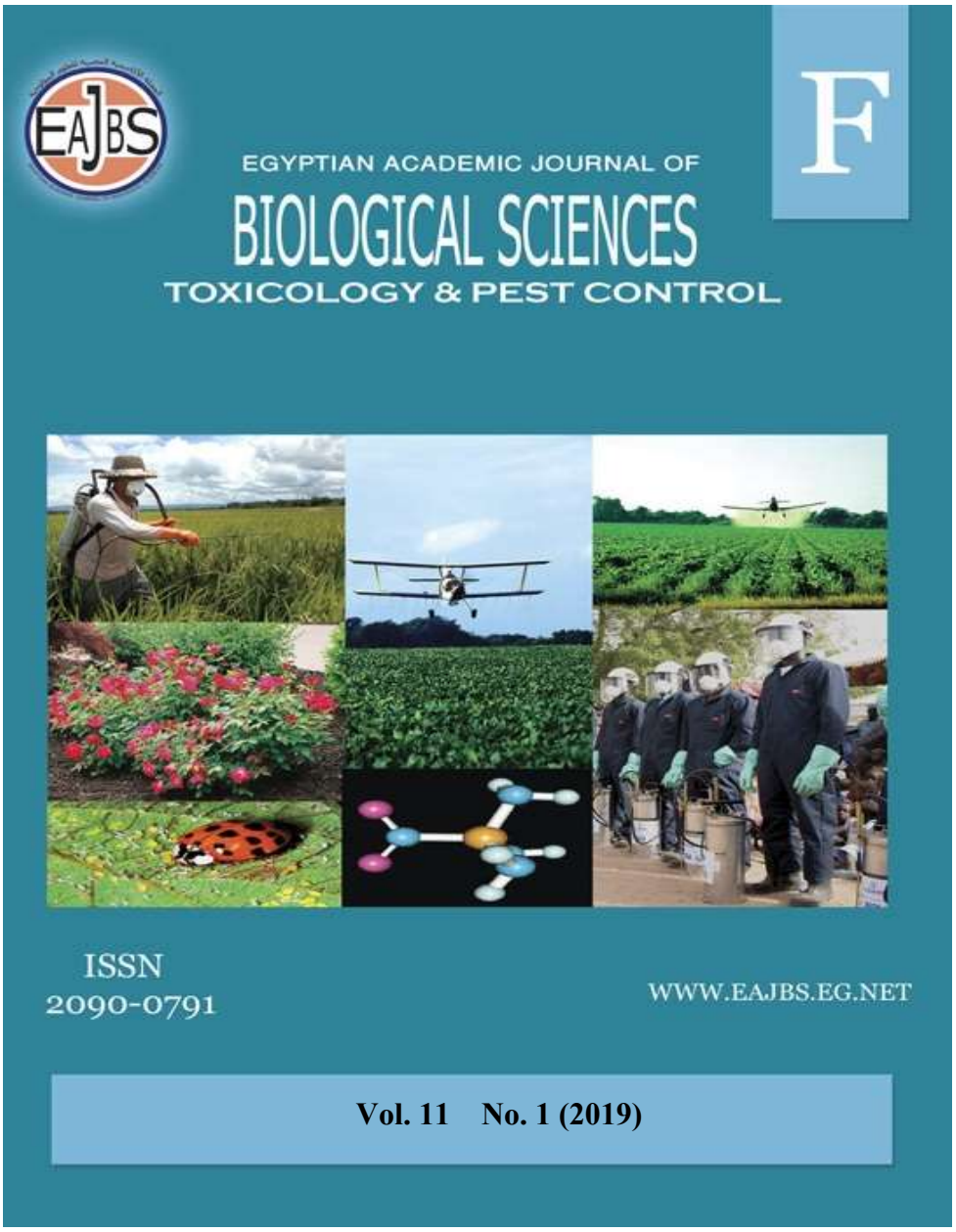

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Toxicity of some Essential oils and its Biochemical Effect against Red Flour Beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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ABSTRACT

Fumigant activities for four essential oils; Garlic oil (*Allium sativum*L); Basil oil(*Ocimum basilicum*); Pine (*Pinus longifolia* L.) and Eucalyptus (*Eucalyptus oblique* L.*)* at different concentrations after 1, 2, 3, 5 and 7 days at 28±1° C were tested against 4th instar larvae and adults of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) in the laboratory. The results showed that mortality increased with increasing oil concentration and time of exposure, also the higher essential oils toxicity at the LC_{50} after 7 days post-treatment was Garlic oil. Effects of Garlic essential oils on some enzymes activity in 4th instars' larvae of *T. castaneum* were investigated. From this study, we conclude that these essential oils have a potential for applications in IPM programs for stored-grain pests because of its high volatility and fumigant activity.

INTRODUCTION

Red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is one of the most destructive beetle species to several grains and flours. The progeny production rate of *T. castaneum* is so high. The fourth instars larvae are highly active in the rainy season and cause a very high infestation. This insect pest had a long association with individual stored food and has been found in a relationship with an array of commodities including grain, flour, peas, beans, cacao, nuts, dried vegetables, and spices, but milled grain products such as flour seem to be their preferred food. Good (1936); Campbelland Runnion (2003). Control of these insects depends on the use of synthetic insecticides and fumigants, which has generated problems such as disturbances of the environment, increasing costs of application, pest resurgence, pest amount of resistance to pesticides and deadly effects on non-target organisms in addition to direct toxicity to users Muniz *et al.*, 2008; Jembere *et al.*, 1995; Boyeret 's., 2012 and Simoniello et al., 2008. Fumigation was being the most effective method for controlling stored grains pest pests.

Utilized of plants for infestations control on stored grain seems to offer desired solutions, especially in the developing tropical countries where plants are found in abundance everywhere throughout the 12 months. Moreover, there have been growing interest in the use of both plant extracts and their essential oils since they exhibit low mammalian toxicity and low persistence in environmental surroundings Fouad *et al.*,

(2012); Papachristos and Stamopoulos(2002). Indicated that the essential herbal oils produced in various external and internal glands of these plants are containing a very complex combination of terpenes, sesquiterpenes, their oxygenated derivatives and other aromatic compounds Ogendo *et al.*, (2008). Many studies of the fumigant activity of such natural substances have been undertaken to ascertain new control practices with lower mammalian toxicity and low persistence in environmental surroundings Isikber *et al.*,)2006); Erler (2005) and Mishra *et al.*,)2014). Most natural plant compounds and microorganisms employed in controlling pests are proven to affect the enzymatic profiles (Nathan, *et al.*, 2005). Cytochrome P450 monooxygenases (CYPs), glutathione-S-transferases (GSTs) and esterase (ESTs) are 3 major detoxifying enzymes in most organisms. At least one of them is involved with detoxification of insecticides in insects (Bull, 1981). Insect GSTs have recently been implicated in resistance to insecticides, organophosphorus and pyrethroid, through direct insecticide metabolism (Wei *et al.*, 2001) or by protecting against secondary harmful effects, such as raises in lipid peroxidation, caused by insecticide exposure (Dou *et al.*, 2009). A member of the esterase cluster probably plays a role in the detoxification of xenobiotic esters. (Gacarand Tasksn, 2009). Alkaline phosphatase (ALP) is a brush border membrane gun enzyme and is especially active in tissues with active membrane transport, such as intestinal epithelial cellular material (Etebari and Matindoost, 2004a), Malpighian tubules (Etebari and Matindoost, 2004b) and hemolymph(Etebari *et al.*, 2007). This study investigates toxic effectiveness of three essential herbal oils against the Red flour beetle, *Tribolium castaneum* (Herbst) for protection of stored-grain from insect infestation also we had investigated their biochemical effects against some nutrients process of the 4th stretcher larvae.

MATERIALS AND METHODS

The experiments were conducted at the stored product pests' laboratory of the Plant Protection Dept. Faculty of Agric., Moshtohor, Benha University

Insect Culture:

Tribolium castaneum was reared in a glass container (250ml) containing wheat flour covered with a fine mesh cloth for ventilation. The cultures were maintained in the dark in an incubator at 28 ±1 ºC and 60 ± 5% RH. Adults were obtained from laboratory stock cultures maintained at the Plant Protection Dept. Faculty of Agric., Moshtohor, Benha University, Egypt.

Essential Oils:

Four essential oils in Table (1) belonging to different families; Amaryllidaceae; Lamiaceae; Myrtaceae; and Pinaceae were used during these investigations. All the essential oils were bought from Al-Gomhuria Company of drugs, chemicals and medical supplies in Egypt. Garlic oil (*Allium sativum*L); Basil oil (*Ocimumbasilicum*);Eucalyptus (*Eucalyptus globulus*); and Pine (*Pinus pinaster* L.) The fumigant toxicity of these oils was tested to the adults and 4th instar la**r**vae of *T. castaneum* .

Table (1): The essential oils used in the investigation.

	Scientific name	Family	Common name		Used part
			English	Arabic	
1	*Allium sativum*L.	Amaryllidaceae	Garlic oil	الثوم	Seeds
2	*Ocimum basilicum*	Lamiaceae	Basil oil	الريحان	Leaves
3	*Eucalyptus globulus*	Myrtaceae	Eucalyptus	الكافور	Leaves
4	*Pinus pinaster* L.	Pinaceae	Pine	الصنوبر	Fruits

Fumigant Toxicity of Essential Oils:

Ten grams of each pure oil was diluted with 50 ml. acetone to obtain 20% (w/v) stock concentration which diluted to obtain 10,50, 2.5,1.25,and .625% (w/v)

concentrations. In this experiment, 200 ml glass jars with tied covers were used as fumigation chambers for the plant oil. The tested dosages of oil inside the jars were 62.5, 125, 250, 500, and 1000 mg/l.air. Six jars were taken in each treatment. Inside every jar one filter paper was inserted at the bottom. Then one ml. from each oil concentration of the different prepared concentrations (10; 5; 2.5,1.25 and 6.25 % w/v) was taken and added to every glass jar on a filter paper for achieving the mentioned oil conc. inside the well-closed jars. Thirty adults and larvae were put inside each jar in cotton bags (2×1 cm) with a few amounts of crushed wheat. The jars well closed and incubated at 28 ±1 ºC and 60±5% R.H. The same steps were followed in the control treatment using only acetone without oil. Mortality was calculated after 1, 2, 3, 5 and 7 days post-treatment. Percentage of insect mortality was calculated using Abbott formula Abbott (1925). The fumigation of sub-lethal-time of insect was done as described above, using the method with concentrations viz. LT_{50} , LT_{90} and LT_{95} after 3, 5 and 7 days from treatment.

Biochemical Studies:

A known weight of 4thinstar larvae of *T. castaneum*(0.5g) which was treated with LC_{50} of garlic oil and the same weight of untreated ones were kept in the deep freezer until used for the certain physiological purpose as follows: frozen larvae were homogenized in 1ml. distilled water by using chilled glass Teflon homogenizer (ST- 2 Mechanic-Preczyina, Poland). Homogenates were centrifuged at 8000 r.p.m. for 15 min at 5 ºC and the supernatant was used for biochemical parameters assay.

Determine the Effect of Different Treatments on the Activity of some Insect Enzymes:

1-Acetylcholinesterase Determination (AChE):

Acetylcholinesterase (AChE) activity was estimated according to the method described by Simpson *et al.*(1964) using acetylcholine bromide (AChBr) as substrate . The reaction mixture contained 200 ul enzyme solution / 0.5 ml 0.067 M phosphate buffer (PH_7) and 0.5 ml AchBr (3 mM). The test tubes were incubated at 37°C for exactly 30 min. 1 ml of alkaline hydroxylamine (equal volume of 2 M hydroxylamine chloride and 3.5 M NaOH) was added to the test tubes , Then 0.5 ml of Hcl (1 part of cone. Hcl and 2 parts of Δ H_2O) was added. The mixture was shaken vigorously and allowed to stand for 2 min. 0.5 ml of ferric chloride solution (0.9 M Feels in 0.1M Hcl) was added and mixed well . The decrease in AChBr resulting from hydrolysis by AChE was read at 515 nm .

2-Determination of Phosphatases Enzymes (AcP&AlkP):

Acid phosphatases (AcP) and alkaline phosphatases (AlkP) were determined according to the method described by Powell and Smith (1954). In this method, the phenol released by enzymatic hydrolysis of disodium phenyl phosphate reacts with 4-amino antipyrine, and by the addition of potassium ferricyanide, the characteristic brown color is produced .The reaction mixture consisted of 1 ml carbonate buffer (pH10.4) for alkaline phosphatase or 1 ml citric buffer (pH 4.9) for acid phosphatase, 1 ml of 0.01 M disodium phenyl phosphate (substrate), mix with 0.1 ml sample and incubate for exactly 30 min at 37° C. At the end of incubation period, 0.8 ml of 0.5 N NaOH was added to stop the reaction .Then add 1.2 ml of 0.5 N NaHC03, followed by the addition of 1 ml of 4-aminoantipyrine solution (1%) and 1 ml potassium ferricyanide (0.5%). The produced color was measured immediately at 510 nm. The enzyme activity is expressed by unit (U) where 1 unit will hydrolyze 1.0 u mole of p-nitophenyl phosphate per minute at 37 C°/ and pH 10.4 and 4,8 for alkaline and acid phosphatases , respectively .

3-Transaminase Enzymes (GOT & GPT):

Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) enzyme activities were determined colorimetrically according to the method of Reitman and Frankle (1957). GOT transfer the amino group from L-aspartate to α-ketyo

acid (α-ketoglutaric acid) producing a new amino acid (L-glutamate) and a new keto acid (oxaloacetic acid). GPT transfer the amino group from D,L alanine to α-keto acid (α-ketoglutaric acid), resulting in a new amino acid (L-glutamate) and a new keto acid (pyruvic acid). Oxaloacetate or pyruvate reacts with 2, 4-dinitrophyenylhedrazine forming oxaloacetate or pyruvate hydrazone which in alkaline medium form a brown colour which can measure by spectrophotometerically. The reaction mixture consisted of 1ml of a mixture of phosphate buffer (PH 7.4) 0.2 mM α-ketoglutaric and 200 mM D-L alanine or L-aspartate, 0.2 ml of larval homogenate was then added to the reaction mixture. The mixture was incubated for 30 min. then after, 10 ml of 0.4 N NaOH was added. The optical density of the produced brown color is measured after 5 min using a spectrophotometer at 520 nm. The enzyme activity is expressed as M Pyruvate/gm body weight/min.

4-Determination of Non-Specific Esterases Activities:

Alpha esterases (α-esterases) and beta esterases(|3-esterases)were determined according to Van Asperen (1962) using a-naphthyl acetate or β-naphthyl acetate as substrates ,respectively.Napthol produced as a result of substrate hydrolysis can be measured by the addition of diazoblue sodium lauryl sulphate solution which produces a strong blue colour in case of α-naphthol or strong red color in the case of β-naphthol. The color was measured by spectrophotometrically. The reaction mixture consists of 5 ml substrate solution ($3x10^{-4}$ M α-or β-naphthylacetate, l% acetone and 0.1M phosphate buffer,pH7) and 20 ul of larval homogenate . The mixture was incubated for exactly 15 min at 27°C, then 1 ml of diazoblue color reagent (prepared by mixing 2 parts of l% diazoblue B and 5 parts of 5% sodium lauryl sulphate) was added. The developed color was read at 600 or 555 nm for a- and P-naphthol produced from hydrolysis of the substrate respectively.

5-Carbohydrate Hydrolyzing Enzymes:

The methods used to determine the digestion of trehalose, starch and sucrose by trehalase, amylase and invertase enzymes respectively, were similar to those described by Ishaaya and Swiriski (1976). The free aldehydic group of glucose formed after trehalose, starch and sucrose digestion was determined using 3, 5 dinitrosalicylic acid reagent.The trehalase reaction mixture consisted of 0.2 ml of 3% trehalose (substrate), 0.2 ml phosphate buffer (pH 5.4) and 0.2 ml larval homogenate. The invertase reaction mixture consisted of 0.2 ml of 4% sucrose (substrate), 0.1 ml phosphate buffer (pH 5.4) and 0.2 ml of larval homogenate. The amylase reaction mixture consisted of 0.2 ml of 2% starch (Substrate), 0.160 ml phosphate buffer (pH 5.4) and 0.2 ml of larval homogenate. The dinitrosalicylic acid reagent was prepared by dissolving one gram of 3, 5-dinitrosalicylic acid in 20 ml of NaOH and 50 ml of distilled water with the aid of a magnetic stirrer. Potassium sodium tartar ate (30 gm.) was added, and magnetic stirring was continued until a clear solution was obtained. Distilled water then added to bring the final volume to 100 ml. All test tubes were incubated at 37°C for exactly 60 min, 0.8 ml of 3, 5 dinitrosaleylic acid reagent were then added. The reaction mixture was heated 5 min at 100°C in a boiling water bath followed by immediate cooling in an ice bath. The optical density (OD) of the produced color is measured at 550 nm using a spectrophotometer. The enzymatic activity was expressed as mg glucose released/gm. body weight/min.

Determination of the Main Metabolites:

The main metabolites (total proteins, total lipids and total carbohydrates) were determined in the body homogenates.

1-Total Proteins:

Total proteins were determined by the method of Bradford (1976). Protein reagent was prepared by dissolving 100 mg of Coomassie Brilliant blue G-250 in 50 ml of 95%

ethanol.100 ml of 85% (W/V) phosphoric acid were added to this solution . The resulting solution was diluted to a final volume of one liter. Sample solution (50 ul) or for preparation of standard curve 50 ul of serial concentrations containing 10 to 100 ug bovine serum albumin were pipetted into test tubes. The volume in the test tube was adjusted to one ml with phosphate buffer (0.1M, PH 6.6), Five millimeters of protein reagent were added to test tube and the contents were mixed either by inversion or vortexing. The absorbance at 595 nm was measured after two min and before one hr against blank prepared from one ml of phosphate buffer and five ml protein reagent.

2-Total Lipids:

Total lipids were estimated by the method of Knight *et al.* (1972) using phosphovanillin reagent prepared by dissolving of 0.6 gm pure vanillin in 10 ml ethanol and completed to 100 ml with distilled water. Then 400 ml of cone. Phosphoric acid was added.

3-Total Carbohydrates:

The method was based on the digestion of trehalose. starch and sucrose by trehalase, amylase, and invertase, respectively, according to the method described by Ishaaya and Swiriski (1976). The free aldhydic group of glucose formed after trehalose, starch and /or sucrose digestion was determined using 3,5 dinitrosalicylic acid reagent. The trehalase reaction mixture consisted of 0.2 ml 3% trehalose (substrate) , 0.18 ml (0.2 M) acetate buffer (pH 5.4) and 20µl of adults homogenate. The amylase reaction mixture consisted of 0.2 ml of 2% starch (substrate), 0.16 ml (0.2 M) phosphate buffer (pH 6), and 20 µl of adults homogenate. The invertase reaction mixture consisted of 0.2 ml of 4% sucrose (substrate), 0.18 ml (0.2 M) acetate buffer (PH 5.4) and 20 µl of adults homogenate.

RESULTS AND DISCUSSION

1-Fumigant Toxicity of some Essential Oils against the Adults and 4th instar Larvae of *T. castaneum* :

The results of the effect of fumigation toxicity of plant oils (Garlic oil (*Allium sativum*), Basil oil (*Ocimum basilicum*), Eucalyptus oil(*Eucalyptus globulus*), and Pine oil (*Pinus pinaster*) on 4th instar larvae and adult of *T. Castaneum*. The results showed that mortality was increased by increasing the plant oil concentration and period of exposure. The lethal concentration of Garlic and Basil essential oils to adult and larval stage of *T. castaneum* are shown in Table (2).The results showed that after 3 days post-treatment for the adult stage the LC_{50} values were 126 and 1667% mg/l. air. The corresponding values at 7 days were significantly lower and amounted 47and 209% mg/l. air. For Garlic and Basil essential oils, respectively. While 4thinstar larvae the LC_{50} value was 7, and 881 % mg/l. air. The corresponding values at 7 days were significantly lower and amounted 41and 205% mg/l. air. For Garlic and Basil essential oils, respectively. The LC_{90} value of adult stage was 4436and 75088 % mg/l. air at 3 days and declined to 198and 1590% mg/l. air at 7 days post-treatment Garlic and Basil essential oils, respectively. while The LC_{90} value of larvae stage were 839and 41849% mg/l. air at 3 days and declined to 162and 7010% mg/l. air at 7 days post-treatment Garlic and Basil essential oils, respectively. The LC_{95} value were 12163and 221062 % mg/l. air at 3 day and reduced to 297and 2827% mg/l. air at 7 days from treatment for Garlic and Basil essential oils, respectively. while The LC_{95} value of larvae was 1639and 128027 % mg/l. air at 3 days and reduced to 238, 276 and 19081% mg/l. air at 7 days from treatment for Garlic and Basil essential oils, respectively. The lethal concentrations of Eucalyptus and Pine essential oils to the adult and 4th larvae stage of *T. castaneum* are shown in Table

(3). The results showed that the lethal concentrations are exposure period dependent. The higher the exposure period was the lower the LC values. At 3 days post-treatment for adult stage the LC_{50} value was 861, and 857 % mg/l. air. The corresponding values at 7 days were significantly lower and amounted 55 and 86 % mg/l. air. For Eucalyptus and Pine essential oils, respectively. while4th larvae the LC_{50} value was 238 and % mg/l. air. The corresponding values at 7 days were significantly lower and amounted 70 and 51 % mg/l. air. For Eucalyptus and Pine essential oils. respectively. The LC_{90} value of the adult stage was 37081 and 37815 % mg/l. air at 3 days and declined to 1030 and 9507% mg/l. air at 7 days post treatment Eucalyptus and Pine essential oils, respectively. while The LC_{90} values of 4th larvae stage were 2617 and 105547 % mg/l. air at 3 days and declined to 415 and 1377% mg/l. air at 7 days post treatment Eucalyptus and Pine essential oils, respectively. The LC_{95} values were 107787, 110681 and 548427 % mg/l. air at 3 days and reduced to 2365 and 36104 % mg/l. air at 7 days from treatment for Eucalyptus and Pine essential oils. respectively. While, the LC_{95} values of 4th larvae were 5163 and 486788 % mg/l. air at 3 days and reduced to 687 and 3504 % mg/l. air at 7 days from treatment for Eucalyptus and Pine essential oils. Respectively

Table (2): Lethal concentrations of some essential oils in the fumigant toxicity against adult and 4th larval stage of *T. castaneum* and various exposure periods.

Exposure period (days)	Stage	Lethal concentrations and their confidence limits			Slope ±SD	R
		LC_{50}	LC_{90}	LC_{95}		
Garlic oil(*Allium sativum*)						
3 days	**adult**	126 (69-232)	4436 (855-23006)	12163 (1400-105663)	0.82±0.009	0.992
	larvae	79 (47.34-132)	839 (427-1649)	1639 (661-4062)	1.25±0.06	0.978
5 days	**adult**	53 (26-104)	627 (326-1206)	1265 (508-3147)	1.19±0.008	0.996
	larvae	62 (43.72-88)	227 (167-307)	327 (222-483)	2.28±0.30	0.969
7 days	**adult**	47 (28-78)	198 (145-271)	297 (199-444)	2.06±0.34	0.956
	larvae	41 (24-70)	162 (116-225)	238 (154-367)	2.17±0.03	0.996
Basil oil(*Ocimumbasilicum*)						
3 days	**adult**	1667 (501-5538)	75088 (2542-2217906)	221062 (3947-132380)	0.77±0.03	0.971
	larvae	881 (340-1934)	41849 (1989-880209)	128027 (3165-5177340)	0.74±0.01	0.985
5 days	**adult**	416 (286-605)	4143 (1474-11642)	7947 (2262-27913)	1.28±0.05	0.981
	larvae	450 (231-876)	25532 (1567-415867)	80224 (2524-2549327)	0.73±0.02	0.976
7 days	**adult**	209 (153-284)	1590 (807-3132)	2827 (1211-6597)	1.45±0.04	0.988
	larvae	205 (123-342)	7010 (1132-43417)	19081 (1856-196166)	0.83±0.01	0.989

R= Correlation Coefficient of regression line SD= Standard deviation of the mortality regression line.

2-Toxicity Index of Various Essential Oils against Adults of *T. castaneum* .

The toxicity effect of various essential oils against adults of *T. castaneum*at the L_{C50} after 7 days post-treatment at 28±1° C could be arranged in descending order as follows: Garlic oil, Eucalyptus oil, Chili pepper oil, Pine oil, Basil oil and Nigella oil which was the least effective. Table (4) The toxicity effect of various essential oils against larvae of *T. castaneum*at the L_{C50} after 7 days post-treatment at 30±1° C could be arranged in descending order as follows: Garlic oil, Pine oil, Eucalyptus oil and Basil oil which was the least effective**.** Table (5).The results indicated that Garlic oils were much more effective as fumigants than other used oils against ***T. castaneum*** adults**.** Meanwhile, this results demonstrated the probability of using these essential plant oils as grain protestants' as well as or as Fumigants to control *T. castaneum* stages. According to the results obtained from the present study insects mortality increased with increasing concentration levels and exposure time.

Table (3): Lethal concentrations of some essential oils in the fumigant toxicity against adult and 4th larval stage of *Tribolium castaneum* and various exposure periods.

Exposure period (days)	Stage	Lethal concentrations and their confidence limits			Slope ±SD	R
		LC_{50}	LC_{90}	LC_{95}		
Eucalyptus oil (*Eucalyptus globulus*)						
3 days	**adult**	861 (364-2035)	37081 (2151-639158)	107787 (3446-639158)	0.78±0.08	0.934
	larvae	238 (168-338)	2617 (1016-6738)	5163 (1580-16868)	1.23±0.008	0.996
5 days	**adult**	177 (115-270)	2886 (929-8970)	6370 (1490-27229)	1.05±0.0009	0.999
	larvae	113 (72-178)	1367 (598-3123)	2770 (935-8199)	1.18±0.03	0.987
7 days	**adult**	55 (25-120)	1030 (414-2563)	2365 (658-8492)	1.00±0.03	0.982
	larvae	70 (46-107)	415 (266-648)	687 (381-1238)	1.66±0.04	0.991
Pine oil(*Pinuspinaster*)						
3 days	**adult**	857 (364-2015)	37815 (2137-669152)	110681 (3414-3587856)	0.77±0.01	0.982
	larvae	481 (194-1192)	105547 (905-106223)	486788 (1283-1845)	0.54± 0.015	0.972
5 days	**adult**	332 (174-633)	26869 (1285-561728)	93372 (2042-4268589)	0.67±0.02	0.971
	larvae	149 (80-278)	7516 (953-59237)	22831 (1552-335752)	0.75±0.002	0.997
7 days	**adult**	86 (32-227)	9507 (673-134217)	36104 (1037-1256877)	0.62±0.001	0.998
	larvae	51 (20-126)	1377 (452-4193)	3504 (727-16873)	0.89±0.011	0.992

R= Correlation Coefficient of regression line SD= Standard deviation of the mortality regression line.

This kind of is in agreement with Sahaf and Moharramipour (2008). However, generally, the effectiveness of gas decreased over time that can be related to their high volatility and low stability (Mikhaiel, 2011; Ogendo *et al.*, 2008; Rozman *et al.*, 2007; Sahaf *et al.*, 2007). Isman (2006) Observation of *T. granarius* exposed to *C. copticum* essential oil indicated their knockdown, hyperactivity, convulsion, paralysis; and finally insect death and mentioned that fast effect of essential oils is because of their neurotoxic mode of action. Insect's susceptibility to the same essential oil differs from species to species. In line with the Sahaf and Moharramipour (2008), LC50 values of *C. copticum*

essential oil against adults of C. maculates after 24 h exposure time was 0. 90? L-1 air. In the other study Sahaf et 's., (2007) explained that zero. 91? L-1 air of *C. copticum* oil was required to obtain 50% mortality of *S. oryzae,* and 33. 14? L-1 air in the case of *T. castaneum*. Shojaaddini *et al.,* (2008) also estimated 257. 83 and 91. 36? L-1 air of *C. copticum* oil to control 50% of *G. interpunctella* adults and larvae, respectively. Based on LC50 values, S. granariusseems to be more tolerant of *C. copticum* oil than other Coleopteran species. Most of the plants and herbal products are locally available and is applied for pest control programs in small scales. However , there is a need to conduct further studies to examine their insecticide efficacy against stored-product insect pests.

Table (4): Toxicity index of various essential oils for the adults of *T. castaneum* after 7 days post-treatment**.**

Essential oils	LC_{50} after 7 days (mg/l.air)	Toxicity index at LC_{50}	Slope ± SD	R.
Garlic oil	47.49	100	2.06±0.34	0.956
Eucalyptus oil	55.04	86.28	1.00±0.03	0.982
Pine oil	86.03	55.20	0.62±0.001	0.998
Basil oil	209.23	22.69	1.45±0.04	0.988

R= Correlation Coefficient of regression line SD= Standard deviation of the mortality regression line

Table (5): Toxicity index of various essential oils for 4thinstar larvae of *T. castaneum* after 7 days post treatment

Essential oils	LC_{50} after 7 days (mg/l.air)	Toxicity index at LC_{50}	Slope ± SD	R.
Garlic oil	41.67	100	2.17±0.03	0.996
Pine oil	51.12	81.51	0.89±0.011	0.992
Eucalyptus oil	70.35	59.23	1.66±0.04	0.991
Basil oil	205.29	20.29	0.83±0.01	0.989

R= Correlation Coefficient of regression line SD= Standard deviation of the mortality regression line

3-Effect of Garlic Essential Oil on the Total Proteins, Lipids and Carbohydrates of 4th larval of *T. castaneum*:

Data on the total proteins, carbohydrates and lipids are shown in Table (6) revealed that, no significant increase was found under treatment garlic oil which recorded 26.66 mg/g.b.w., in compared to the control, the rate of increase was 21.49%. On the other hand, the obtained results in the same table indicated that the rate of total protein was of decrease was -63.14% meanwhile insignificant reduced under garlic oil which recorded 211.33 mg/g.b.w. in compared to the control. Total lipids content were insignificantly reduced to garlic oil treatments which recorded 16.76 mg/g.b.w., compared to control treatment which recorded 16.96 mg/g.b.w. these of decrease was -1.19% for garlic oil.

4-Effect of Different Treatments on the Activity of the Enzymes:

The treatments of controlling *T. castaneum* have significantly affected the activity of different enzymes.Table (6) indicated that, the decrease of Alkaline phosphatase was -8.79% while on the contrary no significant increase of enzyme activity under garlic oils

treatment was recorded (491.66) in compared to the control treatment, this increase was 1.02%. On the other hand, the obtained results of Acid phosphatase indicated that the decrease of enzyme was -70.94%, while no significant increase of the enzyme activity under garlic oils treatment was recorded (116.76 $Ux10^3$/g.b.wt) in compared to the control.

Table (6): Activity of certain enzymes in *T. castaneum* larvae exposed to different treatments.

Treatment	Activity (Mean ± S.E.)	percent of increase or decrease (%)	Activity (Mean ± S.E.)	percent of increase or decrease (%)
	Alkaline phosphatase $Ux10^3$/g.b.wt		**Acid phosphatase** $Ux10^3$/g.b.wt	
Garlic oil	491.66±33.42 a	1.02	116.0±10.76 a	0.29
Control	486.66±33.42 a	-	115.66±10.76 a	-
	α-esterase mg/g.b.w.		**β-esterase** mg/g.b.w.	
Garlic oil	3404.33±351.66 b	5.33	1230.0±39.79 a	14.22
Control	3222.66±351.66 b	-	1055.0±39.79 b	-
	(GOT) uM/g.b.wt.		**(GPT)** uM/g.b.wt.	
Garlic oil	4722.7±414.13 b	-53.72	808.67 ±40.19 ab	15.16
Control	7260.0 ±414.13 a	-	686.00 ±40.19 a	
	Acetylcholinesterase µg/ Br/g/min.		**Trehalase** µgGlu./g/min	
Garlic oil	290.33 ±8.01 a	26.52	1157.00±92.22 a	4.84
Control	213.33 ±8.01 b	-	1101.00±92.22 a	-
	Invertase µgGlu./g/min		**Amylase** µgGlu./g/min	
Garlic oil	1786.66±130.91 b	19.51	140.66±19.50 b	9.00
Control	1438.00±130.91 b	-	128.00±19.50 b	-

Total carbohydrates			**Total proteins**		**Total lipids**	
Garlic oil	26.66±1.98b	21.49	211.33±8.71a	-11.67	16.76±0.84a	-1.19
Control	20.93±1.98b	-	236.00±8.71a	-	16.96±0.84a	-

This increase was 0.29%.A Significant increase in activity of Alpha esterases and Beta esterases with garlic oils treatments was recorded 3404.33 mg/g.b.w., compared to control treatment which recorded 3222.66 mg/g.b.w., the increase was 5.33 for the activity of Alpha esterases for garlic oil,. while an increase to Beta esterases for garlic oils treatments was 14.22%.A high significant decrease in the activity of Glutamic Oxaloacetic Transaminase (GOT) and Glutamic Pyruvic Transaminase (GPT)for garlic

oil treatments was recoded 4722.7uM/g.b.wt. in compared to control treatment which was 7260.0 uM/g.b.wt. this decrease was -53.72% for garlic oil. The activity of Acetylcholinesterase enzyme showed significant greatest under garlic oils treatments was290.33 µg/ Br/g/min.,the increase of this enzyme was 26.52%.Trehalase activity showed no significant increase in garlic oil treatments which were 1157.00 µg Glu./g/min. in compared to control treatment 1101.00 µgGlu./g/min. the increase was 4.84 % for garlic oil. No significant increase in the activity of Invertase enzyme with garlic oil treatments which was 1786.66 µg Glu./g/min compared to control treatment. The rate of increase was 19.51%.Amylase activity showed no significant increase with garlic oil treatments was recorded (140.66 µgGlu./g/min.) compared to control treatment, the increase was 9.00%. These results were in harmony with **(**Nathan, *et al.,* 2005) indicated that the Cytochrome P450 monooxygenases (CYPs), glutathione-S-transferases (GSTs) and esterases (ESTs) are three major detoxifying enzymes in most organisms. Abd El-Raheman (2013) Found that the activity of trehalase, acid phosphates, acetyl cholinesterase, phenoloxidase and lactate dehydrogenase enzymes increased in treated *Sitotroga cerealella* larvae with modified atmospheres while the activity of amylase, alkaline phosphatase enzymes decreased. Abotaleb (2013) showed that total proteins, lipids and carbohydrates contents were significantly decreased in *Sitophilus oryzae* adults feeding in wheat grain treated with different oils compared to control. Furthermore, different levels of significant changes in the carbohydrase's, protease, phosphatases and acetylcholine esterase activity, The decrease in the protein content in treated larvae might be due to inhibition of DNA and RNA synthesis as suggested by Mitlin and Haynes (1977).

REFERENCES

Abbott, W.S. (1925): A method of computing the effectiveness of an insecticide. J.Econ. Entomol. 18: 265–267.

Abd El-Raheman, S.F. (2013): Ecological and biological investigations on two wheat insect pests with special reference to the effect of CO_2 on *Sitotrogacerealella*(Olivier)larvae. Ph.D. thesis, Cairo Univ., Egypt,147 p.

Abotaleb, O. A. (2013): Evaluation of some natural plant oils for the control of the rice weevil *Sitophilusoryzae* (Coleoptera:Curculionidae) on wheat. M.S. thesis, Ain Shams Univ., Egypt.

Boyer, S.; Zhang, H. and Lempérière, G. (2012): A review of control methods and resistance mechanisms in stored product insects. Bulletin of Entomological Research. 102:213-229.

Bradford, M. M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Ann. Biochem., 72: 248- 254.

Bull, D. L. (1981): Factors that influence tobacco budworm, *Heliothisvirescens*, resistance to organophosphorous insecticides. Bull. Entomol. Soc. Amer27: 193-197.

Campbell, J. F. and Runnion, C. (2003): Patch exploitation by female red flour beetle, *Triboliumcastaneum*. J. Insect Science; 3:20-24.

Dou, W.; Shuang Wu.; Hassan, M. W. and Jin-Jun, W. (2009): Purification and biochemical characterization of glutathione S-transferases from three strains of *Liposcelisbostrychophila*Badonnel (Psocoptera: Liposcelididae): Implication of insecticide resistance. Pestic.Biochem.Physiol. 94: 10–14.

Erler, F. (2005): Fumigant activity of six monoterpenoids from aromatic plants in Turkey against the two stored-product pests confused flour beetle, *Tribolium confusum* and Mediterannean flour moth, *Ephestia kuehniella*. Journal of Plant Diseases and

Protection; 112: 602-611.

Etebari, K. and Matindoost, L. (2004a): The study on effects of larval age and starvation stress on biochemical macromolecules abundance of haemolymph in silkworm *Bombyxmori*, in: Proceedings of the Sixteenth Iranian Plant Protection Congress, General Entomology Symposium, August 28–September 1, Univ. of Tabriz, Iran, pp. 435.

Etebari, K. and Matindoost, L. (2004b): Effects of hyper vitaminosis of vitamin B3 on silkworm biology, J. Biosci. 29: 417–422.

Etebari, K.; Bizhannia, A.R.; Sorati, R. and Matindoost, L. (2007): Biochemical changes in haemolymph of silkworm larvae due to pyriproxyfen residue. Pestic. Biochem. Physiol. 88: 14–19.

Fouad; HA; Faroni; LRDR; Ribeiro; C; Tavares; WD and Petacci; F. (2012): Extraction and repellent activity of Lepidoploaaurea and Memoranodosa against stored grain and byproduct pests. Vie Milieu-life and environment; 62:11-15.

Gacar, F. and Tasksn, V. (2009): Partial base sequence analysis of MdaE7 gene and ali-esterase enzyme activities in field collected populations of housefly (*Muscadomestica*L.) from Mediterranean and Aegean Regions of Turkey. Pestic. Biochem. Physiol., 94: 86–92.

Good, N.E. (1936): The flour beetles of the genus Tribolium. USDA Technical Bulletin. 5:27- 28.

Ishaaya, I. and Swiriski, E. (1976): Trehalase, invertase and amylase activities in the black scale *Saissetiaoleae*, and their relation to host adaptability. J. Insect Physiol., 22: 1025-1029.

Isikber, AA; Alma, MH; Kanat, M. and Karsil, A.(2006): Fumigant toxicity of essential oils from Laurusnobilis and *Rosmarinus officinalis* against all life stages of *Tribolium confusum*. Phytoparasitica.; 34:167-177.

Isman MB (2006): Botanical insecticides, deterents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol 51: 45-66.

Jembere, B.; Obeng-Ofori, D.; Hassanali, A. and Nyamasyo, G. N..(1995): Products derived from the leaves of *Ocimumkilimands charicum* (Labiatae) as post-harvest protectants against the infestation of three major stored product insect pests. Bulletin of Entomological Research. 85:361-367.

Knight, J. A.; Anderson, S. and Rawle, J. M.(1972): Chemical basis of the sulfophospho-vanillin reaction for estimating total serum lipids. Clin. Chem.,18: 199-202.

Mikhaiel AA (2011): Potential of some volatile oils in protecting packages of irradiated wheat flour against *Ephestia kuehniella* and *Tribolium castaneum*. J Stored Prod Res 47: 357- 364.

Mishra, BB; Tripathi, SP. and Tripathi, CPM. (2014): Chronic activity of plant volatiles essential oils in management of rice weevil *Sitophilus oryzae* (Coleoptera: Curculionidae). Journal of Entomology; 11:76-83.

Mitlin, N. G. W. and Haynes, J. W. (1977): Inhibition of DNA synthesis in boll weevil *Anthonomus grandis* Boheman sterilized by Dimilin. Pestic. Biochem. &Physiol. ,7: 559-563.

Muniz, J.F, McCauley, L; Scherer, J; Lasarev, M; Koshy, M; Kow, YW *et al.*(2008): Biomarkers of oxidative stress and DNA damage in agricultural workers: a pilot study. Toxicology and Applied Pharmacology. 227(1):97-107.

Nathan, S. S.; Kalaivan, K. and. Chung, P. G. (2005):The effects of azadirachtin and nucleopolyhedrovirus on midgut enzymatic profile of *Spodoptera litura*Fab. (Lepidoptera: Noctuidae) Pestic.Biochem. Phys., 83 (1): 46-57.

Ogendo, JO.;Kostyukovsky, M.; Ravid, U.; Matasyoh, JC.; Deng, AL. and Omolo, EO. (2008): Bioactivity of *Ocimum gratissimum* L. oil and two of its constituents

against five insect pests attacking stored food products. J. Stored Products Research; 44:328- 334.

Papachristos, DP. andStamopoulos, DC. (2002): Repellent, toxic and reproduction inhibitory effects of essential oil vapours on *Acanthoscelide sobtectus* (Say) (Coleoptera: Bruchidae). Journal of Stored Product Research; 38(2):117-128.

Powell,M.E.A. and Smith, M.J.H. (1954): The determination of serum acid and alkaline phosphatase activity with 4-aminoantipyrine. J.Clin .Pathol., 7:245-248.

Reitman, S. and Frankel, F. (1957):Colourimetric method for aspartate and alanine transminases. Amer J. Clin. Pathol., 28-56

Rozman V, Kalinovic I, Korunic Z (2007): Toxicity of naturally occurring compounds of Lamiaceae and Lauraceae to three stored-product insects. J Stored Prod Res 43: 349-355.

Sahaf, BZ, Moharramipour, S, Meshkatalsadat, MH (2007) :Chemical constituents and fumigant toxicity of essential oil from Carumcopticum against two stored product beetles. Insect Sci 14: 213-21

Sahaf BZ, Moharramipour S (2008): Fumigant toxicity of Carumcopticum and Vit expseudonegundo essential oils against eggs, larvae and adults of *Callosobruchus maculatus*. J Pest Sci 81: 213-220.

Shojaaddini M, Moharramipour S, Sahaf BZ (2008): Fumigant toxicity of essential oil from Carumcopticum against Indianmeal moth, *Plodia interpunctella*. J Plant Prot Res 48:411-419.

Simoniello, MF.;Kleinsorge, EC.; Scagnetti, JA.; Grigolato, RA.; Poletta, GL. and Carballo MA. (2008): DNA damage in workers occupationally exposed to pesticide mixtures. Journal of Applied Toxicology; 28(8):957-65.

Simpson, D.R.; Bull, D.L. and Lindquist, D.A. (1964): A semimicro technique for the estimation of cholinesterase activity in bull weevil. Ann. Entomol. Soc. Am. 57(3):367-377.

Van Asperen, K. (1962): A study of house fly esterase by means of sensitive colourimetric method. J.Insect physiol.,8:401-416.

Wei, S.H.; Clark, A.G. and Syvanen, M. (2001):Identification and cloning of a keyinsecticide -metabolizing glutathione *S*- transferase (MdGST-6A) froma hyper insecticide -resistant strain of the housefly *Muscadomestica*, Insect Biochem. Mol. Biol., 31: 11451153.

ARABIC SUMMARY

سمية بعض الزيوت العطرية وتاثيرها البيوكيميائى ضد حشرة خنفساء الدقيق الصدئية

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****قسم بحوث النبات – مركز البحوث النووية - هيئة الطاقة الذرية – ابو زعبل – مصر**

تم اختبار فاعلية ست زيوت نباتية هما زيت الثوم، الفلفل الاحمر، الريحان، الصنوبر، الكافور، حبة البركة. وذلك بتركيزات مختلفة بعد 1، 2، 3، 5، 7 ايام عند درجة حرارة °1±28 درجة مئوية ضد العمر اليرقى الرابع وطور الحشرة الكاملة لخنفساء الدقيق الصدئية وذلك بالمعمل. واظهرت النتائج ان نسبة الموت تزداد بزيادة تركيز الزيت وطول مدة التعريض وكان اكثر الزيوت النباتية سمية هو زيت الثوم للحشرة تحت الدراسة وذلك بعد اسبوع من المعاملة ايضا تم اختبار تاثير زيت الثوم على فاعلية نشاط بعض الانزيمات للعمر اليرقى الرابع. من خلال تلك الدراسة نوصى باستخدام الزيوت النباتية داخل برامج المكافحة المتكاملة لافات الحبوب المخزونة نظرا لسمية ابخرتها العالية.