Journal of Stored Products Research 119 (2026) 103152

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Botanical innovation in grain storage: Dual insecticidal and acaricidal activity of *Commiphora myrrha* essential oil in stored wheat

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ARTICLE INFO

Keywords:
Botanical insecticide
Stored-product pests
Insects
Mite
Furanosesquiterpenes

ABSTRACT

The search for effective botanical alternatives to synthetic insecticides in stored-grain protection is intensifying due to resistance development and regulatory restrictions of the latter. The current study evaluated the acaricidal and insecticidal effectiveness of *Commiphora myrrha* essential oil (EO), rich in furanosesquiterpenes, against three stored-product pests of wheat: *Acarus siro* adults and nymphs, *Trogoderma granarium* larvae and adults, and *Rhyzopertha dominica* adults. The myrrh EO main constituents were furanoeudesma-1,3-diene, curzerene, lindestrene, and isofuranodiene. Hard wheat was treated with two concentrations of *C. myrrha* EO (500 and 1000 ppm) and compared with pirimiphos-methyl at the label concentration (5 ppm). Mortality was assessed from 4 h to 7 days of exposure. The EO demonstrated species- and stage-dependent efficacy, while outperforming the synthetic insecticidal agent in many cases. *Trogoderma granarium* adults were the more susceptible developmental stage (100 % mortality at 6 d at 1000 ppm), followed by *A. siro* adults (77.8 % mortality at 7 d at 1000 ppm). In contrast, *R. dominica* adults (71.1 % mortality at 7 d at 1000 ppm) and *A. siro* nymphs (68.9 % mortality at 7 d at 1000 ppm) showed moderate mortalities and *T. granarium* larvae (25.6 % mortality at 7 d at 1000 ppm) high tolerance. The pronounced adulticidal activity against *T. granarium* and the documented acaricidal effect against *A. siro* indicate that *C. myrrha* EO may serve as a multi-targeting grain protectant. This dual action enhances the potential integration of *C. myrrha* EO into sustainable stored-product pest management programs.

1. Introduction

Numerous arthropods, i.e., insects and mites, reduce the amount and quality of stored products. While these pests attack and consume the stored commodities, their presence contaminates and reduces their commercial value (Karabörklü and Ayvaz, 2023). Among these pests, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), *Acarus siro* L. (Sarcoptiformes: Acaridae), and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) are commonly found in storage facilities, posing a continuous threat worldwide (Win et al., 2020; Barbosa et al., 2022; Rosentrater, 2022). *Rhyzopertha dominica*, a primary pest of stored grains globally, infests numerous types of grains, such as rice, wheat, oat, sorghum, and peanuts (Hagstrum and Subramanyam, 2009; Rosentrater, 2022). Nonetheless, grains and particularly wheat, are substrates where *R. dominica* performs the greatest reproductive success (Bashir, 2002; Edde and Phillips, 2006; Edde, 2012; Win et al., 2020). One of the most destructive stored-product insect pests with invasive behavior is *T. granarium* (Papanikolaou et al., 2019; Rosentrater, 2022; EPPO, 2025). Several countries throughout the world currently handle *T. granarium* as a quarantine pest (EPPO, 2025). This is because unfavorable abiotic and/or biotic factors, such as food availability, relative humidity, temperature, and high density of population, can induce *T. granarium* larvae to undergo diapause (Bell, 1994; Bell and Wilson, 1995; Dageri, 2024). One of the most common mite pests found in humid environments of food processing and storage is *A. siro* (Hubert et al., 2003; OConnor, 2009; Barbosa et al., 2022). This species promotes fungal infection, decreases food weight, and modifies organoleptic properties and nutritional values (ŽĎárková and Reška, 1976;

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https://doi.org/10.1016/j.jspr.2026.103152
Received 4 May 2026; Received in revised form 8 June 2026; Accepted 27 June 2026
Available online 19 July 2026

Hubert et al., 2003; Yassin, 2015; Brückner and Heethoff, 2016). Additionally, the contact with this mite can cause allergies and skin infections (Halliday, 2003). Interestingly, it has been previously reported that *Trogoderma* sp., *R. dominica*, and *A. siro* can co-exist on stored wheat (Athanassiou et al., 2003).

Fumigants and synthetic insecticides are the mainstay of traditional pest management techniques in storage environments (Rajendran, 2020). However, their frequent usage has resulted in resistance development, regulatory limitations, environmental issues, and consequently in efficacy limitations against various pest species and life stages (Oberemok et al., 2017; Lalah et al., 2022; Araújo et al., 2023; Jayaprakas et al., 2023). These challenges have prompted research into alternate, eco-friendly management strategies (e.g., botanical substances, entomopathogens, natural products) that can work well in integrated pest management (IPM) systems (Said-Al Ahl et al., 2017). Due to their low mammalian toxicity, biodegradability, and several modes of action that might interfere with insect physiology, behavior, and metabolism, plant essential oils (EOs), have become attractive options to control stored-product pests (Arab et al., 2022; Annaz et al., 2024; Kavallieratos et al., 2024a, b; Li et al., 2025). EOs can have insecticidal, repellent, and growth-regulating effects against various storage pests, according to recent studies (Nenaah, 2014; Singh et al., 2021; Gupta et al., 2023; Li et al., 2025). The EO obtained from the *Commiphora myrrha* (Nees) Engl. oleo-gum-resin, also referred to as myrrh, is a popularity-gaining EO. Myrrh, derived from *Commiphora* spp., is a mixture of substances with ecological role secreted from the trunk and chemically composed of three main fractions, namely polysaccharides, resin (triterpenes), and a volatile fraction (EO) (Spinozzi et al., 2025). The latter is dominated by furanosesquiterpenes (such as furanoeudesma-1,3-diene, isofuranodiene, and curzerene), which may provide sustained bioactivity and unique mechanisms of action. When compared to a synthetic organophosphate insecticide, *C. myrrha* EO induced species-specific metabolic disruptions and caused significant mortality in major stored-product pests like bostrychid, curculionid, and tenebrionid stored-product pests (Kavallieratos et al., 2025a, 2025b). Spinozzi et al. (2025) showed that the bioactivity of myrrh EO against the culicidans *Anopheles gambiae* Giles, *Aedes albopictus* (Skuse), *A. aegypti* L., and *An. stephensi* Liston larvae, is mainly given by the furanosesquiterpenes fraction, among which furanoeudesma-1,3-diene was proven to be the compound with the most powerful larvicidal action.

Despite the growing body of work, *C. myrrha* EO has been tested against a limited number of stored-product pests and to our knowledge there is no data concerning the efficacy of this EO against mites. Thus, the present study endeavors to evaluate the insecticidal and acaricidal potency of *C. myrrha* EO against various stored-product pests (*R. dominica*, *T. granarium*, and *A. siro*) examining different EO concentrations, exposure intervals, and life stages.

2. Materials and methods

2.1. *C. myrrha* EO chemical analysis

The myrrh EO employed in this study derived from the same batch of the one previously analyzed and tested by Kavallieratos et al. (2025a, b). The consistency of the chemical composition was verified and confirmed by GC-MS (Kavallieratos et al., 2025a, 2025b) to the assays, confirming that no degradation or alterations had occurred. In detail, the EO consisted of, in decreasing order of relative percentage as determined by GC-MS, furanoeudesma-1,3-diene (40.2 %), curzerene (26.1 %), lindestrene (12.9 %), (*R*)(*5E,9E*)-8-methoxy-3,6,10-trimethyl-4,7,8,11-tetrahydrocyclodeca[b]furan (4.6 %), β-elemene (3.4 %), germacrene B (1.8 %), and isofuranodiene (1.8 %).

2.2. Insect and mite species

Colonies, of the species used in the experiments, are maintained in the AUA (Agricultural University of Athens), especially in the Laboratory of Agricultural Zoology and Entomology, under complete darkness. *Trogoderma granarium* and *R. dominica* were reared on whole wheat grains, at steady temperature (30 °C) and relative humidity (RH) (65 %). *Acarus siro* was cultured at 80 % RH, and 25 °C, on oat flakes, wheat germ, and dried yeast extract at a ratio of 10:10:1 (Kavallieratos et al., 2024a). The insect individuals were of indeterminate sex; adults of *R. dominica* were <14 days old and adults of *T. granarium* were <24 h (newly emerged). Regarding the *T. granarium* larvae used in the experiments, they were 5–6 mm (large size) (Lindgren et al., 1955; EPPO, 2013). The *A. siro* individuals were also of indeterminate sex and were collected from 1 to 21 days-aged colonies. External features were used to distinguish between adults and nymphs, such as the ability to identify nymphs by their shorter body setae (Kavallieratos et al., 2024a).

2.3. Grain commodity

Clean *Triticum durum* Desf. (var. Claudio), pesticide- and pest-free, was used for the mortality bioassays. Using a PCE-PEL 20 moisture meter (Organametrisis, Athens, Greece), grain moisture was determined to be 12.2 % prior to experimentation.

2.4. Mortality bioassays

Following preliminary experiments that assessed 500 ppm (500 μL EO per kg wheat) and 1000 ppm (1000 μL EO per kg wheat), the bioassays were initiated. First, 125 and 250 μL of the EO were dissolved in pure ethanol at 1:1 (v/v) ratio for 500 and 1000 ppm, respectively, to prepare the test solutions. The solutions were then adjusted to 750 μL total volume, using aqueous solution of Tween 80 0.3 % (v/v) (Kavallieratos et al., 2022). Using the B-Knight airbrush X301 (Airbrushstudio, Athens, Greece), 250 g of grain were equally sprayed with the EO solutions on trays; each tray was used as a treatment replication. Extra lots were also sprayed with supplementary solutions: (1) ethanol 99.8 %, (2) Tween 80 combined with ethanol and water (carrier control), (3) water, and (4) Actellic EC containing pirimiphos–methyl (50 %) at the label concentration (5 ppm = 5 μL/kg wheat) (positive control). Separate airbrushes were used to spray each solution. The treated grain lots were transferred into glass containers of 1 L and carefully shaken by hand for 10 min, to guarantee equal distribution of the solutions, throughout the wheat lots and left to air-dry. Then, using separate spoons, from each treated 1 L container of wheat (EO or controls), three sub-samples of either 10 g of wheat for insects or 1 g of wheat for mite, were taken. The XB 3200 D Toploading Balance electronic balance (Ttequipment, Eatontown, NJ, USA) was used to precisely weigh the sub-samples. Weighings were conducted on filter paper and between each weighing, different filter paper was used. The sub-samples were then transferred into glass vials that were intended especially for the mite (60 × 27 mm height × diameter) or the insect species (125 × 75 mm height × diameter). The caps of the bigger vials had a sealed aperture with a diameter of 15 mm with cloth to allow for air circulation, whereas the tops of the smaller vials had perforations (Kavallieratos et al., 2021, 2024a). To keep the arthropods on wheat, polytetrafluoroethylene dispersion at 60 % by weight (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) in water was applied to the inside walls close to the vial lids. Each species of arthropod was added to the designated vials in groups of ten larvae, adults, or nymphs. For the duration of the experiment, the vials were then kept in incubators set at 65 % RH and 30 °C for *R. dominica* and *T. granarium*, and at 80 % RH and 25 °C for *A. siro* (Kavallieratos et al., 2024a). The ZEISS Stemi 355 stereomicroscope (ZEISS, Oberkochen, Germany) was used to evaluate mortality after exposure periods ranging from 4 to 16 h and then from 1 to 7 days. To evaluate mortality of the arthropods, specimens were meticulously examined using separate fine brushes reserved for controls or EO treatments. The entire procedure was carried out three times (three replications), using fresh arthropods (groups of ten larvae, adults, or nymphs), vials, and grain kernels per time. For each EO concentration, a different pirimiphos-methyl solution was prepared.

2.5. Statistical analysis

Since mortality rates of negative controls were less than 5 %, no adjustments were required. The data was log (x + 1) transformed prior to the analysis to standardize variance (Zar, 2014; Scheff and Arthur, 2018). Each arthropod species' data was analyzed using the repeated measures model, with exposure serving as the repeated factor and mortality serving as the response variable. EO concentration and formulation (EO and pirimiphos-methyl) were the main effects considered (Sall et al., 2001). JMP 16.2 software was used for every analysis (SAS Institute Inc, 2021). The Tukey HSD test was used for mean separation at a significance level of 0.05 (Sokal and Rohlf, 1995). Additionally, each EO concentration was compared with the positive control of each exposure period for each species/life stage at the n – 2 df and 0.05 level using a two-tailed *t*-test (Snedecor and Cochran, 1989).

3. Results

3.1. MANOVA main effects and interactions for all the tested species/stages

Across all insect and mite species and their stages, exposure was significant. For *T. granarium* larvae and *R. dominica* adults, all main effects with their respective interactions were significant within and between exposures. With the exception of concentration × formulation, all main effects and respective interactions between and within exposures were significant for *T. granarium* adults. Formulation and concentration × formulation were significant for *A. siro* adults and nymphs, respectively (Table 1).

3.2. C. myrrha EO vs. R. dominica adults

The EO resulted in no mortality against *R. dominica* adults until day 3, and 8 h at 500 and 1000 ppm, respectively. On day 7, mortality reached 46.7 % at 500 ppm and 71.1 % at 1000 ppm. Positive control was more effective, achieving 80.0 % mortality by day 7. Mortality rates caused by the EO and the positive control significantly differed from day 1 to day 7 for the 500 ppm of the EO, while from day 2 to day 4 for the 1000 ppm of the EO. For both EO concentrations and the positive control, mortality increased significantly during the experimental duration (4 h to 7 days) (Table 2).

3.3. C. myrrha EO vs. T. granarium adults

Mortality rates of both EO concentrations significantly exceeded those of the positive control, from day 1 and 16 h, for 500 and 1000 ppm, until the end of the experimentation. On day 7, EO at 500 ppm achieved 87.8 % mortality of *T. granarium* adults, while at 1000 ppm achieved 100 % mortality by day 6. Pirimiphos-methyl mortality rates did not exceed 68.9 %. Over the duration of the trial (4 h to 7 days), mortality increased significantly for both the positive control and the EO concentrations (Table 3).

3.4. C. myrrha EO vs. T. granarium larvae

The first mortality records appeared at day 6 for 500 ppm and day 4 for 1000 ppm. Thus, *T. granarium* larvae showed a delayed initial lethal response to the EO treatments. Similarly, positive control achieved low mortality rates by day 1. The 500 ppm-EO treatment did not achieve mortality rates >6.0 % even by day 7, while 1000 ppm of the EO and the positive control mortality rates remained below 28.0 % at the end of the experimentation (Table 4).

3.5. C. myrrha EO vs. A. siro nymphs

No mortalities of *A. siro* nymphs were recorded until day 1 of both EO concentrations and the positive control. For both EO concentrations and pirimiphos-methyl, mortality increased significantly during the experimental duration (4 h to 7 days). Between each EO concentration (500 and 1000 ppm) and the positive control, no significant differences were observed in the mortality rates of *A. siro* nymphs. On day 7, both EO concentrations (500 and 1000 ppm) achieved higher, but not significantly different, mortality rates (60.0 and 68.9 %, respectively), compared to pirimiphos-methyl (52.2–53.3 %) (Table 5).

3.6. C. myrrha EO vs. A. siro adults

Adult *A. siro* mortality caused by EO was initially noted on day 1 for both 500 and 1000 ppm. Mortality rates were significantly increased during the experiment duration for all the treatments. Positive control caused significantly higher mortality rates at days 2, 5, and 6 (12.2, 55.6, and 63.3 %, respectively), compared to the 500 ppm of the EO (6.7, 35.6, and 48.9 %, respectively). On day 7, the EO achieved 60.0 % mortality at 500 ppm and 77.8 % mortality at 1000 ppm (Table 6).

Table 1
MANOVA parameters showing the main effects and their interactions leading to the observed mortalities of *Rhyzopertha dominica* adults, *Trogoderma granarium* adults and larvae, and *Acarus siro* adults and nymphs, between and within exposures (error df = 32 for all species).

		Between exposures				Within exposures			
Source		Intercept	Concentration	Formulation	Concentration × formulation	Exposure	Exposure × concentration	Exposure × formulation	Exposure × concentration × formulation
Pest species/stage	df	1	1	1	1	9	9	9	9
R. dominica adults	*F*	1731.2	35.2	45.4	31.8	4,860.1	82.1	136.3	78.3
	P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
T. granarium adults	*F*	4591.3	5.2	57.6	2.5	5845.2	3.5	37.8	11.0
	P	<0.01	0.03	<0.01	0.12	<0.01	0.01	<0.01	<0.01
T. granarium larvae	*F*	438.9	24.3	57.2	13.2	290.3	27.3	33.7	24.8
	P	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01
A. siro adults	*F*	1717.0	1.5	5.5	1.7	1482.1	0.7	1.8	2.1
	P	<0.01	0.22	0.03	0.21	<0.01	0.69	0.12	0.07
A. siro nymphs	*F*	585.8	0.4	0.2	4.0	631.3	0.2	0.7	0.6
	P	<0.01	0.55	0.67	0.05	<0.01	0.99	0.69	0.76

Table 2
Mean (%) mortality ± standard errors (SE) of *Rhyzopertha dominica* adults after 4–16 hours (h), and 1–7 days (d) in wheat treated with *Commiphora myrrha* essential oil (EO) and with pirimiphos-methyl (positive control). For each concentration of EO and positive control, within each row, asterisks indicate significant differences between EO and positive control group (df = 16; two-tailed *t*-test at *P* = 0.05). Within each column, means followed by the same uppercase letter are not significantly different (df = 9, 89; Tukey HSD test at *P* = 0.05). No significant differences were recorded where no asterisks exist. No statistical analysis was performed where dashes exist.

	EO	Pirimiphos-methyl			EO	Pirimiphos-methyl		
Concentration	500 ppm	Label (5 ppm)			1000 ppm	Label (5 ppm)		
Exposure			*t*	*P*			*t*	*P*
4 h	0.0 ± 0.0 D	0.0 ± 0.0 D	-	-	0.0 ± 0.0 F	0.0 ± 0.0 E D	-	-
8 h	0.0 ± 0.0 D	2.2 ± 1.5 CD	1.5	0.15	0.0 ± 0.0 F	2.2 ± 1.5 CD	1.5	0.17
16 h	0.0 ± 0.0 D	3.3 ± 1.7 CD	2.0	0.06	2.2 ± 1.5 F	2.2 ± 1.5 CD	0	1.00
1 d	0.0 ± 0.0 D	6.7 ± 2.9 C*	2.5	0.02	8.9 ± 1.1 E	6.7 ± 2.4 C	−1.4	0.19
2 d	0.0 ± 0.0 D	16.7 ± 2.9 B*	17.9	<0.01	12.2 ± 1.5 DE	20.0 ± 2.4 B*	2.9	0.01
3 d	0.0 ± 0.0 D	30.0 ± 2.9 AB*	39.2	<0.01	22.2 ± 1.5 CD	31.1 ± 2.0 AB*	3.7	0.01
4 d	8.9 ± 1.1 C	38.9 ± 3.1 AB*	5.4	<0.01	28.9 ± 1.1 BC	42.2 ± 3.6 AB*	3.6	0.01
5 d	17.8 ± 2.2 BC	50.0 ± 4.4 A*	3.3	0.01	46.7 ± 1.7 AB	52.2 ± 4.0 AB	1.0	0.35
6 d	28.9 ± 2.6 AB	66.7 ± 2.9 A*	6.5	<0.01	62.2 ± 1.5 A	65.6 ± 4.1 A	0.6	0.58
7 d	46.7 ± 2.4 A	80.0 ± 5.3 A*	5.8	<0.01	71.1 ± 1.1 A	78.9 ± 4.2 A	1.7	0.13
F	127.7	48.6			133.0	59.5		
P	<0.01	<0.01			<0.01	<0.01		

Table 3
Mean (%) mortality ± standard errors (SE) of *Trogoderma granarium* adults after 4–16 hours (h), and 1–7 days (d) in wheat treated with *Commiphora myrrha* essential oil (EO) and with pirimiphos-methyl (positive control). For each concentration of EO and positive control, within each row, asterisks indicate significant differences between EO and positive control group (df = 16; two-tailed *t*-test at *P* = 0.05). Within each column, means followed by the same uppercase letter are not significantly different (df = 9, 89; Tukey HSD test at *P* = 0.05). No significant differences were recorded where no asterisks exist. No statistical analysis was performed where dashes exist.

	EO	Pirimiphos-methyl			EO	Pirimiphos-methyl		
Concentration	500 ppm	Label (5 ppm)			1000 ppm	Label (5 ppm)		
Exposure			*t*	*P*			*t*	*P*
4 h	0.0 ± 0.0 F	0.0 ± 0.0 F	-	-	0.0 ± 0.0 G	0.0 ± 0.0 E	-	-
8 h	0.0 ± 0.0 F	1.1 ± 1.1 EF	1.0	0.33	0.0 ± 0.0 G	1.1 ± 1.1 E	1.0	0.35
16 h	7.8 ± 1.5 E	5.6 ± 2.4 DE	−1.3	0.22	17.8 ± 1.5 F*	5.6 ± 1.8 D	−3.6	0.01
1 d	22.2 ± 2.2 D*	10.0 ± 2.9 CD	−2.8	0.03	37.8 ± 2.2 E*	11.1 ± 2.0 C	−4.5	0.01
2 d	33.3 ± 2.4 CD*	17.8 ± 2.8 BC	−4.2	0.01	52.2 ± 1.5 D*	17.8 ± 2.8 BC	−7.4	<0.01
3 d	50.0 ± 1.7 BC*	30.0 ± 2.9 AB	−5.6	0.01	66.7 ± 1.7 C*	31.1 ± 2.6 AB	−8.6	<0.01
4 d	60.0 ± 2.9 AB*	40.0 ± 3.3 AB	−4.6	0.01	77.8 ± 1.5 BC	38.9 ± 2.6 AB	−10.2	<0.01
5 d	71.1 ± 2.6 AB*	51.1 ± 2.6 A	−5.4	0.01	83.3 ± 1.7 B*	52.2 ± 3.2 A	−7.5	<0.01
6 d	73.3 ± 2.4 AB*	62.2 ± 3.2 A	−2.9	0.01	100.0 ± 0.0 A*	60.0 ± 3.5 A	−9.8	<0.01
7 d	87.8 ± 2.2 A*	67.8 ± 4.0 A	−4.0	0.01	100.0 ± 0.0 A*	68.9 ± 3.5 A	−7.2	<0.01
F	216.9	48.8			2247.5	65.8		
P	<0.01	<0.01			<0.01	<0.01		

Table 4
Mean (%) mortality ± standard errors (SE) of *Trogoderma granarium* larvae after 4–16 hours (h), and 1–7 days (d) in wheat treated with *Commiphora myrrha* essential oil (EO) and with pirimiphos-methyl (positive control). For each concentration of EO and positive control, within each row, asterisks indicate significant differences between EO and positive control group (df = 16; two-tailed *t*-test at *P* = 0.05). Within each column, means followed by the same uppercase letter are not significantly different (df = 9, 89; Tukey HSD test at *P* = 0.05). No significant differences were recorded where no asterisks exist. No statistical analysis was performed where dashes exist.

	EO	Pirimiphos-methyl			EO	Pirimiphos-methyl		
Concentration	500 ppm	Label (5 ppm)			1000 ppm	Label (5 ppm)		
Exposure			*t*	*P*			*t*	*P*
4 h	0.0 ± 0.0 B	0.0 ± 0.0 C	-	-	0.0 ± 0.0 D	0.0 ± 0.0 D	-	-
8 h	0.0 ± 0.0 B	0.0 ± 0.0 C	-	-	0.0 ± 0.0 D	0.0 ± 0.0 D	-	-
16 h	0.0 ± 0.0 B	0.0 ± 0.0 C	-	-	0.0 ± 0.0 D	0.0 ± 0.0 D	-	-
1 d	0.0 ± 0.0 B	1.1 ± 1.1 BC	1.0	0.35	0.0 ± 0.0 D	2.2 ± 1.5 CD	1.5	0.17
2 d	0.0 ± 0.0 B	2.2 ± 1.5 BC	1.5	0.17	0.0 ± 0.0 D	3.3 ± 1.7 CD	2.0	0.08
3 d	0.0 ± 0.0 B	6.7 ± 2.9 B*	2.6	0.04	0.0 ± 0.0 D	6.7 ± 1.7 BC*	4.0	0.01
4 d	0.0 ± 0.0 B	12.2 ± 2.2 A*	7.5	<0.01	8.9 ± 1.1 C	13.3 ± 2.4 AB	0.7	0.50
5 d	0.0 ± 0.0 B	16.7 ± 1.7 A*	26.2	<0.01	12.2 ± 1.5 BC	20.0 ± 2.4 A*	2.9	0.01
6 d	4.4 ± 1.8 A	20.0 ± 2.4 A*	4.4	0.01	18.9 ± 1.1 AB	23.3 ± 1.7 A	2.1	0.05
7 d	5.6 ± 1.8 A	27.8 ± 1.5 A*	4.7	0.01	25.6 ± 1.8 A	26.7 ± 1.7 A	0.5	0.65
F	7.3	34.3			229.7	33.7		
P	<0.01	<0.01			<0.01	<0.01		

Table 5
Mean (%) mortality ± standard errors (SE) of *Acarus siro* nymphs after 4–16 hours (h), and 1–7 days (d) in wheat treated with *Commiphora myrrha* essential oil (EO) and with pirimiphos-methyl (positive control). For each concentration of EO and positive control, within each row, asterisks indicate significant differences between EO and positive control group (df = 16; two-tailed *t*-test at *P* = 0.05). Within each column, means followed by the same uppercase letter are not significantly different (df = 9, 89; Tukey HSD test at *P* = 0.05). No significant differences were recorded where no asterisks exist. No statistical analysis was performed where dashes exist.

	EO	Pirimiphos-methyl			EO	Pirimiphos-methyl		
Concentration	500 ppm	Label (5 ppm)			1000 ppm	Label (5 ppm)		
Exposure			*t*	*P*			*t*	*P*
4 h	0.0 ± 0.0 D	0.0 ± 0.0 E	-	-	0.0 ± 0.0 C	0.0 ± 0.0 E	-	-
8 h	0.0 ± 0.0 D	0.0 ± 0.0 E	-	-	0.0 ± 0.0 C	0.0 ± 0.0 E	-	-
16 h	0.0 ± 0.0 D	0.0 ± 0.0 E	-	-	0.0 ± 0.0 C	0.0 ± 0.0 E	-	-
1 d	1.1 ± 1.1 D	2.2 ± 1.5 DE	0.6	0.56	3.3 ± 1.7 BC	1.1 ± 1.1 E	−1.1	0.29
2 d	1.1 ± 1.1 D	4.4 ± 1.8 D	1.6	0.13	7.8 ± 3.2 BC	3.3 ± 1.7 E	−0.7	0.47
3 d	6.7 ± 2.4 C	11.1 ± 2.0 C	1.6	0.13	13.3 ± 4.1 B	7.8 ± 2.2 DE	−0.5	0.65
4 d	21.1 ± 5.4 B	20.0 ± 2.4 BC	0.7	0.51	32.2 ± 5.2 A	15.6 ± 2.9 CD	−2.1	0.06
5 d	30.0 ± 6.2 AB	27.8 ± 3.6 AB	0.3	0.80	44.4 ± 5.3 A	24.4 ± 4.1 BC	−2.0	0.07
6 d	45.6 ± 7.5 AB	42.2 ± 4.0 AB	0.2	0.86	55.6 ± 6.7 A	40.0 ± 3.3 AB	−1.6	0.13
7 d	60.0 ± 8.0 A	52.2 ± 4.3 A	−0.6	0.54	68.9 ± 8.2 A	53.3 ± 3.7 A	−1.4	0.20
F	46.5	62.0			39.0	36.9		
P	<0.01	<0.01			<0.01	<0.01		

Table 6
Mean (%) mortality ± standard errors (SE) of *Acarus siro* adults after 4–16 hours (h), and 1–7 days (d) in wheat treated with *Commiphora myrrha* essential oil (EO) and with pirimiphos-methyl (positive control). For each concentration of EO and positive control, within each row, asterisks indicate significant differences between EO and positive control group (df = 16; two-tailed *t*-test at *P* = 0.05). Within each column, means followed by the same uppercase letter are not significantly different (df = 9, 89; Tukey HSD test at *P* = 0.05). No significant differences were recorded where no asterisks exist. No statistical analysis was performed where dashes exist.

	EO	Pirimiphos-methyl			EO	Pirimiphos-methyl		
Concentration	500 ppm	Label (5 ppm)			1000 ppm	Label (5 ppm)		
Exposure			*t*	*P*			*t*	*P*
4 h	0.0 ± 0.0 D	0.0 ± 0.0 E	-	-	0.0 ± 0.0 D	0.0 ± 0.0 E	-	-
8 h	0.0 ± 0.0 D	0.0 ± 0.0 E	-	-	0.0 ± 0.0 D	0.0 ± 0.0 E	-	-
16 h	0.0 ± 0.0 D	1.1 ± 1.1 DE	1.0	0.35	0.0 ± 0.0 D	1.1 ± 1.1 DE	1.0	0.35
1 d	2.2 ± 1.5 CD	3.3 ± 1.7 D	0.5	0.62	3.3 ± 1.7 D	3.3 ± 1.7 D	0.0	1.0
2 d	6.7 ± 2.4 C	12.2 ± 1.5 C*	2.5	0.04	10.0 ± 2.9 C	13.3 ± 1.7 C	1.6	0.15
3 d	15.6 ± 3.8 B	24.4 ± 1.8 BC	2.1	0.07	24.4 ± 4.1 B	25.6 ± 2.4 BC	0.6	0.54
4 d	30.0 ± 5.0 AB	40.0 ± 2.4 AB	2.1	0.07	37.8 ± 6.0 AB	38.9 ± 3.1 AB	0.7	0.52
5 d	35.6 ± 5.3 AB	55.6 ± 3.8 A*	2.8	0.02	53.3 ± 5.8 AB	50.0 ± 3.3 AB	−0.1	0.92
6 d	48.9 ± 5.4 A	63.3 ± 2.4 A*	2.3	0.04	66.7 ± 9.1 A	62.2 ± 3.6 A	0.1	0.91
7 d	60.0 ± 6.0 A	70.0 ± 2.4 A	1.5	0.17	77.8 ± 6.4 A	71.1 ± 2.6 A	−0.6	0.55
F	53.9	130.9			75.2	124.6		
P	<0.01	<0.01			<0.01	<0.01		

4. Discussion

The results of the present study demonstrated a species- and stage-dependent efficacy of *C. myrrha* EO. Adults of *T. granarium* were the most vulnerable developmental stage, followed by adults of *A. siro*, while adults of *R. dominica* and nymphs of *A. siro*, and larvae of *T. granarium* showed moderate and high tolerance, respectively. Such specific insecticidal responses were also observed for pirimiphos-methyl (positive control). The stage- and species-specific effect is a common feature of plant-based and synthetic pesticides (Spochacz et al., 2018; Morrison III et al., 2019; Kavallieratos et al., 2024a, b; Stejskal et al., 2025). The various responses detected in this study could be ascribed to the variability of the cuticular composition (Mewis and Ulrichs, 2001), but also to differences in the uptake of the insecticidal compounds (Pedersen et al., 2020) among the different developmental stages.

The most noteworthy outcome of the present study is the high effectiveness of *C. myrrha* EO against adults of *T. granarium*, where both tested concentrations significantly outperformed the positive control pirimiphos-methyl. By day 6, complete adult mortality was achieved at 1000 ppm, whereas by day 7, the synthetic insecticide was unable to surpass 70% mortality. The strong adulticidal activity, observed here, is consistent with previous studies reporting high susceptibility of *T. granarium* adults to various EOs used as grain protectants (Küçüktopcu and Saruhan, 2024; Kavallieratos et al., 2024a, 2024b). Interestingly, *C. myrrha* EO shares a common characteristic with two of the EOs examined in the aforementioned studies. Both *Smyrnium olusatrum* L. EO, that achieved complete mortality of *T. granarium* adults by day 4 at 1000 ppm (Kavallieratos et al., 2024b), and *C. myrrha* EO, that caused 100 % mortality to *T. granarium* adults at 1000 ppm by day 6 (this study), are rich in furanosesquiterpenes (Kavallieratos et al., 2024b; Spinozzi et al., 2025). Although the precise mode of action of myrrh EO has not yet been fully elucidated, its bioactivity is likely attributable to the high concentration of furanosesquiterpenes, mainly furanoeudesma-1,3-diene, which has already been reported as the most active compound of *C. myrrha* EO (Spinozzi et al., 2025). In contrast to acetylcholinesterase inhibitors, such as pirimiphos-methyl, which exert toxicity through a well-defined single molecular target (Eleršek and Filipič, 2011), furanosesquiterpenes appear to act through interference with multiple physiological systems simultaneously (Spinozzi et al., 2025; Kavallieratos et al., 2025a, 2025b). Previous metabolomic analyses conducted on different mosquito species (Spinozzi et al., 2025) demonstrated that furanosesquiterpenes significantly disrupted amino acid metabolism, influencing the pathways related to glutamate, alanine, and aspartate. Conversely, the study of Kavallieratos et al. (2025b) on tenebrionids revealed that the metabolic pathways affected were those involved in neurotransmission, energy homeostasis, sugar metabolism, and oxidative stress. Multi-targeting mode of action of various insecticidal agents may reduce the efficiency of detoxification enzymes such as esterases and cytochrome P450 monooxygenases, which are frequently upregulated in resistant insect populations (Nauen et al., 2022; Drosdoski et al., 2024). Insecticides successfully neutralize the natural chemical defense of the insect by physically attaching to the enzyme, preventing it from degrading or neutralizing the toxin (El-Sayed et al., 2023; Gargi and Paul, 2026). While the exact molecular targets remain to be fully characterized, the pronounced lipophilicity of furanosesquiterpenes likely enhances their penetration and distribution within insect tissues. These physicochemical properties may contribute substantially to their bioavailability and could help explain the interspecific and developmental-stage differences observed among treated insects (Benelli et al., 2017). On the opposite side of the furanosesquiterpene-rich EOs are the monoterpene-rich EOs (*Monodora myristica* (Gaertn.) Dunal, *Xylopia aethiopica* (Dunal) A. Rich., and *Citrus reticulata* Blanco), that demonstrated moderate adulticidal efficacy against *T. granarium* (Kavallieratos et al., 2025c). The high adulticidal efficacy of *C. myrrha* EO (furanosesquiterpene-rich) may be also due to the relatively low volatility of furanosesquiterpenes (Spinozzi et al., 2025), in contrast to monoterpene-rich EOs (Malik et al., 2023). Reduced evaporation could prolong the residual activity of the EO on grain kernels. This characteristic is advantageous in stored-grain systems, where long-lasting protection is essential, as insecticidal agents degrade over time (Bantz et al., 2018). Whether *C. myrrha* EO is suitable for longer periods than those tested here remains to be investigated.

Larvae of *T. granarium* were the least affected by all treatments, including the positive control, confirming that this developmental stage represents a major challenge for stored-product pest control (Edde et al., 2012; Ghimire et al., 2017; Dageri, 2024). Probably, the presence of hairs in the whole larval body (tuffs of arrow-shaped hairs on the last four segments and long hairs in the remainder of the body), which become denser over time (Lindgren et al., 1955; Hadaway, 1956; Hill, 2003; Rosentrater, 2022), reduces its physical contact with the applied insecticidal agent.

Rhyzopertha dominica adults exhibited only moderate mortality, even at the higher EO concentration. Previous studies have revealed that *R. dominica* is more tolerant to botanical compounds, when applied to grain substrates, compared to other stored-product pests (Wuttiwong et al., 2019; Wicochea-Rodríguez et al., 2021; Wijerathna et al., 2023; Kavallieratos et al., 2024a, 2024b, 2025c; Mostafa and Abotaleb, 2024). *Rhyzopertha dominica* adults have detoxification mechanisms, related to a series of genes, that enable them to combat multiple insecticidal agents, including chemical (phosphine) and natural (inert dusts) insecticides (Yang et al., 2018; Bai et al., 2025). Thus, these mechanisms may have been activated and resulted in the moderate mortality that *C. myrrha* EO induced in this study. However, molecular investigation is needed to support this hypothesis.

One other novel contribution of this study is the documentation of acaricidal activity of *C. myrrha* EO against *A. siro*. Nymphs and adults showed moderate mortality rates, with EO treatments exceeding the positive control (pirimiphos-methyl). Similar mortalities were observed when the sesquiterpenes-rich *S. olusatrum* EO was tested as grain protectant, against *A. siro* nymphs and adults (Kavallieratos et al., 2024a). In contrast, the polyacetylene carlina oxide-rich *Carlina acaulis* L. EO achieved 91.1 and 95.6 % mortalities to *A. siro* adults and nymphs, respectively, 7 days post exposure. These contrasting outcomes underscore the critical importance of the chemical composition of EOs in determining acaricidal efficacy (Kavallieratos et al., 2022).

Overall, from an applied perspective, the observed efficacy of *C. myrrha* EO, mainly against *T. granarium* adults and *A. siro* nymphs or adults, supports its potential role as a component of the protection strategies of stored grains. The simultaneous presence of insect and mite species in stored wheat has been well documented (Athanassiou et al., 2003, 2005; Stejskal et al., 2003; Collins, 2012). In such multi-pest environments, control measures targeting only insect pests are insufficient, as mite populations could persist in the treatments. The dual activity (insecticidal and acaricidal) of *C. myrrha* EO observed in the present study suggests that it could contribute to broader-spectrum suppression of different pests, reducing the need for separate control interventions.

CRediT authorship contribution statement

Nickolas G. Kavallieratos: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Demeter Lorentha S. Gidari:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Maria C. Boukouvala:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Constantin S. Filintas:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Anna Skourti:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Vasiliki Panagiota C. Kyrpislidi:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Dionysios Ntinokas:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Eleonora Spinozzi:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. **Riccardo Petrelli:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Filippo Maggi:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The project 80375 (Agricultural University of Athens, Special Account for Research Funds) supported this study in part.

Data availability

Data will be made available on request.

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