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Research Article

Larvicidal activity of essential oils from *Cuminum cyminum* and *Anethum graveolens* against *Ephestia kuehniella*: toxicity and biochemical assays

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Abstact

Botanical compounds are increasingly investigated for their potential in pest control. Several botanical compounds, including essential oils (EOs), are recognized to affect the digestion and metabolic processes of insect herbivores. This study examines the effects of plant EOs on mortality, digestive physiology, and storage macromolecules of *Ephestia kuehniella*. Oral toxicity of *Cuminum cyminum* and *Anethum graveolens* EOs were investigated against the fourth instar larvae of *E. kuehniella*. The LC_{50} and LT_{50} values were recorded 13.26 µL/mL and 15.28 h for *C. cyminum* and 6.15 µL/mL and 8.39 h for *A. graveolens*, respectively. In the present study, *A. graveolens* EO at lower concentrations and shorter times caused higher larval mortality. Activities of digestive enzymes, except for lipase, were significantly reduced in the insects fed on treated diet by both EOs. Additionally, the amounts of protein, triacylglycerol, and glycogen as energy reserves were markedly lower in the exposed insects, indicating depletion due to energetic costs imposed by this two EOs. The findings suggest that the *A. graveolens* EO is more effective than *C. cyminum* on the *E. kuehniella* larvae and disrupt critical digestive processes in, which may contribute to their insecticidal properties. However, further studies are needed to expand advanced plant derivative compounds-based encapsulation techniques to increase the stability, and efficacy.

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Introduction

Ephestia kuehniella Zeller (Lepidoptera: Pyralidae) is a destructive pest in stored products, such as cereals and grains, and is widely distributed globally (Shahriari *et al*. 2018). The larvae produce thin webs throughout their developmental period, and in cases of severe infestation, these can cover the entire surface of the affected food remnants. Furthermore, the larvae leave behind floss and food remnants, causing additional damage (Jallouli *et al*. 2013). Currently, synthetic chemical compounds are the most effective control method against flour moth; however, their use should be reconsidered due to detrimental environmental effects on human, non-target organisms, and the ecosystem (Senthil-Nathan 2013; Isman 2020). The emergence of resistance to synthetic pesticides has heightened the need for alternative pest control compounds. In recent years, researchers have increasingly emphasized the use of novel natural-derived compounds that inhibit the biological and physiological activities of insects while posing low environmental risk (Bouayad *et al*. 2012; Shahriari *et al*. 2017a). Available reports clearly demonstrate that botanical compounds, including essential oils (EOs), can considerably contribute to the decrement in the population of insect pests (Isman 2020). Among aromatic plants, EOs from the Apiaceae plants, such as ajwain, anise, fennel, persian hogweed, cumin and dill EOs, exhibit a broad spectrum of effect on the various pests (Shahriari *et al*. 2017b; Pavela *et al*. 2020; Shahriari *et al*. 2024). Cumin (*Cuminum cyminum* L.) and dill (*Anethum graveolens* L.) are among the most recognized and utilized plants for their rich EO content (Chaubey 2012; Benelli *et al*. 2018; Pavela *et al*. 2020). The primary constituents of these EOs (monoterpenes, phenylpropanoids and sesquiterpenes) possess high toxicity against insect pest (Pavela *et al*. 2018, 2020). Benlembarek *et al*. (2022) reported carvone (34.33%) and α-phellendrene (22.03%) as the major constituents of *A. graveolens*. The major components of *C. cyminum* EO are cuminlaldehyde (39.48 %) and γ-Terpinene (15.21 %) (Hajlaoui et al. 2010). Both EOs have already demonstrated toxicity against various insects; fumigant toxicity of *A. graveolens* EO against the larvae of *E. kuehniella* larvae (Najafzadeh *et al*. 2019), contact toxicity of *A. graveolens* EO against the larvae and adults of the confused flour beetle (*Tribolium confusum* Jacquelin du Val) (Guesmi *et al*. 2024), fumigant toxicity of *C. cyminum* EO against the grain weevil (*Sitophilus granarius* L.) (Ziaee *et al*. 2014), fumigant toxicity of *C. cyminum* and *Mentha piperita* EOs against the rice weevil (*Sitophilus oryzae* L.) and the red flour beetle (*Tribolium castaneum* Herbst) (Lashgari *et al*. 2014) and contact toxicity of *Artemisia herba-alba, Cinnamomum camphora, C. cyminum,* and *Salvia rosmarinus* EOs against nymphs and adult of the prickly pear cochineal (*Dactylopius opuntiae* Cockerell) in laboratory and greenhouse (Naboulsi *et al*. 2023). These EOs also exhibit repellent and antifeedant properties and can also disturb various physiological processes in insect pests (Lashgari *et al*. 2014; El-Sayed and Yousef 2021; Guesmi *et al*. 2024). Biochemical investigations demonstrated that EOs and their constituents affect the digestive system of insects by altering enzyme activities (Talepour *et al*. 2021). Digestive enzymes play a crucial role in providing nutrients through food digestion and absorption, as well as supplying energy for metabolic activities (Zibaee 2012). These enzymes facilitate energy production via nutrient intake and, in the absence of food, through the breakdown of stored macromolecules with the aid of intermediary enzymes (Shahriari *et al*. 2019, 2020). Zibaee & Bandani (2010), Nasr *et al*. (2017), Shahriari *et al*. (2020), and Oftadeh *et al*. (2020) demonstrated negative effects of plant EOs and their components on the digestive physiology of insect pests. Oftadeh *et al*. (2020) showed that *Artemisia annua* EO statistically decreased α-amylase activity in the lesser mulberry pyralid (*Glyphodes pyloalis* Walker). Shahriari & Sahebzadeh (2017) also demonstrated the lower digestive enzymes activities in *E. kuehniella* after fed on treated diets by diallyl disulfide®. Disrupting the digestive system using plant compounds represents an effective strategy for pest management (Aghaee Pour *et al*. 2022).

Despite growing interest in the insecticidal activities of EOs, their mode of action remains incompletely understood. A comprehensive understanding of the mechanisms by which these compounds affect insect biology and physiology is essential for their safe and efficient application in the pest control. Therefore, this study aims to evaluate the potential effects of *C. cyminum* and *A. graveolens* EOs on the larvicidal activity, digestive physiology, and content of macromolecules of *E. kuehniella*.

Materials and methods

Insect Rearing

Larvae of *E. kuehniella* were provided from the infected wheat flours in Zabol (Iran). The larvae were reared on a semi-artificial diet containing wheat flour (43 g), yeast (6 g), and glycerin (20 mL) (Lima *et al*. 2001). Insects were maintained in transparent plastic containers (17 × 9 × 9 cm3) at 26 ± 2 °C, 65 ± 5% RH and a 16L: 8D photoperiod (Shahriari *et al*. 2020).

EOs Preparation

Seeds of *C. cyminum* and *A. graveolens* (collected from medicinal plant farms at the Sistan Agricultural and Natural Resources Research Center, Zabol, Iran) were ground into a powder, and 50 g of powdered samples were subjected to hydrodistillation for 4 h using a Clevenger-type apparatus according to the method described by Shahriari *et al*. (2017b).

Larvicidal assay

The bioassay tests were performed under identical rearing conditions. According to primary experiments concentrations with mortality effects ranging from 10-90 obtained. Five hundred µL of each concentration of EOs individually was added to 500 mg of semi-artificial diet and allowed to dry for 30 min under laboratory conditions. Fourth-instar larvae were placed on treated and control diets (experiment in 3 replicates and each replication including 10 insects). Control larvae were fed on semi-artificial diet containing alone acetone. After 24 h, mortality of insects was recorded. To estimate the LT_{50} for both EOs, the concentration of 20 µL/mL (as highest concentration) was used by the above procedure. The insect mortality was recorded at 3, 6, 12, 24, and 48 h post-treatments.

Biochemical assays

C. cyminum and *A. graveolens* EOs were separately dissolved in acetone, and 0.5 mL of LC_{30} concentration of each EO was added to 500 mg of semi-artificial diet. For controls, 0.5 mL of acetone was added to 500 mg of diet. Then, 90 larvae per treatment which were starved for eight h prior to exposure, were added to each treatment. Exposure lasted 24, 48, and 72 h, with 30 larvae randomly selected at the end of each time interval and their midguts were dissected out in an ice-cold saline solution (NaCl, 10 mM). The midguts and fat bodies samples were separately homogenized in 1 mL of distilled water by a glass pestle, and then were transferred to 2 mL of microtubes prior to centrifugation at 20,000 rpm for 15 min at 4^0C. The supernatants were stored at -20^0C for biochemical assays (Shahriari & Sahebzadeh 2017).

Enzyme's activity assays

Activity of α-amylase was assessed by 20 µL soluble starch (1%) in 50 µL Tris-HCl buffer (20 mM, pH 7.1) and 20 µL of midgut extraction. The given reaction was kept for 25 min at 30°C. Afterward, 80 µL of DNS was added and the mixture was heated in boiling water for 10 min prior to recording the absorbance at 540 nm (Bernfeld 1955). Activity of lipase was assayed using the procedure of Tsujita *et al*. (1989). Twenty µl of midgut extraction, 100 µL of Tris-HCl buffer (20 mM, pH 7.1), and 27 mM of p-nitrophenyl butyrate were mixed completely and kept at 35 °C for 10 min. Afterward, 100 µL of NaOH (1 M) was added to samples and absorbance was recorded at 405 nm. General proteolytic activity was measured using the method of Elpidina *et al*. (2001). Twenty µL of the midgut solution, 100 µL of Tris-HCl buffer (20 mM, pH 8) with 40 µL of azocasein (2 %) were kept for 1 h at 35 °C. Reaction was stopped by adding TCA (10 %). The reaction mixture was centrifuged at 20000 rpm for 12 min. Afterward, 100 µL of NaOH (2 M) was added to the same volume of the obtained supernatant and the absorbance recorded at 440 nm.

Activities of trypsin, chymotrypsin and elastase proteases were determined using the specific substrates including BApNA (1 mM), SAAPFpNA (1 mM) and SAAApNA (1 mM). The reaction mixture contained 35 µL of Tris-HCl buffer (20 mM, pH 8), 5 µL of each forenamed substrate and 5 µL of midgut solution. The reaction mixture was maintained for 10 min at 30°C. Then, the absorbance was read at 405 nm (Oppert *et al*. 1997). Carboxypeptidase and aminopeptidase activities were assayed using the procedure of Oppert *et al*. (1997). Briefly, 5 µL of hippuryl-L-arginine or hippuryl-L-phenilalanine were separately added into 35 µL of Tris-HCl buffer (20 mM, pH 8) and the incubation was initiated by adding 5 µL of midgut extraction for 10 min at 30 °C. Finally, the absorbance was recorded at 340 nm.

Determination of macromolecules content

Protein concentrations were measured according to the procedure described by Lowry *et al*. (1951). In the assay, 10 µL of solution was added into 50 µL of Folin-Ciocalteu reagent, and incubation was made for 30 min prior to recording the absorbance at 545 nm (diagnistic kit, Ziest Chem. Co., Tehran-Iran).

Triglyceride content was measured according to the procedure described by Fossati & Prencipe (1982). Reagent solution contained 4-chlorophenol (4 mM), 4-aminoantipyrine (0.5 mM), glycerol-3-phosphate-oxidase (0.5 kU/l), adnosine triphosphate Mg2+ (15 mM), (2 mM), glycerokinase 0.4 kU/l), lipoprotein lipase (2 kU/l), phosphate buffer (50 mM, pH 7.2) and peroxidase (2 kU/l). Ten µL of samples were added to 70 µL of reagent and kept for 15 min at 30 °C. The absorbance was recorded at 546 nm.

For glycogen measurement, the fat bodies of twenty larvae were cut and incorporated into 1 mL of 30% KOH and Na2SO4 solution. The samples were covered with foil and boiled for 30 min. Then, the samples were shaken and cooled in ice for 10 *A. graveolens*. No significant difference was observed in lipase activity between treated and control larvae at 24 h, but lipase activity was increased by adding *A. graveolens* EO to semi-min. Two mL of EtOH (95 %) were added to the precipitated glycogen from the digested solution. The samples were then shaken again and kept on ice for 30 min. The samples were centrifuged at 13,000 rpm for 20 min. Afterward the supernatant was deleted and pellets (glycogen) were re-dissolved in 1 mL of distilled water before being re-shaken and phenol (5 %) was added to the mixture. Incubation was done in the ice bath for 30 min. The samples were recorded at 490 nm (Chun &Yin 1998).

Statistical Analysis

Data were analyzed using ANOVA and Tukey’s test ($p < 0.05$) using the Minitab (Version 19) software. LCs and LT$_{50}$ values were estimated using Polo Plus software.

Results

Larvicidal assay

Sublethal and lethal concentrations (LC) and lethal time (LT) of *C. cyminum* and *A. graveolens* EOs on *E. kuehniella* are presented in Tables 1 and 2, respectively. Larvae exposed to *A. graveolens* EO exhibited LC$_{10}$, LC$_{30}$, and LC$_{50}$ values of 1.23, 3.19, and 6.15 µL/mL, respectively, while those exposed to *C. cyminum* revealed the LC values of 1.81, 5.86, and 13.26 µL/mL, respectively (Table 1). The LT$_{50}$ values (concentration of 20 µL/mL) were 8.39 h for A. graveolens and 15.28 h for *C. cyminum* (Table 2).

Biochemical assays

Incorporation of *C. cyminum* and *A. graveolens* EOs at LC$_{30}$ into the semi-artificial diet significantly altered digestive enzyme activities compared to the control (Figure 1). α-amylase activity was lower in larvae treated with both EOs than in control at all the time intervals. At 24 h post treatment, the α-amylase activity was significantly lower in the larvae fed on treated diets with *C. cyminum* but the activity at 48 and 72 h was lower in larvae fed on treated diet with

artificial diet, compared to the control at 48 and 72 h intervals. General protease activity was statistically lower in all the time intervals in the larvae fed on treated diets with *C. cyminum* and *A.*

graveolens EOs, compared to the control. However, *C. cyminum* EO revealed more inhibitory activity than *A. graveolens* EO on general protease activity at all time intervals (Figure 1).

Table 1. Oral toxicity of *Cuminum cyminum* and *Anethum graveolens* essential oils against *Ephestia kuehniella* larvae.

Essential oil	**LC$_{10}$ (95% Cl) (µL/mL)**	**LC$_{30}$ (95% Cl) (µL/mL)**	**LC$_{50}$ (95% Cl) (µL/mL)**	**X^2 (df)**	**Slop ± SE**
Cuminum cyminum	1.81 (0.42 - 2.34)	5.86 (3.32 - 8.18)	13.26 (9.52 - 22.74)	2.274 (3)	1.480 ± 0.349
Anethum graveolens	1.23 (0.39 - 2.14)	3.19 (1.71 - 4.49)	6.15 (4.35 - 8.07)	2.347 (3)	1.837 ± 0.347

X2 (Chi Squared), Cl (confidence limits).

Table 2. Lethal time of *Cuminum cyminum* and *Anethum graveolens* essential oils at concentrations of 20 µL/mL against *Ephestia kuehniella* larvae.

Essential oil	**LT$_{50}$ (95% Cl) (h)**	**X^2 (df)**	**Slop ± SE**
Cuminum cyminum	15.28 (12.27 - 18.97)	3.603 (4)	2.526 ± 0.299
Anethum graveolens	8.39 (6.60 - 10.44)	1.337 (4)	2.564 ± 0.328

The activity of trypsin-like proteinase was statistically reduced in the larvae exposed to *C. cyminum* and *A. graveolens* EOs at all time intervals, in case of *C. cyminum* EO was more obvious (Figure 2). Chymotrypsin activity was considerably lower at all the time intervals in the larvae exposed to *C. cyminum* EO and at 72 h in the larvae exposed to *A. graveolens* EO. However, chymotrypsin activity increased in larvae exposed to *A. graveolens* at 24 h post-treatment. No significant difference was observed in chymotrypsin activity between larvae exposed to *A. graveolens* EO and control at 48 h (Figure 2). The activity of elastase-like proteinase was statistically reduced in the larvae fed on the diet containing EOs at all time intervals. However, *A. graveolens* EO demonstrated more inhibitory activity than *C. cyminum* EO on elastase activity at all time intervals (Figure 2). The larvae fed on diets treated with EOs demonstrated lower activities of aminopeptidases in comparison with control at different time intervals, in case of *A. graveolens* EO was more obvious (Figure 3). The larvae fed on diets treated with both EOs demonstrated lower activities of carboxypeptidases in comparison with control at all time intervals (Figure 3).

Finally, the contents of energy reserves (protein, glycogen and triacylglyceride) in the larvae fed on diets treated by *C. cyminum* and *A. graveolens* EOs were lower than in the control larvae at all time intervals (Figure 4). Total protein amount was significantly lower at all the time intervals in the larvae exposed to *A. graveolens* EO. Similar results were observed in case of glycogen contents. The amount of glycogen was significantly lower at 24 and 72 h time intervals in the larvae exposed to *A. graveolens* EO. The larvae fed on diets treated with EOs demonstrated lower amount of triacylglyceride in comparison with control at different time intervals, in case of *A. graveolens* EO was more obvious at 48 h post-treatment (Figure 4).

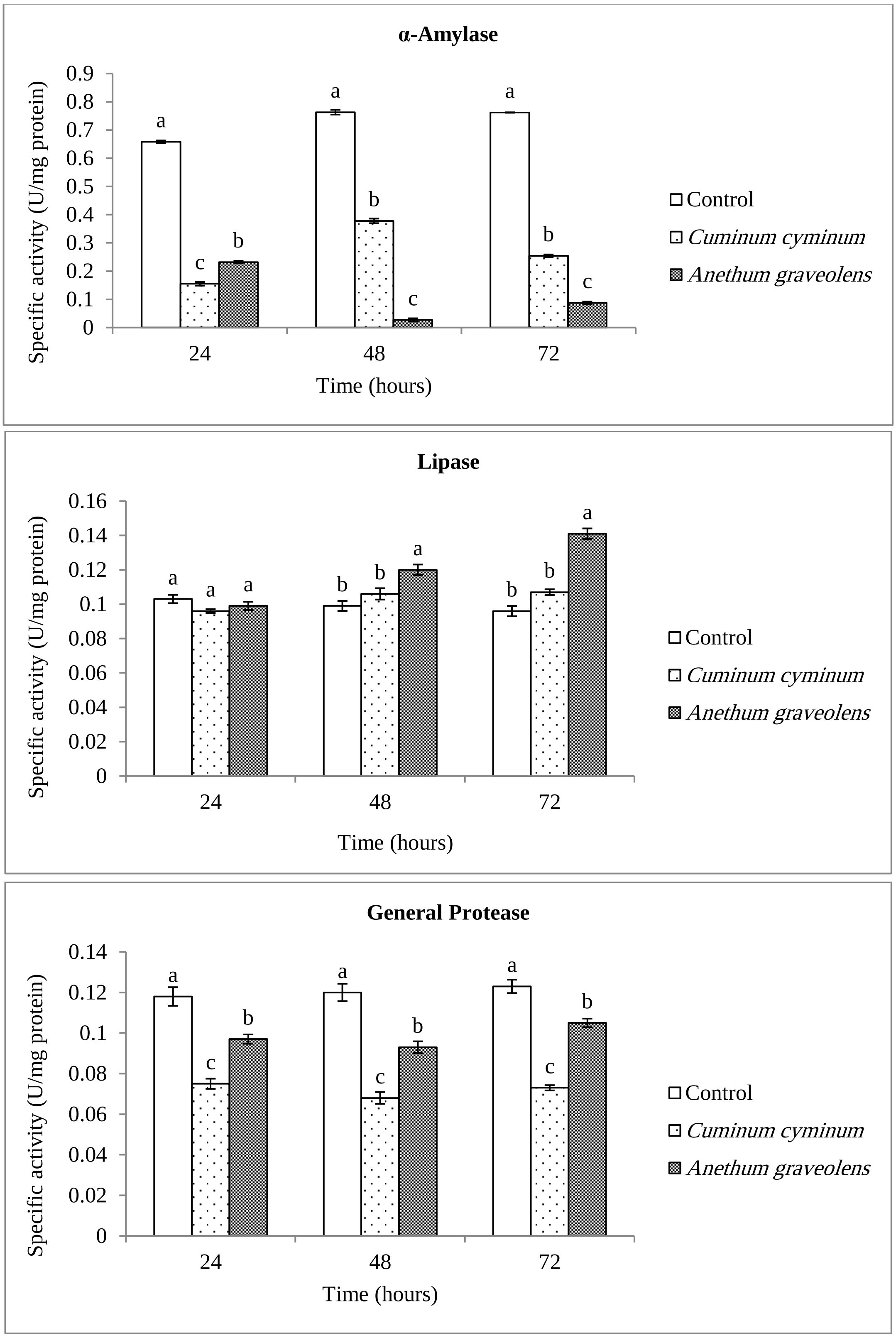

Figure 1. Activity of digestive enzymes in fourth instar larvae of *Ephestia kuehniella* at 24, 48 and 72 h after feeding on treated diets by LC_{30} value of *Cuminum cyminum* and *Anethum graveolens* essential oils. Statistical analysis has been done within each time intervals and significant differences were marked by different letters (Tukey test, $p < 0.05$).

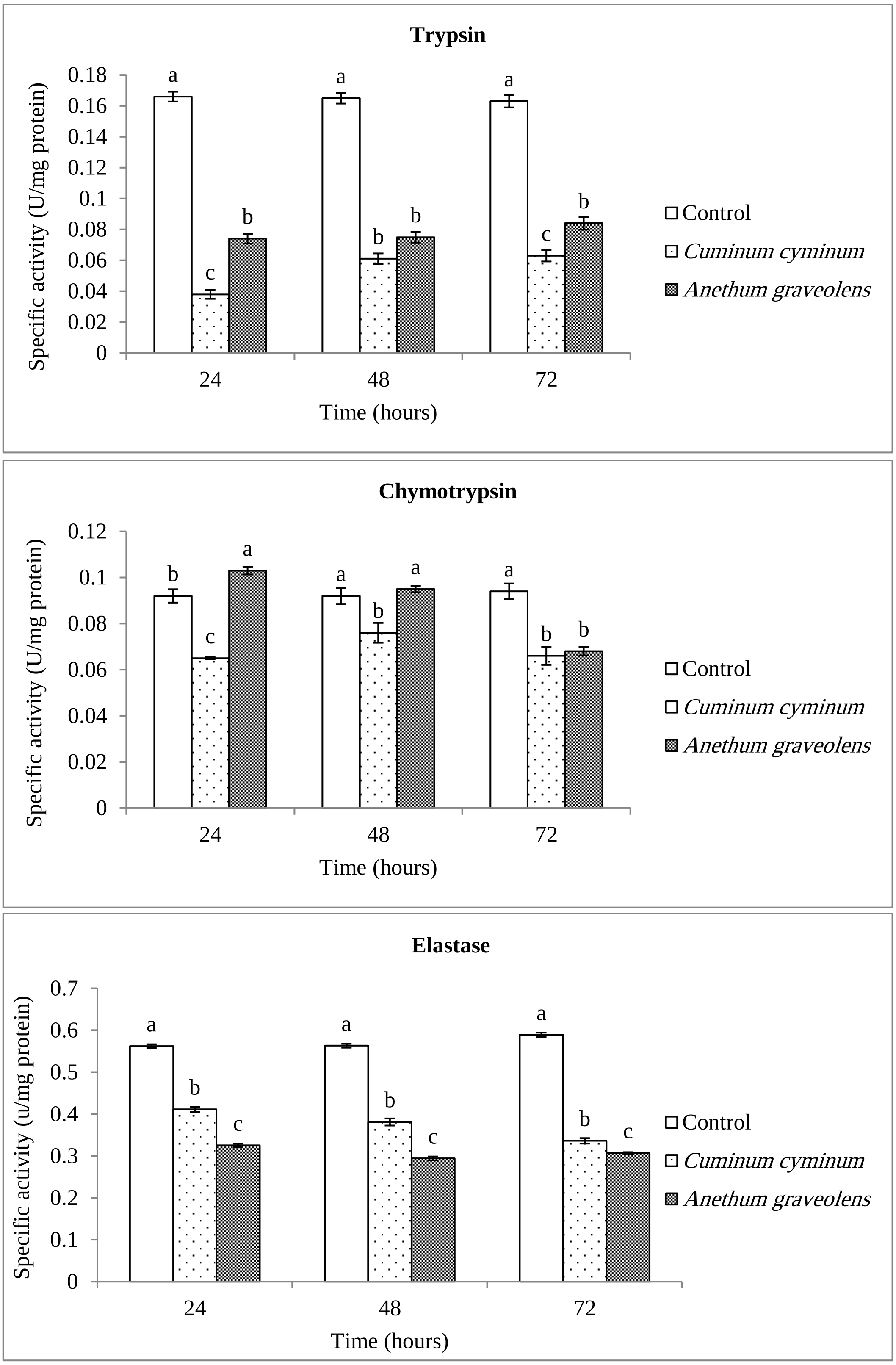

Figure 2. Activity of endoproteases in fourth instar larvae of *Ephestia kuehniella* at 24, 48 and 72 h after feeding on treated diets by LC$_{30}$ value of *Cuminum cyminum* and *Anethum graveolens* essential oils. Statistical analysis has been done within each time intervals and significant differences were marked by different letters (Tukey test, $p < 0.05$).

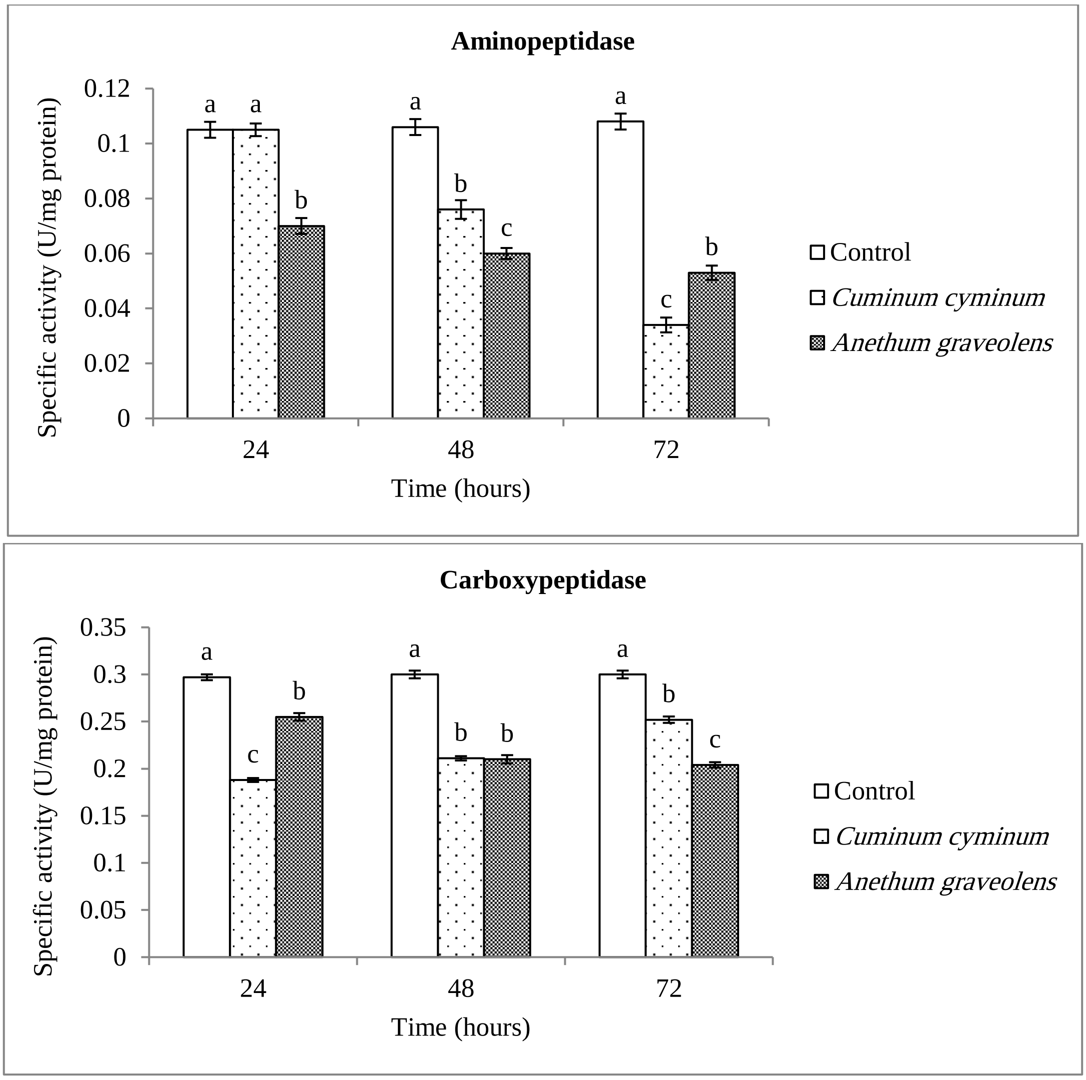

Figure 3. Activity of exopeptidases in fourth instar larvae of *Ephestia kuehniella* at 24, 48 and 72 h after feeding on treated diets by LC_{30} value of *Cuminum cyminum* and *Anethum graveolens* essential oils. Statistical analysis has been done within each time intervals and significant differences were marked by different letters (Tukey test, $p < 0.05$).

Discussion

Plant EOs and their active derivatives offer promising alternatives to synthetic chemicals for controlling pests in stored products, owing to their rapid degradation, low residual effects, and multiple modes of action (Isman 2020). Plant EOs possess multiple pest-control properties including toxicity, repellency, feeding deterrence (Isman 2020). This activity has been attributed to neurotoxic activity, and there is evidence that some oils interfere with the neuromodulator octopamine while others interfere with GABA-gated chloride channels (Priestley *et al*. 2003). Such mechanisms of action make EOs neurotoxic poisons with a rapid contact-based effect on the insects (Pavela 2014). In the present study, the strong larvicidal activities of *A. graveolens* and *C. cyminum* EOs against *E. kuehniella* were observed. Both EOs demonstrated a positive correlation between increasing time and concentration with higher insect mortality. The susceptibility of other insect pests to these EOs have been reported in other studies. For example, *A. graveolens* EO was reported to kill 76.1% larvae and 74.3% adult of *T. confusum* at 24 h, and the LD_{50} of its was determined 1.23 and 2.14 µl/cm2 for larvae and adults, respectively (Guesmi *et al*. 2024). Lashgari *et al*. (2014) demonstrated that the mortality rate of *T. castaneum* and *S. oryzae* adults was increased with increase in *C. cyminum* EO concentration.

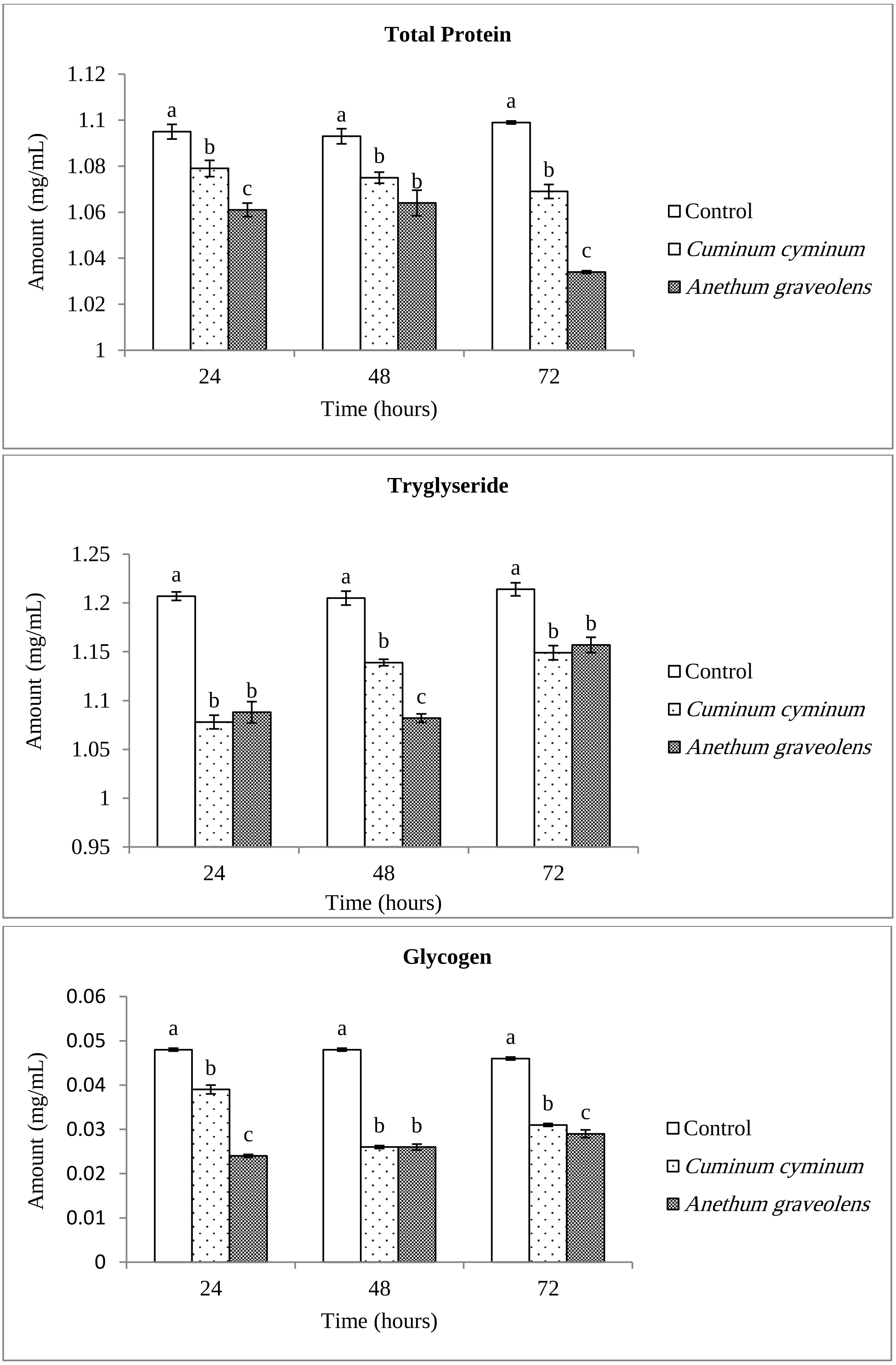

Figure 4. Amounts of storage macromolecules in fourth instar larvae of *Ephestia kuehniella* at 24, 48 and 72 h after feeding on treated diets by LC_{30} value of *Cuminum cyminum* and *Anethum graveolens* essential oils. Statistical analysis has been done within each time intervals and significant differences were marked by different letters (Tukey test, $p < 0.05$).

The highest insect mortality rate in the case of *M. piperita* and *C. cyminum* on *T. castaneum* and *S. oryzae* was 64, 68, 82 and 78%, respectively. Our results indicated that *A. graveolens* EO is more effective than *C. cyminum* EO against *E. kuehniella*, achieving higher mortality at lower concentrations and shorter times. The efficacy of EOs is due especially to some monoterpenes contained therein in various combinations. Insecticidal potency has been known to depend on the composition and mutual ratios of main components (Basij *et al*. 2023). Similar to our results, carvone (as main constituents of *A. graveolens* EO, 18 μg/larvae) showed the higher insecticidal activity than cuminaldehyde (as main constituents of *C. cyminum* EO, 32 μg/larvae) against the cotton leafworm (*Spodoptera littoralis* Boisd., Lepidoptera: Noctuidae) (Pavela 2014). Therefore, it may be concluded that the higher toxicity of *A. graveolens* EO compared to *C. cyminum* EO attributed to the higher toxicity of its components on pests.

Digestive enzymes are vital for nutrient utilization and energy supply in insects (Zibaee 2012). α-Amylase is a hydrolytic enzyme responsible for catalyzing many types of carbohydrates like starch and glycogen (Zibaee 2012). Results of the present study showed statistically lower activity of α-amylase in *E. kuehniella* larvae exposed to *C. cyminum* and *A. graveolens* EOs than in the control. This finding is similar to earlier reports on the insects treated with other plant secondary metabolites. Oral exposure of *E. kuehniella* larvae to *Allium sativum* L., *Teucrium polium* L., and *Eucalyptus globulus* Labill EOs decreased α-amylase activity compared to the control (Shahriari *et al*. 2017a, 2020). Decreased α-amylase activity of *E. kuehniella* larvae is most likely due to cytotoxic effects of *C. cyminum* and *A. graveolens* EOs on epithelial cells of the midgut of larvae or direct inhibitory properties on the enzyme. Lipase plays an important role in the digestion of lipids as well as detoxification of xenobiotic compounds (Zibaee & Bandani 2010; Zibaee 2012). Here, fed of *E. kuehniella* larvae on the treated diet containing *A. graveolens* EO significantly increased activity of lipase. These results may demonstrate its involvement in detoxification and metabolism of EO because some research demonstrates lipases are the third class of esterases (Terra & Ferreira 2005). Similarly, Shahriari & Sahebzadeh (2017) reported that diallyl disulfide increased lipase activity in *E. kuehniella* larvae. Proteases have critical roles in the digestive physiology of insects by converting proteins into amino acids. Different types of proteases can be produced in the midgut of insects. Trypsin, chymotrypsin, and elastase are the main subclasses of serine proteases in lepidopteran larvae (Zibaee 2012). Carboxypeptidases and aminopeptidases hydrolyze single amino acids either from the C-terminus or from the N-terminus at the end of a polypeptide chain, respectively (Zibaee 2012). In our study, exposure of *E. kuehniella* to *C. cyminum* and *A. graveolens* EOs in the laboratory reduced general and specific proteolytic activities, which is similar to other research (Shahriari & Sahebzadeh 2017; Shahriari *et al*. 2017a; Aghaee Pour *et al*. 2022). Shahriari *et al*. (2017a) reported that plant EOs may affect the construction of some kinds of proteases and inhibit the digestion and ingestion of proteins. Mojarab-Mahboubkar & Jalali-Sendi (2016) also demonstrated low general protease and specific proteolytic (trypsin, chymotrypsin, elastase and exopeptidases) activities in the cotton bollworm (*Helicoverpa armigera*) larvae fed on the diets containing LC_{30} (16.12%) and LC_{50} (19.32%) concentrations of *Artemisia annua* extract in comparison with the control. After exposure to botanical insecticides, the function of digestive enzymes of insects becomes imbalanced; this can lead to a decrease in normal utilization and digestive processes (Zibaee & Bandani 2010; Nasr *et al*. 2017; Shahriari *et al*. 2020; Oftadeh *et al*. 2020). Shahriari & Sahebzadeh (2017) reported that binding plant toxins to membrane proteins resulting death of the midgut epithelial cells in insects that are responsible for secretion of digestive enzymes. Hence, any decrease in the number of midgut epithelial cells leads to lower activities of digestive enzymes (Aghaee Pour *et al*. 2022). Therefore, inhibition of digestive enzyme activities can be either due to destroying epithelial cells or through inhibition of the enzymes by plant EOs.

Key macromolecules in insect fat bodies-glycogen, triacylglycerides and proteins- support metabolism and energy production (Shahriari & Sahebzadeh 2017; Oftadeh *et al*. 2021). Glycogen is converted to trehalose and released into hemolymph when the amounts of sugars reduce to low crucial levels (Aghaee Pour *et al*. 2022). Triacylglycerols are the types of lipids that play important roles in synthesizing phospholipids and provide fatty acids for intermediary metabolism of insects (Shahriari & Sahebzadeh 2017). Proteins have major roles in the compensatory mechanisms of insects during stress (Bouayad *et al*. 2012). In our study, *C. cyminum* and *A. graveolens* EOs caused decreased contents of storage macromolecules in *E. kuehniella* larvae. Our findings indicated the larval feeding on EOs disrupted nutritional functions and decreased the amounts of storage macromolecules. Shahriari *et al*. (2017a, 2020) reported that the content of protein, glycogen, and triacylglyceride of *E. kuehniella* decreased in the insects after fed on treated diets by *E. globulus*, *T. polium*, and *A. sativum* EOs. Mojarab-Mahboubkar & Jalali-Sendi (2016) showed that methanolic extract of *A. annua* decreased amounts of protein, glycogen, and triacylglyceride of *H. armigera*. In addition, Nasr *et al*. (2017) reported that larvae of the diamondback moth (*Plutella xylostella* L.) treated by *Origanum vulgaris* EO had the lower amount of protein in the fat body which was attributed to the catalysis of proteins into amino acids and their importation into the Krebs cycle as keto acids.

In conclusion, our results displayed the significant toxicity of *A. graveolens* and C. cyminum EOs against the larvae of *E. kuehniella*. Also, these EOs induced alteration in the digestive physiology and storage of macromolecules in *E. kuehniella*, which may help to indicate the potential interference properties of EOs on the larvae. Overall, *A. graveolens* and *C. cyminum* EOs can be promising insecticides in stored products and integrated pest management (IPM) programs for *E. kuehniella* management.

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پژوهش‌های کاربردی در گیاه‌پزشکی

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مقاله پژوهشی

فعالیت لاروکشی اسانس‌های زیره سبز (*Cuminum cyminum*) و شوید (*Anethum graveolens*) علیه بید آرد *Ephestia kuehniella*: سنجش‌های سمیت و بیوشیمیایی

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چکیده

ترکیبات گیاهی به دلیل پتانسیل‌شان در کنترل آفات، به طور فزاینده‌ای مورد بررسی قرار می‌گیرند. برخی ترکیبات گیاهی، از قبیل اسانس‌ها، بر هضم و فرآیندهای متابولیکی حشرات گیاهخوار تأثیر می‌گذارند. در پژوهش حاضر اثرات اسانس‌های گیاهی بر مرگ و میر، فیزیولوژی گوارشی و ماکرومولکول‌های ذخیره‌ای *Ephestia kuehniella* Zeller بررسی شد. سمیت گوارشی اسانس‌های زیره سبز (*Cuminum cyminum*) و شوید (*Anethum graveolens*) علیه لاروهای سن چهارم بید آرد ارزیابی شد. مقادیر LC_{50} و LT_{50} به ترتیب ۱۳/۲۶ میکرولیتر بر میلی‌لیتر و ۱۵/۲۸ ساعت برای زیره سبز و ۶/۱۵ میکرولیتر بر میلی‌لیتر و ۸/۳۹ ساعت برای شوید محاسبه شد. در پژوهش حاضر، اسانس *A. graveolens* در غلظت‌های پایین‌تر و زمان‌های کوتاه‌تر باعث مرگ و میر بیشتری در لاروها شد. فعالیت آنزیم‌های گوارشی (به جز لیپاز) در حشرات تغذیه شده با رژیم غذایی آغشته شده به هر دو اسانس، به طور قابل توجهی کاهش یافت. علاوه بر این، مقادیر پروتئین، تری آسیل گلیسرول و گلیکوژن (به عنوان ذخایر انرژی حشرات در معرض قرار گرفته) به طور قابل توجهی کمتر بود، که نشان دهنده کاهش این ذخایر به دلیل هزینه‌های انرژی تحمیل شده توسط این دو اسانس است. نتایج نشان می‌دهد که اسانس *A. graveolens* نسبت به *C. cyminum* روی لاروهای *E. kuehniella* مؤثرتر است و فرآیندهای حیاتی گوارش را مختل می‌کند، که می‌تواند به خواص حشره‌کشی آنها کمک کند. با این حال، مطالعات بیشتری برای گسترش تکنیک‌های کپسوله کردن ترکیبات مشتق‌شده از گیاهان برای افزایش پایداری و اثربخشی مورد نیاز است.

کلمات کلیدی: آفت‌کش زیستی، زیره، شوید، هضم، *Ephestia kuehniell*