Effects of Five Plant Essential Oils on the Protein Content and Digestive Enzymes of *Ephestia kuehniella*

Mohammad Asadi, Department of Plant Protection, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran (***Iran***)
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ABSTRACT

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The Mediterranean flour moth or mill moth *Ephestia kuehniella* is a common pest of cereal grains, especially flour. In this research, the sublethal effects of essential oils isolated from five medicinal plants including *Allium sativum*, *Glycyrrhiza glabra*, *Rosmarinus officinalis*, *Salvia officinalis*, and *Piper nigrum* were investigated on the protein content and digestive enzymes activity of the fifth instar larvae, under laboratory conditions. The GC-MS analysis of the essential oils showed that tetracosamethyl cyclododeca siloxan, aristolene, α-pinene, β-thujone, and caryophyllene were the dominant constituents for each essential oil, respectively. The enzymatic activity was investigated by the treatment using the LC_{30} concentration against the larvae that were 2.86, 12.03, 2.19, 7.84, and 9.39 µl/l air, respectively. Results revealed that there were significant differences among the treatments on total protein content and enzymatic activities ($F_{5,12}$ = 2.95, 3.56, and 7.07). About the total protein content, the control and treatment with *R. officinalis* essential oil showed the highest and lowest concentrations (1.0288 ± 0.0212 mg/ml versus 0.7333 ± 0.0329 mg/ml). The highest amylolytic activity was also seen in the control (0.0551 ± 0.0025 mU/mg) and the lowest being in *R. officinalis* oil treatment (0.0373 ± 0.0009 mU/mg). Moreover, the highest and lowest proteolytic activities were observed in the control (5.5063 ± 0.1086 U/mg) and *R. officinalis* essential oil (3.3028 ± 0.1077 U/mg). Accordingly, *R. officinalis* essential oil could be applied for the control of *E. kuehniella*.

Keywords: Activity, amylase, enzyme, *Ephestia kuehniella*, protease, protein content

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Protecting of agricultural products from the pests' attacks during their storage is a guarantee for food security and healthy foods for humans. Inability to control pests causes loss of food resources and economic dependence of countries. Regardless of economic aspects, the arrival of pests can be a serious threat to food and health security. For this reason, it seems necessary to create suitable infrastructure for storing primary food outside the growing season and managing pests using new methods. Due to construction method and lack of proper urban infrastructure, food storages are exposed to the invasion of various pests (Jacob and Cox 1976).

The Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), is one of the important pests of stored products especially flour in most parts of the world (Ahmadpour 2021; Asadi et al. 2018a). This pest has global distribution and its adult has a short life

Corresponding author: Mohammad Asadi
Email: assadi20@gmail.com

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span about one week. Females lay an average of 250 eggs in small batches (up to 20) on various food sources. The emerged larvae are reddish pink in color, but feeding type and host has great effect on their color (Jacob and Cox 1976). The number of generations varies according to living environment. In relatively warm storages, this moth continues to feed and reproduce without interruption in all seasons. In these conditions, it can develop six or more generations during a year. In opposite, under cold environmental conditions (temperature less than 10°C), activity of larvae is stopped (Sarmadi et al. 2010).

The effective role of food in growth, reproduction, immune parameters, and the activity of digestive enzymes has been proven. If insects benefit from good food with higher nutritional value, they have stronger immune systems and can better deal with external factors. In addition, sufficient nutrition during the larval period leads to production of insects with appropriate reproductive capacity (Ajamhassani and Amiri Jami 2018). In insects' midgut, the main digestive enzymes included amylases, glycosidases, lipases, and proteases, which are similar in hydrolytic nature. These enzymes play a significant role in digestion and absorption of food. Life of various insects depends on their existence (Terra 1990). Amylases are important enzymes for carbohydrate metabolism in insects (Horie and Watanabe 1980). Many insects, especially larval stages, feed on rich sources of polysaccharides and extensively use these enzymes to break down polysaccharide and starch chains (Valencia et al. 2008). α-amylases are enzymes that are abundant in nature and their main role is to catalyze the joining of α-D-(1,4)-glucan chains in starch and the other carbohydrates (Franco et al. 2000; Stroble et al. 1998). Food sources of insects have a great effect on the activity of their digestive amylases. The activity of this enzyme is very low in wool-eating insects, but it is very abundant in oligophagous and polyphagous insects (Slansky and Scriber 1981). Proteases are also considered as the most important enzymes in insects and their role is to supply necessary amino acids and energy from food sources for growth and development. In many insects, especially in the order Lepidoptera, serine proteases are the most important group (Telang et al. 2005).

Essential oils are a group of plant volatile metabolites, some of which have fumigant, contact, repellent, and anti-feeding properties against pests (Asadi et al. 2019, 2021). Due to high insecticidal activity, they are often recommended to control insect pests. On the other hand, the activity of digestive enzymes in insects, especially herbivorous insects, is influenced by metabolites present in various plants, which are known as plant secondary metabolites (Parsia Aref and Valizadegan 2015). Proper nutrition and getting adequate energy from food resources is ultimately related to egg production and the other biological activities. Therefore, it is necessary to investigate the changes of these enzymes in pest management. One of the obvious effects of plant essential oils is their anti-nutritional activity, which is very intense in some plant species. This characteristic of essential oils is very effective in reducing damage of pests due to low or no production of digestive enzymes (Parsia Aref and Valizadegan 2015; Razmjou et al. 2018). Due to importance of digestive enzymes and nutrition as important features in pests, the present research was performed on the sublethal effects of essential oils isolated from five medicinal plants on the protein content with amylolytic and proteolytic activity of *E.*

kuehniella. The main aim of this research was to determining effective plant compounds on this important pest for applying in pest management designs.

MATERIALS AND METHODS

Rearing of *Ephestia kuehniella*.

The flour moth *E. kuehniella* eggs were obtained from a private insectarium (registered name: Jalilian) from Eslamabad-e Gharb city, Kermanshah province, Iran, during 2018. Then, 0.25 g of the eggs were distributed on 750 g of wheat flour with 250 g of wheat bran, under the laboratory conditions including 23 ± 2°C, 50 ± 5% relative humidity (RH), and 24 h darkness (Abedi et al. 2012, 2014; Mahdavi and Saber 2013).

Extraction of essential oils.

The selected medicinal plants including garlic (*Allium sativum*), rosemary (*Rosmarinus officinalis*), black pepper (*Piper nigrum*), sage (*Salvia officinalis*), and liquorice (*Glycyrrhiza glabra*) that was available in the Iranian flora and contained suitable amount of essential oil, were collected from different regions of Eslamabad-e Gharb city (34.11° N, 46.53° E) in Kermanshah Province, Iran, during May 2017. The aerial parts (leaves and seeds) of the plants were powdered. Then, 50 g of their powder was mixed with 500 ml of distilled water into a 1-liter balloon of the Clevenger apparatus (Asadi et al. 2018a). After 4 h, the essential oil appeared as a pale green layer on the water. To remove excess water and purify the essential oil, sodium sulfate (Na_2SO_4) was used (Asadi et al. 2018b). Finally, the essential oils were stored in special containers covered with the aluminum foil, inside the refrigerator at 5°C, (Asadi et al. 2019; Negahban et al. 2007; Parsia Aref and Valizadegan 2015). To determine the quantitative and qualitative components of the essential oils, it was used the Agilent technologies 7890B (Manufactured by the USA) gas chromatography with HP-5MS column, length 30 m, diameter 0.25 mm, and film thickness 0.25 mm. Temperature program was 350°C. Determining ranges was also done using a data bank of mass, Kovats index, and retention time. Each essential oil components were detected based on patterns of ranges refraction compared to standard by using of reputable sources (Adams et al. 2007).

Bioassay.

To investigate the fumigant toxicity of essential oils on fifth instar larvae of *E. kuehniella*, different concentrations which lead to mortality rate between 20% and 80% were placed on filter paper (2 × 2 cm) using a microapplicator, in 70 ml glass Petri dishes as fumigation chamber. The distilled water was used in the control treatment (Parsia Aref and Valizadegan 2015). Then, 15 larvae were placed inside each Petri dish that were immediately sealed with parafilm tape to prevent evaporation of essential oils. Each essential oil concentration was assayed in 4 replicates. After 24 h of exposure, the number of dead larvae was recorded. Accordingly, insects are considered dead when no movements have been observed when stung with a needle (Shahriari and Sahebzadeh 2016).

Preparation of enzymatic mixture.

Fifth instar larvae of *E. kuehniella* were exposed for 24 h to the LC_{30} values (as sublethal concentration based on bioassay experiments analysis) of the five essential oils (2.86, 12.03, 2.19, 7.84 and 9.39 µl/L air). Then, the alive treated larvae were carefully dissected in distilled water using a stereomicroscope. The digestive tubes of the larvae were carefully separated and placed inside 5 ml microtubules containing NaCl 1.5%. Then, all parts of digestive tube were homogenized with a manual homogenizer on ice and the homogenized mixture was centrifuged at 15,000 rpm for 15 min (4°C). Finally, its supernatant was stored at -15°C for enzymatic analyses (Bidar et al. 2016). For each treatment, ten digestive tubes were isolated.

Protein assay.

Protein concentration is one of the most important components in the formula for determining the specific activity of digestive enzymes (U), which is measured based on the method of Bradford (1976). Based on the studies, absorption rate at 595 nm wavelength indicates protein concentration in each sample. In this study, 600 µl of Bradford's reagent was added to 20 µl of enzymatic mixture inside 5 ml microtubes and three repetitions were considered for each treatment and the control. Finally, the protein concentration was determined using the standard equation and comparing of absorbance in the samples (Bidar et al. 2016).

α-Amylase activity.

To determine the α-amylase activity, each experimental unit containing 20 µl of enzyme mixture, 50 µl of 1% starch substrate, and 250 µl of phosphate buffer was mixed and placed at room temperature for 30 min. Then, 50 µl of 3,5-dinitrosalicylic acid was added as an inhibitor of enzyme activity and then placed in a water bath at a temperature of 90°C (10 min). Finally, after 5 min of centrifugation at 16,000 rpm (4°C), absorbance of the samples was measured at 540 nm wavelength using a spectrophotometer. In the studies, a blank sample was used for all treatments. All assays were performed in triplicate (Bernfeld 1955).

General protease assay.

To study this enzyme in the midgut of *E. kuehniella* larvae, 1.5% azocasein was used as main substrate (Elpidina et al. 2001). Accordingly, 50 µl of the enzyme mixture and 80 µl of its substrate were placed in an appropriate volume of phosphate buffer at room temperature for 60 min. Then, 100 µl of 30% trichloroacetic acid compound was added as enzyme activity inhibitor. Then, the undigested azocasein was removed and the mixture was centrifuged at 15,000 rpm for 15 min (4°C). Finally, 100 µl of 2 M NaOH compound was added to 100 µl of the prepared mixture and its absorbance was measured at 405 nm wavelength by using of spectrophotometer. Each treatment and the control were measured in three replicates (Bidar et al. 2016).

Statistical analysis.

In this research, three repetitions were considered for each essential oil treatment with the control, then the data were examined for normality. Finally, the data were analyzed using one-way ANOVA and their means compared with Tukey's test ($p < 0.05$) using SPSS software, version 22.

RESULTS

Essential oils compounds.

The results of GC-MS analysis from the five essential oils revealed eleven main compounds in *A. sativum*, forty-four compounds in *G. glabra* and *S. officinalis*, and forty-three compounds in *S. officinalis* and *P. nigrum*. These results were previously published in the article by Asadi et al. (2021), but their five dominant combinations are again presented in Table 1.

Table 1. Five dominant constituents in each essential oil of different medicinal plants (Asadi et al. 2021)

Medicinal plant	Family	Compound	Retention Time (Min)	Percentage of Total
		Tetracosamethyl cyclododeca siloxan	38.274	15.824
		Anhydro 5-hydroxy-3-piperonyl-1	38.137	13.344
Allium sativum	Alliaceea	1-Amino-1-prtho-chlorophenyl-2	38.016	12.872
		1, 4-Cyclohexadiene, 1, 3, 6-tris	37.92	10.573
		4-Methoxy-3-(3-methoxyphenyl)	37.802	10.201
		Aristolene	25.58	20.135
		2, 6-Octadien-1-ol, 3, 7-dimethyl	24.704	8.544
Glycyrrhiza glabra	Fabaceae	Butanoic acid, 3, 7-dimethyl-2	23.588	7.337
		Lavandulyl acetate	18.293	6.257
		3-Hexen-1-ol, benzoate	23.889	4.373
		α-Pinene	5.448	9.985
		Bicyclo [2.2.1] heptan-2-one	9.847	7.775
Rosmarinus officinalis	Lamiaceae	Bicyclo [3.1.1] hept-3-en-2-one	11.768	7.242
		1, 8-Cineole 2-oxabicyclo	7.256	6.111
		Bicyclo [2.2.1] heptan-2-ol	14.503	5.71
		β-Thujone	8.903	25.626
		Bicyclo [2.2.1] heptan-2-one	9.865	16.463
Salvia officinalis	Lamiaceae	1, 8-Cineole 2-oxabicyclo	7.248	10.556
		Thujone	9.109	6.538
		Veridiflorol	24.33	4.705
		Caryophyllene	19.512	36.031
		L-Limonene cyclohexene	7.191	7.02
Piper nigrum	Piperaceae	3-Carene	6.836	5.167
		Cyclohexene, 1-methyl-4-(5-Methyl)	22.082	5.125
		2-β-Pinene bicyclo	6.222	4.343

Bioassay.

The obtained values of LC_{30}, LC_{50}, and LC_{90}, probit line, slope slope ± error with $\chi 2$ value for five plant essential oils on *E. kuehniella* larvae are shown in Table 2. Results from bioassays revealed that the acute toxicity of *R. officinalis* essential oils on the larvae was more than others. Also, *G. glabra* essential oil showed the lowest acute toxicity in this research.

Table 2. Acute toxicity of the essential oils on the last instar larvae of *Ephestia kuehniella* (Insect number: 360)

Medicinal plant	Slope ± E	LC_{30} µl/l air (95% CL)	LC_{50} µl/l air (95% CL)	LC_{90} µl/l air (95% CL)	χ^2
Allium sativum	1.03 ± 0.21	2.86 (0.91 – 4.88)	9.14 (5.58 – 12.69)	156.39 (74.65 – 815.48)	5.47 ns
Glycyrrhiza glabra	0.83 ±0.13	12.03 (4.76 – 20.63)	51.18 (32.42 – 77.49)	1761.33 (715.73 – 9656.30)	7.63 ns
Rosmarinus officinalis	1.71 ± 0.28	2.19 (0.95 – 3.41)	4.48 (2.65 – 5.98)	24.92 (18.70 – 40.69)	7.67 ns
Salvia officinalis	1.10 ± 0.21	7.84 (3.06 – 12.42)	23.41 (15.76 – 31.77)	339.23 (164.31 - 1652.50)	4.62 ns
Piper nigrum	0.91 ± 0.15	9.39 (3.57 - 15.73)	35.32 (29.97 - 51.58)	905.44 (379.59 - 5356.93)	4.14 ns

CL: Confident limit, LC: Lethal Concentration, E: Error, $\chi 2$: Chi-Square value, ns: non-significant.

Protein concentration in the digestive tube.

The results of determining protein concentration in the digestive tube of *E. kuehniella* larvae are shown in Table 3. The highest protein concentration in the control group was 1.0288 ± 0.0212 mg/ml and the lowest being in *R. officinalis* treatment as 0.7333 ± 0.0329 mg/ml. Also, there was no significant difference among the essential oils of *A. sativum*, *G. glabra*, *S. officinalis*, and *P. nigrum* ($F_{5,12} = 2.95$). In this case, the decreasing trend for all treatments was as follows: the control, *P. nigrum*, *S. officinalis*, *G. glabra*, *A. sativum*, and *R. officinalis*.

Table 3. Protein content in the digestive tube of *Ephestia kuehniella* under essential oils treatment

Treatment	Protein (mg/ml)
Control	1.0288 ± 0.0212 a
Allium sativum	0.7695 ± 0.0.0663 ab
Glycyrrhiza glabra	0.8153 ± 0.0397 ab
Rosmarinus officinalis	0.7333 ± 0.0329 b
Salvia officinalis	0.8424 ± 0.0688 ab
Piper nigrum	0.8446 ± 0.0955 ab

Different letters following the values indicate significant differences (Tukey's test, $p < 0.05$).

Amylolytic activity assay.

The results of amylolytic activity in the digestive tube of *E. kuehniella* larvae are shown in Table 4. Accordingly, the highest activity was observed in the control (0.0551 ± 0.0025 mU/mg) and the lowest being in *R. officinalis* essential oil (0.0373 ± 0.0009 mU/mg). Also, there was no significant difference between the control and *S. officinalis* essential oil, as well as among the essential oil of *A. sativum*, *G. glabra*, and *P. nigrum* ($F_{5, 12}$= 3.56). Besides, the decreasing trend in all treatments was as follows: the control, S. officinalis, G. glabra, A. sativum, P. nigrum, and R. officinalis.

Table 4. Amylolytic activity in digestive tube of *Ephestia kuehniella* under treatment of essential oils

Treatment	Protein (mg/ml)
Control	0.0551 ± 0.0025 a
Allium sativum	0.0471 ± 0.0047 ab
Glycyrrhiza glabra	0.0491 ± 0.0032 ab
Rosmarinus officinalis	0.0373 ± 0.0009 b
Salvia officinalis	0.0547 ± 0.0027 a
Piper nigrum	0.0435 ± 0.0056 ab

Different letters following the values indicate significant differences (Tukey's test, p <0.05).

Proteolytic activity in the alimentary canal.

The results of proteolytic activity in the digestive tube of *E. kuehniella* larvae are shown in Table 5. Based on this, the highest and lowest activity of this enzyme was determined in the control and *R. officinalis* essential oil (5.5063 ± 0.1086 to 3.3028 ± 0.1077 U/mg). Also, the differences among the control, *G. glabra*, and *S. officinalis*, as well as the difference between *A. sativum* and *P. nigrum* were not significant ($F_{5,12}$ = 7.07). The decreasing trend of all treatments was as follows: the control, *S. officinalis, G. glabra, A. sativum, P. nigrum,* and *R. officinalis.*

Table 5. Proteolytic activity in the digestive tube of *Ephestia kuehniella* under essential oils treatments

Treatment	**Protein (mg/ml)**
Control	5.5063 ± 0.1086 a
Allium sativum	4.5347 ± 0.3238 ab
Glycyrrhiza glabra	5.4353 ± 0.2540 a
Rosmarinus officinalis	3.3028 ± 0.1077 b
Salvia officinalis	5.6223 ± 0.4775 a
Piper nigrum	4.4516 ± 0.5100 ab

Different letters following the values indicate significant differences (Tukey's test, $p < 0.05$).

DISCUSSION

The secondary compounds of plants known as essential oils are the most popular group. They have different effects with important role especially in defense mechanisms of plants against the herbivores (Parsia Aref and Valizadegan 2015; Rafiee-Dastjerdi et al. 2013; Razmjou et al. 2018). These compounds are present in most plant families, especially in the Asteraceae, Lamiaceae, Lauraceae, and Myrtaceae, in which the variety and amount of these volatile compounds are greater. For this reason, these families are called aromatic plants (Bakkali et al. 2008). In the present study, *R. officinalis* and *S. officinalis* are from the family Lamiaceae and the three other plants, *A. sativum*, *G. glabra*, *P. nigrum*, belong to the families Alliaceae, Fabaceae, and Piperaceae, respectively. Based on essential oil analysis, number of secondary compounds in *S. officinalis* and *P. nigrum* was more than the other investigated plants. The essential oils that have more effective compounds made greater insecticidal effects (Asadi et al. 2018b). In this study, this was well observed in the case of *R. officinalis*. In addition, essential oils with less insecticidal compounds showed insignificant effects on this pest. This was observed in the case of essential oils isolated from *S. officinalis* and *G. glabra* (Asadi et al. 2018a, 2018b, 2019, 2021).

Regarding the direct effects of plant essential oils on the digestive enzymes of *E. kuehniella*, Teodoro Martinez et al. (2012) studied the insecticidal effect of labramin which is a lectin-like protein isolated from the seeds of the *Labramia bojeri*, on *E. kuehniella* when reported that it caused 90% mortality on larvae under 1% (w/w) treatment. The presence of 0.25% labramin in the diet caused high larval period, less pupal development, and low percentage of emerged adults. The treatments in increased levels of trypsin indicated that labramin could affect enzymatic mechanisms by disrupting the peritrophic membranes in the midgut of *E. kuehniella* larvae. Results of nutrition experiments with *E. kuehniella* larvae revealed a decrease in conversion efficiency of ingested and digested food with an increase in approximate digestibility and metabolic cost (Teodoro Martinez et al. 2012). In the present study, purification and investigation of dominant constituents from the essential oils on the digestive properties of larvae were not investigated, but the main effect of essential oils due their presence is argued. Further work on the purification of the dominant constituents from five medicinal plants and the investigation of their effects on different pest will be planned.

Shahriari and Sahebzadeh (2016) investigated the effects of diallyl disulfide on physiological characteristics of *E. kuehniella* and found that the concentration (0.31%) was determined as LC_{50}. Also, activity of digestive enzymes including α-amylase and proteases significantly decreased in the treated larvae. Their results confirmed that diallyl disulfide interrupted larvae digestion with less digestive enzymes activity which showed a significant effect on its metabolism. Our results are in agreement with this research because with application of various treatments, significant changes have been made on various enzymes, which indicate effect of these factors on the digestion process. In another study, Shahriari et al. (2022) studied the response of *E. kuehniella* larvae to toxicity of trans-anethole and found that its LC_{50} value on the larvae was determined as 7.03 $\mu l/g^{-1}$. Also, a significant decrease in activity of digestive enzymes (α-amylase, α and β-glucosidase, and lipase) and proteases (trypsin, chymotrypsin, elastase, amino, and carboxypeptidase) was observed.

Zallaghi et al. (2020) studied the combined effect of *Lavandula angustifolia* essential oils with γ irradiation treatment on some biological aspects of *E. kuehniella* when concluded that there was a significant increase in conversion effect of food consumed and feed inhibition index due to irradiation and essential oil application. The experiment showed that insecticidal effect of essential oils can be increased by primary irradiation. Ajamhassani (2021) studied the effects of ethanolic extracts from *Ferula assa-foetida* and *Aloysia citriodora* on mortality and physiological characteristics of *E. kuehniella* larvae and concluded that *F. assa-foetida* extract significantly reduced the activity of lipase and β-glucosidase enzymes compared to the control, but the effect of *A. citriodora* extract on activity of these enzymes was insignificant than the control. The extracts had also no significant effects on α-glucosidase enzyme activity. The results of the present study are in agreement with the above work because changes in the physiological characteristics of the pest have been observed with the application of treatments. However, chemical compounds of medicinal plants were different.

Studies related to the direct effects of plant essential oils or their isolated compounds on the digestive enzymes of *E. kuehniella* are very limited. Therefore, this study provided some additional data in this field. The Mediterranean flour moth is one of the most important storage pests. For this, use of plant essential oils is a good solution due to their low effects on foods.

RESUME

Asadi M. 2023. Effets de cinq huiles essentielles végétales sur les modifications de la teneur en protéines et des enzymes digestives chez les larves d'*Ephestia kuehniella*. Tunisian Journal of Plant Protection 18 (1): 29-39.

La teigne méditerranéenne de la farine ou teigne du moulin *Ephestia kuehniella* est un ravageur commun des grains de céréales, en particulier de la farine. Dans cette recherche, les effets sub-létaux des huiles essentielles isolées de cinq plantes médicinales, *Allium sativum*, *Glycyrrhiza glabra*, *Rosmarinus officinalis*, *Salvia officinalis* et *Piper nigrum* ont été étudiés concernant la teneur en protéines et l'activité des enzymes digestives chez les larves de ce ravageur, sous des conditions de laboratoire. L'analyse GC-MS des huiles essentielles a montré que le tétracosaméthyl cyclododéca siloxane, l'aristolène, l'α-pinène, la β-thuyone et le caryophyllène étaient respectivement les constituants dominants de chaque huile essentielle. L'activité enzymatique a été étudiée par le traitement utilisant la concentration LC30 contre des larves de troisième stade qui étaient respectivement de 2,86, 12,03, 2,19, 7,84 et 9,39 µl/l d'air. Les résultats ont révélé qu'il y avait des différences significatives entre les traitements concernant la teneur en protéines totales et les activités enzymatiques ($F_{5,12}$ = 2,95, 3,56 et 7,07). Concernant la teneur en protéines totales, le témoin et le traitement avec l'huile essentielle de *R. officinalis* ont été associés aux concentrations les plus élevées et les plus faibles (1,0288 ± 0,0212 mg/ml contre 0,7333 ± 0,0329 mg/ml). L'activité amylolytique la plus élevée a été observée avec le témoin (0,0551 ± 0,0025 mU/mg) et la plus faible avec le traitement à l'huile de *R. officinalis* (0,0373 ± 0,0009 mU/mg). De plus, les activités protéolytiques les plus élevées et les plus faibles ont été observées avec le témoin (5,5063 ± 0,1086 U/mg) et l'huile essentielle de *R. officinalis* (3,3028 ± 0,1077 U/mg). En conséquence, l'huile essentielle de *R. officinalis* pourrait être appliquée pour lutter contre *E. kuehniella*.

Mots clés: Activité, amylase, enzyme, *Ephestia kuehniella*, protéase, teneur en protéines

ملخص

أسدي، محمد. 2023. تأثير خمسة زيوت نباتية أساسية على التغيرات في محتوى البروتين والإنزيمات الهاضمة لدى حشرة *Ephestia kuehniella*.

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تعتبر عثة الطحين المتوسطية أو عثة المطحنة *Ephestia kuehniella* من الآفات الشائعة على بذور الحبوب، وخاصة على الدقيق. في هذا البحث، تم التعرف على التأثيرات شبه المميتة للزيوت الأساسية المستخلصة من خمس نباتات طبية هي *Allium sativum* و *Glycyrrhiza glabra* و *Rosmarinus officinalis* و *Salvia officinal*is و *Piper nigrum* حيث تم تقييم محتوى البروتين ونشاط الإنزيمات الهاضمة لدى هذه الآفة تحت الظروف المختبرية. أظهر تحليل GC-MS للزيوت الأساسية أن tetracosamethyl cyclododeca siloxane و aristolene و α-pinene و β-thujone و caryophyllene كانت على التوالي المكونات المهيمنة لكل زيت أساسي. تمت دراسة فعالية الإنزيمات باستخدام تركيز LC_{30} ضد يرقات الطور الثالث والتي كانت 2.86 و 12.03 و 2.19 و 7.84 و 9.39 ميكرولتر/لتر من الهواء، على التوالي. أوضحت النتائج وجود فروق معنوية بين المعاملات فيما يتعلق بمحتوى البروتين الكلي وأنشطة الإنزيمات ($F_{5,12}$ = 2.95 و 3.56 و 7.07). فيما يتعلق بمحتوى البروتين الكلي، كان الشاهد والمعاملة بالزيت الأساسي لنبتة *R. officinalis* مرتبطان بأعلى وأدنى تركيزات (1.0288 ± 0.0212 مغ/مل مقابل 0.7333 ± 0.0329 مغ/مل). ولوحظ أعلى نشاط للمحلل النشوي مع الشاهد (0.0551 ± 0.0025 ميليوحدة/مغ) والأدنى مع زيت النبتة *R. officinalis* (0.0373 ± 0.0009 ميليوحدة/مغ). زيادة على ذلك، لوحظ أعلى وأدنى نشاط للمحلل البروتيني مع الشاهد (5.5063 ± 0.1086 وحدة/مغ) والزيت الأساسي لنبتة *R. officinalis* (3.3028 ± 0.1077 وحدة/مغ). بالتالي، يمكن استخدام الزيت الأساسي لنبتة *R. officinalis* لمكافحة البكتيريا *E. kuehniella*.

كلمات مفتاحية: الإنزيم، المحتوى البروتيني، المحلل البروتيني، المحلل النشوي، النشاط، عثة الطحين المتوسطية، *Ephestia kuehniella*

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