

Article

Behavioral Responses, Fumigant Toxicity, and Contact Toxicity of Eugenol and Cinnamaldehyde Against *Sitobion avenae* Under Controlled Conditions

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Abstract

Traditionally, the control of *Sitobion avenae* (Fabricius) relies heavily on chemical insecticides; however, the long-term use of chemical insecticides has caused hazards to the environment and human health. Eugenol and cinnamaldehyde are important active components of plant essential oils. In this study, the toxicity of these two compounds against *S. avenae* was determined via fumigation and contact bioassays, and repellent activity tests were conducted to clarify their control effects. The results showed that the contact KD_{50} values of eugenol and cinnamaldehyde against *S. avenae* through cuticular penetration were 0.25 $\mu\text{L/mL}$ and 0.11 $\mu\text{L/mL}$, respectively (applied onto a treated surface area of 30 cm^2 per replicate), while their fumigant KD_{50} values were 7.04 $\mu\text{L/mL}$ and 2.55 $\mu\text{L/mL}$, respectively (tested in a sealed 125 mL glass vial). Both compounds exhibited repellent activity. At a concentration of 0.75 $\mu\text{L/mL}$, the repellency rate of cinnamaldehyde was 24.4%, while that of eugenol was 15.6%. However, both compounds displayed a certain attractive effect at the lowest concentration of 0.375 $\mu\text{L/mL}$. These findings demonstrate that eugenol and cinnamaldehyde exhibit high insecticidal activity against the wheat aphid under laboratory conditions, with dose-dependent repellency and toxicity in simple laboratory assays.

Keywords: *S. avenae*; eugenol; cinnamaldehyde; contact toxicity; fumigation toxicity; repellent activity

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1. Introduction

Wheat aphids are major piercing and sucking pests in wheat production. They damage wheat by absorbing sap from the phloem, leading to nutrient loss, shriveled grains, and subsequent yield reduction. Wheat aphids mainly cause damage during the flowering and maturity stages; they are mainly concentrated on the back of leaves during flowering and on the ears during maturity [1,2]. Furthermore, wheat aphids can spread various plant viruses, including barley yellow dwarf viruses (BYDVs) and wheat streak mosaic virus (WSMV), further exacerbating losses in wheat production [3]. The honeydew secreted by the wheat aphid provides a favorable environment for the growth of

microorganisms and may also promote the occurrence of wheat diseases such as wheat powdery mildew, resulting in a reduction in the photosynthetic area of wheat [4].

The reproductive cycle of the wheat aphids is complex and can adjust according to the environmental conditions. They mainly reproduce by parthenogenesis, but also have a sexual reproduction stage. Generations overlap, and populations can increase significantly within a short period of time [5]. When environmental conditions deteriorate or the host is not suitable, some wingless aphids develop into winged forms and migrate to other crops such as corn and rice to maintain population survival and reproduction [6]. Among them, *Sitobion avenae* (Fabricius) is the main species in wheat-growing regions across China, and exhibits distinct stage-specific damage characteristics [7].

At present, chemical control is still the main method for managing wheat aphids because it can quickly and effectively reduce pest population density [8]. The commonly used chemical insecticides mainly include neonicotinoids and pyrethroids [9,10]. However, long-term, excessive, and unreasonable use of these insecticides has led to the development of resistance in *S. avenae* [10,11]. It has been reported that from 2007 to 2019, resistance of wheat aphids to neonicotinoid insecticides increased by 1.4 to 14.7 times [12]. By 2023–2024, moderate resistance to several insecticides, including imidacloprid and related compounds, has been observed in many regions [11]. Overall, *S. avenae* has developed varying degrees of resistance to chemical pesticides. In this situation, exclusive reliance on conventional chemical pesticides has become unsustainable [13]. Therefore, it is necessary to develop and promote environmentally friendly control strategies, such as novel biopesticides, to reduce pesticide use while effectively controlling aphid populations [14–16].

Plant essential oils are complex mixtures present in various tissues of plants, containing a variety of volatile components, including aromatic, aliphatic, and terpenoid compounds. They are important secondary metabolites and exhibit a range of biological activities such as antibacterial, antifungal, insecticidal, and antiviral effects [17–19]. Due to their short persistence and relatively low toxicity to non-target organisms, plant essential oils have been widely used in medical, cosmetic, and food industries [20]. A large number of studies have shown that plant essential oils and their main components have insecticidal activity [21]. Because they contain a variety of volatile substances with unique odors, they can regulate various physiological activities of insect pests, including behavior, feeding, and oviposition [22–25].

To date, numerous studies have focused on the repellent, fumigant, and oviposition-inhibitory effects of plant essential oils against pests [21,26–28]. For example, the essential oil of the Neotropical plant *Siparuna guianensis* Aubl., commonly known as *Negramina*, shows high toxicity and repellent effects against the green peach aphid *Myzus persicae* (Sulzer) [29]. Similarly, *Syzygium aromaticum* essential oil exhibits high toxicity against *Rhopalosiphum maidis* ($LC_{95} = 0.17 \mu\text{L}/\text{cm}^2$), while causing less than 18% mortality in ladybird predators. Moreover, its active components selectively bind to the TRP, octopamine, and GABA receptor targets of aphids, without affecting the movement or predatory behaviour of ladybugs [30]. The essential oil (EO) of *Lavandula dentata* also exhibits strong contact toxicity against *Aphis spiraeicola*, with a 72 h LD_{90} as low as $3.39 \mu\text{L}/\text{mL}$ [31]. Furthermore, a 1:1 binary mixture of β -camphor alcohol and linalool exhibits high insecticidal activity against the wheat aphid (*Metopolophium dirhodum*) and significantly reduces its reproductive capacity [32]. Therefore, plant essential oils have been widely used as insecticides, repellents, fumigants, and insect growth regulators [33,34]. In contrast to conventional synthetic insecticides with single targets, plant essential oils generally exert comprehensive effects on insect pests through multiple pathways, which not only expands pest control strategies but also provides new insights into insecticide resistance management [35–40].

Eugenol and cinnamaldehyde belong to phenylpropanoid compounds and are the main active components of essential oils from *Cinnamomum*, *Laurus*, and other aromatic plants [41,42]. These compounds have long been studied for their biological activities such as antibacterial, anti-inflammatory, and anticancer properties [41–43]. In recent years, with the continuous advancement of research on plant pesticides, the insecticidal activity of eugenol and cinnamaldehyde has received increasing attention [44–46]. In terms of insecticidal activity, both compounds have broad-spectrum activity against pests. For example, they show high toxicity to larvae and pupae of houseflies, and can cause deformities in adults [47]. In studies on the control of fleas, mosquitoes, and aphids, both have shown excellent contact, fumigation, repellent, and oviposition inhibition effects, with cinnamaldehyde showing particularly strong fumigant activity against mosquitoes [48–50].

Previous studies have mainly focused on evaluating the biological activity of crude extracts or individual compounds. However, a comparative study assessing the toxicological effects of eugenol and cinnamaldehyde against the same target pest is still lacking. Moreover, despite their known efficacy against other pest species, no systematic effort has been made to elucidate their toxicity or differences in biological activity specifically in relation to *S. avenae*. Therefore, this study serves as an extension of the toxicological data for a specific aphid species, rather than a major conceptual breakthrough in the field of grain aphid control. Its aim is to fill the aforementioned research gap and lay a preliminary theoretical foundation for the development of environmentally friendly plant-based insecticides. The study targeted *S. avenae* as the target pest, mainly due to its severe damage to wheat crops and high risk of resistance. Effective control measures urgently need to be sought. The results of this study are expected to provide basic toxicological data for the screening of candidate pesticides.

2. Materials and Methods

2.1. Insects and Chemicals

In 2024, *S. avenae* were collected from the Anhui Academy of Agricultural Sciences in Hefei, Anhui Province, China (31°49′21.30″ N, 117°13′18.25″ E). After collection, the aphids were transferred to a rearing room in a greenhouse for continuous rearing. According to morphological criteria, the key characteristics such as the body color, the ratio of antennal segments, the shape of abdominal tubes, and the number of bristles on the tail wings of the aphids were identified under a microscope, and the specimens were confirmed as *S. avenae*. During the breeding process, the aphids reproduced multiple generations under greenhouse conditions. The fifth generation of aphids was uniformly used for subsequent biological measurement experiments. Aphids were maintained at constant temperature of 20 ± 1 °C, with relative humidity maintained between 60% and 80%, and the photoperiod was set to 16 h of light and 8 h of darkness (light:darkness = 16 h:8 h). This was done to ensure the stability and reproducibility of the experimental conditions. The “Zhongmai 175” variety of wheat was used as the host plant. The test compounds eugenol (99% purity, CAS number: 97-53-0) and cinnamaldehyde (98% purity, CAS number: 104-55-2) were purchased from Anergy Chemical (Anhui Zesheng Technology Co., Ltd. Shanghai, China). Both compounds were stored in sealed containers at 4 °C under light-protected conditions and were restored to room temperature before use.

2.2. Repellent Test

The behavioral responses of *S. avenae* were evaluated using a Y-tube (Y-type tube selection experiment), following the method of Geedi et al. with slight modifications [51,52]. Essential oils were diluted with 0.1% aqueous solution of Tween 80 to prepare

concentrations of 6.000 µL/mL, 3.000 µL/mL, 1.500 µL/mL, 0.750 µL/mL, and 0.375 µL/mL. The Tween 80 solution served as the control (CK).

The Y-tube olfactometer (diameter 2.5 cm) (Beijing Fangguo Technology Co., Ltd., Beijing, China), consisted of a 10 cm base tube with two 15 cm arms positioned at an angle of 75°. A constant airflow (100 mL/min), filtered through activated charcoal and humidified, was introduced into each arm. Before each experiment, the apparatus was cleaned with 75% anhydrous ethanol and dried at 37 °C to remove any remaining odors or impurities.

For each run, a strip of filter paper (5 mm × 15 mm) treated with 200 µL of essential oil was placed in one arm (treatment) of the Y-tube as the test arm, while another strip (5 mm × 15 mm) treated with 200 µL of 0.1% Tween 80 was placed in the other arm (control). Untreated wheat seedlings were provided as food. To eliminate the non-specific interference of the host plant odors on the distribution of aphids, fresh wheat seedlings were placed at the ends of both arms of the Y-shaped tube, ensuring that both arms provided the same background host odor. Aphids were released from the opposite end of the Y-shaped tube.

In the Y-tube behavior experiment, 30 aphids were introduced for each repetition. After 24 h, the number of individuals in the treated arm and the control arm was recorded. Individuals that did not enter either arm were classified as part of the control group (Nc). The position of the treated arm was swapped between each repetition to eliminate positional bias. Three biological replicates were conducted at each concentration. The observation period was set to 24 h to assess the long-term host selection preference of aphids for the tested compounds, rather than the instantaneous avoidance response. The entire experiment was conducted under dark conditions, and fresh wheat seedlings were provided at the ends of both arms to reduce stress. It should be noted that the distribution of aphids under the longer observation period may be affected by resting behavior or micro-environmental factors. Therefore, the results of this study are classified as “long-term selection preference” to avoid over-interpretation of the instantaneous behavioral mechanism. In the results, all negative values will be expressed as “attraction”.

$$\text{Percentage Repellency (PR\%)} = [(Nc - Nt)/(Nc + Nt)] * 100,$$

where Nc is the number of insects present in the negative control and Nt is the number of insects present in the treatment.

2.3. Fumigation Toxicity

Fumigant toxicity was assessed using an airtight wide-mouth bottle assay (125 mL), with slight modifications [53]. Eugenol and cinnamaldehyde (with purity of 99% and 98% respectively) were selected as the test essential oil components. They were diluted with a 0.1% Tween 80 aqueous solution and then thoroughly shaken to prepare a stable micro-emulsion. Subsequently, the concentrations were diluted to 10.000, 5.000, 2.500, 1.250, and 0.625 µL/mL. The 0.1% Tween 80 aqueous solution without the test compounds was used as the control (CK). A 50 µL aliquot of the essential oil was applied to a 2 cm × 2 cm filter paper strip, which was then suspended in the center of the bottle to avoid direct contact with aphids. Based on the densities of cinnamaldehyde (1.05 g/mL) and eugenol (1.06 g/mL), the nominal concentrations of cinnamaldehyde in the air headspace of each group were calculated to be 4.00000, 2.00000, 1.00000, 0.50000, and 0.00025 µL/L (equivalent to 4200.00, 2100.00, 1050.00, 525.00 and 0.26 µg/L), while the nominal concentrations of eugenol were 4.00000, 2.00000, 1.00000, 0.50000, and 0.00025 µL/L (equivalent to 4240.00, 2120.00, 1060.00, 530.00, and 0.27 µg/L). Each treatment was replicated three times using at least 20 wingless adult aphids per replicate. After 24 h of treatment, the test aphids were taken out, and their survival status was assessed under an optical microscope. The

judgment criteria were as follows: Aphids were gently touched with a soft brush; aphids showing no movement or only weak movement (two or fewer legs) were considered dead. The number of dead aphids in each bottle was recorded, and the corrected mortality rate, toxicity regression equation, and other biological indicators of each treatment group were calculated accordingly.

2.4. Direct-Contact Toxicity

The dosages of eugenol and cinnamaldehyde used in this study were determined by preliminary bioassays. The contact toxicity assay was conducted using the residual film method, with acetone as both the solvent and the control, following slight modifications of the method described in [54]. Eugenol was tested at five concentrations (1.000, 0.500, 0.250, 0.125, and 0.063 $\mu\text{L/mL}$), and cinnamaldehyde was tested at five concentrations (0.250, 0.200, 0.150, 0.100, and 0.050 $\mu\text{L/mL}$). Using a calibrated micropipette, 150 μL of each solution was applied to the inner surface of a transparent glass tube (diameter 1.5 cm, height 6 cm; internal surface area: 30.0 cm^2). Based on the surface area and the densities of the test compounds (1.06 g/mL for eugenol and 1.05 g/mL for cinnamaldehyde), the corresponding surface doses were calculated. For eugenol, the five solution concentrations yielded surface doses of 0.00500, 0.00250, 0.00125, 0.000625, and 0.000315 $\mu\text{L/cm}^2$ (equivalent to 5.30, 2.65, 1.33, 0.66, and 0.33 $\mu\text{g/cm}^2$), respectively. For cinnamaldehyde, the corresponding values were 0.00125, 0.00100, 0.000750, 0.000500, and 0.000250 $\mu\text{L/cm}^2$ (equivalent to 1.31, 1.05, 0.79, 0.53, and 0.26 $\mu\text{g/cm}^2$). Three independent glass tubes were prepared for each concentration (three replicates). The tubes were rotated on a roller mixer for 2 h to accelerate the evaporation of acetone, during which the essential oil components gradually and uniformly deposited onto the inner wall, forming a thin and homogeneous oil film. All operations involving acetone were performed in a fume hood to ensure operator safety and to avoid interference from residual solvent. After the acetone had completely evaporated, at least 20 healthy wingless adult aphids were placed in each glass tube. The aphids had no access to food during the exposure period. All tested aphids were from the same breeding population and were handled gently to avoid mechanical injury. After placement, the tubes were maintained under standard rearing conditions (temperature, humidity, and photoperiod identical to those used for aphid rearing) for 3 h. Following the treatment, aphid mortality was assessed under a microscope using the same criteria described above.

2.5. Data Analysis

Excel 2021 from Microsoft Office was used to organize the trial data. The corrected mortality rate was determined using the Abbott formula; the virulence regression equation and the knockdown concentration (KD_{50}) were calculated using the DPS 9.0 program, and the 95% confidence interval of the KD_{50} was recorded to confirm the accuracy and reliability of this value. Data were statistically analyzed using SPSS 26.0 software. Comparisons between the two compounds at the same concentration were performed using independent-samples *t*-tests. When Levene's test indicated unequal variances ($p < 0.05$), the *t*-test results with unequal variances assumed (i.e., Welch's correction) were reported. For concentration-response comparisons within each compound, homogeneity of variances was evaluated using Levene's test. When both assumptions were satisfied ($p > 0.05$), one-way ANOVA was employed to detect overall differences among concentration groups, followed by Tukey's HSD post hoc test for pairwise comparisons. When the homogeneity of variance assumption was violated (Levene's test, $p < 0.05$), Welch's ANOVA was applied as a robust alternative, followed by Games–Howell post hoc tests for multiple comparisons. Data plotting was done using GraphPad Prism 10.0 software.

3. Results

3.1. Repellent Test

Under closed conditions, the repellent effects of eugenol and cinnamaldehyde against *S. avenae* were evaluated at different concentrations (Figure 1, Table 1 and 2). The results showed that cinnamaldehyde exhibited concentration-dependent repellent effects. At a concentration of 1.500 $\mu\text{L/mL}$, its repellent effect was significantly higher than that of eugenol. At concentrations of 6.000 $\mu\text{L/mL}$, 3.000 $\mu\text{L/mL}$, and 1.500 $\mu\text{L/mL}$, the repellency rates were 91.11%, 84.44%, and 75.56%, respectively. There were no significant differences among these three treatments ($p > 0.05$).

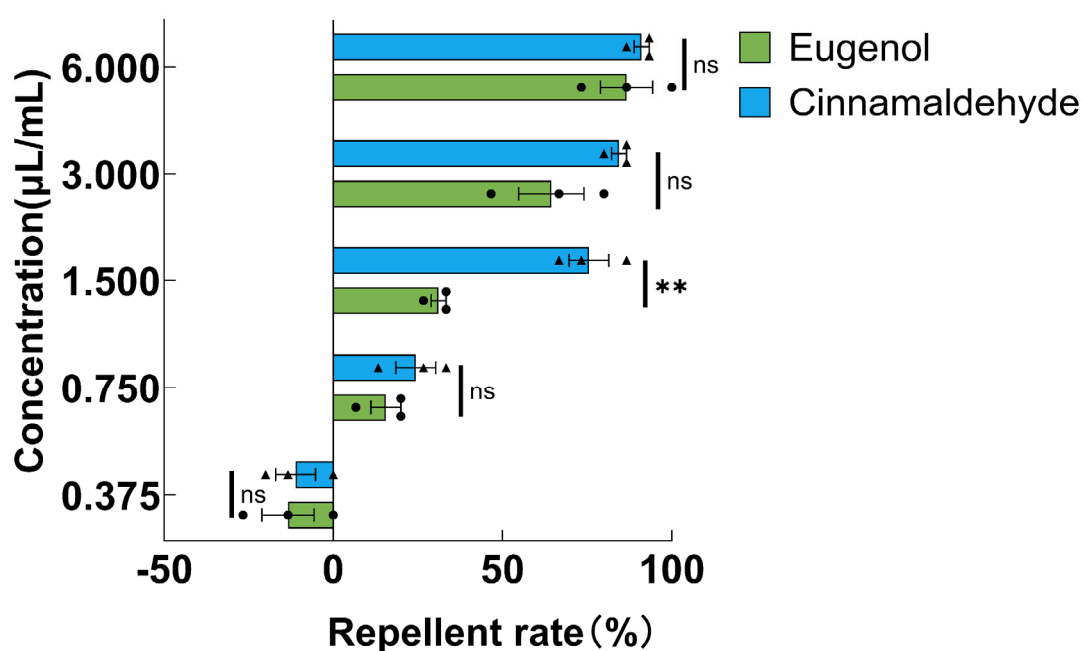


Figure 1. The repellent effects of eugenol and cinnamaldehyde on the *S. avenae*. Note: The significant difference was analyzed by Student's *t* test (**, $p < 0.01$; ns indicates no significance). Clearly stated sample size ($n = 3$), data presentation format (the values are the mean $\pm$ standard error, SE), and it is indicated that negative values represent attractiveness to the target. Levene's test confirmed homogeneity of variances ($p > 0.05$ for all). The corresponding *t* and *p* values are as follows: [0.375]: $t_{(4)} = 0.229$, $p = 0.830$; [0.750]: $t_{(4)} = 1.789$, $p = 0.148$; [1.500]: $t_{(4)} = 7.071$, $p = 0.002$; [3.000]: $t_{(4)} = 2.012$, $p = 0.114$; [6.000]: $t_{(4)} = 0.555$, $p = 0.609$.

Table 1. The repellent effects of cinnamaldehyde on *S. avenae* (mean $\pm$ SD).

Concentration ($\mu\text{L/mL}$)	Nc	Nt	Cinnamaldehyde	95% CI
0.375	40	50	$-10.00\% \pm 0.14$ c	-36.41 – 14.19
0.750	56	34	$24.44\% \pm 0.10$ b	14.88 – 34.01
1.500	79	11	$75.56\% \pm 0.10$ a	50.26 – 100.85
3.000	83	7	$84.44\% \pm 0.04$ a	74.88 – 94.01
6.000	86	4	$91.11\% \pm 0.04$ a	81.55 – 100.01

Note: Nc = total number of insects in the negative control (solvent only) and Nt = total number of insects in the treatment arm, both pooled from three independent replicates. Levene's test confirmed homogeneity of variances across all concentration groups ($F_{(4,10)} = 1.862$, $p = 0.194$). One-way ANOVA revealed a highly significant concentration effect ($F_{(4,10)} = 117.265$, $p < 0.001$). Different lowercase letters denote significant differences among concentrations (Tukey's HSD, $p < 0.05$).

Repellency declined sharply at lower concentrations, with a rate of 24.44% at 0.750 $\mu\text{L/mL}$. At the lowest concentration (0.375 $\mu\text{L/mL}$), cinnamaldehyde displayed an attractant effect. Similarly, eugenol also demonstrated concentration-dependent repellent activity. At concentrations of 6.000 $\mu\text{L/mL}$ (86.67%) and 3.000 $\mu\text{L/mL}$ (64.44%), respectively; higher repellency rates were observed, with no significant difference between these two concentrations ($p > 0.05$). At concentrations of 1.500 $\mu\text{L/mL}$ and 0.750 $\mu\text{L/mL}$, repellency rates decreased to 31.11% and 15.56%, respectively. At a concentration of 0.375 $\mu\text{L/mL}$, eugenol also exhibited an attractant effect.

Table 2. The repellent effects of eugenol on *S. avenae* (mean $\pm$ SD).

Concentration ($\mu\text{L/mL}$)	Nc	Nt	Repellent Effect	95% CI
0.375	39	51	$-13.33\% \pm 0.19$ d	-46.46 – 19.79
0.750	54	36	$15.56\% \pm 0.08$ bc	-3.57 – 34.68
1.500	59	31	$31.11\% \pm 0.04$ b	21.55 – 40.67
3.000	74	16	$64.44\% \pm 0.17$ a	22.77 – 100.06
6.000	84	6	$86.67\% \pm 0.13$ a	53.54 – 100.20

Note: Nc = total number of insects in the negative control (solvent only) and Nt = total number of insects in the treatment arm, both pooled from three independent replicates. Levene's test confirmed homogeneity of variances across all concentration groups ($F_{(4,10)} = 0.867$, $p = 0.516$). One-way ANOVA revealed a highly significant concentration effect ($F_{(4,10)} = 32.948$, $p < 0.001$). Different lowercase letters denote significant differences among concentrations (Tukey's HSD, $p < 0.05$).

The difference between the two compounds was significant only at 1.500 $\mu\text{L/mL}$ ($p < 0.01$). No significant differences were observed between the two compounds at other concentrations tested ($p > 0.05$).

3.2. Fumigation Toxicity

Eugenol and cinnamaldehyde exhibited concentration-dependent fumigant toxicity against *S. avenae*. (Figure 2, Table 3). The knockdown rate of *S. avenae* increased with concentration, reaching 97.01%, 64.38%, and 33.56% at 10.000, 5.000, and 2.500 $\mu\text{L/mL}$, respectively, with significant differences among these concentrations ($p < 0.05$), while the knockdown rate at 1.250 and 0.625 $\mu\text{L/mL}$ decreased to 14.37% and 6.98%, respectively ($p > 0.05$). Similarly, eugenol caused knockdown rates of 78.60%, 43.69%, and 18.99% at 10.000, 5.000, and 2.500 $\mu\text{L/mL}$, respectively, while the knockdown rate at 1.250 and 0.625 $\mu\text{L/mL}$ decreased to 6.96% and 4.49%, respectively ($p > 0.05$). At equivalent concentrations, cinnamaldehyde showed significantly a higher toxicity than eugenol. Toxicity bioassays were analyzed by probit analysis, and KD_{50} values were calculated. These results showed that cinnamaldehyde ($\text{KD}_{50} = 2.55$ $\mu\text{L/mL}$) was more toxic than eugenol ($\text{KD}_{50} = 7.04$ $\mu\text{L/mL}$) (Table 4). These results demonstrate that cinnamaldehyde exhibited higher knockdown rate of *S. avenae* at similar concentrations than did eugenol.

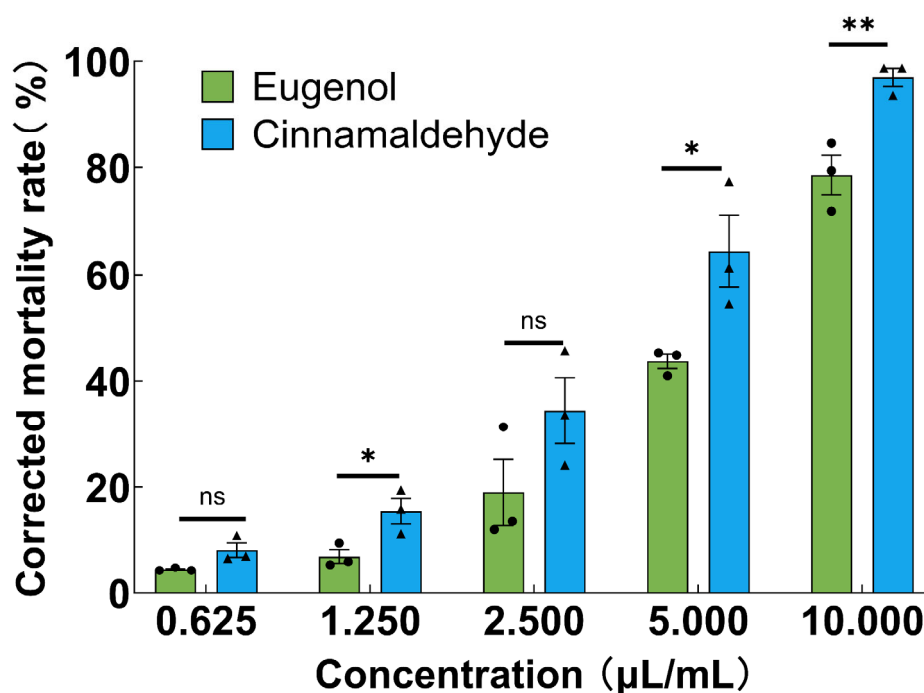


Figure 2. The fumigation effects of eugenol and cinnamaldehyde on the *S. avenae*. Note: Knockdown rates were corrected using Abbott's formula. Statistical significance was determined by Student's *t* test (*, $p < 0.05$; **, $p < 0.01$; ns, not significant). Data are presented as corrected knockdown rates, with error bars indicating the mean $\pm$ standard error (SE). Levene's test indicated homogeneity of variances for most comparisons, except for the 0.625 $\mu\text{L/mL}$ concentration ($F = 11.391$, $p = 0.028$), for which Welch's correction was applied. The corresponding *t* and *p* values are as follows: [0.625]: $t(2.055) = -2.692$, $p = 0.111$; [1.250]: $t(4) = -3.160$, $p = 0.034$; [2.500]: $t(4) = -1.756$, $p = 0.154$; [5.000]: $t(4) = -2.981$, $p = 0.041$; [10.000]: $t(4) = -4.552$, $p = 0.010$.

Table 3. The fumigation effects of eugenol and cinnamaldehyde on *S. avenae* (mean $\pm$ SD).

Treatment	Concentration ($\mu\text{L/mL}$)	Cinnamaldehyde	Eugenol
Fumigation effect	0.625	6.98% $\pm$ 2.40 d	4.49% $\pm$ 0.28 c
	1.250	14.37% $\pm$ 4.17 cd	6.96% $\pm$ 2.22 c
	2.500	33.56% $\pm$ 10.96 c	18.99% $\pm$ 10.72 bc
	5.000	64.38% $\pm$ 11.79 b	43.69% $\pm$ 2.35 b
	10.000	97.01% $\pm$ 2.92 a	78.60% $\pm$ 6.37 a

Note: Knockdown rates were corrected by the Abbott's formula. Cinnamaldehyde: Levene's test confirmed homogeneity of variances across all concentration groups ($F_{(4,10)} = 2.343$, $p = 0.125$). One-way ANOVA revealed a highly significant effect of concentration ($F_{(4,10)} = 70.739$, $p < 0.001$), indicating that the treatments differed significantly among the tested concentrations. Different lowercase letters denote significant differences among concentrations (Tukey's HSD, $p < 0.05$). Eugenol : Levene's test indicated heterogeneity of variances ($F_{(4,10)} = 6.042$, $p = 0.010$), and Welch's ANOVA confirmed a highly significant concentration effect ($F_{(4,4.062)} = 205.909$, $p < 0.001$). Different lowercase letters denote significant differences among concentrations (Games–Howell post hoc, $p < 0.05$).

Table 4. Fumigation of eugenol and cinnamaldehyde on *S. avenae*.

Sample	(Slope $\pm$ SE)	KD ₅₀ (95% Fiducial Limit) $\mu\text{L/mL}$	R	LCR ₅₀ (95% CIs)
Cinnamaldehyde	2.74 $\pm$ 0.50	2.55 (1.80–3.61)	0.95	
Eugenol	1.65 $\pm$ 0.16	7.04 (5.36–9.25)	0.99	2.77 (1.78–4.30) *

Note: LCR = KD₅₀ (eugenol)/KD₅₀ (cinnamaldehyde), representing the relative knockdown potency between the two compounds. The 95% confidence intervals (CIs) for LCR₅₀ were estimated using Fieller's theorem; a significant difference between the two compounds is indicated when the 95% CI excludes 1. The correlation coefficient (r) is also provided. Asterisks (*) indicate statistically significant differences between treatments ($p < 0.05$). Data are presented as mean $\pm$ standard error (SE), with sample size $n = 3$ per concentration.

3.3. Contact Toxicity

The concentration-response relationships of eugenol and cinnamaldehyde were examined using the residual film method, with acetone as the solvent and control (Tables 5 and 6). The results showed that both compounds exhibited concentration-dependent toxicity. For cinnamaldehyde, the knockdown rate reached 95.86% at the highest concentration tested. At 0.200 and 0.150 $\mu\text{L/mL}$, the knockdown rates were 81.97% and 78.10%, respectively, with no significant difference between these two concentrations ($p > 0.05$). The knockdown rates at 0.100 and 0.050 $\mu\text{L/mL}$ decreased to 29.66% and 14.16%, respectively, also showing no significant difference ($p > 0.05$). For eugenol, the knockdown rates at 1.000, 0.500, and 0.250 $\mu\text{L/mL}$ were 96.01%, 82.92%, and 37.01%, respectively, with significant differences among these three concentrations ($p < 0.05$). At lower concentrations (0.125 and 0.063 $\mu\text{L/mL}$), the knockdown rates decreased to 7.95% and 2.95%, respectively, with no significant difference between them ($p > 0.05$). The toxicity regression equations (Table 7) revealed that cinnamaldehyde (KD₅₀ = 0.11 $\mu\text{L/mL}$) was significantly more toxic than eugenol (KD₅₀ = 0.25 $\mu\text{L/mL}$). In conclusion, both compounds possessed contact toxicity against *S. avenae*.

Table 5. The contact killing effect of cinnamaldehyde on *S. avenae* (mean $\pm$ SD).

Treatment	Concentration ($\mu\text{L/mL}$)	Cinnamaldehyde (%)
Contact killing effect	0.050	14.16 $\pm$ 2.32 b
	0.100	29.66 $\pm$ 12.15 b
	0.150	78.10 $\pm$ 5.95 a
	0.200	81.97 $\pm$ 4.95 a
	0.250	95.86 $\pm$ 7.14 a

Note: Knockdown rates of *S. avenae* after 24 h exposure to various concentrations of cinnamaldehyde. Data are presented as Abbott-corrected percentages (mean $\pm$ SE, $n = 3$). Levene's test confirmed homogeneity of variances ($F_{(4,10)} = 2.023$, $p = 0.167$), and one-way ANOVA showed a significant effect of concentration ($F_{(4,10)} = 72.758$, $p < 0.001$). Different lowercase letters indicate significant differences among concentrations (Tukey's HSD, $p < 0.05$).

Table 6. The contact killing effect of eugenol on *S. avenae* (mean $\pm$ SD).

Treatment	Concentration ($\mu\text{L/mL}$)	Eugenol (%)
Contact killing effect	0.063	2.95 $\pm$ 0.76 d
	0.125	7.95 $\pm$ 3.64 d
	0.250	37.01 $\pm$ 9.81 c
	0.500	82.92 $\pm$ 4.65 b
	1.000	96.01 $\pm$ 1.84 a

Note: Knockdown rates of *S. avenae* after 24 h exposure to various concentrations of eugenol. Data are presented as Abbott-corrected percentages (mean $\pm$ SE, $n = 3$). Levene's test confirmed homogeneity of variances ($F_{(4,10)} = 2.350$, $p = 0.125$), and one-way ANOVA showed a significant concentration effect ($F_{(4,10)} = 203.603$, $p < 0.001$). Different lowercase letters indicate significant differences among concentrations (Tukey's HSD, $p < 0.05$).

Table 7. Contact toxicity of eugenol and cinnamaldehyde against *S. avenae*.

Sample	(Slope $\pm$ SE)	KD ₅₀ (95% Fiducial Limit) $\mu\text{L/mL}$	R	LCR ₅₀ (95% CIs)
Cinnamaldehyde	3.99 $\pm$ 0.63	0.11 (0.01–0.13)	0.96	
Eugenol	3.39 $\pm$ 0.37	0.25 (0.20–0.30)	0.98	2.33 (1.76–3.08) *

Note: LCR = KD₅₀ (eugenol)/KD₅₀ (cinnamaldehyde), representing the relative knockdown potency between the two compounds. The 95% confidence intervals (CIs) for LCR₅₀ were estimated using Fieller's theorem; a significant difference between the two compounds is indicated when the 95% CI excludes 1. The correlation coefficient (r) is also provided. Asterisks (*) indicate statistically significant differences between treatments ($p < 0.05$). Data are presented as mean $\pm$ standard error (SE), with sample size $n = 3$ per concentration.

4. Discussion

Plant essential oils and their active components have attracted considerable attention as potential biopesticides, owing to their multiple modes of action, including repellency, contact toxicity, and antifeedant activity, as well as their favorable environmental compatibility [14,55,56]. Their volatile and lipophilic nature facilitates penetration through the insect cuticle, respiratory system, and digestive tract, thereby disrupting normal physiological and behavioral functions [37,57–59]. In the present study, both eugenol and cinnamaldehyde exhibited strong contact toxicity, fumigant toxicity, and repellent effects against *S. avenae*, consistent with the multi-target activities of plant essential oils reported previously. Notably, cinnamaldehyde consistently showed higher activity than eugenol in all three bioassays. The contact KD₅₀ values of both compounds were approximately two orders of magnitude lower than their corresponding fumigant KD₅₀ values, clearly indicating that contact action is the primary route of insecticidal activity, with fumigation playing a secondary role. This differential potency may be attributed to the physicochemical properties of these volatile phenylpropanoids, such as their relatively low vapor pressure and high lipophilicity, which favor cuticular penetration over respiratory uptake. The superior activity of cinnamaldehyde over eugenol is likely related to their structural differences [60,61], which can influence volatility, lipophilicity, and binding affinity to potential target sites in *S. avenae* [62]. However, further mechanistic studies (e.g., molecular docking and enzymatic assays) are required to verify these possibilities.

Meanwhile, low concentrations of eugenol and cinnamaldehyde have shown significant attractive effects, suggesting that these two compounds could serve as potential components in an “attract-and-kill” strategy. In the future, low-dose attractants might be applied to lure aphids into areas already sprayed with lethal doses, or they could be combined with attractants for natural enemies, thereby potentially further improving overall pest control efficacy.

Some discrepancies exist between the findings of this study and previous reports, possibly reflecting differences in the target insect species. For example, eugenol and trans-cinnamaldehyde showed strong contact toxicity and repellency against *Tribolium castaneum*, but no fumigant activity has been reported against bed bugs [63]. In addition, eugenol displayed strong area repellent properties, while a 24 h residual effect of eugenol elicited avoidance in bed bugs; but this effect has not been studied with trans-

cinnamaldehyde [64]. Differences in the activities of these compounds against the same insect at different life stages have also been reported, with eugenol being more active against *Dermanyssus gallinae*, while cinnamaldehyde showed greater activity against *Musca domestica* [47,65]. Some of these differences might stem from variation in cuticular permeability, metabolism and target site sensitivity among insect species, as well as the intrinsic properties of the components in the oils themselves [60, 66, 67]. Target species- and life stage-specific approaches using extracts would be beneficial if more economical options for formulating insecticidal agents from plant essential oil compounds can be identified.

The present study confirms that eugenol and cinnamaldehyde possess dual insecticidal and repellent activities against *S. avenae* under laboratory conditions. These findings add to the growing body of evidence that certain plant-derived phenylpropanoids may serve as useful tools for aphid management. However, the practical relevance of these laboratory-based observations remains to be established.

The relatively short persistence of essential oil constituents, while advantageous for rapid degradation and low environmental residue, poses a practical limitation that may necessitate frequent applications or the development of sustained-release formulations to ensure prolonged field efficacy. If appropriately formulated, these compounds could potentially be integrated with conventional insecticides to achieve effective control, a prospect conceptually supported by earlier reports that plant essential oils synergize the activity of pyrethroid, organophosphate, and neonicotinoid insecticides against various arthropod pests [68–72]. However, synergism studies targeting *S. avenae*, as well as evaluations of non-target safety and phytotoxicity to wheat, would be required before any field application could be recommended. Furthermore, formulation and application technology would be critical for any practical use.

Several limitations of this study should be acknowledged. First, the immobilization criterion employed in our bioassays may include reversible knockdown effects; therefore, the reported KD_{50} values should be interpreted as median knockdown concentrations rather than true lethal endpoints, and verification through post-exposure recovery observations would be needed to distinguish irreversible toxicity from transient paralysis. Second, all experiments were conducted under controlled laboratory conditions, and the performance of eugenol and cinnamaldehyde against natural *S. avenae* populations in the field, as well as their persistence, volatility, and potential phytotoxicity to wheat, has not been evaluated. Third, the specific molecular mechanisms by which these compounds exert their toxicity after cuticular penetration or respiratory uptake remain unclear. Fourth, the biological activity of individual compounds may not fully reflect the overall efficacy of their corresponding essential oils, which typically contain multiple constituents with potential synergistic interactions [73]. Future research should also focus on formulation optimization, assessment of field persistence, and elucidation of the molecular targets of these compounds, in order to better evaluate their potential role in sustainable aphid management practices.

5. Conclusions

In conclusion, eugenol and cinnamaldehyde, as plant-derived natural products, exhibit both insecticidal activity and behavioral regulation effects on *S. avenae* under laboratory conditions. Among them, the contact toxicity and fumigation toxicity of cinnamaldehyde are significantly superior to those of eugenol. The repellent effect shows a clear concentration-dependent relationship, and at low concentrations, there is a reverse attraction phenomenon. This relationship suggests that in practical applications, the appropriate dosage should be carefully selected to avoid adverse behavioral responses caused by low doses.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture16141538/s1>, Figure S1: Dose-response curve of fumigation effect; Figure S2: Dose-response curve of the contact killing effect of cinnamaldehyde. Figure S3: Dose-response curve of the contact killing effect of eugenol. Table S1: Fumigation data-cinnamaldehyde. Table S2: Fumigation data- eugenol; Table S3: Contact data- cinnamaldehyde; Table S4: Contact data- eugenol; Table S5: Repellent data- cinnamaldehyde; Table S6: Repellent data-eugenol.

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