Arch Biol Sci. 2026;78(1):69-79 https://doi.org/10.2298/ABS251229003H

Fumigant toxicity and sublethal effects of *Thymus munbyanus* essential oil on biomarkers, reproduction, and locomotion in the Mediterranean flour moth *Ephestia kuehniella*

Zahia C. Hamida[1,*], Hadjira Bendjedid[1], Mohamed C. Oulhaci[1,2], Amina Yezli[1,3], and Samira Yezli-Touiker[1]

[1]*Department of Biology, University Badji Mokhtar Annaba, Faculty of Sciences, Applied Animal biology Laboratory*, Annaba, Algeria

[2]*Department of Organismal Biology, Faculty of Nature and Life Sciences, Mustefa Ben Boulaïd Batna 2 University*, Batna, Algeria

[3]*Teacher Education College of Setif*, Algeria

***Corresponding author**: zahia-cirine.hamida@univ-annaba.dz

Received: December 29, 2025; **Revised**: February 11, 2026; **Accepted**: February 15, 2026; **Published online**: February 23, 2026

Abstract: This study evaluated the fumigant toxicity of *Thymus munbyanus* subsp. *coloratus* essential oil against adult *Ephestia kuehniella* (Zeller). To determine sublethal effects, we integrated biochemical biomarkers with assessments of reproductive performance and locomotor activity. Adults were exposed via fumigation to concentrations between 0.05 and 0.8 µL/mL of air, with lethal concentrations calculated after 24 h of exposure. The essential oil showed strong fumigant activity, with LC_{25} and LC_{50} values of 0.153 and 0.26 µL/mL of air, respectively. Sublethal exposure at these concentrations was associated with changes in biomarker responses in adults, including increased glutathione S-transferase (GST) and catalase (CAT) activities, along with decreased acetylcholinesterase (AChE) activity. In addition, fumigation affected adult reproductive performance by reducing oviposition, as reflected by a decrease in the number of eggs laid and hatched. Locomotor activity was also impaired, with reductions in distance traveled, movement duration, and movement speed. Overall, these findings suggest that *T. munbyanus* essential oil has potential as a botanical fumigant against *E. kuehniella* adults.

Keywords: *Ephestia kuehniella*, *Thymus munbyanus*, essential oil, fumigant toxicity, biomarker

INTRODUCTION

Stored cereals and their products are essential for ensuring global food and nutritional security [1,2]. However, these commodities are highly susceptible to losses during storage, primarily due to infestations by various insect species, which can cause significant quantitative losses and deterioration of their nutritional and sanitary quality [3]. According to the Food and Agriculture Organization of the United Nations, these losses amount to up to 29.6% of global production [4]. Among damaging insects, the Mediterranean flour moth ***Ephestia kuehniella Zeller*** (Lepidoptera: Pyralidae) is one of the most destructive pests of grain, particularly affecting flour [5]. The larvae of ***E. kuehniella*** not only cause direct damage by feeding on grains but also reduce product quality by contaminating them with excrement and webbing that favor fungal growth, and, consequently, mycotoxin production [6].

Chemical control strategies for this pest have primarily relied on fumigants such as methyl bromide, cyanogens, and phosphine [7]. However, the use of these fumigants may lead to insect resistance, residues in food products, environmental contamination, occupational threats, and toxicity to non-target organisms [8,9]. Therefore, the search for safer and more sustainable alternatives has gained increasing attention over the last few decades. Essential oils (EOs) have emerged as promising alternatives due to their high volatility and potent bioactivity against insect pests [10,11]. ***Thymus munbyanus*** (Boiss. & Reut.), an aromatic species of the Lamiaceae family endemic to North Africa [12], possesses diverse biological activities,

How to cite this article: Hamida ZC, Bendjedid H, Oulhaci MC, Yezli A, Yezli-Touiker S. Fumigant toxicity and sublethal effects of *Thymus munbyanus* essential oil on biomarkers, reproduction, and locomotion in the Mediterranean flour moth *Ephestia kuehniella*. Arch Biol Sci. 2026;78(1):69-79.

including antimicrobial, antioxidant, antibacterial, and antiproliferative effects on human cells [13]. ***T. munbyanus*** EO has demonstrated significant potential for controlling ***E. kuehniella*** by disrupting key physiological processes and enzymatic activities. Bendjedid et al. [14] reported that topical application of this EO to pupae inhibited AChE and activated detoxification systems in a concentration-dependent manner (15.38 and 25.22 μL/mL). This bioactivity is likely attributed to its major constituents, carvacrol (39.11%) and paracymene (15.47%), highlighting its potential as a natural alternative to synthetic insecticides for stored-product protection. However, insect susceptibility to bioactive compounds varies significantly based on developmental stage and exposure route, both of which are linked to distinct physiological and biochemical differences across life cycles [15]. While pupae represent a transitional, non-feeding phase, adults exhibit distinct metabolic activity, detoxification capacity, and respiratory function [16,17]. Monoterpenes, which are major constituents of many essential oils, are characterized by high volatility and lipophilicity, allowing rapid penetration into insects and preferential uptake via inhalation, particularly in adults [18,19]. Consequently, adult fumigation exposure occurring primarily through the tracheal system may elicit toxicological and enzymatic responses distinct from those observed following topical application during the pupal stage [20,21]. Essential oils may exhibit variable activity across developmental stages, reflecting differences in sensitivity and cuticular properties [22,23]. In this context, the present study examines the effects of ***T. munbyanus*** essential oil on adult ***Ephestia kuehniella*** using fumigation exposure by assessing mortality, biochemical biomarkers (GST, CAT, and AChE), and behavioral parameters (oviposition and mobility).

MATERIALS AND METHODS

Ethics statement

This research did not involve human participants or vertebrate animals. The experimental model used was the insect ***Ephestia kuehniella***, an invertebrate species for which ethical committee approval is not required according to applicable regulations. The study was conducted following good laboratory practices.

Insect rearing

Ephestia kuehniella (Zeller 1879) is a lepidopteran pest of stored products that causes damage mainly to flour and wheat stocks; it is a convenient biological laboratory model [24]. ***E. kuehniella*** was reared at 27°C and 80% relative humidity in almost continuous darkness to assure their development [25]. Last instar larvae were collected from a stock colony, separated according to sex, and deposited in jars containing flour until pupation. Newly emerged adult females (<8 h old) were used in all experiments.

Plant collection

The aerial parts of Thymus munbyanus subsp. coloratus (Boiss. & Reut.) were collected from Annaba, Northeast Algeria (36°56’47.4”N 7°23’43.9”E). Plant material was identified by the taxonomist Prof. T. Hamel (Department of Biology, Badji-Mokhtar University, Algeria). The essential oil was extracted by hydrodistillation using a Clevenger-type apparatus. The chemical composition of the EO was previously determined by GC/MS analysis [14]. A total of 54 chemical compounds were identified, representing 93.27% of the total oil content. The EO was characterized by a high proportion of carvacrol (39.1%), followed by ***p***-cymene (15.4%), while linalool (2.3%) and D-limonene (2.3%) were identified as minor components [14].

Fumigant assay

The fumigant toxicity of ***T. munbyanus*** EO was tested on newly emerged adults (< 8 h old). Whatman No. 1 filter paper discs (2 cm diameter) were impregnated with concentrations of 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.8 μL/mL [25]. Adults were placed in 100 mL plastic jars at a density of 10 individuals per jar. Three independent replicates were performed for each concentration, with each replicate comprising 10 jars, resulting in 100 adults per replicate and a total of 300 adults per concentration. Applied concentrations were converted based on the jar volume and expressed as concentrations in μL/mL of air. For the 100 mL experimental jars, the corresponding applied volumes were 5, 10, 20, 30, 40, 50, 60, and 80 μL/100 mL of air. The experiment consisted of three replicates of 10 adults per jar, including the control. Mortality was recorded hourly until death, and the observed rates were adjusted using Abbott's correction formula [26]. Lethal concentrations (LC_{50} and LC_{90}) and their 95% confidence limits were determined using GraphPad Prism 10.3.1 software.

Biomarker activities

Adult ***Ephestia kuehniella*** from control and treated groups (LC_{25} and LC_{50}) were collected 1, 2, and 3 days post-emergence for biomarker analyses. Six independent biological replicates were analyzed for each treatment and sampling interval.

Glutathione S-transferase (GST) activity was determined according to the method of Habig et al. [27] and expressed as μM/min/mg protein. Samples were homogenized under cold conditions in 1 mL of phosphate buffer (0.1 M, pH 6) and centrifuged at 17,968 × g for 30 min. An aliquot of 200 μL of the resulting supernatant was mixed with 1.2 mL of substrate solution containing reduced glutathione (GSH, 5 mM) and 1-chloro-2,4-dinitrobenzene (CDNB, 1 mM) prepared in phosphate buffer (0.1 M, pH 6). Absorbance was recorded at 340 nm every minute for 5 min.

Catalase (CAT) activity was assessed following the method of Claiborne et al. [28], which monitors the decrease in absorbance associated with the decomposition of hydrogen peroxide (H_2O_2). Samples were homogenized in phosphate buffer (100 mM, pH 7.4), sonicated, and centrifuged at 20,627 × g for 10 min. The reaction mixture consisted of 50 μL of supernatant added to 750 μL of phosphate buffer (0.1 M, pH 7.4) and 200 μL of H_2O_2 (0.5 M). The decrease in absorbance was recorded at 240 nm after an initial delay of 15 s and then every 5 s for a period of 30 s. Catalase activity was expressed as μmol/min/mg protein.

Acetylcholinesterase (AChE) activity was assessed according to the method of Ellman et al. [29], using acetylthiocholine as substrate. Samples were homogenized in 1 mL of detergent buffer (1 mM EGTA, 1% Triton X-100, 1 M NaCl, 0.01 M Tris-HCl, pH 7). After centrifugation at 2,292 × g for 5 min, an aliquot of 100 μL of supernatant was mixed with 100 μL of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and 1 mL of Tris buffer (0.1 M, pH 7). After a 5-min incubation period, 100 μL of acetylthiocholine was added to initiate the reaction. Absorbance was measured at 412 nm every 4 min for 20 min. AChE activity was expressed as μM/min/mg protein.

Total protein content was determined according to the method of Bradford [30] using Coomassie Brilliant Blue G-250 and bovine serum albumin (BSA) as standard. Absorbance was measured at 595 nm, and protein concentrations were calculated from a standard calibration curve. All enzymatic activities were normalized to total protein content to ensure accurate comparison among treatments.

Reproductive events

To evaluate the potential effects of the essential oil on oviposition, newly emerged adults of ***E. kuehniella*** (<8 h old) were subjected to fumigation at LC_{25} and LC_{50} concentrations. Untreated adult males were paired with surviving treated females and allowed to mate. Mating and oviposition were conducted in glass Petri dishes lined with dark paper to facilitate egg counting. Six pairs were used per treatment, and adults were kept together for three days. The number of eggs laid per female, fertility, and hatching percentage were subsequently recorded.

Mobility of adults

Each newly emerged ***E. kuehniella*** adult was tested individually in a transparent glass arena (20 × 20 × 10 cm). Insects were placed in the center of the arena and allowed to acclimatize for 2 min. Locomotor activity was then recorded for 5 min using a camera positioned above the arena. Distance traveled was estimated in millimeters using a calibrated grid placed at the bottom of the arena, providing a semi-quantitative assessment of displacement, while movement duration (s) was determined from the video recordings. Movement speed (mm s^{-1}) was subsequently calculated based on the total distance traveled and the duration of movement. The arena was cleaned between trials to prevent chemical or olfactory contamination. For each treatment, 15 independent individuals were used as replicates.

Data analysis

All data were first assessed for normality using the Shapiro-Wilk test, and homogeneity of variances was evaluated using the Brown-Forsythe test, which is robust to deviations from normality and suitable for small sample sizes. For datasets meeting the assumptions of normality and homogeneity, parametric one-way ANOVA followed by Tukey's post hoc test was applied. For datasets not meeting these assumptions, non-parametric Kruskal-Wallis tests followed by Dunn's post hoc test were used. Differences were considered statistically significant at $P<0.05$. All statistical analyses were performed using GraphPad Prism 10.3.1 for Windows 11 (GraphPad software, La Jolla, CA, USA, www.graphpad.com/).

RESULTS

Toxicity assays

Fumigation with ***Thymus munbyanus*** EO for 24 h significantly impacted ***Ephestia kuehniella*** survival. Corrected mortality rates for adults ranged from 15% at the lowest concentration (0.05 µL/mL air) to 100% at the highest concentration (0.80 µL/mL air). The EO induced adult mortality in a concentration-dependent manner (Fig. 1). Table 1 presents the lethal concentrations (LC) and their corresponding Hill slope values, both accompanied by 95% fiducial limits. Statistical analysis revealed a significant concentration effect ($F(6,15)=545.6$; $P<0.0001$). Ranking the concentrations using Tukey's HSD test allowed for the classification of concentrations based on their toxicities (Fig. 2). The results revealed 7 distinct groups, indicating varying levels of EO efficacy on adult mortality percentages.

Table 1. Lethal concentrations of *Thymus munbyanus* essential oil against *Ephestia kuehniella* adults

Concentrations	Values (µL/mL air)	Fiducial limits (95%)	R^2
LC_{10}	0.087	0.047 to 0.138	0.97
LC_{25}	0.153	0.104 to 0.207	
LC_{50}	0.269	0.215 to 0.326	
LC_{90}	0.829	0.582 to 1.321	
Hill slope	1.949	1.358 to 2.871	

Values represent lethal concentrations (LC_{10}, LC_{25}, LC_{50}, LC_{90}) expressed as µL/mL of air. Fiducial limits correspond to 95% confidence intervals calculated from probit regression. The Hill slope describes the steepness of the concentration–response relationship, and R^2 indicates the goodness of fit of the model.

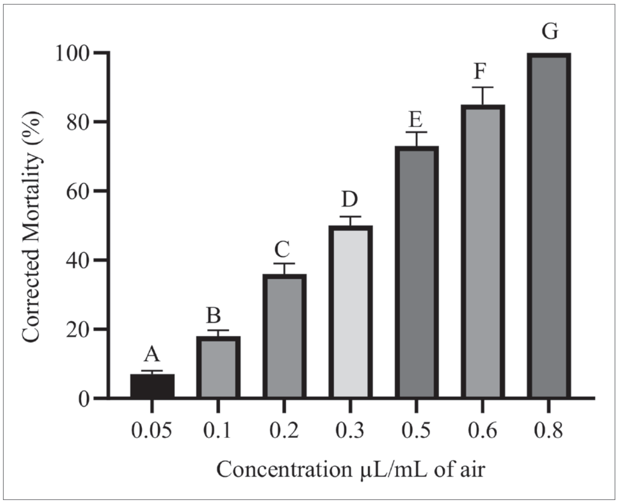

Fig.1. Effect of *Thymus munbyanus* essential oil on adult mortality of *Ephestia kuehniella* after 24 h fumigation exposure. Mortality (%) was recorded 24 h post-exposure. Values are means of 3 independent replicates. Bars with different letters indicate significant differences (one-way ANOVA followed by Tukey's HSD test, $P<0.0001$).

Effect of *Thymus munbyanus* essential oil on the specific activity of GSTs

As shown in Fig. 3A, GST activity was significantly stimulated in adults exposed to sublethal concentrations (LC_{25} and LC_{50}) of ***T. munbyanus*** EO throughout the three-day monitoring period. The highest stimulation was observed on day 1, with increases of +23.7% (LC_{25}) and +57.4% (LC_{50}) compared with the control group. Two-way ANOVA revealed significant effects for concentration ($F(2,45)=899.3$; $P<0.0001$), time ($F(2,45)=549.4$; $P<0.0001$), and their interaction ($F(4,45)=27.76$; $P<0.0001$).

Effect of *Thymus munbyanus* essential oil on the specific activity of CAT

Catalase is a key antioxidant enzyme involved in the elimination of hydrogen peroxide. As shown in Fig. 3B, CAT activity increased in EO-exposed adults compared with controls. The strongest response was recorded on day 3, with CAT activity increasing by +95.1% at LC_{25} and +124.4% at LC_{50} relative to the control group. Statistical analysis confirmed significant effects

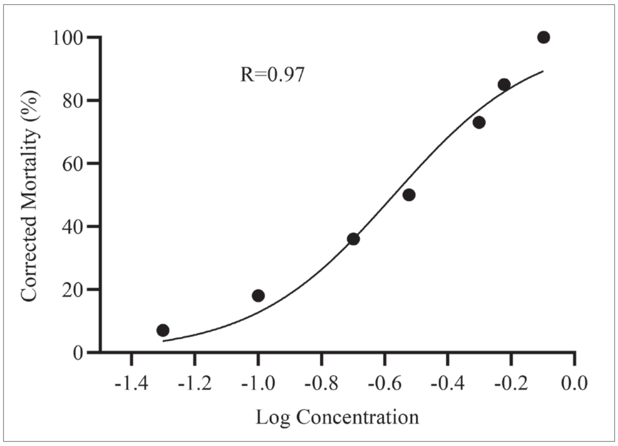

Fig. 2. Effect of *Thymus munbyanus* essential oil on adult *Ephestia kuehniella* at different concentrations (µL/mL). The concentration-response curve expresses the relationship between corrected mortality and the logarithm of essential oil concentrations (µL/mL). R^2 indicates the goodness of fit of the regression model.

of concentration (F(2,45)=228,096; P<0.0001) and time (F(2,45)=116,761; P<0.0001), as well as a significant concentration × time interaction (F(4,45)=4,586; P<0.0001). The extremely high F values observed are attributable to minimal within-group variance coupled with substantial differences between group means, as evidenced by the small standard deviations across replicates.

Effect of *Thymus munbyanus* essential oil on the specific activity of AChE

AChE is a major target enzyme of neurotoxic insecticides. Fig. 3C shows that EO exposure strongly inhibited AChE activity throughout the experiment. On day 1, AChE activity decreased by 73.8% at LC_{25} and 78.6% at LC_{50} compared with controls, and this inhibition persisted throughout the experimental period. Two-way ANOVA indicated significant effects of concentration (F(2,45)=3,856; P<0.0001), time (F(2,45)=287.4; P<0.0001), and their interaction (F(4,45)=104.4; P<0.0001).

Effect of *Thymus munbyanus* essential oil on oviposition

Regular monitoring of mating pairs allowed the determination of fecundity, egg hatching, and hatching rate. Exposure to *T. munbyanus* EO significantly reduced female fecundity, with decreases of 28.1% at LC_{25} and 66.4% at LC_{50} compared with the control group (one-way ANOVA followed by Tukey's HSD test: F(2,5)=2644; P<0.0001). Egg viability was also negatively affected, as the number of hatched eggs declined by 48.7% at LC_{25} and 78.2% at LC_{50} relative to the control (one-way ANOVA followed by Tukey's HSD test: F(2,15)=3742; P<0.0001). Similarly, the hatching rate decreased by 30.9% at LC_{25} and 37.5% at LC_{50} compared with the control group (one-way ANOVA followed by Tukey's HSD test: F(2,15)=314.6; P<0.0001) (Table 2).

Effect of *Thymus munbyanus* essential oil on the mobility of *Ephestia kuehniella*

Exposure of adult *Ephestia kuehniella* to *Thymus munbyanus* EO induced pronounced, concentration-dependent impairments in mobility (Fig. 4). Distance

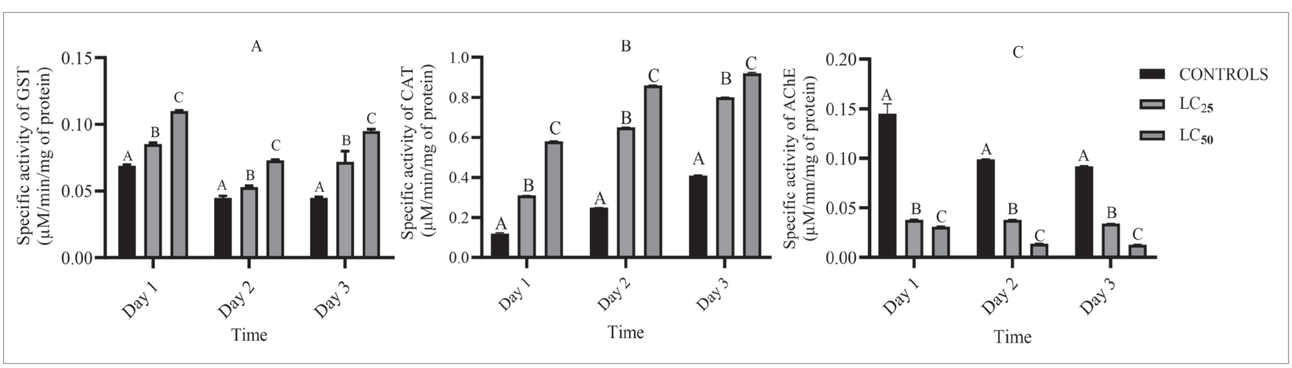

Fig. 3. Effects of *Thymus munbyanus* essential oil on glutathione S-transferase (GST) (A), catalase (CAT) (B), and acetylcholinesterase (AChE) activities (C) in adult *Ephestia kuehniella* exposed to sublethal concentrations (LC_{25} and LC_{50}). Data are expressed as mean±SD (n=6). Different letters indicate significant differences among treatments (two-way ANOVA followed by Tukey's HSD test, P<0.0001).

Table 2. Effects of sublethal exposure to *Thymus munbyanus* essential oil on the reproductive traits of adult *Ephestia kuehniella*

Treatment	Number of eggs laid	Number of eggs hatched	Hatching rate (%)
Control	194.25±3.50 A	137.5±3.10 A	73.44±2.09 A
LC_{25}	139.75±4.11 B	70.5±2.60 B	50.76±2.84 B
LC_{50}	65.33±4.75 C	30±2.16 C	45.89±1.72 C

Data are presented as mean±SD (n=6). Mean values followed by different uppercase letters are significantly different among treatments (P<0.0001) according to one-way ANOVA followed by Tukey's HSD multiple-comparison test.

travelled (mm) decreased by 78.8% at LC_{25} and by 100% at LC_{50} compared with the control group (Kruskal-Wallis test: H=40.63; P<0.0001) (Fig. 4A). Similarly, movement duration (s) was reduced by 43.8% at LC_{25} and 100.0% at LC_{50} relative to controls (Kruskal-Wallis test: H=35.94; P<0.0001) (Fig. 4B). Movement speed (mm s^{-1}) also declined in a concentration-dependent manner, decreasing by 61.1% at LC_{25} and approaching 100% at LC_{50} compared with controls (Kruskal-Wallis test: H=39.07; P<0.0001) (Fig. 4C).

DISCUSSION

EOs offer several advantages over synthetic chemical pesticides due to their medicinal properties, low mammalian toxicity, and rapid biodegradability. Consequently, they represent a promising natural alternative for integrated pest management [31]. Furthermore, the high volatility of many plant extracts and EOs allows them to function effectively as fumigants [32].

In the present fumigant bioassays, *T. munbyanus* EO exhibited potent toxicity against the Mediterranean flour moth, *Ephestia kuehniella* (Lepidoptera: Pyralidae). These findings align with previous studies reporting the fumigant efficacy of various plant essential oils against different developmental stages of *E. kuehniella*. Specifically, essential oils from *Satureja thymbra*, *Origanum onites*, and *Myrtus communis* have demonstrated high efficacy against adults, achieving 100% mortality within 24 h [33, 34]. Similarly, exposure of adult *E. kuehniella* to *Ocimum basilicum* and *Zingiber officinale* EOs resulted in significant mortality [35]. Recent findings indicate that *Schinus molle* EO, applied via fumigation, was the most effective, followed by *Rosmarinus officinalis* [36]. Furthermore, Wang et al. [37] demonstrated the potent toxicity of *Artemisia annua* EO and its primary constituent, 1,8-cineole, against *E. kuehniella* larvae. Additionally, EOs from *T. argaeus* and *T. sipyleus* proved effective against both larval and egg stages [38]. The pronounced insecticidal activity of *T. munbyanus* EO observed in this study may be attributed to its high concentration of monoterpenes, including carvacrol, *p*-cymene, and thymoquinone [39]. Fumigant exposure to volatile compounds imposes significant metabolic and neurophysiological constraints on insects. These stressors manifest as measurable alterations in detoxification and antioxidant enzymes, such as GST and CAT, alongside neurophysiological targets like AChE. The magnitude of these responses often fluctuates according to the physiological context and specific exposure

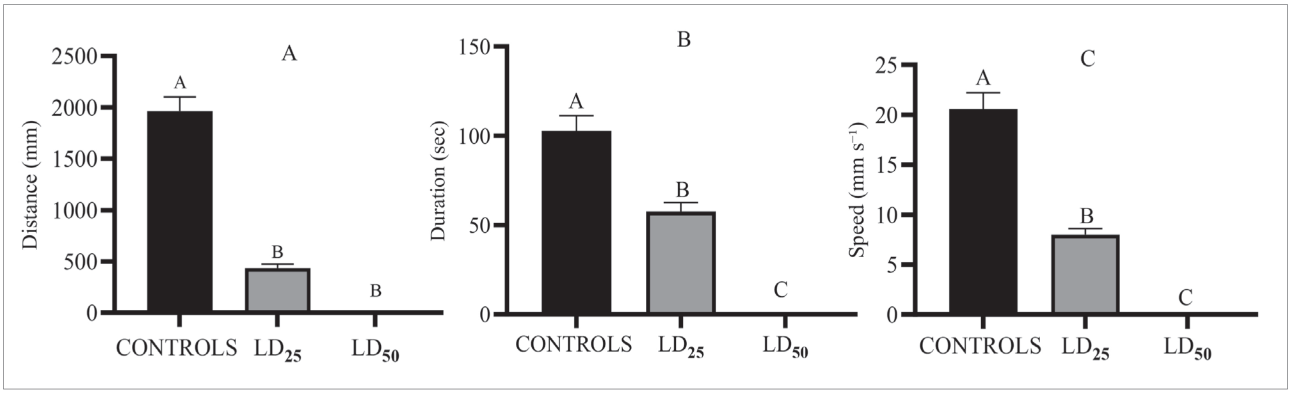

Fig. 4. Effects of *Thymus munbyanus* essential oil on locomotor performance of adult *Ephestia kuehniella*: distance travelled (A), movement duration (B), and movement speed (C) after exposure to sublethal concentrations (LC_{25} and LC_{50}). Data are expressed as mean±SD (n=15). Different letters indicate significant differences among treatments (Kruskal-Wallis test followed by Dunn's post hoc test, P<0.0001).

conditions [40, 41]. Glutathione S-transferases (GSTs) are key phase II detoxification enzymes that protect insects against toxic plant metabolites by facilitating xenobiotic conjugation and contributing to cellular defense under oxidative stress [42]. In the present study, fumigation with *T. munbyanus* EO significantly increased GST activity as early as 24 h post-treatment, an effect that persisted through the third day compared to controls. Similar inductions of GST activity have been reported following the treatment of larvae with α-pinene, trans-anethole, and thyme EO [43], as well as in *Culex pipiens* exposed to *Myrtus communis* EO [44]. This elevated GST activity in the Mediterranean flour moth likely represents a response to oxidative stress induced by the botanical compounds; specifically, GST facilitates the inactivation of lipid peroxidation products that accumulate during oxidative damage [45].

Catalase (CAT) is a major antioxidant enzyme involved in the decomposition of hydrogen peroxide and is widely used as a biomarker of oxidative stress responses in insects [46]. In the present study, CAT activity was significantly elevated in *E. kuehniella* larvae following EO exposure. Similar increases in CAT activity have been reported in *E. kuehniella* larvae after exposure to EO constituents such as α-pinene and thymol [47], as well as after short-term exposure to *Artemisia annua* EO [37]. Comparable enzymatic changes have also been observed in *Plutella xylostella* treated with *Chenopodium ambrosioides* EO and its major compounds, *p*-cymene and α-terpinene [48], and in *Hyphantria cunea* larvae exposed to *A. annua* EO [49]. This increase in enzyme activity may indicate an adaptive response to stress induced by the applied compounds to eliminate the negative effects of reactive oxygen species (ROS) [37].

Acetylcholinesterase (AChE) is a primary target of neurotoxic insecticides and is therefore widely used as a biomarker to assess neurophysiological impairment in insects [50]. In the present study, AChE activity in adults was significantly reduced 24 h after fumigation with *T. munbyanus* EO. This inhibitory effect is consistent with previous reports demonstrating that essential oils rich in terpenoid compounds can interfere with AChE activity [51]. In particular, *Thymus zygis* EO, characterized by a high carvacrol content, has shown strong AChE inhibitory potential [52], and topical application of carvacrol significantly reduced AChE activity in adults of *Diaphorina citri* [53]. The inhibition of AChE activity observed in this study may be related to the high monoterpenoid content of *T. munbyanus* EO, particularly carvacrol. Previous studies have reported neuroactive effects of carvacrol in insects, including interactions with neural targets such as octopaminergic and GABAergic receptors [54].

In *E. kuehniella*, vitellogenesis occurs during the pupal stage, while mating and fertilization take place within the first 12 h post-eclosion. The subsequent pre-oviposition and oviposition periods typically span three to four days [55]. The results obtained show that *T. munbyanus* EO significantly affects fertility, viability, and hatching percentage. Furthermore, in a previous study, *Satureja thymbra* EO had an inhibitory effect on the egg-laying of *E. kuehniella* [56]. Recently, essential oils from *S. sahendica*, *S. khuzistanica*, and *S. macrantha* were shown to significantly inhibit oviposition in *E. kuehniella*. Among these, *S. khuzistanica* oil exhibited the highest efficacy, primarily due to its high carvacrol content [57]. A recent study revealed that the EOs of anise, basil, chamomile, cumin, dill, garlic, thyme, and yarrow inhibit the egg-laying of *Bactrocera tryoni* [58]. *M. communis* EO was tested on fourth-instar larvae of *Cx. pipiens* and showed a reduction in fertility and egg numbers [59].

Insect mobility is widely regarded as a key indicator of an insect's overall physiological state and a critical factor influencing its environmental interactions [60]. In the present study, fumigation with *T. munbyanus* EO significantly affected the locomotor activity of *E. kuehniella* adults. Exposure to LC_{25} and LC_{50} concentrations resulted in reduced distance traveled and movement duration, whereas the highest concentration caused prolonged periods of immobility. Similar locomotor effects following EO exposure have been described in several insect species. Lopes et al. [61] reported modifications in walking and flying activity in *Sitophilus zeamais* after sublethal exposure to *Piper hispidinervum* EO. Reduced locomotor activity has also been observed in *Drosophila melanogaster* following fumigation with *Chamaecyparis obtusa* EO [62], and in *D. melanogaster* and *D. suzukii* exposed to monoterpenes, with these impairments linked to the modulation of tyramine receptor function [63]. These behavioral changes may be associated with physiological disturbances induced by EO exposure.

Previous research has established a clear correlation between AChE inhibition and impaired locomotor performance in insects [64]. In this context, the reduction in AChE activity observed following *T. munbyanus* EO treatment likely contributes to the recorded mobility alterations, potentially acting in synergy with other disrupted physiological processes.

Despite these consistent behavioral patterns, certain methodological limitations of the mobility assay warrant acknowledgment. The assay employed here provides a semi-quantitative estimation of locomotor activity based on grid displacement. While this approach facilitates the comparison of relative mobility across treatments, it does not capture fine-scale movement dynamics. Consequently, the observed shifts in mobility should be interpreted as indicative behavioral trends rather than precise measurements of locomotor performance.

CONCLUSIONS

Fumigation with *Thymus munbyanus* essential oil showed strong toxicity against adult *Ephestia kuehniella*, causing high mortality at low concentrations. The treatment increased GST and CAT activities and inhibited AChE, suggesting physiological stress and possible neurotoxic effects. It also reduced oviposition and egg viability and impaired adult mobility. Overall, these findings support the potential of *T. munbyanus* EO for stored-product pest management, although further semi-field and field validation is still required.

Funding: The authors received no specific funding for this work. The study was carried out using institutional resources.

Acknowledgments: This research was funded by the Algerian Fund for Scientific Research (Excellence Laboratory of Applied Animal Biology) and the Ministry of Higher Education and Scientific Research of Algeria. (PRFU Project N° D01N01UN230120230005 to Pr. S. TOUIKER).

Author contributions: ZCH: conception and design of the study, data collection, data analysis, and drafting of the manuscript. HB: contribution to data collection and analysis. MCO: data analysis, software, and critical revision of the manuscript AY: contribution to data collection and manuscript preparation. SY: supervision, study conception and design, and critical revision of the manuscript. All authors have read and approved the final version of the manuscript.

Conflict of interest disclosure: The authors declare no conflicts of interest.

Data availability: The data supporting this article are available in the online dataset: https://figshare.com/s/376195310cb2b16b25b7

REFERENCES

1. Yu J. Chemical Composition of Essential Oils and Their Potential Applications in Postharvest Storage of Cereal Grains. Molecules. 2025;30(3):683. https://doi.org/10.3390/molecules30030683
2. Elbrense H, El Husseiny I, Abo El Makarem HA, Abo Arab R, El Kholy S. Insecticidal, antifeedant and repellent efficacy of certain essential oils against adult rust-red flour beetle, Tribolium castaneum. Egypt J Chem. 2022;65(1):167-78. https://doi.org/10.21608/ejchem.2021.79263.3897
3. Bendjedid H, Yezli-touiker S, Taffar A. Yezli A, Souilah N. Impact of Infestation of Flour by Stored Food Pest Insects Ephestia Kuehniella on Quality Flour: Physico-Chemical Analyses. J Bioresour Manag. 2024;11(1):12.
4. FAO. The four betters and leaving no one behind [Internet]. Rome (IT): Food and Agriculture Organization of the United Nations; 2022 [cited 2025 Dec 1]. Available from: https://www.fao.org/faostat/en/#home
5. Yezli-Touiker S, Kirane-Amrani L, Meskache R, Soltani N. Effect of captopril on growth development and cuticular secretion in pupae of the Mediterranean flour moth, Ephestia kuehniella Zeller. Fresenius Environ Bull. 2019;28(2):733-8.
6. Taffar A, Yezli-Touiker S, Bendjedid H, Soltani N. Evaluation of azadirachtin, a biopesticides, on growth, development and cuticle secretion of Mediterranean flour moth, Ephestia kuehniella Zeller. J Entomol Res. 2021;45(3):436-43. https://doi.org/10.5958/0974-4576.2021.00068.2
7. Kathirvelu C, Maline AS, Sivasankari S. Effect of essential oils as repellent against coleopteran pests of stored produce. J Entomol Res. 2020;44(3):371-6. https://doi.org/10.5958/0974-4576.2020.00063.8
8. Drabo F, Doumbia S, Sangaré L, Konaté A, Sissoko F, Sanogo Y. Résistance des insectes ravageurs des denrées stockées aux insecticides: cas de Sitophilus zeamais et Tribolium castaneum au Mali. J Appl Biosci. 2019;133:13543-50.
9. Rezende-Teixeira P, Dusi RG, Jimenez PC, Espindola LS, Costa-Lotufo LV. What can we learn from commercial insecticides? Efficacy, toxicity, environmental impacts, and future developments. Environ Pollut. 2022;300:118983. https://doi.org/10.1016/j.envpol.2022.118983
10. Gad HA, Al-Anany MS, Atta AA, Al-Ayat AA, Abdelgaleil SA. Efficacy of abamectin and emamectin benzoate alone or in binary combinations with carvone, p-cymene and menthone for the control of Callosobruchus maculatus. J Nat Pestic Res. 2025;11:100106. https://doi.org/10.1016/j.napere.2024.100106
11. Karabörklü S, Ayvaz A. A comprehensive review of effective essential oil components in stored-product pest management. J Plant Dis Protect. 2023;130(3):449-81. https://doi.org/10.1007/s41348-023-00712-0
12. Bendif H, Boudjeniba M, Miara MD, Biqiku L, Bramucci M, Caprioli G, Maggi F. Rosmarinus eriocalyx: An alternative to Rosmarinus officinalis as a source of antioxidant compounds. Food chemi. 2017;218:78-88.

https://doi.org/10.1016/j.foodchem.2016.09.063
13. Sadou H, Aouad M, Ghalem S. Chemical composition, antioxidant and antiproliferative activities of essential oil from Thymus munbyanus from Algeria. S Afr J Bot. 2020;130:246-52.
14. Bendjedid H, Yezli-Touiker S, Taffar A, Soltani N. Phytochemical composition and insecticidal activities of essential oil of Thymus munbyanus (Lamiales: Lamiaceae) aerial parts and its properties against biomarkers of Ephestia kuehniella Zeller (Lepidoptera: Pyralidae). J Essent Oil bear Plants. 2021;24(4):792-807. https://doi.org/10.1080/0972060X.2021.1984315
15. Pandır D, Baş H. Compositional analysis and toxicity of four plant essential oils to different stages of Mediterranean flour moth, Ephestia kuehniella Zeller (Lepidoptera: Pyralidae). Turk J Entomol. 2016;40(2):185-95. https://doi.org/10.16970/ted.82833
16. Chapman RF. The insects: structure and function. 5th ed. Cambridge: Cambridge University Press; 2013.
17. Nation JL. Insect physiology and biochemistry. 4th ed. Boca Raton:CRC Press; 2022.
18. Regnault-Roger C, Philogène BJR, Vincent C. Biopesticides d'origine végétale. Tropicultura. 2006;24(2):124.
19. Lee SE. Biochemical mechanisms conferring cross-resistance to fumigant toxicities of essential oils in a chlorpyrifos-methyl resistant strain of Oryzaephilus surinamensis L.(Coleoptera: Silvanidae). J Stored Prod Res. 2002;38(2):157-66. https://doi.org/10.1016/S0022-474X(01)00013-3
20. Isman MB. "Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol. 2006;51(1):45-66. https://doi.org/10.1146/annurev.ento.51.110104.151146
21. Rajendran S, Sriranjini V. Plant products as fumigants for stored-product insect control. Stored Prod Res. 2008;44(2):126-35. https://doi.org/10.1016/j.jspr.2007.08.003
22. Bostanian NJ, Akalach M, Chiasson H. Effects of a Chenopodium based botanical insecticide/acaricide on Orius insidiosus (Hemiptera: Anthocoridae) and Aphidius colemani (Hymenoptera: Braconidae). Pest Manag Sci. 2005;61:979-84. https://doi.org/10.1002/ps.1065
23. Ngamo LS, Hance TH. Diversité des ravageurs des denrées et méthodes alternatives de lutte en milieu tropical Tropicultura. 2007;25:215-20.
24. Yezli-Touiker S, Soltani-Mazouni N. Profil des ecdystéroïdes durant la métamorphose et rapport avec le cycle cuticulaire chez Ephestia kuehniella (Insecta, Lepidoptera, Pyralidae). Synthèse. 2011;22:49-55.
25. Titouhi F, Amri M, Messaoud C, Haouel S, Youssfi S, Cherif A, Mediouni Ben Jemâa J. Protective effects of three Artemisia essential oils against Callosobruchus maculatus and Bruchus rufimanus (Coleoptera: Chrysomelidae) and the extended side-effects on their natural enemies. J Stored Prod Res. 2017;72:11-20. https://doi.org/10.1016/j.jspr.2017.02.007
26. Abbott WW. A method for computing the effectiveness of an insecticide. J Econ Entomol.1925;18:265-7. https://doi.org/10.1093/jee/18.2.265a
27. Habig WH, Pabst MJ, Jackoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem. 1974;249(22):7130-9. https://doi.org/10.1016/S0021-9258(19)42083-8
28. Claiborne Stephens J, Nei M. Phylogenetic analysis of polymorphic DNA sequences at the Adh locus in Drosophila melanogaster and its sibling species. J Mol Evol. 1985;22(4):289-300. https://doi.org/10.1007/BF02115684
29. Ellman GL, Courtney KD, Andres Jr V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharm. 1961;7(2):88-95. https://doi.org/10.1016/0006-2952(61)90145-9
30. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72(1-2):248-54. https://doi.org/10.1016/0003-2697(76)90527-3
31. Polano Ody L, Ferraz AD, de Mello E, Ugalde G, Mazutti, MA, Tres MV, Zabot GL. Trichilia claussenii (Meliaceae): A Review of Its Biological and Phytochemical Activities and a Case Study of Composition. Processes. 2025;13(10):3058. https://doi.org/10.3390/pr13103058
32. Elbehery HH, Ibrahim SS. Potential fumigant toxicity of essential oils against Sitotroga cerealella (Olivier) (Lepidoptera:Gelechiidae) and its egg parasitoid Trichogramma evanescens (Hymenoptera: Trichogrammatidae). Sci Rep. 2024;14(1):6253. https://doi.org/10.1038/s41598-024-56611-3
33. Ayvaz A, Sagdic O, Karaborklu S, Ozturk I. Insecticidal activity of the essential oils from different plants against three stored-product insects. J Insect Sci. 2010;10(1):21. https://doi.org/10.1673/031.010.2101
34. Moazeni N, Khajeali J, Izadi H, Mahdian K. Chemical composition and bioactivity of Thymus daenensis Celak (Lamiaceae) essential oil against two lepidopteran stored-product insects. J Essent Oil Res. 2014; 26(2):118-24. https://doi.org/10.1080/10412905.2013.860412
35. Mikhaiel AA. Potential of some volatile oils in protecting packages of irradiated wheat flour against Ephestia kuehniella and Tribolium castaneum. J Stored Prod Res. 2011;47(4):357-64. https://doi.org/10.1016/j.jspr.2011.06.002
36. Ben Chaabene R, Martinez M, Bonavoglia A, Maco B, Chang YW, Lentini G, Soldati-Favre D. Toxoplasma gondii rhoptry discharge factor 3 is essential for invasion and microtubule-associated vesicle biogenesis. PloS Biol. 2024;22(8):e3002745. https://doi.org/10.1371/journal.pbio.3002745
37. Beshkoufe S, Azizi R, Afrazeh Z, Nemati A, Mojarab-Mahboubkar M, Sendi JJ. The effect of Artemisia annua essential oil and one of its main components on the biology and enzymatic and non-enzymatic activities of Ephestia kuehniella Zeller (Lepidoptra: Pyralidae). Invertebr Survival J. 2025;22(1):64-77.
38. Ercan F, Baş H, Ercan N, Vural C, Ozcan S. Fumigant toxicitiy of essential oils from Thymus argaeus Boissier & Balansa and Thymus sipyleus Boissier (Lamiaceae) againts Ephestia kuehniella Zeller (Lepidoptera: Pyralidae). Int J Sci Technol Res. 2018;4(3):54-60.
39. Eltalawy HM, El-Fayoumi H, Aboelhadid SM, Al-Quraishy S, El-Mallah AM, El-Ela FI, Abdel-Baki AA. Insecticidal

activity and systematic insights of carvacrol against Tribolium castaneum: Acetylcholinesterase inhibition, oxidative stress, and molecular docking. J Stored Prod Res. 2025;112:102638. https://doi.org/10.1016/j.jspr.2025.102638
40. Krzyżowski M, Baran B, Łozowski B, Francikowski J. The effect of Rosmarinus officinalis essential oil fumigation on biochemical, behavioral, and physiological parameters of Callosobruchus maculatus. Insects. 2020;11(6):344. https://doi.org/10.3390/insects11060344
41. Lazarević J, Jevremović S, Kostić I, Vuleta A, Manitašević Jovanović S, Kostić M, Šešlija Jovanović D. Assessment of sex-specific toxicity and physiological responses to thymol in a common bean pest Acanthoscelides obtectus Say. Front Physiol. 2022;13:842314. https://doi.org/10.3389/fphys.2022.842314
42. Jing TX, Wu YX, Li T, Wei DD, Smagghe GG, Wang JJ. Identification and expression profiles of fifteen delta-class glutathione S-transferase genes from a stored-product pest, Liposcelis entomophila (Enderlein) (Psocoptera: Liposcelididae). Comp Biochem Physiol. 2017;206:35-41. https://doi.org/10.1016/j.cbpb.2017.01.008
43. Shahriari M, Zibaee A, Mirhaghparast SK, Pour SA, Ramzi S, Hoda H. Mortality and physiological impacts of the tea saponin against Ephestia kuehniella Zeller (Lepidoptera: Pyralidae). Toxin rev. 2022;41(4):1077-85. https://doi.org/10.1080/15569543.2021.1974042
44. Yezli A, Boudjelida H, Arroussi D. Components and toxicological effects of Myrtus communis L. (myrtales: myrtaceae) essential oil against mosquito Culex pipiens L. (diptera: culicidae). J Appl Ecol. 2024;22(3):2164. https://doi.org/10.15666/aeer/2203_21492164
45. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alex J Med. 2018;54(4):287-93. https://doi.org/10.1016/j.ajme.2017.09.001
46. Shahriari M, Zibaee A, Sahebzadeh N, Shamakhi L. Effects of α-pinene, trans-anethole, and thymol as the essential oil constituents on antioxidant system and acetylcholine esterase of Ephestia kuehniella Zeller (Lepidoptera: Pyralidae). Pestic biochem physiol. 2018;150:40-7. https://doi.org/10.1016/j.pestbp.2018.06.015
47. Wei H, Liu J, Li B, Zhan Z, Chen Y, Tian H, Gu X. The toxicity and physiological effect of essential oil from Chenopodium ambrosioides against the diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae). Crop prot. 2015;76:68-74. https://doi.org/10.1016/j.cropro.2015.06.013
48. Mojarab-Mahboubkar M, Sendi JJ. Evaluating the potential of Artemisia annua essential oil for controlling Hyphantria cunea (Lepidoptera: Erebidae): effects on larval development, immune function, and enzymatic activities. J Econ Entomol. 2025;118(4):1680-93. https://doi.org/10.1093/jee/toaf120
49. Farhadi B, Amiri A, Pourabad RF, Bandani AR. Transgenerational effects of peppermint essential oil on life table parameters and antioxidant enzymes in Callosobruchus maculatus. Sci Rep. 2025;15(1):20721. https://doi.org/10.1038/s41598-025-08563-5
50. Lonare M, Kumar M, Raut S, Badgujar P, Doltade S, Telang A. Evaluation of imidacloprid-induced neurotoxicity in male rats: a protective effect of curcumin. Neurochem Int. 2014;78:122-9. https://doi.org/10.1016/j.neuint.2014.09.004
51. Jankowska M, Rogalska J, Wyszkowska J, Stankiewicz M. Molecular targets for components of essential oils in the insect nervous system-a review. Molecules. 2017;23(1):34. https://doi.org/10.3390/molecules23010034
52. Dandlen SA, Miguel MG, Duarte J, Faleiro ML, Sousa MJ, Lima AS, Pedro LG. Acetylcholinesterase inhibition activity of Portuguese Thymus species essential oils. Essent Oil-bear Plant. 2011;14(2):140-50. https://doi.org/10.1080/0972060X.2011.10643914
53. Rizvi SA, Ling S, Tian F, Xie F, Zeng X. (2018). Toxicity and enzyme inhibition activities of the essential oil and dominant constituents derived from Artemisia absinthium L. against adult Asian citrus psyllid Diaphorina citri Kuwayama (Hemiptera: Psyllidae). Ind Crops Prod. 2018;121:468-75. https://doi.org/10.1016/j.indcrop.2018.05.031
54. Benelli G, Pavela R, Petrelli R, Cappellacci L, Bartolucci F, Canale A, Maggi F. Origanum syriacum subsp. syriacum: From an ingredient of Lebanese 'manoushe'to a source of effective and eco-friendly botanical insecticides. Ind Crops Prod. 2019;134:26-32. https://doi.org/10.1016/j.indcrop.2019.03.055
55. Yezli-Touiker S, Soltani-Mazouni N, Kirane-Amrani L, Soltani N. Delayed effects of caproptil on the mediterranean flour moth: reproductive events, of ovaries. Biochemical composition and molting hormone contents of ovaries. Fresen Environ Bull. 2016;25(4):1190-205.
56. Sabbour MM, Abd El-Aziz SE. Impact of certain nano oils against Ephestia kuehniella and Ephestia cutella (Lepidoptera-Pyralidae) under laboratory and store conditions. Bull Natl Res Cent. 2019;43(1):80. https://doi.org/10.1186/s42269-019-0129-3
57. Gorji M. Oviposition deterrent effects and ovicidal activity of three species of Iranian endemic savory on Mediterranean flour moth (Ephestia kuehniella Zeller (Lepidoptera: Pyralidae). Iran J Med Aromat Plants Res. 2024;40(2):388-99.
58. Hossain M.S, Akter S, Hossain M.F, Rizvi S.Z, Mendez V, Taylor P, Park S.J. Essential oils as potential insecticides and behavior-modifying agents against Bactrocera tryoni (Diptera: Tephritidae). J Insect Sci. 2025;25(5):ieaf073. https://doi.org/10.1093/jisesa/ieaf073
59. Yezli A, Arroussi D.E.R, Boudjelida H, Bensouici C. Repellency and activity evaluation of Myrtus communis essential oil on physiology and reproduction of mosquito species Culex pipiens. Fresen Environ Bull. 2024; 33(5):407-17.
60. Bayley M. Basic behaviour: The use of animal locomotion in behavioural ecotoxicology. In: Dell'Omo G, editor. Behavioural Ecotoxicology. London, UK: John Wiley; 2002. p 211-30.
61. Lopes L.M, Sousa A.H, Faroni L.R, Silva M.V, Ferraz M.S, Santos V.B. Toxicity and Sublethal Effects of Piper hispidinervum Essential Oil on Behavioral and Physiological Responses of Sitophilus zeamais Populations. Molecules. 2024;29(17):4116. https://doi.org/10.3390/molecules29174116

62. Lee S.H, Do H.S, Min K.J. Effects of essential oil from Hinoki cypress, Chamaecyparis obtusa, on physiology and behavior of flies. PloS one. 2015;10(12):e0143450. https://doi.org/10.1371/journal.pone.0143450
63. Finetti L, Tiedemann L, Zhang X, Civolani S, Bernacchia G, Roeder T. Monoterpenes alter TAR1-driven physiology in Drosophila species. J Exp Biol. 2021;224(1):jeb232116.
64. Ramin M, Azizi P, Motamedi F, Haghparast A, Khodagholi F. Inhibition of JNK phosphorylation reverses memory deficit induced by β-amyloid (1-42) associated with decrease of apoptotic factors. Behav Brain Res. 2011;217(2):424-31. https://doi.org/10.1016/j.bbr.2010.11.017