How to Get Rid of the two spotted spider mites?

Sabrine Attia[a*], Kaouthar Lebdi Grissa[b], Stéphanie Heuskin[c], Georges Lognay[c], Anne Catherine Mailleux[a], Thierry Hance[a]

[a]Earth and Life Institute, Biodiversity Research Centre, Université Catholique de Louvain, 4-5, Place Croix du sud, B- 1348 Louvain-la-Neuve, Belgium.

[b]Laboratoire d'Entomologie-acarologie. Institut National Agronomique de Tunisie, 1082 Cité Mahrajène, Tunis, Tunisia.

[c]Université de Liège, Gembloux Agro-Bio Tech, Laboratoire de Chimie Analytique, Passage des Déportés, 2, B-5030 Gembloux, Belgium.

Corresponding author: sabrine.attia@uclouvain.be, sabine_bio5@yahoo.fr

SUMMARY

Two essential oils were tested for their toxicity against eggs and adults of *Tetranychus urticae* Koch as well as adults of *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 *Citrus* oil were linalyl acetate (41.95%), sabinene (18.60%) and linalool (18.14%) whereas pulegone (41.86%) and menthone (28.33%) were most prevalent in the *Mentha* oil.

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

Key words: *Tetranychus urticae, Phytoseilus persimilis*, essential oils, fumigation, toxicity, pest management.

RESUME

Deux huiles essentielles ont été testées par fumigation sur les œufs etles adultes de *Tetranychus urticae* Koch ainsi que sur *Phytoseilius 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 *Citrus aurantium* suivi par le sabinene (18.60%) et le linalool (18.14%) tandis que le pulegone était le composé le plus abondant chez l'huile essentielle de *Mentha pulegium* avec 41.86%, suivi par le menthone avec 28.33%. 15 concentrations des deux huiles (variant de 0.01 à 8 µl/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 *T. urticae* et a augmenté avec l'augmentation de la concentration avec LC_{50} de 5.39 et 4.09 µl/l d'air pour *C. aurantium* et *M. pulegium* respectivement. Cependant peu de mortalité a été observée dans le cas de *P. persimilis* avec LC_{50} de 0.46 et 0.26 µl/l d'air pour *C. aurantium* et *M. pulegium*. Une réduction de fécondité à été observée chez *T. urticae* suite au traitement par les deux huiles à une concentration de 0.01 µl/l. Ceci nous laisse envisager, la possibilité de l'utilisation de ces huiles essentielles par fumigation dans la lutte intégrée contre *T. urticae*.

Mots clés: *Tetranychus urticae, Phytoseilus 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 intemperate and subtropical zoneswith temperaturesranging from 7.5to 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; Basta & 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 effect on the invertebrate octopamine pathway (Saxena, 1989; Isman, 2000; Enan, 2001).

The aim of this work is to assess the potential acaricidal activities of *Mentha pulegium* and *Citrus aurantium* essential oils as fumigants against *T. urticae* and *P. persimilis*.This studyis in line withprevious workthat showedthe toxicityof several plant extracts on the two spotted spider mite (Attia *et al.*, 2011a, 2011b, 2011c; Attia, 2012; Attia *et al.*, 2013). EL-Khodary *et al.* (2007) highlighted the contact toxicity of these two essentials 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 *Phytoseilus 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 mitres

Predatory mites *P. persimilis* were purchased from Koppert Biological Systems (Netherlands). They were transferred to a spider mite colony maintained on beans 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 Clevenger-type apparatus. The oil yield was 0.4 % and 0.1 % of the dry weight of *C. aurantium* and *M. pulegium* respectively (Attia *etal.*, 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 *etal.*, 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 EI 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.): UFC-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 differentdoses of essential oils were introduced into theglass,outside thePetri dishin order toavoid contact withmites. Just after, the glass receptacle was closed above with a metal cover on which 5 holes were drilled to allow air exchange (Kouninki *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) femaleswere 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 $Y=M^* X^h/(K' + X^h)$, 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_{50}, 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 1. Major constituents in *C. aurantium* essential oil and their relative proportions in the pure oil. Components were identified by GC-MS and quantified by Fast GC-FID (Area %)).

Components	Retention time (min)	Retention index (measured)	%
α-pinene	9.29	950	0.3
sabinene	10.19	972	18.6
myrcene	10.56	990	2.66
limonene	11.30	1029	1.95
β-ocimene	11.47	1038	5.21
terpinolene	12.40	1090	0.72
linalool	12.58	1097	18.14
terpineol	13.90	1182	1.13
α-terpineol	14.10	1195	3.57
pulegone	14.86	1246	0.77
linalyl acetate	15.03	1257	41.95
neryl acetate	16.54	1365	1.47
gernayl acetate	16.80	1384	2.56
E- caryophyllenne	17.45	1434	0.73

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.

3.3. Effect of essential oils on Fecundity of T. urticae

M. pulegium and *C. aurantium* affected fecundity of *T. uricae* at a dose of 0.01µl/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. aurantium* 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.

95% Confidence Intervals	*C. aurantium* oil	*M. pulegium* oil	Control
Top	19.62 to 23.84	7.959 to 9.723	69.05 to 83.87
LogEC50	4.168 to 5.798	-2.764 to 6.223	5.461 to 6.604
h	0.1442 to 0.4490	0.03195 to 0.4464	0.1621 to 0.3571
EC50	14722 to 627585	0.001724 to 1.671e+006	289324 to 4.022e+006

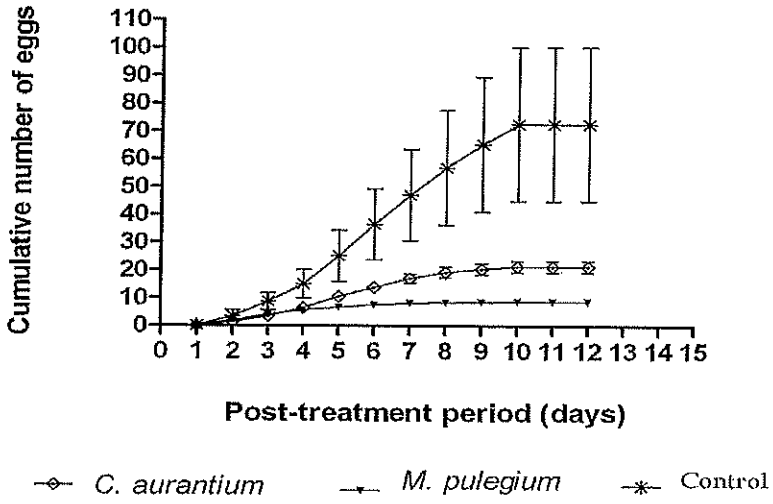

Figure 1. Cumulative number of eggs laid by the females treated with both 0, 01µl/l *M. pulegium* and *C. aurantium* essential oils

4. DISCUSSIONS

Our study showed that *M. pulegium* and *C. aurantium* exhibit a high mortality rate when applied as fumigants on *T. urticae* females with LC_{50} at 4.09 µl/l and 5.39 µl/l , LC_{90} at 5,67µl/l and 8.07µl/l and LC_{100} 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 LC_{50} at 0.26 µl/l and LC_{90} of 19.96 µl/l with *M. pulegium* and with LC_{50} at 0.46 µl/l and LC_{90} of 25.49 µl/l with *C. aurantium*, 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. aurantium* causes 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 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 (0.01µl/l), both oils reduced the number of the eggs laid 20 and 10 for *C. aurantium* 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 LC_{50} value of 1.63 µl/l .

In Attia *et al.* (2011a), 31 plant extracts obtained from Tunisia and two synthetic acaricides (spirodiclofen and fenbutatin oxide) were assessed on *T. urticae* (Koch). Field experiments showed that the extracts of seven plant species (*Haplophyllum tuberculatum, Deverra scoparia, Mentha pulegium, Chrysanthemum coronarium, Hertia cheirifolia, Citrus aurantium and Santolina africana*) are 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 Spirodiclofen ® and 0.53 mites per leaf for Fenbutatin 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 LD_{50} and LD_{90} values of 1.79 mg/l and 3.2 mg/l respectively and a reduction in fecundity had already been observed for concentrations of 0.064, 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%), limonene (9.02%) 3-octanol (6.93%) (Attia *et al.*, 2011c) while linalyl acetate (41.95%), linalool (18.14%) and sabinene (18.6%) were the most abundant constituents in *C. aurantium* essential oil. This is in accordance with several other authors (Boussaada & Chemli, 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 (Telci *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, sabinene) 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 , sabinene, 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; Karabörklü *et al.*,2010; Rim& Jee, 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 (Soylu *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 *Dermanyssus. 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 toxicityagainst 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 Abamectine ®), 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 7.1×10^{-3} $\mu l.ml^{-1}$ 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.

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