Bioactivities and Biochemical Effects of Marjoram Essential Oil used against Potato Tuber Moth *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae)

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Abstract: The bioactivities of marjoram essential oil against immature stages and adults of potato tuber moth *Phthorimaea operculella* Zeller were evaluated. The essential oil showed significant contact and fumigation insecticidal activities against different stages. The oil revealed strong contact toxicity and moderate fumigant activity against immature stages. Both adult males and females showed high susceptibility to the fumigation. Oviposition deterrent effects were found to be insignificant. Furthermore, the results showed that treatment of immature stages with the essential oil produced adult deformations. The essential oil tested had some biochemical effects on the last larval instar treated by the contact method, based on LC_{50} during metamorphosis to the adult. The results showed increases in the total protein and triacylglycerol content of most post-treatment days. Insignificant increases were found in the activities of acetylcholinesterase and chitinase**.** These results suggested that marjoram essential oil could be used as a potential control agent for potato tuber moth in storage facilities.
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1. Introduction:

The potato tuberworm, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is an important and ubiquitous pest of potato, *Solanum tuberosum* L. (Solanaceae), in both field and stores in the subtropical and tropical zones (Golizadeh & Razmjou 2010). It is wide spread in Egypt, especially in the northern areas of Lower Egypt (Sharaby *et al.,* 2009).

An integrated pest management (IPM) strategy for potato tuber pests has been developed and promoted by various institutions. The main component of this IPM package is a biopesticide that is applied to the surface of the tubers in farm storage (Zeddam *et al.*, 2008). Among biopesticides, botanical pesticides have received a great deal of attention because of their favourable eco-toxicological properties, e.g., low human toxicity, rapid degradation and reduced environmental impact. These properties make them suitable insecticides for organic agriculture. Aromatic plants are among the most effective insecticides of botanical origin. Essential oils often constitute the bioactive fraction of plant extracts (Shaaya *et al,*. 1991; 1997; Regnault-Roger 1997). Their have lipophilic nature facilitates their interference with basic metabolic, biochemical, physiological and behavioural functions of insects (Nishimura, 2001). They have potential as ovicides, fumigants, insect growth regulators and insecticides against various insect species (Regnault-Roger, 1997 and Shaaya *et al,*. 1997). In addition, most of these substances are volatile and can act as fumigants, thus offering the option of use against stored-product insects (Stamopoulos *et al*, 2007). Sweet marjoram *Majorana hortensis* Moench (family: Lamiaceae) is an Old World perennial aromatic herb that was cultivated and used as flavouring in foods. The leaves and stems yield an essential oil. Its volatile constituents have previously been found to have a broad spectrum of biological activities, including antifeedant, repellent and insecticidal properties. It is used against a number of agricultural and stored-product pests (Pavela, 2004; Mohamed and Abdelgaleil, 2008). Numerous studies have addressed the general use of essential oils against *P. operculella* (Guerra *et al.*, 2007; Sharaby *et al.*, 2009).

Compounds extracted from plants, or the derivatives of such compounds may affect insect physiology in various ways (Shekari *et al.,* 2008). This investigation aimed to investigate the repellency, toxicity and some biochemical effects of crude oil extracted from *M. hortensis* for use against *P. operculella*.

2. Material and Methods:

2.1. Insects:

A culture of *P. operculella* was maintained in our laboratory over 3 years without exposure to insecticides. Larvae were kept in wire cages and reared on potato tubers. The bottoms of cages were furnished with a thin layer of clean sand (previously exposed to a high temperature in an oven) for pupation (El-Sinary, 1995). Culture and experiments

were maintained at 29±1º C, and 12L: 12D photoperiod.

2.2. Essential oil:

Sweet marjoram *(Majorana hortensis)* essential oil (EO) was purchased from El-captain Company (CAP. PHARM., Egypt) for extracting natural oils, herbs and cosmetics, Cairo, Egypt.

2.3. Contact bioassay:

The insecticidal activities of the essential oil against larval (4^{th} last larval instar), prepupal and pupal stages were evaluated using the contact method in a sandy soil. Ten grams of clean sand was placed in 250 ml glass jars and treated with different doses (0.2, 0.1, 0.05, 0.025 and 0.012 ml) of oil solutions diluted in 1 ml of acetone. The sand was stirred continuously for 1 min to ensure the even spread of the oil over the surface. The solvent was allowed to evaporate for 10 min. Twenty individuals of each test stage were placed in the jars and then covered with a thin layer of the treated sand. In the control group, the sand was treated only with acetone. Each jar was covered with nylon mesh held in place with rubber bands. Mortality percentage was recorded 24 hours later. The experiments were observed until the emergence of the adults to assess total inhibition of metamorphosis and adult malformations.

2.4. Fumigant bioassay:

The fumigant activity of tested oil was determined according to the method described by Prates *et al.,* (1998). Twenty test insects (last larval instars, prepupae, pupae and adult males or females) were put into separate 250 ml glass jars. Marjoram essential oil at doses of (0.2, 0.1, 0.05, 0.025 and 0.012 ml) was diluted in 1 ml of acetone and applied to 5 cm diameter filter paper. The filter papers were attached to the underside surface of the screw caps of the glass jars after solvent evaporation (10 min). The jars were first covered with nylon mesh. The caps were then attached. This measure was taken in order to prevent a direct contact between insects and the bioinsecticide. Another group of filter papers was treated only with acetone and used for the control group. Mortality percentage was recorded 24 h later. Experiments with the immature stages (larvae, prepupae and pupae) were followed until adult emergence to assess total inhibition of metamorphosis and adult malformations.

2.5. Ovicidal bioassay:

The toxicity of marjoram essential oil to eggs was examined with contact and fumigant bioassays. Adult insects (males and females) were collected from the stock culture after emergence and put together in galss jars covered with muslin (5 cm diameter) for oviposistion. Muslin-egg batches of 1 day-old were collected, numbered and divided into two groups. In order to test the contact toxicity of essential oil, the first group of eggs was dipped in different concentrations of test oil (0.2, 0.1, 0.05, o.025 and 0.012 ml) diluted in 1 ml of acetone. Acetone solution was used only for control group. After drying for 20 minutes, egg batches were inserted in Petri dishes and subsequently covered. In order to test the toxicity of essential oil vapours, the second group of egg batches was inserted into 250 ml glass jars covered with filter papers attached to the under surface of the screw cap. The filter papers were treated previously with different concentrations of EO diluted in 1 ml of acetone (0.2, 0.1, 0.05, 0.025 and 0.012 ml) and allowed to dry. Another group of filter papers treated with acetone only were used for the control group. Hatchability percent was recorded after 3 days in all groups.

2.6. Oviposition repellency bioassay:

Muslin pieces (5 cm diameter) were treated with doses (0.008, 0.004, 0.002 and 0.001 ml) of EO diluted in 1 ml of acetone and dried for 20 minutes. Ten (one day old) sexed adults were placed in 250 ml glass jars. The muslin were attached to the under surface of the glass jar screw caps. The caps were screwed tightly on the jars. Another group of the muslin was treated with acetone only and used for the control group. The percent effective oviposistion repellency was recorded 24h later for three successive days.

2.7. Biochemical analysis:

Last instar larvae of *P. operculella* were treated with LD_{50} of the EO *M. hortensis* by the contact method previously described, in order to estimate the activities of chitinase and acetylcholinesterase (AChE), and total protein and triacylglycerol (TAG) content. The results were recorded during the metamorphosis of larvae to the adult stage at intervals of 0 and 1 day for larvae, 1, 3 and 6days for pupae and 1 day for adults.

Assays of AChE and chitinase activities were performed according to Waterhouse *et al.,* (1961) and Simpson *et al.,* (1964), respectively. The method described by Bradford (1976) was applied to measure total protein content. TAG was measured by using a kit produced by Randox Laboratories LTD. (United Kingdom BT294QY).

2.8. Statistical Analysis

LC_{50} value was determined according to Finney (1971) for the contact method. Means were tested for significance by the one way analysis of

variance (ANOVA). When the ANOVA statistics were significant ($P < 0.05$), means were compared using Duncan's multiple range test. Percent of insect mortality was calculated using the corrected Abbott's formula (Abbott, 1925).

The percent effective oviposition repellency for each dosage was calculated using the following formula: ER (%) = NC – NT / NC x 100,
where ER (%) = percent effective repellency, NC = number of eggs of control, and NT = number of eggs of treated group.

3. Results:

3.1. Contact toxicity:

Analysis of the toxicity data showed that marjoram essential oil exhibited strong toxic activity against larvae and prepupae after 24 h exposure (Table 1). All larvae and prepupae (100%) died at the highest dose (0.2 ml/10gm). The mortality percent decreased significantly with decreasing the dosage. The lowest dose induced insignificant mortality. On the other hand, the pupal stage was more tolerant than other stages. The highest dose induced a significantly higher mortality of 16.67% relative to the control. The other doses produced insignificant effects.

The data in Table (2) showed that *M. hortensis* affected the emergence of adult insects from treated larvae and prepupae. The highest dose (0.2 ml) induced significant reductions in the emergence of adults from all treated immature stages, relative to the control. Although insignificant effects of the contact bioassay on treated pupae were observed within 24 h of application, a significant reduction ($P< 0.05$) in adult emergence was observed at most dose levels. Some deformed adults emerged from immature stages treated by the contact method (Table 2). Some doses induced various degrees of adult deformation. Permanently dumpy fore-wings, expanded membranous wings, retained pupal skin, and failure of wing formation were observed.

3.2. Fumigant toxicity

These experiments were conducted in order to determine whether the insecticidal activity of marjoram oil against *P. operecculella* was attributable to fumigant action (Table 3). The oil exhibited strong insecticidal activity against larval and prepupal stages at the highest doses. The lowest doses (0.025 and 0.012 ml/250 ml) had insignificant effects against the same stages. As shown in the contact method, the pupal stage was more tolerant to fumigation than other stages. The mortality percentage of pupae was not significantly different from the control in all treatments. Fumigant efficacy tests of EO against adult males and females showed very high susceptibility in almost all treatments after 24 h exposure. The oil induced 100% mortality of in both males and females at s 0.2 and 0.1ml doses. The male was more susceptible to lower doses than was the female.

Some adults emerged from immature stages treated by the fumigant method (Table 4). Some doses induced various degrees of adult deformation. Permanently dumpy fore-wings, expanded membranous wings, retained pupal skins, and failure of wing formation were observed.

3.3. Ovicidal activity

In contact bioassay (Table 5), all doses had significant effects compared with the control. The strongest adverse effect on egg hatchability was observed at 0.2, 0.1 and 0.05 ml doses. Hatchability increased gradually as the dosage decreased. The same table shows that fumigation had significantly lower effects ($P< 0.05$) on egg hatchability. The lower doses seem to have moderate or insignificant effects on this biological parameter.

3.4. Oviposition repellency:

The data (Table 6) revealed that *M. hortensis* had insignificant oviposition deterrent activity. The data showed that even at the highest dose (0.008 ml), the effective oviposition repellency was only 11.5 %. This value does not differ significantly ($P< 0.05$) from those found in other treatments and control values. The hatchability percentage of eggs oviposited by the treated females did not differ significantly from the corresponding value for control females.

3.5. Biochemical analyses:

The effects of LC_{50} doses (0.037 ml / 10 gm) of EO applied by the contact method to the last larval instar were tested for biochemical changes during the metamorphosis to the adult stage.

Figure (1) shows that chitinase activity increased gradually with time in control and treated larvae. However, treated larvae had slightly increased enzyme activity. Chitinase could not be detected in adults emerged from both treated and control larvae.

AChE activity did not changed in treated larvae, relative to the control (Fig. 2). Insignificant inhibition of AChE activity was observed in treated larvae for all days during metamorphosis to the adult, relative to the control.

Triacylglycerol content (TAG) decreased gradually in both treated and control insects during the metamorphosis to the adult stage (Fig. 3). The amount of reduction in treated larvae was significantly higher ($P< 0.05$) than in untreated larvae. The highest reduction was observed after one day of treatment and 3 days of pupation. No

difference in TAG content was observed between the treated and control insects at the 1st day of pupation. TAG content increased suddenly on the 1st day for newly emerged adults, both in control and in treated insects. The degree of elevation was more marked in the treated insects.

Hyperproteinemia was observed in infected larvae during metamorphosis to the adult stage relative to the corresponding control (Fig. 4). The exposure of potato tuber larvae to the LD_{50} of *M. hortensis* resulted in elevation of the total protein content on the 1st day after treatment. The protein content of the day-one pupa decreased significantly ($P< 0.05$) and irreversibly. The total protein content of treated insects increased again at the 3rd and 6th days of the pupal stage relative to the values for the control. The day-one treated adult did not show a significant difference in total protein content, relative to the control. Generally, total protein content decreased in treated and control larvae.

Table (1): Contact toxicity of marjoram essential oil against different stages of *P. operculella* after 24h of exposure.

Dose (ml/10gm)	Mortality % ± SE		
	Larva	Prepupa	Pupa
0.2	100 ± 0.0[a]	100± 0.0[a]	16.67± 3.3[a]
0.1	90.0± 5.8[a]	66.67 ± 8.82[b]	6.67±3.3[b]
0. 05	61.7± 4.4[b]	50.0± 5.7[b]	6.67±3.3[b]
0.025	30.0± 5.8[c]	23.33± 3.3[c]	0.0±0.0[b]
0. 015	6.7± 3.3[d]	11. 67± 4.4[d]	0.0±0.0[b]
0	0.0±0.0[d]	0.0±0.0[d]	0.0±0.0[b]

Means within a column followed by the same lower case letter are not significantly different ($P< 0.05$).

Table (2): Percent reduction and deformation in adult emergency from immature stages of *P. operculella* treated with marjoram essential oil by contact method.

Dose (ml/10gm)	% Reduction in adult emergence ±SE and % Deformations					
	Larva		Prepupa		Pupa	
	% Reduction	% Deformation	% Reduction	% Deformation	% Reduction	% Deformation
0.2	100±0.0[a]	-	100± 0.0[a]	-	41.7± 4.4[a]	-
0.1	100±0.0[a]	-	95. 0 ±2.9[a]	-	25.0± 2.9[b]	7.6
0. 05	86.7± 1.6[b]	20	71.7±6.0[b]	25	16.7± 1.7[b]	10.5
0.025	55.0± 5.7[c]	6.9	36.0± 6.0[c]	-	10.0 ± 2.9[c]	-
0. 012	38.3± 1.6[d]	3.9	13.3± 6.7[d]	10	5.0± 0.0[c]	-
0	1.6± 1.7[e]	-	0.0±0.0[d]	-	0.0±0.00[c]	-

Means within a column followed by the same lower case letter are not significantly different ($P<0.05$).

Table (3): Fumigant toxicity of marjoram essential oil vapours against different stages of *P operculella* after 24 h of exposure.

	Mortality % ± SE				
	Larva	Prepupa	Pupa	Adult	
				male	female
0.2	100 ±0.0[a]	100±0.0[a]	11.3±1.7[a]	100±0.0[a]	100 ±0.0[a]
0.1	88.6± 3.7[a]	63.6±4.4[b]	5.0± 2.9[a]	100± 0.0[a]	100±0.0[a]
0. 05	45.7±5.1[b]	34.3±3.4[c]	0.0± 0.0[a]	93.3± 3.3[a]	85.0± 2.9[a]
0.025	11.4± 0.9[c]	9.3±4[d]	0.0±0.0[a]	70.0± 5.0[b]	64.0± 5.8[c]
0. 012	3.8±1.5[c]	2.3±1.3[d]	0.0±0.0[a]	25.7±6.0[c]	19.3±4.9[d]
0	0.0±0.0[c]	0.0±0.0[d]	0.0±0.0[a]	0.0±0.0[d]	0.0±0.0 [e]

Means within a column followed by the same lower case letter are not significantly different ($P< 0.05$).

Table (4): Percent reduction and deformation in adult emergency from larvae of *P. operculella* treated with marjoram essential oil by fumigation method.

Dose (ml/250ml)	% Reduction in adult emergence ±SE and % Deformation					
	Larva		Prepupa		Pupa	
	% Reduction	% Deformation	% Reduction	% Deformation	% Reduction	% Deformation
0.2	100.0 ± 0.0 [a]	-	100.0±0.0[a]	-	13.3± 1.7[a]	-
0.1	100.0±0.0[a]	-	100.0±0.0[a]	--	9.0± 0.0[a]	-
0. 05	71.7 ±3.6[b]	22.2	63.3± 1.7[b]	21.3	7.3± 1.7[a]	-
0.025	46.3± 7. 3[c]	-	28.6 ±1.7[c]	9.5	0.00±0[a]	-
0.012	18.3± 1.7[d]	6.8	12.5 ± 2.4[d]	-	1.7± 3.3[a]	-
0	0.0±0.0[d]	-	0.0±0.0[d]		0.0±0.0[a]	-

Means within a column followed by the same lower case letter are not significantly different (P< 0.05).

Table (5): Ovicidal effects of marjoram essential oil against egg stages of *P. operculella.*

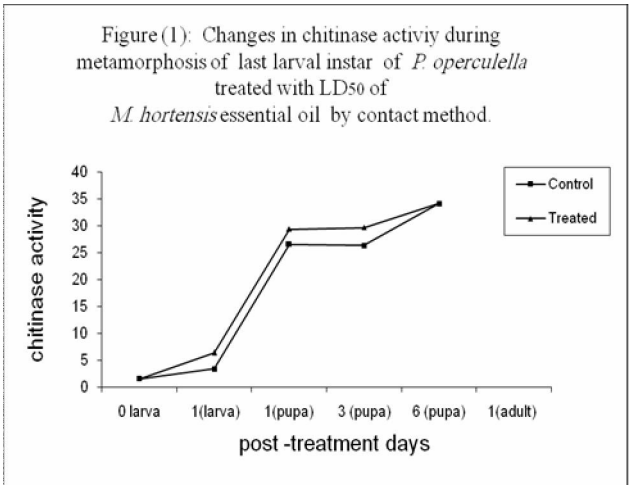
	Contact Bioassay		Fumigation Bioassay	
	Total No. of eggs	% hatched eggs ±S.E.	Total No. of eggs	% hatched eggs ±S.E.
0.2	146.8± 4.3	0 .0 ± 0.0[d]	147.8 ±4.3	67.3 ± 5.8[c]
0.1	158.6 ± 5.21	0 .0 ± 0.0[d]	159.2 ± 5.7	77.7 ± 6.1 [ac]
0. 05	155.2± 3.9	8.1 ± 1.8[d]	150 ± 4.2	88.3±6.4[ab]
0.025	153.4 ± 3.44	48.1± 2.1[c]	146.8 ±4.	95.2 ± 3.1[a]
0.012	165.4 ± 6.3	73 ± 7.6[b]	152 ± 4.2	94.7 ± 3.2[a]
0	145.4 ± 4.1	98.0 ± 1.5[a]	149.75± 3.8	99.0 ± 1.5[a]

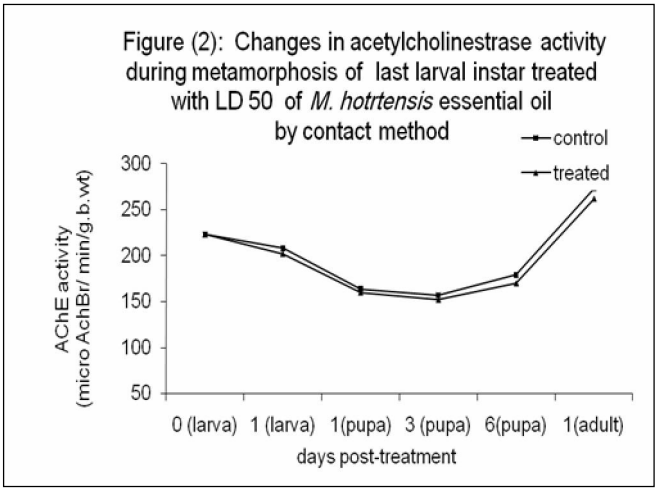
Means within a column followed by the same lower case letter are not significantly different (P< 0.05).

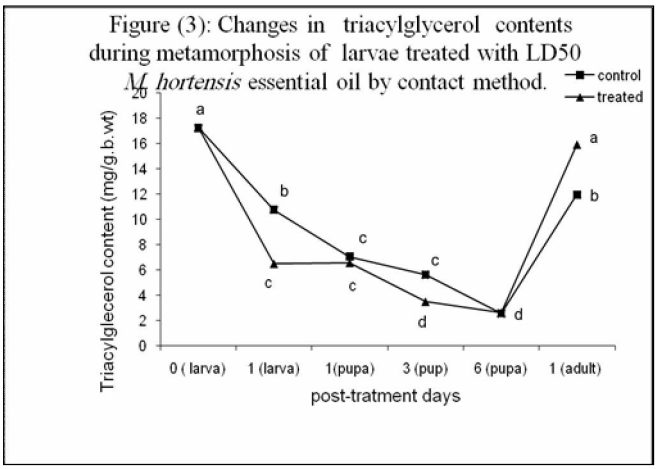
Table (6): Oviposition deterrent activity of marjoram essential against *P. operculella* adult and the percent of egg hatchability.

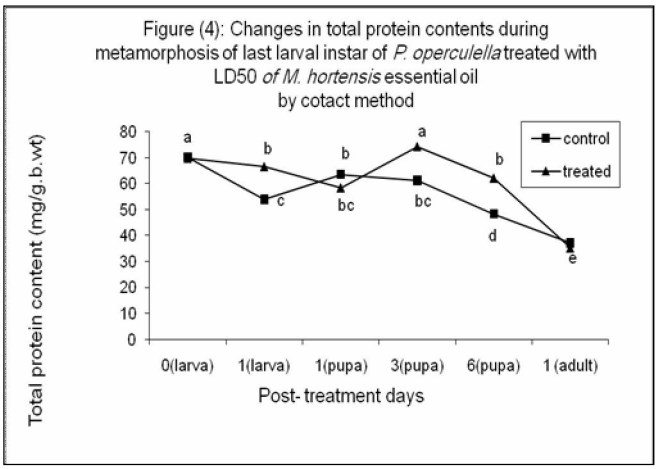
Dose (ml)	Mean eggs laid per female ± S.E.	Effective repellency (ER%)	% hatched eggs ± S.E.
0.008	77.3±3.2[a]	11.5	88.4 ±8.6[a]
0.004	83.7± 2.9[a]	2.9	91.7 ±4.8[a]
0.002	83.3 ± 2.2[a]	3.5	96.6 ±1.1[a]
0.001	84.6 ±6.4 [a]	1.9	95.3 ±7.6[a]
0	86.2 ± 3. 3[a]	-	99.3± 2.4[a]

Means within a column followed by the same lower case letter are not significantly different (P< 0.05).









Means bearing different subscripts are significantly different ($p < 0.05$)

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4. Discussion:

The most effective botanical oils would be those offering a broad spectrum of activity against various life stages of the pest. The control agent should reduce the insect population at all stages, and it should decrease the incidence of the pest (Lamiri *et al.*, 2001). The present investigation showed that *M. hortensis* essential oil exhibited strong contact toxicity and moderate fumigant activity against test stages. Furthermore, adult males and females showed high susceptibility to the fumigation. In a related study, Mohamed & Abdelgaleil (2008) stated that *M. hortensis* displayed strong contact toxicity and did not cause fumigant toxicity in *Sitophilus oryzae* and *Tribolium castaneum.* Furthermore, Shaaya *et al.*, (1991) and Prates *et al,* (1998) showed that essential oils produced contact toxicity through the insect cuticle and produced fumigant toxicity through the respiratory and digestive systems.

The results of the current study demonstrated that the pupa was the most tolerant stage and that the adult was the most sensitive one. It is well known that for fumigants, the active stages (adults and non-diapausing larvae) of insects are more susceptible than the sedentary stages (eggs and pupae), owing to differences in their respiratory rates (Rajendran and Sriranjini 2008).

The major constituents of the essential oil of *M. hortensis* plant growing in Egypt are 4-terpineol (29.96%) and β-terpinene (11.34%) (Mohamed, Abdelgaleil 2008). Lee *et al,*. (2001) and Koschier *et al.*, (2002) stated that 4-terpineol and β-terpinene exhibited insecticidal effects. Moreover, Lamiri *et al.*, (2001) demonstrated that the insecticidal activity of an essential oil could be attributed either to the major compound present in the oil or to the synergistic and/or antagonistic effects of all the components of the oil.

In the ovicidal bioassay, the test oil exhibited weak fumigant toxicity and strong contact activity against egg hatchability. Ability of the monoterpenoid vapours, especially those of terpinen-4-ol and 1,8-cineole, to reduce fecundity and hatchability of the eggs laid, recalls analogous properties of IGRs (Semple, 1992). It is likely that oil vapours diffused into eggs and thereby affected the physiological and biochemical processes associated with embryonic development (Raja *et al.*, 2001). Hence, in the fumigation bioassay the very low vulnerability of the eggs to vapours at the beginning of embryogenesis results from the fact that the permeability of the egg's external surface is lower at the start of embryogenesis. This relatively impermeable surface opposes the diffusion of vapours into the young eggs (Maciel *et al.*, 2010). A second explanation offered by Emekci *et al.*, (2002) was that because respiration rates are much lower at the egg stage than at the active stages, the lower rate of air exchange results in less monoterpenoid diffusion into the egg.

In the present study, essential oil of marjoram showed insignificant oviposition deterrent ability. Non-oviposition deterrent toxicity of the insecticide is perhaps because of the absence of corresponding organs or tissues in relation to behavior of oviposition (Hu *et al.*, 2009). Another plausible suggestion is that low doses were not effective. High doses could not be used to test the oviposition repellency because, as previously described, the adult stage showed high susceptibility to high doses.

The contact and fumigation bioassays used against immature stages resulted in some malformed adults. The toxicity of the monoterpenoids has all the characteristics of juvenile hormone activity. The occurrence of deformed adults could be explained by assuming a direct effect on the insect hormonal system similar to that of the insect growth regulators (Schwarz *et al.,* 1970). Blass and Hunt (1980) suggested that the mutation dumpy wing may involve a defect in chitin. The deformations induced by essential oils in other pests have been described by Vardhini *et al.,* (2001); de Mendonc *et al.,* (2005); Shekari *et al.,* (2008).

Along with its larvicidal activity, the effects of EO on insect metamorphosis would decrease the reproductive efficiency of the adult insect and further reduce the population (de Mendonc *et al.,* 2005). Consequently, such IGR-like properties should not be ignored when evaluating a substance exhibiting low direct toxicity, because it could be used in concert with other toxic substances to enhance their insecticidal activity (Stamopoulos *et al.,* 2007).

Furthermore, Christopher *et al.,* (1995) and Turner & Adler (1995) found that increased chitinase activity resulted in dramatic adult morphogenesis. Chitinases are among a group of proteins that digest the structural polysaccharide chitin in exoskeletons and gut linings during the molting process (Fukamizo, 2000). Activity of integumental chitinases is restricted to periods of molt and pupation (Filho *et al.,* 2002). These results are similar to those reported in this paper. The chitinase activity increased in treated and control larvae during morphogenesis to the adult stage. But it was insignificantly higher in treated larvae than the control which resulted in evident adult deformations.

Another reason for the adult deformations observed may be the change in TAG content of treated larvae, relative to the change in untreated larvae, during metamorphosis to the adult stage. TAG is the main form of storage for fatty acids that originate mainly from dietary fats absorbed by midgut epithelium, or from de novo biosynthesis (Kofronova *et al.,* 2009). Lipid accumulation during the larval stages is primarily used to support metamorphosis during the pupal stage and, in many instances, to support the flight demands and reproductive activities of non-feeding adult stages (Canavoso, *et al.,* 2001). The present study found that TAG content decreased gradually in both treated and control insects during metamorphosis to the adult stage. However, the level of reduction in treated larvae was more significant than in untreated larvae. In the tobacco hornworm, *Manduca sextu,* widely used as an insect model, the maximum content of fat body TAG occurs at the end of larval development, as a consequence of the accumulation of reserves during larval feeding. The TAG stores start to decline as a result of lipolysis and of the fatty acid oxidation required to sustain energy metabolism during the subsequent non feeding periods (pupal and adult) (Warnakulasuriya *et al.,* 1988). Most fatty acids are released from the fat body as sn-1,2-diacylglycerols (DG). The DG is carried from the fat body to the sites of utilization, e.g., to flight muscle (Wheeler and Goldsworthy 1985) and ovary (Kawooya *et al.,* 1988). The reduction in TAG content in treated larvae could be due to the energy required by the insect. Gregoire *et al.,* (1998) stated that hydrolysis of TAG occurs in order to generate fatty acids to be used by other organs during periods of energy deprivation. The decrease in TAG content in treated larvae could be due to the energy demands. Such energy demands are associated with increased production of haemocytes following activation of the immune system (Nappi and Ottaviani 2000).

The obtained data showed that AChE activity decreased insignificantly during the metamorphosis to the adult stage. AChE plays an essential role in neurotransmission at cholinergic synapses by catalysing the hydrolysis of the neurotransmitter acetylcholine. It is well known that AChE alteration is one of the main resistance mechanisms in many insect pests (Wang *et al.,* 2004). Several essential oils from aromatic plants, monoterpenes, and natural products act as AChE inhibitors (Shaaya and Rafaeli 2007; L◌opez *et al.,* 2010). It is also known that AChE inhibition is not necessarily related to insect mortality levels. In fumigant toxicity tests with monoterpenes against *Sitophilus oryzae* adults, Lee *et al.,* (2001) did not found a direct correlation between insect toxicity and AChE inhibition. Menthone from *Mentha arvensis* L. was highly toxic to *S. oryzae*, but it had a relatively small inhibitory effect on AChE activity. However, less toxic b-pinene showed high-level inhibition. Therefore, it is suspected that, in addition to producing AChE inhibition, the monoterpenes may act on other vulnerable sites (e.g., cytochrome P450-dependent monooxygenases).

The present study also showed that the total protein content of treated larvae increased, compared with the control, during metamorphosis to the adult stage. However at the 1st pupal , protein content decreased relative to that of the control. The reduction in the amount of total protein at the 1st pupal day and in the control could result because aminotransferase activity prevents the release of free amino acids into the hemolymph (Khanikor *et al.,* 1998). Mukherjee *et al.,* (1998) showed that higher concentrations of azadirachtin increased the amount of protein in the hemolymph of *T. castaneum,*

probably owing to the increased activity of detoxifying enzymes. Shekari *et al.*, (2008) showed that the amount of protein at 24 h after treatment with *Artemisia annua* decreased significantly relative to the value for the control group and increased slightly at 48 h relative to the control.

The present study provides evidence that marjoram essential oil has toxic effects against different stages of *Phthorimaea operculella* and that it also produces considerable biochemical changes. Marjoram essential oil therefore has potential for use in sustainable management of potato tuber moth in storage facilities. This approach is likely to be advantageous, as it is environmentally safe and socially acceptable. However, further studies need to be conducted to evaluate the cost of this essential oil when used in commercial storage applications.

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5. References:

1. Abbott, W.S. (1925): A method of computing the effectiveness of an insecticide. J. Economic Entomology, 18, 265–267.
2. Blass, D.H. and Hunt, D.M. (1980): Pyrimidine biosynthesis in the dumpy mutants of Drosophila melanogaster. Molecular Genetics and Genomics, 178, 437-442.
3. Bradford, M.M. (1976): A rapid and sensitive method for the quotation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72, 248-254.
4. Canavoso, L.E., Jouni, Z.E., Karnas, K.J., Pennington, J.E. and Wells, M.A. (2001): Fat Metabolism in Insects. Annual Review of Nutrition, 21, 23-46.
5. Christopher, M., Turne, P. and Adler, N. (1995): Morphogenesis of Drosophila pupal wings in vitro. Mechanism of Development, 52, 247-255.
6. de Mendonc, F.A.C., da Silva, K.F.S., dos Santos, K.K., Ribeiro, K.A.L., and Sant'Ana, A.E.G. (2005): Activities of some Brazilian plants against larvae of the mosquito Aedes aegypti . Fitoterapia, 76, 629– 636
7. El-Sinary, N.H. (1995): Magnitude and applicability of gamma radiation and controlled atmospheres to minimize the hazards of potato tuber moth, P. operculella Zeller (Lepidoptera: Gelechiidae). Ph. D. Thesis, Cairo Univ, 187 pp.
8. Emekci, M., Navarro, S., Donahaye, E., Rindner, M. and Azrieli, A. (2002): Respiration of T. castaneum (Herbst) at reduced oxygen concentrations. Journal of Stourd Product Research, 38, 413-425.
9. Filho, B.P., Lemos, F.J., Secundino, N.F., Pascoa, V., Pereira, S.T. and Pimenta, P.F. (2002): Presence of chitinase and beta-Nacetylglucosaminidase in the Aedes aegypti: a chitinolytic system involving peritrophic matrix formation and degradation. Insect Biochemistry and Molecular Biology, 32, 1723-1729.
10. Finney, D.J. (1971): Probit Analysis, third ed. Cambridge University Press, London.
11. Fukamizo, T. (2000): Chitilinolytic enzymes: catalysis, substrate-binding and their application. Current Protein & Peptide Science, 1, 105-124.
12. Golizadeh, A., Razmjou, J.(2010): Life Table Parameters of Phthorimaea operculella (Lepidoptera: Gelechiidae), Feeding on Tubers of Six Potato Cultivars. Journal of Economic Entomology, 103(3), 966-972.
13. Gregoire, F.M., Smas, C.M. and Sul, H.S. (1998): Understanding adipocyte differentiation. Physiological Reviews, 78(3), 783–809.
14. Guerra, P.C., Molina, I.Y., Yabar , E. and Gianoli, E. (2007): Oviposition deterrence of shoots and essential oils of Minthostachys spp. (Lamiaceae) against the potato tuber moth. Journal of Applied Entomology. 131(2), 134-138.
15. Hu, Q.B., An, X.C., Jin, F.L., and Ren, S.X. (2009): Toxicities of destruxins against Bemisia tabaci and its natural enemy, Serangium japonicum. Toxicon 53, 115–121.
16. Kawooya, J.K., Osir, E.O., and Law, J.H. (1988): Uptake of the major hemolymph lipoprotein and its transformation in the insect egg. Journal of Biological Chemistry, 263, 8741-8747.
17. Khanikor, D., Unni, B.G., Rai, A.K. and Barauh, R. (1998): Biochemical aspects of protein biosynthesis in the fat body of mug silkworm Antheraea assama. Advanced Biosensors, 17, 89-98.
18. Kofronova, E., Cvacka, J., Vrkoslav, V., Robert, H., Pavel, J., Jiri K., Oldrich H., and Valterova, I.A. (2009): A comparison of HPLC/APCI-MS and MALDI-MS for characterising triacylglycerolsin insects: Species-specific composition of lipids in the fat bodies of bumblebee males. Journal of Chromatography B, 87, 3878-3884

19. Koschier, E.H., Sedya, K.A., and Novak, J. (2002): Influence of plant volatiles on feeding damage caused by the onion thrips Thrips tabaci. Crop Protection, 21, 419-425.
20. Lamiri, A., Lhaloui, S., Benjilali, B., and Berrada, M. (2001): Insecticidal effects of essential oils against Hessian fly, Mayetiola destructor (Say). Field Crop Research, 71, 9-15.
21. Lee, B.H., Choi, W.S., Lee, S.E., and Park, B.S. (2001): Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, S. oryzae (L.). Crop Protection, 20, 17-320.
22. Loֹpez, M.D., and Pascual-Villalobos, M.J. (2010): Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. Industrial Crops and Prodution, 31, 284-288.
23. Maciel, M.V., Morais, S.M., Bevilaqua, C.M.L., Silva, R.S., Barros, R.N., Sousa, L.C., Sousa, E.S. and Brito, Souza-Neto, M.A., (2010): Chemical composition of Eucalyptus spp. essential oils and their insecticidal effects on Lutzomyia longipalpis. Veterinary Parasitology, 167, 1-7.
24. Mohamed, M.I.E., and Abdelgaleil, S.A.M., (2008): Chemical composition and insecticidal potential of essential oils from Egyptian plants against S. oryzae (L.) (Coleoptera: Curculionidae) and T. castaneum (Herbst) (Coleoptera: Tenebrionidae). Applied Entomology and Zoology, 43(4), 599-607.
25. Mukherjee, S.N., Rawal, S.K., Ghumare, S.S. and Sharma, P.N. (1993): Hormetic concentration of azadirachtin and isoesterase profiles in T. castaneum (Herbst) (Coleoptera: Tenebrionidae). Experientia, 94, 557–560.
26. Nappi, A.J., and Ottaviani, E. (2000): Cytotoxicity and cytotoxic molecules in invertebrates. Biography Essays, 22, 469-480.
27. Nishimura, H. (2001): Aroma constituents in plants and their repellent activities against mosquitoes. Aroma Research, 2, 257–267.
28. Pavela, R. (2004): Insecticidal activity of certain medicinal plants. Fitoterapia,
a. 75, 745-749.
29. Prates, H.T., Santos, J.P, Waquil, J.M., Fabris, J.D,, Oliveira, A.B. and Forster, J.E. (1998): Insecticidal activity of monoterpenes against Rhyzopertha dominica (F.) and T. castaneum (Herbst). Journal of Stored ProductResearch, 34, 243-249.
30. Raja, N., Albert, S., Ignacimuthu, S. and Dorn, S. (2001): Effect of plant volatile oils in protecting stored cowpea Vigna unguiculata (L.) Walpers against Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) infestation. Journal of Stored Product Research, 37, 127-132.
31. Rajendran, S., and Sriranjini, V. (2008): Plant products as fumigants for stored-product insect control. Journal of Stored Product Research, 44, 126-135.
32. Regnault-Roger, C. (1997): The potential of botanical essential oils for insect pest control. Integrated Pest Management Reviews, 2, 25-34.
33. Schwarz, M., Sonnet, P.E. and Wakabayashi, N. (1970): Insect juvenile hormone activity of selected terpenoid compounds. Science, 167, 191-192.
34. Semple, R.L. (1992): Insect growth regulators. In: Semple, R.L., Hicks, P.A., Lozare, J.V., Castermans, A. (Eds.), Towards Integrated Commodity and Pest Management in Grain Storage. A Training Manual for Application in Humid Tropical Storage Systems. A REGNET (RAS/86/189) publication in collaboration with NAPHIRE, p. 526.
35. Shaaya, E. and Rafaeli, A. (2007): Essential oils as biorational insecticides potency. In: Ishaaya, I., Ralf, N., Rami, H.A. (Eds.), Insecticides Design Using Advanced Technologies. Springer, Berlin, Heidelberg, pp. 249-261
36. Shaaya, E. , Kostjukovski, M., Eilberg, J. and Sukprakarn, C. (1997): Plant oils as fumigants and contact insecticides for the control of stored-product insects. Journal of Stored Production Research, 33, 7-15.
37. Shaaya, E., Ravid, U., Paster, N., Juven, B., Zisman, U. and Pissarev, V. (1991): Fumigant toxicity of essential oils against four major stored product insects. Journal of Chemical Ecology, 17, 499-504.
38. Sharaby, A.M, Abdel-Rahman, H. and Moawad, S. (2009): Biological effects of some natural and chemical compounds on the potato tuber moth, P. operculella Zeller (Lepidoptera:Gelechiidae). Saudi Journal of Biological Sciences, 16,1-9.
39. Shekari, M., Jalali Sendi, J., Etebari, K and Shadparvar, A. (2008): Effects of Artemisia annua L. (Asteracea) on nutritional physiology and enzyme activities of elm leaf beetle, Xanthogaleruca luteola Mull. (Coleoptera: Chrysomellidae). Pesticide Biochemistry and Physiolog, 91, 66-74.
40. Simpson, D. R., Bull, D. L, and Linquist, D.A. (1964): A semi-micro technique for estimation of cholinesterase activity in boll weevils. Annals of the Entomological Society of America, 57, 367-371.
41. Stamopoulos, D.C., Damosb, P. and Karagianidoub, G. (2007): Bioactivity of five monoterpenoid vapours to T. confusum (du Val)

(Coleoptera: Tenebrionidae). Journal of Stored Product Research, 43, 571–577.
42. Turner, C.M, Adler and P.N. (1995): Morphogenesis of Drosophila pupal wings in vitro. Mechanisms of Development, 52,247-255.
43. Vardhini, D., Raja, S.S., Varalakshmi, K. and M. A. Quddus, (2001): Sujiol, a new potent insect growth regulator from Juniperus communis L. against last instar larvae of Spodoptera litura. Journal of Applied Entomology, 25(8), 425-488.
44. Wang, J.J., Cheng, W.X., Ding, W. and Zhao, Z.M. (2004): The effect of the insecticide dichlorvos on esterase activity extracted from the psocids, Liposcelis bostrychophila and L. entomophila. Journal of Insect Science, 4, 1-5.
45. Warnakulasuriya, F.G., Tsuchida, K. and Wells, M.A. (1988): Effect of dietary lipid content on lipid transport and storage during larval development of Manduca sexta. Insect Biochemistry, 18, 211-214.
46. Waterhouse, D.F., Hochman, R.H. and Mckellar, J.W. (1961): An investigation of chitinase activity in cockroach and termite extract. Journal of Insect Physiology, 6, 96-112.
47. Wheeler, C.H and Goldsworthy, G.J. (1985): Specificity and localization of lipoprotein lipase in the flight muscles of Locusta migratoria. Biological Chemistry Hoppe-Seyler, 366, 1071-1077.
48. Zeddam, J.L., Orbe, K., Léry, X., Dangles, O., Dupas, S. and Jean-François, S. (2008): An isometric virus of the potato tuber moth Tecia solanivora (Povolny) (Lepidoptera: Gelechiidae) has a trisegmented RNA genome. Journal of Invertebrate Pathology, 99, 204-211.

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