Bioactivity of *Artemisia vulgaris* Essential Oil and Two of Its Constituents Against the Red Flour Beetle (Coleoptera: Tenebrionidae)[1]

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J. Entomol. Sci. 58(3): 355–369 (July 2023)
DOI: 10.18474/JES22-49

Abstract *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is one of the most destructive pests of stored products. Although there have been studies on the potential use of essential oils from plants in the *Artemisia* genus as insecticides, no comprehensive bioactivity data are available on the efficacy of *Artemisia vulgaris* L. (Asterales: Asteraceae) essential oil and its chemical constituents on stored-product pests. Therefore, in this study, the bioactivity of *A. vulgaris* essential oil and its chemical constituents, eugenol and terpinen-4-ol, against *T. castaneum* were determined by contact, fumigant, and repellent bioassays. Analysis of contact and fumigant bioassays showed that *A. vulgaris* essential oil, eugenol, and terpinen-4-ol have contact and fumigant toxicities against *T. castaneum*, of which terpinen-4-ol has a strong killing effect on larvae and adults, suggesting that terpinen-4-ol may be the main active component of *A. vulgaris* essential oil in contact and fumigant effects. Additionally, *A. vulgaris* essential oil and eugenol have higher repellent activity against *T. castaneum* larvae and adults, whereas the repellent activity of terpinen-4-ol is low, indicating that the main component of *A. vulgaris* essential oil in repellence may be eugenol. These results further provide relevant theoretical basis for the development of plant essential oil pesticides.

Key Words *Tribolium castaneum*, *Artemisia vulgaris* essential oil, eugenol, terpinen-4-ol, bioactivity

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is an important stored-grain pest. Because of its proven adaptability, high fecundity rates, and short developmental cycle, *T. castaneum* causes billions of dollars of economic losses annually (Zhang et al. 2018). The chemical pesticides methyl bromide and phosphine have been generally used for fumigation to control stored-grain pests including *T. castaneum* (Athanassiou et al. 2015). However, frequent and extensive use of these fumigants causes problems such as the development of insecticide resistance and environmental pollution (Pimentel et al. 2007). Therefore, there is a need to develop environmentally friendly pesticides for the management of stored-grain insect pests.

[1]Received 22 October 2022; accepted for publication 14 November 2022.
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Pest management with insecticides requires the use of chemicals with high specificity, safety, and eco-friendly properties (Caballero-Gallardo et al. 2011). Plant-derived sources of agents pest management are attractive alternatives owing to their low toxicity, low residue, easy biodegradation, high selectivity, and low environmental risk (Isman 2008). Thus, the search for natural products of botanical origin that have insecticidal activity has intensified in the scientific community (Isman 2006) which, in turn, has increased the number of natural products extracted from plants as substitutes for traditional or synthetic compounds (Pavela 2009). In recent years, essential oils of plants have been widely used as bioactive agents including insecticides (Li et al. 2010), ovicides (Tunç et al. 2000), antifeedants (Huang et al. 2000), oviposition inhibitors (Ho et al. 1996), growth regulators (Naseri et al. 2017), and repellents (Ogendo et al. 2008). For instance, the essential oils of *Ocimum basilicum* L. (Lamiales: Laiaceae) (Obeng-Ofori and Reichmuth 1997), *Artemisia annua* L. (Asterales: Asteraceae) (Goel et al. 2007), *Baccharis salicifolia* (Rulz & Pav.) Pers. (Rosales: Rosaceae) (García et al. 2005), *Trachyspermum ammi* L. (Apiales: Apiaceae) (Soni et al. 2016), and *Anethum graveolens* L. (Apiales: Apiaceae) (Jana and Shekhawat 2010) exhibit highly toxic and repellent activity against *T. castaneum* when applied topically or impregnated on filter paper, grains, or glass pebbles. In addition, these essential oils contain terpenes (monoterpenes, biogenetically associated phenols, and sesquiterpenes) and aliphatic and aromatic compounds, of which terpenes are the most represented components (Akhtar et al. 2008, Bakkali et al. 2008). To date, studies also suggest that these terpenes, such as α-terpineol, 1,8-cineole, and linalool, aid in pest management via a broad spectrum of insecticidal activity (Alzogaray et al. 2013, Rozman et al. 2007). Undoubtedly, these terpenes offer another choice of novel insecticides for stored-product insect pest management.

A Chinese herbal medicine of *Artemisia vulgaris* L. (Asterales: Asteraceae) in Tangyin County, Henan Province, possesses rich natural resources (Huang and Qiu 2014, Jiang et al. 2019). The insecticidal and larvicidal properties of *A. vulgaris* essential oil have been attributed to the presence of camphene, a chloro derivative of alpha-pinene, among which eugenol and terpinen-4-ol are relatively high (Pandey and Singh 2017). Although there have been studies on the potential use of essential oils from plants in the *Artemisia* genus as insecticidal fumigants, the molecular mechanisms underlying the toxic effects of *A. vulgaris* essential oil on *T. castaneum* remain unclear (Gao et al. 2020) and no comprehensive bioactivity data are available on the efficacy of essential oil and its chemical constituents on stored-product insects. Therefore, the contact, repellent, and fumigant effects of *A. vulgaris* essential oil and its constituents eugenol and terpinen-4-ol to different developmental stages of *T. castaneum* were measured and compared. These results will further provide a relevant theoretical basis for the development of plant essential oil pesticides.

Materials and Methods

Insects. A laboratory colony of *T. castaneum*, strain Georgia-1 (GA-1) that was not exposed to any insecticides, served as the source of all insects used in this study. The colony was maintained in a jar containing wheat flour + 5% (w/w) brewer's yeast in a growth chamber kept at 30°C and 40% relative humidity under a 14:10-h light:dark photo regime as per the protocol of Zhang et al. (2022b).

Chemical extraction and preparation. Mugwort *A. vulgaris* plants were collected from Tangyin County, Henan Province (N 35°45′ to approximately 36°01′, E 114°13′ to approximately 114°42′). The extraction of *A. vulgaris* essential oil was as described previously (Gao et al. 2020). Briefly, the extraction was performed on the subcritical butane extraction apparatus (Henan Subcritical Biological Technology Co., Ltd., Anyang, China) with the following parameters: liquid:solid ratio = 30:1, temperature = 45°C, extraction time = 34 min, and particle size = 0.26 mm. The extract was subjected to hydrodistillation in a Clevenger-type apparatus for 2 h. To separate the essential oil from residual water, the oil/water emulsion was collected and stored at 4°C overnight. The essential oil was then transferred and stored in a glass bottle at room temperature until further use. Eugenol (99% purity; CAS: 97-53-0) and terpinen-4-ol (97% purity; CAS: 562-74-3) were acquired from the Aladdin Company (Shanghai, China).

Contact bioassay. The *A. vulgaris* essential oil, eugenol, and terpinen-4-ol were dissolved in acetone and diluted to working concentrations of 200, 100, 50, 25, and 12.5 mg/mL). Contact activity against *T. castaneum* larvae and adults was determined as described previously (Gao et al. 2022, Zhang et al. 2022a). Briefly, 30 15-d-old larvae or 10-d-old adults in each group were loaded into 1.5-mL Eppendorf tubes and exposed to 100 μL of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol solutions. After immersion for 1 min, the treated larvae were placed on filter paper and allowed to air-dry for 2 min. Each group was then moved to Drosophila vials (24 × 96 mm) with wheat flour containing 5% brewer's yeast and maintained as previously described. The same volume of acetone was used to treat the control larvae. Each experiment was repeated at least three times. Mortality was recorded at 12, 24, 36, 48, 60, and 72 h after treatment. Larvae were considered dead if they were unable to move or show a response when disturbed with a pair of tweezers or a brush.

Fumigant bioassay. The *A. vulgaris* essential oil, eugenol, and terpinen-4-ol were dissolved in acetone and diluted to working concentrations of 480, 240, 120, 60, 30, and 15 mg/mL. The toxicity of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol against *T. castaneum* was tested as described previously (Abdelgaleil et al. 2009). Briefly, a 300-mL conical flask was used as a fumigation chamber. Different concentrations of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol were individually applied to filter paper (2 × 3 cm) attached to the undersurface of the stoppers of the conical flasks. Stoppers were inserted tightly into the conical flasks containing 30 individuals of *T. castaneum* (early larvae [1 d old], late larvae [18 d old], early adults [1 d old], and late adults [10 d old]) in each one. Acetone was used as the control group under the same conditions. The number of dead insects was counted after 72 h of treatment, with six replicates of each treatment.

Repellent bioassay. The *A. vulgaris* essential oil, eugenol, and terpinen-4-ol were dissolved in acetone and diluted to working concentrations of 200, 100, 50, 25, and 12.5 mg/mL. The repellent effects of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol against *T. castaneum* were evaluated using the area preference method (Tapondjou et al. 2005). Briefly, test arenas contained 9-cm-diameter filter paper discs cut in half. Then, 0.4 mL of different concentrations of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol were uniformly applied to a half-filter paper using a micropipette. Meanwhile, the same volume of acetone was added in the other half of the filter paper as a control. Chemically treated and control half discs were air-dried for 10 min to evaporate the solvent completely. Entire discs were subsequently reconstructed by attaching treated halves to untreated halves with clear adhesive tape. Each reconstructed filter paper disc was placed into a 9-cm-diameter culture dish in which 30 15-d-old larvae or 10-d-old adults were released at the center of the filter paper disc. The culture dishes were subsequently covered. The treatments were replicated five times and the numbers of insects present on the control (N_c) and treated (N_t) areas of the discs were recorded after 12, 24, 36, 48, 60, and 72 h. The values of repellent rate were computed as follows: percentage repellence (PR) $= [(N_c - N_t)/(N_c - N_t)] \times 100$. The PR can be divided into six classes according to the repellent rate (Jilani and Su 1983): class 0 (PR $<$ 0.1%), class I (PR = 0.1–20%), class II (PR = 20.1–40%), class III (PR = 40.1–60%), class IV (PR = 60.1–80%), and class V (PR = 80.1–100%).

Statistical analysis. Concentration–mortality response parameters (e.g., median lethal concentration [LC_{50}], 95% fiducial limits, chi square values, etc.) were calculated by probit analysis using the SPSS statistics program (SPSS Inc., Chicago, IL). The significance of the phytotoxic activity was first examined by analysis of variance and Fisher's LSD test at $P < 0.05$.

Results

Contact toxicity. The contact assay showed that mortality of *T. castaneum* larvae (15 d old) and adults (10 d old) treated with increasing concentrations of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol (12.5, 25, 50, 100, and 200 mg/mL) was concentration dependent regardless of the time interval (12, 24, 36, 48, 60, and 72 h) after treatment. In fact, beetles treated with the same dose of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol showed no significant difference in mortality at the different time intervals (Figs. 1, 2). These results demonstrated that *A. vulgaris* essential oil, eugenol, and terpinen-4-ol exhibited pronounced contact toxicity against *T. castaneum* with a concentration-dependent relationship, but there were no apparent time-dependent responses in mortality.

In addition, the LC_{50} of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol in *T. castaneum* larvae (15 d old) and adults (10 d old) were determined at the 12-, 24-, 36-, 48-, 60-, and 72-h intervals after treatment. The LC_{50} values of *A. vulgaris* essential oil on larvae (15 d old) were 147.546 mg/mL at 12 h, 137.386 mg/mL at 24 h, 132.472 mg/mL at 36 h, 131.206 mg/mL at 48 h, 131.206 mg/mL at 60 h, and 131.206 mg/mL at 72 h. The LC_{50}'s of eugenol on larvae (15 d old) at those respective time intervals were 115.654, 85.035, 78.705, 77.877, 77.877, and 77.877 mg/mL, while the LC_{50}'s of terpinen-4-ol on larvae (15 d old) at those respective times were 84.172, 68.391, 67.211, 67.211, 67.211, and 67.211 mg/mL (Table 1). Similarly, the LC_{50} values of *A. vulgaris* essential oil on adults (10 d old) were 165.195 mg/mL at 12 h, 155.237 mg/mL at 24 h, 149.533 mg/mL at 36 h, 147.757 mg/mL at 48 h, 147.757 mg/mL at 60 h, and 147.757 mg/mL at 72 h. The LC_{50}'s of eugenol on adults (10 d old) for the respective time intervals were 130.556, 123.760, 123.238, 119.489, 119.489, and 119.489 mg/mL, while the LC_{50}'s of terpinen-4-ol on adults at those times were 112.907, 102.250, 99.098, 99.098,

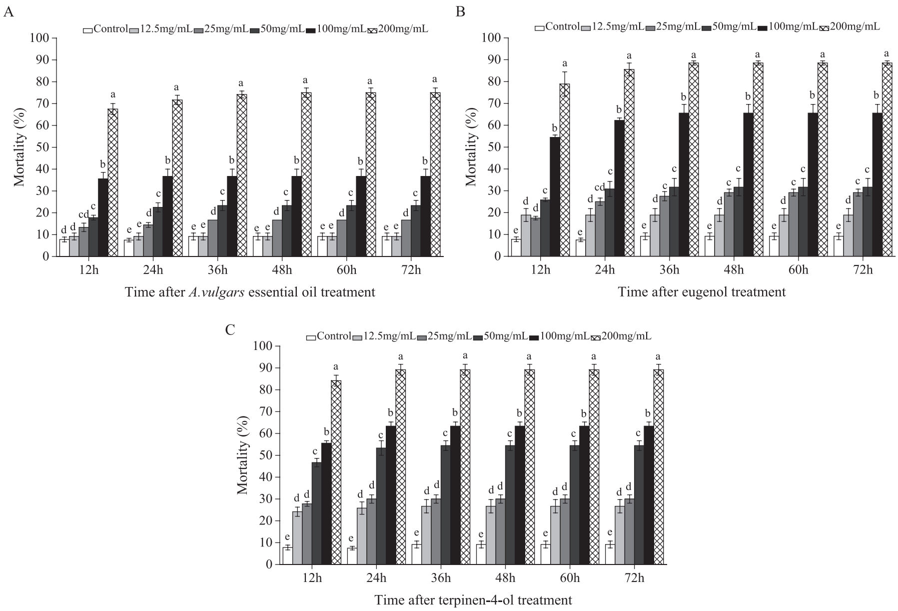

Fig. 1. Contact activity of *A. vulgaris* essential oil (A), eugenol (B), and terpinen-4-ol (C) against *T. castaneum* larvae (15 d old) as indicated by larval mortality at five concentrations of the chemicals at 12, 24, 36, 48, 60, and 72 h after treatment. Vertical bars indicate standard errors of the mean ($n = 6$ independent replicates); different lowercase letters above the bars indicate that the means are significantly different between the control (acetone) and the chemical ($P < 0.05$).

99.098, and 99.098 mg/mL (Table 2). These results indicate that the LC_{50}'s of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol on larvae (15 d old) or adults (10 d old) are apparently time-dependent effects from 12 to 48 h, after which toxicity levels remain relatively constant.

Fumigant activity. We found that the fumigant effect of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol on larvae and adults increased with the increasing concentration, but we saw no differences in effect of fumigation between larvae and adults (Fig. 3). The LC_{50}'s of *A. vulgaris* essential oil on early larvae (1 d old), late larvae (18 d old), early adults (1 d old), and late adults (10 d old) were 64.930, 80.273, 151.826, and 230.219 mg/mL, respectively. The LC_{50}'s of eugenol on early larvae (1 d old), late larvae (18 d old), early adults (1 d old), and late adults (10 d old) were 39.908, 79.470, 150.868, and 116.578 mg/mL, respectively, while the LC_{50}'s of terpinen-4-ol on early larvae, late larvae, early adults, and late adults were 8.254, 18.846, 48.704, and 25.160 mg/mL, respectively (Table 3). These results indicate that the LC_{50}'s and, thus, the toxicity of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol also increased with age or development, providing further evidence

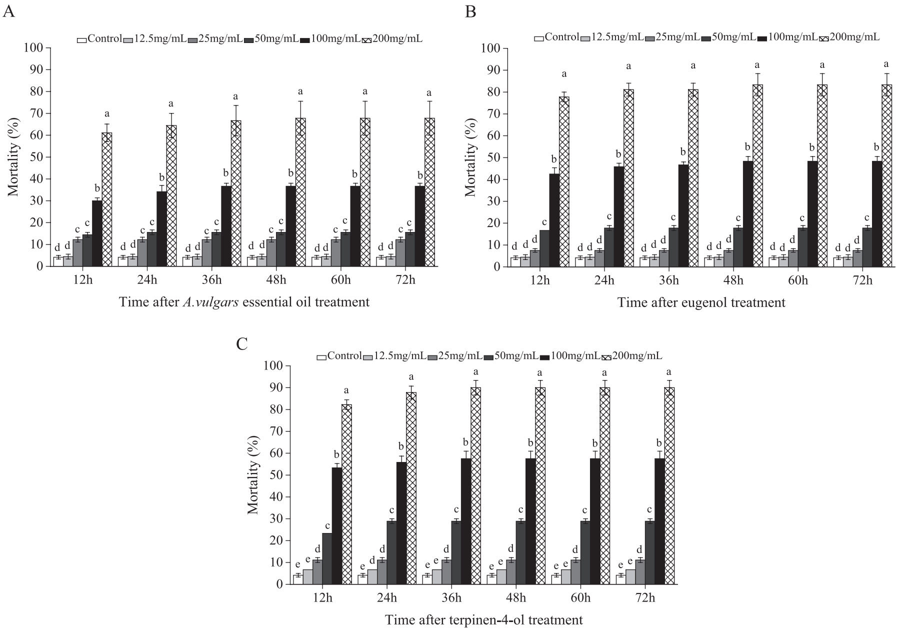

Fig. 2. Contact activity of *A. vulgaris* essential oil (A), eugenol (B), and terpinen-4-ol (C) against *T. castaneum* adults (10 d old) as indicated by adult mortality at five concentrations of the chemicals at 12, 24, 36, 48, 60, and 72 h after treatment. Vertical bars indicate standard errors of the mean ($n = 6$ independent replicates); different lowercase letters above the bars indicate that the means are significantly different between control (acetone) and the chemical ($P < 0.05$).

that *A. vulgaris* essential oil, eugenol, and terpinen-4-ol have fumigant toxicity against *T. castaneum* larvae and adults.

Repellent activity. Repellency of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol against *T. castaneum* larvae (15 d old) and adults (10 d old) was detected in our bioassay (Table 4). There was no significant variation in the repellent effects of *A. vulgaris* essential oil and eugenol against *T. castaneum* larvae (10 d old) with low concentration (PR <80%), but terpinen-4-ol had a significantly lower repellency effect (PR <60%). Similarly, the repellent activity of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol against *T. castaneum* adults have the same effects (Table 5), which indicates that eugenol, one of the main constituents in *A. vulgaris* essential oil, plays a major role in repellent activity.

Discussion

A number of previous studies are available reporting the contact and fumigant toxicities of essential oils and terpenes against stored-product pests. However, it is

Table 1. Contact toxicity of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol against late-instar *T. castaneum* larvae.

Treatment	Time (h)	LC_{50}* (mg/mL)	95% FL** (mg/mL) Lower	Upper	Slope† ± SE	$\chi^{2‡}$	*P*#
A. vulgaris essential oil	12	147.546	131.458	168.353	0.009 ± 0.001	0.417	0.937
	24	137.386	122.505	156.145	0.009 ± 0.001	0.633	0.889
	36	132.472	118.113	150.358	0.010 ± 0.001	1.018	0.797
	48	131.206	117.091	148.696	0.010 ± 0.001	1.024	0.795
	60	131.206	117.091	148.696	0.010 ± 0.001	1.024	0.795
	72	131.206	117.091	148.696	0.010 ± 0.001	1.024	0.795
Eugenol	12	115.654	101.173	133.290	0.009 ± 0.001	4.121	0.249
	24	85.035	73.782	97.297	0.011 ± 0.001	2.642	0.450
	36	78.705	67.936	90.211	0.011 ± 0.001	3.245	0.355
	48	77.877	67.006	89.449	0.011 ± 0.001	3.348	0.341
	60	77.877	67.006	89.449	0.011 ± 0.001	3.348	0.341
	72	77.877	67.006	89.449	0.011 ± 0.001	3.348	0.341
Terpinen-4-ol	12	84.172	70.717	98.773	0.009 ± 0.001	3.936	0.268
	24	68.391	37.419	100.056	0.010 ± 0.001	5.882	0.118
	36	67.211	33.257	101.276	0.010 ± 0.001	6.549	0.088
	48	67.211	33.257	101.276	0.010 ± 0.001	6.549	0.088
	60	67.211	33.257	101.276	0.010 ± 0.001	6.549	0.088
	72	67.211	33.257	101.276	0.010 ± 0.001	6.549	0.088

* LC_{50}, median lethal concentration.
** FL, fiducial limits.
† Slope of the concentration–inhibition regression line ± SE.
‡ Chi square value.
\# $P \geq 0.05$ indicates a significant fit between the observed and expected regression lines in a probit analysis.

difficult to compare those data and findings due to different concentrations of essential oils used for the same insect, *T. castaneum*, or different pest insects (Abdelgaleil et al. 2009, Ogendo et al. 2008, Rozman et al. 2007, Tapondjou et al. 2005). Similar to findings from the previous studies (Borzoui et al. 2016, Naseri et al. 2017), analysis of contact and fumigant activity shows that the *A. vulgaris* essential oil, eugenol, and terpinen-4-ol are toxic to *T. castaneum* larvae and adults (Figs. 1–3). By contrast, eugenol and terpinen-4-ol have higher toxicities against the larvae (15 d old) and adults (10 d old) with concentrations of 50, 100, and 200 mg/

Table 2. Contact toxicity of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol against late *T. castaneum* adults.

Treatment	Time (h)	LC_{50}* (mg/mL)	95% FL** (mg/mL)		Slope† ± SE	$\chi^{2‡}$	$P^{\#}$
			Lower	Upper			
A. vulgaris essential oil	12	165.195	147.574	188.618	0.009 ± 0.001	2.793	0.425
	24	155.237	139.252	175.861	0.010 ± 0.001	3.600	0.308
	36	149.533	134.510	168.549	0.010 ± 0.001	4.265	0.234
	48	147.757	133.075	166.210	0.010 ± 0.001	4.000	0.261
	60	147.757	133.075	166.210	0.010 ± 0.001	4.000	0.261
	72	147.757	133.075	166.210	0.010 ± 0.001	4.000	0.261
Eugenol	12	130.556	106.672	165.109	0.013 ± 0.001	5.534	0.137
	24	123.760	99.513	158.687	0.014 ± 0.001	6.437	0.092
	36	123.238	98.012	160.178	0.014 ± 0.001	7.038	0.071
	48	119.489	95.195	154.307	0.014 ± 0.001	7.016	0.071
	60	119.489	95.195	154.307	0.014 ± 0.001	7.016	0.071
	72	119.489	95.195	154.307	0.014 ± 0.001	7.016	0.071
Terpinen-4-ol	12	112.907	86.535	151.642	0.013 ± 0.001	7.463	0.059
	24	102.250	77.845	136.513	0.014 ± 0.001	7.585	0.055
	36	99.098	77.493	128.311	0.015 ± 0.001	6.441	0.092
	48	99.098	77.493	128.311	0.015 ± 0.001	6.441	0.092
	60	99.098	77.493	128.311	0.015 ± 0.001	6.441	0.092
	72	99.098	77.493	128.311	0.015 ± 0.001	6.441	0.092

* LC_{50}, median lethal concentration.
** FL, fiducial limits.
† Slope of the concentration–inhibition regression line ± SE.
‡ Chi square value.
\# $P \geq 0.05$ indicates a significant fit between the observed and expected regression lines in a probit analysis.

mL, whereas the contact toxicities of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol were not significantly different with concentrations of 12.5 and 25 mg/mL (Figs. 1, 2). In addition, the LC_{50}'s of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol showed that the terpinen-4-ol is more suitable than the other two compounds as a contact insecticide against *T. castaneum*. Similar evidence of insecticidal activity of terpinen-4-ol has been investigated in several insect species, including *Sitophilus oryzae* L. (Lee et al. 2001), *Sitophilus zeamais* (Mochul'skii) (Liao et al. 2016), and *Aedes albopictus* (Skuse) (Seo et al. 2015). In addition, the fumigant effects of *A.*

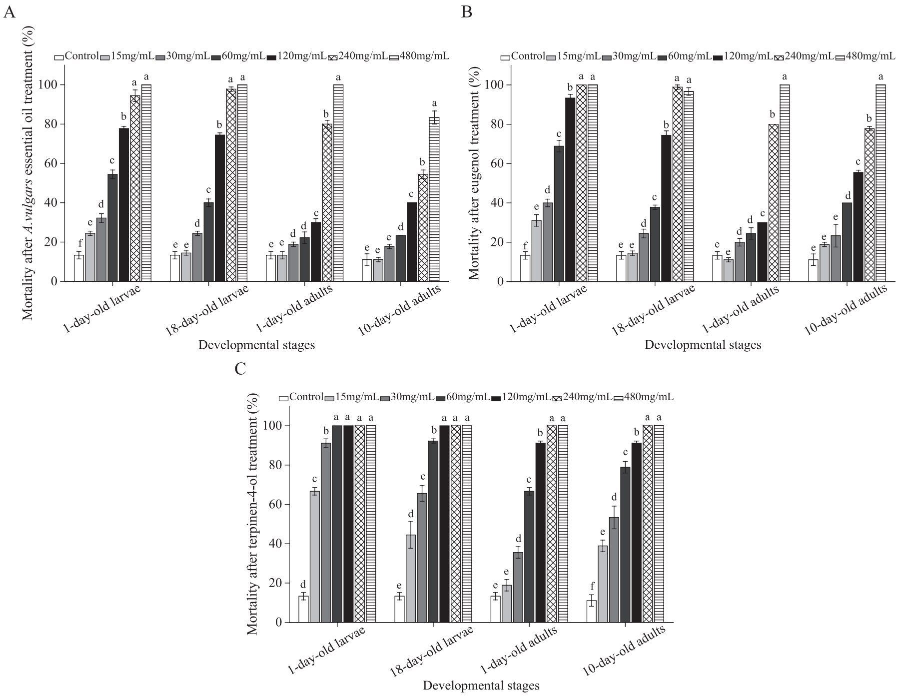

Fig. 3. Fumigation activity of *A. vulgaris* essential oil (A), eugenol (B), and terpinen-4-ol (C) against *T. castaneum* at different developmental stages as indicated by mortality at six concentrations of the chemicals at 72 h after treatment. The determination of developmental stages as follows: early larvae (1 d old), late larvae (15 d old), early adults (1 d old), and late adults (10 d old). Vertical bars indicate standard errors of the mean ($n = 6$ independent replicates); different lowercase letters above the bars indicate that the means are significantly different between control (acetone) and chemical ($P < 0.05$).

vulgaris essential oil and eugenol against early (1 d old) or late larvae (18 d old) were not significantly different with concentrations of 240 and 480 mg/mL, but the fumigant effects *A. vulgaris* essential oil and eugenol against adults were significantly different (Fig. 3A, B). Furthermore, terpinen-4-ol showed consistent fumigant activity at concentrations of 120, 240, and 480 mg/mL (Fig. 3C), indicating that the fumigant effect against larvae was higher than that of adults with the highest concentration of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol. The LC_{50}'s of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol and, thus, their toxicities increased with age or later development of *T. castaneum* (Table 3).

The repellent effects of phytochemicals on insects depend on several factors, among which are the chemical composition of the essential oil and insect susceptibility (Van Nieuwenhuyse et al. 2012). Additionally, the repellent effects

Table 3. Fumigation toxicity of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol against *T. castaneum.*

Treatment	Stage	LC_{50}* (mg/mL)	95% FL** (mg/mL)		Slope† ± SE	$\chi^{2‡}$	$P^{\#}$
			Lower	Upper			
A. vulgaris essential oil	Early larvae	64.930	40.994	87.792	0.011 ± 0.001	7.184	0.126
	Late larvae	80.273	71.049	90.402	0.014 ± 0.001	2.282	0.684
	Early adults	151.826	136.163	170.256	0.008 ± 0.001	5.608	0.230
	Late adults	230.219	180.934	296.820	0.004 ± 0.001	7.180	0.127
Eugenol	Early larvae	39.908	32.442	46.858	0.020 ± 0.002	0.917	0.922
	Late larvae	79.470	70.670	89.292	0.015 ± 0.001	0.762	0.943
	Early adults	150.868	123.214	188.505	0.009 ± 0.001	6.775	0.148
	Late adults	116.578	100.704	133.922	0.007 ± 0.001	5.273	0.260
Terpinen-4-ol	Early larvae	8.254	−1.356	12.804	0.063 ± 0.013	0.072	0.999
	Late larvae	18.846	12.281	23.679	0.035 ± 0.005	0.037	1.000
	Early adults	48.704	42.130	55.341	0.021 ± 0.002	4.428	0.351
	Late adults	25.160	13.799	33.986	0.016 ± 0.002	4.861	0.302

* LC_{50}, median lethal concentration.
** FL, fiducial limits.
† Slope of the concentration–inhibition regression line ± SE.
‡ Chi square value.
# $P \geq 0.05$ indicates a significant fit between the observed and expected regression lines in a probit analysis.

of *A. vulgaris* essential oil and eugenol against *T. castaneum* (PR >80%) are higher than terpinen-4-ol at 200 mg/mL; however, there have been no comprehensive changes of the class of repellence of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol at 100, 50, 25, and 12.5 mg/mL (Tables 4, 5), indicating that *A. vulgaris* essential oil and eugenol are more suitable for use as repellents against *T. castaneum*. In addition, the treatment time of *A. vulgaris* essential oil, eugenol, and terpinen-4-ol had no significant effect on the repellency of larvae and adults, and the repellency effect is concentration dependent. Overall, our results will provide baseline data for the use of these chemistries against *T. castaneum* in stored products as well as indications that appropriately increasing the concentration of the *A. vulgaris* essential oil and eugenol could improve repellency against stored-product pests.

Acknowledgments

This study was sponsored by the Staring Foundation for the Doctor, Anyang Institute of Technology (grant numbers BSJ2019009, BSJ2021040, and BSJ2021041).

Table 4. Repellency class of *A. vulgaris* essential oil (AVEO), eugenol, and terpinen-4-ol (T4OL) to larvae of *T. castaneum*.

Time (h)	Concentration														
	200 mg/mL			100 mg/mL			50 mg/mL			25 mg/mL			12.5 mg/mL		
	AVEO	Eugenol	T4OL	AVEO	Eugenol	T4OL	AVEO	Eugenol	T4OL	AVEO	Eugenol	T4OL	AVEO	Eugenol	T4OL
12	V	IV	III	V	IV	II	IV	IV	II	III	III	II	III	III	II
24	V	V	III	V	IV	II	IV	IV	II	III	III	II	III	III	II
36	V	V	III	V	V	II	IV	IV	II	III	III	II	III	III	II
48	V	V	III	V	V	II	IV	IV	II	III	III	II	III	III	I
60	V	V	II	IV	IV	II	IV	IV	II	III	III	I	III	III	I
72	V	V	II	IV	IV	II	IV	IV	II	III	III	I	III	III	I

Table 5. Repellency class of *A. vulgaris* essential oil (AVEO), eugenol, and terpinen-4-ol (T4OL) to adults of *T. castaneum.*

Time (h)	Concentration														
	200 mg/mL			100 mg/mL			50 mg/mL			25 mg/mL			12.5 mg/mL		
	AVEO	Eugenol	T4OL	AVEO	Eugenol	T4OL	AVEO	Eugenol	T4OL	AVEO	Eugenol	T4OL	AVEO	Eugenol	T4OL
12	V	V	III	IV	V	II	IV	V	II	V	IV	I	IV	V	I
24	V	V	II	V	IV	II	IV	V	II	V	V	I	V	IV	I
36	V	V	II	IV	IV	II	V	V	II	V	V	I	IV	IV	I
48	V	V	III	IV	IV	II	IV	V	II	IV	IV	I	IV	IV	I
60	V	V	II	IV	V	I	IV	V	I	IV	IV	I	IV	IV	I
72	IV	IV	II	IV	IV	I	IV	IV	II	IV	IV	I	IV	IV	I

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