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TOXIC EFFECTS OF ESSENTIAL OILS AND SOME OF THEIR COMPONENTS ON *VARROA DESTRUCTOR* OUD AND *APIS MELLIFERA* L UNDER LABORATORY CONDITIONS.

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Title
The acaricidal effects of essential oils from thyme,
salvia and hyssop plants (from left to right) have been tested
against *Varroa destructor.*

First edition

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TOXIC EFFECTS OF ESSENTIAL OILS AND SOME OF THEIR COMPONENTS ON *VARROA DESTRUCTOR* OUD AND *APIS MELLIFERA* L UNDER LABORATORY CONDITIONS.

Abstract

The essential oils of thyme, salvia and of hyssop, and their main components were tested on *Varroa destructor* Oud. and on honeybees in a dose-effect laboratory test. Thyme and salvia oils, as well as two types of hyssop oil (eucalyptol and pinocamphon type) showed good acaricidal efficiency of more than 80% at concentrations above 500, 300, 500 and 400 µg/l air, respectively. Only salvia oil and *p*inocamphon type hyssop oil were tolerated well by bees, the other two oils caused a relatively high lethality of over 20% for bees at concentrations leading to a good mite toxicity. Apart of the known potent acaricide thymol, the thyme oil components *p*-cymol and γ-terpinene were most toxic for Varroa, while well tolerated by bees at concentartions between 400 to 1000 and 350 to 800 µg/l air respectively. During the application of salvia oil, the concentration of its main components α-thujone, camphor and eucalyptol was not high enough to achieve a good acaricidal effect. On the other hand, when these three components were tested as pure substances at the proper concentrations, a high toxicity against *Varroa* was observed, while they were well tolerated by the bees at concentrations between 150 to 350, 50 to 150 and 240 to 300 µg/l air, respectively.

The acaricidal effects of essential oils have been extensively reviewed (Imdorf et al., 1999). In various studies more than 150 different essential oils have been tested for varroacidal effects under laboratory and field conditions. However, only the oils of thyme, salvia and oregano proved a sufficient efficiency when applied in bee colonies. In addition, wintergreen and marjoram oils showed acaricidal effects when applied with warm air (Hoppe and Ritter, 1989) or in combination with formic acid (Berg et al., 1999).

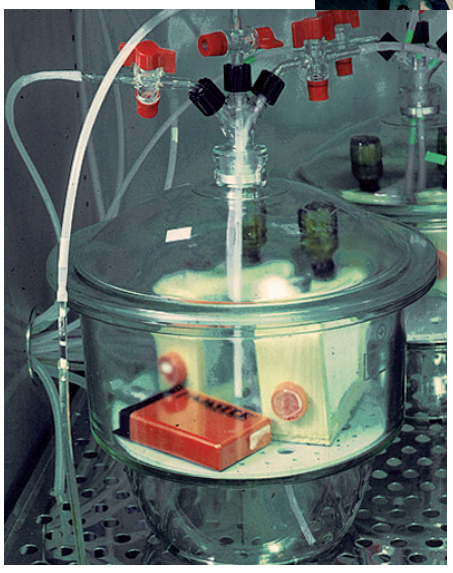
Recently 22 substances were tested in a laboratory essay (Lindberg et al., 2000). Thymol, clove oil, carvacrol, methyl salicylate and Magic 3, a mixture of 5 essential oils, showed a medium to high mite toxicity and a good bee tolerability. In another similar assay thyme, savory, rosemary, marjoram, dillsun and lavender essences were tested (Ariana et al., 2002). At the concentration of 1 and 2 g/100g they showed a mite toxicity of more than 95%, resp. 97%. The bee toxicity of thyme, savory and spearmint essences was comparable to the acetone and water controls. In another investigation 17 monoterpinoids (some synthetic, others naturally occurring) were evaluated for acaricidal effects in a laboratory test (Ali et al., 2002). The compounds exhibited a wide range of toxicity to both *V. destructor* and their honey bee hosts. In field trials only perillyl acetate and myrtenyl acetate showed a significant acaricidal activity. In another work, thymol, menthol, eucalyptol and camphor were tested in a laboratory assay for contact toxicity for *V. destructor* and influence on the behaviour of the mites (Colin, 1999). At the highest dosage applied (4 %) all substances had a only weak lethal effect.

In many of the laboratory tests it is not clear whether the toxic effects observed are due to contact or to the evaporated substance. The experience has shown that it is difficult to use laboratory screening data for the prediction of the optimal oil dosage range of these oils and substances, which is needed in order to develop acaricides for the treatment of bee hives. Only wintergreen oil was studied in a dose-response laboratory study for toxic concentration-dependent effects on mites and bees (Hoppe, 1990).

Most essential oils evaporate to a great extent and their acaricidal effects depend on the biological efficacy of the molecules present in the air. Thus, a test was developed in order to determine the dose-response relationship between the air concentrations of single volatile components of essential oils and their toxic effects on mites and bees (Imdorf et al., 1995). Consequently, it was found, that thymol, menthol, camphor and eucalyptol have a strong acaricidal activity, but eucalyptol was not well tolerated by bees. The acaricidal concentration of thymol was found to be similar both under laboratory (Imdorf et al., 1995) and field conditions (Imdorf et al., 1994). Therefore, this test can be used successfully for screening of essential oils and their components in the laboratory before testing them in bee colonies. Thymol is the most effective natural acaricide, used world-wide for the control of *V. destructor.*

Presently, it is necessary to search for alternatives to thymol, since we do not know if, on the long run, *V. destructor* will develop resistance to this substance. In this study the toxicity of the essential oils of thyme, salvia, two types of hyssop as well as their main components were tested on both *V. destructor* and worker bees by our laboratory test (Imdorf et al., 1995).

Trial setup for screening essential oils and there components under laboratory conditions against *Varroa destructor.*



Two Liebefeld cages, each containing about 100 worker bees, infested with 20-40 *Varroa* mites are placed in an exsiccator to be exposed to different concentrations of essential oils.

2. Material and methods

2.1 Essential oils and components

Essential oils

Thyme oil *(Thymus vulgaris)*, He 555, Salvia oil *(Salvia officinalis)*: B756 were kindly supplied by Marc Colin, INRA, F-89414 Avignon Cedex, France. Eucalyptol and pinocamphon types of hyssop oil *(Hyssopus officinalis)* were both obtained from Phytomed, 3415 Hasle, Switzerland.

Essential oil components

All essential oil components tested were supplied by Fluka (Buchs, Switzerland): thymol, Nr. 89330; camphor, Nr. 21300; eucalyptol Nr. 46900: *p*-cymol Nr. 30040; α+ ß-thujone, Nr. 89230; α-terpinene, Nr. 86475; γ-terpinene, Nr. 86478; camphene Nr. 21290; α-pinene, Nr. 80600; +/- limonene, Nr. 42560.

2.2 Laboratory test for evaluation of mite and bee toxicity

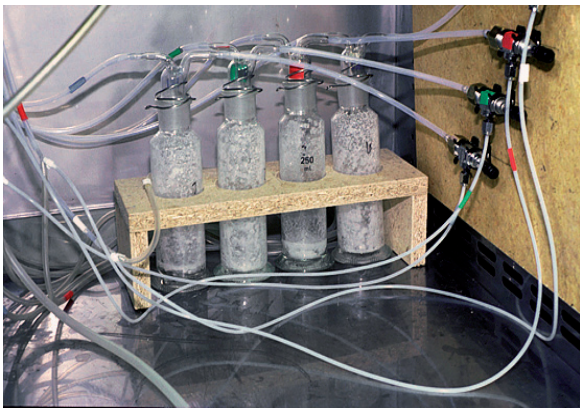
The test used is described in detail in a previous publication (Imdorf et al., 1995). The principle of this method is as follows:

two Liebefeld cages, each containing about 100 worker bees, infested with 20-40 Varroa mites are fed with sterile sugar water (1:1) and placed in an exsiccator at 32°C. Both bees and mites are exposed for 72 hours to air, containing different amounts of essential oils or their components. The air is sampled by pumping it through an adsorption tube (Orbo-101, Supelco). The compounds are extracted from the absorption tubes with toluene and analysed, as described below (2.4). After 72 hours the dead mites and bees are counted and the mortality of bees and mites is determined. On the basis of 10 to 15 tests with different concentrations for each substance, a dose-effect relationship between the air concentration of the essential oils or their components and the toxicity for *V. destructor* and bees was established. Thus, the air concentration is determined that achieves an optimum acaricidal effect (efficacy > 80%) while showing at the same time a low bee toxicity (< 20%). The tests were carried out from August to mid-October, the optimal season for the control of *V. destructor* under central European conditions, during the years 1996 to 1999.

2.3 Determination of essential oils and of their components

A Hewlett-Packard (HP) 5890, Series II gas chromatograph was used. Operating conditions were as follows: carrier gas: helium; inlet pressure 40kPa; flow, approx. 1.6 ml/min; transfer line (from GC to MS): 280 °C; interface: direct inlet; «on column» injection: 0.5 µl of a solution of 60 mg of each essential oil, diluted in 10 ml diethyl ether at 35 °C; temperature program: 2 min. at 35 °C, 20 °C/min to 45 °C, 10 min; 5 °C/min to 275 °C, 11 min; capillary column: 60 m DB5MS, i.d. 0.32 mm, film 1µm, capillary column (Agilent). Components were detected by a mass sensitive detector MSD HP 5972 for the identification of substances.

The MSD operated in scan mode (TIC) from 19 to 250 amu at 2.9 scan/s., ionization by EI at 70 eV by autotuning; MS-scan after 7 minutes. After the mass spectra were acquired and treated with the standard procedure of the GC-MS system (using automated spectra treatment), certain peaks were not sufficiently identified. They were therefore additionally treated using the more sophisticated software package MassLib (Mariaca and Bosset, 1997; Henneberg et al., 2004). In most cases the identification of the peaks was confirmed by taking into consideration the mass spectra and retention indices of authentic substances. Due to the similarity of the mass spectra of several terpenes, a correct 100 % identification was only possible by using their retention indices. In some cases this was not possible and the identity of the substances was determined by the MS libraries only (marked with * in tables I and II). Only peaks present with more than 1 % of the total essential oil components were identified.



Fresh air is contaminated by flowing through flasks containing the active substances. Different volumes of this contaminated air were mixed with fresh air to achieve different concentrations of contaminated air in the exsiccators.

2.4 Determination of concentration of essential oils and their components in the air of the exsiccator

The essential oils and their components were absorbed by the Orbo tubes, extracted with toluene, the solution was filtered and analysed as described above by gas chromatography with FID detection (Imdorf et al., 1995). Chromatographic conditions used in this work are described. If standards were available, e.g. thymol, camphor, eucalyptol, *p*-cymol, γ-terpinene and thujone, the components were determined quantitatively. The chemical nature of other components was determined according to 2.3, while their air concentration was determined semi-quantitatively by comparing the peak area of the substances in the eluate with the total area of essential oils analysed directly. The percentage distribution of essential oil components determined by chromatography with FID and MS detection was the same. As the same chromatographic conditions were used in both detection modes, the chemical nature of the essential oil components in the semi-quantitative determination is certified. For determination of the quantity of essential oil in the air, the sum of the compounds detected in the toluene eluate was compared to the sum of the components of the pure essential oil determined directly by the same method. All concentrations in the air were calculated in µg/l.

3 Resultats and discussion

3.1 Thyme oil

The main components of the thyme oil used are *p*-cymol (32%), thymol (22%) and γ-terpinene (12%), see table I. At a nearly 100% *Varroa* mortality the bee lethality was between 20 and 40% (fig.1). Due to the high toxicity for worker bees this essential oil is probably not suitable as a varroacide. To attain high mite toxicity and also good bee tolerability thymol air concentrations between 5 and 15 µg/l are needed (fig.2, Imdorf et al., 1995). At the highest thyme oil concentration tested (1068 µg/l air) a thymol concentration of only 2.5 µg/l air was registered (fig.3). This means that thymol present during the application of thyme oil is probably not the most important acaricidal ingredient. *p*-Cymol is another major component of thyme oil. A concentration range for this substance lying between 400-800 µg/l (fig.4) was found during the application of thyme oil, causing high *Varroa* toxicity. Pure *p*-cymol in the range between 400 and 1000 µg/l (fig.5) was highly toxic for the mites while well tolerated by the bees (table III). Thus, *p*-cymol is probably the active acaricidal substance of thyme oil. γ-Terpinene, another main component of thyme oil (tab. 1) showed a very good acaricidal effect and a good bee tolerability in the concentration range between 350 and 800 µg/l air (fig.6, table III). During thyme oil application the highest concentration of γ-terpinene measured was only about 180 µg/l air and thus it cannot have an effect on mite mortality. α-Terpinene as a pure substance caused almost no mite and bee mortality at a concentration of 80 µg/l but a very high toxicity of over 90% for both *Varroa* and bees resulted at a concentration of 250 µg/l. When thyme oil was applied, the highest concentration of α-terpinene measured was about 20 µg/l. Consequently, α-terpinene is not responsible for the bee toxicity observed.

3.2 Salvia oil

The main components of salvia oil are α-thujone (27%), camphor (11%), α-humulene (8%), eucalyptol (8%), caryophyllene (6%) and ß-thujone (6%) (table I). A concentration range between 300 and 500 µg/l induced a Varroa mortality of 100% and a bee mortality of 10 to 22% (fig.7). Thus, salvia oil is less toxic for bees than thyme oil. During the application of salvia oil the highest air concentrations of camphor (fig.8), α-thujone (fig.9) and eucalyptol were 25, 90 and 60 µg/l air, respectively. On the other hand, a mite mortality of close to 100% correlated 50 (fig.10), 200 (fig.11) and 150 µg/l (fig.15) of these substances. Therefore, camphor, α-thujone and eucalyptol are not responsible for the high acaricidal effect of salvia oil. α-thujone as a pure substance had an acceptable acaricidal effect and a low bee mortality at concentrations of 150 - 350 µg/l air (fig.11, table III). Salvia oil contains only low amounts of camphene (2.4%, table II), while the highest concentration found in the air of test exsiccators was 80 µg/l. Pure camphene in concentrations between 2000 and 3000 µg/l (fig.12) showed an increasing mortality for both *Varroa* (from 60 to 100%) and bees (from 20 to 40%). Consequently, camphene is not a suitable candidate for an efficient acaricide. α-pinene and limonene showed low effects on both, bees and mites, even at high concentrations (table III). However, we cannot exclude effects of minor compounds which were not tested in this study.

Thus, it cannot be determined which component of salvia oil is the main active ingredient. It is unknown to what extent synergetic or cumulative effects between the different components of salvia oil might be responsible for the high mortality of *Varroa* observed with oil concentrations of 300-500 µg/l (fig.7).

3.3 Hyssop oil

Two hyssop oils were tested: an eucalyptol and a pinocamphone type. The eucalyptol type contains eucalyptol (43%) and ß-pinene (16%) as main components (table II). It's acaricidal effect was nearly 100% at concentrations above 500 µg/l, but caused at the same time a high bee mortality of more than 70% (fig.13). Therefore, this oil is not suitable for *Varroa* treatments. During the application of this hyssop oil the concentration of eucalyptol in a range between 200 and 300 µg/l air (fig.14) was similar to the concentration achieved in a test with pure eucalyptol (fig.15, Imdorf et al., 1995). We thus conclude that eucalyptol is responsible for the toxic effects observed after the application of eucalyptol-type hyssop oil. Pure eucalyptol is neither suitable for *Varroa* treatments because of increasing bee mortality at concentration ranges of high mite lethality (fig.15, Imdorf et al., 1995).

The pinocamphone-type hyssop oil contains isopinocamphone (27%), pinocamphone (14%) and ß-pinene (13%) as main components, table II. Oil concentrations between 300 and 800 µg/l air causing a high mite mortality (nearly 100%) are tolerated well by bees with a mortality of 10% or lower (Fig. 16,17). We could not test isopinocamphone and pinocamphone, as they are not commercially available. Therefore, it is not known which is the active acaricidal substance of this oil.

4 Conclusions

Of all essential oils tested in this study the salvia and the pinocamphone-type hyssop oil had a high efficiency against *V. destructor,* while they were well tolerated by worker honeybees. These oils should be further tested in honeybee colonies. The laboratory tests with thyme oil showed that air concentrations with a good efficiency against the mites result in an increased bee mortality. If further tests with these or other essential oils are carried out, it should be kept in mind that essential oils vary in their composition, depending on various factors. This might be the reason, why conflicting results regarding the efficacy of the same essential oils against *V. destructor* have been reported (Imdorf et al., 1999). These problems could be avoided if oils labelled «EC ANFOR» are used. They are produced under European standards for therapeutic grade oil and certified by the French certifying organisation ANFOR.

On the other hand, when the main components of some oils tested such as *p*-cymol, α-thujone and γ-terpinene were used, a high mortality of mites and a low lethality for bees was observed in our tests. This indicates that single essential oil components, rather than the oils themselves might be more suitable for the control against *V. destructor.* All of these substances, as well as camphor and pinocamphon-type hyssop oil are fairly volatile, which qualifies them mainly for short-term treatments in honeybee colonies without brood. However, before using this components for mite treatments in apicultural practice they should be tested in honeybee colonies. In order to achieve high acaricide effects and low bee mortality rates, the air concentrations indicated in table III should be attained in hives. In addition, new application forms such as heat-induced evaporation (in brood free colonies in November) or aerosol contact application should be tested.

However, before using the components for treatments against *V. destructor* in honey bee colonies, they should be tested for consumer and application safety. Particularly, the user and residue toxicity of α-thujone has to be tested. Toxic effects for this substance have been reported (Höld et al., 2000).

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Zusammenfassung

Varroa- und Bienentoxizität von Thymian-, Salbei- und Ysopöle und deren wichtigsten Einzelkomponenten.

Die Auswirkungen verschiedener ätherischer Öle auf *Varroa destructor* und Bienen wurden bereits von verschiedenen Autoren beschrieben. Bei der Anwendung im Bienenvolk waren aber nur wenige wirksam. In der vorliegenden Untersuchung wurde die Dosis – Wirkungskurve der als interessant erscheinenden, ätherischen Öle von Thymian *(Thymus vulgaris)*, Salbei (Salvia officinalis) und Ysop *(Hyssopus officinalis)* sowie ihrer Hauptkomponenten auf Bienen und *V. destructor* ermittelt.

Zwei Liebefelder Kästchen mit seitlichen Gitterabdeckungen, die je ca. 100 Bienen mit 20 bis 40 Varroamilben enthielten, wurden bei 32 °C in einen Exsikkator gestellt. Während 72 Stunden wurden die Exsikkatoren mit einem Gemisch aus Frischluft und mit ätherischen Ölen angereicherter Luft versorgt. Nach 24, 48 und 72 Stunden wurde aus jedem Exsikkator eine Luftprobe entnommen. Der Mittelwert der drei Luftproben ergab die durchschnittliche Luftkonzentration der ätherischen Öle pro Exsikkator während der Behandlung. Nach 72 Stunden wurden die getöteten *V. destructor* und die toten sowie lebendigen Bienen ausgezählt. Die überlebenden Milben wurden erfasst, indem die Bienen mit CO_2 betäubt, in Alkohol abgetötet, ausgewaschen und anschliessend gezählt wurden. Die Milben- und Bienenmortalität entspricht dem Anteil der während der Behandlung getöteten Tiere an der Gesamtpopulation pro Exsikkator. Mit Hilfe von GCMS wurden die wichtigsten Komponenten von Thymian-, Salbei- und Ysopöl (Typ Eukalyptol und Pinocamphon) bestimmt (Tab.I, II).

Thymianöl (Abb.1, Tab.III):
Bei einer Varroatoxizität von nahezu 100% erreichte die Bienentoxizität des Thymianöls 20 bis 40 %. Der für die varroazide Wirkung des Thymianöls verantwortliche Stoff dürfte *p*-Cymol sein, wie der Vergleich mit der Reinsubstanz (400 - 1000 µg/l Luft) zeigte. Die Thymolkonzentration in der Behandlungsluft lag mit einer Ausnahme unter 1 µg/l und wirkte in der vorgegebenen Zeit kaum. γ-Terpinen, ein weiterer Wirkstoff von Thymianöl, zeigte als Reinsubstanz zwischen 350 und 800 µg/l Luft eine sehr gute, varroazide Wirkung bei einer sehr guten Bienenverträglichkeit. Bei der Anwendung des Thymianöls lag die höchste gemessene Konzentration von γ-Terpinen aber nur bei 180 µg/l Luft. α-Terpinen zeigte bei Konzentrationen von 80 µg/l Luft keine Wirkung. Bei 250 µg/l Luft war dieses Öl jedoch hoch toxisch für *V. destructor* und Bienen. Die bei der Thymianölbehandlung gemessenen Konzentrationen lagen bei maximal 20 µg/l Luft und kommen deshalb kaum als Ursache für die erhöhte Bienentoxizität bei steigender Thymianölkonzentration in Frage.

Salbeiöl (Abb.7, Tab.III):
Das Salbeiöl wies im Vergleich zu Thymianlöl bei einer Varroatoxizität von gegen 100 % eine geringere Bienenmortalität von 10 bis 20 % auf. Die gemessenen Campher-, α-Thujone- und Eukalyptolkonzentrationen in der Behandlungsluft von 25, 90 resp. 60 µg/l können die gute Varroatoxizität nicht erklären. Bei den Tests mit diesen Reinsubstanzen zeigten durchwegs erst höhere Konzentrationen gute Varroatoxizität. Camphen, ein weiterer Wirkstoff, zeigte erst bei 2000 bis 3000 µg/l Luft eine Milben- resp. Bienenmortalität von 60 bis 100% resp. 20 bis 40%. Diese Konzentrationen wurden aber bei der Anwendung von Salbeiöl nie erreicht. α-Pinen und Limonen hatten auch bei hoher Dosierung eine ungenügende Wirkung. Somit ist nicht klar, welche Substanzen bei der Anwendung von Salbei für die Toxizität verantwortlich sind. Synergetische Effekte der verschiedenen Substanzen können nicht ausgeschlossen werden.

Ysopöl (Abb.13 und 16, Tab.III):
Das Ysopöl des Typs Eukalyptol zeigte bei einer guten Varroatoxizität gleichzeitig eine hohe Toxizität für Bienen. Dieses Öl ist deshalb ungeeignet für die Varroabekämpfung. Im Vergleich dazu ermittelten wir für das Ysopöl des Typs Pinocamphon bei gleichzeitiger guter Wirksamkeit gegenüber *V. destructor* eine sehr gute Verträglichkeit bei den Bienen. Anhand des Verlaufes der Mortalitätskurven für dieses Ysopöl (300-800 µg/l Luft) und Isopinocamphon (30-100 µg/l Luft) kann angenommen werden, dass letztere Substanz der Hauptwirkstoff sein könnte.

Résumé

Toxicité pour les varroas et les abeilles des huiles de thym, de sauge et d'hysope de même que de leurs composants les plus importants.

Les effets des différentes huiles essentielles sur *Varroa destructor* et les abeilles ont déjà été décrits par différents auteurs. Cependant, lors de leur application dans les colonies d'abeilles, peu ont été efficaces. Dans la présente étude, nous présentons les courbes doses-réponses des huiles essentielles qui paraissent intéressantes – d'huile de thym (Thymus vulgaris), de sauge (Salvia officinalis) et d'hysope (Hyssopus officinalis) de même que de leurs composants principaux.

Deux cagettes dites «de Liebefeld» avec des parois grillagées contenant chacune 100 abeilles et 20 à 40 *V. destructor* ont été placées à une température de 32°C dans un dessiccateur. Ceux-ci ont été alimentés pendant 72 heures avec un mélange d'air frais et d'huiles essentielles. Après 24, 48 et 72 heures, un échantillon d'air a été prélevé dans chaque dessiccateur. La valeur moyenne des trois échantillons d'air a donné la concentration d'air moyenne des huiles essentielles par dessiccateur pendant le traitement. Après 72 heures, les acariens survivants ainsi que les abeilles mortes et vivantes ont été comptés. Les acariens qui ont survécu ont été dénombrés après avoir étourdis les abeilles avec du CO_2 puis tuées dans de l'alcool et lavées. La mortalité des acariens et des abeilles correspond à la proportion des animaux morts dans chaque dessiccateur pendant le traitement calculé par rapport à l'ensemble de la population. Avec l'aide d'un GC-MS, les composants les plus importants de l'huile de thym, de sauge et d'hysope (type eucalyptol et pinocamphone) ont été déterminés (tab.I, II).

Huile de thym (fig.1, Tab.III):
dans le cas d'une toxicité de près de 100% pour *V. destructor,* la toxicité de l'huile de thym pour les abeilles a atteint 20 à 40 %. La substance à l'origine de l'effet varroacide de l'huile de thym est probablement le *p*-cymol, comme l'a montré la comparaison avec la substance pure (400 - 1000 µg/l d'air). La concentration de thymol dans l'air de traitement se situait, à une exception près, au-dessous de 1 µg/l et a eu peu d'effet pendant la durée du traitement. Le γ-terpène, une autre sustance active de l'huile de thym, a eu un très bon effet varroacide sous la forme de substance pure (350 et 800 µg/l d'air) tout en étant bien tolérés par les abeilles. Cependant, lors de l'utilisation de l'huile de thym, la concentration en γ-terpène la plus élevée s'élevait à seulement 180 mg/l d'air. Le α-terpène n'a eu aucun effet à une concentration de 80 mg/l d'air. Par contre, à une concentration de 250 µg/l d'air, cette huile s'est révélée très toxique tant pour *V. destructor* que pour les abeilles. La concentration maximale mesurée lors du traitement à l'huile de thyms'élevait à 20 µg/l d'air et n'est vraisemblablement pas àl'origine de la toxicité plus élevée pour les abeilles dans le cas d'une augmentation de la concentration de l'huile de thym.

Huile de sauge (fig.17, tab.III):
comparé à l'huile de thym, l'huile de sauge a eu, dans le cas d'une toxicité d'environ 100% pour les acariens, une mortalité des abeilles plus faible de 10 à 20 %. Les concentrations de camphre, d'α-thujone et d'eucalyptol dans l'air de traitement de respectivement 25, 90 et 60 µg/l ne peuvent pas expliquer le bon effet varroacide. Lors des tests avec ces substances pures, seules des concentrations plus élevées ont agi efficacement sur les varroas. Le camphène, une autre substance active, a eu une bonne toxicité uniquement à des concentrations de 2000 à 3000 µg/l d'air engendrant une mortalité des acariens et des abeilles de respectivement 60 à 100% et de 20 à 40%. Ces concentrations n'ont cependant jamais été atteintes lors de l'application de l'huile de sauge. Même à un dosage élevé, les α-pinènes et les limonènes ont eu un effet insuffisant. Ainsi, on ne sait pas quelles substances sont à l'origine de la mortalité dans l'application de l'huile de sauge, mais on ne peut pas exclure des effets synergiques des différentes substances.

Huile d'hysope (fig.13 et 16, Tab.III):
l'huile d'hysope du type eucalyptol a eu non seulement une haute toxicité contre *V. destructor,* mais aussi contre les abeilles. Cette huile ne convient donc pas à la lutte contre les varroas. En revanche, nous avons obtenu avec l'huile d'hysope du type pinocamphone une bonne mortalité des acariens et une bonne tolérance des abeilles. Au moyen des courbes de mortalité de l'huile d'hysope (300-800 µg/l d'air) et d'Isopinocam-phone (30-100 µg/l d'air), on peut supposer que cette dernière substance est la substance active principale.

Table I:
Composition of thyme and salvia oils used in the tests.
Only peaks present as more than 1 % of the total were considered.
RI – Retention Index, * - Identification of substance by MS library only.

Thyme oil			**Salvia oil**		
Substance	%	RI	Substance	%	RI
p-cymene	32.0	1037	α-thujone	27.2	1123
thymol	22.0	1296	camphor	11.0	1173
γ-terpinene	11.8	1070	α-humulene	8.0	1498
ß-caryophyllene	4.7	1464	1,8-cineole (eucalyptol)	7.5	1047
linalool	3.3	1103	ß-thujone	6.1	1134
1,8-cineole (eucalyptol)	2.8	1049	ß-caryophyllene	5.9	1462
1-octene-3-ol	1.7	981	Viridifloral*	5.8	1648
ß-myrcene	1.5	992	α-pinene	3.3	943
caryophyllene oxid	1.3	1640	camphene	3.1	963
α-terpinene	1.3	1029	(+)-borneol	3.0	1196
α-thujene	1.2	934	ß-pinene	2.3	991
(+)-terpinene-4-ol	1.1	1201	bornyl acetate	2.0	1303
(+)-borneol	1.0	1198	caryophyllene oxide	1.5	1636
linalyl acetate	1.0	1251	limonene	1.2	1040
1 unknown	2.8		total	87.9	
total	89.5				

Table II:
Composition of two types of hyssop oils used.
Only peaks present as more than 1 % of the total were considered.
RI – Retention Index, * - Identification of substance by MS library only.

Eucalyptol type			**Pinocamphone type**		
Substance	%	RI	Substance	%	RI
1,8-cineole (eucalyptol)	43.5	1048	isopinocamphone	27.4	1202
ß-pinene	15.9	991	pinocamphone	13.5	1184
limonene	4.0	1041	ß-pinene	12.7	991
isopinocamphone	3.8	1201	ß-phellandrene *	3.1	1045
sabinene	3.8	982	ß-bourbonene *	3.1	1420
α-pinene	3.0	944	ß-caryophyllene	2.7	1462
α-terpineol	2.7	1212	sabinene	1.6	982
p-cymene	2.4	1036	linalyl acetate	1.6	1249
(+)-terpinen-4-ol	1.1	1201	linalool	1.6	1101
6 unknown	10.1		α-humulene	1.6	1498
total	90.3		caryophyllene oxide	1.5	1636
			7 unknown	14.2	
			total	84.6	

Table III:
Optimum range of air concentrations of different essential oils and of some of their main components for achieving a high acaricidal activity, while well tolerated by bees.

Remark

Eucalyptol type hyssop oil, camphene, and α-terpinene showed a high acaricidal effect combined with a high bee mortality at the concentration ranges tested. α-Pinene and limonene showed low effect on *V. destructor* and bees, even at very high dosages of 700 resp. 1400 µg/l.

Essential oil or single components	Air concentration range correlated to a good acaricidal effect (> 80 %) and a low bee toxicity (< 20 %) during the 72 h test*
Substance	µg/litre of air
thymol	5 -15
menthol	20 - 60
camphor	50 - 150
α-thuyon	150 - 350
eucalytol	240 - 300
salvia oil	300 - 500
γ-terpinene	350 - 800
pinocamphone type hyssop oil	400 - 900
p-cymol	400 - 1000
thyme oil	500 - 700

* - see Materials and Methods.

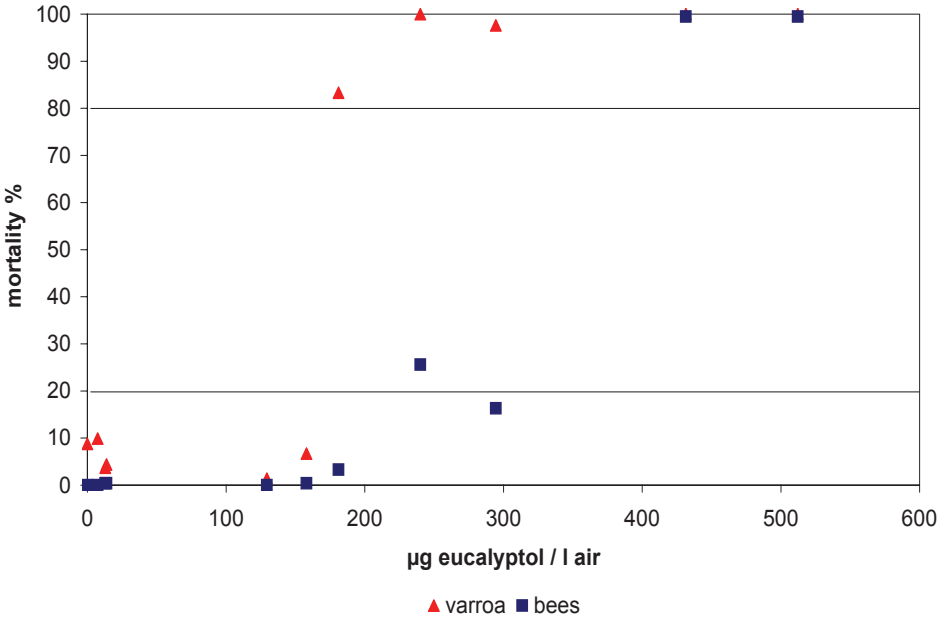


Fig. 1
Mortalities of *V. destructor* and bees presented as dose effect relationships after exposition during 72 hours to different concentrations of thyme oil.

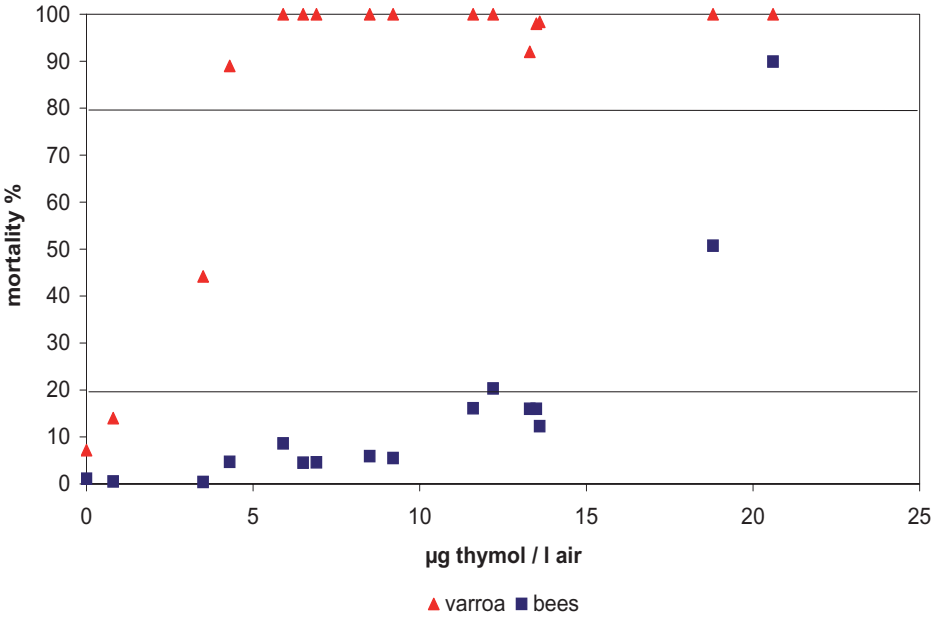


Fig. 2
Mortalities of *V. destructor* and bees presented as dose effect relationships after exposition during 72 hours to different concentrations of thymol.

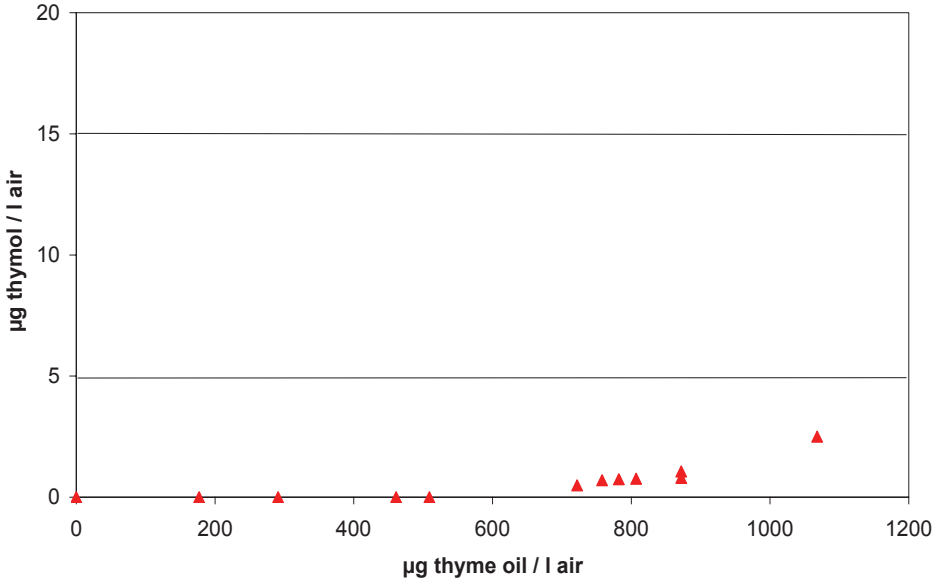


Fig. 3
Thymol concentration in the air of the exsiccator during the application of different concentration of thyme oil.

Fig. 4
p-Cymol concentration in the air of the exsiccator during the application of different concentration of thyme oil.

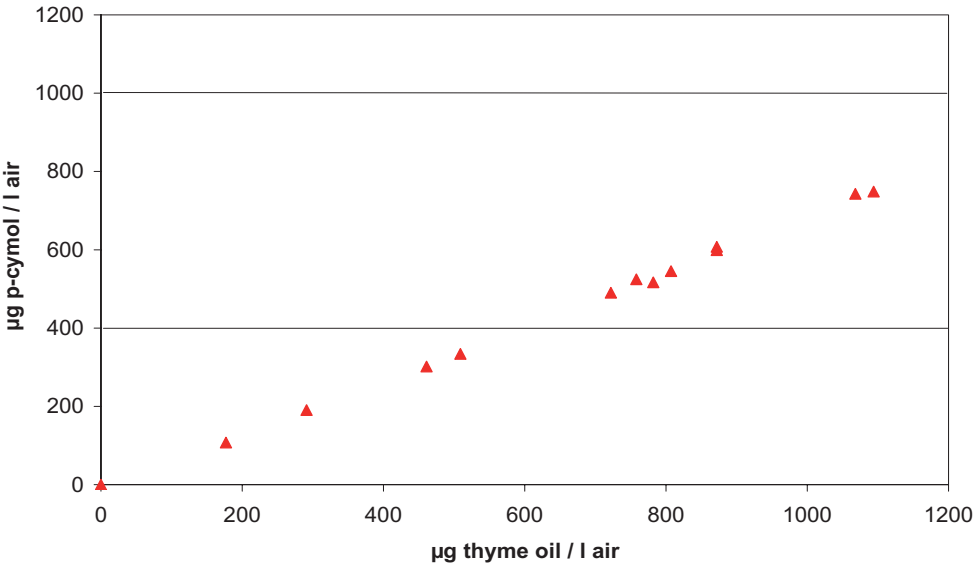


Fig. 5
Mortalities of *V. destructor* and bees presented as dose effect relationships after exposition during 72 hours to different concentrations of *p*-cymol.

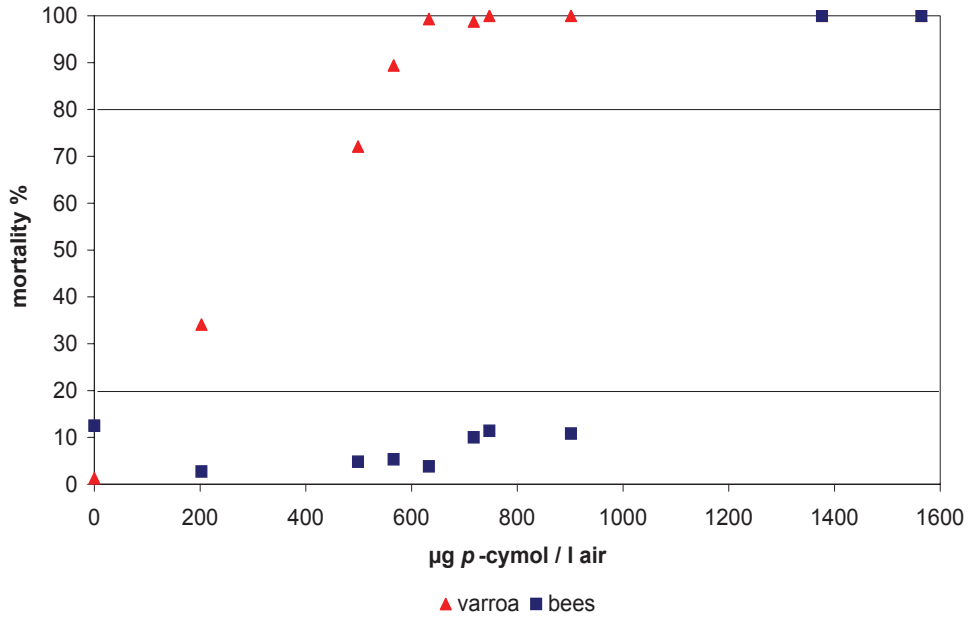
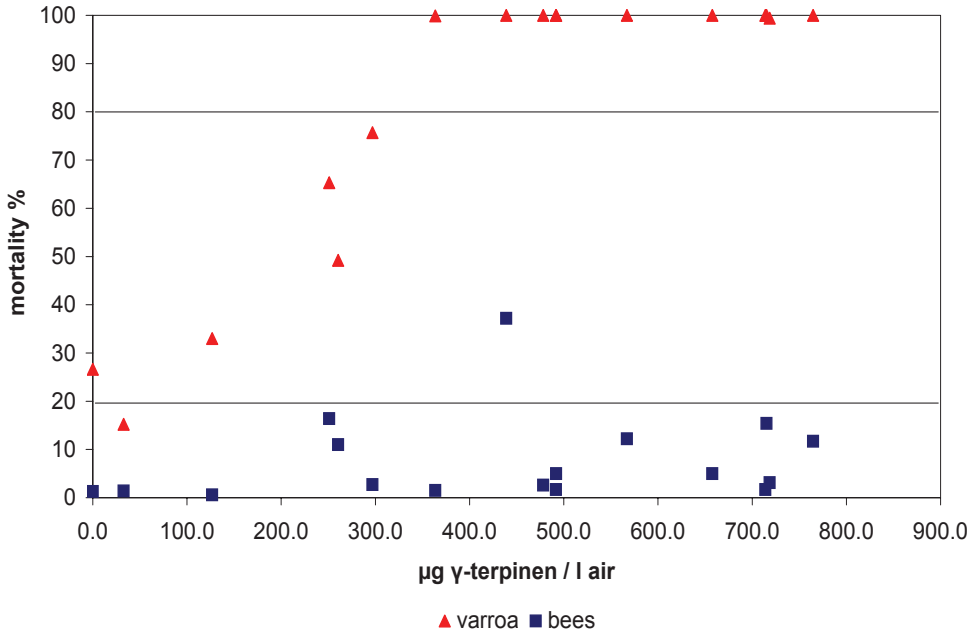


Fig. 6
Mortalities of *V. destructor* and bees presented as dose effect relationships after exposition during 72 hours to different concentrations of γ-terpinene.



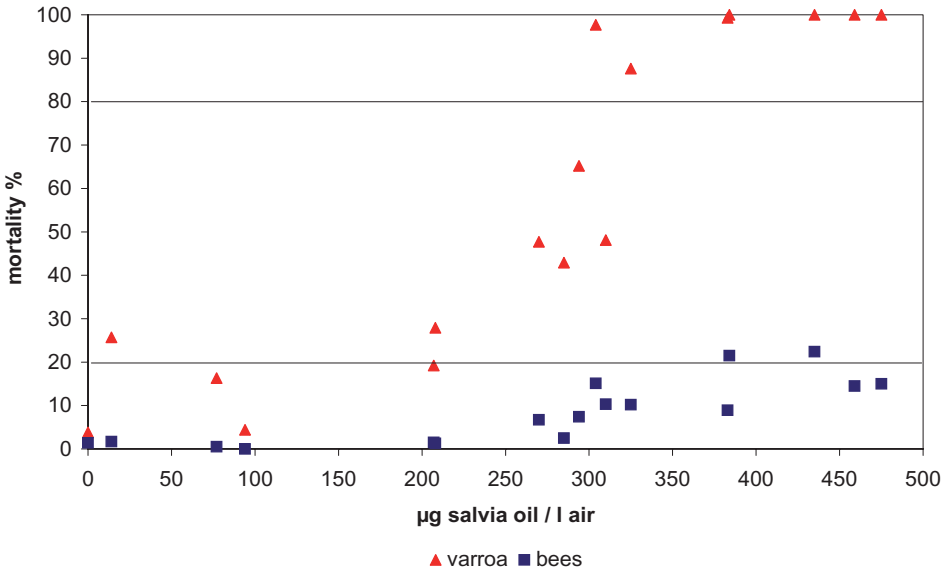


Fig. 7
Mortalities of *V. destructor* and bees presented as dose effect relationships after exposition during 72 hours to different concentrations of salvia oil.

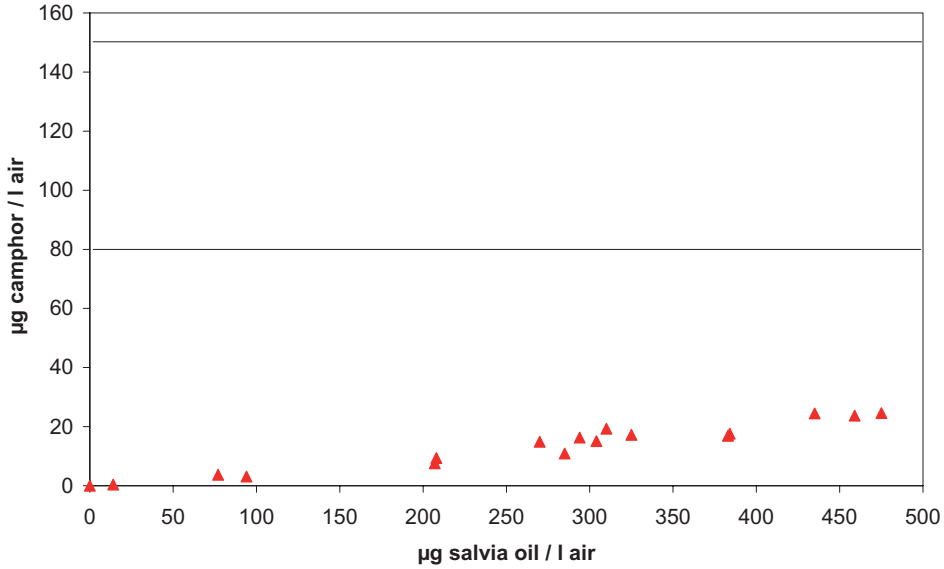


Fig. 8
Camphor concentration in the air of the exsiccator during the application of different concentration of salvia oil.

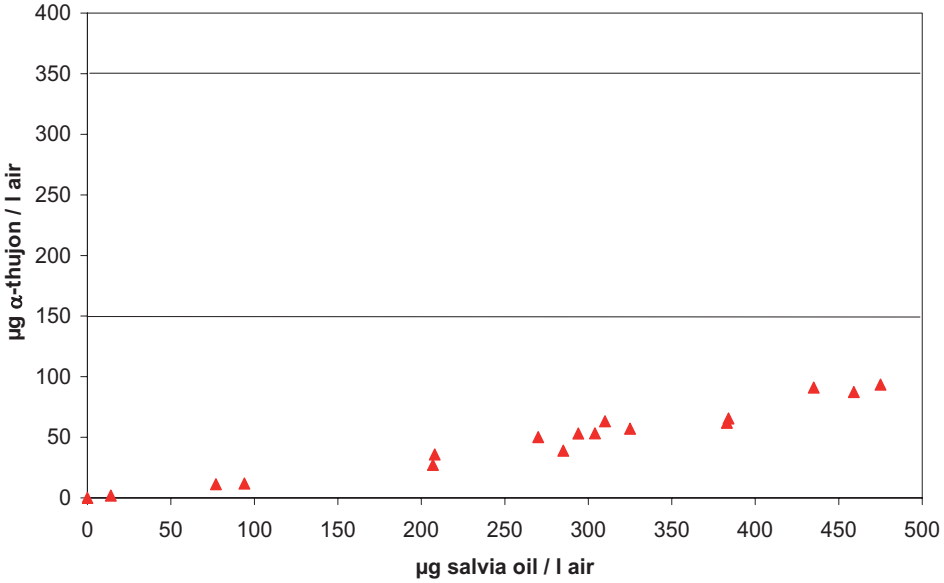


Fig. 9
α-Thujon concentration in the air of the exsiccator during the application of different concentration of salvia oil.

Fig. 10
Mortalities of *V. destructor* and bees presented as dose effect relationships after exposition during 72 hours to different concentrations of camphor.

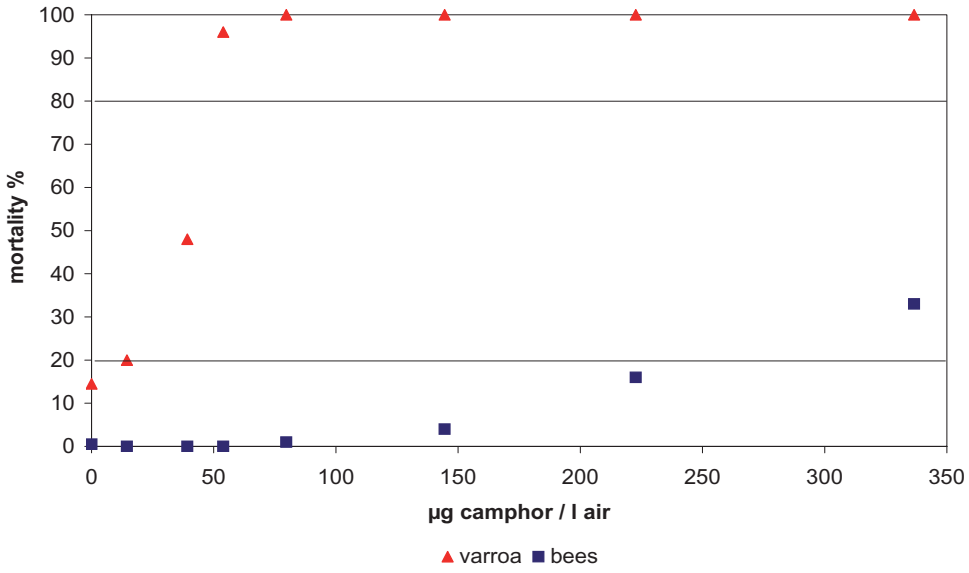


Fig. 11
Mortalities of *V. destructor* and bees presented as dose effect relationships after exposition during 72 hours to different concentrations of α-thujon.

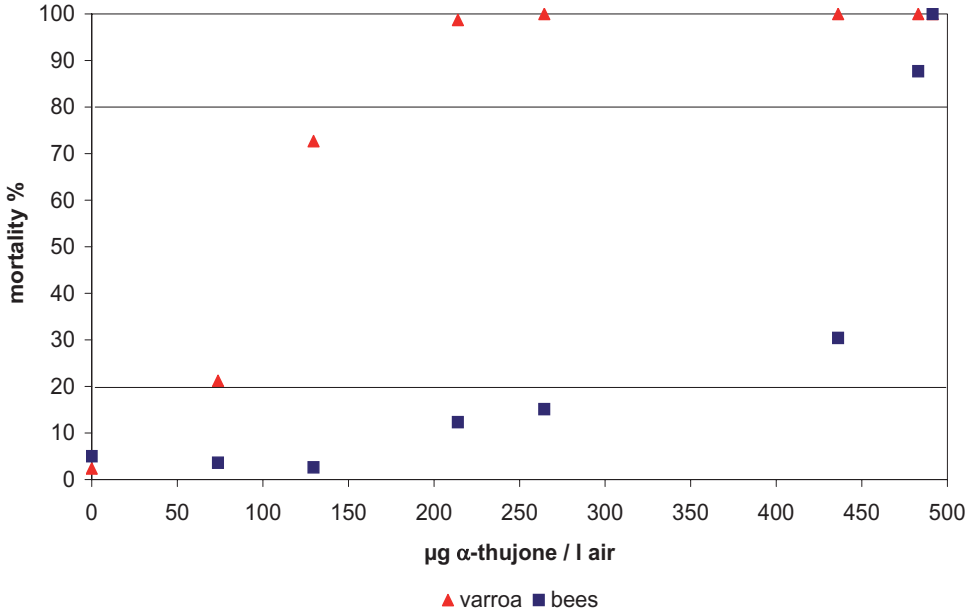
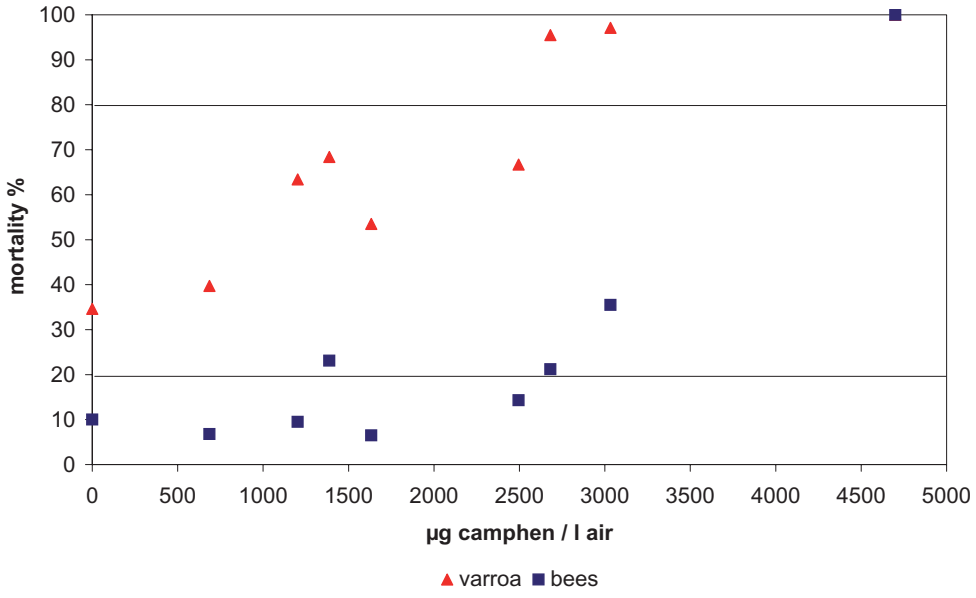


Fig. 12
Mortalities of *V. destructor* and bees presented as dose effect relationships after exposition during 72 hours to different concentrations of camphen.



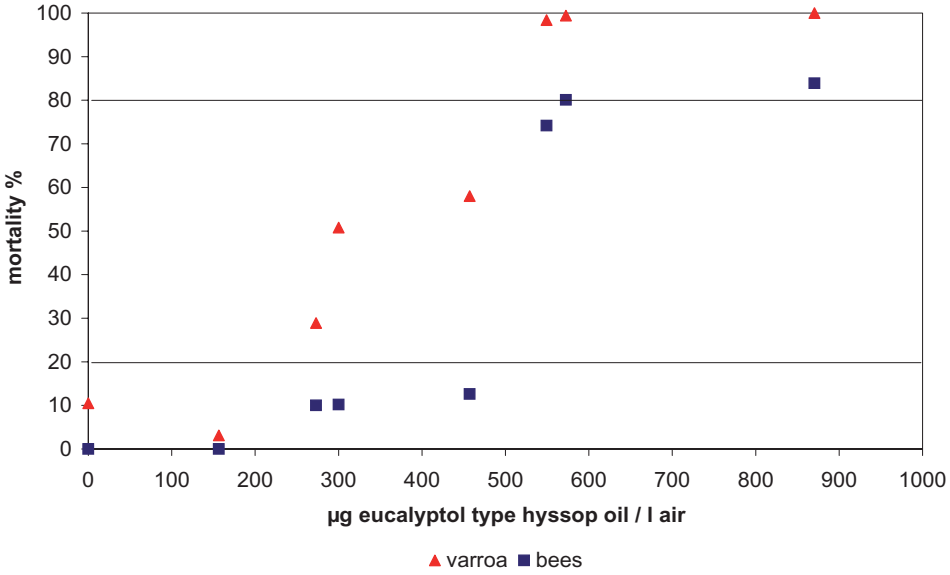


Fig. 13
Mortalities of *V. destructor* and bees presented as dose effect relationships after exposition during 72 hours to different concentrations of eucalyptol type hyssop oil.

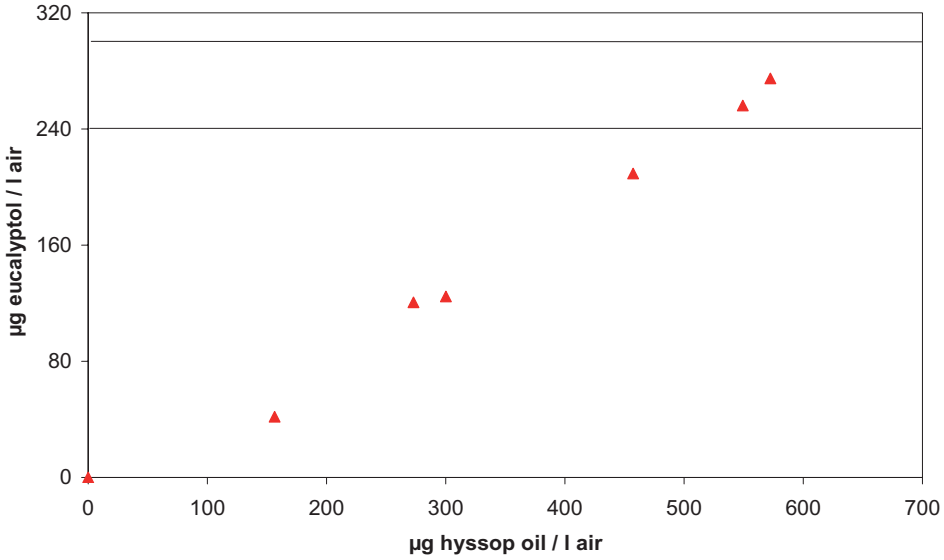


Fig. 14
Eucalyptol concentration in the air of the exsiccator during the application of different concentration of eucalyptol type hyssop oil.

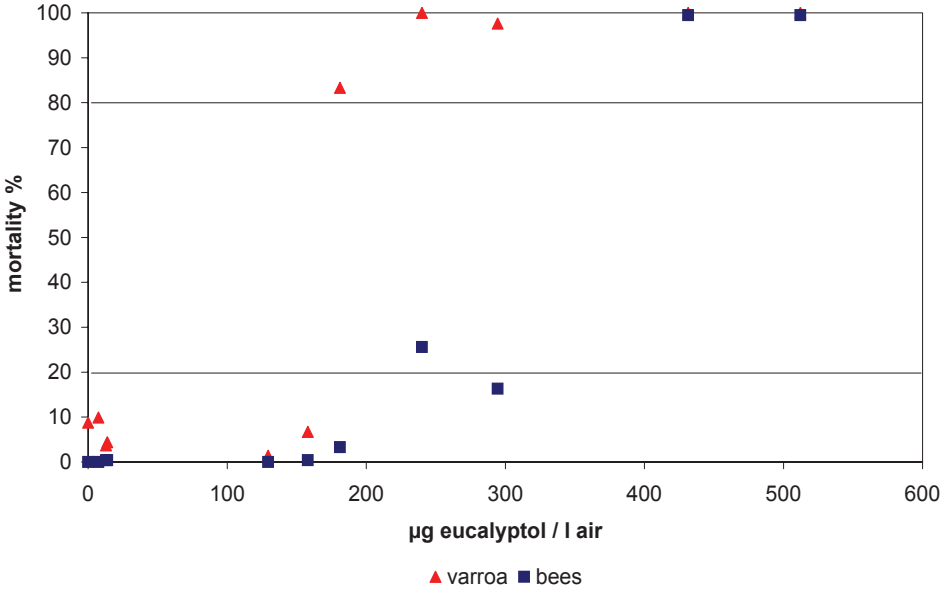


Fig. 15
Mortalities of *V. destructor* and bees presented as dose effect relationships after exposition during 72 hours to different concentrations of eucalyptol.

Fig. 16
Mortalities of *V. destructor* and bees presented as dose effect relationships after exposition during 72 hours to different concentrations of pino-camphone type hyssop oil.

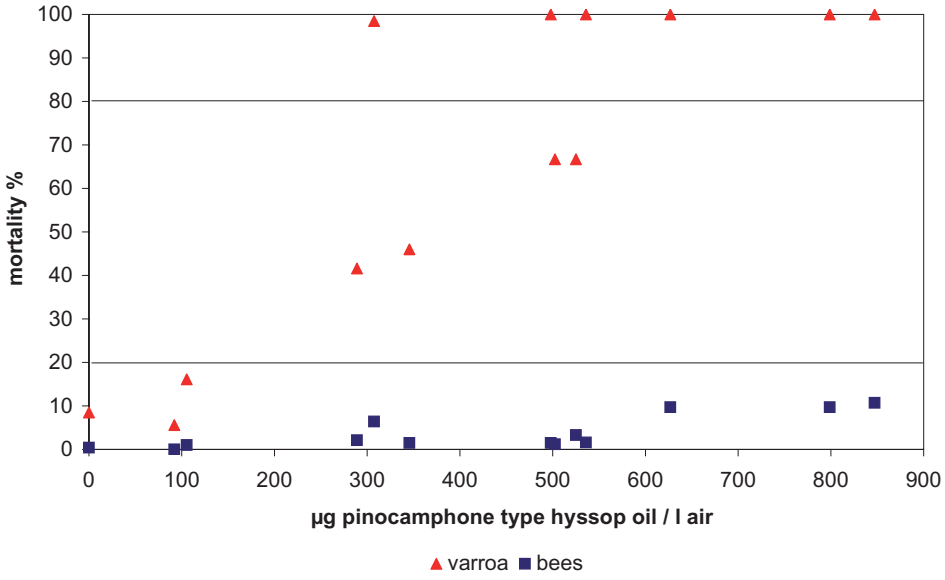


Fig. 17
Mortalities of *V. destructor* and bees presented as dose effect relationships after exposition during 72 hours to different concentrations of isopinocamphone in hyssop oil.