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

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PEER REVIEW

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Comments

Acquired resistance and environmental pollution due to repeated applications of persistent synthetic insecticides have created interest in discovering new natural insecticide products. The activity was assessed based on the interactions between plants and insects. Thus, the use of bioinsecticide may be considered as an important alternative insecticide for the control of *O. trigotephras*.
Details on Page 68

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* was used as referencee and ethanol as control. Group II was used as contact action and Group III was used as fumigant action. For both Groups II and III, Decis was used as reference and ethanol as control. 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. They presented an insecticidal activity. *Rosmarinus officinalis* essential oil was less efficient compared to *Lavandula stoechas*.

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.

KEYWORDS

Rosmarinus officinalis, *Lavandula stoechas*, *Orgyia trigotephras*, Essential oils, Insect control

1. Introduction

Essential oils play an important role 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 significant part of the folklore medicine[2]. These substances are natural and complex, and historically were mainly used for a long time because of 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) (*O. trigotephras*) causes severe damage and sometimes death of the trees. Therefore, this destruction causes the loss of the forest ecological and even economic values. Nowadays, the control methods against this pest are mainly chemicals that have a negative impact on the environment, furthermore, the biological activities by *Bacillus thuringiensis* (*B. 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

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health, leading to an increase in demand for biomolecules. For these reasons, in order to make an application to human health, agriculture and the environmental, the study of biological activities of essential oils remains to be an interesting and useful task[5-8].

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 *O. trigotephras*.

2. Materials and methods

2.1. Plant material and isolation of essential oils

Our study mainly 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 ˚C) for one week and then stored in cloth bags. These two plants were identified according to Nabli. The voucher specimens (RO Hiest01/2014 and LS Hiest01/2014) are available in our institute (High Institute of Environmental Sciences and Technology).

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 ˚C.

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 and an electronic pressure control injector. A polar HP-INNOWax column (30 m×0.25 mm, 0.25 μm 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 μm film thickness) from Agilent were used. Carrier gas flow (N_2) was 1.6 mL/min and the split ratio was 60:1. Analyses were performed by using the following temperature program: oven was kept isothermally at 35 ˚C for 10 min, increased from 35 to 205 ˚C at the rate of 3 ˚C/min and kept isothermally at 205 ˚C for 10 min. Temperatures of injector and detector were held at 250 and 300 ˚C, respectively. The gas chromatography-mass spectrometer (GC-MS) analyses were made by using a HP-5972 mass spectrometer with electron impact ionization (70 eV) coupled with a HP-5890 Series II GC. A HP-5 MS 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 ˚C at a rate of 5 ˚C/min. The transfer line temperature was 250 ˚C. 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 1 second and 40e300 m/z respectively.

Essential volatile compounds were identified by calculating their retention index 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. The relative percentage amount of each identified compound was obtained from the electronic integration of its flame ionization detector 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

The larvae were divided into three groups. Group I was to investigate the effect of extracted essential oils from these aromatic plants as gastric disturbance. *B. thuringiensis* was used as reference and ethanol as control. Group II was used as contact action and Group III was used as fumigant action. Decis was used as reference and ethanol as control. For all of these groups, larvae were distributed in Petri dishes at 10 per box and per concentration. Each treatment was replicated six times.

2.4. Chemical and biological insecticides

The larvicidal effect of essential oils by contact was appreciated by comparison with a chemical insecticide deltamethrin, namely Decis (reference product, provided by Atlas Agro, Tunisia). The product has been diluted with 96% ethanol to prepare for the test solution. Ethanol, used for dilutions was used as control. The larvicidal effect by ingestion of essential oils was assessed by comparison with a biological insecticide *B. 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 *O. 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) ˚C, natural light][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 were distributed in Petri dishes at 10 per box. About 10 μL of each prepared oil solution was deposited on the back of each larva, and 6 replicates for each concentration were performed. The larvicidal effect of the essential oil was appreciated by comparison with deltamethrin (Decis). Ethanol was used for

dilutions and as control.

2.7. Ingestion action of the essential oil

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

2.8. Fumigant action of essential oil

About 100 µL of each prepared solution was spread on filter paper in an empty Petri dish. The Petri dishes were placed in an oven at 21 °C for 20 min. Ten larvae were distributed in each box.

2.9. Assessment of insecticidal effect

Each treatment was replicated 6 times (n=60)[9]. The percentage of dead larvae was calculated by using the formula of Abbott[10] reference:

Corrected (%)=(1-T/C)×100

Where T and C represent numbers of living larvae on experimentation with oil and the standard solution after the observation time respectively.

Treatment efficacy was calculated as the formula:

E=100-[(St-T/St)×100]×2

Where St and T represent dead larvae using standard and experimental solutions respectively. Insecticidal activity was determined by measuring the average time of mortality (ATM), the time required to kill 50% of larvae, and the final time of mortality (FTM), the time required to kill 100% of larvae.

2.10. Statistical analysis

The statistical treatment of data was performed using SPSS (Version 10.0). ATM and FTM were analyzed for variance by the Fisher test to test the hypothesis of equality of means at the threshold 5%. It is complemented by multiple comparisons of means by the least significant difference test.

3. Results

3.1. Component analysis of essential oils

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

Table 1

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

Composition	RT (min)	Area (%)	RO	LS	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
γ-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
(-)-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-Humulene	26.198	0.36	+	-		
Allo-aromadendrene	26.427	0.29	+	-		
γ-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		99.60				91.90

RO: *R. officinalis*; LS: *L. stoechas*. -: Not detected; RT: Retention times.

Average yields of essential oils of selected aromatic plants revealed variable values. *L. stoechas* essential oil yield was the highest with a percentage of 2.17%. Average yield of *R. officinalis* essential oil was about 1.34%.

3.2. Contact action of essential oils

Evaluation of contact action of essential oils against *O. trigotephras* larvae showed a similar effect for different tested oils. For all concentrations, ATM and FTM 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 were proven a high toxicity to the fourth larval instars of *O. trigotephras*. The shortest ATM and FTM were recorded with a concentration of 0.5%, however, all other concentrations showed strong insecticidal activity. The lowest mortality duration was observed with *R. officinalis* oil [ATM=(2.75±0.01) min and FTM=(5.00±0.04 min)] and *L. stoechas* oil [ATM=(2.000±0.030) min and FTM=(2.000±0.050 min)].

Time mortality were analyzed and the results showed that there were no significant differences between the essential oils (P=0.57). By cons, highly significant differences were noted between oils, Decis and control (P<0.05). Furthermore, Student-Newman-Keuls

test showed that ATM and FTM obtained respectively from tested concentrations within same species, were not significantly different (P>0.05). Contact results test showed that the mean time mortality increased when essential oil concentration decreased, with 0.5% *L. stoechas* concentration of FTM=5 min while 0.1% concentration of FTM=6 min and 0.05% concentration of FTM=10 min.

After essential oils exposure, larvae showed similarly behavioral responses to those observed with Decis. Therefore, it was possible to assume that contact action of essential oils was comparable to chemical insecticide which affected 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 spread 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 (Table 2).

Table 2

Bioassays of larvicidal activities, contact action, ingestion action, fumigant action and insecticidal effects (min).

Group	*R. officinalis*		*L. stoechas*	
	ATM	FTM	ATM	FTM
Contact action (P=0.2)	2.75±0.01	5.00±0.04	2.00±0.03	2.00±0.05
Fumigant action (P=0.3)	6.50±0.01	10.50±0.04	22.50±0.03	40.40±0.05
Ingestion action	4.50±0.32	-	8.00±0.40	-

The time of ingestion action is calculated in h. -: Data are not calculated because it takes more than 72 h.

3.3. Ingestion action of essential oils

In this trial, the ingestion action of essential oils was longer than the contact action since the time of death one day beyond the two species of scrub. *R. officinalis* was the species with the highest insecticidal effect. For 0.5% concentration, ATM was recorded as (4.50±0.32) min, which was significantly lower than that of the 0.05% concentration, which was (7.00±0.20) min. *R. officinalis* toxicity was observed for all concentrations; the toxicity was positively related with the concentration. Indeed, comparison of means by Student-Newman-Keuls test showed that differences between *R. officinalis* death time average obtained for 0.05%, 0.1%, 0.5% concentrations were highly significant.

The same results were observed for *L. stoechas*. ATM was around (8.00±0.40) h, which was significantly lower compared to the ATM of *R. officinalis* [(4.50±0.32) h]. On the other side, for 0.05% concentration, ATM was around 12.5 h. Toxicity was observed even lavender for all concentrations. It was even more important that the concentration was high. Comparison of means by Student-Newman-Keuls test showed that differences between lavender death average time obtained for different concentrations were still highly significant. In the case of ingestion treatment, we noted that mortality reached final time for both species.

B. thuringiensis were biopesticide acts only by ingestion. Strains of this bacterium may have a different effect of the diversity of toxins they can produce. Treatment with *B. thuringiensis* was long since its action occurred 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 of tested oils. For all concentrations, ATM and FTM 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 *O. trigotephras* and we noted that the time of death was much lower at higher oil concentrations. The shortest ATM and FTM were registered for the concentration of 0.5%, however, all other concentrations showed strong insecticidal activity. The lowest mortality duration was observed in *R. officinalis* oil. We noted that rosemary oil had a ATM of (6.50±0.01) min and FTM of (10.50±0.04) min with 0.5% dilution; whereas *L. stoechas* had a ATM of (22.50±0.03) min and FTM of (40.40±0.05) min (Table 2).

Student-Newman-Keuls test showed that ATM and FTM, obtained for different concentrations and tested within the same species, were not significantly different (P>0.05).

4. Discussion

The total yields for both aromatic plants are around 1.34% and 2.17% respectively for *R. officinalis* and *L. stoechas*. 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]. It should be noted that this yield is higher than that of reported by Bekkara *et al.* on *R. officinalis* from Algeria (0.8%)[11]. This performance is also better than that obtained from some species of Lamiaceae family, *Mentha rotundifolia* as having an efficiency of 0.8%[12].

Our study showed that major components of *R. officinalis* essential oils are 34.82% of 1.8-cineol, 12.91% of camphor, 11.87% of α-pinene, while the major components of *L. stoechas* are 36.14% of camphor, 25.16% of 1.8-cineol, 11.44% of camphene. 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[16].

1.8-Cineol, which represents the major compound of *R. officinalis* and the second major compound of *L. stoechas* essential oils, have been reported to be toxic to several insect species. Cavalcanti *et al*. mentioned that monoterpenoids eugenol and 1.8-cineol for *Ocimum gratissimum* showed good larvicidal activity against *Aedes aegypti*[17]. Prates *et al*. also reported that monoterpene compound demonstrated insecticidal activity by penetrating the insect cuticle (contact effect), the respiration (fumigant effect) and the digestive system (ingestion effect)[18]. Laborda *et al*. reported that 1.8-cineol among those oils showed a toxicity action on eggs of the louse (*Pediculus humanus capitis*)[19]. In addition, Macedo *et al*. showed that 1.8-cineol in *Eucalyptus globulus* has the best acaricidal action

against *Boophilus microplus*[20]. Camphor, which represents the first major compound of *L. stoechas* and the second major component of *R. officinalis* essential oils, was 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 represents the third major component of *R. officinalis* essential oil, is also known to have insecticidal activities. Simas *et al*. mentioned that monoterpene β-pinene and α-pinene from *Myroxylon balsamum* presented good larvicidal activity against *Aedes aegypti* larvae[21]. Camphene, which represents the third major compound of *L. stoechas* essential oil, is also known to have insecticidal activities. Furtermore, it is also used with great effect to repel insects such as flies and moths[22-25].

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

To conclude, in this study, the two essential oils of selected aromatic plants were tested and shown to be effective on the developmental phases of *O. trigotephras* in the laboratory. Firstly, results from this study demonstrated that the selected aromatic plants essential oils have excellent larvicidal activity and the camphor is the first to response for such activity. Secondary, a direct relationship between mortality rate and concentration was detected. The same trend was observed between mortality rate and exposure time as well[26]. 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, however, understanding the real mechanism of action of these oils will require further investigation[27]. Isman *et al*. suggested 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[28]. Thus, the use of natural products may be considered as an important alternative insecticide for the control of *O. trigotephras*.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Pests are an important agent of trees damage. Chemical drugs are used frequently to develop insects resistance to these substances. The use of plants with insecticidal activity has several advantages over the use of synthetic products: the development of insect resistance to these substances is slow, and the substances do not leave residues in the environment. Therefore there is a need of new and potent bio insecticide.

Research frontiers

The present research work depicts the toxic activities of essential oils obtained from *R. officinalis* and *L. stoechas* against the fourth larval instars of *O. trigotephras*. The larvicidal effect of essential oils by contact is appreciated by comparison with a chemical insecticide deltamethrin Decis and the larvicidal effect by ingestion of essential oils is assessed by comparison with a biological insecticide *B. thuringiensis*.

Related reports

Screening of natural products has received the attention of researchers around the world. The folklore medicine has evidence of effectiveness of herb extract in treating various insect disorders.

Innovations and breakthroughs

R. officinalis has many culinary and medicinal uses: to flavor various foods, to renovate vitality of paralyzed limbs, and to treat gout and to improve the memory. *L. stoechas* has many uses: bactericidal and antiseptic effect, cleans wounds and skin ulcers, eliminates lice, neutralizes the venom in case of viper bite and helping relieve autonomic dystonia. In the present study, authors have demonstrated the toxic activities of essential oils obtained from these aromatic plants against *O. trigotephra* larvae.

Applications

Overall, considering the metabolites of interest, it appears that these aromatic and medicinal plants present a new valuable source of bioactive molecules.

Peer review

Acquired resistance and environmental pollution due to repeated applications of persistent synthetic insecticides have created interest in discovering new natural insecticide products. The activity was assessed based on the interactions between plants and insects. Thus, the use of bioinsecticide may be considered as an important alternative insecticide for the control of *O. trigotephras*.

References

[1] Pimentel D. Pesticides applied for the control of invasive species in the United States. In: Abrol D, editor. *Integrated pest management: current concepts and ecological perspective*. Amsterdam: Elsevier; 2014, p. 111-123.

[2] Roel AR. [The use of plants with insecticide properties: a contribution for Sustainable Rural Development]. *Revista Internacional de Desenvolvimento Local* 2001; **1**: 43-50. Portuguese.

[3] Lawal OA, Ogunwande IA. Essential oil from the medicinal plants of Africa. In: Kuete V, editor. *Medicinal plant research in Africa.* Oxford: Elsevier; 2013, p. 203-224.

[4] Paula JP, Farago PV, Checchia LEM, Hirose KM, Ribas JLC. [Repellent activity of essential oil of *Ocimum selloi* Benth. (variety eugenol) against *Anopheles braziliensis* Chagas]. *Acta Farm*

Bonaerense 2004; **23**: 376-378. Portuguese.

[5] Yaghoobi-Ershadi MR, Akhavan AA, Jahanifard E, Vantandoost H, Amin GH, Moosavi L, et al. Repellency effect of Myrtle essential oil and DEET against *Phlebotomus papatasi*, under laboratory conditions. *Iran J Public Health* 2006; **35**: 7-13.

[6] Gahukar RT. Potential and utilization of plant products in pest control. In: Abrol D, editor. *Integrated pest management: current concepts and ecological perspective*. Amsterdam: Elsevier; 2014, p. 125-139.

[7] Pavela R. Insecticidal activity of some essential oils against larvae of *Spodoptera littoralis*. *Fitoterapia* 2005; **76**: 691-696.

[8] Rajendran S, Sriranjini V. Plant products as fumigants for stored product insect control. *J Stored Prod Res* 2008; **44**: 126-135.

[9] Kostić M, Popović Z, Brkić D, Milanović S, Sivcev I, Stanković S. Larvicidal and antifeedant activity of some plant-derived compounds to *Lymantria dispar* (Lepidoptera: Limantridae). *Bioresour Technol* 2008; **99**: 7897-7901.

[10] Abbott WS. A method of computing the effectiveness of an insecticide. *J Econ Entomol* 1925; **18**: 265-267.

[11] Bekkara FA, Bousmaha L, Taleb Bendiab SA, Boti JB, Casanova J. [Chemical composition of the essential oil of *Rosmarinus officinalis* L. growing in the wild state and cultivated in the region of Tlemcen]. *Biologie & Santé* 2007; **7**(1): 6-11. French.

[12] Brada M, Bezzina M, Marlier M, CarlierA, Logny G. [Variability of the chemical composition of essential oils of *Mentha rotundifolia* in Northern Algeria]. *Biotechnol Agron Soc Environ* 2007; **11**(1): 3-7. French.

[13] Slimane BB, Ezzine O, Dhahri S, Ben Jamaa ML. Essential oils from two *Eucalyptus* from Tunisia and their insecticidal action on *Orgyia trigotephras* (Lepidotera, Lymantriidae). *Biol Res* 2014; **47**: 29.

[14] Saeidi M, Moharramipour S. Insecticidal and repellent activities of *Artemisia khorassanica*, *Rosmarinus officinalis* and *Mentha longifolia* essential oils on *Tribolium confusum*. *J Crop Prot* 2013; **2**(1): 23-31.

[15] Raussel C, Martínez-Ramírez AC, García-Robles I, Real MD. Real M. D. The toxicity and physiological effects of *Bacillus thuringiensis* toxins and formulations on *Thaumetopoea pityocampa*, the pine processionary caterpillar. *Pestic Biochem Physiol* 1999; **65**: 44-54.

[16] Govindarajan M. Larvicidal and repellent properties of some essential oils against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi (Diptera: Culicidae). *Asian Pac J Trop Med* 2011; **4**: 106-111.

[17] Cavalcanti ES, Morais SM, Lima MA, Santana EW. Larvicidal activity of essential oils from Brazilian plants against *Aedes aegypti* L. *Mem Inst Oswaldo Cruz* 2004; **99**: 541-544.

[18] Prates HT, Santos JP, Waquil JM, Fabris JD, Oliveira AB, Foster JE. Insecticidal activity of monoterpenes against *Ryzopertha dominica* (F.) and *Tribolium castaneum* (Herbst). *J Stored Prod Res* 1998; **34**: 243-249.

[19] Laborda R, Manzano I, Gamón M, Gavidia I, Pérez-Bermúdez P, Boluda R. Effects of *Rosmarinus officinalis* and *Salvia officinalis* essential oils on *Tetranychus urticae* Koch (Acari: Tetranychidae). *Ind Crops Prod* 2013; **48**: 106-110.

[20] Macedo IT, Bevilaqua CM, de Oliveira LM, Camurça-Vasconcelos AL, Vieira Lda S, Oliveira FR, et al. [Ovicidal and larvicidal activity *in vitro* of *Eucalyptus globulus* essential oils on *Haemonchus contortus*]. *Rev Bras Parasitol Vet* 2009; **18**(3): 62-66. Portuguese.

[21] Simas NK, Lima EC, Conceição SR, Kuster RM, Oliveira Filho AM. [Natural products for dengue transmission control: larvicidal activity of *Myroxylon balsamum* (red oil) and of terpenoids and phenylpropanoids]. *Quím Nova* 2004; **27**: 46-49. Portuguese.

[22] Mazyad SA, Soliman M. Laboratory evaluation of the insecticidal activity of camphor on the development of *Oestrus ovis* larvae. *J Egypt Soc Parasitol* 2001; **31**(3): 887-892.

[23] Li BT. Bioactivity of camphor against the long-horned beetles (*Anoplophora chinensis* and *Nadezhdiella cantori*). *Acta Phytophy Sin* 2003; **30**(2): 166-170.

[24] Arabi F, Moharramipour S, Sefidkon F. Chemical composition and insecticidal activity of essential oil from *Perovskia abrotanoides* (Lamiaceae) against *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Int J Trop Insect Sci* 2008; **28**(3): 144-150.

[25] Yeh RY, Shiu YL, Shei SC, Cheng SC, Huang SY, Lin JC, et al. Evaluation of the antibacterial activity of leaf and twig extracts of stout camphor tree, *Cinnamomum kanehirae*, and the effects on immunity and disease resistance of white shrimp, *Litopenaeus vannamei*. *Fish Shellfish Immunol* 2009; **27**(1): 26-32.

[26] Ebadollahi A, Safaralizadeh MH, Pourmirza AA. Fumigant toxicity of *Lavandula stoechas* L. oil against three insect pests attacking stored products. *J Plant Prot Res* 2010; **50**(1): 56-60.

[27] Tsukamoto T, Ishikawa Y, Miyazawa M. Larvicidal and adulticidal activity of alkylphthalide derivates from rhizome of *Cnidium officinale* against *Drosophila melanogaster*. *J Agric Food Chem* 2005; **53**: 5549–5553.

[28] Isman MB, Miresmailli S, Machial C. Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. *Phytochem Rev* 2011; **10**: 197-204.