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Insecticidal effects of essential oils from six aromatic and medicinal plants on the pine-processionary caterpillar (*Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775))

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Abstract

The objective of the present study is to test some essential oils in order to recommend a method of effective control of *T. pityocampa*. Essential oils composition, polyphenols content, and antioxidant activity of six aromatic and medicinal plants leaf from Tabarka were investigated. The essential oils obtained by steam distillation from six plant species naturally grown in Tabarka forests were tested at three different concentrations in order to evaluate their effectiveness against the larvae of pine processionary (*T. pityocampa*.Our results revealed the effect of *Pinus pinaster* essential oils which gave the highest mortality rate of80.55% and the fastest on *T. pityocampa* , followed by *Lavandula stoechas* and *Eucalyptus camaldulensis* with 74.81 per cent and 72.81. The other species tested were *Pinus pinea*, *Juniperus oxycedrus* and *Cupressus sempervirens* also had a considerable effect on caterpillar mortality with percentages 66.66, 58.26%, and 40.49.Then, a phytochemical study of the six species tested was carried out which showed that some oils can be used as a spray substance to control *T.pityocampa* due to their compositions in active substances.

Keywords: Activity, Tabarka forests.

Introduction

Tunisian pine forests are affected by various depredation phenomena (drought, anthropogenic activities, and insect pests). In addition to bark beetles, defoliation by the pine processionary (*T. pityocampa* (Thaumetopoeidae: Lepiodoptera) is highly important. Defoliation causes tree growth losses and makes them more susceptible to attack by secondary insect pests and diseases, (FAO, 2010).The continuous use of synthetic chemicals for control apart from their high efficacy and selectivity has raised concerns about their potential safety for human health and the environment. Secondary metabolites constitute a biologically and chemically interesting group of substances extracted from plants. Essential oils of plants show many biological activities in addition to their use in food, flavor, perfumery, cosmetic and pharmaceutical industries as natural antioxidants (Wei and Shibamoto, 2010 and Mothana *et al*.,., 2012).Essential oil has been used since ancient times for

medicinal purposes and known for its anti-rheumatic, anti-inflammatory, and antispasmodic properties (Benincá *et al*., 2011and Zaouali., 2013). It has demonstrated powerful antimutagenic, antibacterial and chemo preventive properties (Okoh *et al*., 2010 and Ayadi, 2011). The secondary metabolites grouped as essential oil impart them the much needed curative properties (Derwich *et al*., 2010).

Secondary metabolites from plants have important biological and pharmacological activities, such as anti-oxidative, anti-allergic, antibiotic, hypoglycemic, and anti-carcinogenic (Stanković, 2011).The essential oils have different kinds of effects for insects: larvicides, autocides, repellents, or growth inhibitors. Most essential oils act through disrupting the structure of the cell membranes. However, for some, neurotoxic effects have been demonstrated, due to interactions with neurotransmitters such as gamma-aminobutyric acid and octopamine or by inhibition of acetyl cholinesterase. Some essential oils can potentiate the action of other molecules by inhibiting cytochromes P450 which normally taxed them. Because of their volatility and small size, many of the constituents of essential oils interact with the odor receptors of insects, triggering various behaviors: flight, attraction, oviposition, etc. (Vidyasagar , 2013).

The aim of this study was to find out whether essential oils from various plants might be used as insecticides for the control of *T. pityocampa*

Materials and methods

Animal material

Estimating the health condition of studied pine forests and their infestation by insects in Tabarka shows that the pine processionary caterpillar is the greatest in the pine forests of Northwestern Tunisia. To study the seasonal development of the pine processionary caterpillar and to test the effectiveness of its control, weekly visits to the study areas were carried out.

Figure 1: The nest(left) and foliage damage by pine processionary caterpillars (right)

During our study, the periods of different stages of the pine processionary caterpillar was refined:

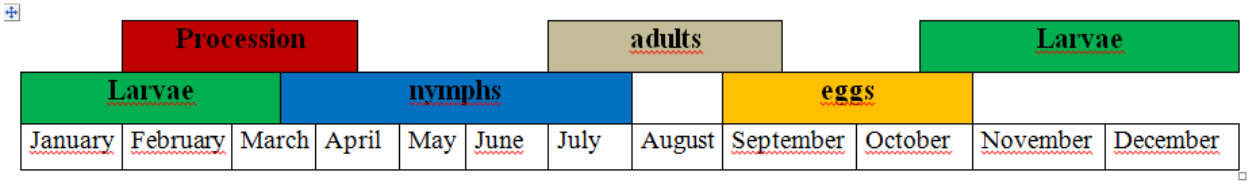

Figure 2: Cycle of *Thaumetopoea pityocampa* (Denis & Schiffermüller,)

It was recognized that the processional phase starts at the beginning of February and may last until the end of March

Plant material

Leaves of some aromatic and medicinal plants (*Pinus pinaster,Lavandula stoechas, Eucalyptus camaldulensis, Pinuspinea,Juniperu soxycedrus,Cupressus sempervirens*) were collected from Tabarka forests, a preserved environment where the plants are vigorously producing very large biomass. The harvested materials were air-dried at room temperature (20±2°C) for one week, ground, sieved through a 0.5 mm mesh screen to obtain a uniform particle size.

Total lipid extraction

Samples of ground powder (1g) in triplicate were weighed and extracted using the modified method of

Bligh and Dyer (1959). A chloroform/methanol mixture (2:1, v/v) was used for total lipid extraction. The mixture was shaken and centrifuged (Eppendorf 5810R) at 3,000 × g for 10 min to allow phase development. The organic layer containing total lipids was collected and filtered. The total extracted lipid material was recovered after the solvent was removed in a stream of nitrogen. The total fat content (TFC) was expressed as a percent of the dry matter and was calculated using the following formula (Gandour *et al*., 2011):

$$\mathbf{TFC}(\%\ \mathbf{ofdw}) = \mathbf{100} \times \frac{\mathbf{m}}{\mathbf{M}} (1)$$

where,

TFC: is the total fat content,
m: the mass of extracted oil (mg),
M: the mass of dry matter (mg)
dw: the dry weight.

Essential oil isolation

The air-dried materials were hydro distilled for 3h. The obtained distillate was extracted using diethyl-ether as solvent (v/v) and dried over anhydrous sulphate sodium. The organic layer was then concentrated at 35°C using a Vigreux column and the essential oil stored at –4°C prior to analysis. The essential oil yield was estimated according to dry leaves matter by using the following equation (Sangun *et al*., 2007).

$$\mathbf{Rhe}(\%) = \mathbf{100} \times \frac{\mathbf{Mhe}}{\mathbf{Ms}} (2)$$

where,

M_{he}: essential oil mass (g),
M_s: dry leaves matter mass (g),
R_{he}: essential oil yield (%).

Essential oil gas chromatography analysis

Gas chromatography analyses were done with Shimadzu HRGC-2010 gas chromatograph equipped with flame ionisation detector (FID), Auto-injector and auto-sampler. An apolar column Rtx-1 (30 m × 0.25 mm, 0.32 µm film thickness) was used. The oven temperature was held at 50°C for 10 min then programmed at 2°C/min to 190°C then held isothermal for 10 min. The injector and detector temperature were programmed at 230°C. The flow of the carrier gas (N_2) was 1.6 ml/min and the split ration was 1:20. Injection volume for all samples was 0.5µl of diluted oils in *n*-pentane. The volatile compounds were identified by comparison of their retention indices (RI) relative to (C_7 –C_{20}) *n*-alkanes with those of literature and/or with those of authentic compounds available in our laboratory. Relative percentage amounts of the identified compounds were obtained from the electronic integration of the FID peak areas.

Preparation of Methanolic extracts

Methanolic extracts of some aromatic and medicinal plants were obtained, as described by Eghdami and Sadeghi (2010),with slight modification by Benhammou *et al*. (2009).

Total Phenolic content

Total phenolics were determined with Folin–Ciocalteu (F-C) assay as per Singleton *et al*.(1999) method and slightly modified by Dewanto *et al*. (2002). The total phenolic content was expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE/g DW) through the calibration curve of gallic acid.

Total Flavonoids content

Total flavonoids content was determined according to the aluminium chloride colorimetric method (Djeridane *et al*., 2006). The total flavonoids content was calculated on the basis of the calibration curve of quercetin and expressed as mg quercetin equivalents per g dry weight (mg QE/g DW).

Antiradical scavenging activity by DPPH method

Plant extracts were tested for the scavenging effect on DPPH This method is based on the measurement of the capacity of antioxidants to trap the 2, 2-diphenyl-1-picrylhydrazil (DPPH.) radical. According to the method described by Braca *et al*. (2001). The DPPH radical scavenging activity in terms of percentage was calculated according to the following equation (Kumaran *et al*., 2006):

$$\boldsymbol{I}(\%) = \left[\frac{\boldsymbol{A0}-\boldsymbol{A1}}{\boldsymbol{A0}}\right] \times \mathbf{100} (3)$$

where,

I was DPPH inhibition (%), A_0 was the absorbance of the control, and A_1 was the absorbance of the extract/standard.
The concentration of the sample required for 50% inhibition was determined and represented as IC_{50} for each test solution which is expressed as µg/ml. All measurements were performed in triplicate.

Biological assays

T.pityocampa Schiff larvae were captured at the fourth and fifth instar, available on the Tabarka pines. The larvae were kept for use in studying the effect of certain oils on their behavior in laboratory. The essential oils were poured on 50 larvae, placed in Petri dishes (Ø 18 mm). The effects of all essential oils were evaluated by measuring the mortality time for killing 50 larvae (100% mortality).The complete test set was replicated four times for each essential oil. The average of four measurements was reported as mean mortality time (TEM).

Statistical analysis

All the analyses were performed in triplicate for each sample and expressed as mean ± standard deviation (SD). One way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test was applied to compare means at the significance level $p < 0.05$. All analyses were performed using SAS v 9.1 software package.

Results and discussion

Total lipid content

The amounts of total lipids in six aromatic and medicinal plants leaves are presented in Table 1.

Table 1: Total lipid content of leaves of six aromatic and medicinal plants

Species	Lipid content (%)
Pinuspinea	2.79^{d}± 0.23
Lavandula stoechas	4.86^{bc}± 0.40
Pinus pinaster	3.74^{cd} ±0.10
Juniperu soxycedrus	5.29^{b} ±1.45
Cupressus sempervirens	4.92^{bc}± 0.58
Eucalyptus camaldulensis	6.64^{a} ±0.26

Variation in the lipid content between species (P> 0.0003), of which *Eucalyptus* has the highest value of 6.64 per cent.

In fact; Lavender, *Juniperus,* and *Cupressus* have close values (4.86%, 5.29%, and 4.92% respectively). However, *Pinus pinea* has the lowest value of 2.79 per cent.

These variations in total lipids content among ecotypes of *Sesamum indicum* L could be mainly due to the effect of genetic factors as well as environmental, edaphic, and cultural conditions (Uzun *et al*., 2002 and Ravi *et al*., 2007).

Yields and essential oils components

Essential oil yields vary considerably among the six plants studied, *L. stoechas*, *C. sempervirens*, *P. pinaster*, *Pi. pinea*, *J. soxycedrus*, *E. camaldulensis*.

Indeed, the highest yield is recorded in *Cupressus* (0.25%) while *L. stoechas* (0.05%) has the lowest yield as shown in Figure 2. Variation in oil yield can be attributed to some factors such as conditions of plant growth, environment, and region. The oil yield during plant growth is particularly susceptible to environmental conditions namely light, nutrient availability, day length, and daily temperature (Skoula *et al*., 2000 and Msaada *et al*., 2009).

The essential oil compositions of Tunisian six aromatic and medicinal plants were determined by GC–FID analysis.

Compounds were identified with a significant variation of the essential oil chemical composition according to studied sites (Table 2).

Table: 2. Chemical composition of essential oils obtained from leaves of six aromatic and medicinal plants

Volatile Compound	Species					
	A	B	C	D	E	F
Hexanol	-	0.4	0.3	0.2	0.9	0.4
Nonane	0.1	-	0.4	0.3	0.6	0.3
α-Thujene	0.15	0.11	0.13	-	0.2	0.25
trans-2-Hexanal	0.18	0.22	-	0.19	0.3	0.4
(z)-2-heptenal	0.55	0.34	0.57	0.63	0.2	0.15
α-Pinene	7.2	6.8	3.35	4.3	2.6	1.9
Camphene	2.32	2.10	1.01	3.1	2.2	1.81
1-Octen-3-ol	-	3.2	4.1	3.1	0.6	0.9
Sabinene	-	-	0.1	1.9	1.1	2.1
β-Pinene	7.01	6.8	3.35	-	0.1	4.3
β-Myrcene	0.11	1.3	-	-	0.3	0.9
Decane	0.18	-	-	0.2	1.3	0.7
α-Terpinene	0.6	0.55	0.3	1.4	0.3	1.3
para-Cymene	-	0.2	1.2	0.7	-	-
γ-terpinene	1.9	1.39	1.75	0.63	3.4	-
para-Cymenene	-	-	-	0.3	0.2	1.3
Nonanal	0.4	0.9	1.3	5.6	4.9	0.2
α-Terpinolene	7.02	14.1	0.1	-	11	1.5
Undecane	1.6	-	-	-	0.3	-
cis-para-Menth-2-ene-1-ol	2.22	1.6	1.92	0.36	1.1	0.55
2-phenylethanol	-	-	0.3	1.8	1.4	-
trans-para-Menth-2-ene-1-ol	-	2.6	1.7	4.6	1.5	-
α-Terpineol	0.5	15.2	14.21	5.6	12	5.9
cis-Piperitol	-	0.9	1.6	2.9	6.7	-
Carvacrylmethyloxide	0.14	0.11	0.58	0.09	0.2	0.78
Carvacrolmethylether	0.12	-	-	-	-	-
Linolylacetate	-	0.6	-	1.4	5	-
Linalylacetate	-	1.3	0.17	4.3	2.3	-
Perillaldehyde	1.5	2.3	2.9	-	-	1.6
Decanol	-	-	0.3	-	1.9	1.8
Thymol	-	4.5	7.1	-	0.3	14
Bornylacetate	-	1.6	8.9	-	-	7.9
Carvacrol	-	4.6	-	-	-	-
Pinocarvylacetate	2.6	-	1.9	-	1.6	-
Tridecane	1.7	-	-	-	0.9	-
α-Terpinylacetate	1.3	-	-	1.6	4.9	1.5
Nerylacetate	2.9	-	1.67	1.3	1.9	1.7
α-cubebene	1.58	-	-	11	1.9	18
Aromadendrene	1.9	-	1.7	0.55	7.3	2.3
β-Ionone	2.3	-	-	0.69	0.3	1.6
Germacrene D	-	1.4	-	-	0.9	1.9
γ-Cadinene	5.3	3	9	1.8	12	-
δ-Cadinene	-	-	-	0.6	1.3	-
α-Calacorene	–	–	–	–	–	–

Legend: (A.)…..(B)…..(C……...)…..(F)*Pnus pinea,*(B) *Lavandula stoechas,*(C) *Pinus pinaster,(*D) *Juniperu soxycedrus,*(E) *Cupressus sempervirens,(F) Eucalyptus camaldulensis*.

The chemical class characterization of leaf essential oil showed the prevalence of monoterpene hydrocarbons. The oxygenated sesquiterpenes formed the second main class and the oxygenated monoterpenes formed the third main class. The remaining fractions, such as aldehydes and sesquiterpene hydrocarbons, formed the minor essential oil chemical classes of six plant leaves studied.

This variability can be explained by the influence of various factors. For instance, it was previously shown for the majority of vegetal species, that primary and secondary metabolism are strongly influenced by plant physiology and genetic, climatic and edaphic factors, the harvest period, inducing changes in chemical qualitative and quantitative composition (Msaada *et al*.,2009; Hosni

et al., 2011;Jemâa, 2014). Thus, the same species may show different chemical compositions from a region to another due to genetic variability influenced by environmental factors (Pritchard *et al*., 2000). The influence of environmental factors in the chemical composition of essential oils has also been reported in *A. absinthium* (Bailen *et al.,* 2013). Variations in the relative amounts of these compounds under different environmental conditions indicate their adaptively ecological value.

Thereby, the variations observed can be explained by the influence of the altitude. Essential oil composition of pepper mint (*Mentha piperita*) was different because of the altitude and irregular daylight (Yazdani *et al*., 2002). Plants are grown and produced in different ecosystems and sites under the influence of different potential factors, including the altitude as one of the vital determinants in the quantity and quality of the plants (Mahdavi *et al*., 2013). Chemical components of the essential oils are cited to vary qualitatively and quantitatively according to geographical location and environmental conditions (Bakkali *et al*., 2008 andOliveira *et al*., 2013).

Kouyokhi *et al*. (2008) reported that phytochemical variations were found not only among samples from different regions but also among samples from the same region with different altitudes, reflecting the effect of environment on essential oil components. Habibi *et al*. (2006) also reported that the essential oils of *T. kotschyanus* differ in different altitudes, but as the altitude increases, the amount of essential oil dropped.

Total Phenolic Content

Total phenolic values of six aromatic and medicinal plant leaves are given in Table 3. This variation can be attributed to several factors climatic and environment, geographical area, drought, diseases (Ebrahimi *et al.,* 2008; Andarwulan *et al.,* 2010), harvest time, and stage of plant development (Miliauskas *et al.,* 2004).

In addition, genetic factors and growing conditions may play a crucial role in the composition of secondary metabolites, including phenolic acids (Islam *et al*., 2003; Hashempour, *et al*., 2010). The method of extraction and quantification also influences the estimation of total phenols content (Lee *et a*l., 2003). Phenols are very important plant constituents not only because of their scavenging ability on free radicals due to their hydroxyl groups. Therefore, the phenolic content of plants may contribute directly to their antioxidant action and it is likely that the activity of the extracts is due to these compounds (Tosun *et a*l., 2009; Ghasemzadeh *et a*l., 2010).This activity is believed to be mainly due to their redox properties, that play a significant role in adsorbing and neutralizing free radicals by donating a hydrogen atom or an electron, chelating metal ions in aqueous solutions (Petti and Scully, 2009), quenching singlet and triplet oxygen, or decomposing peroxides (Zheng and Wang, 2001). Besides, the phenolic compounds possess multiple biological properties such as antitumor, antimutagenic, and antibacterial properties, and these activities might be related to their antioxidant activity (Shui and Leong, 2002).

Total Flavonoid Content

The total flavonoid content of six aromatic and medicinal plant leaves was shown in Table 3. The total phenolic content (mg/g) in methanolic extracts, expressed in quercetin equivalent (QE), varied between 1.83±0.02 and 2.72±0.01. Amjad and Shafighi (2013) reported that annual and geographical climate differences, soil conditions, and pesticide or herbicide use may contribute to variations in antioxidant activity and flavonoid content of plants. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation (Min and Chun-Zhao, 2005). Flavonoids are a class of secondary plant metabolites with significant antioxidant and chelating properties. Besides phenolic compounds; the presence of flavonoids might also influence the antioxidant capacity. Antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups (Sharififar *et al*., 2009; Stanković, 2011). Flavonoids are polyphenolic compounds with low molecular mass, found in leguminous, fruits, flowers, and leaves (Harbone and Williams, 2000), having several biological activities. The polar extracts of the leaves of *Quercus ilex* were abundant in flavonoids, in particular quercetin, isorhamnetin-3-O-glucopyranoside, flavonolacylated glucosides, and the phenolic ellagic acid (Karioti *et al*., 2009).Quantitative analysis of the methanolic extract of the plant revealed that it is a richsource of acylated flavonoid glucosides (Karioti *et al*., 2010). Flavonoids as one of the most diverse and widespread groups of natural compounds are probably the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties (Agbor *et al*., 2005).

Table 3: Total Phenolic and Flavonoid Content and DPPH radical-scavenging activity (IC_{50}) from the leaves of six aromatic and medicinal plants

Species	Total phenolic content (mg GAE/g DW)	Total flavonoids content (mg QE/g DW)	IC_{50} (µg/mL)
~~*Pinus pinea*~~	~~119.73^{b}±1.15~~	~~12.82^{a}±19.24~~	~~1380.13^{b}± 1.00~~
Lavandula stoechas	86.12^{c}±5.78	10.89^{b}±1.33	1865.51^{a}± 0.57
Pinus pinaster	89.06^{c}±2.00	5.48^{c}±0.43	154.14^{c} ±1.00
Juniperus oxycedrus	156.53^{a}±2.80	6.68^{d}± 0.29	27.72^{e}± 1.00
Cupressus sempervirens	64.61^{d}±0.40	3.29^{e}± 0.30	30.42^{e} ±1.00
Eucalyptus camaldulensis	87.64^{c}±2.01	6.14^{d} ±0.73	50.0^{d}± 10.0

Values of the same column with different superscripts (a–c) are significantly different at $p < 0.05$

Effect of essential oils on the mortality rate of caterpillar (*T. pityocampa .)* according to species

As shown in Table 4 that there is a highly significant effect of essential oil depending on the species of which maritime pine has the highest effect which reaches 80.55 per cent. Then the HE of lavender and the eucalyptus have the same effect on the mortality rate of caterpillar that recorded (74.81%, 72.81% respectively) and for juniper has a significant effect that presents 58.26per cent while cypress the weakest effect for a value of 40.49 (Figure 3). According to previous results, the insecticidal effect by fumigation of essential oils depends on the plant material used within the same plant species or even from one species to another, the source of the latter as well as the species of insect and its evolutionary stage. This was also demonstrated by Tunc *et al*. (2000); Chiasson *et al*. (2001); Choi *et al.* (2003); Sedy and Koschier (2003); Negahban *et al.* (2007).

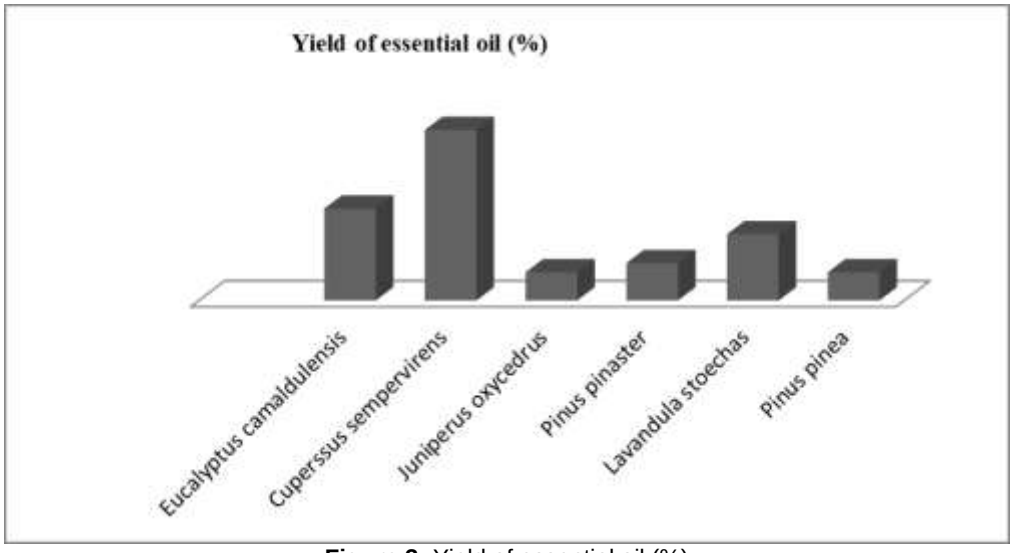

Figure 3: Yield of essential oil (%)

Table 4: Effect of essential oils on the mortality rate of the caterpillar (*T. pityocampa*)

Species	Larvae mortality (%)
Pinus pinaster	80.55^{a}
Lavandulastoechas	74.81^{ba}
Eucalyptus camaldulensis	72.77^{ba}
Pinus pinea	66.66^{bc}
Juniperusoxycedrus	58.26^{c}
Cupressussempervirens	40.49^{d}

Conclusion

The pine processionary caterpillar is a real danger to the forest ecosystem and more specifically to pine forests. This insect continues to damage most of the Northwestern pine species. According to the pine stand infestations map in the regions studied, it is depicted that maritime pine and gable pine are the most affected species by this primary insect: they recorded serious defoliation in the stand and they marked noteworthy growth losses. Analysis of the physicochemical properties of the six species in the laboratory revealed that the difference in physico-chemical composition, secondary metabolites, composition affects the insect's preference. However, the lipid composition seems to have no effect on the pine processionary caterpillar. Study of the interaction of adult larvae and essential oils revealed that maritime pine has an important effect in controlling the caterpillar. The pine processionary caterpillar cycle lasts for a year: it begins in September when the larvae are at instar 1 and ends in August. This phase is spread out between February and March.

Phytochemical analysis of the essential oil of some species has shown encouraging results in order to prepare an effective biological product to control the pine processionary caterpillar and to minimize the pine damage by this insect.

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