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التركيب الكيميائي والنشاط المضاد للأكاروسات زيت اللافندر *Lavandula officinalis* على أكاروس العنكبوت الأحمر ذو البقعتين *Tetranychus urtica*

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الملخص العربي:

الزيوت النباتية الأساسية **(EOs)** تكون مصدرا بديلا لمكافحة العنكبوت الأحمر العادى ذو البقعتين ***Tetranychus urticae*** **(Acari : Tetranychidae) لأنها تشكل مصدرا غنيا للمواد الكيميائية النشطة بيولوجيا. لذا ، هناك حاجة ملحة لتطوير بدائل أكثر أمنا وكفاءة لديها القدرة على أن تحل محل المبيدات المصنعة. فقد تم عمل توصيف للزيت النباتي الأساسي EO الذي تم استخلاصها من الأجزاء النباتية لنبات اللافندر Lavandula تم دراسة تأثيره على الأطوار الكاملة للعنكبوت الأحمر العادى ذو البقعتين *T. urticae* تحت الظروف المعملية ، وبواسطة تحليلGC/MS كانت المكونات الرئيسية للزيت هي مركب linalool (٣٨.٨٥ ٪) ، nerol (٣٢.٢٤ %) ، camphor (١١.٣٠%) ، 1,8-cineole (٧.٦٤%) ، كانت LC_{50} لزيت اللافندر ٢٤.٥٦ ، ١٧.٢٧ و ١٥.٣١ مل/ لتر بعد فترات تعريض كانت ٢٤ ، ٤٨ ، ٧٢ ساعة على التوالي . وأشارت نتائج البحث إلى أن الزيوت النباتية الأساسية أظهرت تأثيرا كبير ضد العنكبوت الأحمر وعند تركيزات مختلفة لم يكن لها سمية نباتية. وأشارت نتائج التقييم المعملية أن الزيت النباتي الأساسي يحتوى على بعض المركبات ذات تأثير فعال جدا وطاردة ضد الأطوار الكاملة للعنكبوت الأحمر *T. urticae* ويمكن أن يعزى هذا التأثير الملحوظ في دراستنا لاحتواء نبات اللافندر لبعض المركبات الرئيسية الفعالة.**

COMPOSITION AND ACARICIDAL ACTIVITIES OF *Lavandula officinalis* ESSENTIAL OIL AGAINST *Tetranychus urticae* (ACARI: TETRANYCHIDAE)

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ABSTRACT: ***Plant essential oils (EOs) may be an alternative source of materials for two-spotted spider mite Tetranychus urticae Koch (Acari: Tetranychidae) control because it constitute a rich source of bioactive chemicals. The EO from aerial parts of lavender Lavandula officinalis were characterized by GC/MS analysis and investigated for its acaricidal activities against the adults of T. urticae under laboratory conditions. The main constituents of the oil were linalool (38.85%), nerol (32.24%), camphor (11.30%) and 1,8-cineole (7.64%). The LC_{50} of lavender oil were 24.56, 17.27 and 15.31 ml/L after exposure periods of 24, 48, and 72 hr respectively. The obtain results indicated that the essential oil exhibited a great potential acaricidal effect against two-spotted spider mite at different concentrations that are not phytotoxic to the host plant. Laboratory bioassay results indicated that the essential oil caused high fumigant and repellent activities against T. urticae. The acaricidal observed activity of L. officinalis in this study may be attributed to the major plant constituents.***

Key words: ***Lavandula officinalis, Essential oil, Acaricidal activity, Tetranychus urticae, GC/MS analysis, Chemical composition***

INTRODUCTION

Over the past decades, plants EOs were widely used against phytophagous pests and ticks (Iori *et al.*, 2005) and mites (Kim *et al.,* 2004). Aromatic plants are among the most efficient pesticides of botanical origin and essential oils often constitute the bioactive fraction of plant extracts (Cosimi *et al.,* 2009). Among bioactive natural compounds, several plant essential oils (Calmasur *et al.,* 2006) were evaluated as acaricides. Moreover, essential oils have a broad spectrum on insect and mite activity due to their several modes of action, including repellent and antifeedant activities, inhibition of moulting and respiration and reduction in growth and fecundity (Enan, 2001; Akhtar and Isman, 2004). Beside native wild species, several medicinal plants are cultivated in Egypt such as *Lavandula officinalis*, it is belong to family Lamiaceae, and it is also known as common or English lavender, native to the western Mediterranean region (Chevallier, 1996). Lavender EO has several medicinal properties and biological activities (Bown, 1995). Its powerful antiseptic properties are able to kill many common microorganisms; it also has antiviral properties and is a powerful antidote to some snake venoms (Phillips and Foy, 1990). The oils are generally composed of complex mixtures of monoterpenes, biogenetically related phenols, and sesquiterpenes. Natural products have been used as templates for semi-synthetic acaricidal agents (Tsukamoto *et al.*, 1997a, b). The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most serious pests in some of agricultural systems (Deligeorgidis *et al.,* 2006, 2007). It ingests leaf cell contents, thus reduces plant photosynthesis (Park and Lee, 2002) and potentially decreases yield quality and quantity (Flaherty and Wilson, 1999). These pests are commonly

controlled by applications of synthetic acaricides (Pontes *et al.,* 2007). Unfortunately, spider mites have developed resistance to most available pesticides and the weakness of acaricidal efficacy is considered result of resistance mite populations in the major problem encountered (Ay, 2005). Alternatives to conventional fumigants and acaricides are needed, because many are being banned from the market. The development of natural or biological pesticides will help to decrease the negative effects (residues, resistance and environmental pollution) of synthetic pesticides.

The aim of this work was to evaluate the acaricidal activities of the EO of *L. officinalis* on two-spotted spider mite *T. urticae* by determine of its LC_{50} under laboratory conditions and recognize the chemical composition of the EO of *L. officinalis.*

MATERIALS AND METHODS

1. Plant material and extraction of the essential oil

Fresh aerial parts of avender, *Lavandula officinalis* were collected during the flowering period of 2010 season from the experimental farm of National Research Center (NRC), Dokki, Giza, Egypt. Collected parts were shaded for 7-9 days at room temperature (25±2°C) until crisp. The EO of lavender was extracted by hydro-distillation using a Clevenger-type apparatus for 3 h as described by Negahban *et al.,* 2006. The oily layer obtained on top of the aqueous distillate was separated and dried with anhydrous sodium sulfate (0.5 g). The extracted EO was kept in sealed airtight glass vials and covered with aluminum foil and stored at 5°C until further analysis.

2. GC/MS analysis of the essential oil

Major constituents of the lavender EO were identified by gas chromatography /mass spectrometry (GC/MS) using a Shimadzu GC-17A gas chromatograph (Shimadzu Corp., Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector (GC-MS QP-5050A). The GC-MS system was equipped with a TRACSIL Meta X5 column (Teknokroma S. Coop. C. Ltd., Barcelona, Spain; 30 m × 0.25 mm i.d., 0.25 µm film thickness). Analyses were carried out according to methods described by Abd-Elhady *et al*., 2011. The identification of individual compounds of essential oil was accomplished using two different analytical methods: (a) KI, Kovats indices in reference to *n*-alkanes (C_8-C_{32}) by National Institute of Standards and Technology (NIST) 2009; and (b) mass spectra (authentic chemicals and Wiley spectral library collection). Identification was considered to be tentative when it was based on mass spectral data only. The relative concentration of each component of the essential oil was quantified according to the peak area integrated by the analysis program.

3. Acaricidal activity

3.1. Origin and rearing of mite

The strain of mite was established from field collections of spider mite *T. urticae* from Sakha Agricultural Research Station, Egypt. Mite cultures were maintained in climatic rooms at 27± 1° C and 60 ± 5 % R.H., with a 16:8 h L: D photoperiod. Spider mites were reared on bean plants (*Phaseolus vulgaris* L.), a more suitable host using Dittrich (1962) technique.

3.2. Toxicity tests

LC_{50} for adult females of *T. urticae:* Leaves of bean plants, *P. vulgaris* L., were punched for preparing leaf discs (3.0 cm diameter). Leaf discs were placed on wet cotton pads in Petri dishes (12 cm diameter). Eight concentrations (from 1.25 to 100.0 ml /L water) of EO, each with nine replicates were prepared using tap water plus the spreader (Tween20, 0.5%). Tested concentrations were sprayed on leaf discs with aid of hand sprayer. Control treatments were made using tap water and spreader only. After shadow

drying of leaf discs, ten adult females of *T. urticae* were transferred to the lower surface of each treated leaf disc. Mortality percentages were calculated after 24, 48 and 72 h from exposing *T. urticae* individuals to treated leaf discs. A slight touch on the mite with a fine haired brush, if they looked black and can not move they considered dead.

Repellent activity: To study the preference response of *T. urticae* when given a choice between EO-treated and untreated plants, the deterrent activity of lavender oil at concentrations from 0.20% to 2.00% against *T. urticae* was assessed. Bean leaves were cut into discs (3.0 cm diameter) of symmetrical portion along the midrib obtained per each disc. Half of each disc was sprayed with tested concentrations, while the other half was sprayed with water plus the spreader (Tween20, 0.5%) as check. The treated discs were left to dry and put on moistened cotton wool in Petri dishes (10.0 cm diameter). Twenty adult females were transferred on the midrib of each disc. Three discs were used as replicates for each concentration. The mites left to move freely across the two portions of the disc. *T. urticae* individuals in each portion were counted after 12, 24 and 48h. The number of eggs laid on both portions was recorded after 48 h. The deterrence index (DI) was calculated according to Pascual and Robledo, 1998 using the equation:

DI = [(C – T) / (C + T)] x 100

Where: T and C represent the mean number of adult female of the treated and control, respectively.

Fumigant activity: The method used to evaluate the fumigant activity of the oil was adapted from Aslan *et al.*, 2004. Glass jars receptacles with a capacity of 3.0 L were used as test chambers. One Petri dish (7.5 cm diameter) was introduced to each chamber. Thirty adult females of *T. urticae*, 10 mites in each leaf disc (2.5 cm diameter) of bean, were put in each Petri dish. Six discs were used as a replicates for each concentration. Filter paper discs saturated with water were used under the leaf discs. Each Petri dish was brought into the glass recipient. The amounts of essential oil without using any solvent applied on Whatman filter paper pieces (2 cm × 3 cm), fixed on the inner surface of the glass recipient, by an automatic pipette were 0.50, 1.00, 2.00, 3.00, 4.00, 5.00, 6.00 and 7.00 ml in each test jar chamber, corresponding to 0.17, 0.33, 0.67, 1.00, 1.33, 1.67, 2.00 and 2.33 ml/L of air, respectively. No material was applied to the control glass recipient. The treatments were kept in a holding chamber of 27 ± 1°C and 60 ± 5 % R.H., with a 16:8 h L: D photoperiod. Exposure periods were 24, 48 and 72 h and the numbers of dead adults were counted daily in every experiment.

4. Phytotoxic effects

Phytotoxicity of the lavender essential oil on greenhouse-grown cotton, bean, tomato and cucumber as host plants with four weeks old were determined. The highest concentration of the essential oil used in the experiment (10%) was dissolved in water plus the spreader (Tween 20, 0.5%) solution. These emulsions (10 ml for each plant) were sprayed uniformly with a hand sprayer on the surface of whole plant leaves. Each plant in control groups was sprayed uniformly with 10 ml of water plus a spreader (Tween 20, 0.5%) solution. The differences in the appearance of treated plants compared with controls were considered as indication of phytotoxicity.

5. Data analysis

The mortality percentage was corrected using Abbott's formula 1925; the observed data were then analyzed by probit analysis PC (Finney, 1971). Means of *T. urticae*t mite numbers among treatments were calculated and compared with a single factor analysis of variance (ANOVA). Duncan's multiple range test was used to determine significant differences ($p < 0.05$) between treatments by CoStat system for Windows, version 6.311, CoHort Software (2006), Berkeley,CA, USA).

RESULTS

1. Essential oil constituents

Lavender oil is extracted from the dried aerial parts by hydrodistillaton and yield 1.3% (w/w). GC/MS analysis indicated that there are twenty-one compounds, representing 98.90% of the essential oil; their retention indices and percentage composition, listed in order of elution in the column, are given in Table (1).

The major components in aerial parts of *L. officinalis* essential oil were identified as linalool (38.85%), nerol (32.24%), camphor (11.30%) and 1,8-cineole (7.64%). The essential oil of *L. officinalis* was rich in monoterpenoids which may provide the acaricidal properties of this oil against *T. urticae*.

2. Acaricidal activity

LC_{50} values of the oil: Mortality results of *T. urticae* were shown in (Table (2) along with their confidence limits (CL). The LC_{50} of lavender EO was 24.56 ml/L (95% CL = 20.72 – 29.36) for adult females of *T. urticae* after 24 h. The rate of mortality was directly proportional to concentration.

Repellent activity: Results in Table (3) showed that adult females of *T. urticae* preferred the untreated section of the leaves to feed and deposit eggs.

Table 1. Chemical composition of essential oil isolated by hydrodistillation from aerial parts of *Lavandula officinalis* analyzed by GC/MS

Number	RI [a]	Compound [b]	Peak Area (%) [c]
1	9.321	alpha pinene	0.27
2	10.716	beta pinene	0.56
3	12.172	para cymene	0.21
4	12.344	1-limonene	0.84
5	12.572	1,8 cineol	7.64
6	14.768	Plinol A	0.13
7	15.029	Linalool	38.85
8	15.621	alpha-pinene oxide	0.15
9	16.349	Plinol C	0.50
10	17.531	Camphor	11.30
11	18.807	Terpineol-4	0.72
12	19.43	1-alpha-terpineol	0.91
13	20.273	Linalyl formate	0.11
14	21.637	Nerol	32.24
15	23.058	Lavandulyl acetate	0.39
16	23.333	Dihydrocarveol	0.15
17	26.621	Neryl acetate	0.30
18	27.535	Geranyl acetate	0.54
19	30.112	trans-caryophyllene	2.77
20	31.025	beta-farnesene	0.16
21	31.739	alpha-humulene	0.16
		Total	98.90

[a] RI, retention index on a TRACSIL Meta X5 column
[b] Compounds are listed into order of their elution from a TRACSIL Meta X5 column
[c] Compound percentage

Table 2. Acaricidal activity of extracted essential oil from *Lavandula officinalis* against adult females of *Tetranychus urticae*

Treatment	LC_{50} * (ml/L)	95% CL	Slope ± SE
24 h	24.56 a	(20.72 – 29.36)	1.4580± 0.0954
48 h	17.27 b	(14.55 – 20.54)	1.4328± 0.0942
72 h	15.31 b	(12.91 – 18.17)	1.4409± 0.0939

* LC_{50} values followed by the same letter in column are not significantly different (95% CL do not overlap).

Table 3. Repellency effect of extracted essential oil from *Lavandula officinalis* on adult females of *Tetranychus urticae*

Period of observation	Conc. (%)	DI	Average number of laid eggs	
			Treatment	Control
12 h	0.20	50.00	n.d.	n.d.
	0.60	56.57	n.d.	n.d.
	1.00	56.57	n.d.	n.d.
	1.20	63.33	n.d.	n.d.
	1.60	66.67	n.d.	n.d.
	2.00	73.33	n.d.	n.d.
24 h	0.20	66.67	n.d.	n.d.
	0.60	73.33	n.d.	n.d.
	1.00	76.67	n.d.	n.d.
	1.20	87.67	n.d.	n.d.
	1.60	93.33	n.d.	n.d.
	2.00	100.00	n.d.	n.d.
48 h	0.20	80.00	3.6 d	5.6
	0.60	83.33	2.2 c	6.8
	1.00	90.00	2.1c	6.5
	1.20	97.67	1.3 b	8.0
	1.60	100.00	1.0 b	9.1
	2.00	100.00	0.0 a	9.8

Values followed by the same letter within the column are not significantly, n.d., not determined.

At the six concentrations of *L. officinalis* essential oil (0.20 to 2.00%) and after 12 h of exposure, low numbers of *T. urticae* were recorded on the treated section. The above concentrations were found to be highly repellent to adult females of *T. utricae.* Females of the mite showed an ovipostion preference for residue-free substrated where the mean number of laid eggs on the water treated control halves of the leaf discs were higher than that on the EO treated halves (Table 3).

Fumigant effect: The LD_{50} values, 95% confidence limits and the regression line are shown in Table 4. The essential oil of lavender has a marked acaricidal activity against mite adults. LD_{50} of the essential oil of lavender were 1.7608 ml/L of air after 24 h of treatment. The LD_{50} value was calculated as 1.0227 and 0.8949 ml/L of air with 95% confidence limits from 0.7731 to 1.3484 and 0.6726 to 1.1537 ml/L of air after 48 and 72 h of treatment, resp.

3. Phytotoxic effects

Phytotoxicity tests were performed on foliage of some host plant species of *T. urticae*. No sign of phytotoxicity was found among the tested host plants at the highest concentration (10%) used in the experiments.

DISCUSSION

1. Essential oil constituents

Like other essential oils, natural *L. officinalis* oil is a complex mixture of terpenoids. Considering that target site resistance is an important problem for mite control, it is more probable that mites will evolve resistance faster to an acaricide based on a single active ingredient than to one based on a mixture of different active compounds. 1,8-cineole was one of the most abundant compound, this compound have activity against a variety of insects, mites, weeds and plant pathogens (Bakkali *et al*., 2008; Koschier, 2008). The essential oils and their major components consisting of 1,8-cineole and camphor were found to be more toxic against *Sitophilus gra*narius (Obeng-Ofori and Reichmuth, 1999; Obeng-Ofori *et al.,* 1998). The primary components of lavender EO are linalool (51%) and linalyl acetate (35%) (Prashar *et al*., 2004). In a study by Perrucci *et al*. (1998) the acaricidal activities of *Lavandula angustifolia* EO and linalool (i.e. its main component), were investigated against the mite, *Psoroptes cuniculi*. Both the EO and linalool exhibited acaricidal properties and linalool was detected in the ether extract of treated mites. The acaricidal activity of *L. officinalis* against *T. urticae* observed in the study might thus be attributed to linalool as the major plant constituents.

2. Lavender oil as an acaricide

The obtained results clearly reported that essential oil from *L. officinalis* showes acaricidal activity against the *T. urticae* adults. These results are confirmed with that of Lis-Balchin and Hart, 1999; Pirali-Kheirabadi and Razzaghi-Abyaneh, 2007 who found that lavender EO geranium and peppermint oils, have acaricidal activity. Plant extracts contain compounds that show ovicidal, repellent, antifeedant, sterilization and toxic effects in insects (Isman, 2006). The results showed that the EO proved to be a high repellent to *T. urticae* females with exposure concentrations from 0.2 - 2.0%. However, synergistic phenomena between the diverse components of the EO may result in a higher bioactivity (an increased repellent response) of the oil as a whole compared to its isolated components (Hori, 2003). Comparisons of the principal components of some EOs suggest that camphor was the main component responsible for the repellent effects (Gillij *et al.,* 2008).

Table 4. Fumigant effect of extracted essential oil from *Lavandula officinalis* against adult females of *Tetranychus urticae*

Exposure period	LD_{50}^{*} (ml/L)	95% CL	Regression equation
24 h	1.7608 a	(1.4851 – 2.1925)	Y = 4.559 + 1.794X
48 h	1.0227 b	(0.7731– 1.3484)	Y = 4.9804 + 2.008X
72 h	0.8949 b	(0.6726 – 1.1537)	Y = 5.107 + 2.213X

* LD_{50} values followed by the same letter within each vertical column are not significantly different (95% CL do not overlap).

Plant essential oils and their constituents invariably have higher boiling points and such plant products that show insect toxicity in the vapour state have been recently reviewed by Rajendran and Sriranjini (2008). The effect of the essential oils on aphid mortality was attributed primarily to starvation or oral and fumigant toxicity (Hori, 1999). Although many studies have demonstrated the contact or volatile efficacy of essential oils, derived from *Rosmarinus officinalis، Lavandula officinalis, L. angustifolia, Artemisia absinthium* and *Thymus vulgaris,* against different mite species in the family Tetranychidae (Amer *et al*., 2001; Momen *et al*., 2001; Refaat *et al*., 2002; Choi *et al*., 2004; Miresmailli *et al*., 2006). Regnault-Roger and Hamraoui (1995) reported that oxygenated monoterpenoids (e.g. carvacrol, linalool and terpineol) are more toxic than non-oxygenated compounds (p-cymene, cinnamaldehyde, anethole) against *Acanthoscelides obtectus* adults. Houghton *et al.,* 2006 indicate that monoterpenoids cause insect mortality by inhibiting acetylcholinesterase enzyme activity.

3. Phytotoxic effects

Ibrahim *et al.* (2001) reported phytotoxicity in limonene-treated plants, and Chiasson *et al.* (2004) did not observe any phytotoxicity among lettuce, roses, and tomatoes that were treated with a *Chenopodium*-based pesticide. In this study no phytotoxic effects of the essential oil with the highest used concentration (10%) were observed on green house-grown cotton, bean, tomato and cucumber with four weeks old.

CONCLUSION

Our results suggested that the *L. officinalis* EO could be used in the control of *T. urticae,* and its content could lead to the development of new classes of acaricidal compounds. In addition, no phytotoxic effects of lavender oil to foliage of some host plants have been observed at high concentration (10%).

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التركيب الكيميائي والنشاط المضاد للأكاروسات لزيت اللافندر *Lavandula officinalis* على أكاروس العنكبوت الأحمر ذو البقعتين *Tetranychus urtica*

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الملخص العربي:

الزيوت النباتية الأساسية (EOs) تكون مصدرا بديلا لمكافحة العنكبوت الأحمر العادى ذو البقعتين *Tetranychus urticae* (Acari : Tetranychidae) لأنها تشكل مصدرا غنيا للمواد الكيميائية النشطة بيولوجيا. لذا ، هناك حاجة ملحة لتطوير بدائل أكثر أمنا وكفاءة لديها القدرة على أن تحل محل المبيدات المصنعة. فقد تم عمل توصيف للزيت النباتي الأساسي EO الذي تم استخلاصها من الأجزاء النباتية لنبات اللافندر Lavandula تم دراسة تأثيره على الأطوار الكاملة للعنكبوت الأحمر العادى ذو البقعتين *T. urticae* تحت الظروف المعملية ، وبواسطة تحليلGC/MS كانت المكونات الرئيسية للزيت هي مركب linalool (٣٨.٨٥ ٪) ، nerol (٣٢.٢٤ %) ، camphor (١١.٣٠%) ، 1,8-cineole (٧.٦٤%) ، كانت LC_{50} لزيت اللافندر ٢٤.٥٦ ، ١٧.٢٧ و ١٥.٣١ مل/ لتر بعد فترات تعريض كانت ٢٤ ، ٤٨ ، ٧٢ ساعة على التوالي . وأشارت نتائج البحث إلى أن الزيوت النباتية الأساسية أظهرت تأثيرا كبير ضد العنكبوت الأحمر وعند تركيزات مختلفة لم يكن لها سمية نباتية. وأشارت نتائج التقييم المعملية أن الزيت النباتي الأساسي يحتوى على

بعض المركبات ذات تأثير فعال جدا وطاردة ضد الأطوار الكاملة للعنكبوت الأحمر *T. urticae* ويمكن أن يعزى هذا التأثير الملحوظ في دراستنا لاحتواء نبات اللافندر لبعض المركبات الرئيسية الفعالة.