

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 possess 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 officinalis* (both Lamiaceae) and *Myrtus communis* (Myrtaceae) against *T. urticae*. The LC₅₀ values of essential oils of *M. longifolia*, *M. communis*, and *S. officinalis* against *T. urticae* were 20.08, 53.22, 60.93 $\mu\text{L L}^{-1}$ air, respectively. This shows that *M. longifolia* possesses the highest lethal activity whereas *S. officinalis* the lowest. Also, essential oils of *M. longifolia*, *M. communis*, and *S. officinalis* were demonstrated to possess repellency effect with ED₅₀s of 147.47, 138.80 and 164.41, $\mu\text{L L}^{-1}$ 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 acaricidal 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 officinalis* (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 $24\pm 3^{\circ}\text{C}$, and $45\pm 60\%$ relative humidity under natural daylight in greenhouse.

Plant Material and Essential Oil Extraction

Mentha longifolia, *S. officinalis* 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 water-distillation for 3 hours using a Clevenger apparatus. Following the essential oil being obtained, the oil was decanted from the water layer, dried through Na_2SO_4 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. officinalis* and *M. communis*, respectively.

Fumigant Toxicity Bioassay

Toxicity of essential oils on the two-spotted 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 µl 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 LT₅₀, 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 LT₅₀

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. urticae* 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 diet.

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 µl were tested for each (treatment) of which three replications each with 20 adult mites were employed.

Data Analysis

The LD₅₀ and LT₅₀ 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 $\alpha=0.05$ (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 *M. longifolia* essential oil at 9.95, 39.81, 69.67, and 159.24 $\mu\text{l L}^{-1}$ 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 LC_{50} of 20.08 $\mu\text{l L}^{-1}$ air, whilst the least potent of the three tested plants was *S. officialis* essential oil with an LC_{50} of 60.93 $\mu\text{l L}^{-1}$ air. Toxicity

(LC_{50}) of *M. communis* essential oil on the mite occurred at 53.22 $\mu\text{l L}^{-1}$ air. Data regarding LC_{10} , LC_{50} , and LC_{90} 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 μl 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*. LC_{10} , LC_{50} , and LC_{90} of *M. longifolia* were found to be 1.76, 20.08, and 223.83 $\mu\text{l L}^{-1}$ air, respectively, whilst LC_{10} , LC_{50} , and LC_{90} of *S. officialis* essential oil on the mite were assessed as 27.79, 60.93, and 133.60 $\mu\text{l L}^{-1}$ air, respectively. Estimation of lethal time shows that when 69.61 $\mu\text{l L}^{-1}$ air essential oil was used the LT_{50} s 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	<i>Tetranychus urticae</i>			
	Source	df	Mean square	F
<i>M. longifolia</i>	Dose($\mu\text{l L}^{-1}$ air)	6	47.60	1.014
	Error	28	0.500	
	Total	34		95.20 **
<i>S. officialis</i>	Dose($\mu\text{l L}^{-1}$ air)	6	61.92	
	Error	28	0.571	
	Total	34		108.37 **
<i>M. communis</i>	Dose($\mu\text{l L}^{-1}$ air)	6	42.64	
	Error	28	0.357	
	Total	34		119.41 **

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.

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

Dose ($\mu\text{l L}^{-1}$ air)	Mortality (%) (Mean)		
	<i>M. longifolia</i>	<i>S. officinalis</i>	<i>M. communis</i>
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 \pm SE	1.213 \pm 0.206	3.759 \pm 0.640	1.518 \pm 0.274
df	4	4	4
X ²	8.20	9.96	6.75

Mean values (n= 5 replicates with ten adult mites per replicate) followed by different letters in the same column differ significantly at $\alpha= 0.05$ according to Duncan's test. The estimated lethal concentration values ($\mu\text{l L}^{-1}$ 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 ED₅₀ 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 $\mu\text{l L}^{-1}$ 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. officinalis*, 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.

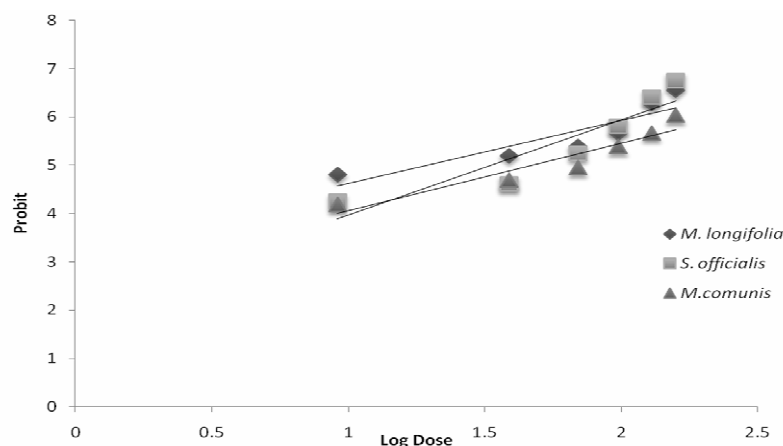


Figure 1. Regression line of *Mentha longifolia*, *Salvia officinalis*, and *Myrtus comunis* essential oils on two- spotted spider mite.

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

Plant species and dose ($\mu\text{L L}^{-1}$ air)	Mortality (%)				LT ₁₀ (h)	LT ₅₀ (h)	LT ₉₀ (h)	X ²
	6	12	18	24				
<i>M. longifolia</i>	39.81	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	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	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
<i>S. officinalis</i>	39.81	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	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	40	56.67	70	3.71(2.28- 5.90)	14.61(12.62- 17.92)	57.48(36.46- 103.90)	0.04
<i>M. communis</i>	39.81	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	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	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

 LT₁₀, LT₅₀, and LT₉₀ estimated, using Polo PC.

Table 4. Repellency Index (RI%), ED₅₀, and ED₉₀ of essential oils *Mentha longifolia*, *Salvia officinalis* and *Myrtus communis* against two spotted spider mite.

Plant species	Dose ($\mu\text{L L}^{-1}$ air)	% RI	<i>Tetranychus urticae</i>			X ²
			ED ₅₀ ($\mu\text{L L}^{-1}$ air)	ED ₉₀ ($\mu\text{L L}^{-1}$ air)	Slope \pm S.E	
<i>M. longifolia</i>	99.59	25	147.47	283.99		
	129.38	45	(134.85- 169.95)	(222.27- 522.80)	4.503 \pm 1.009	0.28
	159.24	57				
<i>S. officinalis</i>	99.59	4	164.41	243.883		
	129.38	31	(147.28- 182.31)	207.60- 261.81)	7.483 \pm 1.295	5.50
	159.24	43				
<i>M. communis</i>	99.59	33	138.80	304.863		
	129.38	48	(125.05- 161.09)	(226.43- 730.76)	3.751 \pm 0.962	0.0018
	159.24	61				

DISCUSSION

The present study demonstrated for the first time that essential oils of three medicinal plants, namely *M. longifolia*, *S. officinalis*, and *M. communis* possess 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 egg-laying activity of the mite (Mansour *et al.*, 1986). Rosemary oil (*Rosmarinus 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 oil-based 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. officinalis*, 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 post-embryonic stage of insects and mites, it has been shown that some essential oils have oviposition-detering 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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چکیده

کنه دونقطه ای (*Tetranychus urticae* Koch (Acari: Tetranychidae) یکی از مهمترین آفات درختان میوه، سبزیجات، و گیاهان زینتی در سرتاسر جهان در فضاهای بسته و باز است. در حال 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 از بخش 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. در نتیجه هدف مطالعه حاضر بررسی اثر اسانس هاس گیاهی استخراج شده از سه گونه گیاه دارویی بنامهای *Mentha longifolia* (Lamiaceae)، *Salvia officinalis* (Lamiaceae) و *Myrtus communis* (Myrtaceae) بر علیه کنه دونقطه ای است. LC₅₀ اسانس سه گونه گیاه *M. longifolia*، *S. officinalis* و *M. communis* بر روی کنه به ترتیب مقدار ۲۰/۰۸، ۵۳/۲۲ و ۶۰/۹۳ میکرولیتر بر لیتر هوا میباشد. این نتایج نشان میدهد که اسانس گونه گیاهی *M. longifolia* دارای بالاترین مقدار سمیت و اسانس گونه *S. officinalis* دارای کمترین مقدار سمیت است. همچنین اسانس گیاهان *M. longifolia*، *S. officinalis* و *M. communis* دارای خاصیت دورکنندگی میباشند که ED₅₀ آنها به ترتیب ۱۴۷/۴۷، ۱۳۸/۸۰ و ۱۶۴/۴۱ میکرولیتر بر لیتر هوا میباشند. بطور کلی، هر چه مقدار دز مصرفی یا مدت زمان بیشتر شود میزان مرگ و میر بالاتر میرود. این داده ها نشان میدهد که اسانس هر سه گونه دارای پتانسیل خوبی در استفاده در برنامه های مدیریت کنه دو نقطه ای در شرایط گلخانه ای را دارند.