

## Article

# Ovicidal and Physiological Effects of Essential Oils Extracted from Six Medicinal Plants on the Elm Leaf Beetle, *Xanthogaleruca luteola* (Mull.)

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**Abstract:** Plant essential oils may serve as safe alternatives to detrimental synthetic pesticides due to relatively lower side effects on the environment and non-targeted organisms. The current study was conducted to investigate the ovicidal toxicity and physiological disruptions of six medicinal plant essential oils, including *Artemisia annua* L., *Lavandula angustifolia* Mill., *Origanum vulgare* L., *Rosmarinus officinalis* Spenn., *Satureja hortensis* L., and *Thymus vulgaris* L., on elm leaf beetle *Xanthogaleruca luteola* (Mull.). The LC<sub>50</sub> (Lethal Concentration to kill 50% of tested insects) values of 122.8, 287.5, 152.8, 180.6, 315.9, and 1366.2 ppm were recorded for *T. vulgaris*, *L. angustifolia*, *A. annua*, *S. hortensis*, *R. officinalis*, and *O. vulgare*, respectively, 72 h after treatment of 3-day-old eggs of the pest. Significant decreases in the amounts of glucose, protein, and triglyceride macromolecules were also observed after treatment. The application of essential oils derived from *T. vulgaris*, *A. annua*, and *S. hortensis* at 400 ppm revealed 100% ovicidal activity. Accordingly, tested essential oils, particularly the essential oil of *T. vulgaris*, have been promising potential as biorational insecticides in the management of *X. luteola*.

**Keywords:** biorational pesticides; elm leaf beetle; essential oils; macromolecules; ovicidal toxicity

## 1. Introduction

The elm leaf beetle, *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae), is a defoliating pest of elm trees (*Ulmus* spp.) widely distributed in Europe, North Africa, Asia, Australia, and temperate areas in North and South America [1]. Elm leaf beetle adults hibernate overwinter in bark fissures and woodpiles and emerge in spring to feed on elm foliage for 1–2 weeks before commencement of egg-laying. Adults feed through leaves, more often in a shot hole pattern. The eggs of elm leaf beetle are bright yellow and laid in clusters, averaging 14–20 eggs per cluster, that are mainly located on the underside of a leaf. Both larvae and adults of *X. luteola* feed on the emergent leaves. The larvae of the elm leaf beetle can feed on elm leaves for 2–4 weeks, undergo three instars, and cause more damage than adults by skeletonizing the leaf surface [2]. Severe infestations by elm leaf beetle larvae can cause physiological stress, defoliation of trees, and enhance the susceptibility of host plants to other pests and diseases [3]. Since elm trees are widely planted in cities and suburbs, the excessive use of chemical pesticides to protect them from the population

impact of *X. luteola* leads to municipal environment pollution, interruption of ecological balance, the mortality of non-target organisms, development of insecticide resistance, as well as toxic waste [3,4].

Natural products derived from plants are potential biorational alternatives to synthetic insecticides for controlling insect infestations. In this regard, botanical insecticides, consisting of naturally occurring chemicals from plant species, are widely studied [5,6]. These compounds comprise a variety of secondary metabolites, including phenols, alkaloids, and terpenoids, and show significant toxicity against insect pests [7,8]. Among botanical compounds, essential oils (EOs), known as volatile secondary metabolites derived from seeds, roots, leaves, and fruits of plants, are low-risk pesticides that showed lethal and sublethal effects on several insect pests [9–11]. Indeed, they have a broad-spectrum of deteriorative effects against insect pests, including acute toxicity [12], antifeedant [13], repellent [14], oviposition deterrent [15], growth regulators [16], and anti-vector [17], activities. As plant EOs have short environmental persistence, they are considered biodegradable agents in the environment [18]. Furthermore, plant EOs have multiple neurotoxic impacts on insects via different mechanisms, including gamma-aminobutyric acid, octopamine synapses, and inhibition of acetylcholinesterase, and accordingly, the chance of insect pests to resistance before them may be very low [19].

Recent studies have explored the insecticidal effect of EOs derived from *T. vulgaris*, *L. angustifolia*, and *Schinus molle* L. on larval and adult stages of *X. luteola* [20], but there has been no attempt to explore the toxicity of EOs on *X. luteola* at the egg stage. It has been reported that oil-based insecticides, such as plant essential oils, penetrate the insect eggshell more readily than water-based insecticides [21]. The ovicidal effect of *T. vulgaris*, *L. angustifolia*, *A. annua*, *S. hortensis*, *R. officinalis*, and *O. vulgare* EOs on silver leaf whitefly (*Bemisia tabaci* Gennadius), common green bottle fly (*Lucilia sericata* Meigen), Angoumois grain moth (*Sitotroga cerealella* Olivier), carob moth (*Ectomyelois ceratoniae* Zeller), and bean weevil (*Acanthoscelides obtectus* Say) have been studied [22–24].

Plant EOs can inhibit the growth and metabolism of insects through physiological processes and enzymatic activities and can have mortal influences on insects by feeding and oviposition deterrence [25,26]. Reduction and disruption in the amounts of biochemical components in the treated larvae with EOs were described in some studies [13,27].

Investigating the biochemical effects of EOs on insects, in addition to their ovicidal effects, can provide a foundation for improving the efficacy of pesticides and efficient pest management. Accordingly, the current study was aimed to assess potential toxicity and ovicidal or egg-hatching inhibition by *T. vulgaris*, *L. angustifolia*, *A. annua*, *S. hortensis*, *R. officinalis*, and *O. vulgare* EOs on *X. luteola*. To gain a better understanding of the physiological interactions between EOs and the *X. luteola* eggs, the metabolic responses of eggs after exposure to different concentrations of EOs were also explored by measuring the amounts of storage macromolecules glucose, protein, and triglyceride.

## 2. Materials and Methods

### 2.1. Mass Rearing of Insects

Adults of *X. luteola* were collected from elm trees (*Ulmus densa* Litw) cultivated in Rasht (37°16'51" N 49°34'59" E), Iran, and were maintained in a rearing chamber at 25 ± 1 °C, 75 ± 5% relative humidity and 16: 8 h (light: dark) photoperiod. The adults were sexed and fed on elm leaves; their eggs were used to maintain the culture and placed in plastic jars 10 × 20 cm for bioassay.

### 2.2. Extraction of Essential Oils

Six different types of aromatic plants, taxonomically related to the Lamiaceae and Asteraceae family, were collected from different growing locations in Iran (Table 1). The aerial parts of plants were dried in the shade. The dried flowers were made into a fine powder by a grinder (354, Moulinex, Paris, France). Essential oils were extracted based on the method described by British Pharmacopoeia [28]. Briefly, 50 g of dried herb powder

was mixed with 1000 mL distilled water and kept for 24 h. Samples were subjected to 2 h hydrodistillation in a Clevenger-type apparatus. This process was repeated several times to receive the required amount of aqueous/oil distillate (about 3 mL). The oil phase was isolated from the obtained solution by dehydration over anhydrous sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>) (Merck Co., Darmstadt, Germany). Extracted essential oils were stored in standing 1.5 mL microtubes covered with aluminum foil and kept at 4 °C.

**Table 1.** List of plants used for exploring the ovicidal effect of their essential oils on elm leaf beetle eggs.

Common Name	Scientific Name	Family	GPS/Location	Part	Essential Oil Yield (% (w/w))
Garden thyme	<i>Thymus vulgaris</i>	Lamiaceae	37.159592, 49.001185	Leaves	1.27 ± 0.02 <sup>c</sup>
Lavender	<i>Lavandula angustifolia</i>	Lamiaceae	38.419401, 48.860797	Leaves	1.61 ± 0.06 <sup>b</sup>
Sweet wormwood	<i>Artemisia annua</i>	Asteraceae	37.198539, 49.653243	Leaves	2.90 ± 0.03 <sup>a</sup>
Summer Savoury	<i>Satureja hortensis</i>	Lamiaceae	35.322589, 51.625637	Leaves	2.11 ± 0.09 <sup>ab</sup>
Rosemary	<i>Rosmarinus officinalis</i>	Lamiaceae	37.193775, 49.641361	Leaves	1.10 ± 0.00 <sup>c</sup>
Oregano	<i>Origanum vulgare</i>	Lamiaceae	36.949912, 50.603010	Leaves	1.72 ± 0.01 <sup>b</sup>

Data are mean ± SE. Different lowercase letters in the same column indicate significant difference at  $p \leq 0.05$  according to Tukey test.

### 2.3. Assessment of the Ovicidal Effect of Essential Oils

To assess the direct ovicidal effect of essential oils on elm leaf beetle eggs, 3-day-old eggs were randomly collected from the mass-reared colonies and exposed to EOs concentrations diluted in distilled water+Tween-80 by egg dipping method. Experiments were performed with 3 replications; 5 eggs-masses (12–15 eggs) per replication were exposed to EOs for 30 sec and left in Petri dishes (10 cm) to hatch. Untreated controls were exposed to only distilled water+Tween-80 for 30 secs. Egg mortality was determined for individual eggs within each egg mass per each treatment using a stereomicroscope. Percent ovicidal activity was assessed after 72 h exposure to EOs by the following formula [29]:

$$\text{Ovicidal Percentage} = \frac{\text{Number of eggs hatched in control} - \text{Number of eggs hatched in treatment}}{\text{Number of eggs hatched in control}} \times 100$$

### 2.4. Preparation of Eggs Homogenates for Biochemical Assay

After 72 h of treatment of *X. luteola* eggs with EOs at the concentrations of 100, 200, 300, and 400 ppm, about 10 g unhatched eggs from each treatment and control groups were weighed and homogenized by a glass pestle in 0.5 mL of distilled water. The homogenate was centrifuged at 13,000× *g* at 4 °C for 10 min. The homogenized egg supernatant was transferred into a 1.5 mL Eppendorf® tube and stored at −20 °C. Biochemical assays were conducted with 3 replicates.

### 2.5. Protein Assay

The protein concentration of the homogenized egg supernatant treated with EOs was determined via Biuret's method [30] using a total protein assay kit (Biochem Co., Tehran, Iran).

### 2.6. Determination of Glucose

Glucose concentration of the homogenized egg supernatant, treated with EOs was determined by automated enzymatic assays using a glucose assay kit (Biochem Co., Tehran, Iran) as described by Siegert [31] with some modifications: reagent A and reagent B were mixed (4:1) followed by addition of samples. Glucose levels were estimated by measuring reduction in absorption at 492 nm in an ELISA reader (Awareness, Temecula, CA, USA).

### 2.7. Determination of Triglyceride

Triglyceride concentration of the homogenized egg supernatant, treated with EOs, was determined by a triglyceride kit (Biochem Co., Tehran, Iran) according to protocol

described by Fossati and Prencipe [32]. The reducing absorption rate for triglyceride analysis was read at 545 nm in an ELISA reader (Awareness, Temecula, CA, USA).

### 2.8. Statistical Analysis

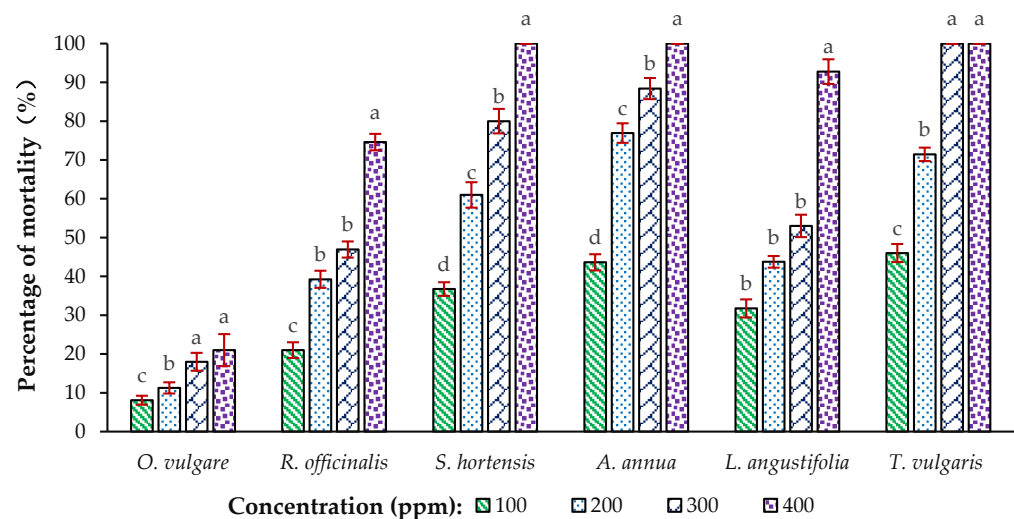
Control mortality was rare, and where corrections for mortality were necessary, these were accomplished using Abbott's formula [33]. Egg mortality and lethal concentrations of EOs were determined 72 h after treatment, and data were analyzed by POLO-PC software to calculate LC<sub>50</sub> values as the effective concentrations to follow the biochemical experiments. Relative potency (RP) for each mixture was determined based on *O. vulgare* (lowest toxicity) and calculated using the following formula: RP = LC<sub>50</sub> of *O. vulgare* essential oil/LC<sub>50</sub> of individual essential oil. Raw data obtained from the biochemical assay and ovidal bioassay were subjected to a one-way statistical analysis of variance test (ANOVA) for significant differences in the measured parameters. The Tukey–Kramer test at a 5% significance level was considered for comparison of means using SAS statistical software.

## 3. Results

### 3.1. The Ovicidal Activity of EOs

The hydrodistillation of aerial parts of *T. vulgaris*, *L. angustifolia*, *A. annua*, *S. hortensis*, *R. officinalis*, and *O. vulgare* gave yellowish oils with a yield of  $1.27 \pm 0.02$ ,  $1.61 \pm 0.06$ ,  $2.9 \pm 0.03$ ,  $2.1 \pm 0.09$ ,  $1.1 \pm 0.00$ , and  $1.7 \pm 0.01\%$  (*w/w*), respectively (Table 1).

Concentration-dependent toxicity was determined for all EOs. Untreated insects in control groups indicated mortality ranging from 5–10%, which were corrected through Abbott formula. All doses of EOs revealed ovicidal activity on *X. luteola* by the maximum mortality of  $100 \pm 00$ ,  $92.76 \pm 3.17$ ,  $100 \pm 00$ ,  $100 \pm 00$ ,  $74.61 \pm 2.31$ , and  $21 \pm 4.13\%$ , for *T. vulgaris* ( $F = 1228.34$ ;  $df = 3$ ;  $p < 0.0001$ ), *L. angustifolia* ( $F = 584.50$ ;  $df = 3$ ;  $p < 0.0001$ ), *A. annua* ( $F = 988.09$ ;  $df = 3$ ;  $p < 0.0001$ ), *S. hortensis* ( $F = 1228.34$ ;  $df = 3$ ;  $p < 0.0001$ ), *R. officinalis* ( $F = 1366.28$ ;  $df = 3$ ;  $p < 0.0001$ ), and *O. vulgare* ( $F = 89.14$ ;  $df = 3$ ;  $p < 0.0001$ ) EOs at the concentration of 400 ppm, respectively (Figure 1).



**Figure 1.** Ovicidal activity of applied essential oils against *Xanthogaleruca luteola* eggs. Statistical differences are shown by different letters according to Tukey's test at  $p \leq 0.05$ .

The LC<sub>50</sub> values were estimated for each EO. *T. vulgaris* essentials showed the highest ovicidal toxicity with the lowest LC<sub>50</sub> of 122.8 ppm, indicating the highest relative potency (Table 2). The LC<sub>50</sub> value estimated for *A. annua* EO was 152.8 ppm, which was much lower than the LC<sub>50</sub> value of *Satureja hortensis* EO (180.6 ppm), indicating a better efficacy and ovicidal activity followed by *T. vulgaris* (Table 2).

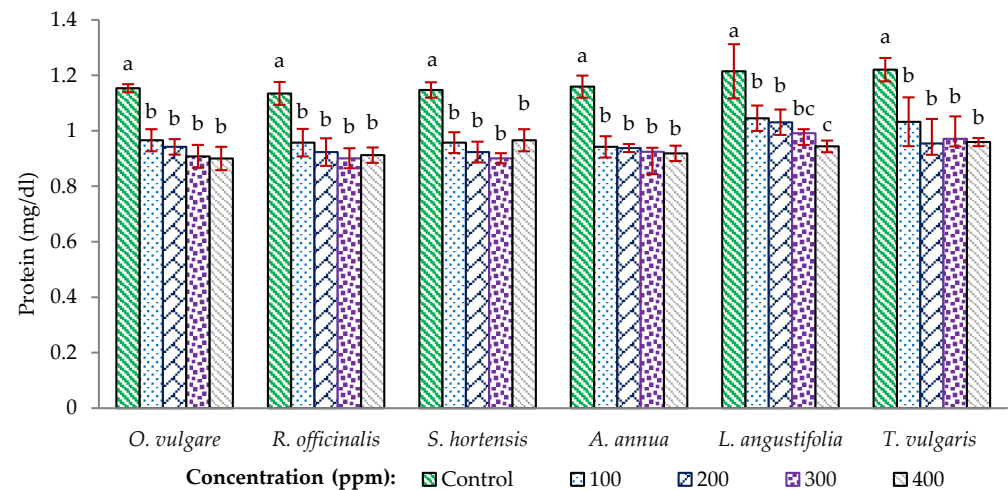
**Table 2.** Estimated LC<sub>50</sub> values and relative potencies of six essential oils on *Xanthogaleruca luteola* eggs after 72 h of exposure.

Essential Oil	Slope ± SE	X <sup>2</sup> (df = 3)	LC <sub>50</sub> (95% CI) <sup>a</sup> (ppm)	Relative Potency <sup>b</sup>
<i>Artemisia annua</i>	1.74 ± 0.26	4.35	152.8 (64.24–291.00)	8.94
<i>Lavandula angustifolia</i>	1.66 ± 0.28	4.62	287.5 (99.81–566.01)	4.75
<i>Origanum vulgare</i>	2.04 ± 0.34	2.55	1366.2 (904.03–1094.6)	1.00
<i>Rosmarinus officinalis</i>	1.70 ± 0.27	4.15	315.9 (117.2–578.5)	4.32
<i>Satureja hortensis</i>	1.31 ± 0.22	4.20	180.6 (53.09–409.29)	7.56
<i>Thymus vulgaris</i>	1.79 ± 0.293	4.31	122.8 (56.2–235.0)	11.12

<sup>a</sup> CI: confidence interval. <sup>b</sup> Relative potency = LC<sub>50</sub> of *O. vulgare* essential oil/ LC<sub>50</sub> of another essential oil.

### 3.2. Total Protein Level in *X. luteola* Eggs Post-Exposure to EOs

Total protein eggs treated were significantly decreased compared to control (F = 27.03; df = 5, 6; *p* < 0.05) (Figure 2).



**Figure 2.** Effect of different concentrations of essential oils on the total protein (mg/dL) of *Xanthogaleruca luteola* eggs. Statistical differences are shown by different letters according to Tukey's test at *p* ≤ 0.05.

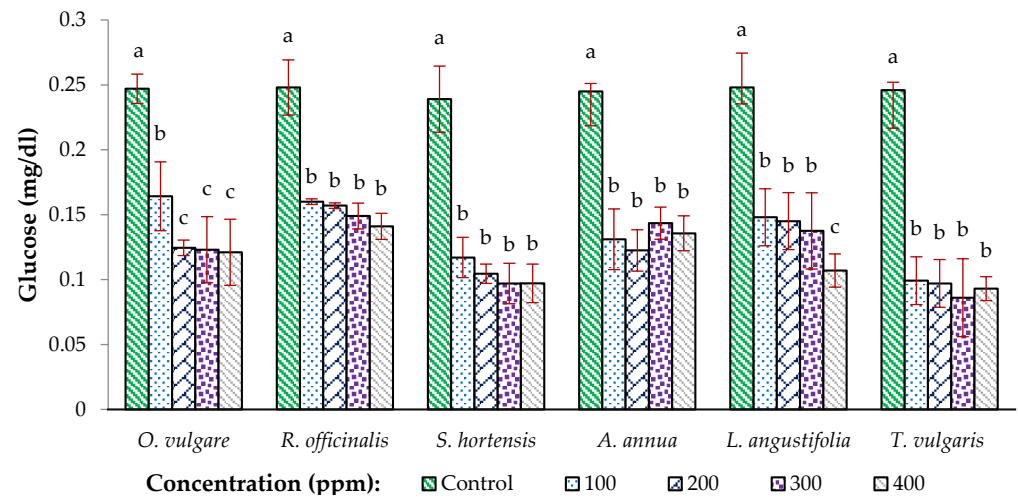
### 3.3. Total Glucose of *X. luteola* Eggs Treated

Profiling the glucose level in eggs per each treatment revealed a higher impact of *O. vulgare* in reducing the total glucose content even at its lower concentrations (Figure 3) (F = 15.97; df = 5, 6; *p* < 0.05).

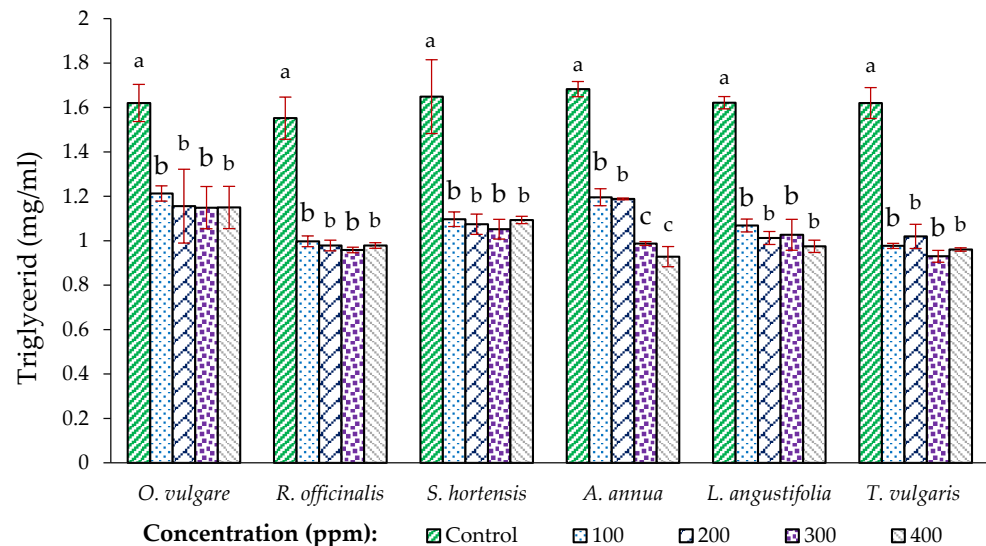
### 3.4. Total Triglyceride of *X. luteola* Eggs Treated

Profiling total triglyceride content of *X. luteola* eggs after exposing with *A. annua* EO at 400 ppm revealed higher reductions by significant differences ranging from 1.68 ± 0.09 mg/dL to 0.92 ± 0.05 mg/dL compared to triglycerides level assessed for control (F = 31.42; df = 5, 6; *p* < 0.001) (Figure 4). This is suggesting that the higher concentrations of *A. annua* were more effective than other applied concentrations of EOs in reducing the lipid contents of the *X. luteola* egg cells because there were no significant differences among the used concentrations.





**Figure 3.** Effect of different concentrations of essential oils on total glucose (mg/dL) of *Xanthogaleruca luteola* eggs. Statistical differences are shown by different letters according to Tukey's test at  $p \leq 0.05$ .



**Figure 4.** Effect of different concentrations of essential oils on total triglyceride (mg/mL) of *Xanthogaleruca luteola* eggs. Statistical differences are shown by different letters according to Tukey's test at  $p \leq 0.05$ .

#### 4. Discussion

The use of plant essential oils to control pests is gaining popularity, as they may provide an eco-friendly alternative to current synthetic insecticides in the control of elm leaf beetle [1,34,35]. Different plant EOs including *A. annua*, *Artemisia sieberi* Besser, *L. angustifolia*, *Melia azedarach* L., *Pteridium aquillinum* L., and *T. vulgaris* have previously been studied for their deterrent, toxic, and developmental inhibitory activities against *X. luteola* at the larval stage. The EOs derived from *A. annua*, *A. sieberi* [1,36], *T. vulgaris*, *L. angustifolia* [34], and *R. officinalis* [37] have shown repellence, toxicity, biochemical, behavioral, and physiological properties against *X. luteola*. However, the present study offers the first evidence of the ovicidal effect of *A. annua*, *L. angustifolia*, *O. vulgare*, *R. officinalis*, *S. hortensis*, and *T. vulgaris* EOs against *X. luteola* eggs. All applied EOs exhibited a degree of ovicidal activity against *X. luteola*, in which ovicidal activity of *T. vulgaris*, *A. annua*, and *S. hortensis* EOs was promising. Within this study, the difference observed in the level of ovicidal activities for each EO is presumably related to the difference in the accumulation level of active substances in each plant, such as tannic acid, which has embryotoxic effects on insects' eggs and

larval specifically at high concentration levels [38]. It has been reported that the phenolic compounds of EOs affect the movement and vital systems of the fetus, and they have inhibitory effects on gas exchange inside the egg, which leads to hardening of the crust and direct influence on protoplasm, causing the death of the fetus inside the egg [38]. The ovicidal effects of thyme and lavender against *Tetranychus urticae* Koch were previously investigated, indicating that lavender oil was the most protective against this spider mite [39]. Bedini [24] reported the oviposition deterrence and toxicity effect of *L. angustifolia* on adults of common green bottle fly (*Lucilia sericata* Meigen). In general, many plant EOs have been reported to have an ovicidal effect and high potential to be developed into oviposition deterrent, ovicidal, and adulticidal agents for controlling populations of insects [40–42].

After the treatment of elm leaf beetle eggs with EOs, total protein, glucose, and triglyceride levels declined. Total protein, glucose, and triglyceride are the three required storage macromolecules closely linked with many metabolic pathways. Reduction in the amounts of these macromolecules as a result of insecticidal toxicity can influence several physiological functions such as energy demand, tissue repair, reproduction, etc. Plant EOs can coagulate the cytoplasm and crashed lipids and proteins [43]. Damage to the cell wall and membrane can lead to the leakage of macromolecules and lysis [44]. The result shows that the amount of total protein decreased in the eggs after exposure to different concentrations of EOs. This phenomenon could be due to the breakdown of proteins into free amino acids, which are transferred into Krebs's cycle [45]. The declines that were observed in the amounts of triglyceride and glucose caused by insecticide could also be attributed to an interruption in the absorption system [46].

The results of recent studies on the chemical profile of *A. annua*, *L. angustifolia*, *O. vulgare*, *R. officinalis*, *S. hortensis*, and *T. vulgaris* essential oils are reviewed and summarized in Table 3. Terpenes including monoterpene hydrocarbons such as  $\alpha$ -pinene and  $\beta$ -pinene, oxygenated monoterpenes such as 1,8-cineole and thymol, and sesquiterpene hydrocarbons such as  $\beta$ -selinene were the main components of all studied essential oils except *S. hortensis*. Phenylpropanoid compounds estragole and eugenol were the main components of *S. hortensis* essential oil.

**Table 3.** Main components of the essential oils of *Artemisia annua*, *Lavandula angustifolia*, *Origanum vulgare*, *Rosmarinus officinalis*, *Satureja hortensis*, and *Thymus vulgaris*.

Essential Oil	Main Components (%)	References
<i>A. annua</i>	Camphor <sup>OM</sup> (13.1%), artemisia ketone <sup>OM</sup> (11.8%), $\beta$ -selinene <sup>SH</sup> (10.7%), pinocarvone <sup>OM</sup> (7.4%), 1,8-cineole <sup>OM</sup> (6.8%), $\alpha$ -pinene <sup>MH</sup> (5.9%), caryophyllene oxide <sup>OS</sup> (5.4%), and $\beta$ -caryophyllene <sup>SH</sup> (3.1%).	[47]
<i>L. angustifolia</i>	Linalool acetate <sup>OM</sup> (35.0%), linalool <sup>OM</sup> (32.7%), 1,8-cineole <sup>OM</sup> (8.1%), camphor <sup>OM</sup> (6.4%), borneol <sup>OM</sup> (2.4%), and lavandulyl acetate <sup>OM</sup> (2.0%).	[48]
<i>R. officinalis</i>	1,8-Cineole <sup>OM</sup> (40.4%), camphor <sup>OM</sup> (11.9%), $\alpha$ -pinene <sup>MH</sup> (8.7%), $\beta$ -pinene <sup>MH</sup> (6.9%), caryophyllene <sup>SH</sup> (5.3%), borneol <sup>OM</sup> (3.9%), and camphene <sup>MH</sup> (3.5%).	[48]
<i>O. vulgare</i>	Thymol <sup>OM</sup> (38.8%), $\gamma$ -terpinene <sup>MH</sup> (13.6%), linalool <sup>MH</sup> (13.0%), <i>p</i> -cymene <sup>MH</sup> (9.5%), caryophyllene <sup>SH</sup> (4.5%), and $\beta$ -myrcene <sup>MH</sup> (3.4%).	[49]
<i>S. hortensis</i>	Estragole <sup>PP</sup> (68.0%), eugenol <sup>PP</sup> (5.6%), (E)- <i>p</i> -methoxycinnamaldehyde <sup>PP</sup> (4.4%), allo-spathulenol <sup>OS</sup> (3.5%), $\alpha$ -pinene <sup>MH</sup> (1.3%), and $\beta$ -pinene <sup>MH</sup> (1.2%).	[50]
<i>T. vulgaris</i>	Thymol <sup>OM</sup> (38.7%), <i>p</i> -cymene <sup>MH</sup> (15.7%), carvacrol <sup>OM</sup> (14.9%), $\gamma$ -terpinene <sup>MH</sup> (13.3%), $\beta$ -caryophyllene <sup>SH</sup> (2.7%), and 3-carene <sup>MH</sup> (2.2%).	[51]

MH = monoterpene hydrocarbons; OM = oxygenated monoterpenes; SH = sesquiterpene hydrocarbons; OS = oxygenated sesquiterpene; PP = phenylpropene.

The insecticidal efficiency of plant-derived essential oils has direct interaction with their chemical components. On the other hand, the significant insecticidal effects of main components identified in *A. annua*, *L. angustifolia*, *O. vulgare*, *R. officinalis*, *S. hortensis*, and *T. vulgaris* essential oils were also reported in recent researches. For example, significant toxicity of terpenes thymol and carvacrol and phenylpropene eugenol against larvae of southern house mosquito (*Culex quinquefasciatus* Say) and adults of the house-

fly (*Musca domestica* L.) was reported by Pavela [52]. In the other study, the significant correlation ( $p \leq 0.01$ ) between 1,8-cineole, camphor, limonene, and  $\alpha$ -terpinene as major components of *T. vulgaris* and *Mentha spicata* L. essential oils and the larval mortality of the Mediterranean flour moth (*Ephesia kuehniella* Zeller), the carob moth (*Ectomyelois ceratoniae* Zeller) and the Indian meal moth (*Plodia interpunctella* Hubner) were found [53]. The acute toxicity and inhibition of the acetylcholine esterase activity of  $\alpha$ -pinene,  $\beta$ -pinene,  $\beta$ -myrcene, 1,8-cineole,  $\gamma$ -terpinene, p-cymene, linalool,  $\beta$ -caryophyllene, and caryophyllene oxide against the adults of rice weevil (*Sitophilus oryzae* (L.)) was approved in the study of Liu et al. [54]. In the other work, the toxicity of 1,8-cineole, p-cymene,  $\alpha$ -terpinene,  $\gamma$ -terpinene,  $\alpha$ -pinene, and  $\beta$ -pinene, along with essential oils from 24 plant species, was evaluated against adults of the maize weevil (*Sitophilus zeamais* Motsch) by Patiño-Bayona et al. [55]. They concluded that the essential oils with higher toxicity were branded by a high content of terpenoids and phenylpropanoids. Along with promising insecticidal activity of several introduced components in the present study, including thymol, carvacrol, p-cymene, eugenol, 1,8-cineole, camphor, myrcene, and  $\gamma$ -terpinene, against the larvae of the tobacco cut-worm (*Spodoptera littoralis* Boisid.), the synergistic and/ or antagonistic effects of some other components such as borneol, linalool, camphene,  $\alpha$ -pinene,  $\beta$ -pinene, and limonene was also reported [56]. Accordingly, it can be said that the promising insecticidal effects of *A. annua*, *L. angustifolia*, *O. vulgare*, *R. officinalis*, *S. hortensis*, and *T. vulgaris* essential oils may be associated with their insecticide components, especially terpenes and phenylpropenes. It can also be concluded that the more insecticidal properties of *T. vulgaris* essential oil than the other oils are probably due to the high concentration of pure insecticide components such as thymol, p-cymene, carvacrol,  $\gamma$ -terpinene,  $\beta$ -caryophyllene, and 3-carene. However, the synergistic and antagonistic effects of other components in the essential oil should also be considered [56,57].

## 5. Conclusions

Due to their volatile nature and lower residual characteristic of plant EOs, there is a much lower level of risk from EOs to the environment and pollinator insects comparing to current synthetic pesticides [58]. However, some EOs, such as thyme, have an impact on the movement and wax production by pollinator insects such as the western honeybee (*Apis mellifera* L.) [59]. Therefore, some EOs, such as thyme, should be applied at suitable times (large infestations) and with the concentrations recommended to reduce the inhibitory impacts on the hive. The reactivity of plant EOs depends upon the condition of their growing environment and their composition, which includes different concentrations of aldehydes, phenolics, terpenes, and other natural compounds and the orientation of their functional groups [60]. Further investigations must be undertaken to find the optimal dosage of *T. vulgaris*, *A. annua*, and *S. hortensis* for controlling target insects with the least mortal impacts on non-target organisms and beneficial insects such as hives. This requires further efforts to explore the molecular mechanisms of these essential oils and the effects of their individual chemical compounds on other insects and organisms. The result obtained from the present study indicates that the EOs of *T. vulgaris*, *L. angustifolia*, *A. annua*, *S. hortensis*, *R. officinalis*, and *O. vulgare* have ovicidal effects on elm leaf beetle *X. luteola* and an irreversible effect on some key biochemical compounds in this pest. It is expected that plant biorational pesticides will find their greatest commercial application in urban pest control. The ovicidal effects of essential oils reveal that these plants may be a good and safe natural toxicant that warrants further investigation.

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