

## EFFICIENCY OF SOME ESSENTIAL OILS AND INSECTICIDES IN THE CONTROL OF SOME *SITOPHILUS* INSECTS (COLEOPTERA: CURCULIONIDAE)

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### ABSTRACT

Current study aimed to investigate the effects of two essential oils of anise (*Pimpinella anisum*) and clove (*Eugenia aromaticum*), diatomaceous earth (DE), spinetoram (S), and malathion (M) on the physiological and biochemical parameters of granary weevil (*Sitophilus granarius* L.), rice weevil (*S. oryzae* L.), and maize weevil (*S. zeamais* Motsch). Additionally, the contents of protein and lipids and the enzymatic activity of aspartate transaminase (AST), alanine transaminase (ALT), and acetylcholinesterase (AChE) were measured. The median lethal concentration values (LC<sub>50</sub>) showed that essential oils were not efficient in controlling the tested pests compared to malathion, spinetoram, and DE. The chemical treatments including the essential oils, spinetoram, and malathion impacted the enzymes and total protein differently with mixed responses. It was clear that the insecticides were more potent against *S. granarius*, *S. oryzae*, and *S. zeamais*. Diatomaceous earth would be a potential candidate to be incorporated in the control approaches of *Sitophilus* species.

### INTRODUCTION

The grain weevils (Curculionidae) are primary pests of cereal grains in storage including wheat, maize and rice. Infestation of stored grain with insects cause significant reduction in their quantity and quality. There are many insect species that infest grains in granaries especially the weevils of the genus *Sitophilus*, such as granary weevil (*Sitophilus granarius* L.), rice weevil (*Sitophilus oryzae* L.), and the maize weevil (*Sitophilus zeamais* Motsch.) (Germinara *et al.*, 2008; Belda and Riudavets, 2010).

Insects of grains in granaries are controlled mainly by direct application of contact insecticides, known as grain protectants. These compounds include many insecticides of the synthetic organophosphorus (OPs) that might cause environmental pollution along with the presence of their residue in the grain, which pose health concerns to human (White and Leesch, 1995; Arthur, 1996). At the same time, several insect pests have become resistant to most of commonly used OPs, which somehow increases the management cost. Also, the use of synthetic pesticides has resulted in toxic effects to living and non-target organisms. Therefore, incorporation of natural and biological control agents would reduce possible side effects. Semi biological insecticides such as spinetoram, which is a natural mixture of spinosyn J and spinosyn L. Spinetoram is a member of the second generation of the spinosads, which are metabolites of the bacterium *Saccharopolyspora spinosa* (Bacteria: Actinobacteridae). Spinetoram has the same mode of action as spinosad. It acts on the insect nervous system at a unique site on the nicotinic acetylcholine receptor, and is active through contact or ingestion (Dripps *et al.*, 2011). In addition, natural essential oils and plant volatiles have been used in the pest management strategies (Piesik and Wenda-Piesik, 2015).

Toxicity of pesticides might be assessed by mortality or their effects on the biochemical and physiological parameters of insects. The transaminase enzymes, aspartate aminotransferase and alanine aminotransferase, are critical in carbohydrate and protein metabolism and altered under stress conditions (Etebari *et al.*, 2007). Moreover, lipid peroxidation is an important mechanism in the oxidative damage to cells and in the toxicity process that lead to cell death (Repetto *et al.*, 2012). Also, acetylcholinesterase (AChE) is inhibited by organophosphate and carbamate insecticides and many other natural substances. Therefore, developing of new active ingredients and/or incorporating different materials that pose less concerns for both humans and environment (eco-friendly), biodegradable, cheap and compatible into the integrated pest management (IPM) approaches of stored-grain is a necessity.

## MATERIALS AND METHODS

### 1- Insect Species

Laboratory strains of rice weevil *Sitophilus oryzae* (L.), maize weevil, *Sitophilus zeamais* (Motschulsky), and granary weevil *Sitophilus granarius* (Linnaeus, 1758) were reared in the laboratory at  $28\pm 2$  °C,  $65\pm 5$  % RH, and the natural photoperiod at Department of Plant Protection, Faculty of Agriculture, Damanhour University, Egypt.

### 2- Rearing of Insects in the Laboratory

The insect species were collected from the main governmental granary storage, Damanhour, Albeheira Governorate, Egypt in 2013. In glass jars, insects of *S. granarius* and *S. oryzae* were reared on wheat grains, while *S. zeamais* was fed corn grains. About 400-500 unsexed adult insects were kept in 500 ml glass jar containing 500 g of grain (Halstead, 1963). Weevils were allowed to oviposit for 7 d and then they were removed by sieving. The newly emerged weevils were returned to the stock cultures Chaisaeng *et al.* 2010). After 1-2 weeks, newly emerged weevils were used for the experimental investigations. All experimental units (stock cultures, medium cultures, and vials) were covered with pieces of muslin cloth fixed by rubber bands to prevent the insects from escaping and facilitate aeration (Ahmed, 1996).

### 3- Chemicals, Extracts, and Essential Oils

Commercial essential plant oil of anise and clove were purchased El-Captain Company, Cairo, Egypt. Diatomaceous earth (DE) formulations were purchased from Al-Ahram Mining Co., Giza, Egypt. Spinetoram (12% SC) is produced by ICI Agrochemical Company, England. Malathion (5% Dust) is produced by Kafr El-Zayat Chemical and Pesticides Company, Egypt. Pesticides were purchased from reputed providers in Egypt.

### 4- Estimation of the Lethal Median Concentration (LC<sub>50</sub>)

To determine the values of LC<sub>50</sub>, series of concentrations of each tested compound were prepared. Eight doses: 0.3, 0.5, 0.8, 1, 2, 3, 4 and 5 ml kg<sup>-1</sup> grain of wheat of anise and clove oils. DE was tested at 0.2, 0.4, 0.6, 0.8, 1, 2, 4 and 8 g kg<sup>-1</sup> grain of wheat. Spinetoram was used at the concentrations of 0.01, 0.1, 0.5, 1, 2, 5, 10 and 20 mg kg<sup>-1</sup>. These concentrations were uniformly applied separately to 30 g of wheat

grains in plastic transparent jars (5 cm diameter × 7.9 cm height). Then, 30 weevils aging 1 to 2 weeks were placed in each jar. Three replicates were used for each concentration and all jars were kept at  $28 \pm 2$  °C and  $65 \pm 5$  % RH. Another three replicates containing untreated grains were used as control. Percentage mortality was estimated 1, 3, 5, 7, 14 and 21 d post-treatment. The lethal concentration that kills 50 % ( $LC_{50}$ ) and toxicity index values were calculated using Log dose Probit ( $LdP$ ) Line software (Ehabsoft, Cairo, Egypt).

#### 5- Effects of $LC_{50}$ of Tested Compounds on the Activity of Some Biochemical Components

The sub-lethal doses equivalent to the  $LC_{50}$  values were used to determine the effects of tested compounds on some biochemical responses. Weevils were exposed separately to the sub-lethal concentration of tested compounds along with their respective controls in triplicate for seven days. Live insects from each treatment were randomly selected, then weighed and used for the estimation of total protein, total lipids peroxidation, and the activities of aspartate transaminase (AST), alanine transaminase (ALT) and acetylcholinesterase (AChE). The biochemical studies were carried out on the whole treated insect tissues (whole body homogenate).

#### 6- Preparation of Whole Body Homogenates for Biochemical Analyses

Samples of treated and untreated adults of the tested insects were collected after seven days of exposure in Eppendorf tubes under freezing conditions ( $- 20$  °C) until used. The adult samples were replicated three times, weighed and homogenized with 10 times volumes (w/v) of phosphate buffer pH 7.2 using a glass homogenizer under ice. The total homogenates were centrifuged at 4000 rpm for 10 min at 4°C. The supernatants were used for biochemical tests. Aspectronic 2D, MILTON ROY COMPANY was used for measuring the density. Data were presented as means  $\pm$  standard errors.

#### 7- Determination of the Content of Total Protein

The total protein content was determined colorimetrically according to the method of Lowry *et al.* (1961). Fifty  $\mu$ L of supernatant were added to 3 ml of reagent C (50 ml of 2%  $Na_2CO_3$  in 0.1N NaOH containing 0.5 g sodium or potassium tartrate plus 1ml of 0.1 g  $CuSO_4$  in a liter of distilled water). The mixture was vortexed and incubated for 10 minutes

at room temperature. Then, 0.3 ml of freshly prepared folin reagent (2.00 N Folin–Ciocalteu reagent diluted 1:1 with distilled water) was added to the mixture and incubated for 30 min. The intensity of the developed blue color product was measured spectrophotometrically at 750 nm against a blank. Protein concentration was calculated and expressed as  $\text{mg mL}^{-1}$  wet tissue.

#### 8- Determination of MDA Content (Total Lipid Peroxidation)

Thiobarbituric acid-reactive substances (TBARS) were used as an index of lipid peroxidation according to Rice-Evans *et al.* (1991) with some modifications. An amount of 250  $\mu\text{L}$  of the supernatant were mixed with 1 ml of 15 % (w/v) trichloroacetic acid (TCA) in 25 ml 1 M HCL and 2 ml of 0.37 % (w/v) Thiobarbituric acid in 25 ml 1 M HCL. The samples were boiled for 10 min in a boiling sand bath, then quickly cooled and immediately centrifuged for 5 minutes at 6500 rpm. Supernatants were subjected to spectrophotometer analyses at 535 nm. MDA was quantified using an extinction coefficient of  $156 \mu\text{M}^{-1}$  and its concentration was expressed in moles  $\text{g}^{-1}$  of wet mass.

#### 9- Determination of Acetylcholinesterase (AChE) Activity

The acetylcholinesterase (AChE) activity was measured according to the method described by Ellman *et al.* (1961). The crude enzyme was used for the determination of the acetylcholinesterase activity. In a 5 ml test tube, 2.9 ml of 0.1M phosphate buffer (pH 8.0), 0.1 ml of 0.1mM DTNB reagent and 20  $\mu\text{L}$  of the tissue supernatant were added. To the above mixture, 20  $\mu\text{L}$  of the substrate (0.075M) acetylthiocholine iodide were added. The optical density of the developed yellow color was measured spectrophotometrically at 412 nm after 10 min against blank. The activity of cholinesterase was calculated as  $\mu\text{mole}$  of substrate hydrolyzed ATChI mg protein per minute.

#### 10- Transaminases Enzymes Activities

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity ( $\text{U mL}^{-1}$ ) were determined colormetrically following the method of Reitman and Frankel (1957).

#### 11- Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using the SPSS version 12 software. Means were separated using SNK

method (Steel and Torrie, 1980) and the results were considered statistically significant when  $P < 0.05$ .

## RESULTS

### Insecticidal Effect of Tested Compounds

The  $LC_{50}$  values at confidence limit 95% 7days post exposure of the tested weevil to the tested compounds are depicted in Table 1. Malathion and spinetoram insecticides were effective in controlling *S. granarius*, *S. oryzae*, and *S. zeamais* compared to anise oil, clove oil, and the diatomaceous earth. Also, diatomaceous earth was more effective against the tested insect pests compared to the essential oils.

Table 1.  $LC_{50}$  values of clove and anise oils, diatomaceous earth, spinetoram, and malathion seven days post-exposure of *S. granarius*, *S. oryzae*, and *S. zeamais*

Tested Compounds	$LC_{50}$ (mg kg <sup>-1</sup> )		
	<i>S. granarius</i>	<i>S. oryzae</i>	<i>S. zeamais</i>
Clove oil	3440	6950	2680
Anise oil	1550	1620	1140
Diatomaceous earth	3250	430	540
Spinetoram	0.14	0.25	0.76
Malathion	0.17	1.02	0.12

### Effects of Tested Compounds on Some Biochemical Components

#### Total Protein Contents

The chemical treatments changed the total protein differently. The contents of total protein in control were 0.17, 0.13 and 0.10 mg g<sup>-1</sup> for *S. granarius*, *S. oryzae*, and *S. zeamais*, respectively (Table 2). For *S. granarius*, a significant reduction in total protein was observed after treating weevils with anise oil (0.06 mg g<sup>-1</sup>) compared with the control. On the other hand, all tested compounds significantly increased the total body proteins (82-88%) compared with the control. In the case of *S. oryzae* and *S. zeamais*, a significant reduction in total protein was observed after the clove oil and DEs treatments. But the other treatments increased the total protein from 69 to 120 % of the control.

Table 2. Changes in total protein content ( $\text{mg g}^{-1}$ ) of *S. granarius*, *S. oryzae*, and *S. zeamais* after 7 days of treatment with the  $\text{LC}_{50}$  concentrations of clove and anise essential oils, diatomaceous earth (DE), malathion, and spinetoram

Tested Compounds	<i>S. granarius</i>	% of D*	<i>S. oryzae</i>	% of D*	<i>S. zeamais</i>	% of D*
Control	0.17 <sup>b</sup> ±0.01		0.13 <sup>c</sup> ±0.00		0.10 <sup>c</sup> ±0.00	
Clove oil	0.31 <sup>a</sup> ±0.01	82.35	0.06 <sup>d</sup> ±0.00	-53.85	0.09 <sup>d</sup> ±0.01	-10
Anise oil	0.06 <sup>c</sup> ±0.00	-64.71	0.22 <sup>a</sup> ±0.00	69.23	0.10 <sup>c</sup> ±0.00	0
DE	0.32 <sup>a</sup> ±0.00	88.24	0.06 <sup>d</sup> ±0.00	-53.85	0.09 <sup>d</sup> ±0.00	-10
Malathion	0.31 <sup>a</sup> ±0.00	82.35	0.22 <sup>a</sup> ±0.01	69.23	0.21 <sup>b</sup> ±0.00	110
Spinetoram	0.31 <sup>a</sup> ±0.00	82.35	0.18 <sup>b</sup> ±0.00	-38.46	0.22 <sup>a</sup> ±0.01	120
LSD <sub>0.05</sub>	0.02		0.01		0.01	

Values are mean ± standard error; n=3; means within each column followed by the same letter were not significantly different; LSD test at 0.05. \*% of D: percent of increase or decrease of control = (Control-Treatment)/Control \* 100.

#### Total Lipid Peroxidation (LPO Assay)

Weevils responded differently to treatments and data were shown in Table 3. For the three insect species, the level of total lipids significantly ( $P < 0.05$ ) increased after the treatment of clove oil; 3.12, 3.13 and 2.39  $\text{nM mg}^{-1}$  for *S. granarius*, *S. oryzae*, and *S. zeamais*, respectively. Meanwhile, total lipid levels were decreased after treating weevils with the others tested compounds.

Table 3. Changes in LPO activity ( $\text{nM mg}^{-1}$ ) of *S. granarius*, *S. oryzae*, and *S. zeamais* after 7 days post treatment with the  $\text{LC}_{50}$  values of clove and anise oils, diatomaceous earth (DE), spinetoram, and malathion under laboratory conditions.

Tested compounds	<i>S. granarius</i>	% of D*	<i>S. oryzae</i>	% of D*	<i>S. zeamais</i>	% of D*
Control	1.53 <sup>b</sup> ±0.33		2.04 <sup>ab</sup> ±0.59		1.50 <sup>b</sup> ±0.09	
Clove oil	3.12 <sup>a</sup> ±0.55	103.92	3.13 <sup>a</sup> ±0.82	53.43	2.39 <sup>a</sup> ±0.34	59.33
Anise oil	1.16 <sup>b</sup> ±0.43	-24.18	1.41 <sup>b</sup> ±0.11	-30.88	1.28 <sup>b</sup> ±0.42	-14.67
DE	1.14 <sup>b</sup> ±0.37	-25.49	1.26 <sup>b</sup> ±0.48	-38.24	1.08 <sup>b</sup> ±0.37	-28.00
Malathion	1.34 <sup>b</sup> ±0.68	-12.42	1.02 <sup>b</sup> ±0.16	-50.00	1.41 <sup>b</sup> ±0.56	-6.00
Spinetoram	1.31 <sup>b</sup> ±0.22	-14.38	1.39 <sup>b</sup> ±0.29	-31.86	1.42 <sup>b</sup> ±0.42	-5.33
LSD <sub>0.05</sub>	1.07		1.31		0.75	

Values are mean ± standard error; n=3; means within each column

followed by the same letter were not significantly different; LSD test at 0.05. \*% of D: percent of increase or decrease of control = (Control-Treatment)/Control \* 100.

#### Transaminases Enzymes Activities Aspartate Transaminase (AST)

The results in Table 4 revealed that 7 days post application of the tested compounds, the activity of the AST enzyme increased in *S. granarius* from 6 to 30% compared to control. Mixed results were obtained with *S. oryzae*, where AST activity was decreased after DE and malathion treatments and increased after clove oil, anise oil, and malathion. Similar to *S. oryzae*, AST activities of *S. granarius* were decreased or increased after 7 days of treatment. DE and clove oil increased the AST activity, but anise oil, spinetoram, and malathion concentrations significantly reduced its activity.

Table 4. Changes in aspartate transaminase (AST) activity (U mL<sup>-1</sup> of AST) of *S. granarius*, *S. oryzae*, and *S. zeamais* after 7 days of treatment with the LC<sub>50</sub> of clove oil, anise oil, diatomaceous earth (DE), spinetoram, and malathion under laboratory conditions

Insecticides	<i>S. granarius</i>	% of D*	<i>S. oryzae</i>	% of D*	<i>S. zeamais</i>	% of D*
Control	29.53 <sup>d</sup> ±1.21		14.34 <sup>c</sup> ±0.99		27.65 <sup>b</sup> ±1.31	
Clove oil	38.35 <sup>b</sup> ±0.51	29.87	37.81 <sup>a</sup> ±2.27	163.67	27.61 <sup>b</sup> ±0.99	0.14
Anise oil	42.99 <sup>a</sup> ±0.72	45.74	17.41 <sup>b</sup> ±1.19	21.41	22.47 <sup>c</sup> ±1.46	-18.73
DE	31.17 <sup>d</sup> ±0.30	5.55	13.24 <sup>c</sup> ±1.58	-7.67	33.74 <sup>a</sup> ±1.07	22.02
Malathion	34.95 <sup>c</sup> ±1.36	18.35	14.29 <sup>c</sup> ±1.70	-0.35	13.34 <sup>d</sup> ±1.95	-51.75
Spinetoram	36.74 <sup>b</sup> ±0.08	24.42	17.43 <sup>b</sup> ±0.84	21.55	11.75 <sup>d</sup> ±0.24	-57.50
LSD <sub>0.05</sub>	2.04		2.24		2.53	

Values are mean ± standard error; n=3; means within each column followed by the same letter were not significantly different; LSD test at 0.05. \*% of D: percent of increase or decrease of control = (Control-Treatment)/Control \* 100.

#### Alanine Aminotransaminase (ALT)

It was apparent from the results shown in Table 5 that *Sitophilus* species responded differently to the treatments. *S. granarius* showed an increase in the ALT activity after malathion treatments compared to control. No significant differences were observed between other tested



compounds and the control. For *S. oryzae*, only the DE and spinetoram treatments reduced the ALT activity compared to control. There were no significant differences between all tested materials and the control. In case of *S. zeamais*, with the exception of anise oil, all the tested materials elevated the enzymatic activity as compared to the control.

Table 5: Changes in alanine transaminase (ALT) activity (U mL<sup>-1</sup> of ALT) of *S. granarius*, *S. oryzae*, and *S. zeamais* after 7 days of treatment with the LC<sub>50</sub> of clove oil, anise oil, diatomaceous earth (DE), spinetoram, and malathion under laboratory conditions

Tested compounds	<i>S. granarius</i>	% of D*	<i>S. oryzae</i>	% of D*	<i>S. zeamais</i>	% of D*
Control	7.89 <sup>b</sup> ±0.88		6.51 <sup>a</sup> ±0.71		8.47 <sup>d</sup> ±0.54	
Clove oil	7.44 <sup>b</sup> ±0.71	-5.70	6.98 <sup>a</sup> ±0.87	7.22	12.67 <sup>b</sup> ±0.65	49.59
Anise oil	8.47 <sup>ab</sup> ±1.04	7.35	6.04 <sup>a</sup> ±0.37	-7.22	8.16 <sup>d</sup> ±1.33	-3.66
DE	6.69 <sup>b</sup> ±0.10	-15.21	2.31 <sup>b</sup> ±0.10	-64.52	10.51 <sup>c</sup> ±0.25	24.09
Malathion	9.33 <sup>a</sup> ±0.62	18.25	5.58 <sup>a</sup> ±0.10	-14.29	15.69 <sup>a</sup> ±0.52	85.24
Spinetoram	7.49 <sup>b</sup> ±0.94	-5.01	2.84 <sup>b</sup> ±0.60	-56.37	9.87 <sup>cd</sup> ±1.00	16.53
LSD <sub>0.05</sub>	1.99		1.52		1.97	

Values are mean ± standard error; n=3; means within each column followed by the same letter were not significantly different; LSD test at 0.05. \*% of D: percent of increase or decrease of control = (Control-Treatment)/Control \* 100.

Table 6. Changes in acetylcholinesterase (AChE) activity (µmole. mg<sup>-1</sup>. min<sup>-1</sup>) of *S. granarius*, *S. oryzae*, and *S. zeamais* after 7 days of treatment with the LC<sub>50</sub> of clove and anise oils, diatomaceous earth (DE), malathion, and spinetoram under laboratory conditions.

Tested compounds	<i>S. granarius</i>	% of D*	<i>S. oryzae</i>	% of D*	<i>S. zeamais</i>	% of D*
Control	0.05 <sup>b</sup> ± 0.01		0.10 <sup>b</sup> ± 0.01		0.08 <sup>b</sup> ± 0.01	
Clove oil	0.03 <sup>bc</sup> ± 0.00	-40	0.13 <sup>a</sup> ± 0.03	30	0.06 <sup>c</sup> ± 0.01	-25
Anise oil	0.12 <sup>a</sup> ± 0.02	140	0.03 <sup>d</sup> ± 0.00	-70	0.10 <sup>a</sup> ± 0.00	25
DE	0.03 <sup>bc</sup> ± 0.01	-40	0.06 <sup>c</sup> ± 0.02	-40	0.06 <sup>c</sup> ± 0.01	-25
Malathion	0.02 <sup>c</sup> ± 0.00	-60	0.03 <sup>d</sup> ± 0.00	-70	0.03 <sup>d</sup> ± 0.01	-62.5
Spinetoram	0.02 <sup>c</sup> ± 0.00	-60	0.02 <sup>d</sup> ± 0.01	-80	0.03 <sup>d</sup> ± 0.00	-62.5
LSD 5%	0.03		0.03		0.02	

Values are mean ± standard error; n=3; means within each column followed by the same letter were not significantly different; LSD test at 0.05. \*% of D: percent of increase or decrease of control = (Control-Treatment)/Control \* 100.

### Acetylcholinesterase (AChE) Specific Activity

The specific activity of acetylcholinesterase of untreated weevils were 0.05, 0.10, and 0.08  $\mu\text{mole mg}^{-1} \text{min}^{-1}$  for *S. granarius*, *S. oryzae*, and *S. zeamais*, respectively 7 days post treatment (Table 6). For the three insect species, except for the anise oil, all tested materials reduced the AChE activity with presents of decrease ranged from -25 to -80 compared to the control. The LC<sub>50</sub> concentrations of clove resulted in a pronounced inhibition of AChE for *S. granarius* and *S. zeamais*. Meanwhile, it caused a significant increase in the activity of AChE for *S. oryzae*.

## DISCUSSION

Results reported herein revealed significant dissimilarities in the efficiencies and effects of the tested compounds on the biochemical parameters. Somehow, these data highlight different mode of action and potency of these compounds against stored grain insects. For example, anise oil significantly reduced the total protein content of *S. granarius* weevils. While the same oil, increased the total protein in *S. oryzae*. Effects on reduction or increase of the level of total soluble protein may also be attributed to changes in DNA synthesis, reduced protein synthesis, low assimilation of food, and low uptake of amino acids during the protein synthesis after the stress of exposure to insecticides (DeLoach *et al.*, 1981; Ribeiro *et al.*, 2001). But the increase in protein content after clove oil treatment to adults of *S. granarius* might be a result of an elevation in tissues metabolic activity (Shakeet and Bakshi, 2010). Spinosad reduced the total protein contents in *S. oryzae* (Hamza *et al.*, 2014). Current results are in agreement with those of El-Bassiony *et al.* (2005) in the camel nasal botfly, *Cephalopina titillator* and El-Shershaby *et al.* (2008) in cotton leafworm, *Spodoptera littoralis*.

Worth mentioning that lipids serve as essential sources of energy to provide the needs of insects and to cup with stress caused by insecticides (Klowden, 2007; Abo El-Makarem *et al.*, 2015). Additionally, lipid peroxidation is a main cellular mechanism in the oxidative damage of cell structures and in the toxicity process and eventually leads to cell death. Specifically, it involves the production of

lipid radicals, oxygen uptake, rearrangement of the double bonds in the unsaturated lipids, and subsequent destruction of membrane lipids, with the formation of alcohols, ketones, alkanes, aldehydes and ethers (Dianzani and Barrera, 2008).

In the light of results presented in Table 3, data showed that the application of clove oil to the adults of the three *Sitophilus* species increased total lipid levels. But, the LC<sub>50</sub> of anise oil, DEs, malathion, and spinetoram decreased it. So, obtained result are in disagreement with Abo El-Makarem *et al.* (2015) who revealed an increase in total lipids. But it was in agreement with the results of Alimohamadi *et al.* (2014) who reported that spirodiclofen increased lipase enzyme activity and consequently lipid metabolism resulting in decrease lipid content in *Hippodamia variegata*. Similar reduction in the total lipid was reported by the acetone extract of *Melia azedarach* (Abou El-Ghar *et al.*, 1996; Abd El-Wahab, 2002; El-Shiekh, 2002). The decrease in tissue lipid under the insecticide stress could be due to the formation of lipoproteins, which are utilized to repair cell damage, energy source, increased lypolyses, and/or damage to cellular organization and adipokinetic hormone (Steele, 1985; Lohar and Wright, 1993).

Transaminase enzymes (AST and ALT), mitochondrial enzymes, transfer the amino groups from amino acids to keto acids (Ali *et al.*, 2013). The increasing concentration in the hymph of AST and ALT is an indication of cell damage. In the present study, clove and anise oils significantly increased AST activity for all the three tested insect species with the exception of *S. zeamais*, where an inhibition of AST activity was observed. Also, results of the current study showed that the two plant essential oils significantly increased ALT activity for the stored grain insect species, except with *S. granarius*. These results are in agreement with Abo El-Makarem *et al.* (2015). Basil and clove oils significantly increased AST activity, while basil oil only increased the activity of ALT. Increased transaminase activity might have been required by weevils to metabolize amino acids to obtain energy under stress of poisoning. Fluctuating effects of plant essential oils, DE, spinetoram, and malathion on AST and ALT activities might be due to their effects on function of these enzymes or cytomorphology of cells (Nath, 2000). Other insecticides (indoxacarb, emamectin benzoate, and pyridalyl) gave the same effects to the transaminases (Abd El-Aziz *et*

*al.*, 2007).

Acetylcholinesterase stops nerve communication by catalyzing the break-down of the acetylcholine at the neuromuscular junction in the nervous system (Ryan and Byrne, 1988; Lopez and Pascual-Villalobos, 2010). The overall results indicated that the activities of AChE in treated insects increased and/or decreased by the tested compounds. The LC<sub>50</sub> concentrations of clove oil significantly inhibited the activity of AChE for *S. granarius* and *S. zeamais*. Meanwhile, anise oil also inhibited the activity of AChE but only for *S. oryzae*. Maybe due to that anise and clove essential oil interfere with the passage of pulses in the insect nervous system. The inability of AChE to hydrolyze acetylcholine, the buildup of concentration of the acetylcholine in the synapse and excessive neuro excitation are the results of prolonged binding of ACh to its postsynaptic receptor (Lopez and Pascual-Villalobos, 2010; Rajashekar *et al.*, 2014). Previous works indicated that monoterpenoids in most plant essential oils cause insect mortality by inhibiting acetylcholinesterase enzyme (Gracza, 1985; Grundy and Still, 1985; Lopez and Pascual-Villalobos, 2010). Also, Picollo *et al.* (2008) reported that the monoterpenoid, 1, 8-cineole as a potent AChE inhibitor. Malathion, the organophosphorus insecticides, showed higher inhibiting effect than plant essential oils because it's a specific inhibitor of cholinesterase, this was in agreement with hundreds of researchers for example O'Brien (1967) and Mosleh *et al.* (2011).

## CONCLUSIONS

Efficiency of essential oils, DE, malathion and spinetoram were tested against *S. granarius*, *S. oryzae*, and *S. zeamais* along with the study of their biochemical effects. Malathion and spinetoram insecticides were effective in controlling the *S. granarius*, *S. oryzae*, and *S. zeamais* followed by the diatomaceous earth compared to anise oil and clove oil. Total protein content was reduced in *S. granarius* after the anise oil treatment but, all tested compounds significantly increased the total body proteins (82-88%). Mixed results were obtained with *Sitophilus* species in lipid peroxidase, AST and ALT activity. AST activities of *S. granarius* were different from increase and reduction after 7 days of treatment. DE and clove oil increased the AST activity, but anise oil,

spinetoram, and malathion concentrations significantly reduced its activity. *S. granarius* showed increased activity of ALT after malathion treatments compared to control. For the three insect species, except for the anise oil, all tested materials reduced the AChE activity with presents of decrease ranged from -25 to -80 compared to the control. The LC<sub>50</sub> concentrations of clove resulted in pronounced inhibition of AChE for *S. granarius* and *S. zeamais*. Meanwhile, it caused a significant increase in the activity of AChE for *S. oryzae*. However, essential oils were not efficient but DE might be effective in controlling the studied stored grain weevils. They exert complex and mixed effects on the tested biochemical parameters.

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كفاءة بعض الزيوت الطيارة والمبيدات الحشرية ضد آفات الحبوب المخزونة من جنس الـ  
**SITOPHILUS (COLEOPTERA: CURCULIONIDAE)**

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هدفت الدراسة الحالية الي اختبار تأثير الزيوت الطيارة من نباتات الينسون والقرنفل والتربة الدياتومية و السببينيورام والمالاتيون ضد آفات الحبوب المخزونة: *S. granarius* و *S. oryzae* و *S. zeamais* . أيضا هدفت الدراسة الي دراسة تأثير تلك المركبات علي بعض المكونات الحيوية والانزيمية لتلك الحشرات. تم تقدير التركيز النصف قاتل للحشرات ثم استخدامه لدراسة التأثيرات البيو كيميائية. تم دراسة المحتوي البروتيني ومعدل نشاط إنزيمات الـ *lipid peroxidase* و *aspartate transaminase (AST)* و *alanine transaminase (ALT)* و *acetylcholinesterase (AChE)*. أضحت النتائج أن المبيدات (المالاتيون والسببينيورام) أكثر سمية ويليها التربة الدياتومية ثم الزيوت الطيارة وتمثل ذلك في قيم التركيزات النصف قاتلة (LC<sub>50</sub>) لتلك المواد ضد الـ *S. granarius* و *S. Oryzae* و *S. Zeamais*. وكانت التأثيرات ضد النظم الانزيمية المختبرة وكمية البروتين متضاربة النتائج، ففي بعض الأحيان إزداد تركيز البروتين ونشاط الانزيمات وفي بعض الاحيان تنخفض تلك المكونات بعد إضافة التركيزات النصف قاتلة للحشرات. وكان من الواضح أن الزيوت الطيارة مفردة لا تعطي مستوي مقاومة مناسبة لافات الحبوب المخزونة بالمقارنة بالمبيدات والتربة الدياتومية.