



Article Exploring the Efficacy of Four Essential Oils as Potential Insecticides against *Thrips flavus*

Yulong Niu ^{1,†}, Tianhao Pei ^{1,†}, Yijin Zhao ^{1,2,†}, Changjun Zhou ³, Bing Liu ³, Shusen Shi ¹, Meng-Lei Xu ^{4,*}

- Key Laboratory of Soybean Disease and Pest Control (Ministry of Agriculture and Rural Affairs), College of Plant Protection, Jilin Agricultural University, Changchun 130118, China
- ² Dalian City Investment Asset Management Co., Ltd., Dalian 116021, China
- ³ Daqing Branch, Heilongjiang Academy of Agricultrual Science, Daqing 163316, China
- ⁴ State Key Laboratory of Supramolecular Structure and Materials, College of Food Science and Engineering, Jilin University, Changchun 130062, China
- * Correspondence: xumenglei@jlu.edu.cn (M.-L.X.); gaothrips@jlau.edu.cn (Y.G.)
- ⁺ These authors contributed equally to this work.

Abstract: Plant essential oils are important alternatives in green integrated pest management. This study examined the chemical composition, bioactivity, and control efficacy of four Lamiaceae essential oils (EOs) against Thrips flavus Schrank in laboratory conditions with the goal of exploiting plantderived insecticides to control Thrips flavus. The four EOs tested were marjoram oil (Origanum majorana L.), clary sage oil (Salvia sclarea L.), perilla leaf oil (Perilla frutescens (L.) Britt.), and spearmint oil (Mentha spicata L.). All these EOs exhibited a certain degree of insecticidal activity against Thrips *flavus.* The median lethal concentration (LC_{50}) was determined after treatment by the leaf-dipping method in laboratory bioassays, and its values were 0.41 mg/mL for marjoram oil, 0.42 mg/mL for clary sage oil, 0.43 mg/mL for perilla leaf oil, and 0.54 mg/mL for spearmint oil. In the pot experiment, the number of dead insects was recorded at 1, 3, and 7 days post-application, and the control efficacy of EOs against Thrips flavus was calculated. The concentration of 900.00 g a.i. hm⁻² of spearmint oil was 100% lethal against Thrips flavus after treating potted plants for seven days. The Y-tube olfactometer method was used to test for the attraction or repellent response of EOs against Thrips flavus. The spearmint oil significantly attracted female adults in the olfactory test. Furthermore, gas chromatography-mass spectrometry (GC-MS) was used to examine the chemical composition of the EOs. Linalool (24.52%), isopropyl myristate (28.74%), (+)-limonene (32.44%), and (+)-carvone (70.3%) were their primary ingredients. The findings suggest that all four EOs are highly effective against Thrips flavus and may be a possible alternative in the management of Thrips flavus, especially when considering reducing the use of synthetic pesticides.

Keywords: Thrips flavus; Lamiaceae; essential oil; plant-derived insecticides; insecticidal toxicity

1. Introduction

Thrips flavus Schrank (Thysanoptera, Thripidae) is an important pest affecting cash crops, a member of the Thripidae (Thysanoptera), which is found worldwide and can seriously damage crops at various stages of growth [1]. *Thrips flavus* is regarded as a major pest of flowering plants of the Asteraceae (Compositae) and Fabaceae (Leguminosae) families, among others, in Northern China. This pest typically causes premature senescence or deformation of flowers and curling, distortion, and wilting of leaves [2,3]. Various ecological factors have been shown to affect *Thrips flavus* survival and development. Recent research indicates that *Cucumis sativus* L. and *Glycine max* (L.) Merr. were two potentially suitable host plants for *Thrips flavus* [1]. The ambient CO₂ concentrations can accelerate the development of thrips but reduce their survival rate [4]. The survival of thrips gradually



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Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). decreased with increasing temperature from 19 °C to 31 °C [5]. Currently, chemical insecticides are predominantly used to control *Thrips flavus*, which shows greater sensitivity to imidacloprid, avermectin, and lambda-cyhalothrin emulsifiable concentrate compared to *Frankliniella occidentalis* (Pergande) (Thripidae) in the Yunnan region [6]. However, concerns over the increasing resistance of Thripidae to chemical insecticides and their environmental and ecological impacts have led to a search for safer, environmentally friendly alternatives [7,8]. Essential oils (EOs), secondary plant metabolites, offer such an alternative. These substances possess a range of bioactivities, including oviposition inhibition, avoidance, egg hatching inhibition, larval development suppression, antifeedant and repellent effects, as well as the capacity to knock down, poison, and fumigate phytophagous pests [9,10]. EOs also exhibit attractive behavior to certain pests [11]. They are characterized by their diverse bioactivities, safety for non-target organisms, availability from various sources, environmental friendliness, natural degradation, the ability to delay the development of pest resistance, and the potential to replace chemical insecticides [12–14]. The target pests, thrips, are unlikely to become resistant to these EOs, as products containing EOs are more complex chemically than traditional insecticide products with a single active ingredient [15]. *Thrips flavus* can potentially be controlled using EOs and products derived from EOs.

Lamiaceae, the sixth largest angiosperm family, comprises more than 7000 species across approximately 230 genera [16]. Many of these species are well-known as ornamental and medicinal plants; examples include lavender (Lavandula angustifolia Mill.), basil (Ocimum basilicum L.), peppermint (Mentha \times piperita), rosemary (Rosmarinus officinalis L.), thyme (Thymus mongolicus (Ronniger) Ronniger), etc. The EOs are synthesized and accumulated in the leaves, stems, and epidermal glands of the reproductive structures [17]. Numerous EOs have been widely utilized in the food, cosmetic, pharmaceutical, and crop protection industries [18–20]. Lamiaceae EOs consist of a diverse array of chemical components, including aliphatic and aromatic molecules, with general compounds such as β -caryophyllene, linalool, limonene, β -pinene, 1,8-cineol, α -pinene and thymol [21–23]. For instance, terpenoids and isoprenoids are a class of organic substances found in peppermint oil that are among the most diverse naturally occurring compounds derived from plants [24]. The chemical composition of Lamiaceae EOs determines their mechanism of action and their target applications, and their active ingredients with insecticidal properties show promise for developing plant-derived insecticides [25,26]. Numerous studies have demonstrated the versatile effects of EOs derived from plants of the Lamiaceae family against various agricultural pests. Marjoram oil showed significant antifeedant activity against Hylobius abietis (L.) (Curculionidae) and significant toxicity as a fumigant against Tribolium castaneum (Herbst) (Tenebrionidae) [27,28]. Rosemary oil contains 1,8-eudesmol, which demonstrated attractant activity against Frankliniella occidentalis (Pergande), and lavandula oil exhibited attractant activity against Drosophila suzukii (Matsumura) (Drosophilidae) [29,30]. Conversely, thyme oil showed remarkable repellent activity against Sitophilus zeamais Motsch (Curculionidae) [31]. Culex pipiens L. (Culicidae) oviposition is strongly inhibited by the EOs of mint and basil [32]. However, no studies have yet explored the use of Lamiaceae EOs to control soybean (*Glycine max* (L.) Merr.) pest thrips. This study aimed to assess the insecticidal potential of EOs against Thrips flavus, providing insights for the development of botanical insecticides and offering practical guidelines to protect crops from Thrips flavus infestations.

2. Materials and Methods

2.1. Insects

The thrips were collected from a soybean field of the Ministry of Agriculture and Rural Development (Jilin) (located at 125°24′19″ E, 43°48′17″ N, approximately 225 m above sea level). No pesticides are applied to this experimental field during the experimental period. The region is characterized by a temperate continental semi-humid monsoon climate. Thrips were brought back to the laboratory and placed in a rearing cage using the sweeping method. Thrips were identified using the key of Y.F. Han (1997) [33] and Mound

et al. (2018) [34] and continuously reared three generations. The insect cage was then kept in an incubator with soybean plants for two to three days at 25 ± 1 °C, $70 \pm 5\%$ relative humidity, and a 16:8 h light: dark photoperiod. The soybean plants were cultivated for two to three days under a 16:8 h light–dark photoperiod and were watered three to five times weekly [35].

2.2. Essential Oils

The four EOs assessed were perilla leaf oil (*Perilla frutescens* (L.) Britt.), marjoram oil (*Origanum majorana* L.), clary sage oil (*Salvia sclarea* L.), and spearmint oil (*Mentha spicata* L.), which were obtained from Lamiaceae species by Ji'an Zhongxiang Natural Plants Co. Ltd. (Ji'an City, Jiangxi Province, China) The EOs were extracted by steam distillation, and their purity was >98%.

2.3. Chemical Composition Analysis

Gas chromatography–mass spectrometry (GC–MS) (QP2010 plus, Shimadzu, Japan) was utilized to analyze the chemical composition of the four EOs [36–38]. The heating procedure was according to the methodology adopted by Pei et al. [36]. GC–MS software (version 2.53) tools (NIST 147 and NIST 27) were used for compound identification.

2.4. Laboratory Bioassay

The leaf-dipping method was used to determine the toxicity of the four EOs [36]. Fresh soybean leaves of the same size that were undamaged and free of pests and diseases were selected, washed in water, and allowed to dry naturally. Serial dilutions of the four EOs (0.2, 0.4, 0.6, 0.8, 1.0 mg/mL) were prepared in acetone to be tested. The leaves were then immersed in the different EOs, removed after ten seconds, and dried again naturally. One leaf (4 cm \times 2 cm) was then placed in a centrifuge tube (50 mL) with moistened filter paper inside. Thirty female thrips (3-day-old) were introduced into the tubes. Subsequently, the tube opening was promptly sealed with Parafilm sealing film. Approximately 70 micro-holes were punched into the film using a 2[#] insect pin, ensuring an even distribution of the micro-holes. Leaves from the control group were treated without EOs. The 30% thiamethoxam SC was purchased from Hebei Zhongbaolvnong Science & Technology Co., Ltd. (Langfang City, Hebei Province, China) and used as a control group of commonly used insecticides, with serial dilutions of 0.005, 0.008, 0.012, 0.015, 0.018 mg/mL. This procedure was repeated for each EO. Each group was replicated three times. After 24 h of treatment at room temperature, thrips mortality was assessed. After counting the number of dead and surviving insects, the mortality and adjusted mortality rates were calculated [39]. The mortality rate was calculated by Equation (1):

$$M_0 = \frac{M_1}{M_2} \times 100$$
 (1)

where M_0 is the mortality rate (%); M_1 is the number of dead thrips; M_2 is the total number of thrips in each treatment.

The adjusted mortality rate was calculated by Equation (2):

$$AM = \frac{N_1 - N_2}{1 - N_2} \times 100 \tag{2}$$

where *AM* is the adjusted mortality rate (%); N_1 is the mortality rate of the treatment group (%); N_2 is the mortality rate of the control group (%).

2.5. Olfactory Test

The ability of each EO to attract or repel adult *Thrips flavus* was assessed by an olfactory test. A Y-tube olfactometer was used to test the attraction or repellent rate [40]. Prior to the olfactory tests, both female and male adults were removed and cultivated separately.

Lights were positioned in parallel to avoid light interference, and humidified air was used as a control. The EO was diluted to 1.0 mg/mL with acetone and tested with a 1 μ L volume dropped on filter paper in the odor chamber. Clean, humid air was passed through each arm of the odor chamber and Y-tube at a flow rate of 300 mL/min. An adult *Thrips flavus* was placed in the front end inside the olfactometer. Its position in the tube was recorded after five minutes. Female and male adults were tested separately, with at least thirty individuals of each sex being treated with each EO. The response criteria were as follows: the adult was considered to have selected that odor source if it crawled more than half-length into either of the tubes and remained there for at least one minute; if the adult did not select or remained motionless after five minutes, it was considered not to have selected the odor source (no response) [36]. The selection rate was used as an informative indicator to evaluate the level of activity.

2.6. Pot Experiment

A pot experiment was conducted to assess the control efficacy of four EOs against *Thrips flavus*. The pot experiment procedure was performed as described by Pei et al. [36]. The schematic diagram of the pot experiment is shown in Figure S1. Soybean plants were planted in pots in batches, and when the 2nd compound leaf was grown, plants with uniform growth were selected, and one soybean seedling free of pests and diseases was kept in each pot. Five concentrations of four EOs were set at 180.00, 360.00, 540.00, 720.00, and 900.00 g a.i.·hm⁻². The control treatment was acetone without the EOs. Each concentration and control treatment was replicated three times. After spraying 5 mL evenly per pot with a spray bottle, the potted plants dried naturally in a windless area, and then thirty adult thrips were introduced per pot. The treated pots were arranged in randomized blocks one meter apart. At one, three, and seven days after treatment, the number of insects that died was counted, and the control efficacy was calculated. The control efficacy was calculated using Equation (3):

$$CE = \left(1 - \frac{P_1 \times P_2}{P_3 \times P_4}\right) \times 100 \tag{3}$$

where *CE* is the control efficacy (%); P_1 is the number of thrips in the treatment group after treatment with EOs; P_2 is the number of thrips in the control group before treatment with EOs; P_3 is the number of thrips in the treatment group before treatment with EOs, and P_4 is the number of thrips in the control group after treatment with EOs [36].

2.7. Statistical Analysis

The laboratory bioassay results were used to calculate the 95% confidence intervals, the median lethal concentration (LC_{50}), and the bioactivity regression equation using DPS 9.50 (Hangzhou Ruifeng Information Technology Co., Ltd.) [41]. One-way analysis of variance (ANOVA) was performed using IBM SPSS 20.0, and Tukey's test was used to compare significant differences between treatments [42]. The olfactory behavior response test results were processed using IBM SPSS 20.0, and the chi-square test was used to determine the main differences between treatments. The results were plotted using Origin 2021.

3. Results

3.1. Laboratory Bioassay

In the toxicity test, although the LC_{50} value of marjoram oil was slightly lower than those of the other three EOs (perilla leaf oil, spearmint oil, and clary sage oil), the 95% confidence intervals overlapped, and, therefore, there was no difference in the toxicity of these four essential oils (Table 1). The four EOs exhibited similar bioactivity for *Thrips flavus*. The LC_{50} of thiamethoxam used in this experiment was only 0.0077 mg/mL, significantly lower than those of the four EOs above. This indicates that the toxicity of these EOs is significantly lower than 30% thiamethoxam.

Essential Oils	95% Confidence Interval	LC ₅₀ (mg/mL)	Regression Equation	Correlation Coefficient	χ^2	df
Perilla leaf oil	0.35~0.51	0.43	y = 6.2134 + 3.3226x	0.89	7.03	3
Marjoram oil	0.17~0.60	0.41	y = 6.5178 + 3.9312x	0.88	17.87	3
Clary sage oil	0.35~0.48	0.42	y = 6.1932 + 4.1952x	0.99	0.94	3
Spearmint oil	0.47~0.62	0.54	y = 6.1934 + 4.4029x	0.94	7.41	3
30% thiamethoxam	0.0053~0.0095	0.0077	y = 10.4813 + 2.5941x	0.92	3.1347	3

Table 1. The toxicity of four essential oils to adult Thrips flavus.

Note: LC_{50} = concentration to kill 50% of thrips.

3.2. Pot Experiment

After one day of treatment, there were no significant differences in the control efficacy against *Thrips flavus* between the 180.00 g a.i.·hm⁻² (F = 2.818, p = 0.107), 360.00 g a.i.·hm⁻² (F = 1.699, p = 0.244), and 540.00 g a.i.·hm⁻² (F = 3.792, p = 0.058) application rates. At 720.00 g a.i.·hm⁻² (F = 5.684, p = 0.022), the control efficacy of spearmint oil was not significantly different from that of the other two EOs and was significantly higher than that of clary sage oil. In addition to this, marjoram oil at 900.00 g a.i.·hm⁻² (F = 4.605, p = 0.037) concentration was significantly more effective than clary sage oil against thrips, and there was no significant difference between the former and the other two EOs (Figure 1).

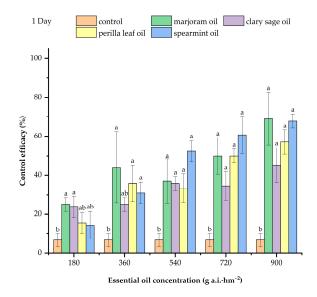


Figure 1. The control efficacy of four essential oils against *Thrips flavus* after one day of application in pot experiment. Different lowercase letters indicated significant differences (p < 0.05) among the control and essential oil treatments at the same concentration.

The control efficacy of perilla leaf oil at 900.00 g a.i.·hm⁻² did not differ significantly from the other two EOs three days after application, although it was significantly more effective against thrips than clary sage oil (F = 6.624, p = 0.015). The control efficacy of the four EOs against thrips did not differ significantly at concentrations of 180.00 g a.i.·hm⁻² (F = 3.841, p = 0.057), 360.00 g a.i.·hm⁻² (F = 0.406, p = 0.753), 540.00 g a.i.·hm⁻² (F = 0.649, p = 0.605), and 720.00 g a.i.·hm⁻² (F = 1.230, p = 0.361) (Figure 2).

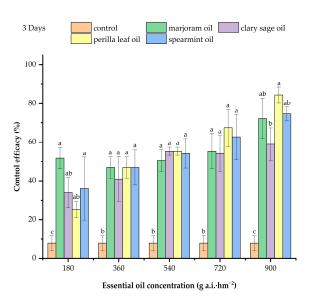


Figure 2. The control efficacy of four essential oils against *Thrips flavus* after three days of application in pot experiment. Different lowercase letters indicated significant differences (p < 0.05) among the control and essential oil treatments at the same concentration.

After seven days of treatment at 180.00 g a.i.·hm⁻² (F = 3.054, p = 0.092), 720.00 g a.i.·hm⁻² (F = 1.203, p = 0.369), and 900.00 g a.i.·hm⁻² (F = 1.562, p = 0.273), there was no significant difference in the control efficacy against *Thrips flavus*. In contrast, both marjoram oil and spearmint oil had significantly higher control efficacy compared to perilla leaf oil at 360.00 g a.i.·hm⁻² (F = 5.870, p = 0.020). Moreover, spearmint oil and clary sage oil were significantly higher than perilla leaf oil at 540.00 g a.i.·hm⁻² (F = 5.428, p = 0.025), and marjoram oil was not significantly different from the first two EOs (Figure 3).

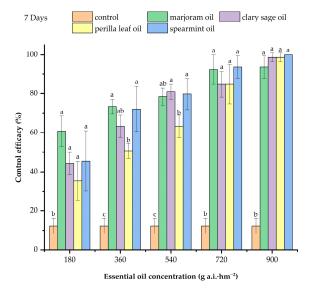


Figure 3. The control efficacy of four essential oils against *Thrips flavus* after seven days of application in pot experiment. Different lowercase letters indicated significant differences (p < 0.05) among the control and essential oil treatments at the same concentration.

The control of thrips by the four EOs, marjoram oil, clary sage oil, perilla leaf oil, and spearmint oil, was significantly improved with longer application times and higher concentrations. Spearmint and marjoram oil had a higher efficacy at all concentrations, one day, three days, and seven days after application. Perilla leaf oil had a better control efficacy at one day and three days after application. However, seven days after application, perilla leaf oil was inferior to the other EOs at low concentrations. One day and three

days after application, perilla leaf oil applied at a low concentration had a significantly lower control efficacy than the other oils, but at seven days, at the higher concentrations, it was not significantly different compared to the other oils. Clary sage oil showed better efficacy seven days after application and was not significantly different from spearmint and marjoram oil. Clary sage oil applied at a high concentration was significantly less effective than the other EOs after one day of application and three days after application. In contrast, the low concentration of clary sage oil did not differ in efficacy from the other EOs. The four EOs reached the highest efficacy seven days after application, with no significant differences observed between the different concentrations.

3.3. Olfactory Test

Female adults of *Thrips flavus* showed different olfactory behavioral responses to the EOs of different Lamiaceae species. Female adult thrips were significantly attracted to spearmint oil ($\chi^2 = 4.948$, p = 0.026) with an attraction rate of 71.05%. There were no significant differences in attraction between clary sage oil ($\chi^2 = 0.178$, p = 0.673), marjoram oil ($\chi^2 = 0.530$, p = 0.467), and perilla leaf oil ($\chi^2 = 0.166$, p = 0.684). *Thrips flavus* showed 67.86%, 84.09%, 56.45%, and 81.25% selectivity for the four EOs (Figure 4). Among the four EOs tested, spearmint oil had a significant attractant effect on adult females.

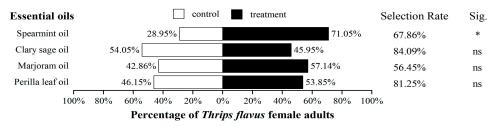


Figure 4. Olfactory behavioral response of female adult *Thrips flavus* to four essential oils. The symbol "ns" indicated 'no significance' (p > 0.05), while an asterisk "*" indicated significance (p < 0.05).

Male adults of *Thrips flavus* show no significant attraction response to spearmint oil ($\chi^2 = 0.000$, p = 1.000), clary sage oil ($\chi^2 = 2.381$, p = 0.123), marjoram oil ($\chi^2 = 0.617$, p = 0.432), and perilla leaf oil ($\chi^2 = 0.617$, p = 0.432). *Thrips flavus* exhibited a selectivity of 65.22%, 80.00%, 65.96%, and 63.27% for the four EOs (Figure 5).

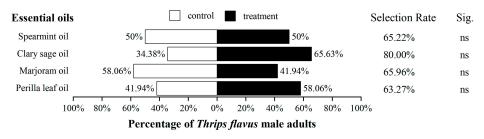


Figure 5. Olfactory behavioral response of male adult *Thrips flavus* to four essential oils. The symbol "ns" indicated 'no significance' (p > 0.05).

3.4. Chemical Analysis of Essential Oils

Marjoram oil consisted of seventeen major compounds, ranging in concentration from 24.52% to 0.26%, the most abundant being linalool (24.52%), followed by benzyl acetate (16.42%) and α -hexylcinnamaldehyde (14.28%), and the least abundant leaf alcohol (0.26%). Of these, five belong to esters, representing 29.27% of the constituents. They were followed by three types of terpene alcohols, accounting for 25.35% of the constituents. There were also aldehydes (14.28%), alcohols (8.87%), phenols (3.99%), amides (2.95%), and ketones (0.96%) (Table 2).

No.	Retention Time (min) Retention Index Con		Compounds	Relative Percentage (%)
1	8.14	1085	linalool	24.52
2	3.308	843	leaf alcohol	0.26
3	7.637	1069	methyl benzoate	2.95
4	9.765	1145	benzyl acetate	16.42
5	11.575	1215	ethyl phenylacetate	4.31
6	11.96	1240	nerol	0.57
7	13.012	1306	methyl aminobenzoate	2.95
8	13.135	1317	2-tert-butylcyclohexanol	3.09
9	13.307	1332	phenol, 2-methoxy-3-(2-propenyl)	1.64
10	13.579	1355	4-tert-butylcyclohexanol	5.78
11	13.758	1370	2,6,6-trimethyl-2,4-cycloheptadien-1-one	0.29
12	15.226	1494	butylated hydroxytoluene	2.35
13	16.93	1618	methyl dihydrojasmonate	4.65
14	17.368	1645	cis-3-hexenyl salicylate	0.94
15	18.666	1720	α-hexylcinnamaldehyde	14.28
16	19.198	1747	1-phenyl-1-nonen-3-one	0.67

Table 2. Chemical constituents of	marjoram oi	il (<i>Origanum</i>	majorana L.).
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Clary sage oil consisted of nineteen major chemical constituents, ranging in concentration from 28.74% to 0.34%. The most abundant component of clary sage oil was isopropyl myristate (28.74%), followed by linalyl acetate (20.07%), and the least abundant was linalyl anthranilate (0.34%). Of these, six belong to the ester compound group, representing 62.14% of the content. They were followed by two types of terpene alcohols, accounting for 22.49% of the total constituents. There were also alcohols (4.74%), ketones (4.13%), terpenes (3.07%), phenols (2.23%), and aldehydes (1.2%) (Table 3).

Table 3. Chemical constituents of clary sage oil (Salvia sclarea L.).

Number	Retention Time (min)	Retention Index	Compounds	Relative Percentage (%)
1	14.557	1438	α-guaiene	0.44
2	15.348	1504	<i>α</i> -bulnesene	0.57
3	17.518	1654	patchouli alcohol	1.10
4	6.142	1013	<i>p</i> -cymene	2.23
5	4.554	933	α-pinene	1.24
6	5.261	973	β-pinene	0.37
7	14.357	1420	bicyclo[5.2.0]nonane, 4-ethenyl- 4,8,8-trimethyl-2-methylene-	0.45
8	6.336	1021	1,8-cineole	7.31
9	10.482	1171	terpinen-4-ol	1.12
10	10.731	1179	α-terpineol	2.52
11	12.056	1246	linalyl acetate	20.07
12	13.476	1346	geranyl acetate	0.52
13	8.132	1085	linalool	15.18
14	9.286	1126	DL-camphor	4.13
15	11.479	1208	7-methoxy-3,7-dimethyloctanal	1.20
16	13.382	1338	linalyl anthranilate	0.34
17	13.687	1364	neryl acetate	0.6
18	18.819	1728	benzyl benzoate	11.87
19	20.475	1813	isopropyl myristate	28.74

Perilla leaf oil consisted of nine major chemical constituents ranging in concentration from 32.44% to 0.59%. The most abundant was (+)-limonene (32.44%), followed by γ -terpinene (23.92%), and the least abundant was cis-linalool oxide (0.59%). Of these, three belong to the terpene group, representing 56.97% of the total constituents. Aldehydes followed with 15.61% of the total. There are also phenols (13.25%), terpene alcohols (10.53%), olefins (2.34%), ketones (0.70%), and alcohols (0.59%) (Table 4).

Number	Retention Time (min)	RI	Compounds	Relative Percentage (%)
1	6.139	1013	<i>p</i> -cymene	13.25
2	6.375	1023	(+)-limonene	32.44
3	7.097	1051	γ -terpinene	23.92
4	7.956	1079	terpinolene	10.53
5	6.783	1039	1,3,6-octatriene, 3,7-dimethyl-, (z)-	2.34
6	7.2	1054	3-carene	0.61
7	7.33	1059	cis-linalool oxide	0.59
8	7.608	1068	(-)-fenchone	0.70
9	11.87	1234	cinnamaldehyde	15.61

Table 4. Chemical constituents of perilla leaf oil (Perilla frutescens (L.) Britt.).

Spearmint oil consists of five major chemical constituents ranging in concentration from 70.34% to 1.05%. The most abundant is (+)-carvone (70.34%), followed by (+)-limonene (26.22%), and the least abundant is α -pinene (1.05%). Of these, three belong to the terpene group, representing 28.64% of the total constituents. There are also ketones (70.34%) and alcohols (1.20%) (Table 5).

Table 5. Chemical constituents of spearmint oil (Mentha spicata L.).

Number.	Retention Time (min)	RI	Compounds	Relative Percentage (%)
1	4.557	934	<i>α</i> -pinene	1.05
2	6.373	1023	(+)-limonene	26.22
3	5.263	973	β -pinene	1.19
4	10.382	1167	3-p-menthol	1.20
5	11.614	1217	(+)-carvone	70.34

4. Discussion

EOs have several advantages when used as biocontrol agents for pest control. Firstly, they are made from natural plant components and can be broken down in the environment, making them environmentally friendly. Secondly, the extraction of EOs can effectively use excess peelings, twigs, and leaves from agricultural production, thus minimizing the waste of resources. Thirdly, EOs have specific insecticidal activity against certain pests and are safer against non-target species [43,44]. This study investigated the toxicity against Thrips *flavus* of four EOs: marjoram oil ($LC_{50} = 0.41 \text{ mg/mL}$); clary sage oil ($LC_{50} = 0.42 \text{ mg/mL}$); perilla leaf oil ($LC_{50} = 0.43 \text{ mg/mL}$); and spearmint oil ($LC_{50} = 0.54 \text{ mg/mL}$) under laboratory conditions. All four EOs exhibited some degree of toxicity. The use of Lamiaceae EOs for pest control has been studied extensively. Spearmint oil was shown to be toxic as a fumigant to *Reticulitermes dabieshanensis* Wang and Li (Rhinotermitidae) with an LC_{50} of 0.194 μ L/L [45]. At a concentration of 5 mg, spearmint oil and basil oil could control 100% of male Blattella germanica L. (Blattidae) [46]. Similar results in the Lamiaceae reported that pennyroyal oil (Mentha pulegium L.) and Thymus mastichina L. essential oil exhibited fumigant effect against Frankliniella occidentalis with LC₅₀ of 3.1 mg/L and 3.6 mg/L [47]. Mentha pulegium essential oil treatment also showed a significant fumigant effect on Thrips tabaci Lindeman [48] and was the most toxic fumigant to Thrips palmi Karny (Thripidae) [49]. The EOs of Syzygium aromaticum Merr. and L.M. Perry, Cinnamomum bejolghota (Buch.-Ham.) Sweet and Cymbopogon citratus (Dc.ex.Nees) showed high fumigant toxicity against Frankliniella schultzei (Trybom) (Thripidae) [50]. Whether the four EOs in this study have fumigant activity against *Thrips flavus* will be further investigated by fumigation toxicology assays. To further confirm the insecticidal activity of the four EOs, the pot experiment was conducted using live soybean potted plants. Spearmint oil showed 100% lethality against *Thrips flavus* at a concentration of 900.00 g a.i. hm⁻² after seven days of treatment. At the same time, after seven days of treatment, the other EOs showed more than 90% lethality against *Thrips flavus* at a concentration of 900.00 g a.i. hm^{-2} . These

results suggest that the exploitation and development of Lamiaceae EOs into plant-derived insecticides holds great potential. Because the insecticidal mechanism of these EOs is not yet clear, further research will be conducted exploratively.

EOs can be found in large quantities and with various chemical compositions. Several factors influence the biological activity, content, and composition of EOs. These include the plant growth stage [51], the extraction site and technique [52-54], and the geographical environment [55]. The use of innovative technologies to increase extraction efficiency can also improve the quality of EOs [56,57]. The chemical composition of the four EOs used in this study varied significantly based on GC-MS analysis. The highest concentration of (+)-carvone was found in spearmint oil, while isopropyl myristate was most abundant in clary sage oil, limonene in perilla leaf oil, and linalool in marjoram oil. Previous studies have shown that the main constituents of perilla leaf oil are 2-furyl methyl ketone (71.83%), decahydro-1-methyl-2-methylene-naphthalene (10.47%), limonene (5.16%) and caryophyllene (1.66%) [58]. On the other hand, β -terpineol and γ -terpinene were shown to be the main constituents of marjoram oil [27]. Similarly, limonene (+1,8-cineole; 14.3%) and carvone (67.1%) were identified as the major constituents of spearmint oil by GC-MS analysis [59]. When EOs are used as insecticides for pest control, one of the problems to be solved is that the production and quality properties of plant material cannot be standardized, and the control efficacy varies widely. Therefore, further research is needed to study the efficacy of individual chemical constituents in controlling pests and to refine the component with the best control efficacy to be used in the development of plant-derived insecticides.

This study found that of the four EOs tested, spearmint oil was a particularly strong attractant for mature female Thrips flavus. Recent studies have shown that some Lamiaceae EOs have a significant attractant effect on certain pests [60]. Rosmarinus officinialis L. was attractive to *Frankliniella occidentalis*, which is attracted by the major component 1,8cineole [30]. Rosemary oil with a certain concentration of β -caryophyllene and limonene was found to be an attractant for Bemisia tabaci (Gennadius) [61]. The EO of Tetradenia *riparia* (Hochst.) Codd, of which the primary compounds are fenchone, δ -cadinene, 14hydroxy-β-caryophyllene, and *tau*-cadinol, is an attractant for *Ceratitis capitata* (Wiedemann) (Tephritidae) [62]. In addition, some pests can be repelled by Lamiaceae EOs. For example, patchouli oil has significantly repelled Tribolium castaneum [63]. EO of Origanum majorana L. captured 87% fewer Thrips tabaci (Thripidae) than that in the control treatment, and this EO is a promising onion thrips repellent [64]. *Cinnamomum verum* Presl showed repellent activity against Hercinothrips femoralis (Reuter) (Thripidae) [65]. Rosmarinus. officinalis EO showed repellent activity against female adults of Thrips tabaci [66]. Rosmarinus officinialis EO can also inhibit the oviposition of Frankliniella occidentalis (Thripidae), Frankliniella *intonsa* (Thripidae), and *Thrips palmi*. α -Pinene was repellent to *Frankliniella occidentalis* and Frankliniella intonsa. Eucalyptol showed significant repellent activity in these three thrips species [67]. Moreover, carvacrol, cinnamaldehyde, and thymol, common constituents of Lamiaceae EOs, can be combined with nanogel technology to repel mosquitoes [68]. The EOs can be utilized as attractants or repellents for pest management by exploiting their ability to attract or repel pests [69]. In the olfactory test, spearmint oil had a different effect on the male and female Thrips flavus. This may be due to the chemical composition of the spearmint oil affecting adult male and female thrips differently. This phenomenon was reported in some thrips species [66,67]. The volatile dihydrotagetone alone attracted females *Megalurothrips sjostedti* (Trybom) (Thripidae) but had neither repellent nor attractive activity to males [70]. Tagetes minuta (L.) flower oil resulted in different olfactory responses to different sexes of *Ceratitis capitata*, causing attraction to males and avoidance to females. This suggests that the composition of EOs influences their olfactory properties [71]. In addition, their different effects on males and females may be due to other environmental factors. At a 0.01% concentration, the EO derived from celery seeds attracted both male and female adult Tribolium castaneum. However, at a 0.1% concentration, the EO had the opposite effect, repelling male adults but attracting female adults. In addition, basil oil at 0.01% repelled adult males but did not affect adult females [72]. This suggests that

the olfactory effects of EOs are concentration-dependent and can have different effects on different sexes of insects of the same species. The behavioral responses of *Thrips flavus* to single compounds and different compound blends will be investigated.

It has been shown that changing the application method could effectively improve the control efficacy of EOs against pests [73–75]. The utilization of nanomaterials is an important part of the implementation of novel application methods. When rosemary essential oil was incorporated into biodegradable poly (epsilon-caprolactone) nanoparticles and administered topically to Drosophila suzukii, it effectively prolonged and enhanced the plant's resistance to the insect. The results of this study will provide useful inspiration for our subsequent research [76,77]. In addition, nanoemulsion technologies are also being investigated; research is underway to use the EOs of Ayapana triplinervis (Vahl) R.M. King and H. Robinson to create more stable and long-lasting nanoemulsions to inhibit the reproduction of Aedes aegypti L. (Culicidae) larvae [78]. In addition, the stability of EOs has been improved through the use of microencapsulation technologies. An advantage of this method is that bioactive components' release, solubilization, and protection can be controlled [79]. The application method used in the pot experiment in this study was spraying, which is proven to be almost safe for soybean potted plants (Table S1). The appropriate application method requires further testing and screening. In this study, the main aim is to screen the EOs for their potential insecticidal activities against Thrips flavus under laboratory conditions. Further studies will encompass greenhouse or field trials aimed at further assessing the effectiveness of the efficacy of different EOs for thrips control. The proven potential for control of Thysanoptera pests should be fully exploited in management strategies that include combined approaches [80].

5. Conclusions

Four Lamiaceae EOs showed significant control efficacy against thrips. The EOs caused more than 90% mortality at the concentration of 900.00 g a.i.·hm⁻² and higher after 7 days. Spearmint oil caused a significant attraction response in adult females. The other EOs showed no significant attraction or repellent effects toward adult *Thrips flavus* of either sex. Linalool (24.52%), isopropyl myristate (28.74%), (+)-limonene (32.44%), and (+)-carvone (70.3%) were the primary chemical constituents of the EOs. The results of this preliminary study demonstrate that the four Lamiaceae EOs have the potential to develop plant-based insecticides and may be a possible alternative in the management of *Thrips flavus*, especially when considering reducing the use of synthetic pesticides.

Supplementary Materials: The following supporting information can be downloaded at https: //www.mdpi.com/article/10.3390/agronomy14061212/s1, Figure S1: Schematic diagram of the structure of a Y-tube olfactometer. Table S1 The phytotoxicity grades of four essential oils to soybean potted plants.

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