevalent environmental conditions in these regions contribute significantly to the reproduction and development of *O. afrasiaticus* on date palm fruits (Al-Doghairi 2004). As fruit formation begins, *O. afrasiaticus* infests palm trees, where it weaves complex webs around date clusters. As a result, the entire fruit set is covered in densely woven webs, causing damage and scarring of the fruit (Arbabi et al. 2017). Management practices such as the release of biological control agents are ineffective because the dense webbing inhibits their activity and is impervious to chemical acaricides. Lack of effective treatment leads to increased infestation by these mites and greater economic losses (Alatawi 2020, El-Shafie 2022). The webs persist until the later stages of date maturation, resulting in a reduction in the growth and development of date palm fruits and ultimately in harvest losses because the fruits are unsuitable for human consumption (Ben Chaaban and Chermiti 2010).

During the winter, *O. afrasiaticus* is found in date fronds and crown fibers, grasses, and other alternative host plants (Alatawi 2020, Ben Chaaban et al. 2017). *Oligonychus afrasiaticus* takes refuge on other host plants as an alternative to date palms until the new fruiting season. Plants in the Arecaceae and Poaceae families are among these refuges that provide shelter from unfavorable conditions (Alatawi 2020).

In the absence of management measures, date production losses due to *O. afrasiaticus* infestation can reach 100% (El-Shafie 2022). Management of *O. afrasiaticus* on date palms relies mainly on the use of chemical acaricides (Al-Dosary 2010). However, residues of synthetic acaricides pose a risk to consumers and contribute to environmental pollution, and the nonselective use of these products is restricted because of their harmful effects on nontarget species.

The main advantage of many plant-based acaricides is that they have no significant direct impact on agricultural ecosystems. Botanical acaricides containing essential oils may be a viable alternative to synthetic acaricides with longer persistence than other control agents (Assouguem et al. 2022). Numerous plant extracts have been assessed for their acaricidal properties. These essential oils comprise volatile secondary compounds, predominantly terpenes (Hussain et al. 2019). For example, essential oils extracted from plants of the *Lantana* genus (Verbenaceae) and the *Ruta* genus (Rutaceae) have acaricidal properties (de Carvalho et al. 2015, De Sousa et al. 2020).

Alternative management strategies to mitigate the impact of this date palm mite are critical. This study was conducted to extract and characterize the components of two essential oils, one from *Ruta chalepensis* L. and the other from *Lantana camara* L., to assess their acaricidal properties against *O. afrasiaticus* females and to identify more environmentally friendly control alternatives.

Materials and Methods

Collection of plant material. *Lantana camara* and *R. chalepensis* plant specimens were collected from Janoura and Bazma, respectively, in Kebili, southern Tunisia in April 2021. These plants were harvested during their flowering phase and transported to the laboratory of the Institut des Régions Arides (Medenine, Tunisia) for the extraction process.

Essential oil extraction. The essential oils were extracted from the plants with the Clevenger hydrodistillation method (Zouari et al. 2014). The aerial portions of fresh specimens of each plant species were treated with boiling water. The steam was passed through the plant and condensed in a condenser, and the essential oil floating on top of the condensate was recovered.

Gas chromatography–mass spectrometry analysis. Volatile compounds were identified by gas chromatography–mass spectrometry with a GC2010Plus gas chromatograph coupled with a QP2010 ULTRA mass spectrometer (Shimadzu Corp., Kyoto, Japan). The RTX5MS capillary column had a stationary phase of 5% diphenyl and 95% dimethylpolysiloxane and dimensions of 30 m by 0.25 mm (inside diameter) by 0.25 μm. The oven temperature was programmed at 50°C for 2 min, increased at 7°C/min to 250°C, and then left at 250°C for 5 min. The split/splitless injector temperature was 250°C (split ratio of 1/50). The carrier gas was helium (99.9995% pure) with a flow rate of 1 ml/min. For the mass spectrometer conditions, the ionization energy was 70 eV, the ionization source temperature was 200°C, the ionization mode was electron impact, and the mass range was 35–500 *m/z*. Compounds were identified by comparing their mass spectra with those recorded in the National Institute of Standards and Technology library (https://www.nist.gov).

Mite rearing. Date palm fruits (var. 'Deglet noor') heavily infested by *O. afrasiaticus* were collected from the oases of Dawia El-Oued, Algeria on 22 June 2021. After collection of all stages of mites, a reserve colony was created. Females and males mites were selected and placed with the Deglet noor date fruits to lay eggs in rearing units at the Biodiversity and Environmental Protection Laboratory (Faculty of Life Sciences, Elchahid Hamma Lakhdar University, El-Oued, Algeria). Adult female mites from this colony were used for the bioassay experiments to ensure that the female mites were of the same age. All colonies were maintained in a room at 25 $\pm$ 1°C and relative humidity of 60 $\pm$ 10% with a photoperiod of 16 h of light and 8 h of dark.

Preparation of essential oil concentrations. Preliminary trials were conducted to determine appropriate concentrations for the bioassay of each essential oil. For each essential oil, the concentrations to be tested were prepared by dilution in distilled water with Tween 80 to 50, 25, 10, 5, and 1 μl/ml. The negative controls consisted of distilled water and Tween 80 without the oil.

Bioassays. For each concentration of each essential oil, five date palm leaf discs (7.5 $\times$ 4 cm) were submerged in the essential oil solution for 30 s, ensuring even coverage with tiny droplets (Pinto et al. 2020). Control leaf discs were soaked in 0.05% Tween 80 (Hussain et al. 2020). The soaked leaf discs were air-dried for 1 h. Ten fertilized adult *O. afrasiaticus* females of the same age were placed with a brush on a single leaf disc. The disk was then placed on filter paper inside a transparent plastic box (10 $\times$ 5 cm). These leaf discs were surrounded by damp cotton. Each box treatment had five replicates. Dead and surviving *O. afrasiaticus* females were counted with a binocular microscope at 4, 8, 12, 24, 48, and 96 h posttreatment. Female *O. afrasiaticus* were considered dead when they did not walk or move when prodded with the tip of a brush.

Statistical analysis. The corrected mortality of adult female *O. afrasiaticus* was calculated with Abbott's formula (Abbott 1925). Data were standardized prior to analysis and subjected to a one-way analysis of variance. The significance of differences in mean mortality levels at 96 h after treatment was determined using Tukey's honestly significant difference test at $P < 5\%$. Mortality response data also were subjected to probit analysis (Finney 1971) using SPSS 22.1 software.

Results

Chemical composition of the essential oils. The chemical components of the two essential oils and their percentages and retention times are listed in Table 1. The major compounds identified in the essential oil extracted from *L. camara* were caryophyllene (26.33%), humulene (26.65%), and γ-muurolene (14.22%). A few other minor compounds such as γ-elemene (4.68%), β-pinene (3.19%), α-farnesene (3.06%), α-cedrene (2.31%), α-zingiberene (2.30%), and copaene (2.16%) also were identified in measurable amounts. Of the total compounds identified in the *L. camara* leaf extract, 8.57% were monoterpenes and 88.95% were sesquiterpenes. The main components identified in the *R. chalepensis* essential oil were 2-undecanone (50.52%), 2-nonanone (11.27%), and 2-octanol, acetate (9.17%). Most of the components of this essential oil were ketones (73.91%).

Acaricidal effect of the essential oils. The acaricidal activity of the two essential oils was assessed in contact toxicity bioassays against adult *O. afrasiaticus* females (Fig. 1). The corrected mortality of *O. afrasiaticus* females increased significantly with increasing concentration and duration of exposure to the two essential oils (*L. camara*: $F = 86.07$, df $= 4$, $P < 0.0001$; *R. chalepensis*: $F = 209.81$, df $= 4$, $P < 0.0001$). The *L. camara* essential oil treatment resulted in 79.16% mortality after 96 h of contact exposure at a concentration of 50 μl/ml (Fig. 1A). At the same concentration, the *R. chalepensis* essential oil treatment resulted in 83.33% mortality after 96 h of exposure (Fig. 1B).

Adult *O. afrasiaticus* females were more sensitive to the acaricidal effect of *R. chalepensis* essential oil than to that of *L. camara* essential oil. The median lethal concentrations of these two essential oils were 3.329 and 5.259 μl/ml, respectively. This mite apparently has greater tolerance to *L. camara* essential oil than to that of *R. chalepensis* (Table 2).

The most effective essential oil was that with the lowest median lethal time (LT_{50}) (Table 3). The lowest LT_{50} (9.951 h) occurred with the *L. camara* essential oil at a concentration of 50 μl/ml. The LT_{50} for the *R. chalepensis* essential oil was 5.530 h, and the LT_{50} decreased with decreasing concentration.

Discussion

We identified 26 constituents of the essential oil extracted from the leaves of *L. camara* and 23 constituents of the essential oil extracted from *R. chalepensis* foliage. Our identification of caryophyllene, humulene, and murolene in the *L. camara* extract corroborates the identification of sesquiterpenes, such as (E)-β-caryophyllene and α-humulene, in hydrodistillates of *L. camara* by Nea et al. (2020). Sarma et al. (2020) detected 22 compounds, accounting for 88.97% of the total chemical constituents of *L. camara* extract. They listed β-caryophyllene (24.96%) and δ-selinene (17.46%) as main constituents of *L. camara* foliage. According to De Sousa et al.

Table 1. Volatile compounds identified from the essential oils of *L. camara* and *R. chalepensis*.

Compound(s)	Retention Time (min)	% Composition	
		L. camara	*R. chalepensis*
α-Pinene	6.86	1.08	
Camphene	7.22	0.38	
β-Pinene	7.81	3.19	
2-Octanone	8.18		0.16
β-Myrcene	8.2	1.04	
α-Phellandrene	8.54	0.17	
3-Carene	8.69	0.94	
p-Cymene	9.02	0.11	
Limonene	9.136	0.86	
Eucalyptol	9.21	0.12	
γ-Terpinene	9.856	0.76	
2-Nonanone	10.66		11.27
Nonanal	10.89		0.11
1,3-Cycloheptadiene	11.84		2.14
2-Decanone	12.92		1.36
Acetic acid, octylester	13.3		0.18
2-Octanol, acetate	13.89		9.17
2-Undecanone	15.28		50.52
2-Undecanol	15.32		0.25
Acetic acid, heptylester	15.46		1.08
6-Dodecanone	16.6		2.85
Acetic acid, nonylester	16.73		0.13
Copaene	16.97	2.16	
2-Propenoic acid, 3-phenyl-methylester	17.05		0.36
2-Dodecanone	17.19		2.69
β-Elemene	17.28	1.93	
5-Tetradecanol acetate	17.89		3.27
Caryophyllene	17.99	26.33	
cis-β-Farnesene	18.46	1.75	
Humulene	18.67	26.65	

Table 1. Continued.

Compound(s)	Retention Time (min)	% Composition	
		L. camara	*R. chalepensis*
α-Farnesene	18.99	3.06	0.10
2-Tridecanone	19.15		3.49
γ-Muurolene	19.16	14.22	0.38
α-Zingiberene	19.24	2.30	
γ-Elemene	19.41	4.68	0.44
α-Cedrene	19.53	2.31	
δ-Cadinene	19.82	1.48	
Alloaromadendrene	20.53	1.50	0.56
Caryophyllene oxide	21.47	0.53	
2-Heptanone, 7-phenyl-	22.38		1.54
1,3-Benzodioxole, 5-propyl-	24.74		5.14
n-Hexadecanoic acid	26.9		0.42
3,4-Methylenedioxyphenyl acetone	28.12		2.88
Phytol	29.03	1.4	0.34
Monoterpenes		8.57	
Hydrocarbons		0.11	2.14
Sesquiterpenes		88.95	1.50
Ketones			73.91
Esterification			14.22
Acids			0.42
Alcohols		1.40	0.59
Aldehydes			0.11
Aromatic compounds			8.03

(2020), the essential oil of *L. camara* was mainly composed of bicyclogermacrene, isocaryophyllene, valencene, and D-germacrene.

The predominant compound we identified from the essential oil of *R. chalepensis* foliage was 2-undecanone (50.52%). In a similar study on the essential oil of dried aerial parts of *R. chalepensis* harvested in northern Tunisia, 13 major compounds were found, primarily 2-undecanone (77.18%), 2-decanone (8.96%), and 2-dodecanone (2.37%). The 2-undecanone was the sole component (100%) of the essential oil obtained from *R. chalepensis* flowers (Mejri et al. 2010). Those results revealed that leaf and flower extracts from *R. chalepensis* were dominated by

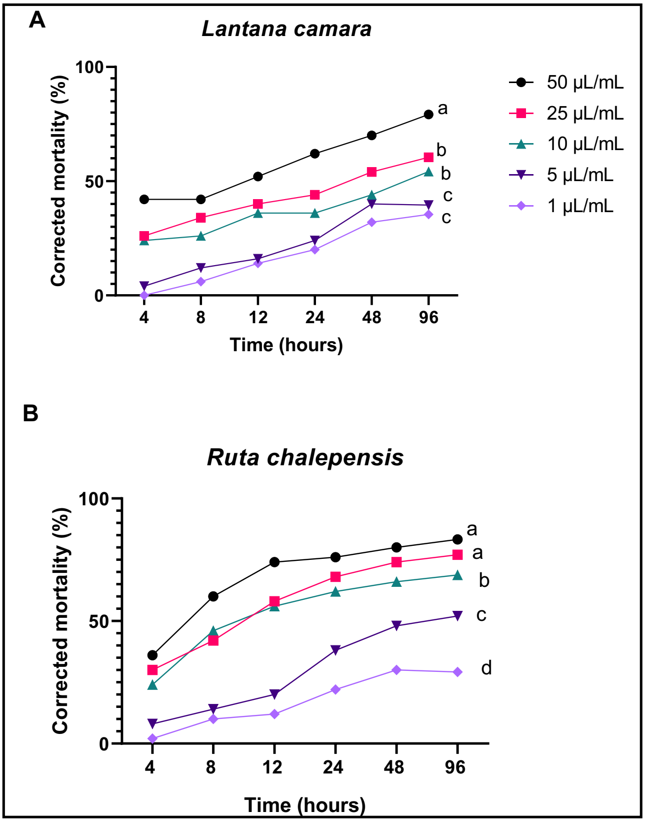

Fig. 1. Corrected mortality of adult females of *O. afrasiaticus* at various concentrations of foliar extracts of (A) *L. camara* and (B) *R. chalepensis.*

ketones, in particular the 2-undecanone derivative, accounting for 85.94 and 89.89% of leaf and flower oils, respectively. *R. chalepensis* essential oil collected from the Oulmès region (Central Plateau) of Morocco was characterized by the dominance of 2-undecanone (64.35%) and piperonyl piperazine (11.9%) (Najem et al. 2020).

These differences in the composition of the two essential oils might be influenced by edaphic and climatic factors, the phase and stage of plant development at harvesting, and the extraction and analysis techniques used (Bammou et al. 2016). Plants harvested at different growth stages also differed in all the chemical classes of essential oils (Khalid 2019).

Treatment with the essential oils of *L. camara* and *R. chalepensis* significantly impacted the mortality of adult *O. afrasiaticus* females compared with the untreated controls. Mite mortality increased as the concentration of each essential oil increased. The acaricidal effect observed with these two essential oils against adult *O. afrasiaticus* females can be attributed to the active chemical compounds in these plants or to the major compounds. Bakkali et al. (2008) reported that the biological properties of essential oils are generally determined by their main components. Similar studies

Table 2. Concentration-mortality response of *O. afrasiaticus* adult females to foliar extracts from *L. camara* and *R. chalepensis* after 96 h of exposure.

Essential oil	LC_{50} (μl/ml) (95% CL)*	Probit Equation	Linear Slope (SE)	χ^2 (df)
L. camara	5.259 (2.287–9.472)	$Y = 0.627 \log C - 0.452$	−0.452 (0.156)	3.482 (3)
R. chalepensis	3.329 (1.708–5.253)	$Y = 0.884 \log C - 0.462$	−0.462 (0.158)	0.443 (3)

*LC_{50} = median lethal concentration; CL = confidence limit (calculated with 95% confidence).

Table 3. Median lethal times (LT_{50}) at various concentrations of foliar extracts of *L. camara* and *R. chalepensis* against *O. afrasiaticus* adult females.

Essential oil	Concentration (μl/ml)	LT_{50} (h)	Fiducial limits (95%)	χ^2
L. camara	1	132.547	81.962–317.357	3.410
	5	112.893	68.528–282.695	1.619
	10	69.575	36.535–344.254	0.948
	25	34.223	20.724–78.241	0.168
	50	9.951	5.217–15.254	1.006
R. chalepensis	1	209.882	104.228–978.155	4.546
	5	61.288	42.469–108.342	1.602
	10	13.761	8.100–21.009	4.546
	25	10.773	6.683–15.361	2.364
	50	5.530	2.352–8.850	4.554

on these two essential oils have shown their effectiveness against several pests. The *L. camara* extract caused significant mortality of the maize grain weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) (Bouda et al. 2001). Dua et al. (2010) also reported the adulticide activity of *L. camara* extract against species of mosquitoes. The methanol extract of *L. camara* leaves had fumigation and contact toxicity against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Callosobruchus chinensis* (F.) (Coleoptera: Bruchidae), and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Rajashekar et al. 2013). The *L. camara* extract also caused contact toxicity and had a repellent effect on second-instar tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Liambila et al. 2021).

Ruta chalepensis essential oil had a contact larvicidal effect on third- and fourth-stage *Orgyia trigotephras* Boisduval (Lepidoptera, Lymantriidae) larvae (Akkari et al. 2015). This essential oil also is highly repellent to *T. castaneum,* largely due to its primary compound, 2-undecanone (Najem et al. 2020). The acaricidal effect of the *Ruta* genus in the present study is similar to that reported against other mite species. A leaf extract of *Ruta graveolens* L. was toxic by fumigation against eggs and adult females of *Tetranuchys urticae* Koch (Acari: Tetranychidae). In addition to the mortality of adult females, this essential oil caused a significant reduction in the fecundity of treated females (Kalmosh et al. 2019). Other studies with the essential oils of *Thymus vulgaris* L. (Lamiaceae), *Salvia mirzayanii* Rechinger & Esfand (Lamiaceae), and *Trachyspermum ammi* L. (Apiaceae) revealed acaricidal activity against *O. afrasiaticus* adults in a laboratory setting (Sohrabi and Kohanmoo 2017).

Conclusion. The essential oils extracted from the foliage of *L. camara* and *R. chalepensis* had significant acaricidal activity against adult females of *O. afrasiaticus*. The effect can be attributed to the high concentration of sesquiterpenes in the *L. camara* extract and ketones in the *R. chalepensis* extract. These results suggest that extracts from these plants could be developed as viable alternatives for the control of the date palm mite. The search for these and other alternatives to control *O. afrasiaticus* with less cost to human health and the environment is necessary.

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