

## Spike Lavender Essential Oil Reduces the Survival Rate and Fecundity of Two-spotted Spider Mite, *Tetranychus urticae* (Acari: Tetranychidae)

R. Laborda<sup>1</sup>, I. Manzano<sup>1</sup>, M. Gamon<sup>2</sup>, I. Gavidia<sup>3</sup>, R. Boluda<sup>3</sup>, and P. Perez-Bermudez<sup>3\*</sup>

### ABSTRACT

The two-spotted spider mite, *Tetranychus urticae* Koch, causes major yield loss in numerous plants. The control of this mite is achieved mainly with synthetic acaricides; other strategies are the use of predatory mites or plant natural products. This study evaluated the effects of *Lavandula latifolia* Medik. (Lamiaceae) essential oil on the survival rate and fecundity of *T. urticae* by slide-dip and leaf-disk bioassays. Acute contact toxicity was provoked by different spike lavender oil concentrations although 95-100% mortality was observed when emulsions contained at least 0.20% (v/v). In the residual toxicity experiments, lavender oil (0.15-0.25%) reduced mite survival and affected its fecundity; laid eggs and emerging larvae were lowered as the oil concentration increased. Incubation temperature determined egg viability; 12°C did not allow larval development, while the highest percentage of hatched eggs was counted at 30°C. Our results confirmed the possibility of using spike lavender oil as an alternative to conventional pesticides.

**Keywords:** Biopesticides, Fecundity, *Lavandula latifolia*, Pesticides, Terpenes.

### INTRODUCTION

Essential oils have received much attention as useful bioactive products, particularly in antimicrobial, antifungal, and pesticidal terms (Dayan *et al.*, 2012; Wu *et al.*, 2012; Miresmailli and Isman, 2014; Kheradmand *et al.*, 2015). Unlike the synthetic pesticides that are usually based on a single ingredient, these oils have a complex mixture of terpenes, which interact synergistically to avoid target-site resistance (Isman *et al.*, 2011). Based on these properties and their toxicity to pests, some natural terpenoids have been commercialized as pest control products (Cantrell *et al.*, 2012; Attia *et al.*, 2013).

One of the main targets to control among agricultural pests is *Tetranychus urticae* Koch (Acari: Tetranychidae), which causes major yield loss in numerous food crops and ornamental plants. Control of this spider mite is achieved by applying synthetic pesticides or is based on biological control agents (Moghadasi *et al.*, 2016). However, these measures entail two major problems: this spider mite develops resistance to many acaricides, and efficacy of biocontrol agents is limited (Miresmailli and Isman, 2006; Bernardi *et al.*, 2013).

The acaricidal activity of essential oils and terpenes is well-documented (Han *et al.*, 2010; Motazedian *et al.*, 2012; Mozaffari *et al.*, 2012; Fatemikia *et al.*, 2014; Laborda *et al.*, 2013; Tak and Isman, 2017), but very

<sup>1</sup> Department of Agroforestry Ecosystems, Polytechnic University of Valencia, 46022 Valencia, Spain.

<sup>2</sup> Agrifood Laboratory, Generalitat Valenciana, 46100 Burjassot (Valencia), Spain.

<sup>3</sup> Department of Plant Biology, University of Valencia, Av. V. A. Estellés s / n, 46100 Burjassot (Valencia), Spain.

\*Corresponding author; e-mail: perezb@uv.es



few studies (further cited and discussed in this study) have been conducted on the toxicity of *Lavandula* spp. against *T. urticae*. No research on this topic in relation to *Lavandula latifolia* Medik. has been reported, and this study aimed to characterize spike lavender essential oil and to determine its acaricidal activity by evaluating its effects on the survival and fecundity of the two-spotted spider mite.

## MATERIALS AND METHODS

### Preparation and Analysis of Spike Lavender Essential Oil

Plant material and extracts were supplied by a cooperative society (located at Rincón de Ademuz, Valencia, Spain), whose activity complies with EU regulations on organic production and preparation. The aerial parts of spike lavender plants, *L. latifolia* (Lamiaceae), were harvested at the beginning of the flowering period from cultivated plantations, which grow at 700–1,100 m above sea level in Rincón de Ademuz. Essential oil was obtained from fresh material (twigs with leaves and flowers) by steam distillation. The chromatographic determinations and quantitative analyses of spike lavender oil were performed as previously described (Laborda et al., 2013). Terpenoids were identified according to their Kovats Retention Indices (RI) (NIST, 2008; Babushok et al., 2011) under the GC-MS experimental conditions with a stationary phase column of dimethylsilicone with 5% phenyl groups.

### Spider Mites and Host Plants

Two-spotted spider mites, *T. urticae*, were obtained from the cultures maintained at the Universitat Politècnica de València (Spain). The mites were reared on bean plants (*Phaseolus vulgaris* L. var. Moradillo) in

greenhouses (18–27°C; 12 hours photoperiod; 60–70% relative humidity).

### Slide-Dip Bioassays

Five female adults of *T. urticae* were placed on double-sided removable tape fixed on the ends of glass microscope slides using a fine brush. Slides with attached mites were immersed for five seconds in 50 mL of distilled water (controls) or in 50 mL of the different oil/distilled water emulsions assayed. Tween 20 (0.1% v/v) was added in all treatments as an emulsifier. Lavender oil was tested at 0.10, 0.15, 0.20, and 0.25% (v/v) concentrations. After dipping, slides were dried for 30 min, put inside a clear plastic box and kept at 25±1°C; 12 hours photoperiod; 60% relative humidity. Four slides, and five mites per slide, were used per treatment (20 spider mites per control and each oil concentration). The complete experiment was repeated twice, with 200 female spider mites employed in these bioassays. Mortality was determined hourly during the first 8 hours, and 24 hours after the test began. Each slide was examined under a microscope; if mites did not move any appendages when prodded with a fine brush they were considered dead.

### Leaf-Disk Bioassays

These tests were carried out to determine the possible effects of residual essential oil on the survival and fecundity of *T. urticae*. Uninfected bean leaves were washed with distilled water and used to prepare leaf disks (2 cm in diameter). Leaf-disks were sprayed with 400 µL of distilled water (control), or with 400 µL of the different tested emulsions; lavender extract was emulsified in water at 0.15 or 0.25% (v/v) concentrations using Tween 20 (0.1% v/v). Leaf-disks were air-dried and deposited in Petri dishes containing a double cotton layer soaked with 40 mL of distilled water. Two adult female mites were transferred to each

leaf-disk. Five replicated Petri dishes with two leaf-disks in each one (5×2×2 mites) were used per treatment, and were kept at 12, 25 or 30°C. The complete experiment was repeated twice. Surviving adults, laid eggs, and emerged larvae were counted on days 1-7 after the test began.

### Statistical Analysis

Data were analyzed using Statgraphics Centurion XVI (Statpoint Technologies). ANOVA was followed by an LSD test to assess the significance of differences among treatments (P-values < 0.05 were considered significant). Abbott's formula (1925) was used for corrections in relation to blank control mortality.

approximately 900 mL per 100 kg of fresh plant material. Terpene Retention Indices (Table 1) were comparable to those previously reported by Babushok *et al.* (2011) and Herráiz-Peñalver *et al.* (2013). The GC-MS analysis indicated that there were around 11 major constituents (Peak areas ≥ 0.8%), which represented 90-95% of the total essential oil (Table 1). Major components were monoterpenes and, within this group, oxygen-containing monoterpenes (~65-85%), represented by linalool (37.8%), 1,8-cineole (24.9%) and camphor (18.7%), which largely dominated over monoterpene hydrocarbons (~5-12%) represented by pinenes, camphene, etc. The other quantitatively important group (~3-8%) comprised hydrogenated and oxygenated sesquiterpenes e.g. caryophyllenes, viridiflorol, etc.

## RESULTS

### Chemical Composition of Spike Lavender Essential Oil

The yield of *L. latifolia* essential oil obtained by steam distillation was

### Slide-Dip and Leaf-Disk Bioassays

Lavender extract was firstly studied for its acaricidal activity against *T. urticae* when applied topically using slide-dip assays. Spike lavender oil showed acaricidal activity (Table 2), and mortality rates of 95-100%

**Table 1.** Main components of *Lavandula latifolia* essential oil determined by GC-MS. Values are the means±SD of three replicates.

| Compound        | % Peak area | Retention index |
|-----------------|-------------|-----------------|
| Linalool        | 37.8 ± 3.8  | 1098 ± 8.6      |
| 1,8-Cineole     | 24.9 ± 2.1  | 1033 ± 6.9      |
| Camphor         | 18.7 ± 3.2  | 1143 ± 8.9      |
| Borneol         | 2.5 ± 0.3   | 1165 ± 7.2      |
| β-Caryophyllene | 2.3 ± 0.6   | 1418 ± 9.8      |
| Terpinen-4-ol   | 1.8 ± 0.4   | 1177 ± 6.1      |
| Camphene        | 1.5 ± 0.3   | 953 ± 8.0       |
| α-Pinene        | 1.2 ± 0.3   | 936 ± 7.0       |
| β-Pinene        | 1.0 ± 0.2   | 980 ± 8.4       |
| Limonene        | 1.0 ± 0.4   | 1031 ± 6.8      |
| Viridiflorol    | 0.8 ± 0.3   | 1591 ± 9.1      |



were observed at 0.20-0.25% oil concentrations. Indeed, this toxicity was even observed at only 1 hour after treatment began. The 0.10 % oil concentration was less efficient since 50-60% of mites survived after this treatment (Table 2). Mortality in the control treatment reached 5%. The  $LC_{50}$  calculated in the slide-dip assays fell within an interval between 0.123 (1 hour) and 0.105% (24 hours), whereas the  $LC_{90}$  values were 0.222 and 0.204%, respectively.

Based on these results, two oil concentrations (0.15 and 0.25%) were selected to study the residual toxicity of the oil on mites growing on sprayed leaf-disks. Although dead female adults were counted on days 1-7 after the test started, only the final data for day 7 are summarized in Table 3. The mortality rates in these experiments were markedly lower than those found in the topical assays. In the slide-dip method, 0.15 and 0.25% oils killed all the mites after 24 hours (Table 2), whereas oil residual toxicity reached only ca. 50-60% mortality in mites exposed for 7 days to 0.25% oil (Table 3). The mortality provoked by residual lavender oil was not affected by varying incubation temperatures.

### Effects on the Fecundity of Two-Spotted Spider Mites

Leaf-disk bioassays were also used to evaluate the effects of *L. latifolia* oil on the mite fecundity, the susceptibility to egg laying, and the resulting offspring. Laid eggs and emerged larvae were counted over a 7-day period on bean leaf-disks, which were

treated with 0, 0.15 or 0.25% oil emulsions. The final effects of spike lavender on spider mite fecundity are summarized in Tables 3 and 4.

Regarding the oviposition pattern (Table 4), no remarkable differences were seen between the control and the treatments performed at 25 or 30°C; approximately 60-70% of the eggs were laid on days 1-3 and 90-100% by day 5. At 12°C, however, the proportion of eggs laid on the first 3 days of incubation was only 20-30%, while 35-67% of all the eggs were laid on days 5-7 (Table 4). Both incubation temperature and oil concentration determined oviposition rates. First, the most favourable temperature for mite oviposition was 25°C, whereas the mean number of eggs was significantly lowered (ca. 50-90%) at 12°C (Tables 3 and 4). Furthermore, the higher the oil concentration, the lower the total number of eggs (Table 4), and rate reductions were significant when lavender extract was used at 0.25%. This deterrent effect on mite oviposition was observed at all incubation temperatures. With the 0.25% oil treatments, the mean numbers of eggs laid per mite were 0.3, 4.0 and 2.8 (at 12, 25 and 30°C, respectively), whereas they were 2.5, 8.1 and 5.5 eggs per mite in the respective control cultures (Table 3).

Larval development started on day 3 in the treatments carried out at 25 and 30°C, while 12°C did not allow larva development. This can be explained by considering that 12°C comes very close to the limiting temperature for *T. urticae* development. The final number of larvae per mite decreased as the

**Table 2.** Mortality caused by *Lavandula latifolia* essential oil on female *Tetranychus urticae* when applied at different concentrations in slide-dip bioassays.<sup>a</sup>

| Treatment essential oil (v/v) | Mean mortality (%)±SE |              |              |              |              |
|-------------------------------|-----------------------|--------------|--------------|--------------|--------------|
|                               | 1 h                   | 2 h          | 4 h          | 8 h          | 24 h         |
| 0.10%                         | 36.8 ± 0.0b           | 36.8 ± 0.0b  | 36.8 ± 0.0b  | 42.1 ± 5.3c  | 47.4 ± 6.1c  |
| 0.15%                         | 42.1 ± 5.3b           | 42.1 ± 5.3b  | 57.9 ± 0.0b  | 63.2 ± 5.3b  | 63.2 ± 5.3b  |
| 0.20%                         | 94.7 ± 5.3a           | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a |
| 0.25%                         | 100.0 ± 0.0a          | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a |
| P value                       | 0.001                 | 0.001        | 0.001        | 0.001        | 0.001        |

<sup>a</sup> In the columns, the data followed by the same letter are not significantly different ( $P$  0.05, LSD test).

**Table 3.** Effects of *Lavandula latifolia* essential oil concentrations and incubation temperature on female *Tetranychus urticae* after 7 days of treatment in leaf-disk bioassays.<sup>a</sup>

| Incubation temp | Essential oil dose (v/v) | Mean mortality (%)±SE | Mean no of eggs/mite±SE | Mean no of larvae/mite±SE | Hatched eggs (%)±SE |
|-----------------|--------------------------|-----------------------|-------------------------|---------------------------|---------------------|
| 12°C            | Control                  | 25.0 ± 14.4a          | 2.5 ± 0.3a              | 0                         | 0                   |
|                 | 0.15%                    | 50.0 ± 0.0a           | 2.0 ± 0.5a              | 0                         | 0                   |
|                 | 0.25%                    | 50.0 ± 0.0a           | 0.3 ± 0.3b              | 0                         | 0                   |
|                 | <i>P</i> value           | 0.100                 | 0.003                   | -----                     | -----               |
| 25°C            | Control                  | 25.0 ± 14.4a          | 8.1 ± 0.4a              | 2.5 ± 0.4a                | 30.8 ± 5.4a         |
|                 | 0.15%                    | 25.0 ± 14.4a          | 5.9 ± 0.6b              | 1.8 ± 0.3ab               | 29.8 ± 3.4a         |
|                 | 0.25%                    | 50.0 ± 0.0a           | 4.0 ± 0.5c              | 1.3 ± 0.1b                | 31.3 ± 2.3a         |
|                 | <i>P</i> value           | 0.274                 | 0.001                   | 0.039                     | 0.895               |
| 30°C            | Control                  | 25.0 ± 14.4a          | 5.5 ± 0.4a              | 3.6 ± 0.5a                | 65.9 ± 4.4a         |
|                 | 0.15%                    | 25.0 ± 14.4a          | 4.3 ± 0.4ab             | 2.0 ± 0.2b                | 47.1 ± 9.2a         |
|                 | 0.25%                    | 62.5 ± 12.5a          | 2.8 ± 0.4b              | 1.4 ± 0.2b                | 50.0 ± 2.9a         |
|                 | <i>P</i> value           | 0.141                 | 0.032                   | 0.004                     | 0.533               |

<sup>a</sup> The data followed by the same letter do not significantly differ (*P* 0.05, LSD test).

**Table 4.** Effects of *Lavandula latifolia* essential oil concentration and incubation temperature in leaf-disk bioassays on *Tetranychus urticae* oviposition pattern. Values are the number of eggs laid by 40 spider mites at each time.<sup>a</sup>

| Treatment essential oil (v/v) | Time after the test started (Days) | Incubation temp |            |            |
|-------------------------------|------------------------------------|-----------------|------------|------------|
|                               |                                    | 12°C            | 25°C       | 30°C       |
| Control                       | 1 d                                | 0               | 80 (25%)   | 80 (36%)   |
|                               | 3 d                                | 24 (24%)        | 204 (63%)  | 152 (69%)  |
|                               | 5 d                                | 56 (56%)        | 304 (94%)  | 220 (100%) |
|                               | 7 d                                | 100 (100%)      | 324 (100%) | -----      |
| 0.15 %                        | 1 d                                | 0               | 84 (36%)   | 56 (33%)   |
|                               | 3 d                                | 16 (20%)        | 160 (68%)  | 100 (58%)  |
|                               | 5 d                                | 52 (65%)        | 220 (93%)  | 172 (100%) |
|                               | 7 d                                | 80 (100%)       | 236 (100%) | -----      |
| 0.25 %                        | 1 d                                | 0               | 76 (47%)   | 40 (36%)   |
|                               | 3 d                                | 3 (25%)         | 104 (65%)  | 60 (54%)   |
|                               | 5 d                                | 4 (33%)         | 160 (100%) | 112 (100%) |
|                               | 7 d                                | 12 (100%)       | -----      | -----      |

<sup>a</sup> In parentheses, the percentages of eggs laid at each counting time.

lavender oil concentration increased, and the reduction was relevant, even for the lowest concentration tested (Table 3). The highest percentages of hatched eggs counted at 30°C. *T. urticae* eggs did not seem to be susceptible to contact with spike lavender

residuals and hatching rates did not vary between the eggs laid on the control leaves and those on leaves with 0.15 or 0.25% oil (Table 3).



## DISCUSSION

Essential oils are mixtures of different plant secondary products, mostly terpenes, which have been identified as being responsible for their biocidal activities. Chromatographic analyses revealed that spike lavender oil primarily consists of three oxygenated monoterpenes, linalool, 1,8-cineole and camphor (Table 1), and the present data on their concentrations corroborated previous findings on the composition of this volatile extract (Salido *et al.*, 2004; Herráiz-Peñalver *et al.*, 2013). These authors reported that the linalool, 1,8-cineole and camphor concentrations fell within the 28-43, 11-35, and 10-23% ranges, respectively. These three terpenes, together with  $\alpha$ -pinene (~1.2%), were the only ones to appear in all the 194 samples analyzed by Herráiz-Peñalver *et al.* (2013) in their survey on spike lavender.

Regarding spike lavender essential oil variability, data showed that, as with other natural extracts from medicinal and aromatic plants, composition is highly influenced by genetic factors, geographical area of origin, environmental conditions, developmental stage of plants at harvest, extraction method, etc. In line with this, the only chemotype described for spike lavender is linalool/1,8-cineole/camphor (Salido *et al.*, 2004). On the contrary, Herráiz-Peñalver *et al.* (2013) did not share this conclusion. These authors, according to the distribution of the major compounds, described three distinct patterns of chemical composition for *L. latifolia*, and concluded that such patterns must be considered ecotypes instead of chemotypes. That is, differences in essential oil components are not due to the genetic differences among wild spike lavender plants, but to the distinct environmental conditions in the areas of origin, particularly altitude, temperature, and light intensity.

The spike lavender extract employed in this study caused significant mortality in two-spotted spider mites within the low oil concentrations range tested (Table 2). The

present findings are similar and comparable to those previously reported by Laborda *et al.* (2013). The comparison of the results obtained in both studies revealed that sage oil provokes greater acute-contact and residual toxicities than rosemary and spike lavender extracts, which had a similar acaricidal effect on *T. urticae*. Camphor, 1,8-cineole and  $\alpha$ -pinene were present in *L. latifolia* (Table 1), *Rosmarinus officinalis* L. and *Salvia officinalis* L. essential oils (Laborda *et al.* 2013); camphor and 1,8-cineole were found at high concentrations (~10-27%) in the three species, whereas the  $\alpha$ -pinene percentage varied from high in rosemary (~18%) to low in spike lavender (~1%), with ~7% in sage. Linalool, a major constituent of lavender oil (~38%), was found in rosemary at a low concentration (~1%), but was not detected in sage. Finally,  $\alpha$ -thujone was found only in sage and is the major constituent of this essential oil (~42%).

Although we did not test acaricidal activity of the constituents of the essential oils, several laboratories have investigated the activity of individual monoterpenes and the synergic effect between active and inactive constituents. For example, as functional groups may determine the toxicity of natural compounds, ketones are often more toxic than aldehydes which, generally, show higher toxicity than alcohols and acids (see Tak and Isman, 2017). Badawy *et al.* (2010) studied the toxicity of 12 monoterpenes against *T. urticae* and suggested that monoterpenes are largely delivered in the vapour phase, and that the fumigant or contact toxicity of each compound depends on access via the respiratory system or hydrogen bond donors, respectively. On the other hand, Miresmailli *et al.* (2006) conducted a research to evaluate acaricidal activity of natural and synthetic rosemary oils on *T. urticae*. The tests done with artificial oils suggested some major active constituents e.g., 1,8-cineole, and some minor active e.g.  $\alpha$ -pinene, and almost inactive constituents e.g. camphor. They observed that inactive constituents are

necessary to achieve full toxicity, and that the active constituents may have an antagonistic effect on each other. The conclusions drawn by Miresmailli *et al.* (2006) have been supported by other studies, which investigated the relationship between essential oil components and toxicity against different pests (Isman *et al.*, 2011).

On the basis of the findings of these research works (Miresmailli *et al.*, 2006; Badawy *et al.*, 2010) and the spike lavender oil composition (Table 1), it is suggested that linalool, 1,8-cineole and limonene are the major active constituents of this oil against adult *T. urticae*. Although information on the exact mode of action of plant essential oils is still lacking, there is evidence that the chemically diverse constituents of these oils present a broad spectrum of activities, related mainly with arthropod nervous systems and detoxification mechanisms. According to the literature (Badawy *et al.*, 2010; Rattan, 2010; Blenau *et al.*, 2012; Modarres-Najafabadi *et al.*, 2012; Attia *et al.*, 2015; Ebadollahi *et al.*, 2017; Zarrad *et al.*, 2017), there is some evidence that, in insects and mites, essential oils may interact with distinct molecular targets, such as tyramine and octopamine receptors, the GABA system (alteration of ionic channels), the cholinergic system (inhibition of acetylcholinesterase), and also with different enzymes e.g. cytochrome P450 monooxygenase, phosphatases, esterases, and glutathione-S-transferase.

To date, only two essential oils distilled from *Lavandula* species, namely *L. vera* DC and *L. officinalis* Chaix, have been tested for their toxicity against *T. urticae*. The true lavender (*L. vera*) extract was included in a large set of 34 commercial essential oils screened for their acaricidal and oviposition deterrent activities by a leaf-dip bioassay (Roh *et al.*, 2011), and for their fumigant activity against two-spotted spider mites (Lim *et al.*, 2011). The results of both studies demonstrated the very poor effectiveness of true lavender since this oil caused less than 30% mortality.

Refaat *et al.* (2002) tested *L. officinalis* oil against two tetranychid mites, *Eutetranychus orientalis* Klein and *T. urticae*, and found a markedly distinct susceptibility of these mites when sprayed directly with oil since mortality was 90-100% or less than 30%, respectively. In 2012, Modarres-Najafabadi *et al.* reported a comparatively greater efficiency of the *L. officinalis* extract against *T. urticae*, although only high concentrations (2-4%) killed 90-100% of adult females in a 24-hour topical treatment.

Choi *et al.* (2004) tested 53 essential oils for their acaricidal activity by means of a filter paper diffusion bioassay. This screening also included the *L. officinalis* extract, as well as twelve other oils from Lamiaceae species. After 24 hours of treatment, they obtained 97% mortality only when lavender was used at  $19 \times 10^{-3} \mu\text{L mL}^{-1}$  air. As oil acaricidal activity was slight, the ovicidal effects were not examined.

The variations noted in the toxic doses and mite responses to *Lavandula* essential oils may be related, among other experimental facts, to these spider mites' distinct susceptibility, and to the chemical compositions of the tested oils, which obviously vary among species of the same plant genus (Choi *et al.*, 2004). It seems that *L. vera* and *L. officinalis* extracts are less active against *T. urticae* than spike lavender essential oil because 95-100% mortality was detected after only 1 h of topical treatment with the 0.20-0.25% oil doses (Table 2).

The spike lavender extract used in the residual toxicity experiments affected *T. urticae* fecundity as both the total number of eggs oviposited and emerging larvae decreased with increasing oil doses (Tables 3 and 4). As in other studies performed with essential oils (Lim *et al.*, 2011) or individual monoterpenes (Badawy *et al.*, 2010), we observed that *T. urticae* eggs were less sensitive than the mite adults. The present findings demonstrate that relatively low concentrations of spike lavender oil applied in residual contact bioassays have the potential to control *T. urticae* populations given its toxicity to adult females and its



deterrent effect on mite oviposition. It is feasible to suggest that reduced fecundity may be the result of an antifeedant effect of residual lavender oil, which could act as a distasteful substance for female spider mites. Moreover, temperature is a key factor for spider mite fecundity. If compared to available data about how temperature affects the fecundity and development of *T. urticae* populations, our data largely corroborate the results reported by Bounfour and Tanigoshi (2001) and Praslicka and Huszár (2004). These authors found that both the development and fecundity of *T. urticae* (eggs laid per female per day, and the total number of eggs) drastically decreased when exposed to temperatures below 15-20°C, whereas mite population increased with temperatures up to 30°C.

Some of the above-cited studies dealing with *L. vera* and *L. officinalis* have reported on the toxicity of their essential oils against adult spider mites, and have also evaluated their effects on mite fecundity. Thus, *L. vera* extract (0.1%) tested using a leaf-dip bioassay (Roh et al., 2011) was almost ineffective to reduce the total number of eggs laid. Roh et al. (2012) found similar results in their study for repellent activities of several oils against *T. urticae*; true lavender was one of the 14 essential oils that lowered the oviposition rates in the initial choice bioassays, but it was ineffective in a subsequent no-choice test.

Modarres-Najafabadi et al. (2012) employed a *L. officinalis* extract at high concentrations to determine its contact toxicity against *T. urticae* eggs. Oil was directly sprayed, at five concentrations, on eggs previously laid on bean leaves. The results demonstrated that hatchability markedly decreased, from 75 to 10%, with an increasing oil dose (from 0.5 to 4.0%), and confirmed that this lavender extract was toxic via direct contact not only for adult mites but also for their eggs. Another experiment with *L. officinalis* (Refaat et al., 2002) employed this essential oil in a repellency and oviposition deterrence test for *T. urticae*. At oil concentrations below

0.5%, some individuals laid their eggs, but females strongly rejected the oil-treated substrates and the total number of eggs decreased by more than 10-fold at high concentrations (1-2%).

These results on *Lavandula* spp. and other reported activities of numerous essential oils demonstrate that essential oils, or essential oil-based pesticides, can prove as effective as conventional synthetic products, particularly against soft-bodied insects and mites (Han et al., 2010; Isman et al., 2011).

In the present study, spike lavender essential oil was analyzed prior to performing the bioassays in order to assess its miticidal activity against two-spotted spider mites. These data indicate the acaricidal power of this natural extract, which had a negative impact on the phytophagous mite over time as it significantly reduced its population development. This effect was due to the combined result of increased mortality (direct toxicity of oil to adult mites) and reduced fecundity of adult females (diminished oviposition and larvae emergence). All this confirms the possibility of its use to control mite pests as an alternative measure to synthetic acaricides.

## ACKNOWLEDGMENTS

We thank Agrobainpa S.L. (Rincón de Ademuz, Valencia, Spain) for providing the spike lavender oil samples. We also thank Helen L. Warburton for checking the English.

## REFERENCES

1. Abbott, W. S. 1925. A Method of Computing the Effectiveness of an Insecticide. *J. Econ. Entomol.*, **18**: 265-267.
2. Attia, S., Grissa, K. L., Lognay, G., Bitume, E. T., Hance, T. and Mailleux, A. C. 2013. A Review of the Major Biological Approaches to Control the Worldwide Pest *Tetranychus urticae* (Acari: Tetranychidae)



- with Special Reference to Natural Pesticides. *J. Pest. Sci.*, **86**: 361–386.
3. Attia, S., Lebdi, K.G., Heuskin, S., Lognay, G. and Hance, T. 2015. An Analysis of Potential Resistance of the Phytophagous Mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) to Four Botanical Pesticides. *Biotechnol. Agron. Soc. Environ.*, **19**: 232-238.
  4. Babushok, V. I., Linstrom, P. J. and Zenkevichb, I. G. 2011. Retention Indices for Frequently Reported Compounds of Plant Essential Oils. *J. Phys. Chem. Ref. Data*, **40**(4): 043101.
  5. Badawy, M. E. I., El-Arami, S. A. A. and Abdelgaleil, S. A. M. 2010. Acaricidal and Quantitative Structure Activity Relationship of Monoterpenes against the Two-Spotted Spider Mite, *Tetranychus urticae*. *Exp. Appl. Acarol.*, **52**: 261-274.
  6. Bernardi, D., Botton, M., Silva da Cunha, U., Bernardi, O., Malausa, T., Silveira Garcia, M. and Edson Nava, D. 2013. Effects of Azadirachtin on *Tetranychus urticae* (Acari: Tetranychidae) and Its Compatibility with Predatory Mites (Acari: Phytoseiidae) on Strawberry. *Pest Manag. Sci.*, **69**: 75-80.
  7. Blenau, W., Rademacher, E. and Baumann, A. 2012. Plant Essential Oils and Formamidines as Insecticides/Acaricides: What Wre the Molecular Targets? *Apidologie*, **43**: 334–347.
  8. Bounfour, M. and Tanigoshi. L. K. 2001. Effect of Temperature on Development and Demographic Parameters of *Tetranychus urticae* and *Eotetranychus carpini borealis* (Acari: Tetranychidae). *Ann. Entomol. Soc. Am.*, **94**: 400-404.
  9. Cantrell, C. L., Dayan, F. E. and Duke, S. E. 2012. Natural Products as Sources for New Pesticides. *J. Nat. Prod.*, **75**: 1231–1242.
  10. Choi, W. I., Lee, S. G., Park, H. M. and Ahn, Y. J. 2004. Toxicity of Plant Essential Oils to *Tetranychus urticae* (Acari: Tetranychidae) and *Phytoseiulus persimilis* (Acari: Phytoseiidae). *J. Econ. Entomol.*, **97**: 553-558.
  11. Dayan, F. E., Owens, D. K. and Duke, S. O. 2012. Rationale for a Natural Products Approach to Herbicide Discovery. *Pest Manag. Sci.*, **68**: 519-528.
  12. Ebadollahi, A., Sendi, J. J., Maroufpoor, M. and Rahimi-Nasrabadi, M. 2017. Acaricidal Potentials of the Terpene-Rich Essential Oils of Two Iranian *Eucalyptus* Species against *Tetranychus urticae* Koch. *J. Oleo Sci.*, **66**: 307-314.
  13. Fatemikia, S., Abbasipour, H., Karimi, J., Saeedizadeh, A. and Gorjan, A. S. 2014. Efficacy of *Elettaria cardamomum* L. (Zingiberaceae) Essential Oil on the Two Spotted Spider Mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). *Arch. Phytopath. Plant Protect.*, **47**: 1008-1014.
  14. Han, J., Choi, B. R., Lee, S. G., Kim, S. I. and Ahn, Y. J. 2010. Toxicity of Plant Essential Oils to Acaricide-Susceptible and –Resistant *Tetranychus urticae* (Acari: Tetranychidae) and *Neoseiulus californicus* (Acari: Phytoseiidae). *J. Econ. Entomol.*, **103**: 1293-1298.
  15. Herráiz-Peñalver, D., Cases, M. A., Varela, F., Navarrete, P., Sánchez-Vioque, R. and Usano-Aleman, J. 2013. Chemical Characterization of *Lavandula latifolia* Medik. Essential Oil from Spanish Wild Populations. *Biochem. Syst. Ecol.*, **46**: 59-68.
  16. Isman, M. B., Miresmailli, S. and Machial, C. 2011. Commercial Opportunities for Pesticides Based on Plant Essentials Oils in Agriculture, Industry and Consumer Products. *Phytochem. Rev.*, **10**: 197-204.
  17. Kheradmand, K., Beynaghi, S., Asgari, S. and Sheykhi Garjan, A. 2015. Toxicity and Repellency Effects of Three Plant Essential Oils against Two-Spotted Spider Mite, *Tetranychus urticae* (Acari: Tetranychidae). *J. Agr. Sci. Tech.*, **17**: 1223-1232.
  18. Laborda, R., Manzano, I., Gamón, M., Gavidia, I., Pérez-Bermúdez, P. and Boluda, R. 2013. Effects of *Rosmarinus officinalis* and *Salvia officinalis* Essential Oils on *Tetranychus urticae* Koch (Acari: Tetranychidae). *Ind. Crops Prod.*, **48**: 106-110.
  19. Lim, E. G., Roh, H. S., Coudron, T. A. and Park, C. G. 2011. Temperature-Dependent Fumigant Activity of Essential Oils against Two-Spotted Spider Mite (Acari: Tetranychidae). *J. Econ. Entomol.*, **104**: 414-419.
  20. Miresmailli, S. and Isman, M. B. 2014. Botanical Insecticides Inspired by Plant–Herbivore Chemical Interactions. *Trend. Plant Sci.*, **19**: 29-35.



21. Miresmailli, S. and Isman, M. B. 2006. Efficacy and Persistence of Rosemary Oil as an Acaricide against Two Spotted Spider Mite (Acari: Tetranychidae) on Greenhouse Tomato. *J. Econ. Entomol.*, **99**: 2015-2023.
22. Miresmailli, S., Bradbury, R. and Isman, M. B. 2006. Comparative Toxicity of *Rosmarinus officinalis* L. Essential Oil and Blends of Its Major Constituents against *Tetranychus urticae* Koch (Acari: Tetranychidae) on Two Different Host Plants. *Pest. Manag. Sci.*, **62**: 366-371.
23. Modarres-Najafabadi, S. S., Taji, M. and Hajihassani, A. 2012. Study on *Thymus vulgaris*, *Lavandula officinalis* and *Eucalyptus camaldulensis* Extracts on the Two-Spotted Spider Mite. *Int. J. Agric. Sci.*, **2**: 228-236.
24. Moghadasi, M., Allahyari, H., Saboori, A. and Golpayegani, A. Z. 2016. Life Table and Predation Capacity of *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) Feeding on *Tetranychus urticae* Koch (Acari: Tetranychidae) on Rose. *J. Agr. Sci. Tech.*, **18**: 1279-1288.
25. Motazedian, N., Ravan, S. and Bandani, A. R. 2012. Toxicity and Repellency Effects of Three Essential Oils against *Tetranychus urticae* Koch (Acari: Tetranychidae). *J. Agr. Sci. Tech.*, **14**: 275-284.
26. Mozaffari, F., Abbasipour, H., Garjan, A.S., Saboori, A. and Mahmoudvand, M. 2012. Efficacy of *Thuja orientalis* L. (Cupressaceae) Essential Oil on the Two Spotted Spider Mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). *J. Essent. Oil Bear. Pl.*, **15**: 550-556.
27. NIST Chemistry Webbook (<http://webbook.nist.gov>). 2008. NIST Standard Reference Database Number 69. "Gas Chromatography—Retention Indices", (Eds.): Linstrom, P. J. and Mallard, W. G. NIST Mass Spectrometry Data Center, (S. E. Stein – Director), Versions: 2005 and 2008, NIST, Gaithersburg, MD.
28. Praslicka, J. and Huszár, J. 2004. Influence of Temperature and Host Plants on the Development and Fecundity of the Spider Mite *Tetranychus urticae* (Acarina: Tetranychidae). *Plant Protect. Sci.*, **40**: 141-144.
29. Rattan, R.S. 2010. Mechanism of Action of Insecticidal Secondary Metabolites of Plant Origin. *Crop Protect.*, **29**: 913-920.
30. Refaat, A. M., Momen, F. M. and Amer, A. A. 2002. Acaricidal Activity of Sweet Basil and French Lavender Essential Oils against Two Species of Mites of the Family Tetranychidae (Acari: Tetranychidae). *Acta Phytopathol. Entomol. Hung.*, **37**: 287-298.
31. Roh, H. S., Lim, E. G., Kim, J. and Park, C. G. 2011. Acaricidal and Oviposition Detering Effects of Santalol Identified in Sandalwood Oil against Two-Spotted Spider Mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). *J. Pest. Sci.*, **84**: 495-501.
32. Roh, H. S., Park, K. C. and Park, C. G. 2012. Repellent Effect of Santalol from Sandalwood Oil against *Tetranychus urticae* (Acari: Tetranychidae). *J. Econ. Entomol.*, **105**: 379-385.
33. Salido, S., Altarejos, J., Nogueras, M., Sánchez, A. and Luque, P. 2004. Chemical Composition and Seasonal Variations of Spike Lavender Oil from Southern Spain. *J. Essent. Oil Res.*, **16**: 206-210.
34. Tak, J. H. and Isman, M. B. 2017. Acaricidal and Repellent Activity of Plant Essential Oil-Derived Terpenes and the Effect of Binary Mixtures against *Tetranychus urticae* Koch (Acari: Tetranychidae). *Ind. Crop. Prod.*, **108**: 786-792.
35. Wu, Y. B., Ni, Z. Y., Shi, Q. W., Dong, M., Kiyota, H., Gu, Y. C. and Cong, B. 2012. Constituents from *Salvia* Species and Their Biological Activities. *Chem. Rev.*, **112**: 5967-6026.
36. Zarrad, K., Laarif, A., Ben Hamouda, A., Chaieb, I. and Mediouni-Ben Jemâa, J. 2017. Anticholinesterase Potential of Monoterpenoids on the Whitefly *Bemisia tabaci* and Their Kinetic Studies. *J. Agr. Sci. Tech.*, **19**: 643-652.

## اسانس سنبله اسطوخودوس باعث کاهش نرخ بقا و بارآوری کنه تارتن *Tetranychus urticae* (Acari: Tetranychidae) دو لکه ای می شود

ر. لابوردا، ی. مانزانو، م. گامون، ی. گاویدا، ر. بلودا، و پ. پرز-برمودز

### چکیده

کنه تارتن دو لکه ای، *Tetranychus urticae* Koch، باعث تلفات سنگین عملکرد در گیاهان مختلف می شود. این کنه بیشتر با استفاده از کنه کش های ساخته شده کنترل می شود و دیگر راه های مقابله با آن استفاده از کنه های شکارگر یا محصولات گیاهی طبیعی است. در این پژوهش، اثرات اسانس *Lavandula latifolia* Medik. (Lamiaceae) روی نرخ بقا و بارآوری *T. urticae* به روش slide-dip و آزمون زیستی دیسک-برگ بررسی شد. غلظت های مختلف اسانس سنبله اسطوخودوس منجر به مسمومیت تماسی شدید شد و در امولسیون هایی با غلظت حد اقل ۲٪ (v/v) مرگ و میر ۹۵-۱۰۰٪ بود. در آزمون های مسمومیت باقیمانده، اسانس اسطوخودوس (۱۵-۲۵٪) باعث کاهش بقای کنه و اثر گذاری روی بارآوری آن شد و تخمگذاری و رشد لاروها با افزایش غلظت اسانس کم شد. درجه حرارت دوره رشد رویانی در پایداری موفق تخم ها نقش تعیین کننده داشت: در حالیکه در ۱۲ درجه سانتی گراد لاروها اجازه رشد نمی یافتند، بیشترین درصد از تخم درآمدن در ۳۰ درجه سانتی گراد شمارش شد. نتایج پژوهش موید این مطلب بود که استفاده از اسانس سنبله اسطوخودوس برای جایگزینی آفتکش های رایج و سنتی امکان پذیر است.