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Aphicidal potential of the essential oil isolated from *Pistacia lentiscus* L. against the larvae of *Aphis spiraecola* P.1914: vector of multiple phytoviruses.

Abstract

This study aims to determine the profiling of the essential oil of *Pistacia lentiscus* leaves by gas chromatography-mass spectrometry and to test its insecticidal properties against the larvae of *Aphis spiraecola*, which represents a serious threat to citrus production and cause most economic loss for the citrus culture. The essential oil of *Pistacia lentiscus* leaves was isolated by hydrodistillation using a Clevenger-type, the chemical composition was determined by GC/MS. The insecticidal activity of essential oil was determined by using the contact method against *Aphis spiraecola* larvae. A total of 74 compounds were identified, corresponding to chromatographic peaks representing 89.59% of the total area of all peaks. The most abundant compounds were monoterpene hydrocarbons (54.23%) with 8.75% p-cymene and 7.18% α-pinene. The insecticidal assay revealed an interesting insecticidal activity against the larvae of *Aphis spiraecola* with an LD50 of 0.2 μL. This investigation introduces and supports the use of the volatile oil from *Pistacia lentiscus* as a biopesticide and paving the path for its future utilization in the phytosanitary sector.

Keywords: Citrus culture, essential oil, biopesticide, *Aphis spiraecola,* insecticidal activity.

Introduction

Citrus is a strategic crop in many countries. In the recent past, Algeria was one of the major citrus-producing countries. However, national production has declined yearly due to several factors, including the damage caused by aphids controlled mainly by the synthetic pesticides. The harmful effects of pesticides on beneficial insects of crops, ecosystems, [1] general environment and human health are previously documented. [2] The World Health Organization's estimation indicates that pesticide poisoning claims the lives of approximately 200,000 individuals each year on a global scale. [3]

In this context, essential oils (EO) can constitute an alternative natural resource that is respectful of the environment and faces the harmful effects of these chemicals. Algerian flora is rich source of aromatic and medicinal plants. The *Pistacia lentiscus* L., vernacularly named Lentisk or Darw, belongs to the *Anacardiaceae* family. It is a wild, thermophilic, aromatic and medicinal species widely distributed in the Mediterranean region, Europe, Asia, and Africa. [4]

The EO of the leaves of *P. lentiscus* is used in the treatment of several diseases by its antibacterial, antioxidant and anti-carcinogenic effects and, on the other hand, as a biopesticide to fight against certain bioaggressors. [5] The aim of this research is to determine the chemical profile of the EO obtained from leaves of *P. lentiscus* as well as its insecticidal effect against the larvae of the aphids of *Aphis spiraecola*. This pest is the most feared of citrus orchards in Algerian producing-zones.

Materials and methods

Plant collection and preparation

The leaves of *P. lentiscus* were harvested in October 2022 in the locality of Ténès, Chlef province, located in the northwest of Algeria (latitude 36° 10′ 26″ North, longitude 1° 20′ 12″ East and altitude 27 m). The climate is warm and temperate, of the Mediterranean type (Köppen classification: Csa). Taxonomic identification was confirmed at the local natural bioresources laboratory of Hassiba Benbouali University in Chlef, Algeria. Following the harvest, the leaves underwent a meticulous process of washing, drying, and crushing.

Insect material

Citrus leaves infested with *A. spiraecola* were taken from an orchard in the town of Medjadja, located northeast of Chlef province, at an altitude of 152 m. The infected leaves were collected in plastic boxes (20×10×5 cm) and covered with fine mesh for ventilation. The identification and isolation of larvae of *A. spiraecola* were carried out under a binocular magnifying glass according to the identification keys of Blackman & Eastop. [6] Larvae were stored at 26 ± 2° C and 40 ± 5% as relative humidity until insecticidal assay.

Essential oil extraction

The essential oil from the leaves of P. lentiscus was obtained through hydrodistillation, employing a Clevenger-type [7] apparatus with a sample-to-water ratio (g/mL) of 1:5. Following a three-hour extraction period, the condensed vapor yielded an organic phase (EO), which was separated from the water through decantation. The isolated EO was subsequently stored in amber bottles at 4°C until analysis.

Determination of chemical composition

The chromatographic analysis was carried out using a Hewlett Packard Agilent 6890 plus GC-MS/MS instrument coupled to an Agilent 5975 mass spectrometer with an electron impact detector. The separation was carried out on an apolar capillary column of the HP-5MS type

consisting of 5% phenyl and 95% dimethylpolysiloxane (30 m×0.25 mm, 0.25 µm). The operating conditions are as follows: the carrier gas is Helium with a flow rate of 1ml/min, and the injector temperature is 250°C with the injection of 0.2 μl in split 1/80 mode. The column temperature was programmed at 60°C for 8 minutes, and then a gradient of 2°C/min to 250°C was maintained for 10 minutes. The total analysis time was 113 min.

A quadrupole detector recorded the mass spectra, and ionization was achieved by electron impact with a filament intensity of 70 eV. The interface temperature was 280°C, and the source temperature was 230°C. Volatile components were identified by matching their recorded mass spectra with those stored in NIST, Wiley, and PAST operating software, the GC-MS Data System Mass Spectra Library, and other published mass spectra. Determining component percentages was based on peak area normalization without correction factors.

Insecticidal activity

The insecticidal activity of the essential oil was determined according to the protocol of Stefanazzi. [8] The test was carried out in Petri dishes of 9 cm in diameter, including sheets of Whatman paper impregnated with 0.25μL of the 5 different concentrations of essential oil tested (1 μL, 2.5 μL, 5 μL, 7.5 μL and 10 μL). The concentrations were achieved by dilution in DMSO, which was used as a negative control. Acetamiprid at 20% was used as the reference insecticide and represented the positive control. 20 aphids were placed in each box which has been covered with perforated plastic tape and incubated at temperature of 26 ± 2°C and 40 ± 5% relative humidity.

Aphid mortality was recorded after 24h, 48h and 72h. A control (without EO application) was used as corrected factor in each repetition according to the formula of Abbott, [9] which is expressed as follows:

$$Mc = \frac{Me - Mt}{100 - Mt} X100$$

Mc = corrected mortality in percentage.

Me = mortality of the sample tested.

Mt = mortality in the untreated control.

The protocol was repeated in triplicata, and the LD50 values (lethal concentration) were calculated by Probit analysis.

Statistical analysis

Statistical analyzes were performed with SPSS IBM software version 26.0. Results were expressed as mean ± SD. The One-Way ANOVA test followed by the Tukey post-hoc test were used to compare the results of the insecticidal activity of the essential oil of the plant studied with the two controls. The level of statistical significance was set at $P<0.05$.

Results and discussion

Yield and chemical composition of essential oil

The EO obtained from the leaves of *P. lentiscus* is characterized with pale-yellow color, possessing a robust scent, and a specific density of 0.86. The extraction yield reached 0.19±0.02 %.

The yield is influenced by various factors such as nature and components of the soil, the temperature, the altitude, the climate, the cultivation region and the individuals' genetic composition. [10] In addition, other factors can also influence the yield, such as the organ

used, the stage of development, the degree of freshness, the method, and the extraction equipment used. [11].

The EO was analyzed by GC-MS/MS (Figure 1). A total of 74 chromatographic peaks were identified and recorded (Table 1). These compounds were associated with the chromatographic peaks that represented 89.59% of the total composition of the EO.

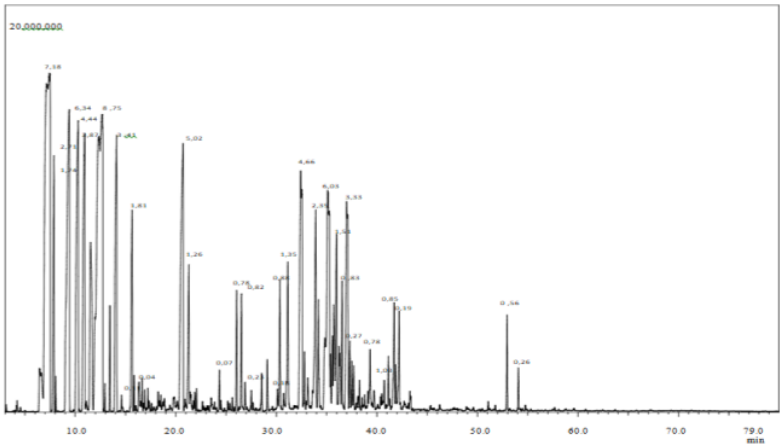

Figure. 1. Chromatogram of the essential oil of *Pistacia lentiscus* L.

In this oil, the main compounds are p-cymene (8.75%), α-pinene (7.18%), 2(10)-pinene (6.34%), γ-Muurolene (6.03%), D-limonene (5.13%), Bicyclo[5.2.0]nonanel (4.66%), β-pinene (4.44%), α-phellandrene (3.87%), γ-Terpinene (3.41%), δ-cadinene (3.33%), α-terpinene (2.78%) andα- tricylene (2.71%) (Table1).

Table 1: The compounds detected in the essential oil obtained from the leaves of *Pistacia lentiscus* L.

N°	Compounds	RT	%
1.	Tricyclene	6.450	0.22
2.	Tricyclene	6.475	0.29
3.	α-thujene	6.625	0.48
4.	α-pinene	7.130	7.18
5.	Myrtenyl format	7.265	1.39
6.	α-Tricyclene	7.375	2.71
7.	Cyclofenchene	7.436	0.96
8.	2-Pinene	7.494	1.74
9.	Camphene	7.878	1.95
10.	2,4-Thujadiene	8.058	0.14
11.	2(10)-Pinene	9.403	6.34
12.	β-pinene	10.278	4.44
13.	α-phellandrene	10.928	3.87
14.	3-Hexen-1-ol, acetate, (E)-	11.035	0.11
15.	α-terpinene	11.522	2.78
16.	p-cymenene	12.323	8.75
17.	D-Limonene	12.676	5.13
18.	α-Ocimene	12.950	0.12
19.	β-Ocimene	13.446	0.52
20.	γ-Terpinene	14.099	3.41
21.	α-Terpinolene	15.646	1.81
22.	2-Nonanone	15.843	0.18
23.	Pinane	16.015	0.04
24.	2-Norbornanol, 1,3,3-trimethyl-	16.907	0.16
25.	alpha.-Campholenal	17.584	0.05
26.	Acetaldehyde, (3,3-dimethylcyclohexylidene)-, (E)-	17.818	0.06
27.	Sabinol	18.246	0.18
28.	Camphor	18.496	0.15
29.	trans-3-Pinanone	19.415	0.12
30.	Borneol	19.840	0.22
31.	trans-3-Pinanone	20.147	0.13
32.	Terpinen-4-ol	20.705	5.02
33.	2-Cyclohexen-1-one, 4-(1-methylethyl)-	20.923	0.14
34.	alpha.-Terpineol	21.292	1.26
35.	Myrtenal	21.428	0.22
36.	(+)-2-Bornanone	21.872	0.16
37.	Levoverbenone	22.056	0.12
38.	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-	22.657	0.11
39.	cis-p-mentha-1(7),8-dien-2-ol	23.241	0.09
40.	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-acetate, (1S-endo)-	26.049	1.40
41.	2-Undecanone	26.530	1.36
42.	delta.-Elemene	28.541	0.28
43.	Copaene	29.105	0.33
44.	Globulol	29.613	0.06
45.	Ylangene	30.111	0.18
46.	Copaene	30.351	0.88
47.	Cyclobuta[1,2:3,4]dicyclopentene	30.727	0.17
48.	(-)-cis-beta-Elemene	31.142	1.35

49.	Bicyclo[5.2.0]nonane	32.423	4.66
50.	β-Copaene-4α-ol	32.786	0.36
51.	1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene	33.911	2.35
52.	Neoalloocimene	34.184	0.91
53.	γ.-Muurolene	35.099	6.03
54.	Longifolene-(V4)	35.716	1.48
55.	alpha.-Muurolene	35.980	1.51
56.	beta.-Cadinene	36.228	0.8
57.	γ-Cadinene	36.532	0.83
58.	Δ.-Cadinene	36.998	3.33
59.	Cadinadiene-1,4	37.313	0.45
60.	α-Amorphene	37.509	0.28
61.	alpha.-Calacorene	37.693	0.27
62.	β-Germacrene	38.264	0.36
63.	Caryophyllene oxide	39.321	0.78
64.	Agarospirol	39.713	0.18
65.	Junenol	40.728	0.26
66.	4a(2H)-Naphthalenol, 1,3,4,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)	41.142	0.49
67.	tau.-Muurolol	41.717	1.03
68.	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-,	41.870	0.30
69.	alpha.-Cadinol	42.209	0.85
70.	alpha.-Bisabolol	43.290	0.25
71.	geranyl-.alpha.-terpinene	51.072	0.07
72.	p-Camphorene	51.779	0.06
73.	p-Camphorene	52.949	0.56
74.	p-Camphorene	54.074	0.26
	Total compounds identified		89.59
	Hydrocarbon monoterpenes		54.23
	Oxygenated monoterpenes		8.4
	Hydrocarbon sesquiterpenes		23.02
	Oxygenated sesquiterpenes		7.99
	Diterpenes		0.95
	Other compounds		2.99

RT: retention time, %: percentage of the compound from the total identified

Recent studies in different Mediterranean countries have noted a large chemical variability involving the main compounds and the total amounts of terpene classes. [12, 13] Monoterpene hydrocarbons generally represented the main fraction: 75% in Egypt [13], 68% in Greece [14], and 59% in Tunisia. [15] However, in Tunisia, *P. lentiscus* EOs were rich in monoterpene hydrocarbons (41%) and sesquiterpene hydrocarbons (40%). [16] The main factor contributing to this chemo-variability is generally attributed to the environmental conditions. No data exists regarding relationship between genetic traits and HE profiles. [17]

The chemical profile of EO isolated from *Pistacia lentiscus* L. is dominated by monoterpens with 61.63% (53.23% are hydrocarbonated monoterpens). The p-cymenene (8.75%), α-pinene (7.18%), and 2(10)-pinene (6.34%) are the main components. The sesquiterpens class represents 31.01%, the major components are Bicyclo[5.2.0]nonanel and delta.-Cadinène representing respectively 4.66% and 2.54% of the total mixture (Table 2).

Previous investigations in the Mediterranean region revealed important quantitative variability of the EO's constituents of *Pistacia lentiscus* L. [12, 13]However, the qualitative composition demonstrated less variability. In comparison to *Pistacia lentiscus* L. collected from Tunisia

[14] , limonène (10,3-43,8%), α-pinène (2,9-34,2%), terpinène 4-ol –terpinène β (8,2- 34,7%), α-terpinéol (10,4-11,0%) represented the main components. From Greece, *Pistacia lentiscus* L. showed dominance of α -pinène (63%), β – myrcène (25%), β -pinène (3,3%) [15]. The Morrocan *Pistacia lentiscus* L. is marked with myrcene (39.2%), limonene (10.3%), and β -gurjunene (7.8%) as main constituents. [16]

The variability between different Algerian localities is mentioned previously. [17] The *Pistacia lentiscus* L. EOs obtained from Algiers, Tizi-Ouzou, and Oran provinces showed dominance of α-pinène in Algiers and Tizi-Ouzou samples, whereas the Oran's sample was dominated by P-Cymenene.

The findings of our study are in accordance with the previous literature in terms of qualitative feature of the EO. The quantitative variability characterizing the chromatographical profiles is attributed the local environmental conditions as well as the genetic characteristics of the *Pistacia lentiscus* L. varieties [18]. Unfortunately, the chemical composition of EOs relationships with the genetic factors is not fundamentally documented contrarly to their dependence to epigenetic factos.

Aphicidal activity

The essential oil of *P. lentiscus* showed interesting larvicidal activity (Table 3). The mortality rate is concentration-dependent. The ANOVA test indicated a significant variability ($P<0.001$) between various EO's concentrations and the synthetic insecticide used as positive control. After 24 h of exposure, the essential oil of *P. lentiscus* caused a mortality rate of 73.4% at a concentration of 1 μL; on the other hand, acetamiprid at 20% caused only 5.08% mortality in aphids larvae.

Table 2: Average corrected mortality of individuals of *A. spiraecola* as a function of the concentration of the essential oil of *P. lentiscus* (ANOVA, $P<0.001$).

Concentration	Corrected mortality (%)
1μL	73.4 ± 5.11^{b}
2.5μL	74.65 ± 6.06^{b}
5μL	79.8 ± 3.08^{b}
7.5μL	$85.08 \pm 6.01^{b,a}$
10μL	96.88 ± 3.07^{a}
Negative control (DMSO)	0.00 ± 0.00^{c}
Acetamiprid 20%	$5.08 \pm 1,02^{c}$

The Tukey post-hoc test separated the results of the mortality rate of aphids in contact with EO into three variable homogeneous groups (labeled "a, b, c" in Table 3). Thus, there is no significant difference between the concentrations of 1, 2.5, 5, and 7.5 μL of EO with regard to the larvicidal effect. Similarly, there is no significant difference between the concentrations 10 μL and 7.5μL, with which the mortality rates reached 96.88% and 85.08%, respectively. In the other hand, the probit analysis indicated that the lethal doses DL20, DL50 and DL90 of the EO were respectively 0.02, 0.2, and 10.5 μL, these values indicate that EO of *P. lentiscus* is very toxic.

The larvicidal activity is attributed to the chemical mixture, which is dominated by monoterpene compounds known for their larvicidal effects. [18, 19] GC-MS/MS analysis of the tested EO showed the richness of EO with monoterpenoids and sesquiterpenoids known as potential insecticidal agents against wide range of insects. [20, 21, 22] Additionally, several

compounds recorded in *P. lentiscus* EO profile, such as α-pinene, β-pinene, limonene and p-cymene, are well known for their larvicidal activity. [23]

Previous investigations depicted the mechanism of some volatile constituents of *P. Lentiscus*. As example, (E)-β-caryophyllene is an active component that act by contact on the integument of insects, [11] moreover, α-terpineol has been found to possess a high toxicity [24] and δ-cadinene has proven to be highly toxic against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinque fasciatus*.[25] Generally, the bioactivity of EOs is dependent on their chemical composition and thus, the determination of their profiles is an important aspect before a recommendation is made in a control program.

Conclusion

The current investigation, focusing on the aphicidal activity of EO extracted from *Pistacia lentiscus* against *A. spiraecola*. The findings indicated that this bioresource is a rich source of monoterpenes and sesquiterpenes demonstrating important larvicidal properties. The findings of our research represent useful data on the biological activities of the medicinal herb *P. lentiscus*, thus supporting the future exploitation of the EO as a biopesticide and creating opportunities for its future utilization in the phytosanitary sector.

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