agronomy

MDPI

Article

Antifeeding, Toxic, and Growth-Reducing Activity of *trans*-Anethole and *S*-(+)-Carvone against Larvae of the Gypsy Moth *Lymantria dispar* (L.)

Igor Kostić [1], Slobodan Milanović [2,3], Miroslav Kostić [4], Darka Šešlija Jovanović [5], Dušica Ćalić [5], Libor Jankovský [3] and Jelica Lazarević [5,*]

[1] Institute for Multidisciplinary Research, University of Belgrade, Kneza Višeslava 1, 11030 Belgrade, Serbia
[2] Faculty of Forestry, University of Belgrade, Kneza Višeslava 1, 11030 Belgrade, Serbia
[3] Department of Forest Protection and Wildlife Management, Faculty of Forestry and Wood Technology, Mendel University, Zemědělská 3, 61300 Brno, Czech Republic
[4] Institute for Medicinal Plant Research "Dr Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia
[5] Institute for Biological Research "Siniša Stanković"—National Institute of Republic of Serbia, University of Belgrade, Bulevar Despota Stefana 142, 11060 Belgrade, Serbia
* Correspondence: jellaz@ibiss.bg.ac.rs

Abstract: Botanicals, such as essential oils (EO) and their compounds, are considered a viable eco-friendly alternative to synthetic insecticides, which threaten human health and ecosystem functioning. In the present study, we explored the potential use of two EO compounds, *trans*-anethole (phenylpropanoid) and *S*-(+)-carvone (monoterpene ketone), against gypsy moth larvae (GML), a serious pest of deciduous forests and orchards. GML feeding, survival, molting, and nutritional physiology were assessed at different compound concentrations and compared with the effects of the commercial botanical product NeemAzal®-T/S (neem). The impact of botanicals on GML feeding was assessed by the leaf-dipping method and showed the highest antifeeding activity of neem in the no-choice assay. GML that were offered a choice were deterred by anethole and attracted by low concentrations of carvone and neem. Ingestion of botanicals was more effective in inducing mortality and reducing molting than residual contact exposure. Anethole and carvone were better toxicants but worse growth regulators than neem. Assessing nutritional indices revealed reduced growth, consumption, and food utilization in larvae fed on botanical-supplemented diets. The highest metabolic cost of food processing was recorded in carvone-fed larvae, which exhibited a negative growth rate. The results suggest that anethole and carvone might be used as control agents against GML.

Keywords: *Lymantria dispar*; pest control; botanicals; deterrent; attractant; residual contact toxicity; digestive toxicity; molting; nutritional indices

Citation: Kostić, I.; Milanović, S.; Kostić, M.; Šešlija Jovanović, D.; Ćalić, D.; Jankovský, L.; Lazarević, J. Antifeeding, Toxic, and Growth-Reducing Activity of *trans*-Anethole and *S*-(+)-Carvone against Larvae of the Gypsy Moth *Lymantria dispar* (L.). *Agronomy* **2022**, *12*, 3049. https://doi.org/10.3390/agronomy12123049

Academic Editors: Chunqing Zhao and Yueping He

Received: 18 October 2022
Accepted: 28 November 2022
Published: 1 December 2022

1. Introduction

Without appropriate management, insect pests cause enormous losses in forestry and agriculture [1–3]. Since the 1930s, inorganic (e.g., lead arsenate) and organic (e.g., DDT) synthetic insecticides, as well as insecticides of plant origin (rotenone, nicotine, and pyrethrum) have been extensively used against many pests and proved to be efficacious, persistent, and easy to apply [4,5]. However, their application raised concerns due to toxicity to non-target organisms, environmental pollution, threats to domestic animals and human health, as well as the evolution of pest resistance [6].

It was estimated that, despite spending USD 10 billion for plant protection in the USA, insects still caused up to 13% crop losses, and more than USD 9 billion had to be spent to manage environmental and health damages [7].

Therefore, other means of pest control have been explored to overcome the disadvantages of synthetic insecticides [8,9]. Among them, botanicals such as essential oils (EO)

and EO compounds are considered as the most viable alternatives. Their biodegradability lowers environmental and health risks, whereas diverse modes of action slow down the evolution of pest resistance [10]. Botanicals can provoke avoidance or attractant behavior that can be used in push–pull management strategies or induce physiological toxicity leading to reduced fitness and impairment of pest population dynamics [11,12]. Their efficacy might be comparable to chemical insecticides and repellents. For instance, triterpenoid azadirachtin, isolated from the neem tree *Azadirachta indica,* exhibited higher contact toxicity to several aphid species than malathion, carbosulfan, cypermethrin, and imidacloprid [13]. In addition, *Lippia origanoides* EO was ten times more repellent to a storage grain pest *Sitophilus zeamais* than the commercial product IR3535 [14]. Similarly, a higher antifeeding effect of *Cymbopogon martinii* EO than IR3535 was shown in two lepidopteran pests of African oil palm [15].

The gypsy moth (GM) (*Lymantria dispar* (L.) (Lepidoptera: Erebidae)) is a serious pest of tree species in forests, urban environments, and orchards [16–18]. During the outbreak, gypsy moth larvae (GML) defoliated and damaged trees in large areas in Europe, Australasia, and America [19,20]. In Serbia, in the period from 2011 to 2014, more than 300,000 ha of forests were attacked by GML, leading to the complete defoliation of not only optimal oak hosts but also of beech trees and conifer plantations [21]. Additionally, leaves of fruit trees, such as apricot, apple, blueberry, and plum, are suitable food for GML development and, thus, can suffer from defoliation during outbreaks [22,23].

The use of botanicals against tree pests in forests and orchards has been evaluated in numerous papers [24–26]. Furthermore, an azadirachtin-based formulation was developed and registered for use against many forest pest insects, including GM [4]. Several papers also addressed insecticidal and antifeeding effects of plant extracts [27,28], EOs [29–34]), and EO compounds [27,35] on the GM. Here, we evaluate the efficacy of two botanicals against GML. Phenylpropanoid *trans*-anethole is a major compound of *Foeniculum vulgare*, *Clausena austroindica*, *Pimpinella anisum*, and *Illicium verum* EOs [33,36–39], whereas (*S*)-(+)-carvone, an oxygenated monoterpene ketone, is present in *Carum carvi*, *Anethum graveolens*, *Mentha longifolia*, and *Lippia alba* EOs [33,38,40–42]. Toxicity, deterrent, and growth-reducing activity of these compounds have been confirmed in many stored products [43–49] and other crops and fruit pests [50–53].

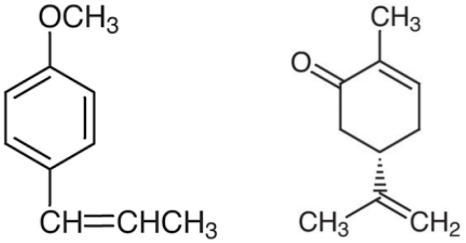
In the present paper, we assessed the impact of two EO compounds (anethole and carvone) on the GML feeding, survival, molting, and growth. Our study aimed to explore the different modes of action of the two botanicals. First, the antifeeding activity of anethole and carvone was estimated in choice and no-choice assays to estimate the relative importance of their influence on GML behavior and physiology. Second, to determine how different routes of entrance into larvae affect compounds toxicity, we compared larval mortality and molting after residual contact exposure and feeding on anethole-/carvone-supplemented diets. Third, we determined various nutritional indices related to food consumption and utilization to evaluate the contribution of pre- and post-ingestive physiological processes to changes in larval growth.

2. Materials and Methods

2.1. Chemicals

Phenylpropanoid *trans*-anethole (cat. no. 117870) and oxygenated monoterpene (S)-(+)-carvone (cat. no. 435759) (anethole and carvone in further text) were purchased from Sigma-Aldrich, St. Louis, MO, USA (Figure 1).

We also used a botanical insecticide NeemAzal®–T/S (neem in further text) developed by Trifolio–M GmbH, Lahnau, Germany, as a standard (positive control) compound to evaluate the efficacy of anethole and carvone on GML performance. Neem contains triterpenoid azadirachtin, known as a feeding deterrent and growth disruptant for most insects [54].



***trans*-Anethole** **(S)-(+)-Carvone**

Figure 1. Chemical structure of *trans*-anethole and (S)-(+)-carvone.

2.2. GML Rearing

Thirty GM egg masses were collected from natural populations in the oak (*Quercus robur* L.) forest Lipovica, Serbia (44°38′34″ N; 20°26′13″ E), during the autumn and maintained at 4 °C until the following spring. Eggs were processed according to Markovic et al. [55] by cleaning hairs mechanically, disinfecting by soaking in 0.1% NaOCl (sodium hypochlorite) solution for 5 min, washing with distilled water for 10 min, and air-drying. Approximately 100 cleaned eggs per egg mass were mixed and kept at 25 ± 1 °C, Rh = 65 ± 5%, and a photoperiod of 15:9 L:D. Ten hatched 1st instar larvae were transferred to each Petri dish (90 × 14 mm) and fed an artificial high wheat germ GM diet (MP Biomedicals, Inc., cat. no. 296029304) at the same temperature, humidity, and photoperiod. For diet preparation, agar (1.5 g) was dissolved in 45 mL of distilled water, and other ingredients (wheat germ 12 g, casein 2.5 g, salt mix 0.8 g, sorbic acid 0.2 g, methyl paraben 0.1 g, and vitamins 1 g) were mixed with another 45 mL of water. Agar was brought to a boil and mixed with other components.

2.3. Antifeeding Activity

The antifeeding activity was evaluated by no-choice and choice tests, with the 2nd instar larvae being starved for 24 h. A 2% agar–water layer, 2 mm thick, was poured into each Petri dish (90 × 14 mm), and after it became solid, the agar was covered with moistened filter paper. In the no-choice test, we placed one oak leaf disc (30 mm diameter) in the center of the Petri dish, whereas in the choice test, we placed two leaf discs (one treated and one control) on opposite sides of the Petri dish. Leaf discs were treated by the leaf-dipping method [56]. In the no-choice test, as described in Kostić et al. [33], leaf discs were immersed either in a 50% ethanolic solution of botanicals (anethole, carvone, or neem) at three different concentrations (0.1, 0.5, and 1.0%, which corresponds to 1, 5, and 10 µL/mL) or in a solvent (50% ethanolic solution, control disc) for 3 s. In the choice test, one leaf disc was treated with a botanical (at the same concentrations) and the other disc with the solvent. After the evaporation of solvent for 30 min, leaf discs were fixed to the agar layer with pins and one larva was placed into the center of each Petri dish. After 48 h, the remains of the consumed oak leaf discs were scanned at 200 dpi in jpg format by using Mustek A3 1200S scanner. ImageTool software [57] was used for quantification of the area of the remains (R) and the average area of uneaten discs (U). The consumed areas (CA) were calculated as CA = U − R. Each experimental group consisted of 25 replicates (larvae).

Data on the consumed areas of treated and control leaf discs were used for the calculation of absolute deterrence coefficients (ADC) in the no-choice assay and relative deterrence coefficients (RDC) in the choice test [58]:

$$ADC = (CC - TT)/(CC + TT) \times 100 \quad (1)$$

$$RDC = (C - T)/(C + T) \times 100 \quad (2)$$

where CC is the average area of consumed parts of the control leaf disc, and TT is the area of consumed parts of the treated leaf discs in the no-choice test; C is the consumed part of

the control leaf disc, and T is the consumed part of the treated leaf disc in the choice test. Negative values of coefficients suggest attractant properties of applied compounds.

2.4. Toxicity and Molting after Digestive and Contact Application of Chemicals

In the digestive toxicity test, the 2nd instar larvae, previously starved for 24 h, were fed for one day GML diet cubes with incorporated botanicals at concentrations 0 (control), 0.05, 0.1, 0.25, 0.5, and 1%, which corresponds to 0, 0.05, 0.1, 0.25, 0.5, and 1 mL of compounds per 100 g of artificial diet. Compounds were dissolved in 2 mL of 96% ethanol and thoroughly mixed with the diet before solidification. In the control diet, 2 mL of ethanol was added. The concentration range was chosen on the basis of the preliminary test in order to fit the probit analysis. In the residual contact toxicity test, the bottoms of the Petri dishes were covered with 0.5 mL ethanolic solutions of anethole, carvone, or neem at the same concentrations. After the evaporation of the solvent for 30 min at room temperature, larvae were placed into Petri dishes and fed the control diet cubes for one day.

After 24 h of exposure to anethole, carvone, and neem, larvae from both toxicity assays were transferred into clean Petri dishes and fed control diets for another 96 h (i.e., 120 h from the beginning of exposure). During the experiment, two fresh cubes of artificial diet per Petri dish were provided daily. Within each experimental group, five replicates with 10 larvae per replicate were analyzed. Larval mortality and molting into the 3rd larval instar were observed daily, up to 120 h, and percentages of mortality and molting were calculated.

2.5. Growth and Nutritional Indices

After molting into the 4th larval instar, larvae were transferred to clean Petri dishes (one larva per dish) and starved for 24 h. After starving, their masses were measured individually. GML were daily fed cubes of the artificial diet with incorporated botanicals (Bot). The chosen concentrations were 0 (control), 0.1, 0.25, and 0.5% (0, 0.1, 0.25, and 0.5 mL of compounds per 100 g of artificial diet) according to the preliminary results that revealed that larvae fed on Bot-supplemented diets decreased their mass at the concentration of 0.5% and higher. Cubes of artificial diet were weighed before and after the feeding trial, the excrements were weighed at the end of the trial, and larvae were weighed again after 48 h of feeding. All indices were assessed on a dry mass basis: larvae, uneaten cubes, and excrements were weighed after being dried at 65 °C for 72 h. A regression of dry on fresh mass in a random sample of 30 larvae and 30 cubes of artificial diet was used to estimate the dry mass of larvae and cubes of artificial diet at the beginning of the trial. The obtained data were used to calculate the growth and nutritional indices according to the standard formulae [59–61] (Table 1).

2.6. Statistical Analysis

Statistical analyses were carried out by software package Statistica 13 (TIBCO Software Inc., Palo Alto, CA, USA). After appropriate data transformations, Kolmogorov–Smirnov and Levene's tests were used to assess whether the data satisfied the assumptions of parametric ANOVA and *t*-test for normality and homoscedasticity, respectively. All analyzed traits had a normal distribution. Equal sample size and normal distribution of data in all experimental groups also enabled valid parametric ANOVA and *t*-test for traits with non-homogeneous variances where the ratio of the largest and smallest variance was less than four [62]. For variance ratios larger than four, we carried out non-parametric analyses (RDC, growth, and nutritional traits). Two-way ANOVA tested the significance of main (botanical type—Bot, botanical concentration—C) and interaction (Bot × C) effects on the trait variation, whereas one-way ANOVA and Dunnett's test compared the control with each treatment group.

Table 1. Formulae for calculation of growth and nutritional traits.

Traits	Formulae	Units
Mass gain	$MG = m_2 - m_0$	mg
Amount of consumed food	$m_c = d_2 - d_0$	mg
Amount of assimilated food	$m_a = m_c - m_e$	mg
Amount of metabolized food	$m_m = m_a - MG$	mg
Relative growth rate	$RGR = MG/(2 \times m_0)$	mg/mg/day
Relative consumption rate	$RCR = m_c/(2 \times m_0)$	mg/mg/day
Relative metabolic rate	$RMR = m_m/(2 \times m_0)$	mg/mg/day
The efficiency of conversion of ingested food (gross growth efficiency)	$ECI = MG/m_c \times 100$	%
Approximate digestibility (assimilation efficiency)	$AD = m_a/m_c \times 100$	%
The efficiency of conversion of digested food (net growth efficiency)	$ECD = MG/m_a \times 100$	%
Metabolic cost	$MC = 100 - ECD$	%

m_0—initial dry larval mass at the beginning of the experiment; m_2—dry larval mass at the end of the experiment (2 days of feeding); d_0—dry mass of uneaten diet cubes; d_2—dry mass of remains after 2 days feeding; 2—duration of the experiment expressed in days; m_e—dry mass of excrements.

For ADC, we applied parametric two-way ANOVA on untransformed data followed by LSM contrast. Untransformed values of consumed areas in each treatment group were compared with the control group by using Dunnett's test.

For RDC, we carried out two-way ANOVA on ranks with untransformed trait values. ANOVA was followed by LSM contrasts with Bonferroni adjustment to compare RDC among different concentrations within each compound and among compounds within each concentration. Consumed areas in the choice assay were sqrt(X+0.5)-transformed and the significance of the difference was tested by *t*-test for dependent samples. These comparisons revealed significant antifeeding/attractant activity within each treatment.

Mortality was analyzed with parametric two-way ANOVA on arcsine–square-root-transformed percentages of mortality. Groups with zero variances were omitted from analyses. For example, comparisons between control and treatment groups could not be performed because there was no mortality in the control group during the 5 days of the examination (zero variance). Median lethal concentrations (LC_{50}) for mortalities after 24, 48, 72, 96, and 120 h were calculated by using the probit model [63]. Non-overlapping/overlapping confidence intervals indicated a significant/non-significant difference in LC_{50} between anethole- and carvone-treated larvae within each period of observation. Due to low mortality, LC_{50} could not be determined on neem in the digestive toxicity assay or all botanicals in the contact toxicity assay. Kaplan–Meier survival probability, depending on the duration of exposure, was calculated for larvae fed diets containing anethole and carvone, and a log-rank test was applied to estimate the significance of survival distribution differences among botanicals within each concentration and among concentrations within each botanical.

Percentages of larvae molted from the 2nd to the 3rd instar after 120 h were also arcsine–square-root-transformed and analyzed by parametric two-way ANOVA and LSM contrasts, whereas Dunnett's test showed which treatment groups significantly differed from the control.

Since none of the growth and nutritional traits satisfied the assumption of homogeneity of variances, they were analyzed by non-parametric ANOVAs on ranks and appropriate planned (LSM contrasts) and post hoc comparisons (Dunnett's test).

3. Results

3.1. Feeding Deterrence

Feeding deterrent activity in no-choice assay was significantly affected by the applied compound (significant Bot term in two-way ANOVA, Figure 2A). The consumed area was approximately 1.8–2.1 times lower in neem-fed larvae than in the control group, and hence, deterrence was significant at all examined neem concentrations (Table A1). On average,

ADC did not differ between anethole- or carvone-fed larvae (Bonferroni test, $p = 0.154$), but both ADC values were significantly lower compared with the neem group ($p = 0.001$ and $p < 0.001$, respectively). Deterrence of the botanicals increased with concentration (significant C term in two-way ANOVA). At the highest concentration, anethole was more deterrent than carvone (LSM contrasts, $p = 0.005$) and showed efficacy similar to the neem ($p = 0.868$). Pattern of ADC changes with concentration was similar in the three botanical treatment groups (non-significant Bot × C term, Figure 2A).

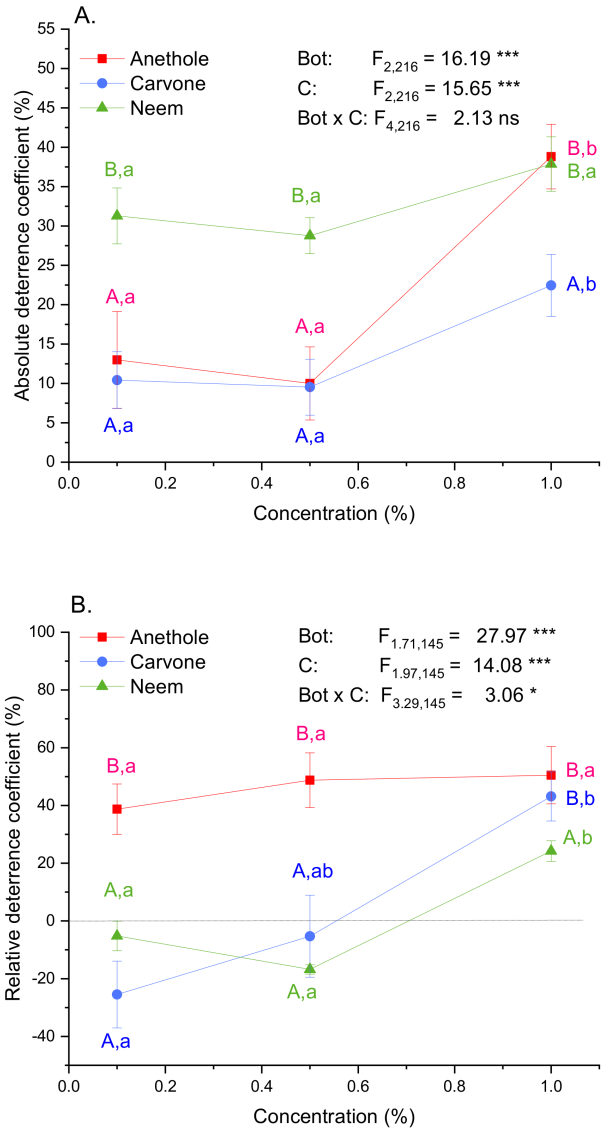


Figure 2. Absolute (**A**) and relative (**B**) deterrence coefficient (mean ± SE) in the 2nd instar GML. F-values indicate significance of the effects of botanicals (Bot), concentration (C), and interaction Bot × C on deterrence obtained by two-way ANOVA (ns non-significant, * $p < 0.05$, *** $p < 0.001$). Different-colored capital letters mark significant differences among botanicals within each concentration, whereas small letters mark significant differences among concentrations within each botanical (LSM contrasts, $p < 0.05$).

When larvae were offered a choice between control and treated leaf discs, anethole showed the most deterrent activity (significant Bot term in two-way ANOVA; Figure 2B). On average, anethole deterrence was higher than deterrence of carvone ($p < 0.001$) and neem ($p < 0.001$). In addition, concentration significantly influenced RDC, and the slope of RDC increase with concentration differed among compounds (significant C and Bot × C terms, respectively; Figure 2B). Activities of the two lowest concentrations were similar

($p = 0.785$), whereas activity at concentration of 1% was, on average, higher compared with 0.1% ($p < 0.001$) and 0.5% ($p = 0.001$). Interestingly, at two lower concentrations of carvone and neem, RDC values were negative, whereas at the highest concentration value, RDC significantly elevated and became positive. Comparisons of consumed areas of control and treated leaf discs revealed that 0.1% carvone and 0.5% neem acted as attractants and exhibited significant antifeeding activity only at the concentration of 1% (Table A2). In contrast, anethole significantly deterred feeding at all examined concentrations (Table A2).

3.2. Toxicity and Molting Reducing Effects

Results presented in Figure 3and Figure A1 show that mortality changes in GML depended on compound type, compound concentration, period of exposure, and mean of application. Compounds incorporated into the diet exhibited much higher toxicity than compounds applied on the bottoms of the Petri dishes (Figure 3A,B, respectively). In the first 48 h, there was no contact toxicity of examined compounds, whereas digestive toxicity of anethole/carvone increased with concentration (significant C term in two-way ANOVA) reaching values 72/78% at the concentration of 1% after 24 h (Figure A1A) and 88/92% after 48 h (Figure 3A). After 72, 96, and 120 h, contact toxicity did not exceed values of 4% at the highest concentration (data not shown), whereas feeding on anethole- or carvone-treated diets led to complete mortality at the highest concentration (Figure A1B–D). Neem exhibited low toxicity and, after 120 h at 1%, reached the value of 8% in contact toxicity assay (Figure 3B) and 14% in digestive toxicity assay (Figure A1D). Because of low toxicity, median lethal concentrations of neem could not be determined. Across all periods of exposure, digestive toxicity of anethole and carvone was similar (Figures 3A and A1). At 96 h and 120 h of exposure, when mortality in the neem group was included in the analysis, it became evident that botanical type significantly influenced mortality and pattern of its changes with concentration (significant Bot and Bot × C terms, Figure A1C,D). Average mortality was lower and increased more slowly with concentration in neem than anethole/carvone groups.

Median lethal concentrations of anethole and carvone decreased after a longer duration of exposure (Table 2). According to non-overlapping confidence intervals, the decrease was statistically significant after 72, 96, and 120 h relative to 24 h. In addition, the digestive toxicity of anethole and carvone was similar across all periods of exposure.

It can be noticed from Figure A2 that survival curves became steeper at higher compound concentrations. Significant influence of compound concentration on survival distribution was recorded both in anethole ($\chi^2 = 232.27$, df = 4, $p < 0.001$) and carvone experimental groups ($\chi^2 = 220.97$, df = 4, $p < 0.001$). It was detected that carvone provoked significantly steeper survival curves than anethole at concentrations of 0.05% ($\chi^2 = 4.76$, df = 1, $p = 0.029$), 0.1% ($\chi^2 = 8.70$, df = 1, $p = 0.003$), and 0.25% ($\chi^2 = 31.83$, df = 1, $p < 0.001$), whereas at higher concentrations, terpenoids were equally effective (0.5%: $\chi^2 = 2.42$, df = 1, $p = 0.120$; 1%: $\chi^2 = 0.28$, df = 1, $p = 0.594$).

All three compounds significantly reduced the percentage of larvae molted after 120 h compared with control larvae (Table A3). After feeding on botanical-supplemented diet, molting was determined up to the concentration of 0.25% because all the 2nd instar larvae died at higher concentrations. On a neem-supplemented diet, although mortality was low, none of the larvae molted into the 3rd instar at 0.5 and 1%. Exposure to compounds by contact showed weaker molting-reducing activity than exposure to a treated diet (Figure 4, Table A3). For example, 34 and 64% larvae successfully molted after contact with 1% anethole and carvone, respectively.

Table 2. Digestive toxicity of anethole and carvone against 2nd instar GML depending on the period of exposure.

Botanical	Period (h)	Slope ± SE (CI)	LC_{30} [a] (%) (CI)	LC_{50} [a] (%) (CI)	LC_{95} [a] (%) (CI)	χ^2 (df)	*p*
Anethole	24	2.25 ± 0.45 (1.36; 3.14)	0.323 (0.216; 0.408)	0.552 (0.441; 0.711)	2.970 (1.744; 9.699)	0.001 (1)	0.980
	48	3.02 ± 0.37 (2.30; 3.74)	0.274 (0.221; 0.324)	0.409 (0.346; 0.483)	1.433 (1.076; 2.228)	0.793 (2)	0.673
	72	4.40 ± 0.53 (3.37; 5.44)	0.220 (0.184; 0.252)	0.289 (0.252; 0.330)	0.683 (0.562; 0.911)	1.857 (2)	0.395
	96	8.42 ± 0.94 (6.58; 10.26)	0.198 (0.170; 0.228)	0.261 (0.231; 0.298)	0.456 (0.401; 0.537)	5.754 (3)	0.124
	120	7.97 ± 1.38 (5.27; 10.67)	0.177 (0.096; 0.264)	0.243 (0.173; 0.375)	0.450 (0.335; 0.805)	7.155 (3)	0.067
Carvone	24	2.35 ± 0.46 (1.45; 3.24)	0.287 (0.188; 0.365)	0.481 (0.382; 0.600)	2.411 (1.503; 6.623)	0.083 (1)	0.774
	48	3.05 ± 0.36 (2.34; 3.76)	0.242 (0.195; 0.287)	0.360 (0.305; 0.424)	1.247 (0.949; 1.884)	0.398 (2)	0.820
	72	4.19 ± 0.49 (3.23; 5.15)	0.205 (0.172; 0.237)	0.274 (0.238; 0.314)	0.677 (0.553; 0.910)	2.802 (2)	0.246
	96	8.34 ± 0.94 (6.50; 10.18)	0.189 (0.161; 0.218)	0.252 (0.223; 0.289)	0.449 (0.395; 0.532)	5.662 (3)	0.129
	120	8.85 ± 1.10 (6.70; 11.00)	0.187 (0.154; 0.219)	0.247 (0.215; 0.283)	0.432 (0.380; 0.514)	3.858 (2)	0.145

a—Lethal concentrations are presented with 95% confidence intervals (CI) and expressed in percentages. χ^2, *p*—results of Pearson's goodness-of-fit test.

Molting percentage significantly depended on the compound type (significant Bot term in two-way ANOVA; Figure 4A,B). Neem was the most effective in both toxicity assays. However, on average, more larvae molted on an anethole- than on a carvone-supplemented diet ($p = 0.001$; Figure 4A), whereas the opposite was obtained after contact application ($p = 0.002$; Figure 4B). Molting percentage gradually decreased with the increase of applied concentration (significant C term in two-way ANOVA; Figure 4A,B). The slope of molting decrease was the steepest in neem-treated larvae (significant Bot × C term in two-way ANOVA). In other words, at lower concentrations, neem exhibited significantly higher activity than anethole and carvone. However, after digestive application, the three compounds were equally efficient at a concentration of 0.25% (Figure 4A). After contact application, compound efficacy was ranked as neem > anethole > carvone at all examined concentrations (Figure 4B).

3.3. Larval Growth and Food Consumption, Assimilation, and Metabolization

GML fed a diet containing anethole, carvone, or neem significantly reduced mass gain, as well as amounts of consumed, assimilated, and metabolized food compared with the control (Figure 5, Table A4). The mass gain was approximately 80% reduced in neem-fed larvae and in larvae fed 0.1 and 0.5% anethole-supplemented diet. Larvae lost their mass, and thus, exhibited negative mass gain in the 1% anethole group and on carvone diet, regardless of concentration (Figure 5A). Reductions in the mass of consumed, assimilated, and metabolized food in treatment groups were within a range of 60–72, 67–77, and 39–67%, respectively.

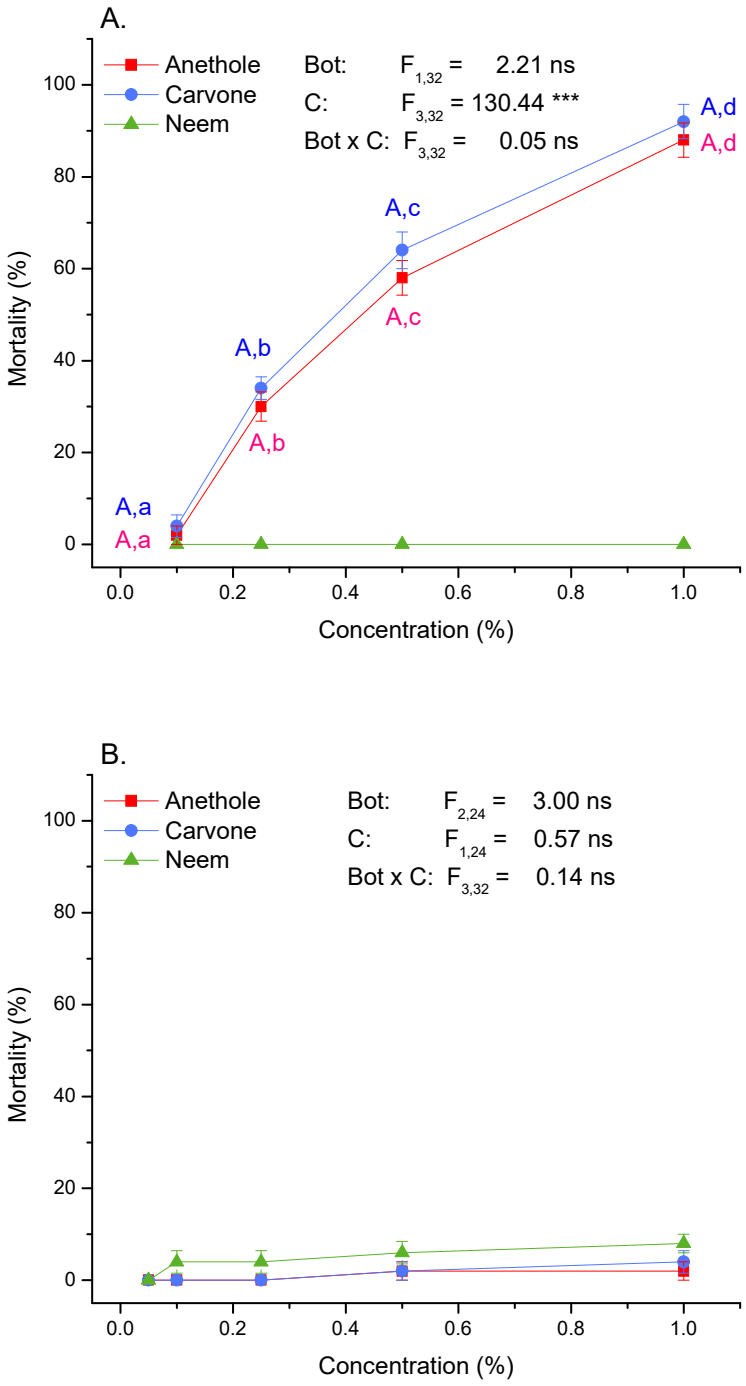


Figure 3. (**A**) Digestive toxicity after 48 h (24 h consumption of Bot-supplemented diet + 24 h untreated diet) and (**B**) contact toxicity after 120 h (24 h exposure to treated glass bottom of Petri dish + 96 h in untreated dishes) in the 2nd instar GML (mean ± SE). F-values were obtained from two-way ANOVA, testing significance of the main (botanicals—Bot, concentration—C) and interaction (Bot × C) effects on mortality (ns non-significant, *** $p < 0.001$). ANOVA for digestive toxicity included 0.1–1.0% concentrations of botanicals, whereas contact toxicity analysis included 0.5 and 1.0% concentrations. Different-colored capital letters mark significant differences among botanicals within each concentration, whereas small letters mark significant differences among concentrations within each botanical (LSM contrasts, $p < 0.05$). There was no mortality in control GML up to 120 h.

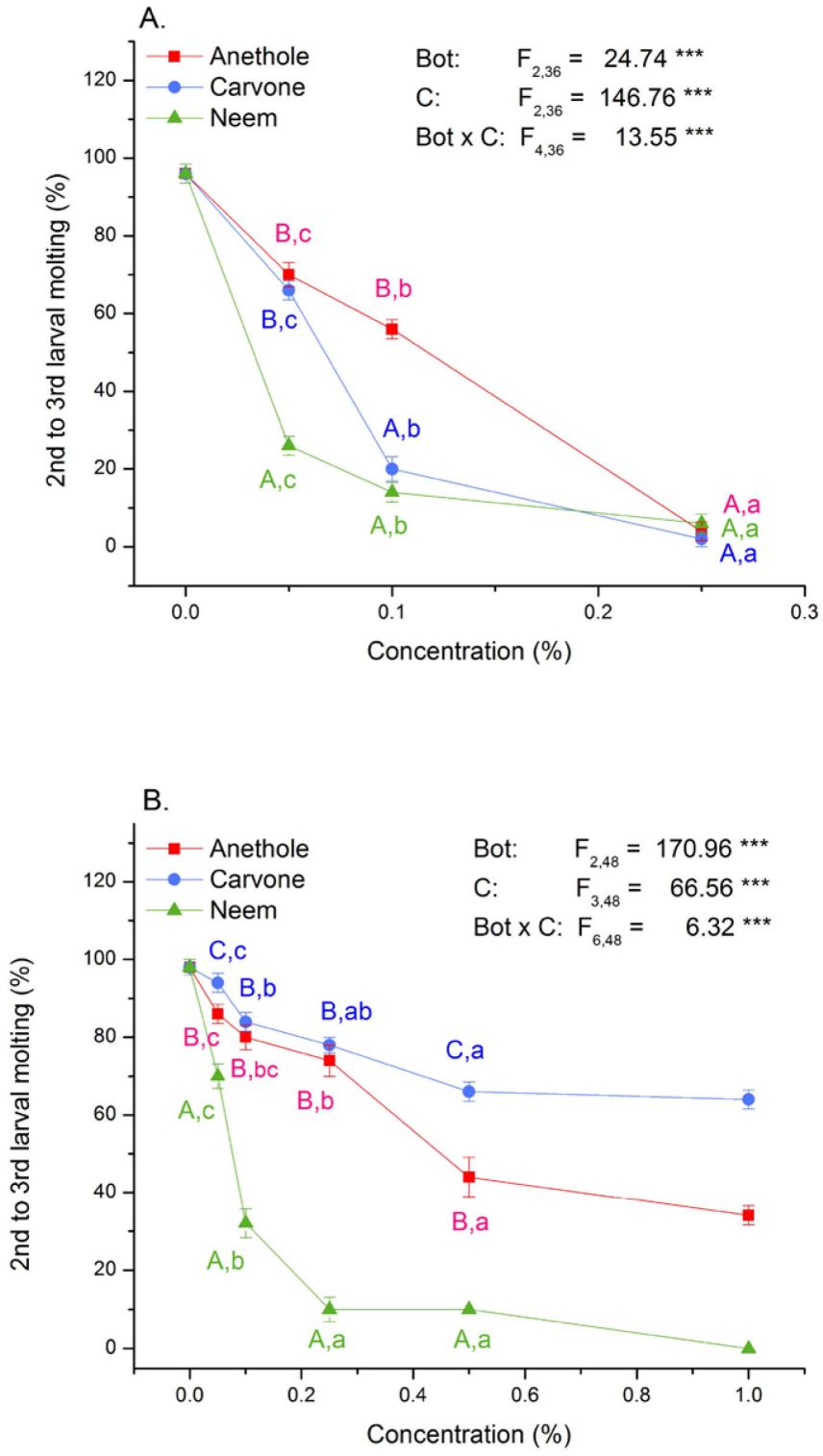


Figure 4. Means (± SE) of the percentages of molted GML after 120 h of (**A**) digestive application of botanicals (24 h consumption of control or treated diet + 96 h on an untreated diet) and (**B**) contact application (24 h exposure to treated glass bottom of Petri dish + 96 h in untreated dishes). F-values were obtained from two-way ANOVA, testing the significance of the main (botanicals—Bot, concentration—C) and interaction (Bot × C) effects on molting percentage (*** $p < 0.001$). Different-colored capital letters mark significant differences among botanicals within each concentration, whereas small letters mark significant differences among concentrations within each botanical (LSM contrasts, $p < 0.05$).

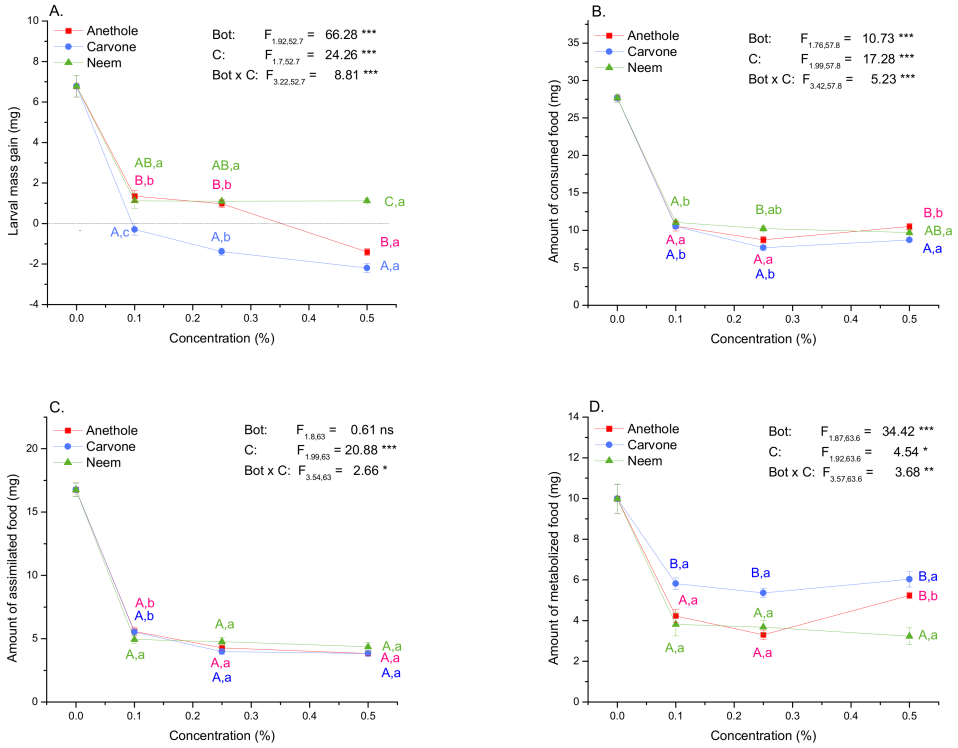


Figure 5. Mass gain (**A**) and amounts of consumed (**B**), assimilated (**C**), and metabolized food (**D**) (mean ± SE) in the 4th instar GML depending on the botanical type (Bot) and concentration (C). F-values indicate significance of the effects of Bot, C, and interaction Bot × C terms in non-parametric two-way ANOVA (ns non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Different-colored capital letters mark significant differences among botanicals within each concentration, whereas small letters mark significant differences among concentrations within each botanical (LSM contrasts, $p < 0.05$).

On average, larval mass gain and amounts of consumed and metabolized food depended on applied botanical type (significant Bot term in two-way ANOVA; Figure 5). Compared with anethole/neem, carvone was more effective in reducing mass gain ($p < 0.001$/mboxemphp < 0.001) and food consumption ($p = 0.025$/$p < 0.001$) and in increasing metabolization ($p < 0.001$/$p < 0.001$). In addition, on average, all nutritional traits were affected by compound concentration (significant C term in two-way ANOVA; Figure 5). However, the shape of trait changes with concentration increase depended on the compound type (significant Bot × C term in two-way ANOVA; Figure 5). For example, mass gain, assimilation, and metabolization remained unchanged in the neem group, whereas in the anethole group, there were lower MG and m_a and higher m_m values recorded at the highest concentration than at the lowest concentration.

3.4. Growth and Nutritional Indices

Performance was mostly significantly reduced in anethole-, carvone-, and neem-fed larvae compared with the control (Figure 6; Table A5). The reduction of RGR, ECI, and ECD at the highest concentration of anethole and all examined carvone concentrations exceeded 100% because larvae lost weight (anethole: 121, 155, and 193%, respectively; carvone: 104–133, 112–203, and 114–244%, respectively). RCR in treatment groups was reduced by 60–70%, indicating primary feeding deterrence. However, the large decrease in ECI revealed that applied compounds also affected post-ingestive processes. ECI decrease can result from the lower proportion of consumed food that was assimilated (AD) and the lower proportion of assimilated food that was allocated to growth (ECD). For anethole/carvone treatment groups and the 0.25% neem group, both AD (12–39% decrease) and ECD (41–244% decrease) significantly contributed to ECI reduction. Due to larger standard error, ECD values in 0.1 and 0.5% neem groups did not differ from the control (Table A5), which led to the

conclusion that changes in digestion were the main cause of reduced ECI on the neem-supplemented diet. RMR was significantly lower (39–63%) in treatment than in control groups due to reduced assimilation (Figure 6C, Table A5). Examined EO compounds imposed an increased metabolic cost of food processing that reached 133 and 168% higher values on 0.5% anethole and carvone, respectively (Figure 6G, Table A5).

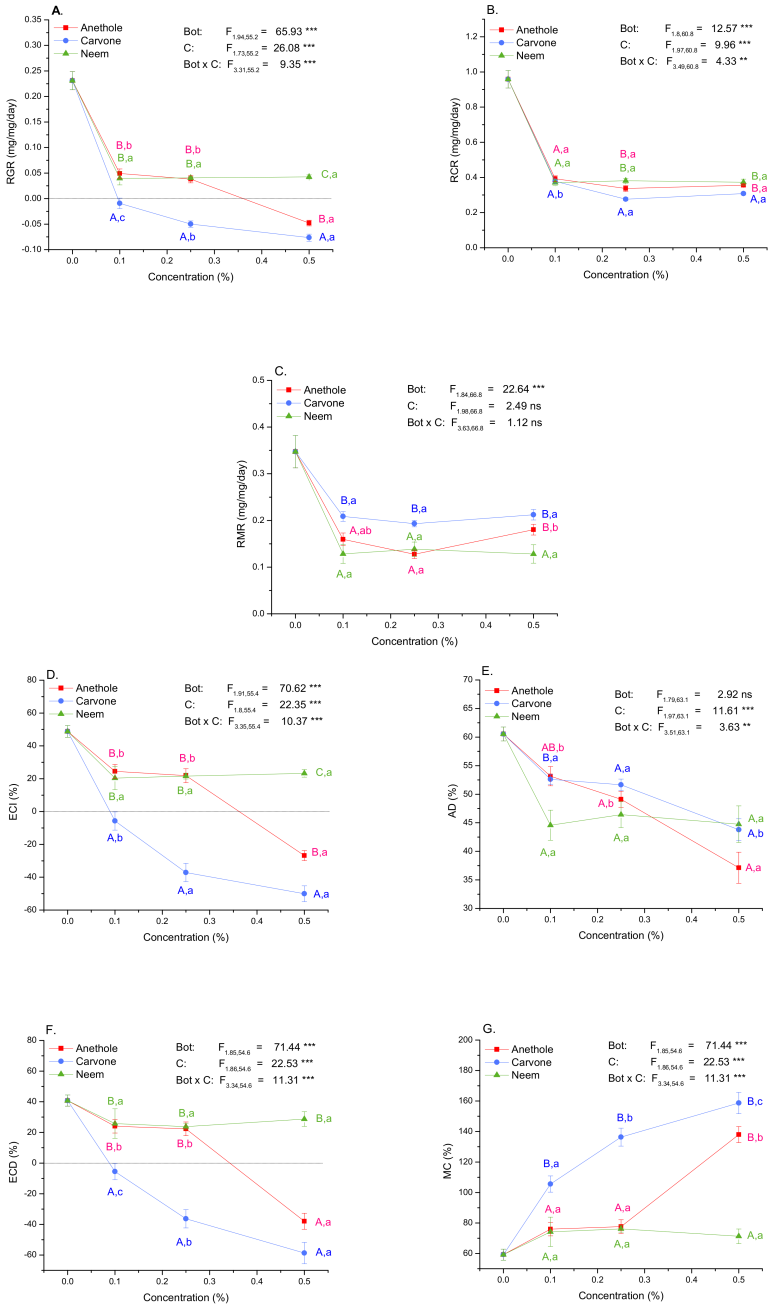


Figure 6. Nutritional indices (mean ± SE) in the 4th instar GML after 48 h of feeding on control and diet treated with botanicals. RGR—relative growth rate (**A**); RCR—relative consumption rate (**B**); RMR—relative metabolic rate (**C**); ECI—efficiency of conversion of ingested food (**D**); AD—approximate digestibility (**E**); ECD—efficiency of conversion of digested food (**F**); and MC—metabolic cost (**G**). F-values obtained by nonparametric two-way ANOVA indicate the significance of the main and interaction effects of botanical type (Bot) and concentration (C) (ns non-significant, ** $p < 0.01$, *** $p < 0.001$). Different-colored capital letters mark significant differences among botanicals within each concentration, whereas small letters mark significant differences among concentrations within each botanical (LSM contrasts, $p < 0.05$).

All indices, except AD, were significantly affected by compound type (significant Bot term in two-way ANOVA, Figure 6). Carvone was the most effective compound in reducing larval performance. On average, compared with anethole/neem, carvone provoked lower RGR, RCR, ECI, and ECD, whereas RMR and MC were higher ($p < 0.001/p < 0.001$). Carvone also showed the steeper change of RGR, RCR, ECI, ECD, and MC with concentration increase (significant Bot × C term in two-way ANOVA, Figure 6). Performance traits did not change with changing neem concentration.

4. Discussion

Terpenoids and phenylpropanoids play diverse roles in plants. In addition to interorganismal signaling, acclimation to stress, UV protection, and floral color/scent, they are involved in direct plant defense from pest insect attacks [64]. Gypsy moth is an extremely polyphagous insect and frequently encounters terpenes in its food. For example, GML feeding on host leaves induces biosynthesis of defensive terpenoids and phenylpropanoids [65]. GML also utilize several conifer species and can successfully metabolize various terpenoids [66]. On the other hand, compounds from non-host leaves [55,67] exhibited negative effects on GML behavior and fitness and were suggested to have the potential for use in gypsy moth control. Similar to results obtained in many other insect species (reviewed in [12,68]), here we showed that anethole (phenylpropanoide) and carvone (monoterpenoid ketone) deterred feeding, induced mortality, retarded molting, and reduced GML growth through influencing both pre- and post-digestive processes.

4.1. Antifeeding Activity Depends on the Applied Assay and Compound Concentration

Ranking feeding deterrence coefficients among anethole, carvone, and neem differed between the two applied assays (choice and no-choice). On average, neem was the most effective deterrent in no-choice assay and anethole was the most effective in the choice test. Higher ADC than RDC values of carvone and neem indicate that their antifeeding activity was based mostly on post-digestive toxicity, whereas higher RDC for anethole points to true antifeedant activity based on its interaction with gustatory receptors. Interestingly, anise and fennel EOs, which contain anethole, were more deterrent in the no-choice situation [33], which possibly resulted from the physiological toxicity of other EO components and/or their synergistic influence on anethole activity.

Compared with deterrence of carvone and anethole detected in the present study, higher deterrence (ADC) against GML was recorded on linalool, linalool-rich fraction of *Ocimum basilicum* EO, ethanol extracts of *Aesculus hipocastannum* [28], and EOs from *O. basilicum*, *Athamantha haynaldii*, and *Myristica fragrans* [27,30,31]. High repellency of S-(+)-carvone and *trans*-anethole was recorded in various pests [69–71].

The antifeeding activity of EO compounds is affected by their physical characteristics and species–specific structure of target molecules in pests [72]. Therefore, distinct responses can be obtained depending on the compound type and pest species [43,48]. Food odor/taste can stop or reduce feeding via interaction with olfactory/gustatory receptors which provide specific odor/taste code [73,74]. In addition, the interaction of compounds with the octopaminergic system underlies deterrence and other adverse effects on insect behavior [50,75]. Since octopamine receptors specifically bind monophenolic amines with a single hydroxyl group, they can bind compounds such as linalool and anethole. Zaio et al. [76] revealed a positive correlation between the physical characteristics of compounds (lipophilicity, polar surface) and their repellency. Therefore, phenolic structure and higher lipophilicity of anethole possibly explain its higher deterrence relative to carvone.

Depending on the concentration, terpenoids and phenylpropanoids can act as attractants or feeding stimulants of pest insects. Our study revealed a low increase in food ingestion (<30%) on neem and carvone, whereas anethole acted as a deterrent at all examined concentrations. In contrast, studies on other pests showed high attraction to neem, carvone [77–79], and anethole [80,81]. The question arises as to how the same compound (carvone) triggers opposite behavioral responses of GML at low and high concentrations.

It seems that a low quantity of volatile botanicals not only may be insufficient to prevent recognition of host leaf disc but also may induce stimulant odor/taste code and lead to higher acceptance of treated than control leaf disc for feeding.

4.2. Mortality and Molting Are Differently Affected by Oral and Contact Application of Compounds

Our results on anethole and carvone indicate that they are more effective digestive toxicants for GML than linalool [27], carvacrol [35], and EOs of *Thymus herba-barona*, *Cinnamomum zeylanicum*, *Helychrisum italicum*, *Myrtus communis*, *Rosmarinus officinalis* [29], and *Tanacetum vulgare* [32]. After 120 h, 0.5% anise, dill, and fennel EOs induced mortality lower than 40% [33], whereas all larvae died on an anethole- and carvone-supplemented diet.

Our finding of a higher mortality and lower percentage of molted larvae in GML fed on a diet supplemented with tested compounds than in GML exposed to their residuals is consistent with the results of the previous study assessing the effects of *T. vulgare* EO on the gypsy moth [32]. Similarly, higher mortality after oral than the topical application of azadirachtin has been recorded in three lepidopteran pests [82]. Characteristics of compounds strongly affect their efficacy after delivery through inhalation, ingestion, or cuticle absorption. Higher vapor pressure is suggested to promote fumigant toxicity, whereas lipophilicity promotes good contact toxicity [83]. For example, thymol, which is more lipophilic and has lower vapor pressure than anethole, led to total mortality of *T. castaneum* at lower concentration in contact and digestive toxicity assays, whereas the advantage of anethole was revealed in the fumigant assays [45,46]. High values of electric polarity and electronegativity of carbonyl carbon contribute to higher contact toxicity of carvone than anethole for *S. zeamais* [76,84].

Regardless of application mode, our study did not reveal differences in anethole and carvone toxicity. On the other hand, reduction of molting was more expressed in larvae fed on the carvone diet, whereas anethole was more effective in contact assay. Higher lipophilicity of anethole might contribute to higher penetration through the cuticle. However, the result that azadirachtin, the least lipophilic compound, was the most effective in both assays in reducing larval molting points out that activity cannot be predicted simply from the compound structure and physical characteristics.

Cuticle composition and interaction with various neurotoxicity and enzymatic targets determine how pests respond to applied compounds [12]. It has been suggested that octopamine receptors mediate anethole toxicity [75]. In addition, anethole [85] and carvone [44,52,86] negatively affect cholinergic systems by inhibiting acetylcholinesterase (AChE). Usually, a weak correlation was found between AChE inhibition and toxicity, implying the involvement of different targets of toxicity. Anethole and carvone or EOs rich in these compounds may inhibit ATPase, alkaline and acid phosphatases, and digestive and detoxification enzymes [26,52,87–89]. Evidently, modes of action may impair different functions in pests, such as the transmission of nerve impulses, locomotion, digestion, and defense from insecticides.

Our result showed that neem standard, which contained azadirachtin, induced negligible mortality but was highly effective in molting reduction after 5 days. In other pests, prolonged exposure to azadirachtin leads to larval/pupal death, increased development time, reduced fecundity, and emergence of smaller and/or malformed adults with thinner cuticle [90,91]. The main mechanism of molting reduction activity is reducing the level of molting hormone through the inhibition of P450-monooxygenase [92]. Anethole and carvone may have similar developmental consequences as azadirachtin [51,93–95], but the mechanism of molting reduction is unknown. It has been found that juvocimene, a fused structure of β-ocimene and *trans*-anethole, possesses strong juvenile hormone activity in the milkweed bug [96].

4.3. Carvone Is the Most Effective Growth-Reducing Compound

All three tested botanicals significantly impaired GML growth, food consumption, and metabolism, but carvone was the most effective. Namely, carvone presence in food provoked negative values of mass gain and RGR at all applied concentrations, which was a consequence of the highest reduction in total consumption/RCR (pre-ingestive effects) and gross/net growth efficiencies (post-ingestive/post-digestive effects). Not only was carvone the best antifeedant for the 4th instar GML, but also feeding on carvone diet imposed high metabolic cost. In anethole-fed larvae, similar negative effects on growth and metabolism were revealed only at the highest concentration.

The reduction of growth and nutritional indices in response to anethole and carvone was mostly similar to the effects of anise and fennel EOs that contained anethole (87.48 and 65.05%, respectively) and dill EO that contained carvone (42.47%) as major compounds [33]. Taking into account the high proportion of anethole in anise EO, we can conclude that other minor components did not contribute significantly to GML nutritional physiology. The fact that fennel EO, with lower anethole content, was still equally effective as a pure major compound implies the important role of other components. Such a relationship was also observed between pure carvone and dill EO, with the exception of ECI/ECD, which were significantly affected by other components only at the highest concentration.

The growth-reducing effect of carvone and anethole was confirmed in various pest species [47,49,50,97–99]. Consistent with our results, mass gain decreased during the feeding period and negative values of gross/net growth efficiencies were recorded in *S. zeamais* exposed to *S*-(+)-carvone [49] and *Pseudaletia unipuncta* exposed to *trans*-anethole [98]. It has been shown that anethole-rich anise EO and carvone cause damages to gut cells of pests [88,100], which, together with the inhibition of digestive enzymes by anethole and carvone [26,88,101,102], might explain reduced assimilation efficiency observed in our experiment.

Post-digestive effects of EO compounds stem from increased utilization of energy resources for reparation processes and defense [35,98,103]. Post-digestive effects of anethole were thoroughly studied [26,85,104]. For example, in *M. persicae*, energy metabolism pathways, ABC transporters critical for detoxification, chaperon protein Hsp40, and cuticle proteins were up-regulated in response to anethole [104]. In addition, carvone-rich EOs elevate activities of antioxidative and detoxification enzymes [89,105]. It has been found that in *S. litura*, detoxification of anethole and carvone is achieved by hydroxylation catalyzed by cytochrome P450 enzymes [106,107]. In total, pest survival and growth in the presence of stressful EO compounds will depend on the pest capability to use reparation and antioxidative and detoxification mechanisms to mitigate the harm.

5. Conclusions

Our results suggest that anethole and carvone have the potential to be used in GM control. At low concentrations, anethole provoked medium feeding deterrence, whereas carvone was a weak attractant for the 2nd instar larvae. Both compounds are good oral toxicants, but molting reduction caused by the presence of carvone in food was more expressed and can be compared to the effects of neem standard. In addition, carvone exhibited the most severe impact on the 4th instar GML growth and food utilization. Further investigations are needed to (1) assess means of deterrence/attraction improvement; (2) understand physiological mechanisms underlying anethole and carvone toxicity, molting, and growth-reducing effects; (3) develop appropriate water-soluble and persistent formulation that can be tested in natural forest systems or nurseries; and (4) evaluate effects of the formulation on non-target organisms.

Author Contributions: Conceptualization, M.K. and S.M.; methodology S.M. and J.L.; formal analysis, I.K., D.Š.J. and J.L.; investigation, I.K., M.K., D.Ć. and J.L.; resources, M.K. and S.M.; data curation, I.K., D.Š.J. and J.L.; writing—original draft preparation, J.L. and D.Š.J.; writing—review and editing, SM., I.K. and L.J.; supervision, M.K. and S.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, grant number 451-03-68/2022-14/200007.

Institutional Review Board Statement: The study was conducted according to the Serbian and European ethical normative (Directive 2010/63/EU) on the protection of animals used for experimental and other scientific purposes. Among invertebrates, ethical protection is granted to cephalopodes by the EU and Serbian legislatives. The national ethical legislative also grants protection to endangered species. However, *Lymantria dispar* does not fall into one of these categories, and this study is in concordance with current state of ethical legislative in the EU and the Republic of Serbia.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Results of the Dunnett test following non-parametric one-way ANOVA (F-value) for comparisons of consumed leaf areas in no-choice assay between untreated control leaf discs and discs treated with different concentrations of botanicals. Significant *p*-values are marked in bold.

		Area Consumed (mm^2)		
Botanical	**Conc (%)**	$\bar{x}$	**SE**	***p***
Anethole	0.1	128.12	13.70	0.7279
	0.5	127.77	9.92	0.7073
	1.0	68.19	6.70	**<0.0001**
Carvone	0.1	123.77	9.17	0.4658
	0.5	125.71	8.73	0.5812
	1.0	98.15	9.17	**0.0020**
Neem	0.1	79.52	6.35	**<0.0001**
	0.5	81.36	3.88	**<0.0001**
	1.0	68.56	6.13	**<0.0001**
Control	0.0	144.08	9.71	
ANOVA			$F_{9,240} = 10.63$	**<0.0001**

Table A2. Results of *t*-test for dependent samples for comparisons of control and treated leaf area consumed in the two-choice assay. Significant *p*-values are marked in bold. **D**—significant feeding deterrent (positive t-values), **A**—significant feeding attractant (negative t-values) and N—neutral activity (non-significant difference).

		Control Area Consumed (mm^2)		Treated Area Consumed (mm^2)					
Botanical	**Conc (%)**	$\bar{x}$	±**SE**	$\bar{x}$	±**SE**	**t**	**df**	***p***	**Activity**
Anethole	0.1	103.91	9.21	51.53	9.03	4.11	24	**<0.001**	**D**
	0.5	140.16	16.07	40.43	7.10	5.15	24	**<0.001**	**D**
	1.0	128.98	13.98	33.43	5.98	5.50	24	**<0.001**	**D**
Carvone	0.1	66.05	11.40	117.43	12.73	−2.32	24	**0.029**	**A**
	0.5	80.40	13.20	85.19	12.90	0.25	24	0.803	N
	1.0	85.30	6.03	40.53	9.29	4.04	24	**0.001**	**D**
Neem	0.1	177.62	7.24	208.96	14.32	1.36	24	0.186	N
	0.5	234.71	6.21	328.93	6.26	−10.33	24	**<0.001**	**A**
	1.0	266.69	7.22	166.70	10.16	6.96	24	**<0.001**	**D**

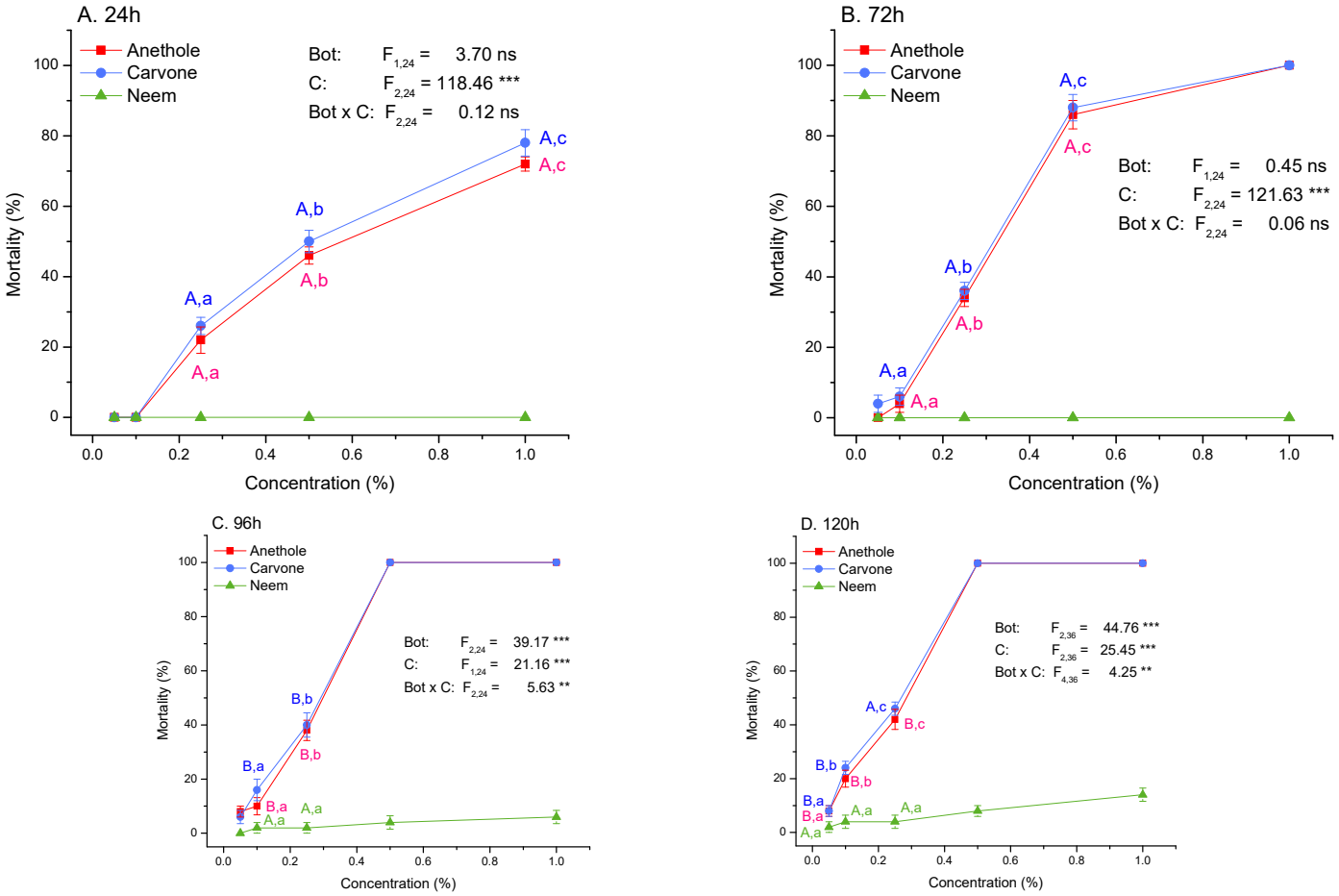


Figure A1. Digestive toxicity after 24 h (**A**), 72 h (**B**), 96 h (**C**), and 120 h (**D**) (24 h consumption of botanical-supplemented diet + 0, 48, 72, and 96 h on the untreated diet, respectively) in the 2nd instar GML (mean ± SE). F-values were obtained from two-way ANOVA, testing the significance of the main (botanical—Bot, concentration—C) and interaction (Bot × C) effects on mortality (ns non-significant, ** $p < 0.01$, *** $p < 0.001$). Different-colored capital letters mark significant differences among botanicals within each concentration, whereas small letters mark significant differences among concentrations within each botanical (LSM contrasts, $p < 0.05$). There was no mortality in control GML up to 120 h.

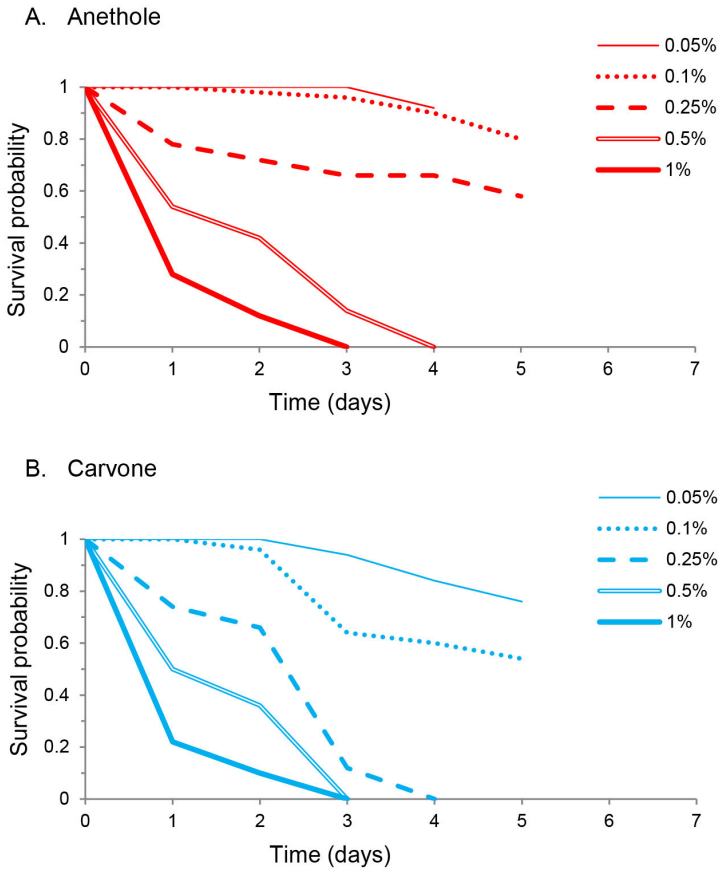


Figure A2. Kaplan–Meier survival probability in GML exposed to anethole (**A**) and carvone (**B**) at different concentrations (0.05–1%).

Table A3. Results of the Dunnett test following one-way ANOVA (F-value) for comparisons of the GML molting percentage between control and treatment groups after 120 h (24 h of contact or digestive application of botanicals + 96h without treatment). Significant *p*-values are marked in bold.

		Contact Application Percentage of Molting			**Digestive Application Percentage of Molting**		
Botanical	**Conc (%)**	$\bar{x}$	±**SE**	***p***	$\bar{x}$	±**SE**	***p***
Anethole	0.05	86	2.45	**<0.001**	70	3.16	**<0.001**
	0.10	80	3.16	**<0.001**	56	2.45	**<0.001**
	0.25	74	4.00	**<0.001**	4	2.45	**<0.001**
	0.50	44	5.10	**<0.001**	0	-	
	1.00	34	2.45	**<0.001**	0	-	
Carvone	0.05	94	2.45	0.403	66	2.45	**<0.001**
	0.10	84	2.45	**<0.001**	20	3.16	**<0.001**
	0.25	78	2.00	**<0.001**	2	2.00	**<0.001**
	0.50	66	2.45	**<0.001**	0	-	
	1.00	64	2.45	**<0.001**	0	-	
Neem	0.05	70	3.16	**<0.001**	26	2.45	**<0.001**
	0.10	32	3.74	**<0.001**	14	2.45	**<0.001**
	0.25	10	3.16	**<0.001**	6	2.45	**<0.001**
	0.50	8	2.00	**<0.001**	0	0	
	1.00	0	0		0	0	
Control	0.00	98	2.00		96	2.45	
ANOVA			$F_{14,60}$ = 57.16	**<0.001**		$F_{9,40}$ = 68.85	**<0.001**

Table A4. Results of the Dunnett test following non-parametric one-way ANOVA (F-value) for comparisons of nutritional traits between control and treatment groups of 4th instar GML fed for 48h on control or botanical-supplemented diets. MG—mass gain, m_c—mass of consumed food, m_a—mass of assimilated food, and m_m—mass of metabolized food. Significant *p*-values are marked in bold.

		MG	**m_c**	**m_a**	**m_m**
Botanical	**Conc (%)**	***p***	***p***	***p***	***p***
Anethole	0.10	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	0.25	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	0.50	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Carvone	0.10	**<0.001**	**<0.001**	**<0.001**	**0.042**
	0.25	**<0.001**	**<0.001**	**<0.001**	**0.001**
	0.50	**<0.001**	**<0.001**	**<0.001**	**0.043**
Neem	0.10	**0.001**	**<0.001**	**<0.001**	**<0.001**
	0.25	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	0.50	**<0.001**	**<0.001**	**<0.001**	**<0.001**
ANOVA		$F_{5.57,53.2}$ = 40.10 **<0.001**	$F_{6.05,58.1}$ = 16.68 **<0.001**	$F_{6.54,63.2}$ = 12.75 **<0.001**	$F_{7.14,69.6}$ = 16.86 **<0.001**

Table A5. Results of the Dunnett test following non-parametric one-way ANOVA (F-values) for comparisons of growth and nutritional indices between control and treatment groups in 4th instar GML fed for 48h on control or botanical-supplemented diets. RGR—relative growth rate, RCR—relative consumption rate, RMR—relative metabolic rate, ECI—efficiency of conversion of ingested food, AD—approximate digestibility, ECD—efficiency of conversion of digested food, and MC—metabolic cost. Significant *p*-values are marked in bold.

		RGR	**RCR**	**RMR**	**ECI**	**AD**	**ECD**	**MC**
Botanical	**Conc (%)**	***p***	***p***	***p***	***p***	***p***	***p***	***p***
Anethole	0.10	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.013**	**0.025**	**0.025**
	0.25	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.014**	**0.014**
	0.50	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Carvone	0.05	**<0.001**	**<0.001**	**0.007**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	0.10	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	0.25	**<0.001**	**<0.001**	**0.030**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Neem	0.05	**0.001**	**<0.001**	**<0.001**	**0.003**	**<0.001**	0.156	0.156
	0.10	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	0.25	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.086	0.086
ANOVA		$F_{5.83,55.8}$ = 40.96 **<0.001**	$F_{6.34,61.1}$ = 14.23 **<0.001**	$F_{7.35,71.9}$ = 11.33 **<0.001**	$F_{5.79,55.4}$ = 38.00 **<0.001**	$F_{6.8,66}$ = 9.45 **<0.001**	$F_{5.66,54.1}$ = 30.75 **<0.001**	$F_{5.66,54.1}$ = 30.75 **<0.001**

References

1. Oerke, E.C. Crop losses to pests. *J. Agric. Sci.* **2006**, *144*, 31–43. [CrossRef]
2. Aukema, J.E.; Leung, B.; Kovacs, K.; Chivers, C.; Britton, K.O.; Englin, J.; Frankel, S.J.; Haight, R.G.; Holmes, T.P.; Liebhold, A.M.; et al. Economic impacts of non-native forest insects in the continental United States. *PLoS ONE* **2011**, *6*, e24587. [CrossRef] [PubMed]
3. Cross, J.; Fountain, M.; Markó, V.; Nagy, C. Arthropod ecosystem services in apple orchards and their economic benefits. *Ecol. Entomol.* **2015**, *40*, 82–96. [CrossRef]
4. Holmes, S.B.; MacQuarrie, C.J. Chemical control in forest pest management. *Can. Entomol.* **2016**, *148*, S270–S295. [CrossRef]
5. Tudi, M.; Daniel Ruan, H.; Wang, L.; Lyu, J.; Sadler, R.; Connell, D.; Chu, C.; Phung, D.T. Agriculture development, pesticide application and its impact on the environment. *Int. J. Environ. Res. Public Health* **2021**, *18*, 1112. [CrossRef] [PubMed]

6. Aktar, W.; Sengupta, D.; Chowdhury, A. Impact of pesticides use in agriculture: Their benefits and hazards. *Interdiscip. Toxicol.* **2009**, *2*, 1–12. [CrossRef]
7. Pimentel, D. Environmental and economic costs of the application of pesticides primarily in the United States. *Environ. Dev. Sustain.* **2005**, *7*, 229–252. [CrossRef]
8. Horowitz, A.R.; Ellsworth, P.C.; Ishaaya, I. Biorational pest control—An overview. In *Biorational Control of Arthropod Pests*; Ishaaya, I., Horowitz, A.R., Eds.; Springer: Dordrecht, The Netherlands, 2009; pp. 1–20. [CrossRef]
9. Cantrell, C.L.; Dayan, F.E.; Duke, S.O. Natural products as sources for new pesticides. *J. Nat. Prod.* **2012**, *75*, 1231–1242. [CrossRef]
10. Pavela, R. History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects—A review. *Plant Protect Sci.* **2016**, *52*, 229–241.
11. Zhang, Z.; Sun, X.; Luo, Z.; Gao, Y.; Chen, Z. The manipulation mechanism of "push–pull" habitat management strategy and advances in its application. *Acta Ecol. Sin.* **2013**, *33*, 94–101. [CrossRef]
12. Abdelgaleil, S.A.M.; Gad, H.A.; Ramadan, G.R.; El-Bakry, A.M.; El-Sabrout, A.M. Monoterpenes: Chemistry, insecticidal activity against stored product insects and modes of action—A review. *Int. J. Pest Manag.* **2021**, 1–23. [CrossRef]
13. Khalequzzaman, M.; Nahar, J. Relative toxicity of some insecticides and azadirachtin against four crop infesting aphid species. *Univ. J. Zool. Rajshahi Univ.* **2008**, *27*, 31–34. [CrossRef]
14. Nerio, L.S.; Olivero-Verbel, J.; Stashenko, E.E. Repellent activity of essential oils from seven aromatic plants grown in Colombia against *Sitophilus zeamais* Motschulsky (Coleoptera). *J. Stored Prod. Res.* **2009**, *45*, 212–214. [CrossRef]
15. Hernández-Lambraño, R.; Caballero-Gallardo, K.; Olivero-Verbel, J. Toxicity and antifeedant activity of essential oils from three aromatic plants grown in Colombia against *Euprosterna elaeasa* and *Acharia fusca* (Lepidoptera: Limacodidae). *Asian Pac. J. Trop. Biomed.* **2014**, *4*, 695–700. [CrossRef]
16. Liebhold, A.M.; Gottschalk, K.W.; Muzika, R.M.; Montgomery, M.E.; Young, R.; O'Day, K.; Kelley, B. *Suitability of North American Tree Species to the Gypsy Moth: A Summary of Field and Laboratory Tests*; U.S. Department of Agriculture Forest Service NE Forest Experimental Station General Technical Bulletin NE-211; U.S. Department of Agriculture: Washington, DC, USA, 1995; p. 34.
17. Arai, T.; Yaginuma, K.; Toyoshima, S.; Ito, T.; Takanashi, M. Damage of *Lymantria dispar* and *Lymantria mathura aurora* in apple orchards. *Annu. Rep. Soc. Plant Prot. North Jpn.* **2010**, *61*, 220–224.
18. Bigsby, K.M.; Ambrose, M.J.; Tobin, P.C.; Sills, E.O. The cost of gypsy moth sex in the city. *Urban For. Urban Green.* **2014**, *13*, 459–468. [CrossRef]
19. Marović, R.; Maravić, M.; Jančić, G.; Lazarev, V. Gypsy moth outbreaks in Serbia. In *Gypsy Moth Outbreaks in Serbia*; Adamović, Ž., Ed.; The Entomological Society of Serbia: Belgrade, Serbia, 1998; pp. 1–12.
20. Davidson, C.B.; Gottschalk, K.W.; Johnson, J.E. Tree mortality following defoliation by the European gypsy moth (*Lymantria dispar* L.) in the United States: A review. *For. Sci.* **1999**, *45*, 74–84.
21. Milanović, S.; Mihajlović, L.; Karadžić, D.; Jankovsky, L.; Aleksić, P.; Janković-Tomanić, M.; Lazarević, J. Effects of pedunculate oak tree vitality on gypsy moth preference and performance. *Arch. Biol. Sci.* **2014**, *66*, 1659–1672. [CrossRef]
22. Miller, J.; Hanson, P.; Dowell, R. The potential of gypsy moth as a pest of fruit and nut crops. *Calif. Agric.* **1987**, *41*, 10–12.
23. Mihajlović, L. The gypsy moth (*Lymantria dispar* L.) (Lepidoptera, Lymantridae) in Serbia. *Forestry* **2008**, *60*, 1–26. (In Serbian)
24. Sundararaj, R. Relevance of botanicals for the management of forest insect pests of India. In *Basic and Applied Aspects of Biopesticides*; Sahayaraj, K., Ed.; Springer: New Delhi, India, 2014; pp. 155–179. [CrossRef]
25. Kovanci, O.B. Feeding and oviposition deterrent activities of microencapsulated cardamom oleoresin and eucalyptol against *Cydia pomonella*. *Chil. J. Agric. Res.* **2016**, *76*, 62–70. [CrossRef]
26. Pour, S.A.; Shahriari, M.; Zibaee, A.; Mojarab-Mahboubkar, M.; Sahebzadeh, N.; Hoda, H. Toxicity, antifeedant and physiological effects of trans-anethole against *Hyphantria cunea* Drury (Lep: Arctiidae). *Pestic. Biochem. Phys.* **2022**, *185*, 105135. [CrossRef] [PubMed]
27. Kostić, M.; Popović, Z.; Brkić, D.; Milanović, S.; Sivčev, I.; Stanković, S. Larvicidal and antifeedant activity of some plant-derived compounds to *Lymantria dispar* L. (Lepidoptera: Limantriidae). *Bioresour. Technol.* **2008**, *99*, 7897–7901. [CrossRef] [PubMed]
28. Gvozdenac, S.M.; Inđić, D.V.; Vuković, S.M.; Grahovac, M.S.; Tanasković, S.T. Antifeeding activity of several plant extracts against against *Lymantria dispar* L. (Lepidoptera: Lymantriidae) larvae. *Pestic. Phytomed.* **2012**, *27*, 305–311. [CrossRef]
29. Moretti, M.D.; Sanna-Passino, G.; Demontis, S.; Bazzoni, E. Essential oil formulations useful as a new tool for insect pest control. *AAPS Pharm. Sci. Tech.* **2002**, *3*, 64–74. [CrossRef] [PubMed]
30. Popović, Z.; Kostić, M.; Stanković, S.; Milanović, S.; Sivčev, I.; Kostić, I.; Kljajić, P. Ecologically acceptable usage of derivatives of essential oil of sweet basil, *Ocimum basilicum*, as antifeedants against larvae of the gypsy moth, *Lymantria dispar*. *J. Insect. Sci.* **2013**, *13*, T161. [CrossRef] [PubMed]
31. Kostić, I.; Petrović, O.; Milanović, S.; Popović, Z.; Stanković, S.; Todorović, G.; Kostić, M. Biological activity of essential oils of *Athamanta haynaldii* and *Myristica fragrans* to gypsy moth larvae. *Ind. Crops Prod.* **2013**, *41*, 17–20. [CrossRef]
32. Devrnja, N.; Kostić, I.; Lazarević, J.; Savić, J.; Ćalić, D. Evaluation of tansy essential oil as a potential "green" alternative for gypsy moth control. *Environ. Sci. Pollut. Res.* **2020**, *27*, 11958–11967. [CrossRef]
33. Kostić, I.; Lazarević, J.; Šešlija Jovanović, D.; Kostić, M.; Marković, T.; Milanović, S. Potential of essential oils from anise, dill and fennel seeds for the gypsy moth control. *Plants* **2021**, *10*, 2194. [CrossRef]
34. Nikolić, B.M.; Milanović, S.D.; Milenković, I.L.; Todosijević, M.M.; Đorđević, I.Ž.; Brkić, M.Z.; Mitić, Z.S.; Marin, P.D.; Tešević, V.V. Bioactivity of *Chamaecyparis lawsoniana* (A. Murray) Parl. and *Thuja plicata* Donn ex D. Don essential oils on *Lymantria dispar*

(Linnaeus, 1758) (Lepidoptera: Erebidae) larvae and Phytophthora de Bary 1876 root pathogens. *Ind. Crops Prod.* **2022**, *178*, 114550. [CrossRef]
35. Chen, Y.Z.; Zhang, B.W.; Yang, J.; Zou, C.S.; Li, T.; Zhang, G.C.; Chen, G.S. Detoxification, antioxidant, and digestive enzyme activities and gene expression analysis of *Lymantria dispar* larvae under carvacrol. *J. Asia-Pac. Entomol.* **2021**, *24*, 208–216. [CrossRef]
36. Huang, Y.; Zhao, J.; Zhou, L.; Wang, J.; Gong, Y.; Chen, X.; Guo, Z.; Wang, Q.; Jiang, W. Antifungal activity of the essential oil of *Illicium verum* fruit and its main component trans-anethole. *Molecules* **2010**, *15*, 7558–7569. [CrossRef]
37. Senatore, F.; Oliviero, F.; Scandolera, E.; Taglialatela-Scafati, O.; Roscigno, G.; Zaccardelli, M.; De Falco, E. Chemical composition, antimicrobial and antioxidant activities of anethole-rich oil from leaves of selected varieties of fennel [*Foeniculum vulgare* Mill. ssp. *vulgare* var. *azoricum* (Mill.) Thell]. *Fitoterapia* **2013**, *90*, 214–219. [CrossRef]
38. Ben-Khalifa, N.E.; Chaieb, I.; Laarif, A.; Haouala, R. Insecticidal activity of six Apiaceae essential oils against *Spodoptera littoralis* Biosduval (Lepidoptera: Noctuidae). *J. New Sci.* **2018**, *55*, 3603–3609.
39. Johnson, A.J.; Venukumar, V.; Varghese, T.S.; Viswanathan, G.; Leeladevi, P.S.; Remadevi, R.K.S.; Baby, S. Insecticidal properties of *Clausena austroindica* leaf essential oil and its major constituent, trans-anethole, against *Sitophilus oryzae* and *Tribolium castaneum*. *Ind. Crops Prod.* **2022**, *182*, 114854. [CrossRef]
40. Mathela, C.S.; Padalia, R.C.; Chanotiya, C.S.; Tiwari, A. Carvone rich *Mentha longifolia* (Linn.): Chemical variation and commercial potential. *J. Essent. Oil Bear. Plants* **2005**, *8*, 130–133. [CrossRef]
41. Porfírio, E.M.; Melo, H.M.; Pereira, A.M.G.; Cavalcante, T.T.A.; Gomes, G.A.; Carvalho, M.G.D.; Costa, R.A.; Júnior, F.E.A.C. In vitro antibacterial and antibiofilm activity of *Lippia alba* essential oil, citral, and carvone against *Staphylococcus aureus*. *Sci. World J.* **2017**, *2017*, 4962707. [CrossRef]
42. Mutlu-Ingok, A.; Karbancioglu-Guler, F. Cardamom, cumin, and dill weed essential oils: Chemical compositions, antimicrobial activities, and mechanisms of action against *Campylobacter* spp. *Molecules* **2017**, *22*, 1191. [CrossRef]
43. Tripathi, A.K.; Prajapati, V.; Kumar, S. Bioactivities of l-carvone, d-carvone, and dihydrocarvone toward three stored product beetles. *J. Econ. Entomol.* **2003**, *96*, 1594–1601. [CrossRef]
44. Abdelgaleil, S.A.; Mohamed, M.I.; Badawy, M.E.; El-arami, S.A. Fumigant and contact toxicities of monoterpenes to *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) and their inhibitory effects on acetylcholinesterase activity. *J. Chem. Ecol.* **2009**, *35*, 518–525. [CrossRef]
45. Mondal, M.; Khalequzzaman, M. Toxicity of naturally occurring compounds of plant essential oil against *Tribolium castaneum* (Herbst). *J. Biol. Sci.* **2010**, *10*, 10–17. [CrossRef]
46. Shahriari, M.; Sahebzadeh, N.; Sarabandi, M.; Zibaee, A. Oral Toxicity of Thymol, α-Pinene, Diallyl Disulfide and Trans-Anethole, and Their Binary Mixtures against *Tribolium castaneum* Herbst Larvae (Coleoptera: Tenebrionidae). *Jordan J. Biol. Sci.* **2016**, *9*, 213–219.
47. Kanda, D.; Kaur, S.; Koul, O. Effect of keto-compounds from essential oils on the growth and reproductive performance of *Tribolium castaneum* (Herbst). *Biopestic. Int.* **2016**, *12*, 37–43.
48. Kanda, D.; Kaur, S.; Koul, O. A comparative study of monoterpenoids and phenylpropanoids from essential oils against stored grain insects: Acute toxins or feeding deterrents. *J. Pest Sci.* **2017**, *90*, 531–545. [CrossRef]
49. Rosa, J.S.; Oliveira, L.; Sousa, R.M.O.F.; Escobar, C.B.; Fernandes-Ferreira, M. Bioactivity of some Apiaceae essential oils and their constituents against *Sitophilus zeamais* (Coleoptera: Curculionidae). *Bull. Entomol. Res.* **2020**, *110*, 406–416. [CrossRef]
50. Hummelbrunner, L.A.; Isman, M.B. Acute, sublethal, antifeedant, and synergistic effects of monoterpenoid essential oil compounds on the tobacco cutworm, *Spodoptera litura* (Lep., Noctuidae). *J. Agric. Food Chem.* **2001**, *49*, 715–720. [CrossRef]
51. El-Minshawy, A.M.; Abdelgaleil, S.A.; Gadelhak, G.G.; Al-Eryan, M.A.; Rabab, R.A. Effects of monoterpenes on mortality, growth, fecundity, and ovarian development of *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). *Environ. Sci. Pollut. Res.* **2018**, *25*, 15671–15679. [CrossRef]
52. Al-Nagar, N.M.; Abou-Taleb, H.K.; Shawir, M.S.; Abdelgaleil, S.A. Comparative toxicity, growth inhibitory and biochemical effects of terpenes and phenylpropenes on *Spodoptera littoralis* (Boisd.). *J. Asia-Pac. Entomol.* **2020**, *23*, 67–75. [CrossRef]
53. Abdelgaleil, S.A.; Al-Nagar, N.; Abou-Taleb, H.K.; Shawir, M.S. Effect of monoterpenes, phenylpropenes and sesquiterpenes on development, fecundity and fertility of *Spodoptera littoralis* (Boisduval). *Int. J. Trop. Insect Sci.* **2022**, *42*, 245–253. [CrossRef]
54. Morgan, E.D. Azadirachtin, a scientific gold mine. *Bioorg. Med. Chem.* **2009**, *17*, 4096–4105. [CrossRef]
55. Markovic, I.; Norris, D.M.; Nordheim, E.V. Gypsy moth (*Lymantria dispar*) larval development and survival to pupation on diet plus extractables from green ash foliage. *Entomol. Exp. Appl.* **1997**, *84*, 247–254. [CrossRef]
56. Mostafiz, M.M.; Shim, J.K.; Hwang, H.S.; Bunch, H.; Lee, K.Y. Acaricidal effects of methyl benzoate against *Tetranychus urticae* Koch (Acari: Tetranychidae) on common crop plants. *Pest Manag. Sci.* **2020**, *76*, 2347–2354. [CrossRef]
57. Wilcox, D.; Dove, B.; McDavid, D.; Greer, D. *Image Tool Copyright UTHSCSA 1996–2002*; University of Texas Health Science Center (UTHSCSA): San Antonio, TX, USA, 1996.
58. Nawrot, J.; Błoszyk, E.; Harmatha, J.; Novotny', L.; Drozdz, B. Action of antifeedants of plant origin onbeetles infesting stored products. *Acta Entomol. Bohemoslov.* **1986**, *83*, 327–335.
59. Waldbauer, G.P. The consumption and utilization of food by insects. *Adv. Insect Phys.* **1968**, *5*, 229–288.
60. Scriber, J.M.; Slansky, F., Jr. The nutritional ecology of immature insects. *Annu. Rev. Entomol.* **1981**, *26*, 183–211. [CrossRef]

61. Farrar, R.R.; Barbour, J.D.; Kennedy, G.G. Quantifying food consumption and growth in insects. *Ann. Entomol. Soc. Am.* **1989**, *82*, 593–598. [CrossRef]
62. Howell, D.C. Simple analysis of variance. In *Statistical Methods for Psychology*, 8th ed.; Wadsworth, Cengage Learning: Belmont, CA, USA, 2013; pp. 325–368.
63. Finney, D.J. *Probit Analysis*, 3rd ed.; Cambridge University Press: Cambridge, UK, 1971.
64. Dudareva, N.; Negre, F.; Nagegowda, D.A.; Orlova, I. Plant volatiles: Recent advances and future perspectives. *Crit. Rev. Plant Sci.* **2006**, *25*, 417–440. [CrossRef]
65. Martemyanov, V.V.; Domrachev, D.V.; Pavlushin, S.V.; Belousova, I.A.; Bakhvalov, S.A.; Tkachev, A.V.; Glupov, V.V. Induction of terpenoid synthesis in leaves of silver birch after defoliation caused by gypsy moth caterpillars. *Dokl. Biol. Sci. Proc. Acad. Sci. USSR Biol. Sci. Sect.* **2010**, *435*, 407–410. [CrossRef]
66. Powell, J.S.; Raffa, K.F. Fate of conifer terpenes in a polyphagous folivore: Evidence for metabolism by gypsy moth (Lepidoptera: Lymantriidae). *J. Entomol. Sci.* **2003**, *38*, 583–601. [CrossRef]
67. Markovic, I.; Norris, D.M.; Phillips, J.K.; Webster, F.X. Volatiles involved in the nonhost rejection of *Fraxinus pennsylvanica* by *Lymantria dispar* larvae. *J. Agric. Food Chem.* **1996**, *44*, 929–935. [CrossRef]
68. Chaudhari, A.K.; Singh, V.K.; Kedia, A.; Das, S.; Dubey, N.K. Essential oils and their bioactive compounds as eco-friendly novel green pesticides for management of storage insect pests: Prospects and retrospects. *Environ. Sci. Pollut. Res.* **2021**, *28*, 18918–18940. [CrossRef] [PubMed]
69. Alkan, M.; Ertürk, S. Insecticidal efficacy and repellency of trans-anethole against four stored-product insect pests. *J. Agric. Sci.* **2020**, *26*, 64–70. [CrossRef]
70. Kłyś, M.; Izdebska, A.; Malejky-Kłusek, N. Repellent Effect of the caraway *Carum carvi* L. on the rice weevil *Sitophilus oryzae* L. (Coleoptera, Dryophthoridae). *Insects* **2020**, *11*, 836. [CrossRef] [PubMed]
71. Cao, D.; Liu, J.; Zhao, Z.; Yan, X.; Wang, W.; Wei, J. Chemical compounds emitted from *Mentha spicata* repel *Aromia bungii* females. *Insects* **2022**, *13*, 244. [CrossRef] [PubMed]
72. Dambolena, J.S.; Zunino, M.P.; Herrera, J.M.; Pizzolitto, R.P.; Areco, V.A.; Zygadlo, J.A. Terpenes: Natural products for controlling insects of importance to human health—A structure-activity relationship study. *Psyche J. Entomol.* **2016**, *2016*, 4595823. [CrossRef]
73. Sanchez, G.D.B.; Giurfa, M. A comparative analysis of neural taste processing in animals. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2011**, *366*, 2171–2180. [CrossRef]
74. Deletre, E.; Schatz, B.; Bourguet, D.; Chandre, F.; Williams, L.; Ratnadass, A.; Martin, T. Prospects for repellent in pest control: Current developments and future challenges. *Chemoecology* **2016**, *26*, 127–142. [CrossRef]
75. Enan, E.E. Molecular and pharmacological analysis of an octopamine receptor from American cockroach and fruit fly in response to plant essential oils. *Arch. Insect Biochem. Physiol.* **2005**, *59*, 161–171. [CrossRef]
76. Zaio, Y.P.; Gatti, G.; Ponce, A.A.; Saavedra Larralde, N.A.; Martinez, M.J.; Zunino, M.P.; Zygadlo, J.A. Cinnamaldehyde and related phenylpropanoids, natural repellents, and insecticides against *Sitophilus zeamais* (Motsch.). A chemical structure-bioactivity relationship. *J. Sci. Food Agric.* **2018**, *98*, 5822–5831. [CrossRef]
77. Wilson, A.; Butler, J.F.; Withycombe, D.; Mookherjee, B.D.; Katz, I.; Schrankel, K.R. Use of D-Carvone as Mosquito Attractant. U.S. Patent 4970068, 13 November 1990. Available online: https://patentimages.storage.googleapis.com/dd/ca/1a/5efd45c9b821cd/US4970068.pdf (accessed on 17 October 2022).
78. Su, T.; Mulla, M.S. Oviposition bioassay responses of *Culex tarsalis* and *Culex quinquefasciatus* to neem products containing azadirachtin. *Entomol. Exp. Appl.* **1999**, *91*, 337–345. [CrossRef]
79. Moon, S.R.; Cho, S.R.; Jeong, J.W.; Shin, Y.H.; Yang, J.O.; Ahn, K.S.; Yoon, C.; Kim, G.H. Attraction response of spot clothing wax cicada, *Lycorma delicatula* (Hemiptera: Fulgoridae) to spearmint oil. *J. Korean Soc. Appl. Biol. Chem.* **2011**, *54*, 558–567.
80. Jaastad, G.; Knudsen, G.K.; Kobro, S.; Bäckman, A.C.; Witzgall, P.; Bengtsson, M. Attractive Plant Volatiles as a Control Method against Apple Fruit Moth (*Argyresthia conjugella* Zell.)? In Proceedings of the Ecofruit—11th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing, Weinsberg, Germany, 3–5 February 2004; pp. 29–34.
81. Tóth, M.; Furlan, L.; Szarukán, I.; Vuts, J. Development of a female-targeted attractant for the click beetle, *Agriotes ustulatus* Schwarz. *Acta Phytopathol. Entomol. Hung.* **2011**, *46*, 235–245. [CrossRef]
82. Simmonds, M.S.J.; Blaney, W.M.; Ley, S.V.; Anderson, J.C.; Toogood, P.L. Azadirachtin: Structural requirements for reducing growth and increasing mortality in lepidopterous larvae. *Entomol. Exp. Appl.* **1990**, *55*, 169–181. [CrossRef]
83. Jiang, Z.L.; Akhtar, Y.; Zhang, X.; Bradbury, R.; Isman, M.B. Insecticidal and feeding deterrent activities of essential oils in the cabbage looper, *Trichoplusia ni* (Lepidoptera: Noctuidae). *J. Appl. Entomol.* **2012**, *136*, 191–202. [CrossRef]
84. Herrera, J.M.; Zunino, M.P.; Dambolena, J.S.; Pizzolitto, R.P.; Gañan, N.A.; Lucini, E.I.; Zygadlo, J.A. Terpene ketones as natural insecticides against *Sitophilus zeamais*. *Ind. Crops Prod.* **2015**, *70*, 435–442. [CrossRef]
85. Shahriari, M.; Zibaee, A.; Sahebzadeh, N.; Shamakhi, L. Effects of α-pinene, trans-anethole, and thymol as the essential oil constituents on antioxidant system and acetylcholine esterase of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). *Pestic. Biochem. Physiol.* **2018**, *150*, 40–47. [CrossRef]
86. López, M.D.; Pascual-Villalobos, M.J. Are monoterpenoids and phenylpropanoids efficient inhibitors of acetylcholinesterase from stored product insect strains? *Flavour. Fragr. J.* **2015**, *30*, 108–112. [CrossRef]

87. Li, S.G.; Li, M.Y.; Huang, Y.Z.; Hua, R.M.; Lin, H.F.; He, Y.J.; Wei, L.L.; Liu, Z.Q. Fumigant activity of *Illicium verum* fruit extracts and their effects on the acetylcholinesterase and glutathione S-transferase activities in adult *Sitophilus zeamais*. *J. Pest Sci.* **2013**, *86*, 677–683. [CrossRef]
88. Murfadunnisa, S.; Vasantha-Srinivasan, P.; Ganesan, R.; Senthil-Nathan, S.; Kim, T.J.; Ponsankar, A.; Kumar, S.D.; Chandramohan, D.; Krutmuang, P. Larvicidal and enzyme inhibition of essential oil from *Spheranthus amaranthroids* (Burm.) against lepidopteran pest *Spodoptera litura* (Fab.) and their impact on non-target earthworms. *Biocatal. Agric. Biotechnol.* **2019**, *21*, 101324. [CrossRef]
89. Thanigaivel, A.; Chanthini, K.M.P.; Karthi, S.; Vasantha-Srinivasan, P.; Ponsankar, A.; Sivanesh, H.; Stanley-Raja, V.; Shyam-Sundar, N.; Narayanan, K.R.; Senthil-Nathan, S. Toxic effect of essential oil and its compounds isolated from *Sphaeranthus amaranthoides* Burm. f. against dengue mosquito vector *Aedes aegypti* Linn. *Pestic. Biochem. Physiol.* **2019**, *160*, 163–170. [CrossRef]
90. Martinez, S.S.; Van Emden, H.F. Growth disruption, abnormalities and mortality of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) caused by azadirachtin. *Neotrop. Entomol.* **2001**, *30*, 113–125. [CrossRef]
91. Taffar, A.; Yezli-Touiker, S.; Bendjedid, H.; Soltani, N. Evaluation of azadirachtin, a biopesticides, on growth, development and cuticle secretion of Mediterranean flour moth, *Ephestia kuehniella* Zeller. *J. Entomol. Res.* **2021**, *45*, 436–443. [CrossRef]
92. Mordue, A.J.; Morgan, E.D.; Nisbet, A.J.; Gilbert, L.I.; Gill, S.S. Azadirachtin, a natural product in insect control. In *Insect Control: Biological and Synthetic Agents*; Gilbert, L.I., Gill, S.S., Eds.; Academic Press: London, UK, 2010; pp. 185–197.
93. Swidan, M.H.; Kheirallah, D.A.; Osman, S.E.I.; Nour, F.E. Impact of certain natural insecticides on the morphological and biochemical characteristics of khapra beetle, *Trogoderma granarium* everts. *Int. J. Zool. Investig.* **2016**, *2*, 147–166.
94. Erler, F.; Tunç, İ. Monoterpenoids as fumigants against greenhouse pests: Toxic, development and reproduction-inhibiting effects/Monoterpenoide als Begasungsmittel gegen Gewächshausschädlinge: Toxizität, Wirkung auf Entwicklung und Reproduktion. *Z. Pflanzenkrankh. Pflanzenschutz J. Plant Dis. Prot.* **2005**, *112*, 181–192.
95. Soonwera, M.; Moungthipmalai, T.; Aungtikun, J.; Sittichok, S. Combinations of plant essential oils and their major compositions inducing mortality and morphological abnormality of *Aedes aegypti* and *Aedes albopictus*. *Heliyon* **2022**, *8*, e09346. [CrossRef]
96. Nishida, R. Chemical ecology of insect–plant interactions: Ecological significance of plant secondary metabolites. *Biosci. Biotechnol. Biochem.* **2014**, *78*, 1–13. [CrossRef]
97. Meisner, J.; Kehat, M. The response of *Earias insulana* Boisd. larvae to phagodeterrent (–)-carvone incorporated in an artificial diet 1. *Z. Angew. Entomol.* **1980**, *90*, 80–82. [CrossRef]
98. Sousa, R.M.O.; Rosa, J.S.; Oliveira, L.; Cunha, A.; Fernandes-Ferreira, M. Activities of Apiaceae essential oils and volatile compounds on hatchability, development, reproduction and nutrition of *Pseudaletia unipuncta* (Lepidoptera: Noctuidae). *Ind. Crops Prod.* **2015**, *63*, 226–237. [CrossRef]
99. Abdelgaleil, S.A.; Abou-Taleb, H.K.; Al-Nagar, N.; Shawir, M.S. Antifeedant, growth regulatory and biochemical effects of terpenes and phenylpropenes on *Spodoptera littoralis* Boisduval. *Int. J. Trop. Insect Sci.* **2020**, *40*, 423–433. [CrossRef]
100. Hashem, A.S.; Awadalla, S.S.; Zayed, G.M.; Maggi, F.; Benelli, G. *Pimpinella anisum* essential oil nanoemulsions against *Tribolium castaneum*—Insecticidal activity and mode of action. *Environ. Sci. Pollut. Res.* **2018**, *25*, 18802–18812. [CrossRef]
101. Derbalah, A.; Keratum, A.; Darweesh, M.; Elebiary, M.; Hegazy, F. New trends for controlling *Sitophilus oryzae* concerning adult mortality, offspring production, mode of action, and grain quality. *J. Verbrauch. Lebensm. J. Consum. Prot. Food Saf.* **2021**, *16*, 343–351. [CrossRef]
102. Shahriari, M.; Sahebzadeh, N.; Zibaee, A. Biochemical response of Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lep.: Pyralidae) to the toxicity of trans-anethole. *Plant Prot. (Sci. J. Agric.)* **2022**, *45*, 121–136.
103. da Cunha, F.A.B.; Wallau, G.L.; Pinho, A.I.; Nunes, M.E.M.; Leite, N.F.; Tintino, S.R.; da Costa, G.M.; Athayde, M.L.; Boligon, A.A.; Coutinho, H.D.M.; et al. Eugenia uniflora leaves essential oil induces toxicity in Drosophila melanogaster: Involvement of oxidative stress mechanisms. *Toxicol. Res.* **2015**, *4*, 634–644. [CrossRef]
104. Ding, C.Y.; Ma, Y.M.; Li, B.; Wang, Y.; Zhao, L.; Peng, J.N.; Li, M.Y.; Liu, S.; Li, S.G. Identification and functional analysis of differentially expressed genes in *Myzus persicae* (Hemiptera: Aphididae) in response to trans-anethole. *J. Insect Sci.* **2022**, *22*, 3. [CrossRef]
105. Petrović, M.; Popović, A.; Kojić, D.; Šućur, J.; Bursić, V.; Aćimović, M.; Malenčić, Đ.; Stojanović, T.; Vuković, G. Assessment of toxicity and biochemical response of *Tenebrio molitor* and *Tribolium confusum* exposed to *Carum carvi* essential oil. *Entomol. Gen.* **2019**, *38*, 333–348. [CrossRef]
106. Passreiter, C.M.; Wilson, J.; Andersen, R.; Isman, M.B. Metabolism of thymol and trans-anethole in larvae of *Spodoptera litura* and *Trichoplusia ni* (Lepidoptera: Noctuidae). *J. Agric. Food Chem.* **2004**, *52*, 2549–2551. [CrossRef]
107. Marumoto, S.; Okuno, Y.; Hagiwara, Y.; Miyazawa, M. Biotransformation of (+)-Carvone and (−)-Carvone by the Common Cutworm *Spodoptera litura* Larvae. *J. Oleo Sci.* **2018**, *67*, 1253–1257. [CrossRef]