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Insecticidal activity of *Lavandula angustifolia* Mill against the pea aphid *Acyrthosiphon pisum*

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Abstract
The essential oil of *Lavandula angustifolia* was investigated for its biocide activity against *Acyrthosiphon pisum* by fumigation. The oil was characterised by GC-MS revealing that linalool was the most abundant component (38.57%), followed by linalyl acetate (29.95%), 1,8-cineole (13,66%), camphor (13,13%), β-pinene (3,14%) and terpinene-4-ol (1,54%). The Mortality was measured upon treatment with oil concentrations ranging from 5 to 25 $\mu l.l^{-1}$ of air. The mortality of aphids increased with oil concentration and LC_{50} values were determined to be 11.2 $\mu l.l^{-1}$ of air. The full mixture was also prepared and toxicity was compared with individual constituents. The results showed that the presence of all constituents were necessary to equal the toxicity of the natural oil. *L. angustifolia* oil can provide valuable pesticide activity with significantly lower LC_{50} values.

Keywords: Insecticidal activity, *Lavandula angustifolia* Mill, *Acyrthosiphon pisum*

1. Introduction
Essential oils from aromatic plants are promising tools for insect control [1]. For instance, they have been used to control pests of the stored products as alternative insecticides in various parts of the world [2, 3] and their toxic activity on mite is also well documented [4]. Previous studies reported also that different concentrations of various plants resulted in different insecticidal activity against larvae of pine processionary moth, *Thaumetopoea pityocampa* Schiff [5]. Various essential oils from plant belonging to the Lamiaceae family may also inhibit the feeding behaviour of aphids and may also present some toxic effect [6]. Perturbation of behaviour are probably the consequence of the high content in (*E*)-β-farnesene of some species [7].
In spite of the intense control strategies applied so far, aphids remain among the major agricultural pests that have expanded their damage to crops all over the world [8]. For example in Europe, direct damage by aphids is responsible for average annual losses of 700,000 t of wheat, 850,000 t of potatoes and 2,000,000 t of sugar beet [9]. Moreover, Due to the excessive use of pesticides and the associated problems of resistance and environmental pollution, there is an increasing demand for sustainable, environmentally friendly control methods. In that context, the European Union (EU Parliament and EU Council) has adopted a directive that all EU countries will convert to the use of integrated pest IMP (Integrated Pest Management) in agricultural production by 2014, by decreasing the use of insecticides. In addition, resistance to insecticide is widespread in aphid [10]. Although biological control of aphids in greenhouses is successful, reduction of high populations with non-persistent chemicals is sometimes necessary before introduction of parasitoids or when the circumstances for biological control are unfavourable [11].
It is therefore important to find new, selective pesticides compatible with the use of natural enemies that can minimise negative effects on the environment, including both fauna and flora. Plant essential oils may be more effective for control in practice. In fact aphid is an important vector of plant viruses [12] and causes growth reduction of plants. Previous studies showed that virus transmission of the Potato Y virus in sweet pepper is inhibited by Neem seed oil [13]. It is unknown whether other formulated essential oils can prevent virus transmission. This still has to be analyzed.
To date, several reports have dealt with the use of essential oils and other extracts from plants to control aphids. It has been found that essential oils of cumin (*Cuminum cyminum* L.), anise (*Pimpinella anisium* L.), oregano (*Origanum syriacum* L.) and eucalyptus (*Eucalyptus camaldulensis* Dehn.) were effective as fumigants against the cotton aphid (*Aphis gossypii* Glover) [14]. From laboratory tests, previous authors underlined the potential of seven essential

oils against *Brevicoryne brassicae* (Hemiptera: Aphididae) [15]. *Lavandula* seems to present interesting properties for pest control [16]. Therefore, the purpose of the present study was to evaluate the effectiveness of *Lavandula angustifolia* essential oil on the mortality rates of *A. pisum* as a function of extract concentration. In addition, because little data is available in the literature on the bioactivities and chemical characteristics of *L. angustifolia,* the chemical composition of this species was analysed directly to determine which compounds could be responsible for the observed effects. Afterwards, to corroborate the role of each constituent in the toxicity to *A. pisum*, we tested them individually and we reconstituted an artificial blend based on the proportion of the different compounds in the natural oil.

2. Materials and Methods

To avoid any side effect due to endosymbionts, the experiments were performed with a strain of *A. pisum* provided by Nancy Moran (University of Texas, Austin, TX, USA). The Tucson uninfected sub-colony was established in 2005 from the Tucson pea aphid originally collected in 1999 from *Vicia faba* in Tucson (Arizona) through killing *S. symbiotica* by heat shock.

Colonies of *A. pisum* (uninfected with *S. symbiotica*) were maintained on bean plant at 19.5±0.6 °C, 40-50% RH, under a 16L: 8D photoperiod in the laboratory of the biodiversity Research Centre, UCL, Louvain-la-Neuve (Belgium) for 5 years without any contact with pesticides before the experiments. The non infected status of aphids was verified using diagnostic PCRs with primers 16SA1 (5'-AGAGTTTGATCMTGGCTCAG-3') and PASScmp (5'GCAATGTCTTATTAACACAT-3') [17].

Essential oil

L. angustifolia individuals were collected locally in Tunisia (Hammamet, North of Tunisia) in June 2011, and were free of any pre-harvest chemical treatments (organic products).

This selection was based on previous work and the use of plant products in traditional medicine in Tunisia [18]. *L. angustifolia* essential oil was obtained through steam distillation for 4 h by using a Clevenger-type apparatus and fresh material. The oil yield was 0.53% of the dry weight of *L. angustifolia.*

Identification of Essential Oil Constituents

The essential oil of *L. angustifolia* was analyzed by GC-MS in the Department of Analytical Chemistry in Gembloux Agro-Biotech, University of Liege, Liege, Belgium.

For quantitative analyses (percentage determination), we used a fast GC, which proved to be powerful enough to analyze the essential oil constituents [19, 20].

GC-MS Analysis. Conventional GC-MS analyses were carried out on a thermo trace MS Finnigan mass selective detector equipped with an Optima 5 MS (Macherey-Nagel) capillary column (30 m by 0.25 mm i.d., 0.25- µm thickness) and a split/splitless injector (splitless mode) at 25 °C. The oven temperature was programed from 40 to 210 °C. Helium was the carrier gas at 1 ml/min. Volatile compounds were identified by comparing the mass spectra obtained with those from the Wiley 275 liters spectral library and with their retention indices. The retention indices were determined relative to the retention times of a series of n-alkane standards (C9-C30, 0.025 g/l in n-hexane, Sigma-Aldrich, Bornem, Belgium), measured under the chromatographic conditions described above, and compared with values in the literature [21].

Fast GC Analysis. Fast GC analyses was conducted on a Thermo Ultra-Fast Trace GC operated with a split/splitless injector and a Thermo AS 3000 autosampler (Thermo Fisher Scientific, Waltham, MA). The GC system was equipped with an ultrafast module (UFM) incorporating a direct resistively heated column (Thermo Fisher Scientific): UFC-5, 5% phenyl (5 m by 0.1 mm i.d., 0.1-m film thickness). The following chromatographic conditions were used to obtain a suitable peak resolution. The UFM temperature program was as follows: initial temperature at 40 °C, held for 0.1 min $^{-1}$, 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 $^{-1}$. Injection temperature was 240 °C; injection volume was 1 l; carrier gas was He, at a constant flow rate of 0.5 ml min $^{-1}$ and split ratio was 1:100. The GC unit had a high-frequency fast flame ionization detector (300 Hz), at 250C; H2 flow was 35 ml min $^{-1}$; air flow was 350 ml min $^{-1}$; and make-up gas flow (N2) was 30 ml min $^{-1}$. Data processing was performed using Chromcard software version 2.3.3. (Louvain-la-Neuve, Belgium).

Fumigant toxicity

Mortality

For evaluation of fumigant toxicity, 1L glass containers with tight lids were used as the test chambers [22]. The insecticidal effect of 5 concentrations of this essential oil was investigated by depositing 5, 10, 15, 20 and 25 µl of the compound to be tested or of the full essential oil on a filter paper inside the 1L glass container that was then closed hermetically.

Aphids were synchronized by placing females on fresh bean leaves during 24h. The females were then removed and larvae were incubated till stage 3 after 3 days. Groups of 25 aphids (L3) non infected with *S. symbiotica* were 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 1 L glass containers. The different doses of essential oil were introduced into the glass outside the Petri dish in order to avoid contact with aphids. Just after, the glass receptacle was closed above with a metal cover on which 5 holes were drilled to allow air exchange [22]. The number of dead individuals was counted after 3 days.

In order to determine mortality after 3 days, a slight touch on the aphid was done 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 [23].

Effect of individual constituents and artificial blend of *L. angustifolia* essential oil on Mortality

The toxicity experiments were repeated with commercially available individual constituents of the essential oil and with

blend of this essential oil at their natural proportions as reported in Table 1. Pure compounds were purchased from Sigma-Aldrich: linalool, linalyl acetate, 1,8-cineole, camphor, β-pinene and terpinene-4-ol. Purities of the compounds varied from 95 to 99%. Then, an artificial blend of the different constituents was made by mixing them at the same proportion that revealed by the GC-MS analyses to reconstitute the oil.

Data analysis

All the data were corrected using Abbott's formula. The LC_{50}, LC_{90} and LC_{100} values were determined by probit analysis using the Statplus program v.2009 (AnalystSoft Inc.). Tests were performed using one-way analysis of variance (ANOVA), and Newman–Keuls tests were used to compare means using Graph Pad Prism v.5.01 for Windows (GraphPad Software, San Diego (CA, 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

Chemical composition of *L. angustifolia* essential oil

Table 1 showed the composition of *L. angustifolia* essential oil. Six components were identified (electron impact mass spectra and retention index comparison) by GC-MS representing 99.99% of the total weight. Linalool was the most abundant compound (38.57%), followed by linalyl acetate (29.95%), 1,8-cineole (13,66%), camphor (13,13%), β-pinene (3,14%) and terpinene-4-ol (1,54%). The essential oil was then analyzed by fast GC on a UFM column of the same polarity as in GC MS (apolar stationary phase). The retention times of the components of interest from the essential oil were compared with those of the reference compounds.

Toxicity of essential oil

After 72 h, all aphids in the control group were still alive. Mortality increased with increasing concentration of essential oil. The experimental distribution of mortality rates was fitted with a sigmoid curve ($R = 0.97$, df = 29, $N = 25$, $P < 0.0001$). The LC_{50} and LC_{90} values were 11.2 and 15 µl/l of air respectively.

Toxicity of single constituents

A significant difference was found in the lethal toxicity of the single constituents of the oil when all treatments were compared: one-way ANOVA: ($F=291.31$, df=54, $P<0.0001$). Indeed, The Newman Keuls tests comparing the toxicity of single constituents revealed that 1,8-cineole was highly toxic, followed by linalool but that they were not as toxic individually as the essential oil and the full mixture.

Three constituents of *L. angustifolia* (camphor, terpinene-4-ol, linalyl-acetate) were slightly toxic, whereas the remaining constituent beta-terpinene was not toxic to *A. pisum* and did not differ significantly from the mortality rate found in the control (Fig. 1).

4. Discussion

As they are volatile, fumigation is probably the most adequate way to analyse the toxicity of essential oil. In the present study, an examination was made of the effect of different concentrations of *L. angustifolia* essential oil, on their efficacy as natural pesticide against *A. pisum*. A significant *A. pisum* mortality appeared at low concentrations, with LC_{50} values of 11.2 µl/l of air. An analysis of the toxicity of the main individual component of the essential oil showed that the activity of the *L. angustifolia* may be explained by the presence of 1,8-cineole (40% of mortality when alone and tested at the same proportion than in the oil) (Fig. 1).

The present results are in agreement with a previous report that 1,8-cineole is responsible for the major toxicity of *Deverra scoparia* essential oil against *T. urticae* [24]. It is also one of the major compound responsible of the toxicity of *Eucalyptus* essential oil against several Arthropod pest [25]. Understanding the role of each constituent to the overall activity of the oil provides an opportunity to create artificial blends that optimise their efficacy against different pests. One interesting aspect of the present study was the difference found in the role of the major constituents in a mixture as opposed to their individual toxicities; thus, 1,8-cineole produced a higher mortality (40%) compared with Linalool (10% of mortality) in spite of the difference in their proportion in the pure oils which correspond to 13.66 and 38.57% for 1,8-cineole and linalool respectively. These differences probably reflect differences in the mechanisms of activity of each component. However, essential oils contain numerous components, and other major and/or minor compound(s) may also play a role in their aphidicidial activity. The highest mortality rates were obtained when all the constituents were present in the mixture (>90% mortality). Knowing the role of each constituent in toxicity of this oil makes it possible to create an artificial blend of different constituents on the basis of their activities and their effect on the pest. In a laboratory bioassay where aphids were placed on mustard cabbage leaf discs dipped in emulsions of blend of eugenol, thymol and phenethyl propionate showed both behavioural effects and toxicity on the Green peach aphid, *Myzus persicae* [2]. The frequency of *M. persicae* feeding and the mortality rate were inversely concentration-dependent. Previous authors tested the biological activity of essential oil volatiles obtained from *Tagetes minuta* L. against aphid species, *Acyrthosiphon pisum* (Harris), *M. persicae, Aulacorthum solani* (Kaltenbach) [26]. They demonstrated that *T. minuta* oil volatiles significantly have reduced the reproduction potential of the tested species. It has been found that application dose of various essential oils resulted in significant differences in mortality rate for *M. persicae* and *A. pisum* [27].

Essential oils from 23 species of plants were mixed with a non-toxic emulsifying agent, dimethyl sulfoxide (DMSO) and tested against *Lipaphis pseudobrassicae* (Davis). Results showed that essential oils from *Bifora, Satureja, Coridothymus, Thymbra* and *Pimpinella* plants are potential candidate as botanical pesticides with additional research into essential oil chemistry and their influence on insects and plants [28].

Several laboratory studies have previously described the acaricidal activity of essential oils on the mite *Tetranychus urticae* Koch [4]. Among the more active essential oils are those from thyme, garlic, rosemary, and mint, but more empirical testing on rarer plant species and a wider array of pest species will undoubtedly reveal particularly valuable biological activities [29]. Recent study investigated the acaricidal effects of four plant extracts collected from Tunisia on the spider mite: *Deverra scoparia, Santolina africana,*

Hertia cheirifolia, and *Allium sativum*. Compared to synthetic acaricides, the efficacy of these four extracts against the two-spotted spider mite is significantly higher with lower LC_{50} values, and greater decreases in fecundity [4].

Considering other control strategies of pests, both efficiency and being environmental friendly reasons makes essential oils much preferable insecticides against different pest groups, particularly against the aphid. Recent studies showed that compared with the other control strategies, essential oil applications have several advantages. Their applications affect aphids and some other pest in a short time by killing them faster and reducing their reproduction potential. Using essential oil as an aphidicide is also safer for the environment and human health because of their low toxicity and shorter degradation time. Little is however known about whether and how these oils disturb the plant-insect interaction. Despite the many unsolved problems related to using plant essential oils to control pest insects, it is expected that they will play an important role in future crop protection.

Table 1: Major constituents of *L. angustifolia* essential oil and their relative proportions in the pure oil (Identification with retention index was obtained by GC-MS. Percentage were obtained by Fast-GC-FID.)

Component	Retention time (min)	Retention index (measured)	%
1,8-cineole	11,36	1033	13,66
Linalool	12,58	1053	38,57
Camphor	13,39	1151	13,13
terpinene-4-ol	13,90	1183	1,54
linalyl acetate	15,03	1258	29,95
β-pinene	15,52	1291	3,14

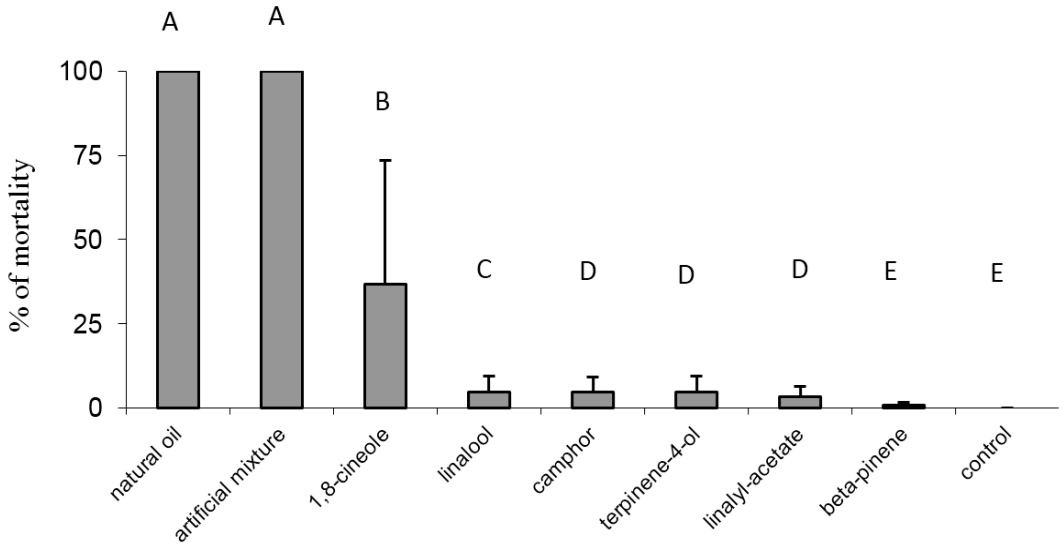

Fig 1: Figure 1. Mortality caused by constituents of *L. angustifolia* oil to *A. pisum* when applied at levels equivalent to those found in the 100% lethal concentration of the pure oil (LC_{100} = 16.51 µl/l of air). Error bars represent the standard error of the mean of ten replicates, each replicate containing 25 aphids. Means corresponding to each treatment with different letters are significantly different from each other (Newman–Keuls test, $P < 0.05$).

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