

Acaricidal activity of *Lantana Camara* (Verbenaceae) and *Ruta chalepensis* (Rutaceae) essential oils against the date palm mite *Oligonychus afrasiaticus* (McGregor) (Acari: Tetranychidae)

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Abstract

Oligonychus afrasiaticus (McGregor) (Acari: Tetranychidae) is a major pest causing substantial economic losses to date palm fruit in southern Tunisia and Algeria, where climatic conditions are very favorable to its development. As part of a study into sustainable management strategies for *O. afrasiaticus*, two essential oils extracted from *Lantana Camara* and *Ruta chalepensis* were tested as adulticides against female *O. afrasiaticus* mites. For this purpose, an analysis of the chemical composition of these two oils was carried out using the GC-MS method in conjunction with laboratory experiments against fertilised *O. afrasiaticus* females. The EO of *L. camara* was mainly composed of caryophyllene (24.33%), Humulene (26.65%) and Muurolene (14.22%). The EO of *R. chalepensis* was mainly composed of 2-Undecanone (50.52%), 2-Nonanone (11.27%) and 2-Octanol, acetate (9.17%). The acaricidal activity of the EOs against *O. afrasiaticus* was assessed by contact with females. The LC 50 after 72 h of treatment was 3.198 $\mu\text{L/mL}$ and 8.312 $\mu\text{L/mL}$ for *L. Camara* and *R. chalepensis*, respectively. According to the LT 50, the acute toxicity of the EOs to *O. afrasiaticus*, from high to low, was the Essential Oils *L. camara* and *R. chalepensis* at the concentration of 200 $\mu\text{L/mL}$. In conclusion, the Essential Oils of *L. Camara* and *R. chalepensis* have a better acaricidal effect and a high application value for the biological control of *O. afrasiaticus*, which provides a basis for the development of botanical acaricides.

Introduction

The date palm, *Phoenix dactylifera* L. (Arecales: Arecaceae), is an economically important crop grown in arid, subtropical and tropical regions. According to the Food and Agriculture Organization of the United Nations (FAO), Algeria ranks fourth and Tunisia tenth in date production, with production reaching 1.188 and 0.345 million tonnes of dates respectively by 2021 (FAOSTAT, 2021). However, this production can deteriorate because of attacks caused mainly by four main phytophagous pests, the carob moth *Ectomyelois ceratoniae*, the white scale insect *Parlatoria blanchardi*, the beetle *Oryctes agamemnon*, and the mite *Oligonychus afrasiaticus* (McGregor) (Acari: Tetranychidae) (Dhouibi 1991). The latter 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 North Africa in Tunisia (Ben-Chaabane et al., 2018) and in Algeria (Lakhdari et al., 2015). The environmental conditions prevailing in these regions have greatly favored the growth and development of *O. afrasiaticus* on date palm fruit (Al-Doghairi, 2004). At the time of fruit formation, infestations of *O. afrasiaticus* begin on date palms by weaving the web around the date clusters. As a result, the entire fruit cluster is covered with very dense and intricate webs of scarred and damaged fruit (Arbabi et al., 2017). Management practices such as the release of biological control agents (the webs hinder the effectiveness of predators) or insecticide applications (the dense webs become impervious to chemicals) become ineffective, leading to increased infestations of this mite and greater economic losses (Alatawi, 2020; El-Shafie, 2022). During the winter season, individuals of *O. afrasiaticus* were observed in fronds, crown fibers, grasses and other alternative host plants (Alatawi, 2020; Ben Chaaban et al., 2017). In recent years, this mite has caused economic damage and reduced yields in many date palm-

growing countries by up to 100% if left uncontrolled (El-Shafie, 2022). These webs remain intact until the later stages of date development, leading to a decline in the growth and development of date palm fruit. Such damage has resulted in crop losses and rendered the fruit unfit for human consumption (Chaaban and Chermiti, 2010). Control of *O. afrasiaticus* on date palms depends mainly on the use of chemical pesticides (Al-Dosary, 2010). However, the residues associated with acaricides are harmful to consumers and are also responsible for environmental pollution. In addition, the indiscriminate use of synthetic acaricides is limited because of the deleterious effects on non-target animals. The main advantages of many plant-based acaricides lie in their low toxicity for agroecosystems. Botanical acaricides composed of EOs can be a good alternative to more persistent synthetic acaricides. (Assouguem et al., 2022). Several EOs have been evaluated for their acaricidal activity. EOs consist of volatile secondary metabolites, mainly terpenes (Hussain et al., 2019). For example, EOs from the genera *Lantana* (Verbenaceae) and *Ruta* (Rutaceae) have acaricidal activity (de Carvalho et al., 2015; De Sousa et al., 2020).

Alternative management methods, therefore, need to be developed to control this palm mite. In order to identify alternatives with less environmental impact for controlling *O. afrasiaticus*, this study aims to extract and identify the constituents of two EOs, *Ruta chalepensis* and *Lantana Camara*, and to assess the acaricidal effect on females of this species.

Materials and methods

Collection of plant material

Two plant species, *L. camara* and *R. Chalepensis*, were collected in southern Tunisia, in the Kebeli region (Table 1). These plants were collected during the flowering phase, and transported to the laboratory of the Arid Regions Institute (IRA) Medenine for extraction.

Table 1
List of plant species tested and their collection sites

Family name	Scientific name	Collecting site	Date
Rutaceae	<i>Ruta chalepensis</i>	Bazma, kebili	23-April-2021
Verbenaceae	<i>Lantana Camara</i>	Janoura, kebili	25-April-2021

Essential oil extraction

The EO was extracted using the Clevenger hydrodistillation method. The EOs were extracted from the aerial part of each plant in a fresh state to test their acaricidal effects on *O. afrasiaticus* females. This method involves boiling water, passing the steam through the plant to extract the EO, condensing it in a condenser and recovering the EO floating on the condensation water.

GC-MS analysis

Volatile compounds were analysed by GC/MS using a SHIMADZU GC2010Plus gas chromatograph coupled with a SHIMADZU QP2010 ULTRA mass spectrometer. The RTX5MS capillary column used had a stationary phase (5% diphenyl, 95% dimethylpolysiloxane) and dimensions (30m, 0.25mm ID, 0.25µm). The oven temperature was programmed at 50°C for 2 min, then at 7°C/min up to 250°C, and then left at 250°C for 5 min. The split/splitless injector temperature was 250°C (split ratio: 1/50). The carrier gas was helium (purity 99.9995%) with a flow rate of 1 ml/min. The mass spectrometer conditions were as follows: The ionisation energy was 70 eV, the ionisation source temperature was 200°C, the ionisation mode was EI electron impact and the mass range was 35 to 500 m/z. Compounds were identified by comparing their mass spectra with those recorded in the NIST 11s library.

Mite rearing

Date palm fruits (Deglet noor) heavily infested by *O. afrasiaticus* were collected from the oases of dawia El-Oued, Algeria on 22 June 2021. After the collection of all stages of spider mites, a reserve colony was started. Females and males of *O. afrasiaticus* were selected and placed with fruits of the Deglet noor date variety to lay eggs in rearing units at the Biodiversity and Environmental Protection Laboratory, Faculty of Life Sciences, Elchahid Hamma Lakhdar University. The eggs were kept until they reached the adult stage and were used for the experiments in this study. This was done to ensure that the female mites used in the experiments were of the same age. All colonies were maintained in a room at a temperature of $25 \pm 1^\circ\text{C}$ and relative humidity of $60 \pm 10\%$ RH, and photoperiod as follows: 16 h light and 8 h dark (L:D).

Preparing essential oils doses

For each EO, the doses to be tested were prepared by dilution in distilled water with Tween 80 and are as follows: 200, 100, 50, 25 and 10 µl/ml. The negative controls are distilled water and Tween 80.

Toxicity of essential oils against *O. afrasiaticus* females

For each EO concentration, five leaf discs were prepared. Untreated date palm leaf discs (7.5 cm long × 4 cm wide) were dipped in each concentration of EO allowing the surfaces of the discs to be uniformly filled with tiny droplets. The control leaf discs were dipped in 0.05% Tween 80 (Hussain et al., 2020). The soaked leaf discs were air-dried for 25 min. Ten fertilised adult females of *O. afrasiaticus* were placed on a single disc using a brush, which was then placed on moist filter paper in a transparent plastic box (10 cm × 5 cm). These leaf discs were surrounded by moist cotton. Each dish contained five discs as replicates. Dead and surviving female mites were counted under binocular loupe after 4, 8, 12, 24, 48 and 96 hours after treatments. Mites were considered dead if they did not walk or move at all after pricking the body with the tip of a brush.

Statistical analysis

The corrected mortality was calculated using the Abbott formula (Abbott, 1925). Mortality data were standardised. *O. afrasiaticus* mortality was analysed by analysis of variance (ANOVA) and comparison of means by Tukey's HSD test. LC50, LT50, and their 95% confidence limits were calculated using probit analysis (Finney, 1971) using the SPSS 22.1 software.

Results

Chemical composition of essential oils

The chemical components of the EOs, their percentage and retention time are listed in Table 2. The major compounds identified in *L. camara* EO were caryophyllene (24.33%), Humulene (26.65%) and Muurolene (14.22%). Few other minor compounds such as γ -Elemene (4.68%), β -Pinene (3.19%), α -Farnesene (3.06%), α -Cedrene (2.31%) and α -Zingiberene (2.30%) and Copaene (2.16%) were also identified in considerable amounts. Of the total compounds identified, 8.57% monoterpenes and 88.95% sesquiterpenes were found in the EO of *L. camara* leaves. For *R. Chalepensis* EO, the main components identified were 2-Undecanone (50.52%), 2-Nonanone (11.27%) and 2-Octanol, acetate (9.17%). The EO of *R. Chalepensis* contains mainly hydrocarbon ketones (73.91%).

Table 2
The chemical components of the two essential oils *Lantana Camara* and *Ruta chalepensis*

Compounds	Retention Time (min)	<i>Lantana camara</i>	<i>Ruta Chalepensis</i>
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
Aceticacid,octylester	13.3	-	0.18
2-Octanol,acetate	13.89	-	9.17
2-Undecanone	15.28	-	50.52
2-Undecanol	15.32	-	0.25
Aceticacid,heptylester	15.46	-	1.08
6-Dodecanone	16.6	-	2.85
Aceticacid,nonylester	16.73	-	0.13
Copaene	16.97	2.16	-
2-Propenoicacid,3-phenyl-,methylester	17.05	-	0.36
2-Dodecanone	17.19	-	2.69
β -Elemene	17.28	1.93	-

Compounds	Retention Time (min)	<i>Lantana camara</i>	<i>Ruta Chalepensis</i>
5-Tetradecanolacetate	17.89	-	3.27
Caryophyllene	17.99	26.33	-
cis- β -Farnesene	18.46	1.75	-
Humulene	18.67	26.65	-
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-Hexadecanoicacid	26.9	-	0.42
3,4-Methylenedioxyphenylacetone	28.12	-	2.88
Phytol	29.03	1.4	0.34
Monoterpenes		8.57	-
Hydrocarbon		0.11	2.14
Sesquiterpene		88.95	1.50
Ketone		-	73.91
Esterification		-	14.22
Acid		-	0.42
Alcohol		1.40	0.59
Aldehyde		-	0.11
Aromatic compound		-	8.03

Acaricidal effect of essential oils

The acaricidal activity of the EOs *L. Camara* and *R. Chalepensis* was assessed by contact against dead adult females of *O. afrasiaticus*, as shown in Fig. 2. The results of the study show that the corrected mortality of adult *O. afrasiaticus* females increased significantly with increasing doses and duration of exposure to these two EOs *L. Camara* ($F = 34.394$; $P < 0.0001$) and *R. Chalepensis* ($F = 18.801$; $P < 0.0001$). *L. Camara* EO caused 95.83% mortality after 96 h of contact exposure at a concentration of 200 $\mu\text{l/l}$ (Fig. 1a). At the same concentration, *R. chalepensis* EO caused 97.91% mortality after 96 h of exposure (Fig. 1b).

Adult females of *O. afrasiaticus* were more sensitive to the effect of *R. Chalepensis* EO than to *L. Camara* EO. The LC50 recorded under the effect of these two oils were 3.413 and 9.813 $\mu\text{l/l}$ respectively. This mite showed greater tolerance to *L. camara* EO, recording the highest LC50 (Table 3).

Table 3
The lethal concentration of the two essential oils *Lantana Camara* and *Ruta Chalepensis* after 96 hours of exposure to adult females of *O. afrasiaticus*

Essential oils	LC50 (95% CL)	Probit equation	Linear Slope (SE)	χ^2 (df)
<i>Lantana camara</i>	9.813 (4.565 ; 15.138)	$Y = 1.263 \log C - 1.253$	-1.253 (0.352)	2.397 (3)
<i>Ruta chalepensis</i>	3.413 (0.336 ; 8.068)	$Y = 0.911 \log C - 0.486$	-0.486 (0.363)	0.437 (3)

CL confidence limit that has been calculated with 95% confidence

The most effective HE recorded the lowest TL50. The EO of *L. camara* had the lowest TL50 (TL50 = 1.951 h) at the concentration of 200 $\mu\text{l/ml}$, followed by *R. chalepensis* representing the TL50 equal to 3.854 h (Table 4). In addition, the TL50 becomes lower the concentration.

Table 4
Lethal times (hours) of the two essential oils *Lantana Camara* and *Ruta Chalepensis* for different concentrations against adult females of *O. afrasiaticus*

Treatments	Concentraion (μ l/ml)	LT ₅₀ (h)	Fiducial limits (95%)	Chi-square
<i>Lantana camara</i>	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
	100	2.975	0.804–5.460	0.744
	200	1.951	0.320–4.100	3.232
<i>Ruta chalepensis</i>	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
	100	4.061	1.827–6.376	2.587
	200	3.854	2.152–5.506	0.114

Discussion

Analysis of the EOs identified 26 constituents for the EO of *L. camara* and 23 constituents for the EO of *R. chalepensis*. In our study, we found that the EO of *L. camara* is composed of caryophyllene, humulene and muurolene. Similarly, the hydrodistilled EOs of *L. camara* are dominated by sesquiterpenes such as (E)- β -caryophyllene and α -humulene (Nea et al., 2020). In another study, twenty-two compounds were detected accounting for 88.97% of the total percentage of *L. camara* EO. The main compounds identified in *L. camara* leaf EO were β -caryophyllene (24.96%) and δ -selinene (17.46%) (Sarma et al., 2020). According to (De Sousa et al., 2020), the EO of *L. camara* was mainly composed of Bicyclogermacrene, isocaryophyllene, valencene and D-germacrene. On the other hand, the predominant compound in the EO of *R. chalepensis* is 2-undecanone (50.52%). A similar study on the EO of dried aerial parts of *R. chalepensis* harvested in northern Tunisia showed the presence of 13 major compounds: 2-undecanone (77.18%), 2-decanone (8.96%) and 2-dodecanone (2.37%). The 2-undecanone was found to be the sole component (100%) of the EO obtained from *R. chalepensis* flowers (Mejri et al., 2010). Likewise, revealed that leaf and flower EO samples from *R. chalepensis* were dominated by ketone, in particular the 2-undecanone derivative, accounting for 85.94 and 89.89% of leaf and flower oils respectively. In addition, the EO of *R. chalepensis* L. from the Oulmès region (Central Plateau of Morocco) obtained was characterized by the dominance of 2-undecanone (64.35%) and piperonyl piperazine (11.9%) (Najem et al., 2020). This difference in the composition of the two EOs can be influenced by several factors. The chemical profile of the EO is influenced by edaphic and climatic factors, as well as by the period and

stage of harvesting, and depends on the extraction and analysis techniques used (Bammou et al., 2016). Harvesting stages led to different variations in all the chemical classes of EO (Khalid, 2019).

In our study, the EOs of *L. camara* and *R. chalepensis* showed a significant effect on the mortality of adult females of *O. afrasiaticus* compared with untreated controls. The corrected mortality of this mite increased as the concentration of EO increased. The acaricidal effect observed with these two EOs on adult females of *O. afrasiaticus* was attributed to the presence of active chemical compounds in these plants or to the major compounds. This was indicated by (Bakkali et al., 2008) that the biological properties of EOs are generally determined by their main components. Similar studies on these two EOs have shown their effectiveness against several pests. The EO of *L. camara* was effective in the mortality of the maize grain weevil *Sitophilus zeamais* (Coleoptera, Curculionidae) (Bouda et al., 2001). Aussi cette huile a une activité adulticide contre différentes espèces de moustiques (Dua et al., 2010). Otherwise, the methanol extract of *L. camara* leaves has a fumigation and contact toxicity against *Sitophilus oryzae* (L.) *Callosobruchus chinensis* (Fab.) et *Tribolium castaneum* (Herbst.) (Rajashekar et al., 2013). In addition, *L. camara* caused contact toxicity and repellent activity of the EO against the 2nd instar larvae of the tomato leaf miner *Tuta absoluta* (Liambila et al., 2021). A study has shown that *R. chalepensis* EO has an appreciable larvicidal effect on contact against third and fourth stage larvae of *Orgyia trigotephras* (Akkari et al., 2015). This EO of *R. chalepensis* is highly repellent to *Tribolium castaneum* Herbst, largely due to its majority compound 2-undecanone (Najem et al., 2020). The acaricidal effect of the *Ruta* genus observed in the present study is similar to that reported for other mite species. One study showed that *R. graveolens* L. leaf EO was toxic by fumigation against eggs and adult females of *Tetranychys urticae* (Acari: Tetranychidae). In addition to the mortality of adult females, this EO caused a significant reduction in the fecundity of these females (Kalmosh et al., 2019). Other studies on EOs of three plant species *Thymus vulgaris* L., *Salvia mirzayanii* Rech. F. & Esfand and *Trachyspermum ammi* L. tested against adults of *O. afrasiaticus* showed desirable acaricidal activity against the pests under laboratory conditions (Sohrabi and Kohanmoo, 2017)

Conclusion

The EOs of *L. camara* and *R. chalpensis* demonstrated a significant acaricidal effect on adult females of *O. afrasiaticus*. This acaricidal activity against *O. afrasiaticus* is linked to the high concentration of Sesquiterpene for *L. camara* and Ketone for *R. chalpensis*. These results suggest that these plants could be used as a viable alternative for the control of the date palm mite. However, the search for other alternatives to control *O. afrasiaticus* with less cost to human and environmental health is necessary. For example, entomopathogenic fungi that are effective in reducing the spider mite *T. urticae* (Chouikhi et al., 2022) could be used successfully in future integrated pest management programs against *O. afrasiaticus* in the Tunisian and Algerian palm groves.

Abbreviations

EO: Essential oil

ANOVA: Analysis of variance

P: P-value

LC: Lethal concentration

LT: Lethal time

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not Applicable

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Conceived and designed the experiments: HG and MSB. Performed the experiments: HG, SC and BHA. Analyzed the data: HG, SC, BHA and MSB. Wrote the paper: HG, SC, KLG, and MSB. All authors read and approved the final manuscript.

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Availability of data and materials

All data of the study have been presented in the manuscript, and high quality and grade materials were used in this study.

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Figures

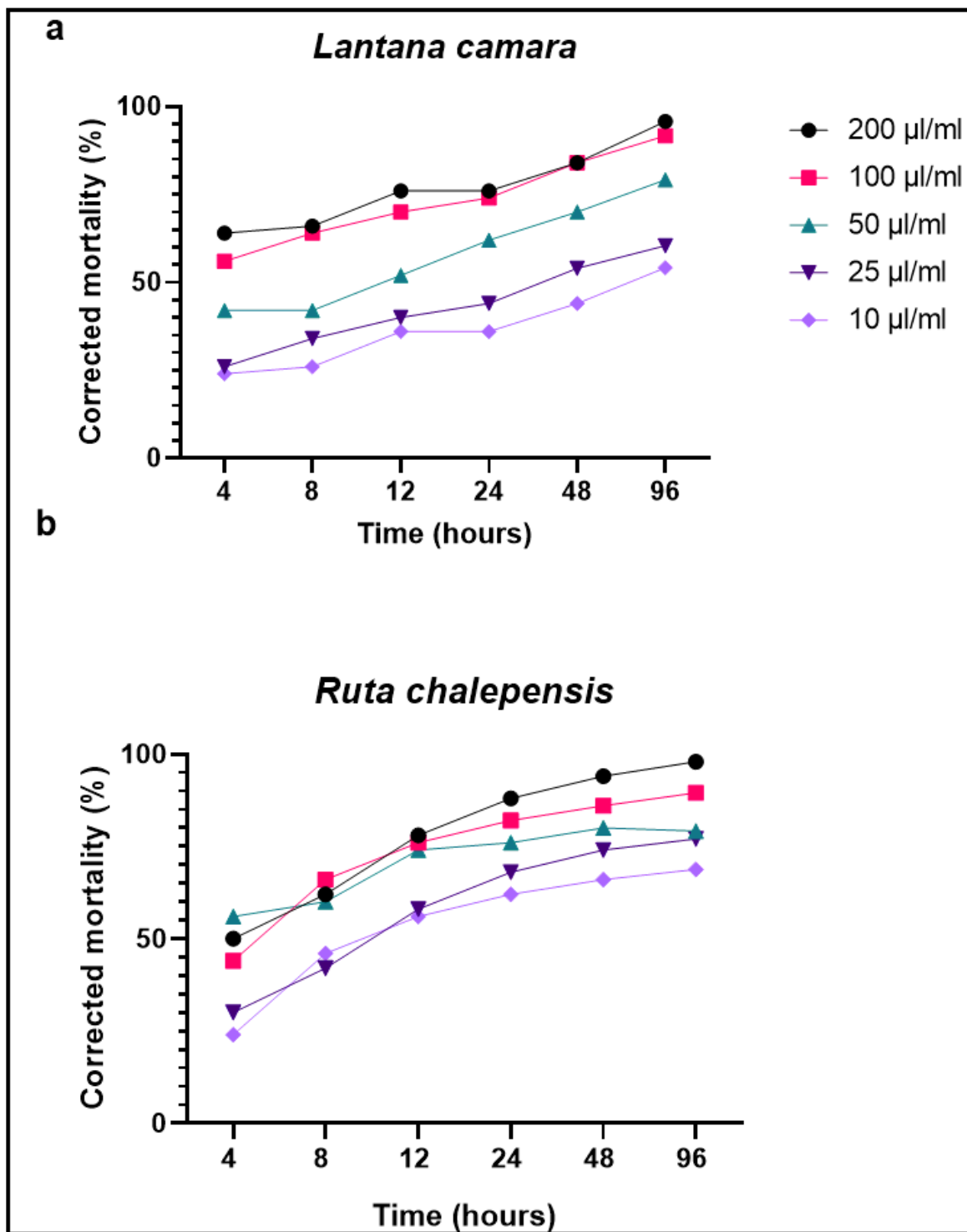


Figure 1

Corrected mortality of adult females of *Oligonychus afrasiaticus* at different concentrations of essential oils; a: *Lantana Camara*, b: *Ruta Chalepensis*