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Bioactivity of *Schinus molle* L. and *Schinus terebinthifolia* Raddi. Essential Oils on *Anticarsia gemmatalis* (Hübner 1818)

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HIGHLIGHTS

- *S. molle* leaf had an EO yield of 0.3% m/v; S*. terebinthifolia* had 0.2% m/v.
- Both EOs presented α-pinene as the major compound.
- *S. molle* EO induced 30% mortality of *A. gemmatalis* in 72 h.
- *S. terebinthifolia* EO induced 70% mortality of *A. gemmatalis* in 72 h.
- The LC_{50} for *S. terebinthifolia* EO was 1.74% v/v (1.58 - 1.97% v/v).

Abstract: *Anticarsia gemmatalis* is one of the main pests of the soybean crop, being controlled mainly with agrochemicals. The environmental and health risks, as well as the development of resistance by the pests, has led to the search for alternative control measures, aiming to use more eco-friendly procedures. The objective of this research was to evaluate the chemical composition and the bioactivity of *Schinus molle* and *Schinus terebinthifolia* essential oils (EOs) on *A. gemmatalis*. The major compound in both EOs was α-pinene (60.04 wt.% for *S. molle* and 38.49 wt.% for *S. terebinthifolia*). Bioassays were carried out with third instar larvae, with five replicates and each replicate with ten larvae, totaling 50 larvae per treatment. The oils were incorporated in the artificial diet (0.1, 0.5, 1.0, 1.5, and 2.0% v/v). The controls were: water, Tween-80® 0.5% v/v, and novaluron 0.075% v/v. According to the Probit method, the *S. terebinthifolia* EO presented a LC_{50} of 1.74% v/v (1.58-1.97% v/v); it was not possible to determine the LC_{50} for the *S. molle* EO. The mortality percentage after 24 and 48 h was 52% and 30% at 2.0% v/v for *S. terebinthifolia* and *S. molle* oil, respectively.

After 72 h, the mortality rate for *S. molle* EO have not changed; for *S. terebinthifolia* EO it increased to 70%; the larvae treated with the chemical control (synthetic insecticide) had a mortality of 100%.

Keywords: insecticidal activity; pest control; soybean caterpillar; terpenes; bioactive compounds.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is a plant of the Fabaceae family, which is native to China. Its grain is very versatile and gives rise to several by-products, which are used in the food, chemical, and agro-industries. Worldwide, soybean is considered one of the most economically important oilseeds, since it is a source of proteins and vegetable oil. It is also a feedstock for food industry, being also used in animal feeding [1].

Soybean cultivation represents the main crop for Brazilian agribusiness and has shown significant growth in the last decades due to advances in technology [2]. The yield in the 2018/2019 harvest was 3,189 kg/ha, with a total production of more than 113 million of tons [3].

Anticarsia gemmatalis (Hübner, 1818) (Lepidoptera: Erebidae) is a tropical and subtropical species restricted to the American continent [4], being also considered a pest of legumes [5]. This pest can hinder the soy production potential, causing up to 100% defoliation and resulting in reduced crop yield [6].

As a result of the constant use of agrochemicals for pest control, these substances are losing their field effectiveness due to the excessive and frequent use of the same product in a certain area. The indiscriminate use of these products, in addition to polluting the environment, can lead to an increase in the selection of resistant insect populations, rendering it difficult to establish their natural enemies and also increasing the production costs [7,8].

The negative impact of the massive use of synthetic pesticides on the environment and on human and animal health is widely reported in the literature and has stimulated the search for environment-friendly practices for controlling pests. The Integrated Pest Management (IPM) utilizes a combination of more environmentally friendly control methods in order to control pest populations in agricultural systems. The advantages of using botanical products include the rapid degradation of these molecules, low mammalian toxicity, high selectivity, and minimal impact on the plants [9].

The genus *Schinus* is native from the Americas, widely distributed throughout the continent; some species are also found in several tropical and subtropical areas of the world. Many of these species are cultivated due to their ornamental value in Australia, California, Mexico, the Canary Islands, and the Mediterranean [10].

Schinus molle L., original from Peru and considered native in Brazil (Rio Grande do Sul and Santa Catarina states), Uruguay, Argentina, and other countries of the Andean region [11], is a perennial, evergreen plant, fast-growing, reaching heights of 15 to 20 m. The leaves are pinnate, distributed alternately; the branchlets and leaves are often pendant, and the small yellow flowers are abundant in terminal clusters. It produces flowers and fruits continually. The *S. molle* growth is greatest in the warm season, until soil moisture is depleted [12].

S. molle is used in its countries of origin in traditional and folk medicine, in beverages, and as a condiment; however, outside of its natural occurrence area, *S. molle* is cultivated for ornamental purposes. There are reports of this species as an invasive plant in Mexico, Australia, and South Africa [11,12].

Schinus terebinthifolia L. is a perennial, evergreen plant, that can be found as a bush or as a tree, its height ranges from 5 to 10 m, the plant trunk has a diameter between 20 and 30 cm, coated by a shell with a thickness of 1 to 5 mm. The shell is impregnated with a resinous material, which often appears on shell surface. The root is well developed, favoring its survival in harsh environments. Its leaves are dark-green, with a length of 10 to 15 cm and width of 2 to 3 cm; the flowers have small size and the color ranges from yellow to white, the flowering period occurs in spring-summer months (September to January). The *S. terebinthifolia* fruits are small drupes, grouped in panicles, with a bright red color. Each fruit holds one seed with dark-brown color and a diameter of 0.3 mm. Each plant generates numerous fruits. The fruiting occurs during the end of the summer and the autumn-winter period (January to July) [13].

Ferriter [14] cited *S. terebinthifolia* as an aggressively invasive pest plant in Hawaii, Florida, South Africa, Mascarene Islands, and Australia, causing several millions of dollars of damage and costs for its control. According to Silva-Luz and Pirani [15], the name *S. terebinthifolius* is a homotypic synonym for *S. terebinthifolia*; thus, the two scientific names correspond to the same species.

The objective of the present work was to determine the chemical composition of the essential oils EOs of *Schinus molle* and *Schinus terebinthifolia* leaves obtained by hydrodistillation and to evaluate their bioactivity on *Anticarsia gemmatalis*.

MATERIAL AND METHODS

Insects

The *A. gemmatalis* larvae used in this study were grown using the artificial diet method proposed by Greene and coauthors [16] and kept in the Laboratory of Pest Control of the University of Caxias do Sul under controlled conditions (26±1 ºC, RH of 75±1%, and photoperiod of 14 h of light and 10 h of dark).

Plant material and obtainment of essential oils

Leaves from five plants of *S. molle* and five plants of *S. terebinthifolia* were collected in the early morning of September 10, 2019, in the city of Caxias do Sul, from the area around the Biotechnology Institute of the University of Caxias do Sul (UCS), and deposited in the herbarium of UCS (HUCS 47634 and HUCS 49320, respectively) [17]. Only one collection of plant material was carried out. The plant material was dried in a drying oven with forced air circulation at a constant temperature of 40 ºC for 3 days.

The EOs from both plants were extracted by steam distillation of the leaves, following the process described by Koketsu and Gonçalves [18], for a period of 90 min. The EOs were collected at the outlet of the condenser and put in amber bottles; a small sample of each EO was sent for GC/MS and GC-FID analyses and the remaining EOs were stored away from sunlight at 4±2 °C. The oils were only brought to room temperature (approx. 25 °C) at the time of the bioassays.

Chemical characterization of the essential oils

The EO samples underwent qualitative analysis by GC/MS and quantitative analysis by GC-FID. The analyses were carried out at the Analytical Center of the Biotechnology Institute of the University of Caxias do Sul.

The GC-FID analysis was performed on a Hewlett Packard 6890 Series gas chromatograph, equipped with a HP-Chemstation data processor, using a HP-5 column (30 m × 320 µm i.d.) with 0.50 µm film thickness (Hewlett Packard, Palo Alto, CA, USA). Temperature program: 40 °C for 8 min, increased to 180 °C at 3 °C/min, then increased to 230 °C at 20 °C/min, then maintained at 230 °C for 20 min; injector temperature of 250 °C; split ratio of 1:50, flow rate of 1.0 mL/min, flame ionization detector with a temperature of 250 °C; hydrogen as carrier gas at 34 kPa; injected volume of 1 µL of EO diluted in hexane (1:10).

The GC/MS analysis was performed on a gas chromatograph coupled to a Hewlett Packard 6890/MSD5973 mass selective detector, equipped with HP-Chemstation software and a Wiley (Hoboken, NJ, USA) 275 spectra library. A HP-5 fused silica capillary column (30 m × 250 µm) with 0.50 µm film thickness (Hewlett Packard). The temperature program used was the same as the one used in the GC-FID; interface temperature of 280 °C, split ratio of 1:100, helium as the carrier gas at 56 kPa, flow rate of 1.0 mL/min, ionization energy of 70 eV, injected volume of 1 µL of EO diluted in hexane (1:10).

The EO components were identified by comparison of their respective mass spectra to those of the Wiley Library, selected by the GC/MS software by match percentage, and comparing the calculated LRI (linear retention index) with the indexes reported by Adams [19]. The linear retention index (LRI) was calculated using the Van den Dool and Kratz equation, using a standard solution of alkanes (C8 to C26). Only the compounds whose LRI and mass spectrum data were concordant were considered as identified, otherwise, were considered as ‘not identified’.

Bioassays

The EOs of *S. molle* and *S. terebinthifolia* were homogeneously diluted in the artificial diet. The concentrations were (% v/v): 0.1, 0.5, 1.0, 1.5, and 2.0. Two negative controls were included: one with distilled water and the other with Tween-80® (0.5% v/v). A positive control with the chemical insecticide Rimon® Supra (0.075% v/v; novaluron as active ingredient) was also added to the experimental design.

Ten *A. gemmatalis* third instar larvae were used for each treatment and each control, with five replicates. The third instar phase was chosen because it is from this stage on that *A. gemmatalis* starts to cause crop losses. The insects were individually placed in 100 mL plastic cups with 1 g of the artificial diet containing the solubilized oil/control and a moist cotton pad to maintain the humidity. The mortality rates were evaluated

after 24, 48, and 72 h. The experiments were carried out at the Laboratory of Pest Control at the University of Caxias do Sul, Caxias do Sul, RS, Brazil.

Statistical analysis

The experimental design was bifactorial (factors: EO type and EO concentration), completely randomized, with five treatments, three controls, and five replicates. Each replicate was composed by ten larvae, totaling 50 larvae per treatment/control. The data underwent two-way analysis of variance (ANOVA), followed by the Tukey's multiple range test ($p < 0.05$). The statistical analyses were carried out using the Statistical Package for the Social Sciences (IBM SPSS for Windows 20.0) [20]. The Probit method [21] was used to determine the median lethal concentration (LC_{50}) values.

RESULTS AND DISCUSSION

Chemical analysis of the essential oils

The chemical constitution of the EOs from different cultivars depends on several factors, such as genetic, abiotic, and climatic factors; water stress; environmental stimulation; nutrition; the growth phase; the parts of the plant used; the collection season; drying conditions; and the EO obtainment method [22-25].

Regarding EO yield, in the present work the leaf oil of *S. molle* had a yield of 0.3% v/m; *S. terebinthifolia* leaf EO yield was 0.2% v/m (Table 1). Cavalcanti and coauthors [26] reported yields of 1.10% v/m and 0.10% v/m for the leaf EOs of *S. molle* and *S. terebinthifolia*, respectively. Machado and coauthors [27] reported a yield of 0.78% v/m for *S. molle* leaf EO obtained by hydrodistillation. Uliana and coauthors [28] reported an EO yield of 1.70% v/m for the leaves of *S. terebinthifolia*.

According to the literature, the leaf EO from *S. molle* is mainly composed by monoterpenes and sesquiterpenes, such as α-pinene, β-pinene, myrcene, limonene, β-caryophyllene, bicyclogermacrene, δ-cadinene, spathulenol, and α-cadinol [11,26].

The EO of *S. molle* contained α-pinene (60.04 wt.%), limonene (11.28 wt.%), and β-pinene (9.24 wt.%) as the major compounds (Table 1). In other studies that characterized this oil, the major components identified were α-pinene, sabinene, limonene, caryophyllene, terpineol, bicyclogermacrene, spathulenol, and γ-muurolene [29]. Cavalcanti and coauthors [26] reported cubenol, caryophyllene oxide and spathulenol as the major compounds of *S. molle* leaf EO, with contents of 27.1, 15.3, and 12.4 wt.%, respectively. Machado and coauthors [27] reported β-pinene, α-pinene, limonene and muurolol as major compounds of the leaf EO of *S. molle*, with contents of 14.7, 14.1, 9.4, and 11.8 wt.%, respectively.

Table 1. Qualitative and quantitative analysis of the *Schinus molle* and *Schinus terebinthifolia* leaf essential oils, obtained by steam distillation.

Compound	Chemical class	Calc. LRI	LRI Lit.[1]	wt.%	
				S. molle	*S. terebinthifolia*
α-thujene	MT	922	924	-	0.32±0.06
α-pinene	MT	932	932	60.04±0.07	38.67±4.45
camphene	MT	944	946	0.25±0.08	0.43±0.21
sabinene	MT	968	969	0.55±0.12	-
β-pinene	MT	973	979	9.24±1.94	12.34±3.75
myrcene	MT	983	988	1.75±0.66	0.72±0.37
α-terpinene	MT	1019	1014	-	1.26±0.89
limonene	MT	1024	1024	11.28±3.27	8.48±2.25
cis-β-ocimene	MT	1037	1032	2.07±0.19	0.21±0.07
terpinen-4-ol	OMT	1180	1174	0.22±0.04	1.21±0.25
α-terpineol	OMT	1185	1186	-	1.88±0.69
linalyl acetate	OMT	1256	1254	-	0.17±0.09
α-terpinyl acetate	OMT	1342	1346	0.22±0.08	-
α-copaene	ST	1354	1348	0.17±0.10	0.15±0.08
β-cubebene	ST	1381	1387	-	3.34±0.58
β-elemene	ST	1393	1389	-	1.32±0.36
β-caryophyllene	ST	1426	1417	2.32±0.74	3.12±0.48
cis-β-farnesene	ST	1444	1440	-	0.15±0.06
α-humulene	ST	1456	1452	0.16±0.03	0.67±0.13
γ-muurolene	ST	1476	1478	-	0.41±0.20
germacrene-D	ST	1487	1484	5.97±1.12	3.14±0.62
bicyclogermacrene	ST	1502	1500	-	3.32±0.94
γ-cadinene	ST	1511	1513	0.22±0.20	0.56±0.19
δ-cadinene	ST	1519	1522	-	0.63±0.32
cis-calamenene	ST	1528	1528	-	1.38±0.26
cis-nerolidol	OST	1563	1561	-	2.16±0.39
spathulenol	OST	1573	1577	-	0.18±0.04
caryophyllene oxide	OST	1584	1582	-	1.53±0.28
globulol	OST	1590	1590	-	1.08±0.61
viridiflorol	OST	1596	1592	-	0.91±0.20
ledol	OST	1604	1602	-	0.19±0.08
β-eudesmol	OST	1644	1649	-	0.33±0.06
α-cadinol	OST	1647	1652	-	2.27±1.12
α-santalol	OST	1670	1674	-	0.92±0.65
Hydrocarbon monoterpenes (MT)				85.18	62.44
Oxygenated monoterpenes (OMT)				0.44	3.26
Hydrocarbon sesquiterpenes (ST)				8.84	18.20
Oxygenated sesquiterpenes (OST)				-	9.57
Not identified				5.54	6.53
Yield (% v/m)				0.3	0.2

RT: retention time; Calc. LRI: calculated linear retention index; Lit. LRI: literature retention time; [1]Adams [19]. The trace (-) indicates absence of the component.

The EO of *S. terebinthifolia* presented α-pinene as the major compound (38.67 wt.%), followed by β-pinene (12.34 wt.%) and limonene (8.48 wt.%) as the other major compounds (Table 1). Other works also cite α-phellandrene, γ-cadinene, and β-phellandrene (34.38 wt.%, 18.04 wt.%, and 10.62 wt.%, respectively) as the major compounds in this EO [30]. Cavalcanti and coauhtors [26] reported β-caryophyllene, α-pinene, and germacrene-D as the major compounds in *S. terebinthifolia* leaf EO, with contents of 35.2, 28.1, and 15.5% wt.%, respectively. Figure 1 presents the chromatograms of both EOs.

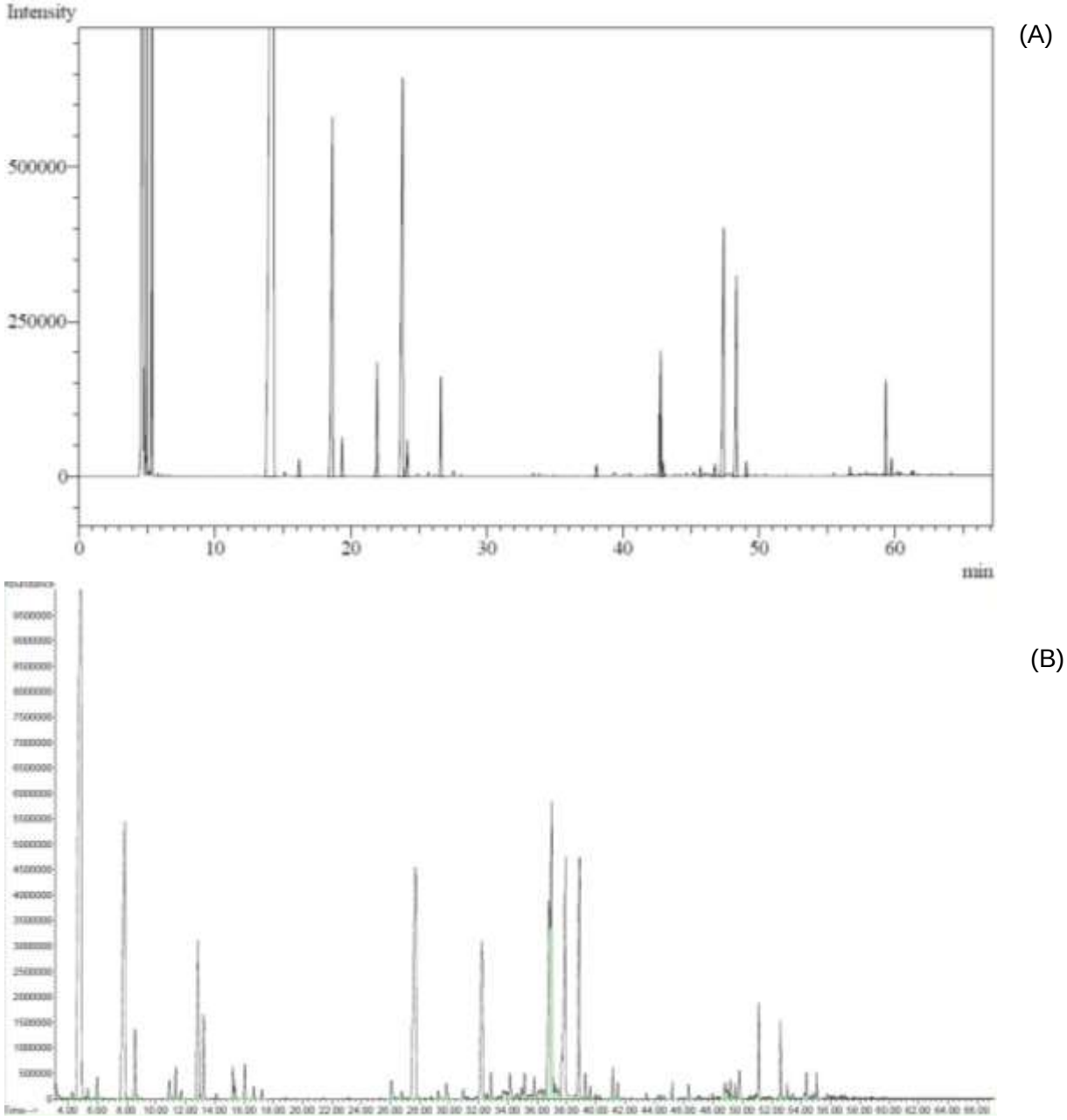

Figure 1. Chromatograms (GC/MS) of *S. molle* (A) and *S. terebinthifolia* (B) essential oils, obtained through steam distillation of the leaves.

Regarding chemical classes, hydrocarbon monoterpenes were the most abundant class (84.59 wt.%) in the *S. molle* EO; it was also noteworthy the absence of oxygenated sesquiterpenes. Cavalcanti and coauthors [26] reported for *S. molle* leaf EO of plants from Southeastern Brazil and obtained after 6 h of hydrodistillation: hydrocarbon monoterpenes content of 4.2 wt.%; a content of oxygenated monoterpenes of 25.6 wt.%; the hydrocarbon sesquiterpenes presented a content of 4.5 wt.%, and oxygenated sesquiterpenes accounted for 63.6 wt.% of the oil. Machado and caouthors [27] reported a hydrocarbon monoterpenes content of 38.8 wt.%, oxygenated monoterpenes content of 14.0 wt.%, hydrocarbon sesquiterpenes content of 10.3 wt.%, and oxygenated sesquiterpenes content of 30.0 wt.% for *S. molle* leaf EO of plants from South Brazil and obtained after 4 h of hydrodistillation.

On the other hand, the *S. terebinthifolia* EO presented higher amounts of hydrocarbon sesquiterpenes (18.20 wt.%) and also a small quantity of oxygenated sesquiterpenes (9.57 wt.%). Cavalcanti and coauthors [26] reported the following composition for *S. terebinthifolia* leaf oil of plants from Southeastern Brazil, after 6 h of hydrodistillation: 41.2 wt.% of hydrocarbon monoterpenes, 8.5 wt.% of oxygenated monoterpenes, and 50.3 wt.% of sesquiterpenes; the authors also reported the absence of oxygenated sesquiterpenes in the oil. Uliana and coauthors [28] reported the following chemical class distribution for *S. terebinthifolia* leaf EO of plants from Southeastern Brazil, obtained after 2 h of hydrodistillation: 85.9 wt.% of hydrocarbon monoterpenes, 0.5 wt.% of oxygenated monoterpenes, 11.3 wt.% of hydrocarbon sesquiterpenes, and 0.2 wt.% of oxygenated sesquiterpenes.

Cavalcanti and caouthors [26], who studied the effect of process time in the extraction kinetics of EO from the leaves and fruits of both *S. molle* and *S. terebinthifolia*, reported an extraction pattern in which the monoterpenes were extracted firstly and more quickly, followed by a slower extraction of the sesquiterpenes. Taking into account that the authors reported a nearly complete extraction of the leaf EO after more than 6 h of process, that the EO yields in the present work for both plants were below the yields reported by the literature, and the differences between the EOs composition, mainly in relation to monoterpene and sesquiterpene contents, the extraction during 90 min have not extracted all of the EO components from the leaves of *S. molle* and *S. terebinthifolia*. Other factors that may have contributed (very probably in a lesser degree) for the differences between the EO compositions were the plant material being collected in different places, with different climates, soil type and nutritional state, and cultural management, since these factors can alter the EO composition and yield [31].

Results of the bioassay

The mortality rates for the individuals of *A. gemmatalis* treated with *S. molle* and *S. terebinthifolia* EOs after 24, 48, and 72 h are presented in the Tables 2, 3 and 4, respectively.

Table 2. Mortality percentages of *A. gemmatalis* after 24 h of exposure to the treatments and controls (CV = 21.49%).

Treatment	Mortality (%) in 24 h	
	S. molle	*S. terebinthifolia*
Water	0.0 Ad	0.0 Ae
Tween-80® 0.5 % v/v	0.0 Ad	0.0 Ae
Novaluron 0.075% v/v	38.0 Aa	38.0 Ab
0.1% v/v	0.0 Ad	0.0 Ae
0.5% v/v	6.0 Ad	0.0 Ae
1.0% v/v	18.0 Ac	8.0 Bd
1.5% v/v	20.0 Ac	18.0 Ac
2.0% v/v	30.0 Bb	52.0 Aa

Means followed by the same letter, uppercase in row (EO type) and lowercase in column (concentration) do not present statistical difference by Tukey's multiple range test at 5% probability (α = 0.05).

Relative to the EO kind, it can be seen in Table 2 that the EO performances differed only at the concentrations of 1.0 and 2.0% v/v in the first 24 h, in which *S. molle* was more effective at 1.0% v/v (18% mortality) and *S. terebinthifolia* was more effective at 2.0% v/v (52% mortality).

Both oils presented no activity at the concentration of 0.1% v/v (Table 2), which have not differed from the negative controls (water and Tween-80® 0.5% v/v). At 0.5% v/v, *S. molle* EO induced a slight mortality (6%), whereas *S. terebinthifolia* oil presented no effect (zero mortality). At the concentration of 1.0% v/v, the mortality induced by *S. terebinthifolia* oil was 8% in the first 24 h of evaluation, the *S. molle* EO performed better at this concentration, with a mortality rate of 18% (Table 2).

At higher concentrations (1.5 and 2.0% v/v) there was a change in the mortality rates. Both oils performed similarly at 1.5% v/v; at 2.0% v/v the EO of *S. terebinthifolia* induced a higher mortality (52%) than the one of *S. molle* (30% v/v), in a reverse trend than the other concentrations (Table 2).

Cole [32] reported that, for contact toxicity on *Aedes aegypti* larvae, *S. terebinthifolia* fruit EO (δ-3-carene as major compound) at the concentration of 200.4 µg/mL (0.024% v/v) induced nearly 100% mortality after 24 h. Silva and coauthors [33] observed, for *S. terebinthifolia* EO, a 100% larvicidal mortality (by contact) of *Stegomyia aegypti* after 24 h of exposure at 862.70 µg/mL (approx. 0.1% v/v). Chopa and coauthors [39] reported that *S. molle* leaf EO had no repellent activity on *Blatella germanica* L.

Table 3. Mortality percentages of *A. gemmatalis* after 48 h of exposure to the treatments and controls (CV = 17.25%).

Treatment	Mortality (%) in 48 h	
	S. molle	*S. terebinthifolia*
Water	0.0 Ac	0.0 Ad
Tween-80® 0.5 % v/v	0.0 Ac	0.0 Ad
Novaluron 0.075% v/v	78.0 Aa	78.0 Aa
0.1% v/v	0.0 Ac	0.0 Ad
0.5% v/v	6.0 Ac	0.0 Ad
1.0% v/v	26.0 Ab	14.0 Bc
1.5% v/v	26.0 Ab	18.0 Bc
2.0% v/v	30.0 Bb	52.0 Ab

Means followed by the same letter, uppercase in row (EO type) and lowercase in column (concentration) do not present statistical difference by Tukey's multiple range test at 5% probability (α = 0.05).

According to Table 3, after 48 h of exposure, there was little difference between the mortality rates regarding the ones in 24 h. There was no difference between the EO types for the concentrations of 0.1 and 0.5% v/v, which have not differed from the negative controls. For the remaining concentrations (1.0, 1.5, and 2.0% v/v), the oils presented different efficiencies; the oil of *S. molle* induced higher mortality rates at 1.0% v/v (26% for *S. molle* and 14% for *S. terebinthifolia*) and 1.5% v/v (26% for *S. molle* and 18% for *S. terebinthifolia*). However, at 2.0% v/v both oils presented the same mortality rate as 24 h, indicating that the oils at this concentration have not killed *A. gemmatalis* individuals in the time between 24 and 48 h. In the other hand, the chemical control (novaluron 0.075% v/v) induced a mortality rate of 78% after 48 h, against 38% in the first 24 h.

Relative to the EO concentrations, 1.0, 1.5, and 2.0% v/v have not differed for *S. molle* EO (Table 3). For *S. terebinthifolia* EO, the concentration of 2.0% v/v differed from 1.0 and 1.5% v/v (Table 3); in the first 24 h the 1.0 and 1.5% v/v differed between themselves (Table 2).

Silva and coauthors [33] reported that *S. terebinthifolia* EO induced a larval mortality of 100% of *Stegomyia aegypti* by contact after 48 h of exposure, at the concentration of 862.20 µL/mL (approx. 0.1% v/v). Abdel-Sattar and coauthors [34], who evaluated the insecticidal activity of *S. molle* EO, observed that the concentration of 1000 µL/10 mL (approx. 10.0% v/v) induced 50% mortality of *T. granarium* individuals after 48 h of exposure.

Table 4. Mortality percentages of *A. gemmatalis* after 72 h of exposure to the treatments and controls (CV = 13.80%).

Treatment	Mortality (%) in 72 h	
	S. molle	*S. terebinthifolia*
Water	0.0 Ad	0.0 Ae
Tween-80® 0.5 % v/v	0.0 Ad	0.0 Ae
Novaluron 0.075% v/v	100.0 Aa	100.0 Aa
0.1% v/v	0.0 Ad	0.0 Ae
0.5% v/v	8.0 Ac	0.0 Be
1.0% v/v	26.0 Ab	14.0 Bd
1.5% v/v	26.0 Ab	24.0 Ac
2.0% v/v	30.0 Bb	70.0 Ab

Means followed by the same letter, uppercase in row (EO type) and lowercase in column (concentration) do not present statistical difference by Tukey's multiple range test at 5% probability (α = 0.05).

Comparing the mortality rates in relation to EO type after 72 h of exposure, the concentrations of 0.5, 1.0, and 2.0% v/v differed between themselves, in which *S. molle* oil induced higher mortality rates at the lower concentrations (0.5 and 1.0% v/v), and *S. terebinthifolia* oil induced it at the highest concentration (2.0% v/v). It is noteworthy to observe that the mortality induced by concentration of 1.5% v/v has not differed between the EOs.

After 72 h, the chemical control (novaluron 0.075% v/v) induced 100% mortality of *A. gemmatalis* individuals, whereas the negative controls (water and Tween-80® 0.5% v/v) presented zero mortality. For *S. molle* EO, there was no difference between the negative controls and the concentration of 0.1% v/v; for *S. terebinthifolia* EO, the concentrations of 0.1 and 0.5% v/v have not differed from the water and Tween-80® treatments.

It is also important to observe that the EO of *S. terebinthifolia* induced 70% mortality after 72 h at the highest concentration (2.0% v/v), whereas the EO of *S. molle*, at the same concentration, has not induced further mortality after 24 h, with the mortality rate remaining as 30% during the entire test period (Table 4).

Ferrero and coauthors [36], evaluating the repellent activity of *S. molle* leaf extract, observed that the extract was effective on nymphs of *Triatoma infestans*. Silva and coauthors [33], who studied the insecticidal effect of *S. terebinthifolia* EO, reported 82% mortality of *S. aegypti* larvae after 72 h at the concentration of 775.68 µg/mL (approx. 0.09% v/v). According to Abdel-Sattar and coauthors [34], the leaf EO of *S. molle* showed insecticidal activity on *Tribolium castaneum*, causing 90% mortality on the sixth day (144 h).

The larvicidal, insecticidal, and repellent activities of *S. terebinthifolia* fruit EO were evidenced against the *Aedes aegypti* mosquito, with concentrations of 169.20 µg/mL (0.02% v/v), 50 µL (1.67 µL/cm² - $1.11 \cdot 10^{-4}$% v/v), and 2.39 wt.% (2.39% v/v) being the most effective, respectively, in each of the performed tests [32]. However, there is little data on the toxicity of *Schinus* spp. leaf EOs against insects.

In the present study, α-pinene (60.04 wt.%), limonene (11.28 wt.%), and β-pinene (9.24 wt.%) were the major compounds identified in *S. mole* EO. In other studies, the major compounds reported were p-cymene (69.39 wt.%), carvotanacetone (2.48 wt.%), and α-terpinene (2.24 wt.%). In the present work, the major compounds identified in the *S. terebinthifolia* oil were α-pinene (38.67 wt.%), β-pinene (12.34 wt.%), and limonene (8.48 wt.%). Santos and coauthors [35] studied the EO of *S. terebinthifolia*, identifying germacrene-D (25.0 wt.%), (E)-β-caryophyllene (17.5 wt.%), and δ-elemene (10.5 wt.%) as the leaf EO major compounds; the authors reported the EO caused 90% mortality of *Hypothenemus hampei*. The observed differences in the mortality rate for each EO might be due to the presence of minor compounds and even due to possible synergisms between EO compounds that enhance the biocidal effect of *S. terebinthifolia* EO on the larvae.

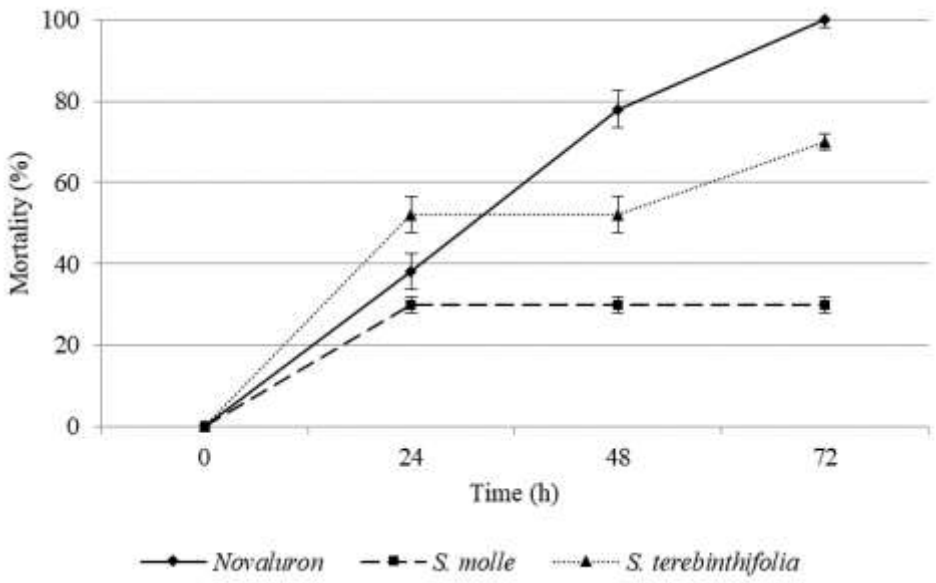

Figure 2. Evolution of *A. gemmatalis* mortality for the positive control (novaluron 0.075% v/v) and the most effective concentration (2.0% v/v) of the essential oils of *S. molle* and *S. terebinthifolia* during the 72 h of bioassay.

It is noteworthy that the mortality rate pattern in function of the exposure time for both EOs at 2.0% v/v when compared to the positive control (novaluron 0.075% v/v), as can be seen in Figure 2. The EO of *S. molle* induced lower mortality rates (30%) than the chemical insecticide (38%) in the first 24 h, unlike *S. terebinthifolia* EO, which induced 52% mortality. The oil of *S. molle* has not induced further increase of the mortality after 48 and 72 h; the oil of *S. terebinthifolia* also presented the same mortality rate in 24 and 48 h, whereas the chemical control presented a mortality rate of 78% after 48 h. After 72 h, the mortality rate induced by *S. terebinthifolia* EO was 72%, whereas the chemical control induced 100% mortality of *A. gemmatalis* individuals.

In general, EOs are composed by terpenoids, which, depending on the chemical composition (mono-, sesqui-, or diterpenes) tend to be more easily oxidized or hydrolyzed. These reactions may reduce the efficiency of the EOs in the long-term [37]. Other factor that is important for the efficiency of EOs to control pests is the synergistic effect that may exist among the EO components. Omolo and coauthors [38] evaluated the repellent activity of some EOs and synthetic mixtures formulated with the major compounds, observing that the mixtures presented lower activity than the EO. This indicated that the minor compounds very probably also present repellent activity, or enhance the repellent activity of the major compounds.

The higher efficiency of *S. terebinthifolia* EO may be result of the higher sesquiterpene content; these compounds have lower volatility and promote a more lasting contact effect on the insects than the oil of *S. molle*, whose large monoterpene fraction is highly volatile and may persist for smaller periods, promoting lower contact toxicity and less exposure time to the insects.

Regarding the LC_{50} of the oils after 72 h, for *S. terebinthifolia* EO the LC_{50} was approximately 1.74% v/v (1.58-1.97% v/v, with a confidence interval of 95%). Taking into account that the maximum mortality rate for *S. molle* EO was 30% at 2.0% v/v, the LC_{50} for this oil could not be determined. This also shows that *S. terebinthifolia* EO has had a superior insecticidal activity than *S. molle* EO, probably due to the greater amounts of sesquiterpenes (27.77 wt.% in *S. terebinthifolia* oil; 8.84 wt.% in *S. molle* oil), which have lower volatility and also present toxic activity on insects.

Other authors have demonstrated that the EO of *S. molle* was effective at controlling *Sitophilus zeamais*, presenting an insecticidal effect during the evaluation of contact toxicity. The LC_{50} observed was 0.25 µL/cm² (2.5 mL/100 dm²) and the LC_{99} was 1.92 µL/cm² (19.2 mL/100 dm²) [39]. Chopa and coauthors [40] studied the effect of the EO of *S. molle* on *Blatella germanica* L. and reported that *S. molle* leaf EO presented no repellent effect on this insect.

For *S. terebinthifolia* EO, Silva and coauthors [33] reported a LD_{50} of 862.20 µg/mL (approx. 0.1% v/v) for *Stegomyia aegypti*. Cole [32] reported, for *S. terebinthifolia* fruit EO, a LD_{50} of 117.34 µg/mL (approx. 0.014% v/v) for *Aedes aegypti*.

According to the IPM method, a pest population control of approximately 50% is desirable to promote environmental equilibrium and avoid major changes in the biologic relations among species [9]. Therefore, *S. terebinthifolia* EO presented an adequate efficiency (52% with 2.0% v/v in 24-48 h and 70% after 72 h); whereas *S. molle* EO was relatively ineffective (30% mortality with 2.0% v/v in 24-72 h). However, it is important to point out that this is an *in vitro* screening, field tests must be carried out to verify the EO insecticidal activity in adverse (non-ideal) conditions.

The low yield of both oils may be a hindrance for an industrial use of these EOs, since the energy cost to obtain these materials are likely to make them economically unfeasible. Bioprospecting studies aiming to find specimens with higher EO yields may be necessary; there is also the issue of genetic variability, which may render several distinct chemotypes among the populations [11]. A possibility is the test and use of the major compound (α-pinene), which is a terpene widely distributed in nature [41]. Nevertheless, synergistic effects between α-pinene and the minor compounds must be evaluated for a proper EO replacement.

Another issue is the potential allergenic and sensitizing effect of some terpenes, which may induce dermatitis and irritation of mucous membranes [9,42]. Considering that the terpenes are volatile and also biodegradable in variable periods, the main issue is relative to EO application; maybe a grace period prior to the manipulation of EO treated materials may be established, as already occurs to synthetic pesticides.

The results obtained suggest the need for further bioguided studies on the efficiency of the larvicidal properties of EOs in order to infer which compounds present insecticidal activity. Additional studies investigating the mechanisms of action, phytotoxicity, and safety to mammals and other vertebrates are also required.

CONCLUSION

The EO of *S. terebinthifolia* was effective (52% mortality at an EO concentration of 2.0% v/v after 24-48 h, and 70% mortality after 72 h) in the control of *A. gemmatalis*, whereas *S. molle* EO presented a lower mortality (30% after 24-72 h at 2.0% v/v). According to the Probit method, the LC_{50} for *S. terebinthifolia* EO was 1.74% v/v (1.58-1.97% v/v for 95% CI); it was not possible to establish a LC_{50} for *S. molle* EO due to the low mortality induced. This demonstrates the potential of *S. terebinthifolia* EO as an alternative to the use of chemical insecticides, whether used alone or associated with other substances, especially when applied using the guidelines provided by the IPM method.

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