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Control of *Thaumetopoea pityocampa* and *T. wilkinsoni* with Essential Oils and Respective Volatile Monoterpenoids †

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Abstract: The pine processionary moth (PPM) is a dangerous parasitic insect pest of several pine species in the Mediterranean basin, causing defoliation and promoting tree decline. Larvae release urticating hairs that cause strong allergic reactions in human and animals. Pest management practices implemented by national health authorities include the eradication of nests and application of chemical insecticides. However, commercial pesticides can induce PPM resistance and be harmful to the environment and human health. Essential oils (EOs) are environment friendlier alternatives to commercial insecticides. The present review analyses the existing body of work on the biological activity against the PPM and highlights the most successful EOs. A total of nine publications were identified reporting on the biological activity of 38 EOs extracted from 31 plant species, against the PPM. The EOs extracted from *Achillea arabica*, *Citrus aurantium*, *Lavandula angustifolia*, *Origanum onites* and *Thymus vulgaris* showed the lowest half maximal lethal concentrations (LC_{50}). *O. onites* EO components with the highest activities were the monoterpenoid isomers carvacrol and thymol. The use of EOs is a potentially eco-friendly alternative for successful PPM pest management, however more extensive studies must be performed to pinpoint highly active and easily accessible EOs and respective volatiles.

Keywords: essential oils; forest health; green pesticides; insecticides; pine processionary moth; Pinus; sustainable pest management; *Thaumetopoea*

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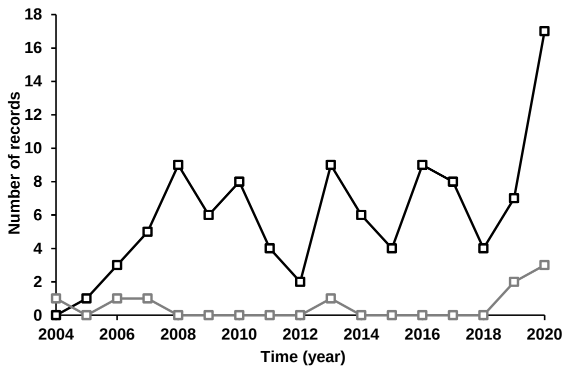
1. Introduction

The environmental alterations that result from climate change and the increase in trading activities have been strong promoters of epidemic events on the conifer forests of the Mediterranean, such as the pinewood nematode or the pine processionary moth (PPM). *Thaumetopoea* species (Lepidoptera: Thaumetopoeidae) that feed on pine needles in the winter are commonly called PPM. While *T. pityocampa* Den. & Schiff. can be found in Europe and North Africa, *T. wilkinsoni* Tams. is common in Asia and the Middle East [1]. The larval stages of the PPM cause pine defoliation and enable attacks from other opportunistic pine pests, that lead to a strong decline in pine vitality and yield of edible pine nuts. PPM life cycle shows two phases, an aerial phase comprising the egg, larvae and adult, and an underground phase, the pupa. The moth emerges from underground in the summer, reproduces and oviposits in the branch tips of the upper crown of pine trees. As eggs hatch, the larvae begin feeding on pine needles and moult. By the fourth instar, the recognizable silken nests that characterize this pest have enlarged to the definitive winter nest. Fully grown larvae (fifth instar) emerge in the beginning of spring and seek out pupation sites in the soil, in long head to tail processions [2]. In addition to the heavy impacts on biodiversity and the environment, the PPM has a dangerous societal impact due to its urticating hairs, produced from the third instar onwards, that cause strong allergic reactions. The allergenic protein thaumetopoein is responsible for causing conjunctivitis, respiratory congestions and asthma, on humans and animals [3]. Several pest management tactics are employed to control PPM populations. Besides the phytosanitary measures applied to pine nurseries, PPM is controlled by mechanical means, through cutting and burning winter nests, by biological means, such as natural enemies and predators and the application of microbial insecticides based on *Bacillus thuringiensis*, or by chemical means employing aerial spraying of commercial insecticides [4]. However, severe adverse effects can be associated to many insecticides, particularly in the elimination of non-target or beneficial species, and environmental and human health issues. The use of plant natural compounds has shown promise, being the source of many highly active phytochemicals. In this respect, essential oils (EOs) can be regarded as potential sustainable chemical control agents [5]. EOs are mostly composed of terpenoids (mainly mono and sesquiterpenes) and phenolic compounds, such as phenylpropanoids, that can often display additive, synergistic and antagonistic component interactions associated to their biological activities. Additionally, these mixtures have the advantage of not accumulating in the environment and having a broad range of activities, which diminishes the risk of developing resistant pathogenic strains [6]. The present review analyses the existing body of work on the biological activity of EOs against the PPM and highlights the most successful plant families and EOs.

2. Reports on Insecticidal EOs Against the Pine Processionary Moth

Research on reported work was performed on the Web of Science® search engine, in all available databases, using the topics "*Thaumetopoea*" and "essential oil". Information on the family and species of the plant source and technique used for EO extraction, EO solubilizer, PPM species and life stage used in bioassays as well as EO activity [half maximal effective concentration (EC_{50}) or mortality], was collected when available.

Nine publications were identified that reported on the activity of EOs on the PPM [1,3–5,7–11]. The reports were published in journals mainly dedicated to the scientific areas of entomology (44%), agronomy (33%), multidisciplinary agriculture (22%) and forestry (22%). The identified publications were cited by over 80 works (77, if publications in the present list are excluded) in a total of 102 citations (89, if citations from works in the present list are excluded), in an average of 11.3 citations per item. The citing publications were mostly from the areas of plant sciences, agriculture, chemistry, environmental sciences, and ecology. The first report was published in 2004 and the last in 2020 (the year with the most publications) (Figure 1a). Citations increased until 2008 and showed a stable average number until 2018 but have since exponentially increased (Figure 1a). Recent scientific interest appears to be rising.



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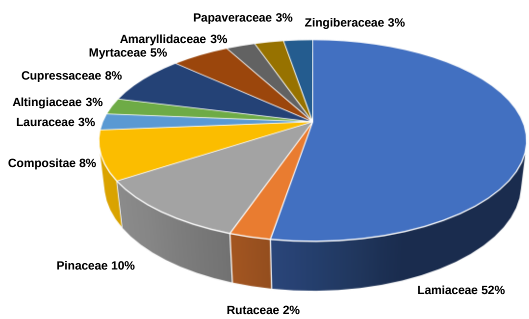
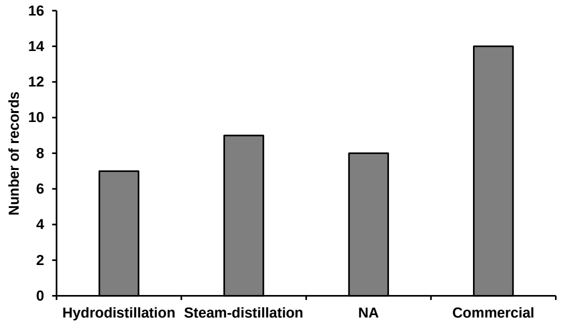


(**b**)

Figure 1. Yearly number of publications on the activity of essential oils on the pine processionary moth (□) and yearly number of citations on these publications (□) (**a**). Percentage of essential oils tested against the pine processionary, distributed by family of plant source (**b**).

3. Active Essential Oils (EOs)

The activity of 38 EOs from various parts of the shoots of 31 plant species were reported against the PPM. Over 50% of the source plants belonged to the Lamiaceae family, the remaining belonging to Altingiaceae, Amaryllidaceae, Compositae, Cupressaceae, Lauraceae, Myrtaceae, Papaveraceae, Pinaceae, Rutaceae and Zingiberaceae (Figure 1b). The EOs were acquired from commercial sources (37%) or obtained by steam-distillation (24%) or hydrodistillation (18%) (Figure 2a). In 21% of EOs the source is not mentioned. The EOs were used in aqueous solutions in their pure form (16%), mixed with 0.3% of the nonionic surfactant Tween 80 (32%) or the organic solvent ethanol (16%). In 21% of EOs, the solubilizer compound is not mentioned. Bioassays were mainly performed on the moth larvae or on the silken nests, and mortality was evaluated in all larval stages, with special incidence in 3rd, 4th, and 5th instars. Over 70% of bioassays were performed in *T. pityocampa* and 26% in *T. wilkinsoni* (Figure 2b).



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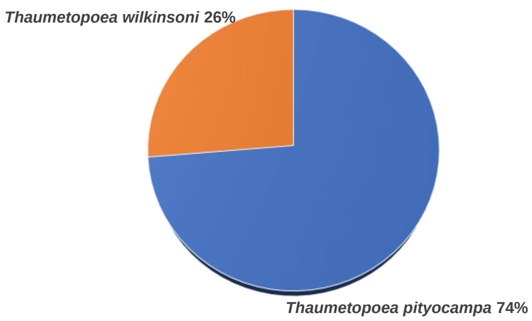


(**b**)

Figure 2. Number of EOs obtained from commercial sources, by steam-distillation or hydrodistillation (**a**) and percentage of bioassays performed on *Thaumetopoea pityocampa* or *T. wilkinsoni* pine processionary moth species (**b**). NA—corresponds to reported EOs where information on extraction methods was Not Available.

The lowest LC_{50} values were reported for the EOs extracted from *Achillea arabica*, *Citrus aurantium*, *Lavandula angustifolia*, *Origanum onites* and *Thymus vulgaris*, that ranged from 0.22 µL/mL, in *T. vulgaris*, to 5.33 µL/mL, in *C. aurantium* [1,4,7,11]. Complete mortalities (100%) were obtained for EOs extracted from *A. gypsicola*, *O. acutidens*, *O. onites*, *O. rotundifolium*, *Satureja hortensis*, *S. spicigera* and *Tanacetum polycephalum* [3,8,10]. Concerning the time needed to reach complete mortality, the EOs extracted from *Laurus nobilis*, *Pinus brutia* and *T. vulgaris* showed the fastest activities, reaching complete PPM mortality between 0.75 and 0.79 min at 25% concentrations [5].

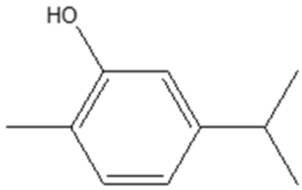
4. Active Volatiles from EOs

In five publications the composition of active EOs was reported. The main EO compounds were identified for *Achillea biebersteinii*, *Laurus nobilis*, *Origanum onites*, *Pinus brutia* and *Thymus vulgaris* (Table 1). EOs were rich in monoterpenoids and sesquiterpenes.

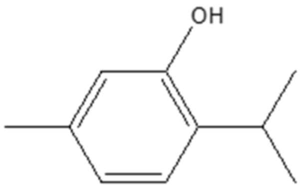
Table 1. Reported main composition of essential oils (≥5%, when mentioned) with activity against the pine processionary moth.

Species	Ref.	Main Compounds (≥5%, when Mentioned)
Achillea biebersteinii	[4]	γ-terpinene 38, camphor 24, borneol 6, α-terpineol 5
Laurus nobilis	[5]	cineole, geraniol, eugenol, several monoterpenes
Origanum onites	[1]	carvacrol, thymol, γ-terpinene, terpinen-4-ol
Pinus brutia	[5]	α-pinene, β-pinene, camphene, δ-3-carene, limonene, β-myrcene
Pinus brutia	[9]	β-pinene 38, α-pinene 25, *trans*-β-caryophyllene 15
Thymus vulgaris	[5]	carvacrol, *p*-cymene, thymol
Thymus vulgaris	[11]	carvacrol 71, *p*-cymene 8, γ-terpinene 6

Cetin et al. [1] identified carvacrol, thymol, γ-terpinene and terpinene-4-ol as the main components of *O. onites* EO, and tested each pure EO compound, acquired from commercial sources, against the PPM. Carvacrol (Figure 3a) and thymol (Figure 3b). showed the highest activities, reaching LC_{50} values of 3.1 and 5.5 μL/mL, respectively, while the original EO showed an LC_{50} value of 3.8 μL/mL. Differential LC_{50} values may indicate an isomeric specificity in activity against PPM that can be explored for the establishment of directed pest management practices.



carvacrol (**a**)



thymol (**b**)

Figure 3. Chemical structure of carvacrol (**a**) and thymol (**b**), the main compounds of *O. onites* EO.

5. Conclusions

The use of EOs can be a valuable tactic for the establishment of sustainable pest management strategies in the control of the pine processionary moth. In comparison with other pine pests, the screening of EOs against the PPM is still in its early stages. A greater number of EOs must be assayed to provide a clearer view of the main plant species and families with highly active phytochemicals and ascertain the most active EO components. The Lamiaceae family appears to be composed of plant species with highly active EOs against the PPM. In particular, the oregano *O. onites* EO shows compound isomers that display different activities. This diversity should be screened in other compound isomers to contribute for the identification of specific mechanisms of action in the PPM, which can be explored in greener precision pest management tactics.

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