Pak. J. Phytopathol., Vol. 35 (02) 2023. 259-267 DOI:10.33866/phytopathol.035.02.0976

Official publication of Pakistan Phytopathological Society

Pakistan Journal of Phytopathology

ISSN:1019-763X(Print), 2305-0284(Online)

http://www.pakps.com

APHICIDAL POTENTIAL OF THE ESSENTIAL OIL ISOLATED FROM *PISTACIA LENTISCUS* AGAINST THE LARVAE OF *APHIS SPIRAECOLA*, VECTOR OF MULTIPLE PHYTOVIRUSES

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ABSTRACT

This study aims to determine the composition of the essential oil of *Pistacia lentiscus* leaves by gas chromatography-mass spectrometry (GC/MS) and to test its insecticidal activity 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 lentisk 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 *A. spiraecola* larvae. A total of 74 compounds were identified, corresponding to chromatographic peaks representing 89.6% of the total area of all peaks. The most abundant compounds were monoterpene hydrocarbons (54.2%) with 8.8% p-cymene and 7.2% α-pinene. The insecticidal assay revealed an interesting insecticidal activity against the larvae of *A. spiraecola* with an LD50 of 0.2 µL. This study introduces and supports the use of the essential oil of *P. lentiscus* as a biopesticide and open new ways for its future exploitation in phytosanitary industries.

Keywords: *Pistacia lentiscus*, *Citrus* tristeza virus(CTV), *Aphis spiraecola*, Biopesticides

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 (Colignon *et al.,* 2003), general environment and human health are previously documented (Batsc, 2009). The world health organization estimates that 200000 people are killed early worldwide due to pesticide poisoning (CAPE, 2009).

Several scientific investigations have proved the efficacy of EO from diverse botanical resources as bio pesticides against wide range of damaging pests (Amara*et al.,* 2019; Behi *et al.,* 2019; Abdelmaksoud *et a.l,* 2023;Hu *et al.,* 2022) including Aphis spiraecola. This aphid (Hemiptera: Aphididae) is aglobally pervasive insect with harmful effects and worldwide distribution in temperate and tropical regions. It is responsible for weakening crops and acts as a vector for multiple phytovir uses such as Citrus tristeza virus (CTV), Cucumber mosaic virus (CMV) and Potato virus Y (PVY) (Hullé *et al.,* 2012). It feeds on apple, citrus, spiraea plants, and on a diverse range of vegetable crops. Globally, it has become the predominant aphid pest affecting citrus, and it expanded its range to include various tropical crops in the1950s (Pfeiffer, 1991).

Aphis spiraecola, ranging from 1.2 to 2.2 mm in length, follows a holocyclic lifecycle and produces sexual morphs. Its typical primary hosts include spirea or citrus fruits, with spirea serving as the primary host

Submitted: June 28, 2023
Revised: July 17, 2023
Accepted for Publication: December 05, 2023
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in North America and Brazil (de Menezes, 1970). In Japan, both spirea and citrus fruits are recorded as primary hosts (Komazaki *etal.,* 1979). Hodjat and Eastop (1983) documented sexual forms on apple in Iran. However, across most of its geographical range, including North Africa, *Aphis spiraecola* exhibits an anholocyclic lifecycle, reproducing entirely through parthenogenesis. As customary, *Aphis spiraecola* undergoes four larval instars.

In the natural environment, essential oils (EOs) serve a significant function in safeguarding plants. They may also attract certain insects, facilitating the dispersion of pollens and seeds, or act as a deterrent to undesirable insects (Bakkali *et al.,* 2008). Consequently, EOs can play a vital role in combating various significant crop pests, offering a plant-based pesticide option with fewer adverse effects and serving as an environmentally friendly product. These botanical pesticides represent an exceptionally promising choice (Pavela and Benelli, 2016). Consisting essentially hydrophobic liquids with volatile active compounds (Burger *et al.,* 2019), EO find application through either contact or fumigation methods justified by their volatility (Ikbaland Pavela, 2019). While contact application remains the more widely adopted method for EOs, fumigation methods are frequently employed in managing stored pest species. This approach enables the homogeneous diffusion of volatile compounds with in confined spaces, as evidenced by the scientific investigations (Kavallieratos *et al.,* 2021; Rajendran and Sriranjini, 2008).

Algerian flora is rich source of aromatic and medicinal plants. The *Pistacia lentiscus* L., commonly called 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 (Rauf *et al.,* 2017).

The EO of the leaves of *P. lentiscus* is used in the treatment of several diseases by its antibacterial, antioxidant and anti-carcinogenic effectsand, on the other hand, as a biopesticide to fight against certain bioaggressors (Amara *et al.,* 2019). The objective of this study is to determine the chemical components of the EOs of the leaves of *P. lentiscu*s 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 27m). The climate is warm and temperate, of the Mediterranean type (Köppen classification: Csa). Botanical identification was carried out at the local natural bio resources laboratory of Hassiba Benbouali University in Chlef, Algeria. After harvesting, the leaves were carefully washed, dried and crushed.

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 152m. The infected leaves were collected in plastic boxes (20×10×5cm) 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 (Blackman and Eastop, 2006). 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 extracted by hydrodistillation using a Clevenger-type apparatus (Clevenger, 1928) with a sample/water ratio (g/mL) equivalent to 1/5. After three hours of extraction, the condensed vapor gave an organic phase (essential oil) separated from the water by decantation. The EO isolated was kept at 4°C in amber bottle until use.

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 (30m×0.25mm, 0.25μm). 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 8min, and then a gradient of 2°C/min to 250°C was maintained for 10minutes. The total analysis time was 113min.

A quadrupole detector recorded the mass spectra, and ionization was achieved by electron impact with a filament intensity of 70eV. 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 *et al*. (2011). The test was carried out in Petri dishes of 9cm in diameter, including heets of Whatman paper impregnated with 0.25µL of 5 different concentrations of essential oil tested (1µL, 2.5µL, 5µL, 7.5µL and 10µL). 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 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 *et al*. (1925), which is expressed as follows:

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

Mc=corrected mortality in percentage.
Me=mortality of the sample tested.
Mt=mortality in the untreated control.

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

STATISTICALANALYSIS

Statisticalanalyzeswereperformedwith SPSSIBMsoftwareversion26.0. Results were expressed as mean ±SD. TheOne-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.

RESULTSANDDISCUSSION

Yield and chemical composition of essential oil: Theessentialoilextractedfromtheleavesof*P.lentiscus*by hydrodistillationproducedpale-yellow oil with a strong fragrance 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 (Bouyahyaoui *et al.,* 2016). In addition, other factor scan also influences the yield, such as the organ used, the stage of development, the degree of freshness, the method, and the extraction equipment used (Tabti*etal.,*2020).

The EO was analyzed by GC-MS/MS (Figure1). A total of 74 chromatographic peaks were annotated (Table 1). These compounds corresponded to chromatographic peaks representing 89.59 % of the total composition of the EO.

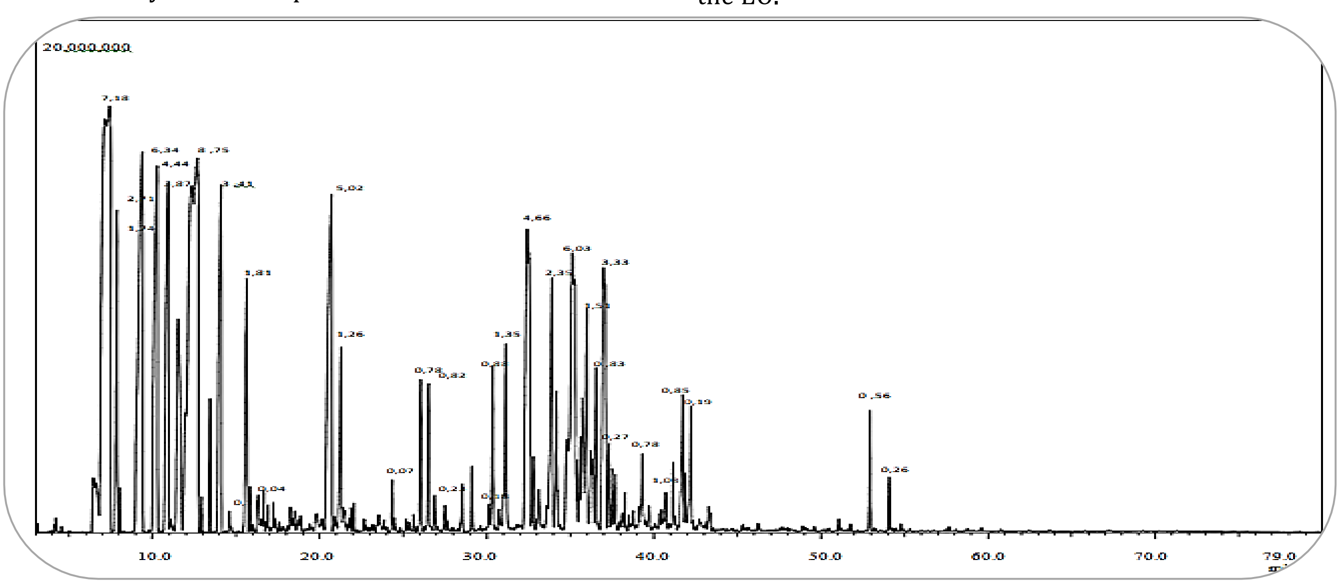

Figure1.Chromatogram of the essential oil of *Pistacia lentiscus* L.

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%) and α-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.	Myrtenylformat	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.-EIemene	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.	Caryophylleneoxide	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 (El Bishbishy *et al.,* 2020; Vidrich *et al.,* 2004). Monoterpene hydrocarbons generally represented the main fraction:75% in Egypt (El Bishbishy *et al.,* 2020), 68% in Greece (Gardeli*etal.,*2008), and 59% in Tunisia (Ben Douissa *et al.,* 2005). However, in Tunisia, *P. lentiscus* EOs were rich in monoterpene hydrocarbons (41%) and sesquiterpene hydrocarbons (40%) (Aissi *et al.,* 2016). 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 (Sehaki*etal.,*2022).

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]nonanelanddelta.-Cadinene 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. (El Bishbishy *et al.,* 2020; Vidrich *et al.,* 2004). However, the qualitative composition demonstrated less variability. In comparison to *Pistacia lentiscus* L. collected from Tunisia (Gardeli *et al.,* 2008), limonene (10.3-43.8%), α-pinène (2.9-34.2%), terpinene4-ol–terpinene β (8.2-34.7%), α-terpineol (10.4-11.0%) represented the main components. From Greece, *Pistacia lentiscus* L. showed dominance of α-pinene (63%), β–myrcene (25%), β-pinene (3.3%) (Ben Douissa *et al.,* 2005). The Morrocan *Pistacia lentiscus* L. is marked with myrcene (39.2%), limonene (10.3%), and β-gurjunene (7.8%) as main constituents (Aissi *et al.,* 2016).

The variability between different Algerian localities is mentioned previously (Sehaki *et al.,* 2022). The *Pistacia lentiscus* L. EOs obtained from Algiers, Tizi-Ouzou, and Oran provinces showed dominance of α-pinene 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 profile is attributed the local environmental conditions as well as the genetic characteristics of the *Pistacia lentiscus* L. varieties (Lucia *et al.,* 2007). Unfortunately, the chemical composition of EOs relationships with the genetic factors is not fundamentally documented contrarily to their dependence to epigenetic factors.

Aphicidal activity: The essential oil of *P.lentiscus* showed interesting larvicidal activity (Table 3). The mortality rate is concentration-dependent. The ANOVA analysis showed a significant difference (P<0.001) between different concentrations used and the synthetic insecticide used as positive control. After 24h of exposure, the essential oil of *P.lentiscus* caused a mortality rate of 73.4% at a concentration of 1µL; 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	Correctedmortality(%)
1µL	73.4±5.11[b]
2.5µL	74.65±6.06[b]
5µL	79.8±3.08[b]
7.5µL	85.08±6.01[b,a]
10µL	96.88±3.07[a]
Negativecontrol(DMSO)	0.00±0.00[c]
Acetamiprid20%	5.08±1,02[c]

The Tukey post-hoc test separated the results of the mortality rate of aphids in contact with EO into three significantly different homogeneous groups (annotated "a, b, c" in Table 3. Thus, there is no significant difference between the concentrations of 1µL, 2.5µL, 5µL and 7.5µL of EO with regard to the larvicidal effect. Similarly, there is no significant difference between the concentrations 10µL 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 10.5µL, these values indicate that EO of *P.lentiscus* is very toxic.

The larvicidal activity can be explained by the chemical composition, which is dominated by monoterpene compounds known for their larvicidal effects (Lucia *et al.,* 2007; Michaelakis *et al.,* 2009). GC-MS/MS analysis of the tested EO showed the richness of EO with monoterpenoids and sesquiterpenoids, which are compounds that possess insecticidal activity against various insect species (Bernays and Chapman, 1998; Papachristos *et al.,* 2002). 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 (Michaelakis *et al.,*2008). Previous investigations depicted the mechanism of some volatile constituents of *P. Lentiscus*. As example, (E)-β-caryophyllene is an active component that acts by contact on the tegument of insects (Tabti *et al.,* 2020), moreover, α-terpineol has been found to possess a high toxicity (Sener *et al.,* 2009) and δ-cadinene has proven to be highly toxic against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinque fasciatus* (Govindarajan *et al.,* 2016). 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 (Khanikor *et al.,* 2013; Tabti *et al.,* 2020).

CONCLUSION

The present study was focused on the aphicidal activity of essential oil extracted from *Pistacia* *lentiscus* against *A. spiraecola*. The findings indicated that this herb is a rich source of monoterpenes and sesquiterpenes as well as other compounds that have strong larvicidal activity. The findings of our research represent useful data on the biological activities of the medicinal herb *P. lentiscus*, thus supporting the future use of this oil as a biopesticide and opening new avenues for its possible exploitation in the phytosanitary industries. Further studies are needed to test the efficiency of this oil on the field.

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