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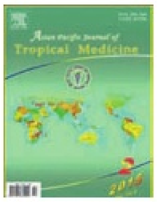

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Chemical composition of *Rosmarinus* and *Lavandula* essential oils and their insecticidal effects on *Orgyia trigotephras* (Lepidoptera, Lymantriidae)

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ABSTRACT

Objective: To evaluate toxic activities of essential oils obtained from *Rosmarinus officinalis* and *Lavandula stoechas* against the fourth larval instars of *Orgyia trigotephras*. **Methods:** A total of 1 200 larvae were divided into three groups Ⅰ, Ⅱ, Ⅲ. Group Ⅰ was to investigate the effect of extracted essential oils from these aromatic plants as gastric disturbance. *Bacillus thuringiensis* and ethanol were used as control group. Group Ⅱ was used as contact action and Group Ⅲ was used as fumigant action. Decis and ethanol were used as control group. During the three experiments, the effect of essential oils on larvae was assessed. **Results:** The chemical composition of essential oils from two medicinal plants was determined and, their insecticidal effects on the fourth larval state of *Orgyia trigotephras* were assessed. The two simples presented an insecticidal activity, nevertheless *Rosmarinus officinalis* essential oil was less efficient compared to *Lavandula stoechas* one are discussed. **Conclusions:** The relationship between the chemical composition and the biological activities is confirmed by the present findings. Therefore the potential uses of these essential oils as bioinsecticides can be considered as an alternative to the use of synthetic products.

1. Introduction

Essential oils play an important in protecting plants against attack[1]. These secondary plant metabolites are extracted from many medicinal and aromatic plants generally located in temperate and warmer regions where they are a significant part of the folklore medicine[2]. These substances are natural and complex substances, historically were mainly used for a long time for their odor properties in cosmetic industries and in the perfume composition[3].

Forests are threatened by many factors of degradation including pest attacks. *Orgyia trigotephras* (Lepidoptera, Lymantriidae) causes severe damage and sometimes death of the trees. Therefore, this destruction causes the loss of the forest ecological and even economic values. In our days, the control methods against this pest are mainly chemicals that have a negative impact on the environment furthermore, the biological one by *Bacillus thuringiensis* is not very effective on the later stages[4]. Thus, the search for new natural substances used for the protection of our forests as well as being environmentally sound arouses great interest. On the other hand, the new attraction for natural products increase the consumer concern about the safety of certain chemical products and their potential effects on health, leading to an increase in demand for biomolecules. For these reasons, the study of biological activities of essential oils in order to their applications to human health, agriculture and the environmental remains an interesting and useful task[5–8].

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The present study was undertaken to evaluate toxic activities of essential oils obtained from *Rosmarinus officinalis* (*R. officinalis*) and *Lavandula stoechas* (*L. stoechas*) against the fourth larval instars of *Orgyia trigotephras*.

2. Materials and methods

2.1. Plant material and isolation of essential oils

Our study focused on two species of aromatic plants, *R. officinalis* and *L. stoechas*. Leaves were collected from each aromatic plant, placed in bags bearing labels on which we noted plant species and then brought to laboratory. The harvested material was air–dried at room temperature (20 ℃ –25 ℃) for one week and then stored in cloth bags.

2.2. Chemical characterization of essential oils

Essential oils were extracted from leaves (100 g of dry matter) subjected to hydrodistillation during 90 min using a modified Clevenger–type apparatus. Anhydrous sodium sulphate was used to remove water after extraction. The extracted oils were stored in Eppendorf tubes, packed by aluminum foil in the dark and stored at –4 ℃.

Essential oils were analyzed by gas chromatography (GC) using a Hewlett–Packard 6890 gas chromatograph (Agilent Technologies, Palo Alto, California, USA) equipped with a flame ionization detector (FID) and an electronic pressure control (EPC) injector. A polar HP–Innowax (PEG) column (30 m×0.25 mm, 0.25 mm film thickness) and an apolar HP–5 column (30 m×0.25 mm coated with 5% phenyl methyl silicone, and 95% dimethyl polysiloxane, 0.25 mm film thickness) from Agilent were used. Carrier gas flow (N_2) was 1.6 mL/min and the split ratio 60:1. Analyses were performed using the following temperature program: oven kept isothermally at 35 ℃ for 10 min, increased from 35 to 205 ℃ at the rate of 3 ℃/min and kept isothermally at 205 ℃ for 10 min. Injector and detector temperatures were held, respectively, at 250 and 300 ℃. The GC/MS analyses were made using an HP–5972 mass spectrometer with electron impact ionization (70 eV) coupled with an HP–5890 series Ⅱ gas chromatograph. An HP–5MS capillary column (30 m× 0.25 mm coated with 5% phenyl methyl silicone, and 95% dimethyl polysiloxane, 0.25 μm film thicknesses) was used. The oven temperature was programmed to rise from 50 to 240 ℃ at a rate of 5 ℃/min. The transfer line temperature was 250 ℃. Helium was used as carrier gas with a flow rate of 1.2 mL/min and a split ratio of 60:1. Scan time and mass range were one second and 40e300 m/z respectively.

Essential volatile compounds were identified by calculating their retention index (RI) relative to (C9–C18) *n*–alkanes (Analytical reagents, Labscan, Ltd, Dublin, Ireland) and data for authentic compounds available in the literature and in our data bank, and also by matching their mass spectrum fragmentation patterns with corresponding data stored in the mass spectra library of the GC–MS data system (NIST). The relative percentage amount of each identified compound was obtained from the electronic integration of its FID peak area.

2.3. Preparation of test solutions

The essential oils dissolved in technical ethanol (96%) were used. Each crude solution was serially diluted with 96% to prepare test solutions of 0.05%, 0.10% and 0.50%. The oil yield was determined by the formula: R% = (mass of essential oil / plant material dry weight)×100

2.4. Chemical and biological insecticides

The larvicidal effect of essential oils by contact is appreciated by comparison with a chemical insecticide Delta–metrine"Decis" (reference product, provided by Atlas Agro–Tunisia). The product has been diluted with 96% ethanol to prepare the test solution. Ethanol, used for dilutions was used as control. The larvicidal effect by ingestion of essential oils is assessed by comparison with a biological insecticide *Bacillus thuringiensis* (reference product, provided by Atlas Agro–Tunisia).

2.5. Larvicidal activities

The larvicidal activity of essential oils was tested against the fourth instar larvae of *Orgyia trigotephras*. Larvae were in Petri dishes (R=9 cm) and were fed daily with fresh leaves of *Erica multiflora*. They were kept under natural conditions (ambient temperature 25°± 2°, natural light). Miroslav *et al*[9]. The oils were diluted with ethanol to obtain three solutions at different concentrations respectively (0.5%, 0.1% and 0.05%).

2.6. Contact action of essential oil

In this test, larvae are distributed in Petri dishes at 10 per box. Ten μL of each prepared oil solution is deposited on the back of each larva and six replicates for each concentration were performed. The larvicidal effect of the essential oil is appreciated by comparison with

Delta methrine "Decis" (reference product, provided by AtlasAgro–Tunisia). Ethanol was used for dilutions and as control.

2.7. Ingestion action of the essential oil

This test is used to determine the action of the essential oil after its ingestion by larvae. 100 μL of each oil concentration are spread over *Erica multiflora*; these leaves left in open air until total absorption of the product and are then placed in Petri dishes each containing 10 larvae fasted for 24 h. *Bacillus thuringiensis* on (provided by AtlasAgro–Tunisia) is used as reference and ethanol as control.

2.8. Olfactory action of essential oil

100 μL of each prepared solution was spread on filter paper in a Petri dish empty. The Petri dishes were placed in an oven at 21 ℃ for 20 minutes. Ten larvae are introduced in each box.

2.9. Assessment of insecticidal effect

Each treatment was replicated six times (total *n*=60)[9]. The percentage of dead larvae was calculated using the formula of Abbott[10] reference: % Corrected=(1–T/C)×100, with T and C are numbers of living larvae on experimentation with oil and the standard solution after the observation time. Treatment efficacy was calculated as the formula: E=100–[(St–T/St)×100]×2 with St and T represent dead larvae using standard and experimental solutions. Insecticidal activity was determined by measuring the average time of death (TMM), the time required to kill 50% of larvae, and the final time of mortality (TFM), the time required to kill 100% of larvae.

3. Results

3.1. Component analysis of essential oils

GC and GC/MS analysis of *R. officinalis* and *L. stoechas* essential oils led to the identification of 32 components were identified, of which 1.8–cineole 34.82%, camphor 12.91% and α–pinene 11.87%, were the major components of essential oil from *R. officinalis*; and Camphor 36.14%, 1.8–Cineole 25.16% and camphene 11.44%, were the major components of essential oil from *L. stoechas*. Among other components, the majority belongs to sesquiterpenes volatile compounds (Table 1).

Average yields of essential oils of selected aromatic plants revealed variable values. *L. stoechas* essential oil yield is the highest with a percentage of 2.17%. Indeed, it has been reported that the essential oils from *L. stoechas* vary between 1% and 6% depending on location and the vegetative stage, moreover this species, in general, has an average higher than other species of lavender[11].

Average yield *R. officinalis* essential oil was 1.34%. It should be noted that this yield is higher than that reported by Bekkara *et al*[11], studying *R. officinalis* from Algeria (0.8%).This performance is also better than that obtained from some species of Lamiaceae family: *Mentha rotundifolia* as having an efficiency of 0.8%[12].

3.2. Contact action of essential oils

Evaluation of contact action of essential oils against *Orgyia trigotephras* larvae showed a similar effect for different tested oils. For all concentrations, average TMM and TFM treated with essential oils were very short compared to the time kills caterpillars treated with Decis. In addition, ethanol used as a solvent for essential oils, produced no toxic effect.

L. stoechas and *R. officinalis* essential oils have proven a high toxicity to the fourth larval instars of *Orgyia trigotephras*. Shortest TMM and TFM were recorded with a concentration 0.5%, however, all other concentrations showed strong insecticidal activity. Lowest mortality duration was observed with *R. officinalis* oil [TTM=(2.75±0.01) min and TFM=(5.00±0.04) min], those *L. stoechas* oil were [TTM=(2.00±0.03) min and TFM=(2.00±0.05) min].

Time mortality were analyzed and the results showed that there are no significant differences between the essential oils used (*P*=0.57). By cons, highly significant differences were noted between oils, Decis and control (*P*=0.000). Furthermore, Student–Newman test showed that TTM and TFM obtained respectively from tested concentrations within same species, are not significantly different (*P*=0.2). Contact results test showed that the mean time mortality increased when essential oil concentration decreased, with 0.5% *L. stoechas* concentration TFM =5 min while 0.1% concentration TFM=6 min and 0.05% concentration TFM=10 min.

After essential oils exposure, larvae showed similarly behavioral responses to those observed with Decis. Therefore, it is possible to assume that contact action of essential oils is comparable to chemical insecticide which affects larvae nervous system. However, high mortality duration observed with Decis action may be attributed to a low transcuticular diffusion of the larvae's body unlike essential oil spreads quickly and easily on the back of the insect. Indeed, species analyzed in our work showed strong insecticidal activity by contact. This strong insecticidal activity can be attributed to the presence of one of these compounds in their essential oils. However, variations between death time probably resulted from change in percentages of these compounds. Statistical analyzes showed that time death differences were not significant between species reflecting a toxic effect level[13].

3.3. Ingestion action of essential oils

In this trial, the ingestion action of essential oils is longer than the contact action since the time of death one day beyond the two species of scrub. *R. officinalis* is the species with the highest insecticidal effect. For a 0.5% concentration, TMM recorded was (4.50±0.32) min, this time is significantly lower than that for a 0.05% concentration, which was (7.0±0.2) min. *R. officinalis* toxicity was observed for all concentrations; it is even more important that the concentration is high[14]. Indeed, comparison of means by Student–Newman test showed that differences between *R. officinalis* death time average obtained for 0.05%, 0.1%, 0.5% concentrations are highly significant.

The same results were observed for *L. stoechas*, TMM is around (8.0±0.4) h, this time is significantly lower compared to the TMM. On the other side, for 0.05% concentration. ATM was around 12.5 h. Toxicity is observed even lavender for all concentrations it is even more important that the concentration is high. Comparison of means by Student–Newman test showed that differences between lavender death average time obtained for different concentrations are

Table 1

Chemical composition (%) of the essential oils of the analyzed *L. stoechas* and *R. officinalis*.

	RT (min)	Area%	*Rosmarinus*	*Lavandula*	RT (min)	Area%
α –Thujene	7.121	0.35	+	–		
α –Pinene	7.401	11.87	+	–		
Camphene	7.910	5.12	+	+	7.928	11.44
Verbene			–	+	8.105	0.48
β –L–Pine	8.975	7.98	+	+	8.935	0.19
β –Myrcene	9.513	1.34	+	–		
1–Phellandrene	10.010	0.34	+	–		
3–Carene	10.233	0.26	+	–		
α –Terpenene	10.497	0.93	+	–		
ρ –Cymene	10.874	2.73	+	+	10.834	1.08
1,8–Cineol	11.200	34.82	+	+	11.160	25.16
Gamma–Terpinene	12.162	1.50	+	+	12.150	0.22
α –Terpinolene	13.306	0.59	+	–		
β –Linalool	13.787	0.62	+	+	13.838	0.36
Trans–Pinocarveol	15.315	0.28	+	+	16.167	0.18
(+–)–Camphor	15.526	12.91	+	+	15.658	36.14
[(15)–Endo]–(–)–Borneol	16.327	5.09	+	–		
1–Terpinen–4–ol	16.734	1.45	+	+	16.728	0.80
α Terpineol	17.272	4.12	+	+	17.237	0.22
Bornyl acetate	20.620	1.56	+	+	20.636	1.97
Eugenol methyl ether	24.607	0.16	+	+	23.074	0.19
Caryophyllene	25.133	3.09	+	+	25.116	0.41
Aromadendrene	25.740	1.70	+	–		
α A.–Humulene	26.198	0.36	+	–		
Allo–Aromadendrene	26.427	0.29	+	–		
Gamma–Muurolene	26.902	0.14	+	–		
2–Camphenilone			–	+	13.106	0.49
Fenchone			–	+	13.335	9.08
Verbenone			–	+	17.890	0.51
(+)–Carvone			–	+	19.143	0.18
Myrtenyl acetate			–	+	21.992	1.37
β –Selinene			–	+	27.222	0.20
β –Cubebene			–	+	27.399	0.23
Total		99.8				91.95

Retention indices calculated using respectively an a polar column (HP–5) and polar column (HP–innwax). (–) means not detected.
The values in bold are to highlight the chemical constituents found in higher percentage in the essential oil.

still highly significant. In the case of ingestion treatment we note that mortality reached final time for both species.

Bacillus thuringiensis is a biopesticide acts only by ingestion. Strains of this bacterium may have a different effect of the diversity of toxins they can produce. Treatment with *Bacillus thuringiensis* is long since its action occurs after ingestion of the toxin release and its binding to specific receptors in the gut of the insect[15].

3.4. Fumigant action of essential oils

Fumigant action of essential oils on larvae showed a similar effect for tested oils. For all concentrations, TMM and TFM treated larvae with essential oils were very short compared to death time of treated larvae with Decis. In addition, ethanol used as a solvent for essential oils, produced no toxic effect on larvae. All oils have proved highly toxic to the fourth stage of *Orgyia trigotephras* and we note that the time of death is much lower than the concentrations are high. The TMM and TFM shortest are registered for the concentration of 0.5%, however, all other concentrations showed strong insecticidal activity. Lowest mortality duration was observed with *R. officinalis oil*. We note that Rosemary oil has a TMM=(6.50±0.01) min and TFM=(10.50±0.04) min with 0.5% dilution; whereas *Lavandula stoechas* has a [TMM=(22.50±0.03) min and TFM=(40.40±0.05) min].

Student–Newman test showed that TTM and TFM obtained for different concentrations tested within the same species, are not significantly different (P=0.3).

4. Discussion

Our study showed that *L. stoechas* and *R. officinalis* essential oils composition are characterized by the presence of 1.8–Cineol 34.82%; Camphor 12.91% α –pinene 11.87% and Camphene 5.12% (*R. officinalis*) and Camphor 36.14%; 1.8–Cineol 25.16%; Camphene 11.44% (*L. stoechas*) as major components. It is clear that essential oils from these aromatic plants are rich in monoterpenoids, compounds that possess insecticidal activity against various insect species. Monoterpenes are known to have insecticidal activity. 1.8–Cineol which represents the major compounds of *R. officinalis*, same result and the second major compound of *L. stoechas* essential oils which have been reported to be toxic to several insect species. Cavalcanti *et al*[16] mentioned that monoterpenoids eugenol and 1.8–Cineol for *Ocimum gratissimum* showed good larvicidal activity against *Aedes aegypti*. Prates *et al*[17] reported also that monoterpene compound demonstrated insecticidal activity by penetrating the insect cuticle (contact effect), by respiration (fumigant effect) and through the digestive system (ingestion effect). Rafael *et al*[18] reported that 1.8 Cineol was among those oils showing a toxicity action on eggs of the louse *Pediculus humanus* capitis. In addition Macedo *et al*[19] showed that 1.8 Cineol in *Eucalyptus globulus* has the best acaricidal action against *Boophilus microplus*.

Camphor which represent the first major compound of *L. stoechas* and the second major component of *R. officinalis* essential oils were also known to have insecticidal activities. Furthermore, it is used with great effect to repel insects such as flies and moths[13]. α –pinene which represent the third major component of *R. officinalis* essential oil is also known to have insecticidal activities. Simas *et al*[20] mentioned that monoterpene β – and α –pinene from *Myroxylum balsamum* presented good larvicidal activity against *Aede aegypti* larvae. Camphene which represents the third major compound of *L. stoechas* essential oil is also known to have insecticidal activities. Furthermore, it is used with great effect to repel insects such as flies and moths[21–24].

All of these data can explain the effectiveness of *L. stoechas* and *R. officinalis* oils on the fourth stage of *Orgyia trigotephras* development.

To conclude, in this study, two essential oils of selected aromatic plants were tested and shown to be effective on the developmental phases of *Orgyia trigotephras* in the Laboratory. Results from this study demonstrated that selected aromatic plants essential oils have excellent larvicidal activity and that camphor is the first responsible for such activity[25]. However, little information exists about the mechanism of action of the essential oils. One of the hypotheses is that the monoterpenes act on other vulnerable sites, such as nervous system, but understanding the real mechanism of action of these oils will require further investigation[26], Isman *et al*[27] suggest that toxicity of rosemary oil, at least to *lepidopteran* larvae, is a consequence of the combined (and possibly synergistic) effects of several chemical constituents, with no individual compound making a dominating contribution. Thus, the use of natural products may be considered as an important alternative insecticide for the control of *Orgyia trigotephras*.

Conflict of interest statement

We declare that we have no conflict of interest.

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