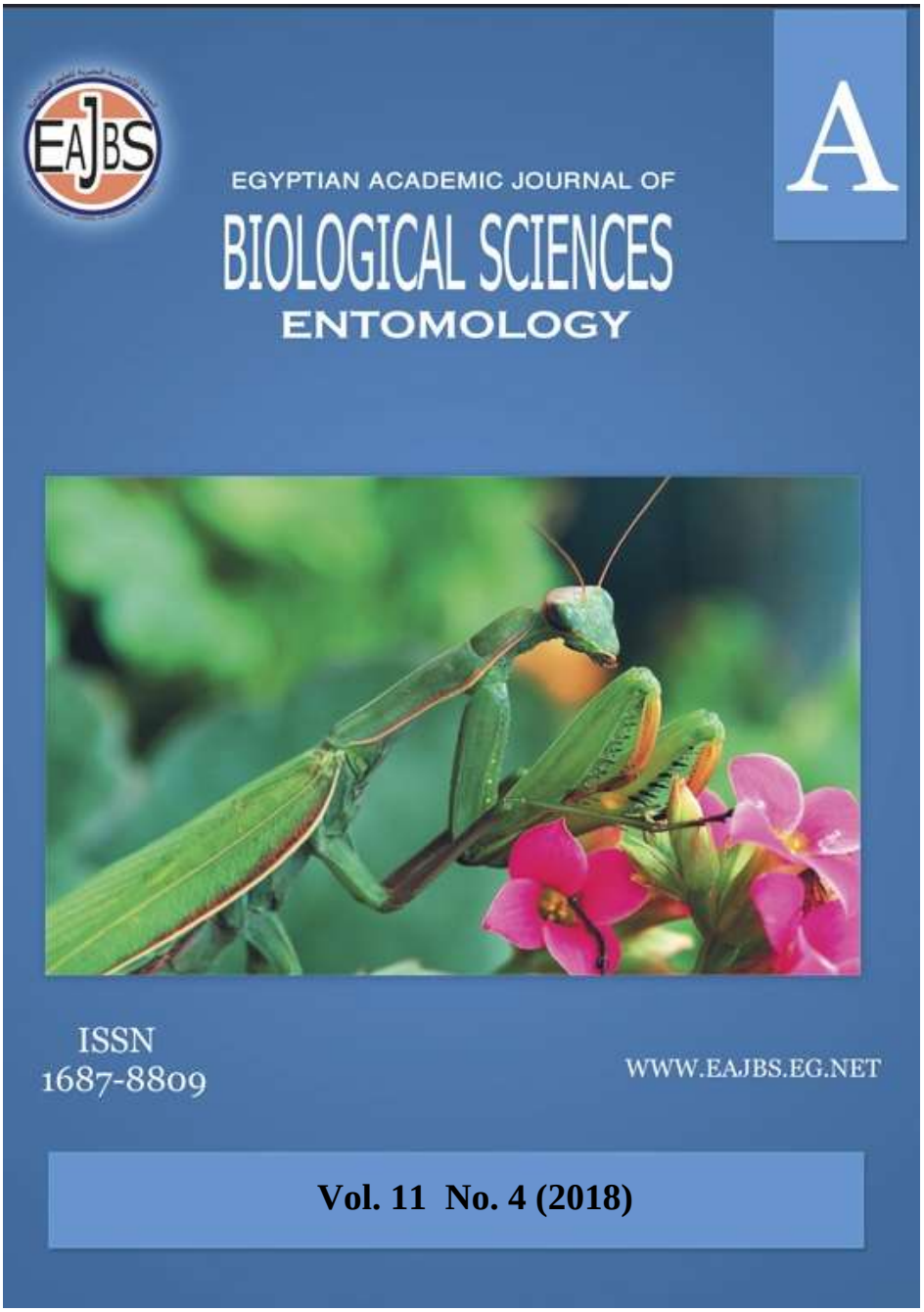

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Toxicity of Some Essential Oils against Myiasis-Producing Fly, *Megaselia scalaris*, and their Impacts on Proteins and Detoxification Enzymes.

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ABSTRACT

Megaselia scalaris (Diptera: Phoridae) is regarded as a medically important insect worldwide because it is the source of different myiasis diseases. Therefore, it should be controlled for human welfare and for economic reasons. Accordingly, the larvicidal activity of six plant essential oils was determined against *M. scalaris* and their impacts on proteins and isozymes were also estimated in whole body homogenate of the third larval instars. The tested essential oils were chamomile (*Matricaria chamomilla*), clove (*Syzygium aromaticum*), orange (*Citrus sinensis*), rosemary (*Rosemarinus officinalis*), spearmint (*Mentha spicata*) and sweet basil (*Ocimum basilicum*). Results exhibited that the clove oil had the highest larvicidal activity, LC_{50} is 2.38 ml/100g media, whereas, chamomile oil was the least one, LC_{50} is 5.37 ml/100g media. Significant concentration-mortality responses of the treated larvae were observed towards all tested oils. Concerning protein estimation, the control protein content was 12.80±2.68 mg/g tissue, and the oil application produced an elevation in total protein contents in treated larvae. This elevation was significant ($p<0.05$) with clove, orange and spearmint oils amounted to be 92.19, 70.31 and 57.58%, respectively, compared to that of the control larvae, but insignificant ($p>0.05$) with chamomile, rosemary and sweet basil oils. The electrophoretic study revealed that the maximum number of native protein bands was seven at approximately rate of flow ranging between 0.14 to 0.72 in the control and clove, orange, spearmint and sweet basil treatments. One newly formed protein band was observed in whole body homogenate of clove, orange, rosemary, spearmint and sweet basil treatments. The profiles of *β*-esterase, alcohol dehydrogenase and aldehyde oxidase showed the same patterns in both control and treated larvae. On the other hand, there was a reduction in the isozymes amount percent in the treated larvae as compared with the control values. The biochemical changes in native proteins and isozymes could be used as a biochemical indicator of toxic stress.

INTRODUCTION

The phorid fly, *Megaselia scalaris* L. (Diptera: Phoridae), is considered as a medically important insect worldwide. It transmits infectious and parasitic diseases of both man and animal when it lands on a source of contamination through direct or indirect contact (Fischer, 2007). *M. scalaris* have been reported to cause Myiasis

disease in a wide variety of cases ranging from the children (Benecke and Lessig, 2001), adults (Nazni *et al.*, 2011) and domesticated and non-domesticated animals (Anderson and Huitson, 2004). Myiasis has been reported also in non-wound cases such as nasopharyngeal (Carpenter and Chastain, 1992), urogenital (Solgi *et al.*, 2017) and intestinal myiasis (Singh *et al.*, 1988). On the other hand, *M. scalaris* is a common species found throughout indoor and outdoor crime locations and plays an important role in the decomposition of human remains. So, it can be used as entomological evidence for the estimation of the post-mortem interval in forensic investigations (Chakraborty *et al.*, 2016). Besides, larvae of *M. scalaris* were recorded infesting some fruits and stored products (Karunaweera *et al.*, 2002 and Yawson *et al.* 2013). Moreover, *M. scalaris* was found as a parasitoid infecting some useful insects like *Apis mellifera* (Cham *et al.*, 2018). All the above-mentioned damage effects because the larvae of *M. scalaris* have been considered as parasite, parasitoid, predator, terrestrial detritivore, phytophagous and coprophagous (Disney, 2008 and Bostock, 2015).

Thereupon, *M. scalaris* should be controlled for human health and for many economic reasons. Even now, the control of myiasis- producing flies is depended mainly on synthetic pesticides (Babar *et al*., 2012) and sometimes insect growth regulators (IGRs) (Levot and Sales, 2004). Nonetheless, these traditional methods are effective against pests, their repeated usance have affected the natural enemies and increase the pest resistance to such insecticides (Ebadollahi, 2013). Therefore, recent researches are being made to develop natural-based pesticides which are safe for human, environment non-target organisms (Khater *et al.*, 2011 and Ismail, 2018).

In Egypt culture from ancient times, people used plants and essential plant oils as insecticides and repellents in homes, because of their natural and pleasant-smelling substances (Bakkali *et al.*, 2008). Essential oils are volatile, natural, complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolites. They are usually obtained by steam distillation first developed by Arabs. Essential oils are effective against a large variety of organisms including bacteria, fungi, protozoa, parasites, different stages of insects (Pavela, 2005and Yazdani *et al.*, 2014). Besides, essential oils have insecticidal effects against some dipterous insects (Pavela, 2008; Govindarajan *et al*., 2013 and Erler and Polat, 2015). Lately, essential oils have become popular because of their specificity to pests, biodegradable nature, and potential for commercial application (Yazdani *et al.*, 2014). Until now, no resistance or adaptation to essential oils has been reported (Bakkali *et al*., 2008).

The aim of the present study was to investigate the possible toxic effects of some essential oils for their potential efficacy to control *M. scalaris* larvae. Besides, their impacts on proteins and detoxification enzymes activities were also studied.

MATERIALS AND METHODS

Insect Rearing:

Adults of *Megaselia scalaris* were allowed free access to sugar and cotton pads soaked in milk powder dissolved in water (10% w/v). Larvae were reared according to the method described by Pavela (2008) on a mixture of sterilized bran (38 g), milk powder (2 g) and water (60 ml) and maintained at 27±2°C and 70±5% relative humidity (rh).

Essential Oils:

The tested oils selected for the present study are listed in Table 1. It includes six botanical oils belonging to four different families. Oils were obtained from local

market of natural herbs. Plant species selected for the present investigation are: chamomile, clove, orange, rosemary, spearmint and sweet basil. The mainline value in the selection of the tested oils were their pesticidal properties against different insects. As well as, their desirable properties as food spiciness and folk and pharmaceutical medicine preparations.

Table 1. General information about the tested botanical oils.

English name	Family name	Scientific name	Arabic name
Chamomile	Compositae	*Matricaria chamomilla*	Babung
Clove	Myrtaceae	*Syzygium aromaticum*	Kuronfil
Orange	Rutaceae	*Citrus sinensis*	Bortokal
Rosemary	Lamiaceae	*Rosemarinus officinalis*	Hasalban
Spearmint		*Mentha spicata*	Naanaa
Sweet Basil		*Ocimum basilicum*	Rehan

Larvicidal Activity of the Essential Oils:

The susceptibility of 1st instar larvae was assayed towards the tested essential oils (chamomile, clove, orange, rosemary, spearmint and sweet basil) using the food contamination method. Six concentrations (0.5, 1, 2, 3, 4 and 5 ml/100g media) of each oil were prepared by using acetone as a solvent. The treatment was done by mixing the appropriate concentrations with rearing media, which in turn was spread on a paper for 30 minutes to evaporate the solvent. Each treatment was divided into 3 equal samples, kept in 250 ml beakers. Control was prepared similarly, without adding the tested oil. Active 1st instar larvae were collected from the laboratory culture. A group of 30 larvae were placed in each beaker. Beakers were labelled, covered with muslin and incubated for 5 days until pupation at laboratory conditions mentioned above. All treatments were observed for the number of formed pupae. The mortality percentages were calculated from the difference between the number of the tested larvae and the number of resulted pupae.

Statistical Analysis:

The results of the bioassay were corrected for control mortality using formula described by Abbott (1925). Then, results were represented graphically as Probit. Log. Regression Lines using Excel 2016 software to obtain the line slope values. Sublethal concentrations LC_{50} and their confidence limits at 95% probability were done according to the method described by Litchfield and Wilcoxon (1949).

Biochemical Studies :

For biochemical analysis, samples of whole-body homogenate *M. scalaris* larvae were prepared according to the method adopted by Selem and El-Sheikh (2015). Larval media were treated with the estimated LC_{50} of each tested oil. Early healthy 1st instar larvae (100 larvae) were transferred from rearing media to each concentration. After three days of treatment, surviving 3rd larval instar from each treatment was collected, washed by distilled water, dried and kept at 4°C to die. One gram of the collected larvae for every treatment was separately homogenised in 2 ml of cold physiological saline solution (NaCl 8.8 g, KCl 0.2 g, and $CaCl_2$ 0.3 g/L, pH 6.7), with traces of phenylthiourea crystals, using a glass homogenizer for 3 min. Homogenates centrifuged at 10000 g for 10 min at 4 °C in a refrigerated centrifuge. Control specimens were obtained by homogenising healthy larvae through the same technique. The supernatants could be used directly or dispensed into 100 µl aliquots and stored at -20°C until needed for the estimation of total protein contents and electrophoretic separation of both proteins and isozymes.

Total Protein Content :

The total protein content of the larval homogenate was assayed according to the method of Bradford (1976), in which Coomassie Brilliant Blue G-250 was used. 0.1 ml of the diluted homogenate samples were mixed with 5 ml of protein reagent. Bovine serum albumin was used as the standard. The absorbance was read at 595 nm.

Statistical Analysis :

Results were expressed as mean ± standard deviations (SD). The statistical significance of differences between means was determined by student "*t*-test" for paired observations (treated and control). The level of significance was stated to be highly significant ($p<0.01$), significant ($p<0.05$) or insignificant ($p>0.05$). The percentages of change were calculated using the following equation: Percentage of change = [(test-control)/control]*100.

Native Proteins and Isozymes Profiles :

Electrophoretic pattern of native proteins and isozymes were studied in larval homogenate for both treated and untreated samples. Pharmacia® gel electrophoresis apparatus GE-2/4 LS, at 10 °C and 100 V per gel slab, was used for the electrophoretic separation of both native proteins and isozymes (*β*-esterase, alcohol dehydrogenase and aldehyde oxidase).

Native Protein Profiles :

Native homogenate proteins of control and treated samples were separated using non-denaturing discontinuous polyacrylamide gel electrophoresis (Disc-PAGE) according to the method of Davis (1964). Separation was carried out with a 4% stacking gel and a 10% separating gel. Samples (150 ug protein) were applied in 40 % solution of sucrose (Akhtar 2000). Run was terminated after about 5 hours. The separated bands of proteins were stained using Coomassie Brilliant Blue R-250. The rate of flow (Rf) and amount percentage (Am%) of each band were accomplished using Gel-Pro Analyzer (Version 3.0) at the Central Lab of Biological and Geological Sciences Department, Faculty of Education, Ain Shams University, Egypt.

Isozymes :

Non-denaturing polyacrylamide gel electrophoresis was conducted to identify isozymes variations. The estimated isozymes were *β*-esterase (*β*-Est), alcohol dehydrogenase (ADH) and aldehyde oxidase (AO). Isozymes were separated in 10% polyacrylamide gel electrophoresis.

***β*-Esterase (*β*-Est):**

Separation of *β*-esterase patterns was achieved by polyacrylamide gel electrophoresis (PAGE) technique (Salama *et al.*, 1992). After electrophoresis, the gel was soaked in 0.5 M borate buffer (pH: 4.1) for 90 min. at 4° C to lower the pH of the gel from 8.8 to 7 at which the reaction proceeds readily (Sims, 1965). The gel then was rinsed rapidly into two changes of bi-distilled water. Then, the gel was stained for ester-lytic activity by incubation at 37° C in a solution of 100 mg *β*-naphthyl acetate (as a substrate) in 2 ml acetone and 100 mg fast blue RR salt in 200 ml of 0.1 M phosphate buffer pH 6.5 (Sell *et al.*, 1974). After incubation, the gel was distained in 7% acetic acid.

Alcohol Dehydrogenase (ADH):

After electrophoresis, the gel was soaked in 100 ml Tris/HCl pH 8.5 which mixed with 20 mg Nitro blue tetrazolium salt **(**NBT), 25 mg Nicotinamide Dinucleotide (NAD) and 4 ml Isopropanol for 30 min., then 5 mg Phenazin

Methosulphate (PMS) were added. The gel was stained in a dark place at 37 °C for 3 h. After incubation, the gel was preserved in 7% acetic acid (Jonathan and Wendel, 1990).

Aldehyde Oxidase (AO):

After electrophoresis, the gel was rinsed in 100 ml Tris/HCl pH 8.5 which mixed with 20 mg NBT, 10 mg Ethylene Di-amino Tetra-Acetic acid (EDTA), 25 mg NAD, 100 mg KCl and 1 ml Benzaldehyde, for 30 min., then 5 mg PMS were added. The gel was stained in a dark place at 37 °C for 3 h. After incubation, the gel was preserved in 7% acetic acid (Ayala *et al.*, 1972).

Gels of native proteins and isozymes were photographed, scanned and analysed using Gel-Pro Analyzer (Version 3.0) at the Central Lab of Biological and Geological Sciences Dept., Fac. of Education, Ain Shams Univ., Egypt.

RESULTS

Larvicidal Activity:

The data obtained from the susceptibility tests of *M. scalaris* larvae to the tested essential oils (chamomile, clove, orange, rosemary, spearmint and sweet basil) were shown in Table 1. The estimated values of the sublethal concentration LC_{50}, at 95% probability, were 5.37, 2.38, 3.16, 3.61, 2.90 and 3.71 ml/100g media, respectively. Results revealed that clove oil was the most effective larvicide (LC_{50} is 2.38 ml/100g media), whereas, chamomile was the least effective one (LC_{50} is 5.37 ml/100g media). Significant concentration-mortality responses of the larvae were observed in all tested oils; as indicated by the positive slope values (Table 1). Clove-oil treatment had the highest slope (17.28), whilst, chamomile had the least one (9.74). Higher slopes indicate greater concentration-mortality response.

Table 2. Larvicidal activities of tested essential oils against *M. scalaris* larvae.

Conc. (ml/100g media)	Chamomile		Clove		Orange		Rosemary		Spearmint		Sweet basil	
	Observed mortality % (obs.)	Corrected mortality % (cor.)	Obs. %	Cor. %	Obs. %	Cor. %	Obs. %	Cor. %	Obs. %	Cor. %	Obs. %	Cor. %
0.5	18.89	3.94	26.67	13.16	22.22	7.89	27.78	14.47	23.33	9.20	25.56	11.84
1	22.22	7.89	41.11	30.26	36.67	25.00	30.00	17.10	37.78	26.32	32.22	19.73
2	24.44	10.52	53.33	44.73	44.44	34.20	38.89	27.63	42.22	31.57	45.56	35.53
3	42.22	31.57	65.56	59.21	54.44	46.05	54.44	46.05	61.11	53.94	47.78	38.16
4	48.89	39.47	83.33	80.26	67.78	61.84	64.44	57.89	74.44	69.73	65.56	59.21
5	52.22	43.81	94.44	93.42	80.00	76.31	68.89	63.16	83.33	80.26	66.67	60.53
control	15.56	00.00										
Slope	**9.74**		**17.28**		**14.15**		**11.87**		**15.52**		**11.14**	
LC_{50} (confidence limits*)	**5.37 (3.65, 7.89)**		**2.38 (1.57, 3.62)**		**3.16 (2.24, 4.46)**		**3.61 (2.62, 4.98)**		**2.90 (2.03, 4.15)**		**3.71 (2.71, 5.08)**	

- Three replicates/treatment, 30 larvae/replicate. *Confidence limits at 95% probability

Total Protein Contents:

Table 3 and Fig. 1 show the effects of the tested plant oils on the total protein contents of *M. scalaris* 3rd instar larvae treated as first instars. The estimated protein content of the control larvae was 12.80±2.68 mg/g tissue. Application of clove, orange and spearmint oils produced a significant increase ($p<0.05$) on the total

protein content of treated larvae, amounted to be 92.19, 70.31 and 57.58%, respectively, compared to that of the control larvae. While, treatment with chamomile, rosemary and sweet basil oils produced insignificant ($p>0.05$) increase on the total protein contents of the treated larvae, amounted to be 9.92, 45.31 and 14.30%, respectively, as compared with the control value.

Table 3. Effect of the LC_{50} of tested essential oils on total protein content of *M. scalaris* third larval instar treated as first larval instar.

Treatment	Mean[(1)] (mg/g tissue)	SD[(2)]	% Change	*t*-test	
				p-value	Significance level[(3)]
Control	12.80	2.68			
Chamomile	14.07	2.35	9.92	0.70	ns
Clove	24.60	1.25	92.19	0.03	*
Orange	21.80	0.85	70.31	0.04	*
Rosemary	18.60	2.65	45.31	0.14	ns
Spearmint	20.17	1.88	57.58	0.04	*
Sweet Basil	14.63	0.81	14.30	0.26	ns

[(1)]Mean of three replicates. [(2)] Standard Deviation. [(3)] (Significance level: ns, insignificant (P>0.05) and *, significant (P<0.05).

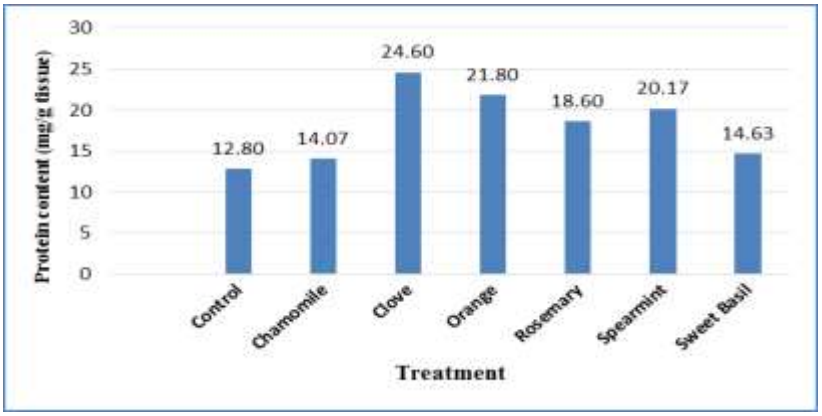

Fig. 1. Effect of the LC_{50} of tested essential oils on total protein content of *M. scalaris* larvae.

Native Protein Profiles

The native protein profiles of *M. scalaris* 3rd instar larvae treated with LC_{50} of each tested oil were illustrated in Fig. 2 and their computer analysis was represented in Table 4. The maximum number of native protein bands was seven at approximate rate of flow (Rf) ranging between 0.14 to 0.72 and observed in the control, clove, orange, spearmint and sweet basil oils treatments, whereas the chamomile and rosemary oils treatments have six bands at the same Rf range.

The obtained results showed that five common native protein bands were observed in both controls and treated larvae with Rf values of 0.14, 0.39, 0.43, 0.58 and 0.72. One newly native protein band (with Rf of 0.47) was detected in whole body homogenate of clove-, orange-, rosemary-, spearmint- and sweet basil- treated larvae which were not found in both control and chamomile- treated ones. Disappearance of protein band (with Rf of 0.37) from larval tissue homogenate of all treated larvae was observed. Also, the disappearance of a native protein band (with Rf of 0.55) was observed with rosemary-treated larvae.

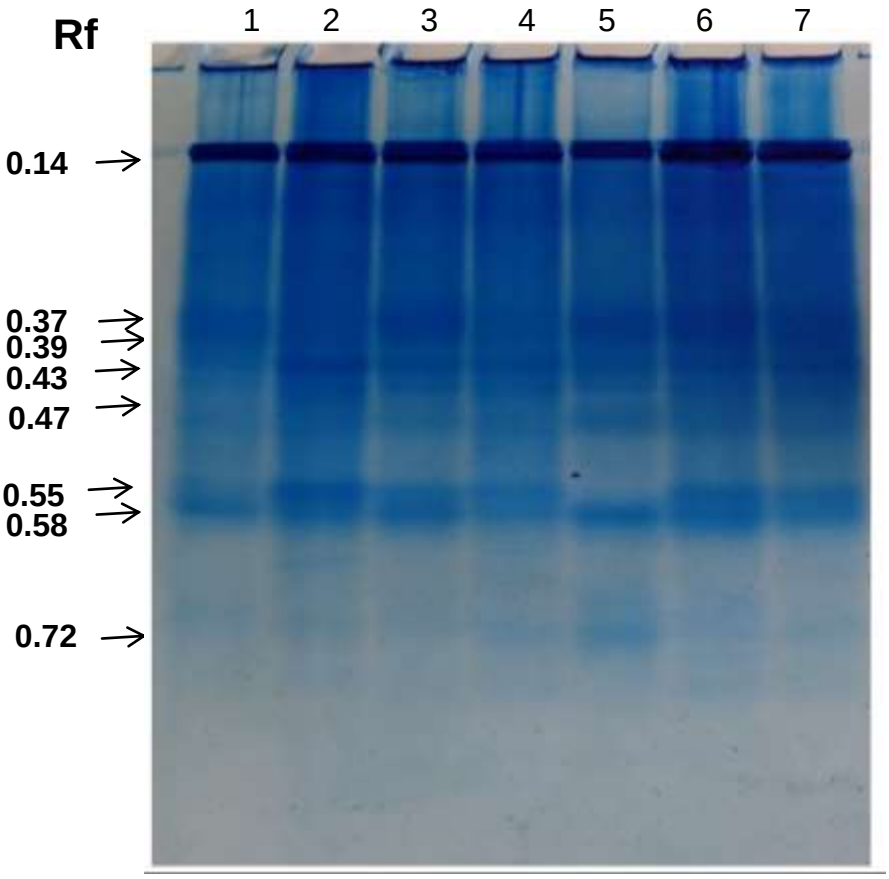

Fig. 2: Electrophoretograms of native protein patterns of control and treated *M. scalaris* 3rd instar larvae after application of essential oils. Lane 1 control larvae; Lanes (2-7) larvae treated with LC_{50} of chamomile, clove, orange, rosemary, spearmint and sweet basil, respectively

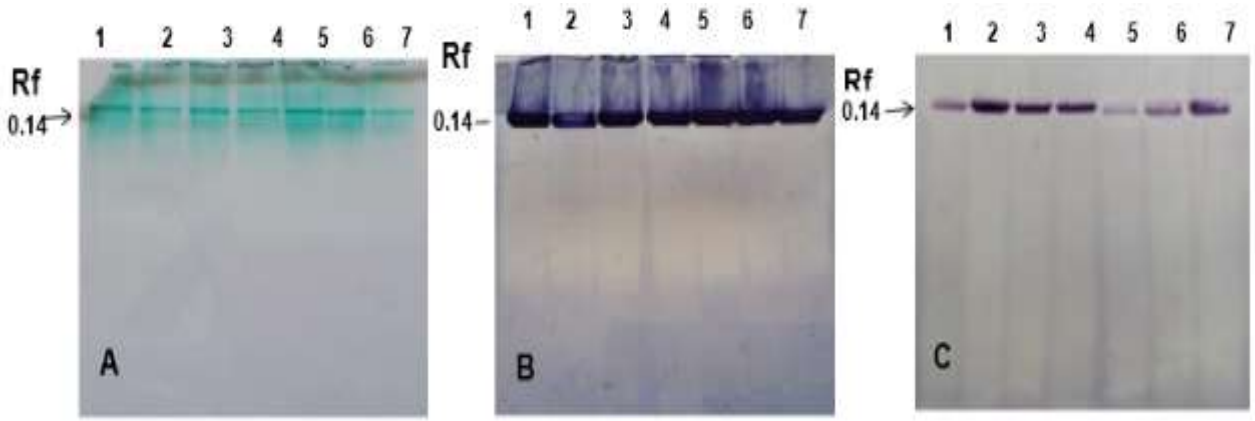

Fig. 3 (A, B and C): Electrophoretograms of *β*-Est, ADH and AO patterns, respectively, of control and treated *M. scalaris* 3rd instar larvae after application of essential oils. Lane 1 control larvae; Lanes (2-7) larvae treated with LC_{50} of chamomile, clove, orange, rosemary, spearmint and sweet basil, respectively

Table 4. Rate of flow (Rf) and amount percentages (Am) of native protein bands detected in electrophoretograms of 3rd larval instar of *M. scalaris* as control and treated with essential oils.

Row.	Rf	Control		Chamomile		Clove		Orange		Rosemary		Spearmint		Sweet Basil	
			Am		Am		Am		Am		Am		Am		Am
1	**0.14**	+	18.10	+	24.11	+	23.65	+	23.57	+	15.58	+	36.64	+	36.51
2	**0.37**	+	33.02	-		-		-		-		-		-	
3	**0.39**	+	12.00	+	21.43	+	21.88	+	21.43	+	15.96	+	13.14	+	17.13
4	**0.43**	+	5.44	+	12.80	+	10.00	+	11.07	+	11.06	+	9.64	+	8.36
5	**0.47**	-		-		+	10.61	+	6.90	+	22.02	+	16.64	+	9.85
6	**0.55**	+	6.47	+	18.33	+	8.01	+	13.63	-		+	8.29	+	13.90
7	**0.58**	+	14.88	+	11.31	+	16.80	+	13.33	+	24.23	+	10.21	+	7.38
8	**0.72**	+	10.10	+	12.20	+	9.01	+	9.88	+	11.35	+	5.42	+	7.18
Total no. of bands		**7**		**6**		**7**		**7**		**6**		**7**		**7**	

Isozymes Profiles:

β-Esterase (*β*-Est), alcohol dehydrogenase (ADH) and aldehyde oxidase (AO) banding patterns are illustrated in Fig. 3 (A, B and C, respectively). Whilst, the rate of flow (Rf) and amount percentages (Am%) of the three isozyme patterns detected in electrophoretograms are tabulated in Table 5. In the present study, the three tested isozymes (*β*-Est-, ADH- and AO-patterns) of whole body homogenate of untreated and treated 3rd instar larvae of *M. scalaris* appeared as only one band (with Rf of 0.14). Essential oils treatments did not affect the isozymes profiles but alter the amount percentages of it. Table 5 shows a general reduction in the amount percentages of the three tested isozymes, except with AO in spearmint-treated larvae.

Table 5: Rate of flow (Rf) and amount percentages (Am%) of *β*-Est, ADH and AO isozyme pattern detected in electrophoretograms of third larval instar of *M. scalaris* as control and treated with essential oils.

Treatment	Amount percentages (Am %)		
	***β*-Est (Rf=0.14)**	**ADH (Rf=0.14)**	**AO (Rf=0.14)**
Control	6.09	21.20	9.34
Chamomile	3.61	15.00	4.84
Clove	3.63	19.90	3.42
Orange	2.79	18.50	8.18
Rosemary	3.83	17.90	8.79
Spearmint	4.62	18.10	12.50
Sweet Basil	3.44	16.00	5.35

DISCUSSION

In view of many well-known problems stick to the use of synthetic insecticides, there was a great need to find alternatives for the control of insect pests. The use of botanical oils was reported by many authors. El-Sabaay (1998) studied the effects of some plant oils against stored product insects. Additionally, Prakash and Rao (1997) reported 866 plant species and identified 256 pesticidal active

ingredients. Nonetheless, the use of botanical oils is still limited to small scales.

Larvicidal Assay:

Larvicidal assay of the tested plant essential oils (Chamomile, clove, orange, rosemary, spearmint and sweet basil) against larvae of *M. scalaris* revealed that there was a positive correlation between the oil concentration and its corresponding mortality. As the oil concentration increased, the corresponding larval mortality percent was also increased. The present results were in accordance with Alrubeai *et al.* (2001) and Reuben *et al.* (2006) who studied the effects of clove extracts against larvae of *Phthorimaea operculella* and *Plutella xylostella*, respectively. Besides, results agreed with Abdel-Rahman *et al.* (2011) who studied twenty of botanical extracts and products against the almond moth, *Cadra cautella*, and with Ismail (2018) who reported the same effects with some medicinal oils against stored products beetles.

The obtained data revealed that plant essential oils had a considerable larvicidal effect. The most effective oil was the clove (LC_{50}: 2.38 ml/100 g media) and the least effective was chamomile oil (LC_{50}: 5.37 ml/100 g media). Several plant essential oils are tested around the world to control a wide variety of pests. Plant components responsible for insecticidal activities are known as "allelochemicals". Research on the site and mechanism of action of such components indicates that many terpenoid compounds are involved in insecticidal and insect growth regulation activities. These substances proved to be important enzymatic, metabolic and metamorphosis inhibitors (Panzuto *et al*., 2002; Kubo *et al.*, 2003 and Cespedes *et al*., 2004). In the same manner, some plant oils and extracts were tested against mushroom scatopsid flies and showed a considerable toxicity against this pest in different degrees (Basbagci and Erler, 2013).

Total Protein Content :

Proteins act as essential components of organisms' structural material, in contrast to that of carbohydrates and fats which are elements for energy supply. Moreover, protein plays an important role in all biological functions as they catalyze biochemical reactions, regulate hormones and share in cell structure (Daas-Maamcha *et al.* 2013). The present results showed that plant oil treatments of the *M. scalaris* larvae resulted in a significant ($p<0.05$) increase in the total protein content in the whole body of third instar larvae treated as first instars with clove, orange and spearmint oils. Whereas, treatments with chamomile, rosemary and sweet basil oils induced insignificant ($p>0.05$) increase in the total protein contents of the treated larvae as compared with the control value. The protein content was also increased in other insect species treated with botanical products: Hewady *et al.* (2002) on their study on *Pectinophora gossypiella* with azadirachtin, and Shoukry *et al.* (2003) on *Plodia interpunctella* treated with oils of *Piper*, *Salvia*, *Rumex*, *Trigonella* and *Acacia*. On the other hand, the present results disagreed with those of Sandhya and Ghule (2003) on *Corcyra cephalonica* treated with azadirachtin, Mostafa (1993) on *Trogoderma granarium* treated with *Thymus*, *Curcuma* and *Piper* extracts, Abou El-Ela *et al.* (1995) on *Musca domestica* treatedwith *Catotropis procera* extract, El-Sheikh (2002) on *Agrotis ipsilon* treated with *Melia azedarach*. Abd El-Wahab (2002) on *Spodoptera litura* treated with *Argemone* and *Nerium* extracts and Khalaf (1998) on *Musca stabulas* treated with *Cymbopogon* and *Rosmarinus* oils. All the later mentioned authors reported a decrease in the total protein contents in the treated larvae. Anyhow, the total protein content varies markedly from insect to another and between different developmental instars of a single insect. It is a general phenomenon that insect structural compositions are altered by diverse factors such as

nutrition, temperature, species susceptibility, pesticide efficacy and diseases (Mullins, 1985).

The possible explanation for the protein elevation could be due to the increase of metabolic activity inside treated-larval tissue to compensate the stress activity caused by the tested oils and hence proteins accumulated in the haemolymph (Abdel-Gawad and Ismail, 2018). Nath *et al.* (1997) suggested that the elevation of protein and amino acid levels may be explained as a preparation for the synthesis of cuticular proteins because of insecticide stress. Another explanation is the hyperecdysonism, a state of higher ecdysteroidal activity in insects, where abnormal moulting processes leading to death may occur. (Smagghe *et al*., 2012; Basiouny *et al.,* 2016 and Su and Monteagudo, 2017).

Native Proteins Profile :

Electrophoresis is one of the best techniques for assessing both pattern and amount percent of different components inside insects. In the present investigation, proteins were prepared in a non-reducing non-denaturing sample buffer, which maintained the proteins secondary structure and native charge density. Generally, each protein reflected the specific gene activity through the enzymatic production which is responsible for a specific biological character (Hassan and Abd El-Hafez, 2009). Thus, changes in protein profiles may mirror proficiency and adaptation in the organism (Kanost *et al*., 1990).

In the present study, electrophoresis was used to evaluate the effects of the sublethal concentration, LC_{50}, of the tested essential oils on *M. scalaris* 3rd instar larvae, treated as 1st instars, to investigate the native protein profiles. During separation, the electrophoretic mobility depends not only on the charge-to-mass ratio, but also on the physical shape and size of the protein.

Results showed that one newly native protein band has appeared in treated larvae with clove, orange, rosemary, spearmint and sweet basil oils treatments. These observations might attribute to the formation of an immune protein as a defence action to the oil treatment (Dimarcq *et al*., 1990 and El-Aziz and Awad, 2010). This also being prompted after the application of insect growth regulators against *S. littoralis* (El-Bermawy, 2005).

Isozymes Profile :

Any set of enzymes that are similar in catalytic properties and occurring in different tissues in a single species but are differentiated by variations in physical properties, such as isoelectric point, was called isoenzyme (isozymes)**.** Isozymes estimation is considered as a good biochemical marker to differentiate between different species and treatments (Elsayed and Amer, 2014). Determination of isozymes has an important role to evaluate the possible effects of tested essential oils treatment against the 3rd instar larvae of *M. scalaris*. In the present study, three isozymes polymorphisms were estimated in the body homogenate of the treated larvae to manifest the biochemical genetic fingerprints of the treatments. The isozymes were: β-esterase (β-Est), alcohol dehydrogenase (ADH) and aldehyde oxidase (AO).

***β*-Esterases (*β*-Est):**

Esterases are very large class of enzymes, all of which can catalyze and hydrolyze the ester bond within lipophilic compounds into its alcohol and acid (El-Bermawy, 2004). Thus, esterases play important roles in the pest resistance towards insecticides (Chang *et al*., 1995). So, it represents one of the major enzyme groups responsible for detoxification of insecticides inside treated insects before these toxic compounds reach the targets (Feng *et al*., 2018). It can also play an important role in

detoxification of xenobiotics (Shen and Dowd, 1991). Esterases can often be separated into isozymes with different substrate specificities. Insect esterases are very diverse and can include monomer, dimers and multimers, which means that their relative molecular mass can cover a wide range (Kao *et al*., 1985).

In the present study, there were no qualitative differences in *β*-Est patterns between control and treated larvae. These results agreed with Abdel-Ghany (2010) who reported that there were no changes in the number of bands in case of castor oil treated larvae of *S. littoralis*.

A reduction in the amount percentages of *β*-Esterase was detected in electrophoretograms of 3rd instar larvae of *M. scalaris*. These results were in accordance with Hu *et al.* (1999) who reported that the esterases were significantly reduced specially acetylcholinesterase in *Pieris rapae* after treating it with the extract of *Myoporum bontioides*. Hussein *et al.* (2002) showed that all the activities of esterase isozymes in the larvae of pink and spiny boll worm treated with neemazal were lower than that of untreated larvae by using PAGE analysis. Besides, Qari *et al*. (2017) reported inhibition of acetylcholinesterase in *rhyzopertha dominica* after treatment with *Zinigiber officinale*. Julio *et al.* (2017) clarified that esterases are involved in multiple resistant phenotypes in *Tribolium castaneum* towards insecticides. The reduction in isozymes amount percentages may be due to the effectiveness of the tested essential oils which affected the detoxification enzymes activities.

On the contrary, the present results disagreed with that obtained by Hassan and Mohamed (2008) who found that in *P. gossypiella* larvae treated with *Coriandrum sativum* and *Cymbapoyon citratus*, more esterase bands than untreated larvae. Also, Abdel-Ghany (2010) reported appearance of a new band of esterases detected by α-NA in *S. littoralis* larvae treated with gossypol.

Alcohol Dehydrogenase (ADH):

Alcohol dehydrogenases are a group of dehydrogenase enzymes that are responsible for converting alcohols to aldehydes or ketones during larval feeding. The alcohols are produced inside the living organism during various metabolic processes. The high concentration of alcohols inside the living organism cause disturbance in different metabolic pathways, so, the living organism must regulate these alcohols through an alcohol metabolizing enzyme such as alcohol dehydrogenase (Parkash and Shamina, 1994; Fry and Saweikis, 2006). Likewise, Edenberg (2007) reported that ADH is essential for breakdown the toxic effects of alcohols and production of useful aldehydes, ketones or alcohol groups during metabolites biosynthesis making it possible to eliminate it from the body. First, ADH metabolizes alcohol to acetaldehyde, a carcinogenic substance, then, acetaldehyde is further metabolized to a less active by-product called acetate, which is finally broken down into water and carbon dioxide for easy excretion (McKechnie and Geer, 1984). Also, Heinstra *et al*. (1983) postulated that ADH is efficient in oxidizing acetaldehyde to acetate.

Dehydrogenases estimation is a vital tool for the studying of different pesticides efficacy against insects (Ghoneim *et al.*, 2014). It was found that ADH plays an important role in alcohol detoxification in *D. melanogaster* larvae (Fry and Saweikis, 2006). In the present study, there were no changes in ADH profile but there was a reduction in its amounts in the treated larvae. So, it is supposed that tested oils interfere with the immune system of the treated larvae, as indicated in the larvicide activity of the oils against the tested insect.

Aldehyde Oxidase (AO):

Aldehyde oxidase (AO) is a member of the molybdenum hydroxylase family. It acts as a catalyst in the conversion and oxidation of aldehydes into carboxylic acid in many organisms. Aldehyde oxidase isozyme patterns were studied in several insects. Garcin *et al*. (1983) demonstrated the capacity of AO in *D. melanogaster* to detoxify acetaldehyde and use it for energy production. Moreover, Barnes (1983) reported that the inversion-allozyme polymorphism of AO is associated with the accommodation to the environment. Also, AO isozyme in adult *Hypera postica* can be influenced by developmental stage and environmental conditions (Romero *et al*., 1986).

In the present work, AO recorded one zone in the whole-body homogenate of control and treated *M. scalaris* larvae. However, treatment with the tested plant oils resulted in a reduction in the amount of AO isozyme in all treatment as compared with the control value. These results disagreed with Bakr *et al.* (2006) who found new band of AO in isozymes pattern of *S. littoralis* larvae treated with *Oryza sativa* and *Citrus aurantium*.

The above results revealed that *β*-Est, ADH and AO recorded as one zone in the whole-body homogenate of control and treated *M. scalaris* larvae but with different densities. The reduction of isozyme activities, as shown in reduction of their amount percent, refers to the effectiveness of the tested oils in disturbance of the internal defence components of oil-treated larvae.

Conclusion

In the present investigation, plant essential oils treatments showed larvicidal activities towards *Megaselia scalaris* larvae in different degrees. Also, tested oils increased the total protein contents and decrease the percentages of the detoxification enzymes in the treated larvae as compared with the control values. These results suggested that plant oils can disturb the internal defence components inside the treated insect larvae. Moreover, these biochemical changes could be considered as biochemical indicators for oil-treatments stress.

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ARABIC SUMMARY

سمية بعض الزيوت الأساسية ضد الذبابة المسببة لأمراض النغاف (Myiasis)، *ميجاسيليا سكالاريس*، وآثارها على البروتينات وإنزيمات إزالة السموم.

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تعتبر *الميجاسيليا سكالاريس* (ذات الجناحين: فوريدى) حشرة مهمة طبيا في جميع أنحاء العالم لأنها مصدر لأمراض النغاف (myiasis) المختلفة. لذلك ينبغي السيطرة عليها من أجل رفاهية الإنسان ولأسباب اقتصادية. تبعا لذلك، تم تحديد النشاط المبيد لليرقات لستة من الزيوت النباتية الأساسية ضد يرقات *الميجاسيليا سكالاريس*، وتم تقدير آثارها على البروتينات وكذلك مشابهات الإنزيمات في أجسام اليرقات للأعمار اليرقية الثالثة. وكانت الزيوت الأساسية المختبرة: البابونج (*Matricaria chamomilla*) والقرنفل (*Syzygium aromaticum*) والبرتقال (*Citrus sinensis*) وإكليل الجبل (*Rosemarinus officinalis*) والنعناع (*Mentha spicata*) والريحان (*Ocimum basilicum*). وأظهرت النتائج أن زيت القرنفل كان لديه أعلى نشاط قاتل لليرقات، LC_{50} is 2.38 ml/100g media ، في حين أن زيت البابونج كان أقلها، LC_{50} is 5.37 ml/100g media. ولوحظ وجود علاقة طردية بين التركيزات والوفيات من اليرقات المعالجة تجاه جميع الزيوت المختبرة. وفيما يتعلق بتقدير البروتين، كان محتوى البروتين في العينات الضابطة 12.80±2.68 mg/g tissue، وأنتج تطبيق الزيوت ارتفاعا ملحوظا في محتوى البروتينات الكلية في اليرقات المعالجة. وكان هذا الارتفاع معنويا ($p<0.05$) مع زيوت القرنفل والبرتقال والنعناع، والتي بلغت ٩٢,١٩، ٧٠,٣١، ٥٧,٥٨%، على التوالي، مقارنة بنتائج المجموعة الضابطة، ولكن كان الارتفاع غير معنويا ($p>0.05$) مع زيوت البابونج واكليل الجبل والريحان. وكشفت دراسة التحليل الكهربي أن العدد الأقصى لنطاقات البروتينات الأصلية كان سبعة عند معدل تدفق بين ٠,١٤ إلى ٠,٧٢ تقريبا، وذلك مع المجموعة الضابطة والمجموعات المعالجة بزيوت القرنفل والبرتقال والنعناع والريحان. ولوحظ تكون مجموعة بروتينية جديدة في أجسام اليرقات المعالجة بالقرنفل والبرتقال واكليل الجبل والنعناع والريحان. وأظهرت بروفايلات -β esterase, alcohol dehydrogenase and aldehyde oxidase نفس الأنماط في كل من اليرقات الضابطة واليرقات المعالجة. ومن ناحية أخرى، كان هناك انخفاضا في النسبة المئوية لمحتوى الإنزيمات في اليرقات المعالجة بالمقارنة بقيم اليرقات الضابطة. هذا ويمكن استخدام التغيرات البيوكيميائية في البروتينات ومشابهات الإنزيمات كمؤشر كيميائي حيوي للإجهاد السام.