How to Get Rid of the two spotted spider mites?

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SUMMARY
Two essential oils were tested for their toxicity against eggs and adults of \textit{Tetranychus urticae} Koch as well as adults of \textit{Phytoseiulus persimilis} Athias-Henriot, by using a filter paper diffusion bioassay without allowing direct contact. Responses varied according to oil type and dose, and mite species. The chemical analyses with GC-MS and GC-FID revealed that the two oils differed in their most abundant components. The most abundant components in the \textit{Citrus} oil were linalyl acetate (41.95\%), sabinene (18.60\%) and limonene (18.14\%) whereas pulegone (41.86\%) and menthone (28.33\%) were most prevalent in the \textit{Mentha} oil.

Mortality and fecundity were measured with 15 oils concentrations ranged from 0.01 to 8\ ml/l of air. Tetranychid mortality increased with increasing concentrations with \textit{LC}_{50} value of 5.39 and 4.09 \textmu l/l for \textit{C. aurantium} and \textit{M. pulegium}, respectively. However few mortality was observed in the case of \textit{P. persimilis} with \textit{LC}_{50} value of 0.46 and 0.26 \textmu l/l for \textit{C. aurantium} and \textit{M. pulegium} respectively. For both oils a reduction of fecundity was observed at 0.01 \textmu l/l in the case of \textit{T. urticae}. The essential oils described herein have potential interest as fumigants for the bio-control of \textit{T. urticae}.

Key words: \textit{Tetranychus urticae}, \textit{Phytoseiulus persimilis}, essential oils, fumigation, toxicity, pest management.

RESUME
Deux huiles essentielles ont été testées par fumigation sur les œufs et les adultes de \textit{Tetranychus urticae} Koch ainsi que sur \textit{Phytoseiulus persimilis} Athias-Henriot. L’effet de ces huiles sur les acariens dépend de l’espèce de plante, de la dose appliquée et aussi de l’espèce d’acarien. L’analyse chimique par GC-MS et GC-FID montre que les deux huiles varient selon leur composition chimique. Le linalyl acétate était le composé le plus abondant (41.95\%) chez \textit{Citrus aurantium} suivi par le sabinène (18.60\%) et le limonène (18.14\%) tandis que le pulegone était le composé le plus abondant chez l’huile essentielle de \textit{Mentha pulegium} avec 41.86\%, suivi par le menthone avec 28.33\%. 15 concentrations des deux huiles (variant de 0.01 à 8\ ml/l d’air) ont été appliquées pour tester la mortalité et la fécondité chez les acariens. L’analyse probit a montré que la mortalité de \textit{T. urticae} a augmenté avec l’augmentation de la concentration avec \textit{LC}_{50} de 5.39 et 4.09 \textmu l/l d’air pour \textit{C. aurantium} et \textit{M. pulegium} respectivement. Cependant peu de mortalité a été observée dans le cas de \textit{P. persimilis} avec \textit{LC}_{50} de 0.46 et 0.26 \textmu l/l d’air pour \textit{C. aurantium} et \textit{M. pulegium}. Une réduction de la fécondité a été observée chez \textit{T. urticae} suite au traitement par les deux huiles à une concentration de 0.01 \textmu l/l. Ceci nous laisse envisager, la possibilité de l’utilisation de ces huiles essentielles par fumigation dans la lutte intégrée contre \textit{T. urticae}.

Mots clés: \textit{Tetranychus urticae}, \textit{Phytoseiulus persimilis}, huiles essentielles, fumigation, toxicité, lutte intégrée

1. INTRODUCTION
The Two spotted spider mite is an important pest in many countries around the world. This ubiquitous mite can live in temperate and subtropical zones with temperatures ranging from 7.5\* to 44\*C (Migeon & Dorkeld, 2007). It is a phytophagous pest that can cause significant yield losses in many agricultural crops, including fruits, cotton, vegetables and ornamentals (Stumpf et al., 2001; Van Leeuwen et al., 2007). To date, 3877 host species have been reported either in outdoor crops or in greenhouse (Migeon & Dorkeld, 2007). From the larval stage to adult, mites feed preferentially on the lower surface of the
leaf (Johnson & Lyon, 1991). The plant could be affected by different ways: decrease in photosynthesis, injection of phytotoxic substances when feeding, accumulation of feces, webbing or defoliation which could affect the plant aspect (Johnson & Lyon, 1991). Yield losses can approach 15% on strawberry in USA, 14% on corn in France, 14 to 44% on cotton (Kreiter, 2011). Common methods to control this pest are cultural, chemicals and biological practices (Powell & Lindquist, 1997; Bethke et al., 2004). Synthetic acaricides have been widely used for the control of T. urticae (Sundaram et al., 1995, Van Leeuwen et al., 2006). However, due to the excessive use of pesticides and the associated problems of resistance and environmental pollution, there is an increasing demand for sustainable, environmental-friendly control methods. So, biological control of spider mites has been tried successfully as an alternative method to chemical methods (Osborne et al., 1985; Kropczynska et al., 1999; Naher & Haque, 2007). which sometimes fail to keep the number of spider mites under economic threshold levels (Duso et al., 2008). It is therefore crucial to find selective pesticides which can integrate the action of natural enemies and guarantee the safety of environment and mammalians (Steiner et al., 2011).

In this context, essential oils are realistic alternatives to synthetic acaricides because of their selectivity, biodegradability and few side effects on non-target organisms and the environment (Hay & Waterman, 1993; Isman, 2000, 2001; Chiasson et al., 2001; Bastia & Spooner-Haart, 2002; Rasikari et al., 2005; Pontes et al., 2007; Calvacanti et al., 2010). Essential oils have been used to control pests as alternative insecticides in various parts of the world (Attia et al., 2012; Attia et al., 2013; Isman, 2000). Moreover, essential oils may delay the development of resistance (Attia, 2012). This is due to their several modes of action, including repellent and antifeedant activities, inhibition of molting and respiration, reduction in growth and fecundity, cuticle disruption, and effects on the invertebrate octopamine pathway (Saxena, 1989; Isman, 2000; Enan, 2001).

The aim of this work is to assess the potential acaricidal activity of Mentha pulegium and Citrus aurantium essential oils as fumigants against T. urticae and P. persimilis. This study is in line with previous work that showed the toxicity of several plant extracts on the two spotted spider mites (Attia et al., 2011a, 2011b, 2011c; Attia, 2012; Attia et al., 2013). EL-Khodary et al. (2007) highlighted the contact toxicity of these two essential oils. Other route of exposure such as fumigation is useful to test and better understand the acaricidal properties of M. pulegium and C. aurantium. The study presented herein aimed to assess the acaricidal activity of Citrus aurantium and Mentha pulegium essential oils against the two spotted mite Tetranychus urticae and its predator Phytoseiulus persimilis.

2. MATERIALS AND METHOD

2.1. Spider mites

For this experiment, we used a carmine spider mite T. urticae Koch. This population collected from infested plants in citrus orchards (Tunisia) has not come in contact with any chemicals for more than six years. The strain was reared on bean leaves placed on moistened cotton in Petri dishes (Overmeer, 1985) under controlled conditions (26°C, 50-60% RH, 16:8 (L:D)) in the laboratory of the biodiversity Research Centre, UCL, Louvain-la-Neuve (Belgium).

3.2. Predatory mites

Predatory mites P. persimilis were purchased from Koppert Biological Systems (Netherlands). They were transferred to a spider mite colony maintained on bean plants caged in the greenhouse. Only young adult females (24 h old) were chosen for the bioassay.

2.3. M. pulegium and C. aurantium essential oils

M. pulegium and C. aurantium used for this study were selected based on previously reported activity against T. urticae (Attia et al., 2011c). They were collected locally in Tunisia (Hammamet, North of Tunisia) in June 2010, and were free of any pre-harvest chemical treatments (organic products). The essential oils were obtained from 10 kg of flowers by hydrodistillation for 3 hours using a Clever-type apparatus. The oil yield was 0.4% and 0.1% of the dry weight of C. aurantium and M. pulegium respectively (Attia et al., 2011c).
2.4. Chemical analyses
Essential oils were analyzed by GC-MS in the Laboratory of Analytical Chemistry in Gembloux Agro-Biotech (University of Liège (Belgium)). For quantitative analyses (percentage determination), we used a Fast GC according to Heuskin et al., 2009.

2.4.1. GC-MS analyses
GC-MS analyses of the essential oils were performed by using an Agilent GC 7975 coupled with an El mass selective detector (Agilent, United states) and equipped with an HP5-MS capillary column (30m × 0.25mm I.D., 0.25μm film thickness). The oven temperature program was initiated at 40°C, held for 5 min at this temperature, then raised at a rate of 6°C/min to 120°C, held for 5 min, then raised in a second ramp at a rate of 8°C/min to 300°C. Helium was used as a carrier gas at a constant flow rate of 1 mL/min. An injection volume of 1μL was used, in splitless mode. The injection temperature was 250°C. MS detection was performed in electron impact (EI) mode at 70 eV, full-scan acquisition mode from 40-550 amu range. Volatile compounds were identified by comparing their mass spectra with those from the Wiley 275 L spectral library and with retention indices which were determined according to the retention times of a series of C9-C30 n-alkane standards (Sigma-Aldrich, 0.025μg/μL in n-hexane) and compared to literature values (Adams, 2001).

2.4.2. Fast GC Analyses
Fast GC analyses were conducted on a Thermo Ultra-Fast Trace GC gas chromatograph, operated with a split/splitless injector and a Thermo AS 3000 autosampler (Thermo Electron Corp.). The GC system was equipped with an ultra-fast module (UFM) incorporating a direct resistively heated column (Thermo Electron Corp.): UF-5, 5% phenyl, 5 m × 0.1 mm I.D., 0.1 μm film thickness. The following chromatographic conditions were used to obtain suitable peak resolution. The temperature program was as follows: initial temperature at 40 °C, held for 0.1 min, ramp 1 at 30 °C min⁻¹ to 95 °C, ramp 2 at 35 °C min⁻¹ to 155 °C, ramp 3 at 200 °C min⁻¹ to 280 °C, held for 0.5 min. Injection temperature: 240 °C; injection volume: 1 μL; Carrier gas: He, at a constant flow rate of 0.5 mL min⁻¹; split ratio = 1:100. The flame ionization detector (300 Hz), was maintained at 250 °C. Data processing was performed using Chromcard software (version 2.3.3).

The composition of the essential oil of M. pulegium has been reported in another paper by the same authors (Attia et al., 2011c).

2.5. Fumigant toxicity

2.5.1. Mortality
The fumigant tests of the two essential oils were determined in tightly closed glass containers of 1L (Kouninki et al., 2007). The acaricidal effect of 15 concentrations of the two essential oils was investigated which correspond to 0.01, 0.02, 0.04, 0.05, 0.06, 0.08, 0.1, 0.5, 2, 3, 4, 5, 6, 7 and 8 μL/l of air respectively. A group of 25 young females aged 24 h was randomly selected and then transferred to fresh bean leaf discs (diameter 35 mm) placed with the adaxial side up on the moistened cotton in Petri dish (90x15 mm). Each Petri dish was brought into the glass recipient. The different doses of essential oils were introduced into the glass, outside the Petri dish in order to avoid contact with the mites. Just after, the glass receptacle was closed above with a metal cover on which 5 holes were drilled to allow air exchange (Kouniniki et al., 2007). The number of dead individuals was counted daily up to 3 days. Evaluation to determine mortality in each exposure time was made with a slight touch on the mite with a fine haired brush. If they did not move their appendages, they were considered as dead. For each concentration, after using Abbot's corrections, we calculated the mortality rate using this formula: mortality rate = (mean number of deaths with each concentration - mean number of deaths in the control) / total number of females at the beginning of the tests. The data obtained in this experiment were also submitted to a probit analysis (Finney & Stevens, 1948).

3.5.2. Fecundity
Only young adults (24h old) females were chosen for the bioassay. Here, one sub-lethal concentration (0.01 μL/l of air) was used to test the effect of these oils on T. urticae fecundity (25 females). Each individual was transferred to a bean leaf disc (diameter = 15 mm) to check for fecundity. The Petri dish was brought into test chamber, spiked with essential oil and glass closed. The number of eggs laid
by treated females was recorded for a period of 12 days, before being destroyed. The number of eggs was best fit to a sigmoidal curve (GraphPad Prism, Copeland, 2000), using the formula \( Y = M \times X^b / (K + X^b) \), where \( Y \) represents the value of the cumulative number of eggs at age 'X', \( K \) is equal to the inflexion point when \( h=1 \), \( M \) is the maximum number of eggs (plateau value) and \( h \) represents the slope. The fecundity of treated females was compared with a control.

2.6. Data analysis
Probit analysis was used to determine LD_{10}, LD_{90} and DL_{100} values, using the Statplus program version 2009 (AnalystSoft Inc). Tests were performed using One-way Analysis of Variance (ANOVA). Newman-Keuls tests were used to compare means using Graph Pad Prism version 5.01 for windows (Graph Pad Software, San Diego, California, USA, http://www.graphPad.com). All tests were applied under the two-tailed hypothesis, with the level of statistical significance (p) set at 0.05.

3. RESULTS
3.1. Component analysis of the essential oils
The chemical compositions of the essential oil of *C. aurantium* evaluated in this study are shown in Table 1. Experimental retention indices were compared with literature values (Adams, 2001) and EI mass spectra from each peak were compared with the spectral library. Using this approach, it was possible to identify 14 components from *C. aurantium* representing 99.32% of the total constituents. The two oils differed in their most abundant components. The most abundant components in the *Citrus* oil were linalyl acetate (41.95%), sabinene (18.60%) and linalool (18.14%) (Table 1); whereas pulegone (41.86%) and menthone (28.33%) were most prevalent in the *Mentha* oil sample (Attia et al., 2011c).

<table>
<thead>
<tr>
<th>Components</th>
<th>Retention time (min)</th>
<th>Retention index (measured)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-pinene</td>
<td>9.29</td>
<td>950</td>
<td>0.3</td>
</tr>
<tr>
<td>sabinene</td>
<td>10.19</td>
<td>972</td>
<td>18.6</td>
</tr>
<tr>
<td>myrcene</td>
<td>10.56</td>
<td>990</td>
<td>2.66</td>
</tr>
<tr>
<td>limonene</td>
<td>11.30</td>
<td>1029</td>
<td>1.95</td>
</tr>
<tr>
<td>β-ocinene</td>
<td>11.47</td>
<td>1038</td>
<td>5.21</td>
</tr>
<tr>
<td>terpinolene</td>
<td>12.40</td>
<td>1090</td>
<td>0.72</td>
</tr>
<tr>
<td>linalool</td>
<td>12.58</td>
<td>1097</td>
<td>18.14</td>
</tr>
<tr>
<td>terpineol</td>
<td>13.90</td>
<td>1182</td>
<td>1.13</td>
</tr>
<tr>
<td>α-terpineol</td>
<td>14.10</td>
<td>1195</td>
<td>3.57</td>
</tr>
<tr>
<td>pulegone</td>
<td>14.86</td>
<td>1246</td>
<td>0.77</td>
</tr>
<tr>
<td>linalyl acetate</td>
<td>15.03</td>
<td>1257</td>
<td>41.95</td>
</tr>
<tr>
<td>neryl acetate</td>
<td>16.54</td>
<td>1365</td>
<td>1.47</td>
</tr>
<tr>
<td>germacre and</td>
<td>16.80</td>
<td>1384</td>
<td>2.56</td>
</tr>
<tr>
<td>β-caryophylenne</td>
<td>17.45</td>
<td>1434</td>
<td>0.73</td>
</tr>
</tbody>
</table>

3.2. Effect of essential oils on mortality
After 72 hours, few mortalities were observed in control group of *T. urticae* and *P. persimilis*. A few mortalities were observed at 0.01μl/l of air of each essential oil, and acaricidal activity was enhanced with increasing concentrations of oils.

Spider mites: There was a significant difference between the 2 treatments with *M. pulegium* and *C. aurantium* essential oils (F (15,64) = 123.86, P<0.001; F (15,64) = 147.47, P<0.001, respectively, when comparing the different concentrations to control. Interestingly, *M. pulegium* provided better mite control (DL_{50} and DL_{90} values of 4.09 and 5.62 μl/l of air) than *C. aurantium* oil with 5.39 and 8.07 μl/l, respectively.

Predatory mites: There was a significant difference between the 2 treatments with *M. pulegium* and *C. aurantium* essential oils (F (15,64) = 37.31, P<0.001; F (15,64) = 4.61, P<0.001, respectively, when comparing the different concentrations to control. Interestingly, the two essential oils are more toxic against *T. urticae* than *P. persimilis* with (DL_{50} and DL_{90} values of 0.26 and 19.91 μl/l for *M. pulegium* and with 0.46 and 25.49 μl/l for *C. aurantium* essential oil respectively.)

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3.3. Effect of essential oils on Fecondity of *T. urticae*

*M. pulegium* and *C. aurantiun* affected fecundity of *T. urticae* at a dose of 0.01 μl/l (figure 1). Maximum values for the cumulative number of eggs were significantly reduced compared to the control (Table 2), while the other two parameters were not statistically different. Treatments with *C. aurantiun* and *M. pulegium* essential oils reduced the number of eggs laid by females to 20 and 10 eggs respectively. Experimental data were fit to a sigmoidal curve by the method of least squares ordinary fit.

**Table 2.** Comparison between the cumulative numbers of eggs laid by the females treated with the two essentials at 0.01 μl/l and with the control solution. Parameters: Top = the plateau value indicating the maximum number of offspring, h = the hill slope.

<table>
<thead>
<tr>
<th>95% Confidence Intervals</th>
<th><em>C. aurantiun</em> oil</th>
<th><em>M. pulegium</em> oil</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top</td>
<td>19.62 to 23.84</td>
<td>7.959 to 9.723</td>
<td>69.03 to 83.87</td>
</tr>
<tr>
<td>LogECS0</td>
<td>4.168 to 5.798</td>
<td>-2.764 to 6.223</td>
<td>5.461 to 6.604</td>
</tr>
<tr>
<td>h</td>
<td>0.1442 to 0.4490</td>
<td>0.03195 to 0.4464</td>
<td>0.1621 to 0.3571</td>
</tr>
<tr>
<td>ECS0</td>
<td>14722 to 627585</td>
<td>0.001724 to 1.671e+06</td>
<td>289324 to 4.022e+06</td>
</tr>
</tbody>
</table>

**Figure 1.** Cumulative number of eggs laid by the females treated with both 0.01 μl/l *M. pulegium* and *C. aurantiun* essential oils

4. DISCUSSIONS

Our study showed that *M. pulegium* and *C. aurantiun* exhibit a high mortality rate when applied as fumigants on *T. urticae* females with LC50 at 4.09 μl/l and 5.39 μl/l, LC90 at 5.67 μl/l and 8.07 μl/l and LC100 at 5.93 μl/l and 8.56 μl/l respectively. However, these two oils provided a low mortality when applied as fumigants on *P. persimilis* with LC50 at 0.26 μl/l and LC90 of 19.96 μl/l with *M. pulegium* and with LC50 at 0.46 μl/l and LC90 of 25.49 μl/l with *C. aurantiun*, respectively.

This is in agreement with the previous study undertaken by Choi et al. (2004) which demonstrated that several essential oils including *M. pulegium* and *C. aurantiun* cause significant mortality as fumigants on the two-spotted spider mite at very low dose (19 μl/l of air). Our study showed that our essential oils were the most toxic to *T. urticae* at very low doses compared to the study of Choi et al. (2004) but as we know, our study is the first to study the effect of essential oils on the predatory mite *P. persimilis*. In general, higher mortality was observed as the doses of essential oils and exposure time increased. Regarding their effects on fecundity at the tested concentration (0.01 μl/l), both oils reduced the number of the eggs laid 20 and 10 for *C. aurantiun* and *M. pulegium* oils respectively in comparison with control group (75 eggs). Recently, Araujo et al. (2010), studying acaricidal effects of three citrus species *Citrus sinensis*, *C. sinensis*, and
C. aurantium cultivated in North east Brazil underlined the fumigant toxicity of C. aurantium with a LC50 value of 1.63 µl/.

In Attia et al. (2011a), 31 plant extracts obtained from Tunisia and two synthetic acaricides (spiromidofen and fenbubutin oxide) were assessed on T. urticae (Koch). Field experiments showed that the extracts of seven plant species (Haplophylum tuberculatum, Deverra scoparia, Mentha pulegium, Chrysanthemum coronarium, Herta cheirifolia, Citrus aurantium and Santolins africana) were effective and the population density of T. urticae was reduced at 0.30, 0.36, 0.37, 0.46, 0.48, 0.50, and 0.53 mites per leaf respectively for more than 21 days compared with the untreated control (3.7 mites per leaf). They also showed a comparable activity to classical synthetic acaricides (0.50 mites per leaf for Spiromidofen @ and 0.53 mites per leaf for Fenbubutin oxide ™). The evaluation of the potential of biologically active plant volatiles against T. urticae might provide a new approach to the development of natural acaricides to be used both in biological and integrated pest management strategies for controlling two-spotted spider mites in Tunisian citrus orchards (Attia et al., 2011a).

The same authors investigated the essential oil of Deverra scoparia for its acaricidal activity against T. urticae and they showed that female mortality increased with D. scoparia oil concentrations with LD50 and LD99 values of 1.79 mg/l and 3.2 mg/l respectively and a reduction in fecundity had already been observed for concentrations of 0.64, 0.08, 0.26 mg/l (Attia et al., 2011b). Attia et al., 2011a showed that M. pulegium with 91% of mortality were more toxic than C. aurantium (55%) against T. urticae when applied with contact. Similar findings are observed in our study looking lethal concentrations and effects on oviposition. This phenomenon should be explained by the difference in secondary metabolites found in each essential oil. We found that M. pulegium essential oils were mainly composed of pulegone (41.86%) followed by menthone (28.33%), limone (9.02%) 3-octanol (6.93%) (Attia et al., 2011c) while linalyl acetate (41.95%), linalool (18.14%) and sabine (18.6%) were the most abundant constituents in C. aurantium essential oil. This is in accordance with several other authors (Boussada & Chenli, 2007; Elhoussine et al., 2010; Hosni et al., 2010). Another important fact is that, the compounds similar in both oils were very different in percentage so that it could explain the difference in their toxic effects. However, Essential oil accumulation and compositions in aromatic plants depend upon various factors such as genetic structure, environmental factors and agronomic practices (Telesi, et al., 2010; Isman & Machial, 2006).

These secondary metabolites the most probably act synergistically to obtain high toxic effect with multiple modes of action (fecundity and mortality). Individually, some volatiles found in M. pulegium and C. aurantium extracts are known to cause mortality on T. urticae at different rates (α-pinene, pulegone, sabine) and the most toxic constituent, α-pinene, had no effect on fecundity suggesting that oviposition could be reduced by other constituents (Attia et al., 2011b). In the other hand linalool, citral, 1,8-cineole, p-cymene linalyl acetate, thymol, 3-octanol, β-pinene, sabine, pulegone, eugenol, carvacrol, citronellal, menthone, terpineol, geranul acetate are known to act as secondary metabolites in some plant extracts against various pest (Ayvaz et al., 2010; Calmasur et al., 2006; Karabörkli et al., 2010; Rim & Lee, 2006, Palacios et al., 2009). The essential oils tested in this study include one or more of these substances which were reported to be poisonous to insect and mite pests. Essential oils used in our experiment seem to have better results as fumigants than as spray. Indeed, severe lethal concentrations in our study are far below those causing high rate of mortality when applied topically by Attia (2012). It is therefore possible that like in microorganisms (Soysal et al., 2006), the volatile phases of the essential oils could be more toxic than the contact phase to the two spotted spider mites. This is supported by the work of George et al. (2009); their papers reported that when exposed to the vapour phase of three oils (thyme, manuka and pennyroyal) in closed vessels, mortality of the poultry red mite Dermatophagoides gallinae was always significantly greater than if the oil was presented in an open vessel. Other researchers have shown that M. pulegium essential oils present a very high mortality on Ceratitis capitata with over 90% of mortality achieved after 48 hours of exposure (Miguel et al., 2010).Another study highlighted the insecticidal properties of Pennyroyal oil and the compounds pulegone, menthone, 1,8-cineole, and camphor against the pest Drosophila melanogaster and Bactrocera oleae (Diptera : Tephritidae) (Pavlidou et al., 2004).In Ribeiro et al. (2010), the fumigant toxicity of peels essential oils of C. aurantium and C.sinensis cultivated in north east Brazil against Bemisia tabaci Biotype B with lethal concentrations of 380 ml/L and 580 ml/L of air respectively.Larvicidal activities of Greek plants of the Rutaceae family have been underlined by Michaelakis et al. (2009). In their study, essential oils of orange (Citrus sinensis L.), lemon (Citrus
limon L.), and bitter orange (Citrus aurantium L.) exhibited strong toxicity against mosquito larvae of culex pipiens (Diptera: Culicidae), with the LC50 values ranging from 30.1 (lemon) to 51.5 mg/l (orange) depending on citrus species and their composition.

In integrated mite management in greenhouses, the use of chemical pesticides (such as Abaranectine®), and of biological control agents are essential components. These two methods are, unfortunately, incompatible due to toxicity of chemical acaricides to predatory mites (Lee, 1997). In many countries, some predatory mites (including P. persimilis) showed good efficacy in the control of T. urticae. In our study, we found that C. aurantium and M. pulegium essential oils induced a few mortality rate of P. persimilis at very low doses compared to T. urticae. Choi et al. (2004) showed that at 7.1 x 10⁻³ μL.mL⁻¹, essential oils of caraway seed, citronella java, lemon eucalyptus, pennyroyal, peppermint, sage and spearmint were highly toxic to the predatory mite P. persimilis (90% mortality). This suggests that C. aurantium and M. pulegium essential oils could be used in the integrated management against T. urticae.

Until now, because of their mode of action affecting several targets at the same time, generally, no particular resistance or adaptation to essential oils has been described (Van Leeuwen, 2010). These findings support the use of these oils against the two-spotted spider mite and other pest in greenhouses.

RÉFÉRENCES BIBLIOGRAPHIQUES
- Araujo CP, Da Camara CAG, Neves et al. (2010). Acaricidal Activity against Tetranychus urticae and Chemical Composition of Peel Essential Oils of Three citrus Species Cultivated in Brazil: Natural Product Communications, 5, 471-476.


