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# Insecticidal Activity of *Lavandula dentata* L. Essential Oil on *Anticarsia gemmatalis* (Hübner, 1818)

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## HIGHLIGHTS

- The insecticidal activity of *L. dentata* EO was tested on *A. gemmatalis*.
- The calculated LC<sub>50</sub> of *L. dentata* EO was 0.197% v/v for *A. gemmatalis*.
- The EO was applied on soybean leaves to verify its deterrent effect on the insect.
- EO at 0.4% v/v has already had a deterrent effect on the caterpillars.

**Abstract:** *Anticarsia gemmatalis*, commonly known as soybean caterpillar, causes important economic losses in soybean crops. Synthetic pesticides are the standard practice to control this insect. However, the indiscriminate use of these substances has increased the resistance of this pest. Thus, it is necessary to search for different control alternatives that are also more environmentally friendly. The objective of this work was to evaluate the chemical composition of *Lavandula dentata* L. essential oil (EO) and its activity on *A. gemmatalis*. The major compounds of essential oil were 1,8-cineole (31.5 wt.%), camphor (16.6 wt.%), and fenchone (15.9 wt.%). Bioassays were performed with third-instar caterpillars. EO concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0% v/v were diluted in Tween-80® 0.5% v/v and incorporated into the artificial diet given to caterpillars. Water, Tween-80® 0.5% v/v, and novaluron 0.075% w/v were added as negative and positive controls. For the aversion tests, soybean leaf discs were immersed in solutions with

0.4, 0.6, 0.8, and 1.0% v/v of EO, plus a negative control (water), and offered to caterpillars. In the bioassay, 100% mortality was observed from the concentration 0.6% v/v of the essential oil of *L. dentata* in 24 h; without statistical difference from 0.4 and 0.5% v/v. There was no important change in mortality between 24 and 72 h. In the aversion test, all EO treatments caused deterrence of caterpillars when compared to control, but without difference between EO concentrations. It was observed that *L. dentata* EO can be used as an alternative in the control of *A. gemmatalis*.

**Keywords:** alternative control; botanical insecticides; terpenes; soybean caterpillar; Integrated Pest Management.

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## INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) cultivation is one of the major crops worldwide, with a large share of global agribusiness. It is mainly used as a feedstock in the production of vegetable oil for human consumption and the production of biodiesel; the remaining bran is used mostly as a feedstock in animal feed [1-2]. Brazil is the world's largest soybean exporter, being this culture one of the cornerstones of Brazilian agriculture, with several expansion possibilities [3].

The worldwide soybean production in 2019 was 333.67 million tonnes in a cultivated area of 120.50 million hectares, with an average yield of 2.77 t·ha<sup>-1</sup> [4]. The Brazilian production, in 2019, corresponded to approximately one-third of the worldwide production. According to the Brazilian Institute of Geography and Statistics (IBGE), the 2018/2019 harvest has had gross production of 112.61 million tonnes of soybean in a cultivated area of 35.65 million hectares, with an average yield of 3.16 t·ha<sup>-1</sup> [5].

Several pest insects affect the overall yield of soybean crops, causing important economic damage. Among these insects, the velvet caterpillar (*Anticarsia gemmatalis* - Lepidoptera: Erebidae) highlights itself as one of the major soybean pests. This insect is found in tropical and subtropical regions, being restricted to the Americas, and having greater incidence in soybean from the vegetative growth of the plants until the end of flowering [6]. In Brazil, *A. gemmatalis* is considered a key pest of soybean, but this species can also attack other vegetables. Due to the wide diversity of the Brazilian flora, this insect may survive in different host species that are available throughout the year, serving as a food source to this pest in the intercrop period [7-8].

*Anticarsia gemmatalis*, in its growth, passes through six larval instars [7,9]. In the first three instars, its food requirement and consumption are low; the greatest economic impact and damage to the plants occur from the fourth to the sixth instars, in which each individual can consume from 100 to 120 cm<sup>2</sup> of leaf area. At high infestation loads (high population density), this pest limits the productive capacity of soybean, being capable of causing complete defoliation of the plants, rendering huge loss of productivity and severe economic damage [10].

The high productivity and expansion of the cultivated area of soybean directly impact the increasing use of synthetic pesticides. Nowadays, chemical pesticides are losing their effectiveness due to excessive and disorganized use. There are also concerns on the environment and human and animal health caused by exposure to such substances, regardless of the exposure levels. Moreover, there is also the issue of induction of resistance in the pests and the resurgence of secondary pests after the use of synthetic pesticides [11-12].

In order to reduce the economic losses and the expansion of sustainable crops, reducing the effect of synthetic pesticides, there is an increasing interest in the development of new control strategies based on the Integrated Pest Management (IPM) [13-14]. One of the alternative methods that may be used in the control of pest insects is the use of essential oils (EO). In IPM, botanical insecticides are considered a highly effective tactic, enhancing food security, and reducing environmental issues caused by the excessive use of synthetic pesticides [15-16].

The bioactivity of EOs and plant extracts comes from the presence of secondary metabolites called terpenes, which may have antimicrobial, antioxidant, and insecticidal activity, among others. These compounds act as a chemical defense of the plant against several kinds of aggression and stresses, both biotic and abiotic [17]. In addition, EO components may also inhibit oviposition and hinder insect growth, reducing the food uptake by deterrent effect, and increase the mortality percentages of immature and adult individuals due to both toxic and stress-inducing effects [18].

*Lavandula dentata* L., commonly known as 'French lavender', is a perennial, sub-bush with a branching pattern and strong aroma. This species is natural from the Mediterranean region, generally growing in mountainous regions and open savannas, in tropical and subtropical climates. The flowers have a

characteristic aspect and occur at the branch peaks, contrasting with the lignified stems and the light green leaves. The aromatic and medicinal properties of this species are considered as related to the presence of monoterpenes, both hydrocarbon and oxygenated. It is believed that these terpenes assign to *L. dentata* its antispasmodic, antimicrobial, and antioxidant activities [19-20].

The EO of lavender (this includes the *L. dentata* species) is mostly used in the perfumery, being Bulgaria the largest world producer, with a production of 80 t in 2013, followed by France, with 20-30 t. Despite the sparse data, reports indicated that, at least until 2017, Bulgaria remained as the largest world exporter of lavender EO; however, the access to consolidated data relative to gross production and the markets is limited [21-22]. According to Mambri and coauthors [23], the EO of this species is mainly composed of monoterpenes, such as 1,8-cineole, camphor, borneol, fenchol,  $\alpha$ -pinene,  $\beta$ -pinene, trans-pinocarveol, and linalool, among others.

Thus, the objective of this work was to evaluate the insecticidal activity of the leaf EO of *Lavandula dentata* on *Anticarsia gemmatalis* when added to an artificial diet and evaluate the deterrent effect of the EO on this caterpillar when applied on leaves of soybean.

## MATERIAL AND METHODS

### Caterpillar source and breeding

The *A. gemmatalis* caterpillars used in the present study were bred in the Pest Management Laboratory of the University of Caxias do Sul, being grown with an artificial diet [24] under controlled environmental conditions (temperature of  $26\pm 1$  °C, relative humidity of  $75\pm 1\%$ , and photoperiod of 14 h light and 10 h of dark). Third-instar caterpillars (length of 4-6 mm) were used in all experiments. Caterpillar length was measured using a digital pachymeter (TMX, Brazil) with a resolution of 0.01 mm and a measuring range of 0-150 mm.

### Collection of plant material and essential oil isolation

Leaves of *L. dentata* (moisture content of 77 wt.%) were collected at the Experimental Area and Farm School of the University of Caxias do Sul, located in the municipality of Caxias do Sul, South Brazil, at the geographical coordinates of 29°08'27"S; 50°59'28"W and an altitude of 770 m above sea level. The collected material was dried in a kiln with forced air circulation at  $40\pm 5$  °C for 72 h.

The EO was extracted by steam distillation, following the procedures described by Koketsu and Gonçalves [25]. The extraction was carried out for 2 h; the collected EO was stored in an amber glass bottle and kept in a cold chamber ( $4\pm 2$  °C) until the use in the bioassays. A sample of the collected EO (about 0.5 mL) was sent for chromatographic analysis.

### Chromatographic analysis of the essential oil

The qualitative analyses of the EO were carried out using a HP 6890 gas chromatograph, coupled to a HP MSD5973 mass spectrometer, equipped with the HP Chemstation software and the Wiley 275 spectra library. A HP-Innowax (Hewlett Packard, Palo Alto, USA) fused silica column (30 m x 250  $\mu$ m) with 0.50  $\mu$ m of film thickness was used. Starting oven temperature of 40 °C (8 min), increasing to 180 °C at 3 °C $\cdot$ min<sup>-1</sup>, from 180 to 230 °C at 20 °C $\cdot$ min<sup>-1</sup>, ending at 230 °C (20 min). Interface temperature of 280 °C; split ratio of 1:100; helium (He) used as carrier gas at 56 kPa and flow rate of 1.0 mL $\cdot$ min<sup>-1</sup>; ionization energy of 70 eV; injected sample volume of 1.0  $\mu$ L diluted in hexane in a 1:10 proportion.

The quantitative analyses of the EO were carried out using a HP 6890 gas chromatograph with a flame ionization detector (FID), equipped with the HP Chemstation software. A HP-Innowax fused silica column (30 m x 250  $\mu$ m) with 0.50  $\mu$ m of film thickness was used. The temperature program used was the same as GC/MS analysis. Injector temperature of 250 °C; split ratio of 1:50; FID temperature of 250 °C; hydrogen (H<sub>2</sub>) used as carrier gas at 34 kPa and flow rate of 1.0 mL $\cdot$ min<sup>-1</sup>; injected sample volume of 1.0  $\mu$ L diluted in hexane in a 1:10 proportion.

The EO components were identified by comparison of the obtained mass spectra with the ones of the Wiley 275 library (GC/MS equipment) and by comparison of the calculated linear retention indexes (LRI) with the ones reported by the literature (NIST). The LRI values were calculated using the Van den Dool and Kratz equation and the retention times of a standard solution of alkanes C8 to C26.

## Bioassays using artificial diet

The EO of *L. dentata* was dissolved homogeneously in the artificial diet, being emulsified with Tween-80® (0.5% v/v). The EO concentrations tested were 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0% v/v. Two negative controls (distilled water and Tween-80® 0.5% v/v) and a positive control (novaluron 0.075% m/v) were also added to the experiment, being also emulsified and incorporated to the artificial diet.

Third instar caterpillars were used in the experiments. Each caterpillar was put in a 100 mL plastic cup with 1 g of the artificial diet containing the treatment and a moistened cotton pad to keep the humidity inside the cups. The mortality of individuals was evaluated after 24, 48, and 72 h.

## Aversion test

The aversion test was carried out to verify if *L. dentata* EO has a deterrent (aversive) effect on the caterpillars. The aversion test was carried out using soybean (*Glycine max* L. Merrill) plants cultivated in 300 mL plastic cups filled with Carolina Soil® substrate, grown for 30 days under controlled conditions (temperature of 26±1 °C, relative humidity of 75±1%, and photoperiod of 14 h of light and 10 h of dark). Soybean leaves were collected, washed with tap water, and cut as disks with a diameter of 1.5 cm. The disks were individually weighted using a Marte AL500C semi-analytical balance with a measuring capacity of 500 g and resolution of 0.001 g.

Five *L. dentata* EO concentrations were tested: 0.4, 0.6, 0.8, and 1.0% v/v, plus a negative control (water). The EO treatments were emulsified using Tween-80® (0.5% v/v). The weighted disks were immersed in the respective treatments for 10 s and left to dry at room temperature (26±1 °C) and away from sunlight. After drying, the disks were transferred to Petri dishes with a diameter of 13 cm with a moistened paper filter.

Third instar caterpillars kept on fasting for 2 h were used; each Petri dish was occupied by only one individual. The caterpillars were weighted using a semi-analytical balance. The evaluation was carried out after 48 h. The following parameters were evaluated: mortality percentage relative to the ingestion of EO-treated leaves and the relative intake rate (RIR). The measurement of the amount of ingested material was carried out using the formula described by Candy and Baker [26]:

$$RIR = \frac{I}{B \times T}$$

Being 'RIR' the relative intake rate (g·g<sup>-1</sup>·day<sup>-1</sup>), 'T' the time of the feeding period (day), 'I' the amount of food eaten (g) during the time 'T', and 'B' the mass increase of the caterpillars (g) during the time 'T'.

## Experimental design and statistical analysis

EO extraction was carried out in triplicates. The EO samples were also analyzed in triplicates. The bioassays were carried out following a completely randomized design. For all tests, 50 caterpillars were used per treatment, being grouped in five replicates of 10 individuals each replicate. The data underwent Levene's test ( $\alpha = 0.05$ ) and the Shapiro-Wilk test ( $\alpha = 0.05$ ) to verify the homogeneity of the variances and the normality of the residuals, respectively. Mortality data underwent analysis of variance (ANOVA), and the means were compared by Tukey's multiple range test at 5% probability ( $\alpha = 0.05$ ). The Probit method [27] was used to determine the median lethal concentration (LC<sub>50</sub>) and the 99% lethal concentration (LC<sub>99</sub>) with a confidence interval (CI) of 95%. The data on the daily relative intake underwent the Kruskal-Wallis U-test at 5% probability ( $\alpha = 0.05$ ).

## RESULTS AND DISCUSSION

### Essential oil composition and yield

The EO yield obtained in the present work was 0.55% v/w. Dris and coauthors [28] reported an EO yield of 1.18% v/w for the dried aerial part of *L. dentata* plants cultivated in Northeast Algeria. Martins and coauthors [19] reported EO yields of 0.40 and 0.44% v/w for fresh plant and fresh inflorescences, respectively, of *L. dentata* plants cultivated in the Brazilian municipality of Uberaba (MG), Southeast Brazil.

It is important to observe that EO yield is influenced by several factors, especially regarding the environmental factors, such as the presence of stressing effects caused by biotic and abiotic factors (drought or excess rainfall, excessive insolation, attack of herbivores or pests, fires, among others); however, the genetics also play an important role regarding EO production and its yield [17,20,29].

Relative to the chemical composition of the EO, 1,8-cineole was the major compound (31.5 wt.%), followed by camphor (16.6 wt.%) and fenchone (15.9 wt.%). The detailed composition of the *L. dentata* EO is presented in Table 1.

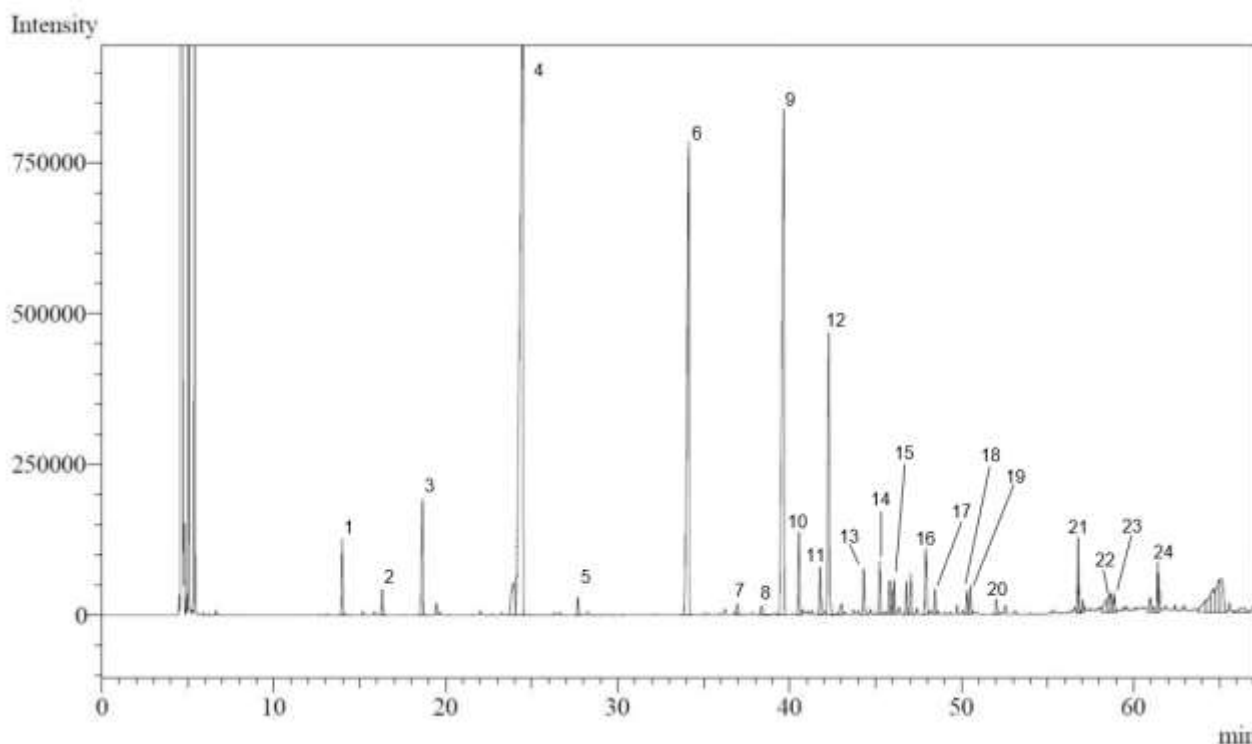
**Table 1.** Chemical composition of the leaf essential oil of *Lavandula dentata*, extracted by steam distillation for 2 h.

Component	Calc. LRI <sup>1</sup>	Lit. LRI <sup>2</sup>	Content (wt.%)
α-pinene	1018	1022	0.33±0.15
camphene	1062	1065	0.49±0.07
β-pinene	1106	1108	3.32±0.68
1,8-cineole	1213	1210	31.52±1.27
p-cymene	1275	1270	0.32±0.09
fenchone	1407	1410	15.93±0.75
cis-β-terpineol	1439	1437	0.24±0.03
α-camphonal	1501	1504	0.29±0.12
camphor	1521	1515	16.63±2.11
linalool	1552	1551	1.54±0.35
pinocarvone	1581	1580	0.92±0.12
fenchol	1593	1597	6.46±0.77
terpinen-4-ol	1607	1600	0.36±0.06
myrtenal	1643	1639	0.97±0.15
trans-pinocarveol	1666	1664	2.59±0.66
α-terpineol	1712	1710	0.77±0.28
β-selinene	1732	1729	3.67±1.15
β-bisabolene	1728	1720	0.51±0.08
cuminal	1798	1794	0.49±0.11
myrtenol	1803	1807	0.58±0.25
cis-carveol	1844	1847	0.38±0.03
caryophyllene oxide	2004	2008	2.04±0.61
perillool	2018	2221	0.24±0.11
α-bisabolol	2222	2215	0.39±0.07
β-eudesmol	2247	2249	0.74±0.08
	Monoterpenes		4.46
	Oxygenated monoterpenes		79.91
	Sesquiterpenes		4.18
	Oxygenated sesquiterpenes		3.17
	Not identified		8.28

<sup>1</sup> - Calc. LRI: calculated linear retention index; <sup>2</sup> - Lit. IRL: linear retention index reported by the literature (NIST) [30].

According to Table 1, the chemotype of the obtained oil was of the 1,8-cineole type. Mambri and coauthors [23], Martins and coauthors [19], and Justus and coauthors [31] reported the same chemotype for the EO of *L. dentata* plants cultivated on a substrate in the municipality of Santa Maria (South Brazil), from plants collected in the region of Uberaba (Southeast Brazil), and from plants collected in the region of Ponta Grossa (South Brazil), respectively. However, Maasada and coauthors [32] reported a linalool chemotype (linalool content of 47.3 wt.%) for *L. dentata* plants cultivated in Northeastern Tunisia.

The GC-FID chromatogram of the EO of *L. dentata* is presented in Figure 1.



**Figure 1.** Chromatogram (GC-FID) of the essential oil of *Lavandula dentata*, obtained by steam distillation for 2 h. 1 –  $\alpha$ -pinene; 2 – camphene; 3 –  $\beta$ -pinene; 4 – 1,8-cineole; 5 – p-cymene; 6 – fenchone; 7 – *cis*- $\beta$ -terpineol; 8 –  $\alpha$ -camphonal; 9 – camphor; 10 – linalool; 11 – pinocarvone; 12 – fenchol; 13 – terpinen-4-ol; 14 – *trans*-pinocarveol; 15 – myrtenal; 16 –  $\beta$ -selinene; 17 –  $\beta$ -bisabolene; 18 – cuminal; 19 – myrtenol; 20 – *cis*-carveol; 21 – caryophyllene oxide; 22 – perillol; 23 –  $\alpha$ -bisabolol; 24 –  $\beta$ -eudesmol.

Relative to the chemical classes, most of the EO was composed of oxygenated terpenes (79.9 wt.%), with similar amounts of mono- and sesquiterpenes (4.5 and 4.2 wt.%, respectively), and smaller content of oxygenated sesquiterpenes (3.17 wt.%). Justus and coauthors [31] reported a total monoterpene (including the oxygenated ones) content of 92.7 wt.% for *L. dentata* EO from plants cultivated in the municipality of Ponta Grossa (South Brazil); total sesquiterpenes accounted for 1.7 wt.% of the EO composition.

However, both the chemotype and the content of the minor compounds of the EO are mainly affected by plant genetics, in which different populations may express different biochemical pathways, favoring the production of a specific terpene to the expense of others [33]. Moreover, the overall chemical composition and yield of the EO are also influenced by the stage of growth of the plant, time of collection, and the process used to extract the EO [34-35].

### Bioassay with artificial diet

The results of mortality percentages of *A. gemmatilis* individuals fed with the artificial diet treated with increasing concentrations of *L. dentata* EO are presented in Table 2.

**Table 2.** Mortality percentage of *Anticarsia gemmatalis* caterpillars fed with artificial diet containing *Lavandula dentata* essential oil at different concentrations.

Treatment	Mortality (%)		
	24 h	48 h	72 h
Water	0.0±0.00 <sup>d</sup>	0.0±0.00 <sup>d</sup>	0.0±0.00 <sup>d</sup>
Tween-80 <sup>®</sup> 0.5% v/v	0.0±0.00 <sup>d</sup>	0.0±0.00 <sup>d</sup>	0.0±0.00 <sup>d</sup>
novaluron 0.075% w/v	0.0±0.00 <sup>d</sup>	93.2±0.82 <sup>a</sup>	100.0±0.00 <sup>a</sup>
0.1% v/v	23.2±0.52 <sup>c</sup>	26.6±0.52 <sup>c</sup>	26.6±0.52 <sup>c</sup>
0.2% v/v	43.2±0.52 <sup>b</sup>	46.6±0.52 <sup>b</sup>	46.6±0.52 <sup>b</sup>
0.3% v/v	56.6±0.41 <sup>b</sup>	56.6±0.41 <sup>b</sup>	56.6±0.41 <sup>b</sup>
0.4% v/v	93.2±0.82 <sup>a</sup>	93.2±0.82 <sup>a</sup>	93.2±0.82 <sup>a</sup>
0.5% v/v	93.2±0.82 <sup>a</sup>	93.2±0.82 <sup>a</sup>	93.2±0.82 <sup>a</sup>
0.6% v/v	100.0±0.00 <sup>a</sup>	100.0±0.00 <sup>a</sup>	100.0±0.00 <sup>a</sup>
0.7% v/v	100.0±0.00 <sup>a</sup>	100.0±0.00 <sup>a</sup>	100.0±0.00 <sup>a</sup>
0.8% v/v	100.0±0.00 <sup>a</sup>	100.0±0.00 <sup>a</sup>	100.0±0.00 <sup>a</sup>
0.9% v/v	100.0±0.00 <sup>a</sup>	100.0±0.00 <sup>a</sup>	100.0±0.00 <sup>a</sup>
1.0% v/v	100.0±0.00 <sup>a</sup>	100.0±0.00 <sup>a</sup>	100.0±0.00 <sup>a</sup>

\*Means followed by the same letter in column do not differ statistically by Tukey's multiple range test at 5% probability ( $\alpha = 0.05$ ).

According to the results in Table 2, in the first 24 h, 100% mortality occurred from EO concentration of 0.6% v/v in the artificial diet. Moreover, the mortality percentages of 0.4 and 0.5% v/v (both 93.3%) have not differed from the mortalities of 0.6% v/v and above. At the same time, the chemical insecticide (novaluron 0.075% w/v) presented zero mortality, indicating a slower action relative to *L. dentata* EO.

It is also noteworthy that there was no important change in the mortality percentages after 24 h. There was a slight increase only in the mortality for the treatment with 0.2% v/v (from 43.2% at 24 h to 46.6% at 48 h), but without significant difference. After 72 h, it was possible to see that EO concentrations of 0.4% v/v and above had statistically the same performance as the chemical control (novaluron).

According with the Probit method, the calculated  $LC_{50}$  of *L. dentata* EO on *A. gemmatalis* was 0.197% v/v (0.165-0.227% v/v for a CI of 95%); the calculated  $LC_{99}$  was 0.795% v/v (0.640-1.087% v/v for a CI of 95%).

There are reports of the insecticidal activity of *L. dentata* EO on some insect species; however, no specific data on *A. gemmatalis* was found. Cossetin and coauthors [36] studied the toxic effect of lavender EO on adult individuals of *Musca domestica* L. (Diptera: Muscidae) and *Chrysomya albiceps* Wiedemann (Diptera: Calliphoridae). The major compounds of the EO were 1,8-cineole, camphor, and linalool oxide. The authors reported that the EO was toxic to these insects, also commenting on a possible use as an option for the control of this species.

Tests with the EO of other species from the *Lavanda* genus are also reported. Delgado [37] tested the insecticidal effect of *L. luisieri* (major compound 1,8-cineole) on larvae of *Spodoptera littoralis* (Lepidoptera: Noctuidae) and adult individuals of *Myzus persicae* and *Rhopalosiphum padi* (Hemiptera: Aphididae). It was reported a strong insecticidal activity of this EO on all species tested.

The insecticidal effect of *L. angustifolia* EO on third-instar larvae of *Lucilia sericata* was tested by immersion (contact). According to the results, the insecticidal effect became stronger with the increase in EO concentration; total (100%) mortality was reached at an EO concentration of 32% v/v [38]. Khosravi and coauthors [39] evaluated the toxic effect of *L. angustifolia* EO on *Xanthogaleruca luteola* pupae by ingestion. The authors reported a  $LC_{50}$  of 0.63% v/v (0.46–0.89% v/v) and a mortality percentage of 62.5% at the concentration of 0.8% v/v. It was also observed that the pupal period was increased by 1.5 days relative to the control. It was also commented that the EO had both a toxic and a disruptive effect on this species.

### Aversion test

The results of the aversion tests with *A. gemmatalis* caterpillars are presented in Table 3.

**Table 3.** Relative intake rate (RIR) and mortality percentage of *Anticarsia gemmatalis* caterpillars fed with soybean leaves treated with different concentrations of the essential oil of *Lavandula dentata*.

Treatment	RIR <sup>1</sup> (g·g <sup>-1</sup> ·day <sup>-1</sup> )	Mortality <sup>2</sup> (%)
Water	6.7±3.87 <sup>b</sup>	20.0±0.05 <sup>d</sup>
0.4% v/v	-60.5±31.63 <sup>a</sup>	46.6±0.55 <sup>c</sup>
0.6% v/v	-22.3±14.87 <sup>a</sup>	53.2±0.57 <sup>b</sup>
0.8% v/v	-36.1±38.20 <sup>a</sup>	73.2±0.62 <sup>a</sup>
1.0% v/v	-186.8±33.23 <sup>a</sup>	73.2±0.48 <sup>a</sup>

<sup>1</sup> – Means in column followed by the same letter do not differ statistically by Kruskal-Wallis U-test at 5% probability ( $\alpha = 0.05$ ); <sup>2</sup> - Means in column followed by the same letter do not differ statistically by Tukey's multiple range test at 5% probability ( $\alpha = 0.05$ ).

Relative to RIR values (leaf consumption), the leaves treated with *L. dentata* EO caused deterrence to the caterpillars when compared to the control leaves (water-only), showing a higher preference of the individuals for the EO-free leaves. However, there was no statistical difference between the RIR values for the EO treatments, indicating that an important deterrent effect may occur at concentrations as lower as 0.4% v/v.

Considering the mortality percentages, the treatments with 0.8 and 1.0% EO have not differed statistically, both with 73.2% mortality. The other two percentages (0.4 and 0.6%) differed between themselves, and between the control and the higher concentrations (Table 3). However, different from the tests with artificial diet, in which the mortality percentages were higher, the lower mortality in the aversion test may be a result of the deterrent effect, hindering leaf tissue consumption and, consequently, the amounts of EO ingested by the caterpillars.

Badredinne and coauthors [40] investigated the insecticidal effect of *Lavandula stoechas* EO on *Orgyia trigotephras* at the concentrations of 0.05, 0.10, and 0.50% v/v. The EO was composed mainly of camphor (36.1 wt.%), 1,8-cineole (25.2 wt.%), and camphene (11.4 wt.%). The results showed that the EO had a strong toxic effect on fourth-instar nymphs of *O. trigotephras* by both ingestion, contact, and inhalation (fumigation), acting in all tested concentrations. However, the toxic effect became stronger with the increase in EO concentration.

Prates and coauthors [41], studying the insecticidal activity of the monoterpenes 1,8-cineole and limonene on *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (H.), related that the insecticidal effect of these substances probably occurs through absorption by the respiratory system (inhalation, fumigation), by the cuticle (contact), and by ingestion (absorption by the digestive system).

The insecticidal effect of *L. dentata* EO may be attributed to the occurrence of monoterpenes, both hydrocarbon and oxygenated ones. According to Regnault-Roger [42], these substances may cause toxic interferences in the biochemical and physiological processes of animals. Also related to other conditions, these toxins may be absorbed through inhalation, direct contact, or by ingestion, acting on several parts of the insect body, such as the nervous system.

## CONCLUSION

According to the observed results, the EO of *L. dentata* had an insecticidal activity on *A. gemmatalis*, both in an artificial diet and in a natural diet (soybean leaves). The EO caused 100% mortality at the concentration of 0.6% v/v and higher, but not differing statistically from the concentration of 0.4 and 0.5% v/v. It was also observed a deterrent effect of this EO on third-instar *A. gemmatalis* caterpillars, with a significant reduction of the RIR relative to the control group. In this sense, the EO of *L. dentata* may be a possible alternative in the management of *A. gemmatalis*, especially when considering the IPM practices and the reduction of the use of synthetic pesticides.

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