Efficiency of *Origanum majorana* essential oil as insecticidal agent against *Rhynchophorus ferrugineus* the red palm weevil (Olivier) (Coleoptera: Curculionidae)

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ABSTRACT

Rhynchophorus ferrugineus is the most dangerous palm pest in Egypt and the Middle East. A lot of efforts have been made since three decades up to date to solve this problem. In this study, the essential oil of *Origanum majorana* was tested *in vitro* against larvae, pupa and adult stages of *R. ferrugineus*. In addition, the chemical composition of the essential oil was elucidated by gas chromatography and mass spectrometry (GC-MS) analysis. Two main toxic components (benzonitrile, 2-hydroxy 23.67%and L-linalool16.32%) were found. A significant mortalities were achieved after exposure to three concentrations (5, 10 and 15%) of *O. majorana* oil. Each concentration contains five replicates with 5 larva, pupa or adult for each replicate. The effect reached 50% for 10% concentration at 96 hrs, 48 hrs and 12 hrs for larva, pupa and adult, respectively. In addition, the cuticle of treated larvae was investigated histopathologically, and corrugation and a thinning surface were found. These results suggest that *O. majorana* essential oil is a promising alternative to chemical pesticides and can be used as a biopesticide against larvae, pupa and adult of *R. ferrugineus*.

Keywords: *Rhynchophorus ferrugineus, Origanum majorana* oil, GS-mass

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INTRODUCTION

Phoenix dactylifera L (date palm) is one of the key plants dry in semidry area. It represents a national wealth in Egypt, where Egypt leads the world in the production of dates equivalent to 18% of global production of dates in 2018 (Chao and Krueger, 2007; FAO 2019).The annual production of Egypt is estimated at 1.5 million tons, equivalent to 17.7 percent of world production estimated at 7.5 million tons. *Rhynchophorus ferrugineus* (*R. ferrugineus*)(Coleoptera: Curculionidae) is the most dangerous insect pest of date palm ever seen in Egypt. Originally from tropical Asia, then *R. ferrugineus* has spread to Africa and Europe. The first infestation with *R. ferrugineus* was recorded in the early 1990s of the 20th century in date palm plantations of Ismailia Governorate, (Saleh, 1992). *R. ferrugineus* has become widespread due to weak quarantine procedures and the transfer of agricultural products among governorates and the ability of the insect to fly and adapt to environmental conditions throughout the country. The insect has a complete metamorphoses life cycle, the eggs are placed individually on the places of pruning and wounds in the palm, Eggs hatched into larvae, which are considered harmful stage, where, they feed on the contents of the trunk of the palm, leaving tunnels to reach the head area of tree and lead to the death of the palm in the end. Many methods and materials have been developed and used in the management and control of this insect, including the use of pesticides (Llácer *et al.,* 2010), cultural and sanitary control, (Azam and Razvi, 2001) pheromone traps. (Vidyasagar *et al.,* 2000), Sterile insect technique (Ramachandran, 1991), and biological control (El Sadawy *et al*., 2020). Management of this insect pest usually involves synthetic insecticides (Mondal and Khalequzzaman, 2006).The chemical control of this pest leads to undesirable effect such the pollution of water courses around areas with palm weevil infestation (Moura *et al.,* 1995; Abuzuhairah *et al.,* 1996). Therefore, effective

and safer control methods should be applied (Nassar and Abdullah, 2001, El Namaky *et al*., 2020; El Sadawy *et al*., 2020). Protection of the environment and preservation of beneficial organisms are important where the natural biopesticides offer desirable alternatives to using synthetic chemicals in agricultural systems (Isman *et al.*, 1990). *Origanum* L. is one out of 200 genera in the family Lamiaceae of 3500 species spread all over the world. The genus consists of over 44 species and includes several types of oregano as well as sweet marjoram (*O. majorana* L.) and dittany of Crete (*O. dictamnus* L.) (Martins *et al*., 1999). Marjoram (*Origanum majorana* L.) is a tender perennial herb native to South West Asia, North Africa and naturalized in Southern Europe and cultivated in France, Hungary, Greece, the United States, Egypt, and several other Mediterranean countries. It is used as a medicinal plant; marjoram has traditionally been used as condiment, stimulant and tonic. Furthermore, natural antioxidant from plants (e.g. marjoram) is generally recognized as safe as an extract .In the other hand, Oregano oil is considered as an excellent antiseptic and insect repellent. It has some active ingredients such as carvacrol, thymol and α-terpinene reported being highly effective in repelling mosquitoes (Park *et al*., 2005). In addition, it showed significant repel activity at concentration range of 2.5–30% against *Su-pella longipalpa* (Sharififard *et al*., 2016). Hippocrates was initially used marjoram as an antiseptic agent. It is a well-liked home remedy for chest infection, sore throat, cough, nervous disorders, rheumatic pain, insomnia, cardiovascular diseases, stomach disorders and skin care (Faleiro *et al*., 2005; Yazdanparast and Shahriyary, 2008). Therefore, this work aimed to evaluate some biological and histopathological effects of *O. majorana* essential oil against larva, pupa and adult of *R. ferrugineus*.

MATERIALS AND METHODS

Insect collection and maintenance

Larvae, pupae and adults of *R. ferrugineus* were obtained during the summer season from naturally infected date palm trees in Abu Rawash, the Giza Governorate, Egypt. Which is located in the western edge of the Nile delta located in northwest of Greater Cairo, the topography of the land is relatively flat (altitude ranging from 10 to 20 m) and the area slopes gently towards the Nile River. Using sugarcane as food, laboratory culture of *R. ferrugineus* was established according to methods of Nassar and Abdullah (2001).

Applied essential oil

O. majorana essential oil was purchased from a local market in Egypt. Serial concentrations of the oil were prepared.

Chemical analysis of the essential oil

GC-MS analysis was carried out using an Agilent 6890 N GC-FID system equipped with a flame ionization detector (FID) on an Agilent capillary column (Agilent Technologies, America), HP-5 (30 m × 0.32 mm; film thickness 0.25 μm).The column temperature was programmed from 40°C to 250°C at a rate of 5°C/min. The column temperatures of injector and detector were set at 250°C. Helium was used as the carrier, in a flow rate of 1.0 mL/min (Liu and Du, 2011).

Dipping test

The efficacy of *O. majorana* essential oil against larva, pupa and adult of *R. ferrugineus* were carried by dipping tests (Khater *et al*., 2013).Three concentrations (5, 10 and 15%) were prepared. Five insects were used per replicate in each test, such that 25 insects were used for each concentration, and the procedures were repeated five times for each concentration. Each group of insects was immersed for 1 min in each concentration, and the mixture was stirred continuously. Control group was treated with distilled water. The immersed insects were placed in a plastic pot with filter papers (Whatman Grade 1) and were kept at 27±2°C and 80±5% RH. Insect mortality was observed (every 12 hrs) for 1 week. Dead and live insects were counted. Insects that did not exhibit movement or any other signs of life were considered dead.

Light microscopic observations

Five larvae, pupa and adults were dipped into 15% *O. majorana* oil for 1 min. After 24 hrs. The exposed specimens were fixed in 10% formalin buffer solution for 24 hrs. The specimens were dehydrated, cleared, and

embedded into paraffin blocks. 5 µm thick of paraffin sections were prepared, stained with hematoxylin and eosin, and examined microscopically.

Statistical analysis

Data on the dose-mortality effects of *O. majorana* oil on larvae, pupae and adults of *R. ferrugineu* were subjected to computerize by ANOVA followed by Duncan test using the SPSS computing program. LC_{50} values were also calculated log-concentrations probit model using Ldp line R software. Values of $P \leq 0.05$ were considered significant. The observed percentage mortality was corrected using Abbott's formula (Abbott, 1925):

$$\text{Observed \% mortality} = \frac{\text{(Test \% mortality - control \% mortality)}}{\text{(100 – control \% mortality)}} \text{x100}$$

RESULTS

GC-MS analysis of the essential oil

The most common compounds are shown in (Table 1).In addition, chemical analysis of essential oil of *O. majorana* oil is shown in (Fig. 1).

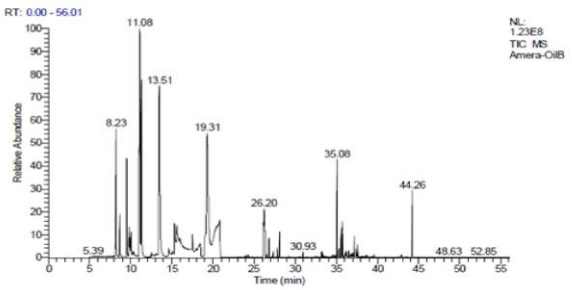

Fig. 1. A typical GC-MS chromatogram showing the chemical analysis of essential oil from *O. majorana* oil.

The GC-Ms analysis of *O. majorana* oil revealed presence of 27 oxygenated and 5 non oxygenated hydrocarbon compounds. The major identified components were 1, 8-Cineole (8.84 %), L-Linalool (16.32 %) and Benzonitrile, 2hydroxy (23.67 %).

O. majorana essential oil against larvae

A comparison with the control indicated that the *Origanum* oil had a significant effect on the larval, pupal and adult stages. The mortality % of *Origanum* oil on the larval stage varied with concentration. The daily mean mortality was presented in (Table 2).

Table 2. Mortality of *R. ferrugineus* larvae treated with different concentrations of *O. majorana* essential oil (means± SEs).

Mean mortality percentages ± SEs				
	Concentrations (%)			
Interval/(days)	**5**	**10**	**15**	**LC_{50}**
1	^{a}0±0	^{a}0.0±0	^{a}0±0.0	-
2	^{a}0±0	^{a}6.6±3.3	^{b}20.0±0.0	9.80
3	^{b}16.6±5.7	^{b} 26.6±3.3	^{c}36±3.3	-
4	^{c}36.6±5.7	^{c}46.6±3.3	^{d}60±0.0	-
5	^{c}56.6±5.7	^{c}66.6±3.3	^{e}80±0.0	-
6	^{d}80±0.0	^{d} 86±3.3	^{f}100±0.0	-
F	**32.108**	**85.1**	**43**	-
P value	**0.0***	**.00**	**0.0****	-

The strongest effect of oil (100% mortality) was observed at day 5 at 15% concentration but zero % mortality at the other concentration after two days. However, other concentration caused mortality as well from 10%. LC_{50} was calculated each day 9.8%.

O. majorana essential oil against pupae

The mortality % of *Origanum* oil on the pupae stage of *R. ferrugineu*, were recorded 100% after variable exposure time. It was found that, the exposure times were decreased as the plant oil extract concentration increased. The % mortality was presented in (Table 3),

Table 3. Mortality of pupa *R. ferrugineus* treated with different concentrations *O. majorana* essential oil (means± SE).

Mean mortality percentages ± SEs				
	Concentrations (%)			
Interval/(days)	**5**	**10**	**15**	**LC_{50}**
1	^{a}10±2.8	^{a}26.6±3.3	^{a}30±2.8	-
2	^{b}40.0±2.8	^{b}43.3±6.6	^{b}60.0±0	9.70
3	^{c}60.0±0.0	^{b}66.6±3.3	^{c} 80.0±0	-
4	^{d}80.0±0.0	^{c}86.6±3.3	^{d}100±0	-
5	^{e}100.0±0.0	-	-	-
F	**220**	**31.9**	**192**	-
P value	**0.0***	**0 .00**	**0.0****	-

ranged from 10 to100%, 20 to 100 % and 30 to 100% at concentrations (5, 10 and15%).

O. majorana essential oil against adult

Comparison with the control revealed significant mortality in the adult *R. ferrugineu* after exposure to oil. The mean mortality was represented in (Table 4). The strongest effect of extract was observed at first day at 15% concentration, which had mortality 100%. However, other concentration caused mortality as well, 60-90%. The LC_{50} was calculated at 12 hrs and was 6.8% of oil concentration. The mean mortality of larvae, pupae and adults increased with increased concentrations also, a significance influence of time and the mortality percentage ($P \leq 0.05$) were observed.

Table 1. Chemical component, retention time and total peak area of *O. majorana* essential oil **35**

Peak No.	Compound Name	Rt (min)	Area %	Mwt	Molecular Formula
1	1-Octen-3-ol(CAS)	9.79	1.22	128	$C_8H_{16}O$
2	Octanone(CAS)	9.91	0.62	128	$C_8H_{16}O$
3	Benzonitrile, 2hydroxy(CAS)	11.08	23.67	119	C_7H_5NO
4	1,8-Cineole	11.30	8.84	154	$C_{10}H_{18}O$
5	L-Linalool	13.51	16.32	154	$C_{10}H_{18}O$
6	Bicyclo[2.2.1]	14.59	0.35	152	$C_{10}H_{16}O$
7	heptan2one, 1,7,7trimethyl, (1S) 2-Pyridylacetate 7	15.31	1.49	137	$C_7H_7NO_2$
8	3-Cyclohexen-1ol, 4-methyl-1-(1methylethyl)(CAS)	15.61	0.96	154	$C_{10}H_{18}O$
9	CARVACROL METHYL ETHER	17.49	0.62	164	$C_{11}H_{16}O$
10	Linalyl propionate 6	18.45	1.34	210	$C_{13}H_{22}O_2$
11	2,4,4trimethyl-3-vinylcy Clopentanone	19.03	0.67	152	$C_{10}H_{16}O$
12	(1RS,4RS,2Z)-Cyclohexyl4dimethyl(phenyl) Silylpent-2-enol	19.31	11.45	302	$C_{19}H_{30}OSi$
13	1-(2-'Amino2'phenylethyl)-4-piperidinol	20.24	0.37	220	$C_{13}H_{20}N_2O$
14	1-{N-(pTolylsulfonyl) amino}indaneEthyltetramethylcyclopent	20.28	0.32	287	$C_{16}H_{17}NO_2S$
15	(-)-Caryophyllene oxide	26.21	6.41	220	$C_{15}H_{24}O$
16	7-Oxabicyclo[4.1.0] heptane, 1methyl4(2methyloxiranyl) (CAS)	26.80	1.16	168	$C_{10}H_{16}O_2$
17	7-(1,3-Dimeethylbuta-1,3-dienyl)-1,6,6 trimethyl-3,8 dioxatricyclo [5.1.0.0(2,4)] octane	35.33	0.30	206	$C_{15}H_{22}O_2$
18	1-diphenylphosphinoyl-3-phenylthiopropan-2-ol	37.21	0.70	368	$C_{21}H_{21}O_2PS$
19	(-)-Caryophyllen oxide	27.80	0.35	220	$C_{15}H_{24}O$
20	2,5-Octadecadiynoic acid, methyl ester (CAS	28.14	1.53	290	$C_{19}H_{30}O_2$
21	4-(2,2,6-Trimethylbicyclo [4.1.0] hept1yl) butan-2-one	35.08	4.06	208	$C_{14}H_{24}O$
22	Cyclohexene,	35.60	0.90	150	$C_{11}H_{18}$
23	a´-iso-methyl ionone	44.26	2.26	206	$C_{14}H_{22}O$
24	Chavicol	37.56	0.51	134	$C_9H_{10}O$
25	Bicyclo[2.2.1]heptanes,2,2-dimethyl-3-methylene-,(1R)	9.51	3.25	136	$C_{10}H_{16}$
26	a´-Myrcene	10.05	0.65	136	$C_{10}H_{16}$
27	7-(1,3-Dimethylbuta-1,3-dienyl)-1,6,6-trimethyl-3,8-dioxtricyclo[5.1.0.0 (2,4)]octane	35.73	1.12	234	$C_{15}H_{22}O_2$
28	a´-PINENE, (-)-	8.23	4.13	136	$C_{10}H_{16}$
29	Camphene (CAS)	8.65	1.35	136	$C_{10}H_{16}$
30	Camphor (CAS)	14.59	0.35	152	$C_{10}H_{16}O$
31	2-Pyridyl acetate	15.31	1.49	137	$C_7H_7NO_2$

Table 4. Mortality of adult *R. ferrugineus* treated with different concentrations of *O. majorana* essential oil (means± SEs).

Mean mortality percentages ± SEs				
	Concentrations (%)			
Interval/(hours)	**5**	**10**	**15**	**LC_{50}**
12	^{a}36.6±3.3	^{a}63.3±3.3	^{a}83±3.3	6.80
24	^{b}46.6±3.3	^{a}66.6±3.3	^{b}93±3.3	-
36	^{c}56.6±3.3	^{a}70±0	^{b}100±0.00	-
48	^{d}66.6±3.3	^{a}76.6±3.3	-	-
60	^{e}76.6±3.3	^{b}90±0.0	-	-
72	^{f}86.6±3.3	^{c}100±0.0	-	-
84	^{g}96.6±3.3	-	-	-
92	**^{g}100±0.0**	-	-	-
F	**55.42**	**37.33**	**9.5**	-
P value	**0.00***	**0.00***	**0.014***	-

A comparison with the control indicated that the *Origanum* oil had a significant effect on the larval, pupal and adult stages.

Light microscopic observations

The normal structure of larvae, pupa and adult stages were illustrated in (Figs. 2 a, b, and c).

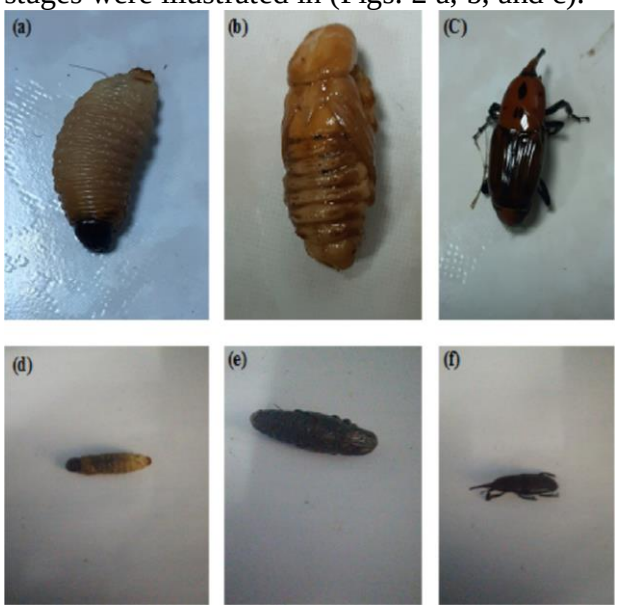

Fig. 2. Deformation of *R. ferrugineus* after essential oil treatment. **a)** Normal larvae, **b)** Normal pupa **, c)** Normal adult**, d**) Deformation of larvae, **e)** Pigmentation of pupa, **f)** dwarfism of adult.

However, as shown in (Figs. 2 d, e, and f) larvae, pupa and adult exhibited dark color and the treated larvae and adult showing many abnormalities in their body and the pupae died inside the cocoon. The normal cuticle of *R. ferrugineus*, as typical for the other insects studied by Wiggle Worth (1947) consist of thin layer of exocuticle , epicuticle followed by endocuticle and the muscles layer are composed of striated fibers (Figs 3, 4 and 5a).In addition, dipping larvae, pupa and adult stages in a 15% concentration of oil induced a corrugated and thinning cuticular surface and a separation of the inner cellular layer of the epidermal cells in some regions of the procuticle (Figs 3, 4 and 5b), while the musculature region showed a disorganized appearance.

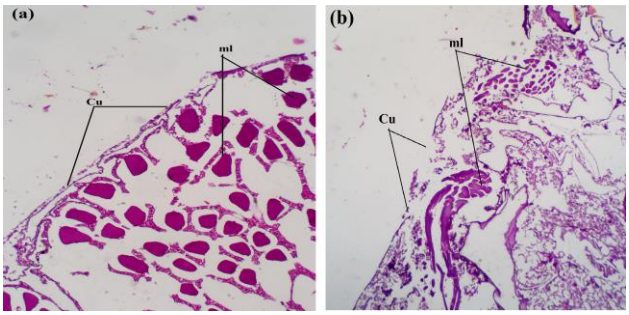

Fig. 3. Transverse H and E-stained sections of **(a)** The cuticle, muscles of normal larvae of RPW, the body wall of normal larvae of the RPW has three layers; an outer electron-dense layer of epicuticle, followed by the procuticle, which is composed of the exocuticle and endocuticle, and then the inner layers of epidermal cells **(b)** Cuticle, muscles 24 hrs after being dipped in 15% *O. majorane* oil (10×) Cuticle (Cu), and muscle layer (mL) (10x).

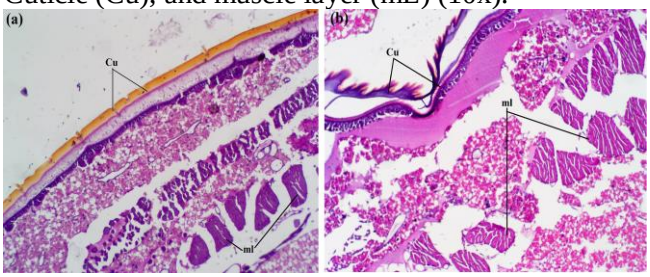

Fig. 4. Transverse H and E-stained sections of **(a)** The cuticle, muscles of normal pupa of RPW, **(b)** Cuticle, muscles 24 hrs after being dipped in 15% *O. majorane* oil. (10×) Cuticle (Cu), and muscle layer (mL) (10x).

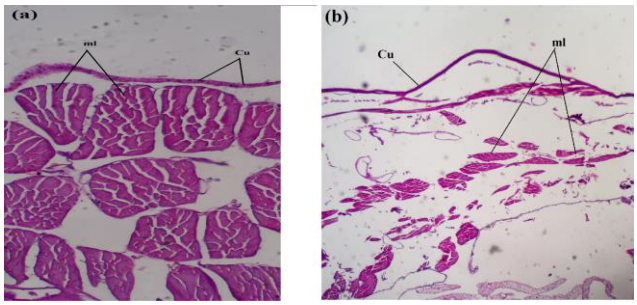

Fig. 5. Transverse H and E-stained sections of **(a)** The cuticle, muscles of normal adult of RPW, **(b)** Cuticle, muscles 24 hrs after being dipped in 15% *O.*

majorane oil. (10×) Cuticle (Cu), and muscle layer (ml) (10x).

DISCUSSION

Insecticides of plant origin are rich sources of bioactive compounds as well as suitable alternative for palm weevil's control. Plants are offer an advantage over synthetic insecticides as are less prone to development of resistance, less toxic to natural environment and easily biodegradable. Although pure components were not tested in this study, over 30 major component have been identified in the essential oil. In the present work, two main toxic components (L-linalool 16.32% and1, 8-Cineole 8.87%) were found. The toxicity of the oil was attributed to these two main compounds. Khalfi *et al.* (2008) identified eighteen components of the essential oil *O. glandulosum* (Desf.), representing 92.6% of the oil. They found that the major components were *γ* –terpinene (5.1%), *p*-cymene (7.9%) carvacrol (32.9%) and thymol (38.8%). Differences in the composition of essential oil of several plants were discussed by several authors. Galleti *et al*. (1998) attributed the differences in composition of 24 samples *of O. vulgare* essential oil to the fact that the synthesis and secretion of oils are influenced by climatic factors, intensity of metabolism of the plants and the secretory activity of the glandular hairs.

In the present study full mortality in adults of *R. ferrugineus* was reached after 24 hrs at 15% concentration. However, pupa and larval **m**ortalities reached full at day 5 at the same concentration. El Namaky *et al*. (2020) found that the ethanolic extract of *Punica granatum* peel leads to significant mortalities in both the larvae and adult stages of RPW after exposure to four concentrations (4.7, 9.3, 18.7, and 37.4 mg/mL). Larval mortality reached only 48% at a 37.4 mg/mL concentration of *P. granatum* at day 6. However, full mortality was found in adults of *R. ferrugineus* at the same time and concentration. Ahmed *et al*. (2018) found that the *O. majorana* essential oil was more potent than *E. globulus* against third instars larvae of *Phlebotomus papatasi* based on its lower LC_{50} value. In addition, Ayvaz *et al*. (2020) reported that the essential oils of *O. onites* L and savory, *Satureja thymbra* L were highly effective against adults of the Indian meal moth *Plodiainter punctella* Hübner (Lepidoptera: Pyralidae) and the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), with 100% mortality obtained after 24 hrs at 9 and 25 μL/l air . The high toxicity of linalool, linalyl acetate and 1.8 - cineole from *Myrtus communis* L. (Rosales: Myrtaceae) was reported against the rice weevil *Sitophilus oryzae* and *Rhyzoper thadominica* (Rozman *et al.*, 2007). Due to their high volatility they have fumigant activity that might be of importance for controlling stored-product insects. The toxic effects of the myrtle *(M. communis)* could be attributed to major constituents such as linalool (31.3%), linalyl acetate (17.8%) and 1.8-cineole (14.7%). Due to the linalool, linalyl acetate and 1.8-cineole constituents, the myrtle could also be used effectively against *S. oryzae* and *R. dominica*. Thymol and Carvacrol are usually extracted from *Origanum* species and have been found to be lethal to turnip aphids (Chiasson *et al*., 2001).There are numerous reports on the insecticidal activity of the essential oils from *Origanum* species, and the major components of this species, such as thymol, γ- terpinen, carvacrol and terpinen-4-ol, are based on repellent activity rather than contact toxicity (Ahn *et al.*, 1998; Tunc *et al*., 2000; Erler and Tunc 2005 and Erler 2005). The verification of the location and form of action on the insect has great importance for the development of an efficient and safe insecticide (Barreto *et al.*, 2006). Therefore, morphological studies are an important tool when trying to understand the form of action of natural products (Dequech *et al.*, 2007). The deleterious physiological effects can be measured by growth reduction and presence of abnormalities (Mordue and Nisbet 2000).

The *O. majorana* oil at a concentration of 15% induced histological damage to the cuticle of the pupa, adult and larvae, while the midgut exhibited a normal appearance. The same result was found in treatment of the larvae of *R. ferrugineus* with a crude plant extract of *P. granatum* L (El Namaky *et al*., 2020). In addition, the ovary and testis of the pre-pupae stage of *R. ferrugineus* exhibited a degenerated

and necrotic appearance after exposure to a range of concentrations of a neem plant extract (El-Bokl *et al*., 2010). Prates *et al*. (1998) and Lee *et al*. (2002) concluded that the insecticidal constituents of many essential oils and plant extracts are mainly monoterpenoids, which are typically volatile and can be toxic by penetrating the insect cuticle or its respiratory system. The primary way oils kill insects is the same by suffocation. Oils block spiracles, reducing the availability of oxygen and interfering with various metabolic processes. In the dipping assay, Shalaby *et al*. (2016) suggested that the main route for entry of oil extract into the *Lucilia sericata* larvae was by transcuticular uptake. In our work, the larvae and pupae were darkening in color as well as the cuticle appeared thinner than normal and the epidermal cell layer was disrupted. The same result was found in treatment of the larvae and adult of *R. ferrugineus* with crude plant extracts of *P. granatum, Carapicheai pecacuanha* and *Eucalyptus camaldulensis* (Hussein *et al*., 2018, El Namaky *et al.,* 2020). In addition, El-Bokl *et al*. (2010) found that the gamete production of the ovary and testis of the pre-pupae stage of RPW were disrupted after exposure to a range of concentrations of a natural plant extract (neem). Also, volatile oils of lufenuron, *Mentha piperita*, *Ocimum basilicum*, *Citrus limon*, and *Citrus sinens* were induced abnormalities in the antennae of adult *C. maculate* and the larvae of the black carpet beetle (*A. fasciatus*) (Baker *et al*., 2010). In conclusion, these results suggest that *O. majorana* oil may be useful for the biocontrol of different stages of *Rhynchophorus ferrugineus* (the red palm weevil) and can be used as a bio-pesticide.

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