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Larvicidal activity of essential oil of *Mentha pulegium* on larvae of *Orgyia trigotephras* Boisduval, 1829 (Lepidoptera, Erebidae)

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Abstract - To ensure better intervention and, in order to contribute to sustainable management of the environment, natural substances such as essential oils are used as insecticides. In this context, *Mentha pulegium* was used for its insecticidal activity against larvae of *Orgyia trigotephras*. Essential oil was diluted in ethanol (96%) to prepare 3 test solutions (S_1=0.05%, S_2=0.10%, and S_3=0.50%). The essential oil was tested by contact action. Larvicidal effect of essential oil was appreciated by comparison to Decis (positif test) and ethanol (negatif test). For each instar a total of 300 larvae were used for the test. Six replications were performed, in each 10 larvae were used. One larva was placed in a Petri dish and 10 µl of each oil solution prepared. The effects of the essential oils, Decis and ethanol were evaluated by measuring the mean mortality time of larvae (MMT). Results showed a highly significant difference between the three concentrations. The 3rd concentration of *M. pulegium* is the most effective of the other concentrations. MMT was 15 min for the 3rd instar, 27 min for the 4th instar and 1 h 15 min for the 5th instar. Larvicidal activity of *M. pulegium* was more efficient on the 3rd instar than the others. A total of 23 compounds of the essential oil of flowers of *M. pulegium* were identified. Polegone was the dominant one at 56.22% seems to be responsible for the larvae mortality. This work should be complemented by histological sections of the caterpillars to identify the insecticidal effect of *M. pulegium* essential oil.

Keywords: contact action, 3 test solutions, chemical composition, mean mortality time, positif test, negatif test

1. Introduction

Among the variety of nature's ecosystem services, the natural pest control is an important aspect (Batish et al. 2008). The application of natural plant products which have antifeedant and/or masking effect and low to moderate toxic effect on larvae can be potential method in integrative, so-called environmental-friendly, control management of pests (Kostic et al. 2008). Several essential oils were used as larvicidal agent in integrative control of forest lepidoptera as *Lymantria dispar* (Kostic et al. 2008; Markovic et al. 1996; Zabel et al. 2002), *Thaumetopoea pityocampa* (Kanat and Alma 2003; Petrakis et al. 2005) and *Orgyia trigotephras* (Akkari et al. 2015; Ben Slimane et al. 2014; Ben Slimane et al. 2015). In Tunisia, larvae of *O. trigotephras* attained such high densities during the outbreak peak in 2005 that cause complete defoliation of more than 500 ha of evergreen shrubs in Mediterranean forest (Ezzine et al. 2010). The high mobility of the larvae allows them to feed on different host plants over the course of the larval stages. This situation led to serious concern for the conservation of the Mediterranean vegetation specific to this region (Ezzine et al. 2015). In this context, this study aim to test the insecticidal effect of essential oil of *M. pulegium* used for contact action on the 3rd, 4th and 5th instars larvae of *O. trigotephras*. Essential oil of *Mentha pulegium* was used for its acaricidal activity (George et al. 2009), insecticidal activity against: coleoptera as *Sitophilus oryzae* (Zekri et al. 2013) and *Bruchus rufimanus* (Amzouar et al. 2016); Lepidoptera as *Plodia interpunctella* (Saeidi and Hassanpour 2014)

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and *Ephestia kuehniella* (Ayvaz et al. 2008). However, there have not been any thorough studies on larvicidal effects of leaf essential oils and their constituents from *M. pulegium* against *O. trigotephras*.

2. Materials and methods

2.1. Plant material and essential oil extraction

Branches of *M. pulegium* from Utique (Bizerte, Tunisia) were cut and separately placed in plastic bags in March 2015. Flowers were carried out, washed with distilled water, dried for 15 days in the shade and stored for essential oil extraction. 100 g of dry matter of flowers were used for Essential oils extraction by hydro-distillation method 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 safe-lock tubes and stored at -4° C until analysis (Riahi et al. 2013).

2.2. Chemical composition

2.2.1. Détermination de la composition chimique des arbustes

Assessment of the chemical composition of flower essential oil of *M. pulegium* was carried out by gas chromatography/mass spectrometry (GC/MS) methods. The GC–MS unit consisted of a PerkinElmer Auto-system XL gas chromatograph, equipped with HP-5MS fused-5% phenyl methyl siloxane capillary column (Agilent, 30 m × 0.25 mm, film thickness 0.25 mm) and interfaced with PerkinElmer Turbo mass spectrometer (software version 4.1). The operating conditions were as follows : the injector temperature was 250°C ; carrier gas helium was adjusted to a linear velocity of 37 cm/s, the flow rate was 1 ml/min; the volume of injected sample was 1 µl; split ratio 50:1; ionization energy was 70 eV; ion source temperature was 200°C; scan mass range was set tom/z 50–550 and that of interface line temperature to 300°C. The temperature gradient started at 110°C, raised to 180°C (4°C/min), then to 220°C (2°C/min) and finally reached 300°C (20°C/min). The total run time was 70 min. The GC/MS analysis was done in triplicate. Oil compounds were identified by comparison to their retention time of n-alkanes standards.

2.3. Preparation of test solutions and Chemical insecticide

Essential oil was diluted in ethanol (96%) to prepare 3 test solutions (S_1=0.05%, S_2=0.10%, and S_3=0.50%). The essential oil was tested by contact action. Larvicidal effect of essential oil was appreciated by comparison to a chemical insecticide Delta-metrine ”Decis” (reference product, provided by Atlas Agro-Tunisia) used as positive control. Ethanol used for dilutions was already used as negative control.

2.4. Larvae collect and larvicidal activity

Third, fourth and fifth instars larvae of *O. trigotephras* were collected from Jebel Beni Oulid (Cap-Bon) in April and May 2015. For each instar a total of 300 larvae were used for the test performed according to Kanat and Alma (2003). Six replications were performed, in each, 10 larvae were used. One larva was placed in a Petri dish (R=9 cm) and 10 µl of each oil solution prepared, ethanol and Decis were deposited on the back of larva (Akkari et al. 2015). The effects of the essential oil, Decis and ethanol were evaluated by measuring the mean mortality time of larvae (MMT).

2.5. Statistical analysis

The statistical analysis was performed using the SPSS-10.0 software package for Windows. Average of the different measurements for insecticidal action was reported as mean mortality time (MMT). Results were statistically evaluated by using analysis of variance (ANOVA) and complemented by multiple comparisons of means by the SNK test (Student–Newman–Keuls).

3. Results and discussion

3.1. Insecticidal activity

Insecticidal activity of the essential oil of *M. pulegium* was more efficient on the young larvae (3^{rd} instar) than the mature larvae (4^{th} and 5^{th} instars). For all instars larvae, caterpillars show the same behavior: severe convulsions, releasing green-colored secretion and sudden death.

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3.1.1. Larvicidal activity of M. pulegium on the 3rd instar larva

MMT of the 3rd instar larva was highly significant among S_1, S_2 and S_3 ($F_{(2, 167)}$=200, p<0.001) and among all tested solutions ($F_{(4, 285)}$=354.74, p<0.001). Contact action of S_3 (15 min) of *M. pulegium* oil was more efficient than S_1 (52 min), S_2 (49 min), Decis (36 min) and ethanol (2 h 30 min) (Fig. 1). Akkari et al. (2015) showed that the MMT obtained after treatment of the 3rd instar larvae of *O. trigotephras* with 0.5 ml of flower oil was 1.40 min being higher than for leaves oil (1.27 min) of *Ruta chalepensis*. It seems that *M. pulegium* is not efficient as *R. chalepensis* against young larvae of *O. trigotephras*.

Figure 1. Mean mortality time of the 3rd instar larvae of *Orgyia trigotephras* (mean±SE)
Within each oil, values labelled with capitals letters are significantly different (p<0.05)
Between tested solution, values labelled with tinies letters are significantly different (p<0.05)

3.1.2. Larvicidal activity of M. pulegium on the 4th instar larva

MMT of the 4th instar larva was highly significant among S_1, S_2 and S_3 $F_{(2, 177)}$=(17.48, p<0.001) and among all tested solutions $F_{(4, 295)}$=(34.60, p<0.001). Contact action of S_3 (26 min) of *M. pulegium* oil was more efficient than S_1 (72 min), S_2 (53 min), Decis (8 h 58 min) and ethanol (10 h 8min) (Fig. 2). Akkari et al. (2015) showed that the MMT of the 4th instar larva of *O. trigotephras* obtained after treatment with 0.5 ml of flower oil and leaves oil of *R. chalepensis* were respectively, 42.53 min and 20.68 min. It seems that flower oil of *M. pulegium* is more efficient than *R. chalepensis*.

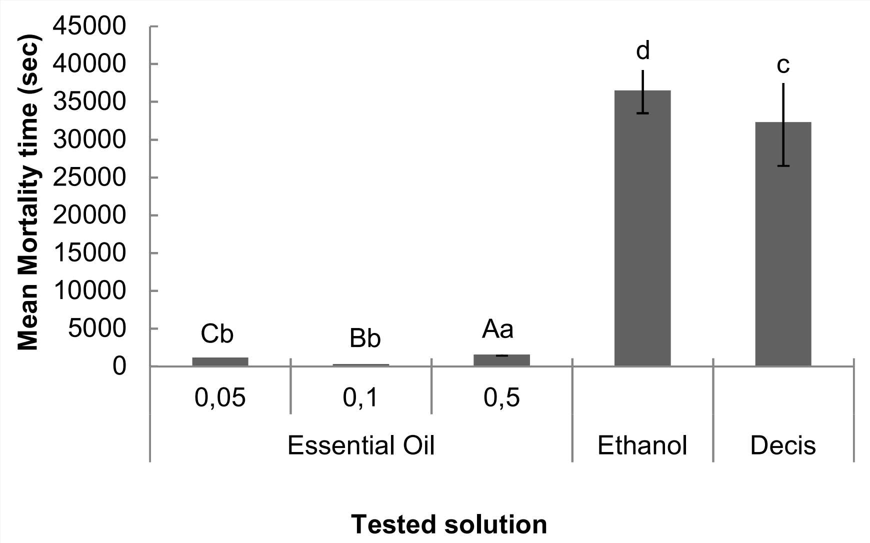

Figure 2. Mean mortality time of the 4th instar larvae of *Orgyia trigotephras* (mean±SE)

Within each oil, values labelled with capitals letters are significantly different (p<0.05)
Between tested solution, values labelled with tinies letters are significantly different (p<0.05)

3.1.3. Larvicidal activity of M. pulegium on the 5th instar larva

MMT of the 5th instar larva was different among S_1, S_2 and S_3 $F_{(2, 177)}$=(3.46, p=0.033) and highly significant among all tested solutions $F_{(4, 295)}$=(40.02, p<0.001). Contact action of S_2 (1 h 24 min) and S_3 (1 h 42 min) of *M. pulegium* oil were approximately the same but were more efficient than S_1 (2 h 13 min) (Fig. 3). Ezzine et al. (2017) showed that Mortality time for killing 100% of larvae was 3 h 33 min for *Eucalyptus kirtoniana* and 1 day18 h 28 min for *E. camaldulensis*. It seems that *M. pulegium* oil is more efficient than *Eucalyptus*.

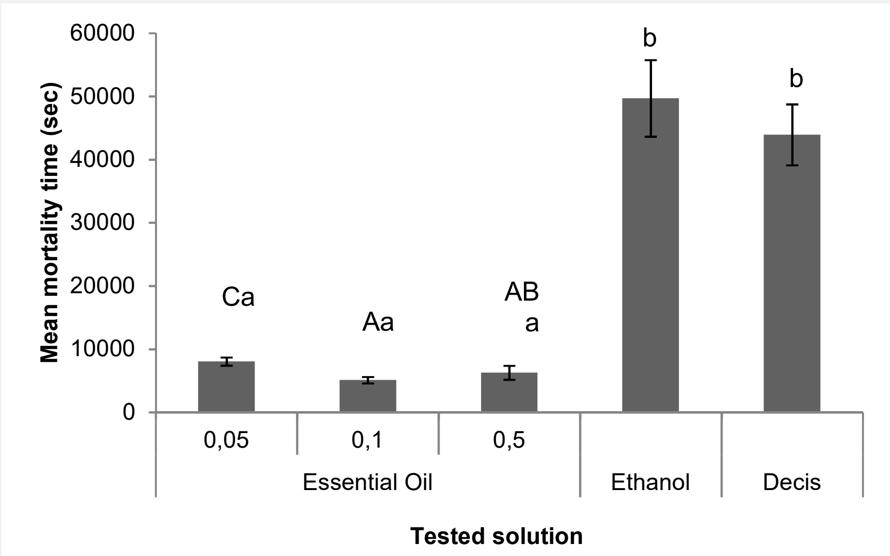

Figure 3. Mean mortality time of the 5th instar larvae of *Orgyia trigotephras* (mean±SE)

Within each oil, values labelled with capitals letters are significantly different (p<0.05)
Between tested solution, values labelled with tinies letters are significantly different (p<0.05)

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3.2. Essential oil analysis

Table 1 shows the chemical composition of the essential oil of flowers of *M. pulegium* with the retention times of the compounds. A total of 23 compounds were identified, which represented 98.98% of the oil extracted. Polegone was the dominant one at 56.22%, followed by Cytohexanone at 32.13% and Cétone at 6.12%. It seems that these compounds and essentially Polegone are responsible for the larvae mortality.

Table 1. Relative percentage of the main constituents of *Mentha pulegium* essential oil

Compounds	Rt	Flower %
α-pinène	7.85	0.17
Β- pinène	9.249	0.18
3-Octanone	9.720	0.08
Β- Mycrene	9.839	0.09
3-Octonal	10.215	1.01
D-Limonene	11.139	0.45
2-Octanol	14,582	0.23
Cytohexanone	16,825	32.13
2-Decanone	17.244	0.42
Cytohexanol	18.116	0.39
Polegone	**20.001**	**56.22**
Cytohexene	20.301	0.17
Cétone	21.087	6.12
4-methyl-1	21.306	0.36
3-N pentamethyl amide	21.501	0.08
2 cyclohexen-1-one	22.344	0.30
2- cyclohexen-1 ol	22.558	0.11
2-Dodecanone	23.825	0.11
α-caryophyllene	25.540	0.04
2-Tridecanone	26.863	0.01
Caryophyllene oxide	29.349	0.10
Coctyl ester	51.521	0.04
3cyclohene1carboxaldehyde	30.125	0.17
All identified components		98.98

4. Conclusion

Results show that application of natural plant products as *M. pulegium* which have a toxic effect against larvae of *O. trigotephras* can be a potential method in environmental-friendly control management. The use of natural products may be considered as an important alternative insecticide for the control of *O. trigotephras*. In addition to *Ruta chalepensis* (Akkari et al. 2015), *Rosmarinus officinalis* and *Lavandula stoechas* (Ben Slimane et al. 2015), *E. kirtoniana* and *E. camaldulensis* (Ezzine et al. 2017) were tested against larvae of *O. trigotephras* and demonstrated an excellent larvicidal activity. It will be interesting to study the insecticidal activity of essential oils of leaves of *M. pulegium* and also to test the insecticidal activity by inhalation and ingestion. Histological section of larvae to identify the insecticidal effect of *M. pulegium* essential oil seems to be interesting.

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