TOXICITY AND BIOCHEMICAL EFFECTS OF CUMIN AND BASIL ESSENTIAL OILS ON *TRIBOLIUM CASTANEUM*

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The essential oils (EOs) of cumin (*Cuminum cyminum* L.) seeds and basil (*Ocimum basilicum* L.) herb were extracted by hydrodistillation and tested against the red flour beetle, *Tribolium castaneum* (Herbst) for insecticidal and biochemical effects on certain enzymes of this insect. Major components of *C. cyminum* EO determined by gass chromatography-mass spectrometry (GC/MS) analysis were γ-terpinene (15.78 %) and benzenemethanol (11.32 %), while those of *O. basilicum* EO were linalool (56.7 %) and epi-α-cadinol (11.4 %). The lethal concentration values for 50% mortality after three days of *T. castaneum* whole body exposure were 678 mg kg^{-1} for cumin oil and 755 mg kg^{-1} for basil oil. The enzymatic activity of treated insects showed a reduction in total protein, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase compared to untreated ones. However, α-amylase activity increased with both tested EOs. Hence, for *T. castaneum* control, these EOs may represent alternatives to conventional insecticides.

Cuminum cyminum, enzymatic activity, *Ocimum basilicum*, stored grain insects

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INTRODUCTION

The red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is a major pest of stored products all over the world, especially in the tropics, causing post-harvest damage of stored food and products. It has been classified as the most significant insect among the stored product insects causing considerable losses to a wide range of stored food products such as cereals, legumes, spices, oilseeds, nuts, flour, milled rice, baked products, etc. (Abou-Taleb et al., 2016).

As reported by Mason, McDonough (2012) and Daglish, Nayak (2018), post-harvest losses due to stored-product insect infestations account for 10 % and 20 % of the total cereal grain and legume production in developed and developing countries, respectively

Today, the stored product pests have been responsible for fundamental food storage grains quality and safety loss. Conventional insecticides, such as organophosphorus and pyrethroids, have still been used in stored product pests control worldwide (Manivannan et al., 2016; Bomzan et al., 2018). The increasing problems of resistance, pesticides residue, and contamination of the biosphere associated with the overuse of broad-spectrum pesticides have led to the search for alternative effective biodegradable pesticides with better selectivity (Mossa, 2016).

All of these problems pushed the researchers to seek a new way to face this danger using natural products, which can offer protection to stored products against pests and are both safe to humans and environmentally friendly. Plant products have long been used as efficient pesticides, even before the onset of the synthetics. They are recognized as safer alternatives for pest management since they have been present in nature for millions of years without any ill effect and have a broad spectrum of biological activity (Dwivedy et al., 2015).

Botanical pesticides based on essential oils (EOs) are promising contenders for commercial stored food protection and integrated pest management programs. Most of them have many advantages such as low mammalian toxicity, work in low concentrations and act at sub-lethal doses. Also, they may work as insect growth regulators inhibiting growth and metabolism without killing. EOs are the principal natural aromatic components rich in terpenoids (monoterpenes and sesquiterpenes), alkaloids, and phenylpropanoids. The bioactivity of EOs is directly related to their chemical composition, which can vary dramatically even within the same species. The variability depends on the plant part, extraction procedure, phenological stage of the plant, season as well as environmental growth conditions (Angioni et al., 2006). These oils have shown various activities against stored product insects such as fumigant, antifeedant, growth inhibitory, repellent, and contact toxic (Isman, 2000; Rajendran, Sriranjini, 2008). They may repel insects, alter their feeding habits, deter oviposition and adult emergence, in addition to effete growth development and moulting.

Basil (*Ocimum basilicum*, Lamiaceae) is a wild plant that grows as a perennial shrub, native to areas in Asia, Africa, Central and South America (Darrah, 1980). Basil contains varying quantities of cinnamate, citronellol, geraniol, linalool, methyl chavicol, myrcene, pinene, ocimene, and terpineol (Bozin et al., 2007). EOs of *Ocimum sanctum* have also exhibited antimicrobial, antifungal, antiaflatoxigenic activity, antioxidant, larvicidal, and repellent activities against mosquito, termites and pulse beetles (Bhatnagar et al., 1993; Kumar et al., 2010; Pandey et al., 2014). Cumin (*Cuminum cyminum*) is used as a food additive with white or pink flowers and small green seeds, it is an aromatic annual plant that grows in Iran, Egypt, Saudi Arabia and some other parts of the world (Boskabady et al., 2006).

However, relatively little attention has been focused on EOs action mode against the stored product pests in vivo. Effects on the biochemical and physiological parameters of insects might reflect the toxicity of pesticides. Carbohydrates, proteins and lipids are significant components of the insect body and play an essential role in the body construction and energy metabolism. Transaminase enzymes, aspartate aminotransferase, and alanine aminotransferase are critical in carbohydrate and protein metabolism and their production is altered under stress conditions like the insects exposure to both natural and synthetic insecticides (Etebari et al., 2007). Numerous compounds of EOs can exhibit insecticidal activity through the effects on octopamine synapses, GABA or by inhibiting acetylcholinesterase (Pavela, Benelli, 2016).

The study objective was to investigate the insecticidal activity of cumin and basil EOs against *T. castameum* and their impact on some biochemical enzymes enabling us with deeper insight into the EOs insecticidal action mode.

MATERIAL AND METHODS

Test insect

The red flour beetles *Tribolium castaneum* were obtained from stock colonies maintained in the laboratory of Stored Product Pests of the Sakha Agricultural Research Station, Agriculture Research Center (ARC), Egypt. Insects were reared on a mixture of cracked wheat grain mixed with wheat flour. Cracked grain was cleaned, sterilized, and put in glass jars containing 400 g of 30% wheat flour each and provided with 100–200 adult insects. The jars were covered and kept at 28 ± 2° C and 65 ± 5 % relative humidity, photoperiod was 16 h light : 8 h darkness. The adult insects (unsexed; 4–7 days old) sieved out of the stock colony were used in the experiments the next day.

Test material

Cumin (*Cuminum cyminum*; seeds) and basil (*Ocimum basilicum*; herb) EOs are widely available in Egypt. The EOs used in the experiment were provided by Hashem Brothers Company for Essential Oils and Aromatic Products in Giza, Egypt as a gift.

Chemicals

All chemical reagents for the analyses (phosphate buffer, chemical reagents for protein, α-amylase, aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT)) were obtained from Egyptian Co. for Biotechnology – Spectrum Diagnostics Egypt, Al Obour City Industrial Area Cairo.

Chemical analysis of the essential oils and identification of their components

The EOs chemical composition was analyzed by gas chromatography-mass spectrometry (GC/MS) system HP5890 (Agilent Technologies, USA) with an HP column (60 m × 0.25 mm, 0.25 μm film thickness) (HP-5 ms). The initial temperature was 60 °C and the maximum temperature was 250 °C for 65.3 min. The injector temperature was 240 °C. Relative percentage amounts were calculated from peaks' total area by the apparatus software. The compounds were identified by matching the mass spectra data with those held in the computer library (Wiley 275.L), according to Swigar, Silverstein (1981) and Adams (1995). The analysis was completely performed in the laboratory of Hashem Brothers Company, Egypt.

Toxicity assessment of the essential oils tested against *Tribolium castaneum* adults

Batches of cracked wheat grains weighing 20 g were introduced into 250 ml jars. The oil concentrations were prepared by diluting 1 ml of crude oil in 100 ml acetone to obtain stock solution. Then, five concentrations of cumin oil (600, 700, 800, 900, and 1 000 mg kg^{-1}) and basil oil (500, 750, 1 000, 1 250, and 1 500 mg kg^{-1}) were prepared from it. These concentrations were subsequently mixed with cracked wheat as 1 ml solutions for each replicate. The treated cracked wheat was left drying for 20 min. Each jar including ten unsexed adults of *T. castaneum* was covered with muslin cloth and kept under the same laboratory conditions as for rearing the insects. Cracked wheat treated with acetone only served as control. Each treatment was replicated three times. Accumulative mortality counts were recorded one, two and three days after treatment, corrected by Abbott's formula (Abbott, 1925). Lethal concentration for 50% mortality (LC_{50}) was determined by log-probit analysis, and the data were analyzed by determining chi-square values and degrees of freedom.

Biochemical analysis

The lethal concentrations, equivalent to the LC_{50} values of tested EOs, were used to examine the effects of the tested compounds on some biochemical responses of *T. castaneum*.

One hundred adult insects were exposed to a derived lethal concentration (LC_{50}) of the tested EO for three days at room temperature (27 ± 2 °C). After treatment, the live insect was removed and homogenized in a phosphate buffer (pH 7.4, 100 mmol l^{-1} in a glass Teflon homogenizer). Three replicates of each treatment were used throughout the biochemical experimentation. Subsequently, the homogenate was centrifuged at 10 000 g for 10 min at 4 °C, and the supernatant was stored in ice for further enzyme activity assay.

Determination of AST and ALT enzyme activity

The AST and ALT activities in insects were determined colorimetrically following the method of Reitman, Frankel (1957): 0.5 ml of the enzyme buffer substrate was incubated for 5 min at 37 °C, then 0.1 ml of the supernatant was added, and incubated at 37 °C for 50 min. Next, 0.5 ml of colour reagent (2,4-dinitrophenylhydrazine) was mixed and left for 20 min at room temperature, then 5 ml of 0.4 N NaOH solution was added and mixed well. The absorbance at 505 nm wavelength was read against distilled water, and then the number of IU per 1 ml of sample was calculated using a standard curve.

Determination of ALP enzyme activity

The ALP enzyme activity was determined spectrophotometrically in the supernatant at 505 nm against blank following the method of Belfield, Goldberg (1971). Totally 0.025 ml of supernatant was mixed with 0.5 ml of buffer substrate (phenyl phosphatase) of pH 10 and incubated for 20 min at 37 °C. Next, 0.25 ml of the enzyme inhibitor EDTA 4-aminophenazone was added, mixed well, then 0.25 ml of potassium ferricyanide was added. The mixture was left in the dark, at room temperature, for 5 min. The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was read against reagent blank at 510 nm.

The quantity of alkaline phosphatase was determined according to the equation:

Alkaline phosphatase concentration (IU l^{-1}) = $A_{sample}/A_{standard} \times 75$

Determination of α-amylase activity.

The α-amylase was determined spectrophotometrically in the supernatant at 660 nm against the blank, according to the method of Caraway (Caraway, 1959). Totally 0.5 ml of the buffered substrate was incubated at 37 °C for 3 min (A_{blank}) in a test tube. In another test tube, 0.01 ml of the supernatant was added, mixed well, and incubated at 37 °C for 7.5 min (A_{sample}). After that, 0.5 ml of working reagent and 4 ml of distilled water were added and mixed well. The absorbance of the sample (A_{sample}) and of the blank (A_{blank}) was measured against distilled water at 660 nm.

The quantity of α-amylase was determined according to the equation:

α-Amylas concentration (IU l^{-1}) = $[(A_{blank} - A_{sample})/A_{blank}] \times 1\,480$

Assay of total protein contents.

The insects whole body total protein content was estimated by Gornall's method (Gornall et al., 1949). One millilitre of biuret reagent was mixed well

Table 1. Components of cumin (*Cuminum cyminum*) essential oil analyzed by gas chromatography-mass spectrometry (GC-MS)

Components	Relative composition ratio (%)	Molecular formula
β-Pinene	10.37	$C_{10}H_{16}$
p-Cymene	7.45	$C_{10}H_{14}$
γ-Terpinene	15.78	$C_{10}H_{16}$
Ethanone	1.99	$C_8H_{12}O$
Isopropylidene	10.48	$C_{11}H_{18}$
Benzenemethanol	11.32	$C_{10}H_{14}O$
α-Pinene	0.87	$C_{10}H_{16}$
β-Phellandrene	0.74	$C_{10}H_{16}$

Table 2. Components of basil (*Ocimum basilicum*) essential oil analyzed by gas chromatography-mass spectrometry (GC-MS)

Components	Relative composition ratio (%)	Molecular formula
Linalool	56.7	$C_{10}H_{18}O$
α-Bergamoten	9.2	$C_{15}H_{24}$
Germacrene D	3.3	$C_{15}H_{24}$
γ-Cadinene	5.4	$C_{15}H_{24}$
Viridiflorol	1.7	$C_{15}H_{26}O$
Epi-α-cadinol	11.4	$C_{15}H_{26}O$

with 0.025 ml of insect supernatant, then incubated for 10 min at 37 °C. After that, the absorbance of the sample and standard was measured against the blank reagent at 550 nm. The quantity of total protein was determined as follows:

Total protein (g dl^{-1}) = ($A_{Sample}/A_{Standard}$) × 5

Statistical analysis

The experiments were performed in triplicate, the data are presented as ± standard error of the means (SEM). The results were analyzed by one-way analysis of variance followed by the Least Significant Difference test for mean separation. P-values ≤ 0.05 were considered significant. The data were analyzed using SPSS software, Version 24.0 for MS Windows.

RESULTS

Chemical composition of the tested essential oils

97 components just for cumin EO were identified. The major ones were γ-terpinene (15.78 %), benzenemethanol (11.32 %), isopropylidene (10.48 %), β-pinene (10.37 %), p-cymene (7.45 %), ethanone (1.99 %), α-pinene (0.87 %) and β-phellandrene (0.74 %) (Table 1). Totally 6 components were identified for the basil EO tested: linalool (56.7 %), epi-α-cadinol (11.4 %), α-bergamoten (9.2 %), γ-cadinene (5.4 %), germacrene D (3.3 %) and viridiflorol (1.7 %) (Table 2).

Toxicity of the essential oils tested against *T. castaneum*

In the present study *C. cyminum* and *O. basilicum* EOs exhibited strong insecticidal activity against *T. castaneum* adults with a significant difference between treated concentrations along the test period.

Data in Table 3 showed that *C. cyminum* EO had the lowest LC_{50} values (765, 713 and 678 mg kg^{-1}) after one, two and three days, respectively opposite to 952, 927 and 755 mg kg^{-1} for *O. basilicum* EO.

The contact toxicity of EOs against *T. castaneum* varied according to the kinds of oils, concentrations and exposure periods. Mortality of *T. castaneum* adults increased with increasing exposure time for both tested EOs, while *C. cyminum* seemed to be more effective. The highest concentration of *C. cyminum* (1 000 mg kg^{-1}) recorded 86.6, 93.3 and 100% mortality after one, two and three days post-exposure, respectively. For *O. basilicum*, mortality was 56.6, 56.6 and 76.6%, respectively, at the highest concentration (1 500 mg kg^{-1}) after the same testing period (see Fig. 1).

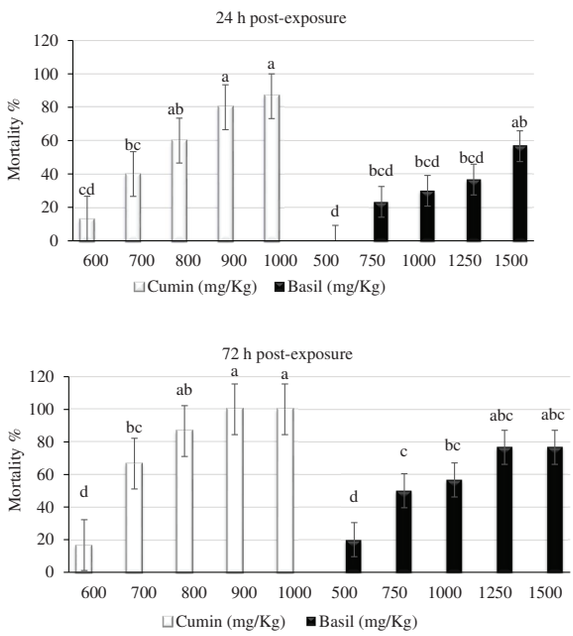
Protein content and enzymatic activities of *T. castaneum*

Concerning biochemical enzymatic activity, there were varied changes in the activity of certain metabolic enzymes of *T. castaneum* (Figs. 2–6) due to treatment with the lethal concentrations of *C. cyminum* and *O. basilicum* after three days. The results showed a reduction in the total protein of *T. castaneum* insect by –30.33 % for cumin oil and by –14.33 % for basil oil if compared to control treatment. This indicates a significant effect of the cumin oil lethal concentrations treatment on *T. castaneum* total protein if compared to the basil treatment and control.

On the other hand, basil oil was more effective than cumin oil over all other enzymes, reduction in ALP was significant compared to control; the changes were –17.99 % for cumin oil and –30.86 % for basil oil compared to control. Also, ALT activity was reduced by –10.99 % for cumin oil and by –20.42 % for basil with no significant difference if compared to control. In addition, AST recorded reduction by –32.25 % for cumin and by –27.5 % for basil oil. On the other

Table 3. Contact toxicity of essential oils from cumin (*Cuminum cyminum*) and basil (*Ocimum basilicum*) against *Tribolium castaneum* adults in contact bioassay 24, 48 and 72 h post exposure

Tested oil	Time (h)	LC_{50} value (mg kg^{-1})	Confidence interval (95 %)		Slope value	Chi-square (X^2)
			lower	upper		
Cumin	24	765	719	806	4.24	1.32
	48	713	665	752	4.43	4.87
	72	678	647	704	7.50	1.72
Basil	24	952	894	1 058	2.89	4.73
	48	927	790	1 006	3.65	6.72
	72	755	685	811	4.03	2.93



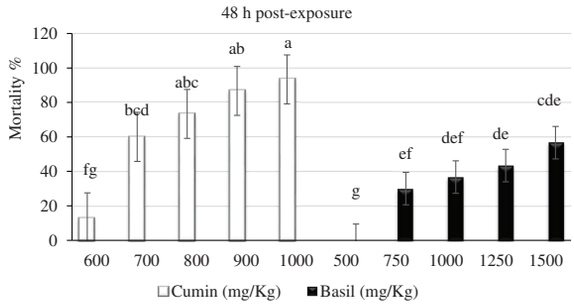


Fig. 1. Toxicity effect of different concentrations of cumin and basil essential oils on *Tribolium castaneum* adults after different exposure times

values are mean ± SEM of 3 independent experiments; means followed by the same letter in the bar diagram are not significantly different according to ANOVA and Duncan's multiple range test

hand, results showed no significant difference in the α-amylase activity for both tested oils (+17.29 % for cumin oil and +13.08 % for basil oil, compared to control).

DISCUSSION

Plant-derived volatile EOs have received much attention in the scientific community in stored grain pest management programs (Chaubey, 2011, 2017). This is due to their fast break-down and readily degradation in sunlight, air, moisture and to detoxification enzymes, hence less persistence and reduced risks to non-target organisms. Therefore, more frequent applications and precise timings are needed. In recent years, the use of botanical insecticides as a replacement for the synthetic ones has gained much importance among scientists.

The toxic effects of plant EOs on insect pests are evidently due to EOs chemical components (Ngassoum et al., 2007; Ko et al., 2009; Wang et al., 2015) including pulegone, linalool, eugenol, thymol, methyl chavicol and others (Park et al., 2006; Thongdon, Inprakhon, 2009). In the present study, the main component in *C. cyminum* was p-cymene (7.45 %),

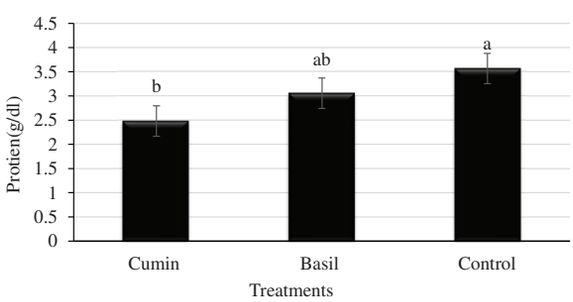


Fig. 2. Protein content in the whole body of *Tribolium castaneum* exposed to LC50 of cumin and basil oils after 24 h of exposure

values are means ± SEM of 3 independent experiments; means followed by the same letter in the bar diagram are not significantly different according to ANOVA and Duncan's multiple range test

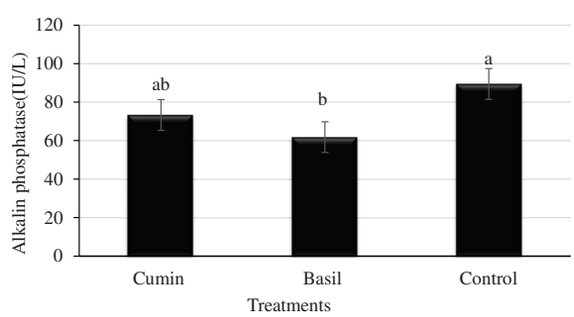


Fig. 3. Alkaline phosphatase activity in *Tribolium castaneum* exposed to LC50 of cumin and basil oils after 24 h of exposure

values are means ± SEM of 3 independent experiments; means followed by the same letter in the bar diagram are not significantly different according to ANOVA and Duncan's multiple range

γ-terpinene (15.78 %), benzenemethanol (11.32 %), isopropylidene (10.48 %) and β-pinene (10.37 %).

The chemical composition of cumin and basil oils determined herein may differ from that reported in other studies due to e.g. plant geographical location, season, environmental conditions, plant nutritional status and other variable factors (P e r r y et al. 1999). In Iran, the major constituents of EO extracted from *C. cyminum* seed were α-pinene (30.12 %), linalool (10.3 %), limonene (10.11 %), 1,8-cineole (11.54 %), γ-terpinene (3.56 %), linalyl acetate (4.76 %) and ethyl acetate (1 %) (E s m a e i l i , 2015).

In our study, the major components of basil (*O. basilicum*) EO were linalool, epi-α-cadinol, α-bergamoten, γ-cadinene and germacrene D, whereas the major components identified in the sweet basil (*O. basilicum*) from Iran included estragole (23.6 %), phthalic acid (20.0 %), geranial (9.43 %), caryophyllene oxide (7.39 %) and linalool (0.18 %) (F a t t a h i et al., 2019).

Our results are in line with those of K e d i a et al. (2014) who reported that natural EOs might show lethal as well as repellent activity at varying doses in integrated pest management programs, as alternative protective substances to conventional pesticides. For *C. cyminum* EO, L e e et al. (2001) reported that its main components – menthol, methonene, limonene, α-pipene, β-pipene and linalool – exhibited toxicity to *Sitophilus oryzae* and inhibited Acetylcholinesterase (AChE) activity.

Also, our results paralleled with those of Z i a e e (2014) reporting 100% mortality of *Tribolium confusum* beetles when exposed to *Carum copticum* and *C. cyminum* EOs for 24 h. Besides, previous studies highlighted that cumin aldehyde can produce larvicidal and adulticidal toxicity together with fumigant effects on several insects such as *Culex pipiens, S. oryzae, Binomial germanica* and *Trichoplusia ni* (Z a h r a n , A b d e l g a l e i l , 2011; Y e o m et al., 2012; K h a n a v i et al., 2017). C h a u b e y (2017) reported that *C. cyminum* EO showed significant repellent, fumigant and contact toxicity effects against *Sitophilus zeamais* in addition to AChE activity inhibition. B e n e l l i et al. (2018) reported that, despite still poor studies on its action mode, the insecticidal activity is presumably related to the aldehyde group presence in the molecule, acting in conjunction with the other two main components of *C. cyminum* EO – γ-terpinen-7-al and α-terpinen-ᵞ-al.

K e d i a et al. (2015) recommended the *C. cyminum* EO application in the stored food products protection

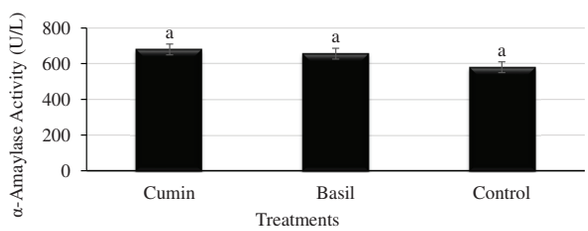


Fig. 4. α-Amylase activity in *Tribolium castaneum* exposed to LC50 of cumin and basil oils after 24 h of exposure

values are means ± SEM of 3 independent experiments; means followed by the same letter in the bar diagram are not significantly different according to ANOVA and Duncan's multiple range test

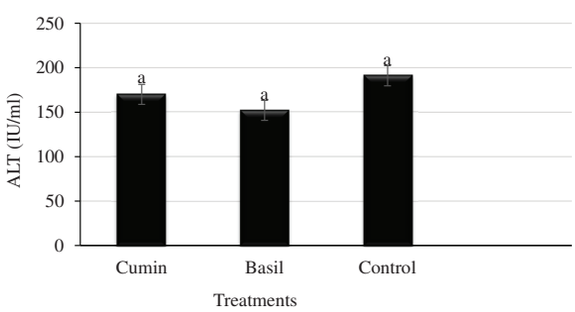


Fig. 5. Alanine aminotransferase (ALT) activity in *Tribolium castaneum* exposed to LC50 of cumin and basil oils after 24 h of exposure

values are means ± SEM of 3 independent experiments; means followed by the same letter in the bar diagram are not significantly different according to ANOVA and Duncan's multiple range test

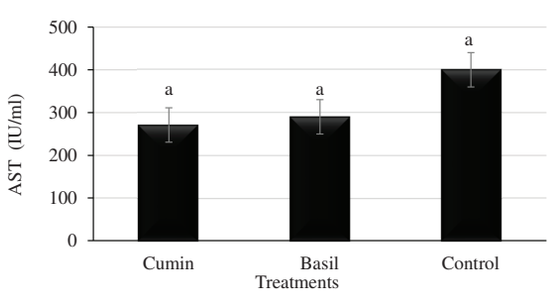


Fig. 6. Aspartate aminotransferase (AST) activity in *Tribolium castaneum* exposed to LC50 of cumin and basil oils after 24 h of exposure

values are means ± SEM of 3 independent experiments; means followed by the same letter in the bar diagram are not significantly different according to ANOVA and Duncan's multiple range test

and considered the natural pesticides based on *C. cyminum* EO a more economical and safer way for pest management programs.

Findings from the present study on *T. castaneum* are in line with other studies demonstrating the toxicity of *O. basilicum* oil and its components against a variety of stored product pests (Jembere et al., 1995; Obeng-Ofori et al., 1999; Popovic et al., 2006; Lopez et al., 2008). Mikhaiel (2011) reported on the *O. basilicum* oil effect in protecting the stored flour against *T. castaneum* insects for up to 45 days. Besides, Kim, Lee (2014) mentioned that a commercial product of *O. basilicum* showed a strong fumigant and contact toxic activity against *S. zeamais* and *T. castaneum* adults after a 24h exposure. *O. basilicum* oil fumigation was highly toxic to adult *S. oryzae*, causing significant mortality overtime (Hossain et al., 2014). The toxicity of *O. basilicum* oil is due to many bioactive components; these chemical components are known to have insecticidal, repellent, nematicidal, fungistatic or antimicrobial properties (Chang et al., 2009).

Lopez et al. (2008) revealed that the components of *O. basilicum* EO, namely linalool, carvone, and methyl eugenol, were toxic to several stored product pests. Stamapoulos et al. (2007) found that linalool exhibited insecticidal activities against *T. confusum*. Studies on the action mode of EOs from citrus peels revealed that they were fast-acting fumigant insecticides, with possible neurotoxic or anti-respiratory properties (Don-Pedro, 1996). During a direct contact, EOs easily penetrate the insect's cuticle and contact the nerve endings in the trachea leading to neurotoxic activity, which may be the reason for the high toxicity of EOs (Bessette et al., 2013; Deletre et al., 2013). The use of botanical EOs has a double advantage due to the binary effects of mechanical action, filling intergranular spaces at high dosages, blocking the insect articulations and chemical action acting primarily on glandular cells (Ramaswamy et al., 1995). Aider et al. (2016) investigated the contact treatments with olive oil on adult *Callosobruchus maculatus*. The formed olive oil film closed the breathing holes or spiracles, thereby depriving the insect of oxygen and causing asphyxia. The advantage of using botanical insecticides is their short persistence in the environment due to rapid degradation (Silva-Aguayo, 2009).

The insects' total body protein reduction after the application of both tested EOs may result from changes in their DNA synthesis, reduced protein synthesis, low assimilation of food and low uptake of amino acids during the protein synthesis triggered by the post-insecticide exposure stress (Deloach et al., 1981; Ribeiro et al., 2001). Also Hussain et al. (2009) or Hamza et al. (2014) reported that Spinosad reduced the total protein contents in *S. oryzae* and *T. castaneum*. Abo El Makarem et al. (2015) reported on a significant total body protein reduction in clove oil-treated granary weevil *Sitophilus granarius* as compared to control weevils.

Acid and alkaline phosphatases were studied as significant enzymes in detoxification. Upadhyay et al. (2011) found that the compound isolated from *Caparis decidua* inhibited the insects' phosphatase enzyme activity and weakened the body defense. Similarly, a total protein reduction was observed in insects treated with two tested EOs, where both glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT), playing an important role in protein metabolism, were inhibited by these EOs (Pant, Morris, 1972).

The increase or reduction of transaminase enzyme concentration in the hemolymph are indicative of a cell damage. Nath (2000) reported that changes in the level of AST and ALT enzymes might be due to the effect on the synthesis of functional levels of these enzymes by altering the cells cytomorphology. In the present study, the transaminase and phosphatase enzyme levels dropped down similarly as described Upadhyay et al. (2011) stating that both sharp decrease or increase in ALP, GPT and GOT enzyme levels affect the insects' oxygen consumption. Similarly, Shakoori et al. (1994) described the GOT and GPT activities inhibition after treating *T. castaneum* with synthetic pyrethroids (sumicidin).

α-Amylase is one of the most important digestive enzymes of many insects. Shekari et al. (2008) reported that natural insecticides showed sublethal effects on insects' physiological parameters. *Artemisia annua* L. extracts decreased the amylase level in *Xanthogaleruca luteola* mull 24 h after treatment, but a significant increase occurred after a 48 h exposure. Mehrabadi et al. (2010) mentioned that when the amylases activity is inhibited, the organism's nutrition is impaired causing shortness in energy as reported by Khosravi et al. (2011). In accord with El-Gizawy et al. (2019), our study confirmed no significant α-amylase activity increase in *T. castaneum* by garlic oil treatment.

CONCLUSION

Natural insecticides are always beneficial to the ecosystem and human health maintenance. Our results emphasized that the use of cumin (*C. cyminum*) and basil (*O. basilicum*) EOs for *T. castaneum* control could be an alternative to usual synthetic insecticides. These oils have an impact on certain metabolic enzymes responsible for the vital process in the insect, which may be an indicator of their mode of action. Being eco-friendly and biodegradable products, botanicals may represent a complementary method to limit chemical control in stored grain pest management. However, further studies of botanical EOs and their components as novel insect control agents are needed, concerning e.g. safety to human and non-target organisms, their

action mode and the effect on the organoleptic properties of stored grain.

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