Toxicity and Repellency Effects of Three Essential Oils against *Tetranychus urticae* Koch (Acari: Tetranychidae)

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ABSTRACT

Two-spotted spider mite, Tetranychus urticae Koch (Acari: Tetranychidae), is one of the most injurious pests of fruits, vegetables and ornamental plants worldwide, both outdoor and indoors. Currently the main method of control of this pest is through application of pesticides which is mostly accompanied by the resistance of the pest against pesticide(s). The resurgence of resistant mite populations brings about further contamination of foodstuff and environment. Essential oils obtained from the aerial parts of plants may have the potential to be an alternative to synthetic pesticides, since they have been demonstrated to posses a wide range of bioactivities against insects and mites. So, the aim of the current study was to investigate the effect of essential oils extracted from three different medicinal plants namely: Mentha longifolia, Salvia officialis (both Lamiaceae) and *Myrtus communis* (Myrtaceae) against T. urticae. The LC_{50} values of essential oils of M. longifolia, M. communis, and S. officialis against T. urticae were 20.08, 53.22, 60.93 µl L^{-1} air, respectively. This shows that *M. longifolia* possesses the highest lethal activity whereas S. officialis the lowest. Also, essential oils of M. longifolia, M. communis, and S. officialis were demonstrated to possess repellency effect with ED₅₀s of 147.47, 138.80 and 164.41, μ L⁻¹ air, respectively. These data suggest that essential oils of all the three plants have the potential to be employed in the pest management programs designed for a control of T. urticae under greenhouse conditions.

Keywords: Essential oils, Medicinal plants, Tetranychus urticae.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is one of the most serious pests of fruits, vegetables and ornamental plants worldwide (Johnson and Lyon, 1991). About 1,200 plant species of which more than 150 are economically important have been reported as the mite's host (Zhang *et al.*, 2003; Jeppson *et al.*, 1975). Development of resistance to pesticides has been widespread in its population mainly due to an irrational use of synthetic pesticides.

The increasing number of resistant insect and mite species to synthetic pesticides is associated with the use of chemicals that indiscriminately affect both natural enemies and pest itself. Spider mites have evolved resistant to more than 80 acaricidals to date, resistance having been reported from more than 60 countries worldwide (Elhag and Horn, 1983; Roush and McKenzie, 1987; Campos *et al.*, 1995; White, 1995).

In greenhouses conditions, their high reproductive potential along with short life cycle on the one hand, and frequent use of synthetic pesticides or acaricides on the other, result in rapid resistance to pesticides in the mite population (Sertkaya *et al.*, 2010). As reported in some areas, mite resistance to some pesticides has been

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recorded as more than 400-times (Wu *et al.*, 1990; Dagli and Tunc, 2001). As a result the efficacy of the pesticides or acaricides being reduced and the cost of chemical control continue to be on the increase. In addition, environmental pollution and food contamination by pesticides is the other scenario that needs serious concern and attention.

Essential oils extracted from aromatic plants have been widely investigated because they are deemed as potentially becoming the alternative to replace synthetic pesticides and because of also being more convenient to use. Many types of spices and herbs are known to possess anti insect and anti mite activity (Tripathi *et al.*, 1999) especially in the form of essential oils (Shaaya *et al.*, 1995).

Generally, essential oils are mostly non toxic to mammals, birds and fish (Isman, 2006). On the other hand, they act as broad spectrum pesticides, that may affect pests, their natural enemies and pollinators due to their several modes of action including repellency and antifeedant activity, disruption of molting and cuticle, as well as retardation of growth and fecundity (Cosimi et al., 2009; Sertkaya et al., 2010). It has also been reported that essential oils possess neurotoxic effects, evident from their rapid action against some insects and mites (Isman, 2006). Thus, there are evidences that plant essential oils affect octopamine pathways and GABA-Gated chloride ion channels (Sertkaya et al., 2010; Isman, 2006). Advantages of pesticides with a diverse mode of action are numerous including delay in resistance development among pests.

So far, the effect of plant essential oils on different insects and mites has been investigated using fumigant toxicity (Lee *et al.*, 2004; Ceferino *et al.*, 2006), repellency activity (Nerio *et al.*, 2009; Cosimi *et al.*, 2009), as well as antifeedant activity (Trigg and Hill, 1996; Chou *et al.*, 1997).

Essential oils derived from different plant species including Lavandula angustifolia ,L. latifolia; Lavandula. angustifolia; Melissa officinalis; Ocimum basilicum; Rosmarinus officinalis, Mentha piperita, Majorana hortensis, Ocimum basilicum, Lavandula officinalis, Origanum onites, Thymbra spicata, Lavandula stoechas have been tested against member/s of Tetranychidae family (Mansour et al., 1986; Momen et al., 2001; Refaat et al., 2002; Choi et al., 2004; Miresmailli et al., 2006; Sertkaya et al., 2010).

However, studies regarding the effect of plant essential oils on phytophagous mites, *T. urticae* are scarce. Thus, the aim of the current study was to investigate the effect of essential oils extracted from three plant species namely: *Mentha longifolia, Salvia officialis* (both Lamiaceae) and *Myrtus communis* (Myrtaceae) on adults of *Tetranychus urticae*.

MATERIALS AND METHODS

Rearing and Maintenance of Mite

Stock colony of mite was received from Plant Protection Department, Shiraz University. The colony had been reared on *Phaseolus vulgaris* at 24±3°C, and 45±60% relative humidity under natural daylight in greenhouse.

Plant Material and Essential Oil Extraction

Mentha longifolia, S. officialis and M. communis at their flowering stage were collected from throughout Shiraz Province, Iran. Essential oils were extracted, basically according to Aslan et al. (2004) and Cosimi et al. (2009). Briefly, aerial parts of plants were shade dried and then ground using a grinder of 2 mm diameter mesh. Plant materials were subjected to waterdistillation for 3 hours using a Clevenger apparatus. Following the essential oil being obtained, the oil was decanted from the water layer, dried through Na₂SO₄ application, and stored in sealed vials at 4°C before the tests being done. From each of the plant species about 500 g of plant aerial parts were taken, with the extracted essential oils obtained being 5, 4.5, and 3 ml for *Mentha longifolia, S. officialis* and *M. communis*, respectively.

Fumigant Toxicity Bioassay

Toxicity of essential oils on the twospotted spider mite was essentially done as based on Pascual-Villalobos and Robledo (1998). Briefly, glass Petri plates (90×20 mm) were used as a chamber for the determination of test materials on the mite.

For determination of LD₅₀, ten adults (a random selection of both sexes) of the same age (1-48 hour of age) from the stock colonies were transferred onto excised bean leaves (2 cm diam.) placed with its dorsal side on four layers of wet (saturated with distilled water) filter paper in a Petri dish using a soft paint brush, and allowed to settle for half an hour before being exposed to the essential oil. The method of application of the essential oil was based on Soylu et al. (2006). To prevent a direct contact between the mites and the tested oils, the desired oil quantities were applied on filter paper (5×2 cm) fixed on the inner surface of the Petri dish. Preliminary tests were done to choose the right doses. Each filter paper received 0.5, 2, 3.5, 5, 6.5, or 8 µl of essential oils using micropipette, which corresponds to 9.95, 39.81, 69.67, 99.59, 129.38, 159.24 μ l L⁻¹ air. Plates were then sealed with parafilm to prevent any loss of essential oils. Each concentration (treatment) was replicated five times with each replicate consisting of 10 adult mites. The control consisted of a similar setup but without essential oils. Mortality was recorded after 24 hours past from exposure. Mites incapable of moving after a slight touch with a fine brush were considered as dead.

For determination of LT_{50} , adult mite was exposed to 39.81, 69.67, and 99.59 µl L⁻¹ air. Then the mortality was recorded at 6, 12, 18, 24 hours past from exposure. In LT_{50} assays, each concentration was replicated three times each with 20 adult mites.

Repellency

The repellency assay was done according to Al-Jaber (2006). Y-tube olfactometer bioassay has been used for the testing of the repellency effect. It consists of a glass tube with a main arm (the stem) along with two other arms one containing the repellent and the other the control. The essential oil sample and the control are placed on P. vulgaris leaf disc (2 cm diam.). Two polyethylene tubes containing the treated and non-treated leaves of P. vulgaris were placed one at each end. A group of 20 adults of T. urtica was placed in the test area between the two joined tubes. Then, the upper end was closed with a piece of muslin cloth and by the aid of a rubber band. After 24 hours past, the number of mites in each tube were counted and repellency index (Pascual–Villalobos and Robledo, 1998) calculated as $RI = (C - T/C + T) \times 100$

Where:

C= The number of insect/mites on control diet.

T = The number of insect/mites on treated died.

RI varying from -100 (total attractancy) to + 100 (total repellency), with 0 meaning no effect.

Following the preliminary test, three doses, namely 5, 6.5 and 8 μ l were tested for each (treatment) of which three replications each with 20 adult mites were employed.

Data Analysis

The LD_{50} and LT_{50} values were obtained through Probit Analysis (Robertson *et al.*, 2007) and by use of POLO-PC software (LeOra Software, 1987). Significant differences among the concentrations were recorded when 95% confidence intervals (CI) not overlapping. Other data were compared by one-way Analysis of Variance (ANOVA) followed by Duncan test when significant differences were found at α = 0.05 (SAS Institute, 1997). Differences among samples were considered as statistically significant (P< 0.0001).

RESULTS

Fumigant Toxicity Assay

The vapors of essential oil from three medicinal plant species were toxic to *T. urticae* and cause more than 90% mortality in high doses, respectively. Six doses for each essential oil were employed with differences between doses found as significant in all the three plants' essential oils (Table 1).

As shown in Table 2, the effect of plant essential oils on the mite is in a dose dependent manner. For example М. longifolia essential oil at 9.95, 39.81, 69.67, and 159.24 µl L⁻¹ air produced 44, 60, 66, and 94% mortality, respectively. The same trends were observed when essential oils of S. officialis and M. communis were tested (Table 2). The most potent of the three tested plants against two spotted spider mite was *M. longifolia* with an LC₅₀ of 20.08 μ l L^{-1} air, whilst the least potent of the three tested plants was S. officialis essential oil with an LC₅₀ of 60.93 μ l L⁻¹ air. Toxicity (LC₅₀) of *M. communis* essential oil on the mite occurred at 53.22 μ l L⁻¹ air. Data regarding LC₁₀, LC₅₀, and LC₉₀ of all three tested plants against the mite is presented in Table 2. Figure 1 compares percentage mortality of *T. urticae* adults caused by different concentrations of *M. longifolia*, *S. officialis* and *M. communis* essential oils. As seen in the figures when low doses of essential was used (e.g. 9.95 μ l of essential oil per L⁻¹ air) *M. longifolia* caused high mortality (44% mortality) whereas the other two plant species' essential oils (at the same concentration) caused about 20% mortality.

Figure 1 shows regression lines of the three plant essential oils on the mite. As seen from the figures, line slopes of the three essential oils are different. The slopes for M. longifolia, S. officialis, and M. communis are 1.3, 1.97, and 1.4, respectively. Thus, it is shown that S. officialis essential oil became more effective with increase in the doses. M. *longifolia* is of the lowest slope showing that its effectiveness on the mite is not as pronounced as S. officialis. LC10, LC50, and LC_{90} of *M. longifolia* were found to be 1.76, 20.08, and 223.83 μ l L⁻¹ air, respectively, whilst LC10, LC50, and LC90 of S. officialis essential oil on the mite were assessed as 27.79, 60.93, and 133.60 μ l L⁻¹ air, respectively. Estimation of lethal time shows that when 69.61 µlL⁻¹air essential oil was used the LT_{50s} of *M. longifolia*, *S. officialis*,

Table 1. ANOVA analysis of the effect of three different medicinal plants, *Mentha longifolia, Salvia officialis* and *Myrtus communis* essential oils against adults of two-spotted spider mite.

Plant species		Tet	ranychus urticae	
	Source	df	Mean square	F
M .longifolia	Dose(µl L ⁻¹ air)	6	47.60 1.014	95.20 **
	Error	28	0.500	
	Total	34		
S. officialis	Dose(µl L ⁻¹ air)	6	61.92	108.37 **
	Error	28	0.571	
	Total	34		
M. communis	Dose(µl L ⁻¹ air)	6	42.64	119.41 **
	Error	28	0.357	
	Total	34		

For each essential oil, six doses were used in three of which there were significant differences between doses for all the three plant species (P < 0.0001).

** Highly significant.

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Dose (µl L ⁻¹ air)		Mortality (%) (Mean)	
	M. longifolia	S. officialis	M.communis
0.00	4e	4d	4g
9.95	44d	26e	24f
39.81	60c	38d	40e
69.67	66c	62c	50d
99.59	76b	80b	66c
129.38	90a	92a	76b
159.24	94a	96a	86a
LC ₁₀ (Fiducial limit)	1.762(0.037-6.25)	27.79 (5.44-44.61)	7.622 (0.62-18.2)
LC ₅₀ (Fiducial limit)	20.08 (5.16-35.60)	60.93(31.98-78.73)	53.22(25.83-79.17)
LC ₉₀ (Fiducial limit)	228.83(121.14-179.12)	133.60(103.53-252.05)	371.69(206.71-1765.34)
Slope±SE	1.213±0.206	3.759±0.640	1.518±0.274
df	4	4	4
X^2	8.20	9.96	6.75

Table 2. The effect of different concentrations of volatile phases of essential oils of the three different medicinal plant species, *Mentha longifolia, Salvia officialis* and *Myrtus communis* on two-spotted spider mite.

Mean values (n= 5 replicates with ten adult mites per replicate) followed by different letters in the same column differ significantly at α = 0.05 according to Duncan's test. The estimated lethal concentration values (μ L⁻¹ air) for each essential oil were given using probit analysis.

and *M. communis* were respectively estimated as 20.19, 21.91, and 19.64 hours (Table 3).

Repellency

Repellency index (RI%) and ED_{50} for the essential oils of the three plant species against the mite was calculated. The data shows that repellency index was increased with increase in doses of the essential oils.

For example *RI*% for *M. longifolia* at doses of 99.59, 129.38, and 159.24 μ l L⁻¹ air were 25, 45 and 57, respectively (Table 4). The same trends were observed for the other two essential oils i.e. with increase in the dose the level of repellency index also increased. RD_{50s} of essential oil obtained from the three plant species, namely *M. longifolia*, *S. officialis*, and *M. communis* were 147.47, 164.41, and 138.86, respectively (Table 4), thus showing that *M. communis* exerts the greatest repellency effect on the mite.

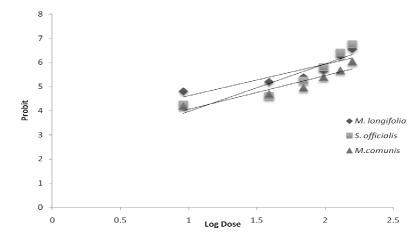


Figure 1. Regression line of *Mentha longifolia*, *Salvia officialis*, and *Myrtus comunis* essential oils on two- spotted spider mite.

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Table 3. Percentage mortality at 6, 12, 18 and 24 hours post-treatment of two-spotted spider mite with Mentha longifolia, Salvia officialis, and Myrtus

(C) HILLS

Plant species and dose (µl L ⁻¹ air)		Mortal	Mortality (%)						
		Exposure	Exposure time (h)		1T (b)	F	т Ю	1T (h)	\mathbf{v}^2
M. longifolia	9	12	18	24	L1 10 (III)	-	L150 (II)	LL 1 90 (III)	۲
39.81	10	26.66	41.67	53.33	6.058(3.4-8.04)		21.855(18.12- 29.75)	78.842(49.13-213.45)	0.05
69.61	13.33	28.33	45	58.33	5.33(2.85-7.26)		20.19(16.79-26.92)	76.53(47.74-206.32)	0.30
99.59	18.33	36.67	53.33	66.67	4.234(2.13-5.98)		16.11(13.54-19.96)	61.32(40.55-141.08)	0.28
S. officialis					,				
39.81	8.33	18.33	26.67	35	7.01(2.69-9.85)		41.91(27.66-146.55)	250.42(91.60-675.42)	0.02
69.61	11.67	26.67	41.67	55	5.72(3.09-7.73)	_	21.91(18.02-30.43)	83.93(50.80- 247.78)	0.19
99.59	21.67	40	56.67	70	3.71(2.28-5.90)		14.61(12.62-17.92)	57.48(36.46-103.90)	0.04
M. communis									
39.81	8.33	16.67	26.67	35	7.28(2.97-10.10)		41.88(27.79-140.65)	240.90(90.22-564.21)	0.12
69.61	11.67	21.67	33.33	45	5.58(3.17-7.45)		19.64(16.52-25.38)	69.13(44.97-165.14)	0.36
99.59	16.67	31.66	45	58.33	4.25(2.28-5.90)		14.91(12.62-17.92)	52.25(36.46-103.90)	0.31
LT_{10} , LT_{50} , and LT_{90} estimated, using Polo PC.	sing Polo	PC.							
Table 4. Repellency Index (R1%), ED ₅₀ , and ED ₉₀ of essential oils <i>Mentha longifolia, Salvia officialis</i> and <i>Myrtus comumis</i> against two spotted spider mite.	cy Index d spider n	(RI%), ED ₅ nite.	0, and ED ₉₀	of essenti	ial oils <i>Mentha</i> .	longifolia, Salv	<i>ia officialis</i> and	Myrtus comumis	
Plant species	Dos	Dose (µl L ⁻¹ air)			Tet	Tetranychus urticae	ae		
4		,	% RI	ED ₅₀ (µ	ED ₅₀ (µl L ⁻¹ air) ED	ED_{90} (µl L ⁻¹ air)	Slope ±S.E	X^2	
M.longifolia		99.59	25	147	147 47	283.99			
		129 38	45	† T		(02) 27-522 80)	4 503+1 009	0.78	

Ë.			Tetranychus urticae	ы	
	% RI	ED_{50} (µl L ⁻¹ air)	% RI $ED_{50}(\mu I L^{-1} air) ED_{90}(\mu I L^{-1} air)$ Slope $\pm S.E$	Slope ±S.E	X^2
	25	<i>LV LV</i> 1	283.99		
	45	112/ 85 160 05)	(222.27 - 522.80)	4.503 ± 1.009	0.28
	57	(66.601 -60.461)			
	4	164 41	113 802		
	31	104.41 /17 70 107 21/	243.003 707 60 761 917	7.483±1.295	5.50
	43	(16.701-07.141)	(10.102-00.102		
	33	120 00	201 862		
	48	100.001 10.001	504.803	3.751 ± 0.962	0.0018
	61	(60.101 -00.021)	(01.061 -64.077)		

DISCUSSION

The present study demonstrated for the first time that essential oils of three medicinal plants, namely M. longifolia, S. and *M. communis* possess officialis. fumigant toxicity as well as repellency effects against two spotted spider mite (T. urticae). It has been shown that different concentrations of the acetone solutions of the essential oils from 14 species of Lamiaceae caused mortality and induced repellency in adult females of the carmine spider mite, T. cinnabarinus Boisd. Also, these plants' essential oils reduced egglaying activity of the mite (Mansour et al., (Rosmarinus Rosemary 1986). oil officinalis) has contact toxicity while the oils of caraway seed, citronella java, lemon eucalyptus (Corymbia citriodora), pennyroyal (Mentha pulegium) and peppermint (Mentha piperita) have fumigant activity against T. urticae (Choi et al., 2004; Miresmailli et al, 2006). More importantly, Isman and Machial (2006) showed that commercial formulation of rosemary oilbased pesticides (Hexicide, Ectrol, Sporam) provides high toxicity against phytophagous mite, T. urticae but it does not have acute toxicity on the predatory mite Phytoseiulus persimilis Athias Henriot (Acari: phytoseiidae), showing that commercial formulation based on plants' essential oils can be effectively used in an integrated pest management program.

In the current study it was found that *M.* longifolia, *S.* officialis, and *M.* communis essential oils not only have fumigant toxicity against *T. urticae* but also repellency effect. Thus, essential oils and their components can be effectively used to dispel both parasitic and free-living ticks as well as mites (Yatagai, 1977; El-Zemmity *et al.*, 2006).

Interestingly, repellency effect was significantly different among the three plant species showing that these plants' essential oils are of great potentials to be used in greenhouses where this mite species causes serious damage and while the application of pesticides produce risk hazard to human health and environment. Thus, when essential oils are employed in a pest management program, concerns regarding pesticide residue would be mitigated since some essential oil constituents taken through diet are actually not harmful, but even beneficial to human health (Haung *et al.*, 1997).

In the present experiment, in some cases where high doses of essential oils were used a 100% mortality was obtained. High percentages of mortality have been reported before, for example Tunc and Sahinkaya (1998) reported 100% mortality of *T. cinnabarinus* and *Aphis gossypii* Glover when essential oils of *Cuminum cyminum*, *Pimpinella anisum* and *Origanum syriacum* were used in greenhouse conditions.

In addition to being toxic against postembryonic stage of insects and mites, it has been shown that some essential oils have oviposition-deterring activities, for example three essential oils extracted from *Laurus nobilis*, *Myrtus communis* and *Artemisia absinthum* showed toxicity effects against adults and eggs of *T. cinnabarinus* under laboratory conditions (Topuz and Erler, 2007).

In conclusion and based on the results obtained from the current research, it can be stated that medicinal plants' essential oils are in possession of a great potential to be used in the greenhouses against *T. urticae*. There are numerous studies reporting resistance against synthetic pesticides as regards this mite species which would inevitably impose great risks on human health, because of accumulation of pesticide residues on the foodstuff and in the environment.

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سمیت و دورکنندگی سه اسانس روی کنه دو نقطه ای

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چکیدہ

کنه دونقطه ای (Tetranychus urticae Koch (Acari: Tetranychidae یکی از مهمترین آفات درختان میوه، سبزیجات، و گیاهان زینتی درسرتاسرجهان در فضاهای بسته و باز است. درحال حاضر روش اصلی مدیریت این آفت استفاده از آفت کشها است که باعث ایجاد مقاومت و افزایش دوباره جمعیت کنه میشود بعلاوه باعث آلودگی محصولات غذایی و محیط زیست هم میشوند.اسانس های بدست آمده ازبخش های هوایی گیاهان پتانسیل خوبی جهت جایگزینی آفت کشهای سنتتیک دارند و آنها نشان داده شده است که دارای اثرات حشره کشی و کنه کشی میباشند. درنتیجه هدف مطالعه حاضر بررسی اثر اسانس هاس گیاهی استخراج شده از سه گونه گیاه دارویی بنامهای Mentha Myrtus communis ₂ Salvia officialis (Lamiaceae) , longifolia (Lamiaceae) (Myrtaceae) برعليه كنه دونقطه اى است. LC₅₀ اسانس سه گونه گياه (Myrtaceae) officialis و M. communis و M. communis و ۵۳/۲۲ میکرولیتر بر لیتر هوا ميباشد. اين نتايج نشان ميدهد كه اسانس گونه گياهي M. longifolia داراي بالاترين مقدار سمیت و اسانس گونه S. officialis دارای کمترین مقدارسمیت است. همچنین اسانس گیاهان M. longifolia ، دارای خاصیت دور کنندگی میباشند که M. communis ی میباشند که ED₅₀ آنها به ترتیب ۱۴۷/۴۷ ، ۱۳۸/۸۰ و ۱۶۴/۴۱ میکرولیتر برلیتر هوا میباشند.بطورکلی، هرچه مقدار دزمصرفی یا مدت زمان بیشتر شود میزان مرگ و میر بالاتر میرود. این داده ها نشان میدهد که اسانس هرسه گونه دارای پتانسیل خوبی در استفاده در برنامه های مدیریت کنه دو نقطه ای در شرایط گلخانه ای را دارند.