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Chemical characterization and acaricidal activity of *Thymus satureioides* C. & B. and *Origanum elongatum* E. & M. (*Lamiaceae*) essential oils against *Varroa destructor* Anderson & Trueman (*Acari*: *Varroidae*)

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ABSTRACT

Thymus satureioides and *Origanum elongatum*, endemic, aromatic and sources of honey in Morocco, are rich in essential oils (EOs). In Southwest, *Varroa destructor* affects less bee colonies foraging in thyme (*Thymus satureioides*) formations. The aim of this study was (i) to determine the composition of *T. satureioides* and *O. elongatum* EOs in relation to origins and phenological stages and (ii) to investigate the acaricidal activity against *Varroa destructor.* Essential oils obtained showed differences in the chemical composition according to plant species but did not vary drastically with the vegetative stage of growth and origins. The main constituents found in *T. satureioides* EOs were borneol (20.07%–48.23%) and α-terpineol (5.12%–18.16%), smaller amounts of camphen (5.10%–14.44%) and α-pinene (2.43%–7.63%), whereas thymol (0.14%–8.64%) or carvacrol (0.88%–15.23%). *O. elongatum* EOs contained carvacrol (67.34%–81.72%), γ-terpinene (3.29%–10.75%), para-cymene (3.62%–7.81%) and thymol (1.79%–9.17%). The apiary tests revealed a variable efficacy from 50 to 94% depending on the composition of essential oils. EOs with carvacrol as major compound produced a better effect than other EOs with dominant borneol. However, the blend containing high levels of carvacrol (55.35%) and borneol (20.60%) exhibited much higher activity than all treatments. A synergistic effect between the compounds of *T. satureioides* and *O. elongatum* EOs was highlighted.

1. Introduction

Varroa destructor Anderson & Trueman (*Arachnida: Acari: Varroidae*), a parasitic bee mite, has become since the 80s, the main pest of apiaries (Le Conte et al., 2010). The heavy parasitic infestations undeniably attracted massive use of chemical insecticides, which eventually contaminate honey productions (Souza Tette et al., 2015).

Currently, there are many preparations and procedures available to treat the mite. The use of synthetic, lipophilic varroacides in colonies is minimized, and the use of organic acids or essential oils increased. The development of highly specific acaricides stays a challenge and alternating between substances with different mechanisms of action to use them as efficiently as possible becomes imperative. This method is the only way to prevent the selection of mite strains that are resistant to a specific active substance (Lipiński and Szubstarski, 2007). Even if the treatment against *Varroa* mites is successful, resistant parasites may survive the treatment. If the pest is not eliminated by a different active substance, it can multiply unchecked in the hive and create a resistant population. Furthermore, bees constantly transport the pest into other hives, helping it to spread. Until we have bees that are resistant to mites, it is extremely important for beekeepers to check how severely a hive has been infected and to treat if needed, repeatedly. Until now, it is the only way to adjust effectively their mite control activities and keep infestation under the damage threshold. In order to minimize the environmental and health problems associated with the application of some synthetic products in beehives, several studies have been conducted around the world to extract and test natural substances as potential acaricides against the parasite (Meikle et al., 2012).

Recently, biopesticides based on EO have become alternative ways to control different types of insects and can have a great impact in the development of IPM programs (Perricone et al., 2015; Pavela and Benelli, 2016). They generally have a broad-spectrum efficacy, but with a specificity for classes or orders of insects and a short afterglow (Pavela, 2009; Moiteiro et al., 2013). EOs may induce fumigant and topical toxicity, as well as antifeedant or repellent effects (Bakkali et al., 2008), but also inhibit reproduction (Regnault-Roger et al., 2012). Moreover, they may act as grooming behavior stimulant of honeybee (Abd El-Wahab et al., 2012).

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EO bioactivity is largely due to its chemical composition and especially to the functional groups of the main constituents and their synergistic effects, but it is likely that minority compounds act synergistically (Lima et al., 2011; Isman et al., 2011). In recent years, research is interested in the synergistic effects exhibited by these substances (Pavela, 2016).

Thus, control of pests of the honeybee (*Apis mellifera* L.) as *Varroa destructor* with EOs is reliable; at least to diversify the range of active molecules and to ensure that honey and main bee products remain without contaminants.

In Morocco, beekeepers have observed that the *Varroa* has less effect on bees' colonies foraging on thyme (*Thymus satureioides*) stands. They also use the plant directly to fumigate beehives to control the pest.

Within this context, bioprospecting of the local flora searching for biopesticides based on EO to be used as acaricides is important. In Morocco, *Thymus satureioides* Coss. and *Origanum elongatum* Bonn., both endemic *Lamiaceae,* represent large wide natural formations, in the central and western High Atlas and in northern (upper eastern Rif) regions, respectively (Fennane and Rejdali, 2016). They are important sources of EOs.

The aim of present study was to investigate the effective using these endemic plants in the control of *Varroa* mites directly in beehives in comparison to a commercial synthetic acaricide. In order to diversify the range of active components, the EOs chemical composition was examined. Different EOs compositions were then assayed to explore acaricidal potential of various active molecules and their combinations against the *Varroa*. Moreover, to check the synergism, blend of EOs was also tested.

2. Materials and methods

The trials reported in this paper were carried out during April 2015 in the experimental apiary of the National Agricultural Research Institute (Station de Koudia) located 38 km South of Rabat, Morocco. The chemical analyzes were performed at Laboratory of phytochemistry, Department of Wood Technology and Forest Products Valorization, Center for Forest Research, Rabat, Morocco.

2.1. Plant harvest, extraction and chemical characterization of essential oil

Samples of *T. satureioides* foliar biomass were gathered on August 2013 (fruiting stage), April 2014 (preflowering) and June 2014 (flowering stage) in Taroudant province (South West Morocco) at six locations (Table 1) using line transect technique, at different altitudes and according to a continentality gradient. We collected Oregano on July 2014 (flowering stage) and August 2014 (fruiting stage) respectively in different localities in North East Morocco (Table 2).

Essential oils were obtained by hydrodistillation using the Clevenger type apparatus. About 200 g of foliar biomass (crushed thyme or oregano) was mixed with one liter of water in a 2 liters flask surmounted by a column of about 60 cm of length connected to a condenser. The mixture was heated to boiling for 3 h. Three replicates per origin have been carried out. Oil yields were estimated on the basis of the dry weight of plant material. The collected essential oil (EO) is dehydrated with anhydrous sodium sulphate and stored in dark vials at 4 °C (Bousbia, 2011).

Table 1
Characteristics of T. satureioides harvest sites in the South West region of Morocco.

Origin	Stand type	Exposition	Altitude (m)	GPS coord.
Argana	Ta	North	1240	N30 42.792, W9 06.194
Aoulouz	As	North	1020	N30 47.812, W8 23.132
Ankrim	Ta, As, Oe	North-east	320	N30 32.812, W9 34.284
Timoulay	Ta, Oe, Pl, Ph, Zs, Qi	East	860	N30 37.443, W9 30.336
Amskrot	Ta	East	1050	N30 38.138, W9 22.921
Amskrot	Ta	West	1050	N30 38.153, W9 22.947

Ta = *Tetraclinis articulata*, As = *Argania spinosa*, Oe = *Olea europaea*, Pl = *Pistacia lentiscus*, Ph = *Pinus halepensis*, Zs = *Zyziphus sp.*, Qi = *Quercus ilex.*

Table 2
Characteristics of *O. elongatum* harvest sites in North East region of Morocco.

Origin	Stand type	Exposition	Altitude (m)	GPS coord.
Bab-berd (Tamelzit)	Qs, Ls, Ci	North	1240	N30 42.792, W9 06.194
Ketama	Qs, Ls, Ci, Cy	North	1020	N30 47.812, W8 23.132
Targuist	Qi, Ci, Gq, Jo, Tr, Ca	North-east	320	N30 32.812, W9 34.284

Qs = *Quercus suber*, Ls = *Lavandula sp.,* Ci = *Cistus* sp., Cy = *Cytisus* sp., Qi = *Quercus ilex*, Gq = *Genista quadriflora*, Jo = *Juniperus oxycedrus, Tr = Thymus riatarum, Ca = Cedrus atlantica.*

For the analysis of volatile compounds we used gas chromatographic (GC) and gas chromatographic coupled to mass spectrometry (GC/MS).

Gas chromatographic (GC) analyses were performed with a Hewlett-Packard type (HP 6890) equipped with a capillary column HP-5 (30 m × 0.25 mm, 0.25 μm film thickness) and a detector FID at 250 °C. Split is the injection mode. Nitrogen was used as the carrier gas at a flow rate of 2 ml/min. The column temperature was programmed from 50 to 200 °C at a rate of 4 °C/min. The injected volume was 1 μl. The device was guided by a computer system type "HP ChemStation", managing the operation of the device and monitoring the changes in chromatographic analysis.

The GC/MS analyses were accomplished on a Hewlett-Packard (HP 6890) equipped with an automatic injector (HP 7683) and coupled with a mass spectrometer (HP 5973). The column used was a capillary column HP-5MS. The carrier gas was Helium (He) with a flow rate set of 2 ml/min and the column temperature was programmed from 50 to 250 °C due to 4 °C/min. The ionization energy was set at 70 eV. The identification of EO components was achieved by comparison of their retention indices relative to (C_8–C_{22}) *n*-alkanes with those of literature and by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC–MS data system and other published mass spectra (Adams, 2007).

2.2. Evaluation of the EO fumigant effect

2.2.1. Apiary trial

The experiments were carried out at apiary on April 2015 with 24 colonies of *Apis mellifera* bees, placed in Dandant-Blatt hives. The daily average ambient temperature and relative humidity (RH) ranged from 17 to 30 °C and 75 to 82%, respectively. All colonies had similar levels of open and sealed brood (max. 1 frame).

Six treatments of *T. satureioides* and *O. elongatum* essential oils (different combinations of compounds) and Bayvarol (2 x strip/hive: 3.6 mg of Flumethrin) were used for testing their efficacy against *Varroa* mite. The treatments (8 including Bayvarol and control) were randomly assigned to the 24 bee colonies with three replicates for each treatment.

Directly in the treated beehive, one strip of absorbent paper pad, soaked with 3 ml of pure EO, was hanged between the frames. The number of mites fallen (mite-drop) from bees has been chosen to be efficiency criterion of substances used against mites (Branco et al., 2006; Dietemann et al., 2013). In order to determine their effectiveness against *V. destructor* (effect of treatment on mite-drop by fumigation) in