

## **Sublethal effects of individual and combined of *Mentha pulegium* essential oil and methanolic extract on life table parameters of *Ommatissus lybicus* (Hemiptera: Tropicuchidae)**

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### **Abstract**

Integrated pest management (IPM) is an ecosystem approach to crop production and protection that combines different management strategies and practices to grow healthy crops and minimize the use of pesticides. Essential oils and extracts are used in the developing world for many purposes including management of agricultural insect pests. This study evaluated the individual and synergistic effects of essential oil and methanolic extract of *Mentha pulegium* against *Ommatissus lybicus* adults. The  $LC_{50}$  and  $LD_{50}$  of *M. pulegium* values are 9.68  $\mu\text{L/L}$  air and 36.97 mg/mL, respectively. To assess the sublethal effects, adult bugs were exposed to the essential oil ( $LC_{25}$ ), methanolic extract ( $LD_{25}$ ) and their combination, and mortality of immature stages and life table parameters of the surviving *O. lybicus* were studied. The highest percent of egg and nymphal mortality were observed, 27.33% and 37.60% respectively, after exposure to  $LC_{25}$  of *M. pulegium* oil. Exposure to sublethal  $LC_{25}$  and/or  $LD_{25}$  of *M. pulegium* negatively affected the life table of *O. lybicus*. All treatments significantly reduced the  $R_0$ ,  $r_m$ ,  $\lambda$ , and  $GRR$  while that increased  $T$  of *O. lybicus* and prolonged the developmental time from egg to adult. In comparison to extract treatment,  $R_0$  and  $r_m$  parameters were reduced to a greater extent in insects exposed to both *M. pulegium* oil and extract, however, a statistically significant difference was not found when compared with essential oil treatment. According to these results, both tested essential oil and methanolic extract has potential applications for the integrated management of *O. lybicus*.

**Keywords:** *Mentha pulegium*, Individual effect, Combined effect, Life table parameters, *Ommatissus lybicus*.

### **Introduction**

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The Dubas bug, *Ommatissus lybicus* Bergevin (Hemiptera: Tropiduchidae), is a key sucking insect pest that attacks date palm in Iran and other countries (Payandeh et al. 2010; Al-dhamin 2015). During severe infestation, honeydew dripped from palms that the upper side of fruit and old leaves are covered with the honeydew and dust, and reduces the quantity and quality of the dates (Klein and Venezian 1985; Mokhtar and Nabhani 2010). The control of this pest is primarily dependent upon continued application of chemical insecticides (Al-dhamin 2015). In recent years, control programs of pests such as *O. lybicus* changed from a chemical-based approach to an integrated pest management, because the use of chemical pesticides has led to several negative effects including environmental disturbances, increasing costs of application, pest resurgence, pest resistance to pesticides, and lethal effects on non-target organisms, in addition to direct toxicity to users (Saber et al. 2004; Sahaf et al. 2008; Borzoui et al. 2016).

Ethnobotany has played an important role in traditional methods of protection against pests in many countries of the world (Isman 2000). Among phytoinsecticides, extract and essential oil of plants have received a great deal of attention in pest management programs because of their favorable ecotoxicological properties, e.g., low human toxicity, rapid degradation and reduced environmental effect (Saber et al. 2004; Halder et al. 2010). *Mentha pulegium* L. (Lamiaceae) grows in many different habitat ranges and is widespread in Europe, North Africa, Minor Asia and the near East (Zargari 1990; Mahdavi et al. 2013). Rafiee-Dastjerdi et al. (2014) reported that *Mentha* species have medicinal, insecticidal, repellent, or antifeedant properties.

The use of reduced rates of tested compounds can lead to the exposure of *O. lybicus* to sublethal dose. Studies of the sublethal effects of plant essential oils and extracts in several important pests have been widely documented, both in larvae and adults. These effects include delayed or accelerated developmental time (Loni and Panahi 2015), induction of supernumerary larval instars, impaired life-table parameters (Borzoui et al. 2016), mortality of immature stages (Al-dhamin 2015), weight loss of both larvae and pupae (Rafiee-Dastjerdi et al. 2014) and wing deformities in adults (Shekari et al. 2008). In addition, Taghizadeh Saroukolai et al. (2014) reported that plant essential oils interfered with the feeding ability of larvae and adults of *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae).

A life table is a convenient and comprehensive method for summarizing the effects that an insecticide has upon the survival, development, reproduction, and rate of increase of an insect population (Zhang et al. 2010; Liu et al. 2016; Borzoui et al. 2016). Because the age-stage, two-sex life table can describe stage differentiation and include both sexes, it can precisely reveal the actual life history of the insect species and life table have been widely applied in the study of various ecological aspects of interest in relation to insect pests, under different environmental conditions (Chi 1988; Chi and Su 2006).

In this study, we investigated the lethal and sublethal effects of essential oil and methanolic extract of *M. pulegium* on *O. lybicus*, the serious date palm pest in Iran, when the adults were continuously exposed to these compounds for 72 h. Specifically, we assess life-table parameter in insects that exposed to sublethal values of essential oil (LC<sub>25</sub>) and methanolic extract (LD<sub>25</sub>) either individually or in combination.

## **Materials and methods**

### **Plant methanolic extract and essential oil**

Medicinal plants used in this study were collected in their flowering stage from natural habitats in Kerman (Iran), on May 2016. First, selected parts of plants were washed with distilled water and left to be air dried for 4–5 days. Then, dried parts were ground and maintained in black plastic in refrigerator.

Dried leaves of *M. pulegium* were grinded to fine powder of 60 mesh. Fifty gr of plant powder was steeped in methanol (300 ml) and placed on shaker in ambient temperature for 48 hours. After this time, the extract was passed through a filter paper and the left over solvent was evaporated on rotary evaporator in 40 °C and 100 rpm and extract was made solvent free in a vacuum desiccators.

The tested essential oil was extracted by water steam distillation using a Clevenger apparatus, from fifty gr of *M. pulegium* leaves powder as described in Borzoui et al. (2016). After extraction, the essential oil was dried over anhydrous sodium sulphate and was stored in a refrigerator at 5 °C.

### ***Insect rearing***

The Dubas bug nymphs and adults were originally collected from infested date palm gardens near Bam, Kerman Province, Iran. The insects were reared into plastic containers (diameter 20 cm, depth 6 cm) with a hole covered by a mesh net for ventilation, containing the date palm leaflets. The *O. lybicus* was reared on date palm leaflets (cultivar Mazafati) at  $30 \pm 1^\circ\text{C}$ ,  $60 \pm 5\%$  RH, and a photoperiod of 16:8 (L:D) h in a growth chamber for three generations. Adult insects (<24 h old) were used for toxicity experiments. All experimental procedures were carried out under the same environmental conditions as the cultures.

### ***Treatment and bioassays***

All following experiments including bioassays and life table parameters were carried out under laboratory conditions inside a growth chamber ( $30 \pm 1^\circ\text{C}$ ,  $60 \pm 5\%$  RH, and a photoperiod of 16:8 (L:D) h) in the Laboratory of Entomology, Islamic Azad University of Bam, Kerman, Iran, from June to November 2016.

### ***Fumigant toxicity test with M. pulegium essential oil***

The fumigant toxicity of *M. pulegium* oil against *O. lybicus* adults was tested as described by Negahban et al. (2007). The main concentrations of *M. pulegium* oil (4.00, 6.16, 9.54, 14.79, and 23.00  $\mu\text{L/L}$  air) were prepared without using any solvent. Whatman filter papers (No. 1, cut into 2-cm-diameter pieces) were each saturated with essential oil, and then placed on the underside of the screw cap of a glass bottle (diameter 20 cm, height 20 cm), each of which contained 15 insects. Five replicates were carried out for all treatments and controls, and they were incubated in the growth chamber set at the above-mentioned conditions. The number of dead *O. lybicus* in each bottle was counted 72 h after exposure to the essential oil. The mean mortality for each concentration of the compound was calculated using the Abbott correction formula for natural mortality in control (Abbott 1925). Also, the  $\text{LC}_{25}$  value was established for sublethal experiments.

### ***Contact toxicity test with M. pulegium methanolic extract***

This experiment was conducted in order to determine whether the insecticidal activity of *M. pulegium* extract was attributable to contact activity. The plant extract was prepared in methanol and water to get doses of 17.00, 24.54, 33.88, 46.77, and 65.00 mg/mL and 2 mL of each dose was applied to test. Leaflets of date palm were randomly selected, dipped in the desired concentration for 15 s and dried in air for 30 min. Control leaflets were dipped in methanol and water and dried as above. Control and treated leaflets were separately placed in foil cages (with a hole in their top covered with fine mesh for ventilation), each of which included 20 adults (<24 h old). After that, all foil cages were kept in the growth chamber set at the above-mentioned conditions for 72 h. After exposure period, the mortality of adults was recorded. Each concentration and control was replicated five times. The mean mortality for each dose of the plant extract was calculated using the Abbott correction formula for natural mortality in control (Abbott, 1925). Also, the LD<sub>25</sub> value was established for sublethal experiments.

#### ***Sublethal effects of M. pulegium oil and extract on life table parameters of O. lybicus***

In order to explore the long-term effects of exposure to *M. pulegium* oil and methanolic extract, life table parameters of *O. lybicus* were determined. About 100 newly mated adults (<24 h post-mating) of *O. lybicus* were treated using essential oil (LC<sub>25</sub>) or methanolic extract (LD<sub>25</sub>) as described earlier. In order to determine the combined effects of these compounds, the newly mated adults (<24 h post-mating) of *O. lybicus* were exposed to both essential oil (LC<sub>25</sub>: 6.84) and extract (LD<sub>25</sub>: 29.37). After 72 h, 15 pairs of *O. lybicus* adults (male and female) were randomly selected and transferred individually (one pair of male and female) into foil cages, and used for life table studies. The foil cages were placed in a growth chamber that was set at the above described standard rearing conditions. The adults were allowed to feed on the untreated leaflets for the duration of the adult longevity. The number of eggs deposited on the leaflets was counted daily. The adult period, fecundity (number of eggs deposited per female), fertility (number of hatched eggs), egg period, nymphal period, and the survival immature stages of emerged insects were recorded.

#### **Data Analysis**

The result of each trial was tested for curve fit using PROC GENMOD procedures (PROC GLM; SAS Institute 2002, Robertson et al. 2007), and the data were subjected to log probit analysis for calculating the LC<sub>25</sub>/LD<sub>25</sub> and LC<sub>50</sub>/LD<sub>50</sub> values with 95% confidence limit by using PROC PROBIT (PROC GLM; SAS Institute 2002). In addition, mortality of *O. lybicus* adults exposed to different concentrations of the tested essential oil or methanolic extract was analyzed by one-way ANOVA. If significant differences were detected, the means were separated at  $\alpha = 5\%$  by Tukey test.

The raw life history data for *O. lybicus* were analyzed based on the theory of the age-stage, two-sex life table (Chi and Liu 1985; Chi 1988) with TWSEX-MSChart computer program (Chi 2015) and were used to calculate population parameters: the intrinsic rate of increase ( $r_m$ ), the finite rate of increase ( $\lambda$ ), the growth reproduction rate ( $GRR$ ), mean generation time ( $T$ ), and the net reproduction rate ( $R_0$ ). Jackknife pseudovalues calculated for life table parameters on different treatments were analyzed by one-way ANOVA (PROC GLM; SAS

Institute 2002) (Meyer et al. 1986; Maia et al. 2000). In addition developmental time and survival of immature stages, adult's longevity and fecundity data were analyzed by one-way ANOVA. If significant differences were detected, the means were separated at  $\alpha = 5\%$  by Tukey test.

## Results

### Adult Bioassays

The  $LC_{25}/LD_{25}$  and  $LC_{50}/LD_{50}$  values recorded after 72 h of exposure are given in Table 1. The  $LC_{25}$  and  $LC_{50}$  values of *M. pulegium* oil were 6.84 and 9.68  $\mu\text{L/L}$  air, respectively. Also, the  $LD_{25}$  and  $LD_{50}$  values of *M. pulegium* extract were 29.37 and 36.97 (mg/mL), respectively.

**Table 1.** Toxicity of essential oil and methanolic extract of *Mentha pulegium* against the adult stage of *Ommatissus lybicus*.

Insecticide	n <sup>a</sup>	$\chi^2$	Slope $\pm$ SE	Lethal concentrations ( $\mu\text{L/L}$ air)		
				$LC_{25}/LD_{25}$ (95% FL)	$LC_{50}/LD_{50}$ (95%FL)	$LC_{90}/LD_{90}$ (95%FL)
Essential oil ( $\mu\text{L/L}$ air)	315	104.22	3.99 $\pm$ 0.39	6.84 (6.00 - 7.58)	9.68 (8.83 - 10.57)	18.71 (16.50 - 22.15)
Methanolic extract (mg/mL)	315	80.25	6.75 $\pm$ 0.75	29.37 (26.42 - 31.74)	36.97 (34.52 - 39.38)	57.22 (52.30 - 65.08)

Lethal concentrations and 95% fiducial limits (FL) were estimated using logistic regression (SAS Institute 2002).

<sup>a</sup> The total number of adult bugs used for bioassay test.

### Sublethal effects of different treatments on biological parameters and mortality

The results of the effect of different treatments on developmental time of *O. lybicus* immature stages are given in Table 2. *O. lybicus* exhibited the longest incubation period ( $F= 1027.391$ ;  $df= 3, 699$ ;  $P < 0.0001$ ) in *M. pulegium* oil treatment ( $31.47 \pm 0.14$  days), while the shortest one was in control ( $30.11 \pm 0.19$  days). Also, significant differences in the duration of the nymphal stage ( $F= 85.69$ ;  $df= 3, 699$ ;  $P < 0.0001$ ), adults longevity ( $F= 32.642$ ;  $df= 3, 699$ ;  $P < 0.0001$ ), and fecundity ( $F= 3413.138$ ;  $df= 3, 407$ ;  $P < 0.0001$ ) was observed in the treatments compared with the control. In general, adults that were exposed to both essential oil and methanolic extract ( $55.62 \pm 4.94$  days) showed lower developmental time than those that were exposed only to essential oil ( $58.90 \pm 4.94$  days) or methanolic extract ( $68.34 \pm 4.42$  days) ( $F= 362.33$ ;  $df= 3, 699$ ;  $P < 0.0001$ ) (Table 2).

The mortality data of *O. lybicus* immature stages after exposure to different treatments are shown in Figure 1 (A-C). Both tested essential oil and methanolic extract had a significant effect on the mortality of *O. lybicus* immature stages ( $F= 224.66$ ,  $df= 2, 8$ ,  $P < 0.0001$ ). The lowest mortality percent ( $F= 101.42$ ;  $df= 2, 8$ ;  $P < 0.0001$ ) of eggs was observed in control ( $7.45 \pm 0.49\%$ ), and the highest mortality percent was seen in *M. pulegium* oil treatment ( $27.33 \pm 1.10\%$ ) (Figure 1-A). Also, control had lowest nymphal mortality percent ( $12.43 \pm 0.78\%$ ), whereas the essential oil treatment has highest nymphal mortality percent ( $37.60 \pm 0.95\%$ ) (Figure 1-B). The mortality percent of egg and nymph was moderate for insect fed on *M. pulegium* methanolic extract ( $20.84 \pm 0.68$  and  $31.08 \pm 0.52\%$ , respectively) when compared to control and *M. pulegium* oil treatment (Figure 1-

A & B). The mortality of immature stages was approximately to 18% in the control. The mortality of immature stages in *M. pulegium* extract and *M. pulegium* oil was 34% and 48% lower than the control (Figure 1-C).

**Table 2.** Sublethal effects of essential oil (LC<sub>25</sub>) and methanolic extract (LD<sub>25</sub>) of *Mentha pulegium* on biological parameters of *Ommatissus lybicus*.

Developmental period	Treatment	Female		Male		Total	
		n <sup>a</sup>	Mean ± SE	n <sup>a</sup>	Mean ± SE	n <sup>a</sup>	Mean ± SE
Incubation period (d)	Control	22	29.91 ± 0.29 b	19	30.16 ± 0.32 c	47	30.11 ± 0.19 b
	Essential oil (LC <sub>25</sub> )	13	31.54 ± 0.18 a	9	31.78 ± 0.40 a	36	31.47 ± 0.14 a
	Metanolic extract (LC <sub>25</sub> )	16	31.19 ± 0.21 a	12	31.08 ± 0.26 b	40	31.35 ± 0.13 a
	Essential oil + Methanolic extract	12	31.33 ± 0.28 a	9	31.22 ± 0.28 ab	35	31.46 ± 0.14 a
Nymphal period (d)	Control	22	43.77 ± 0.11 b	19	43.79 ± 0.16 b	47	43.78 ± 0.10 b
	Essential oil (LC <sub>25</sub> )	13	44.46 ± 0.22 a	9	44.44 ± 0.29 a	36	44.45 ± 0.17 a
	Metanolic extract (LC <sub>25</sub> )	16	44.38 ± 0.20 a	12	44.42 ± 0.26 a	40	44.39 ± 0.16 a
	Essential oil + Methanolic extract	12	43.83 ± 0.17 b	9	43.22 ± 0.28 c	35	43.57 ± 0.16 b
Immature period (egg to adult) (d)	Control	22	73.68 ± 0.30 c	19	73.95 ± 0.39 b	47	73.88 ± 0.24 d
	Essential oil (LC <sub>25</sub> )	13	76.00 ± 0.23 a	9	76.22 ± 0.40 a	36	76.09 ± 0.21 a
	Metanolic extract (LC <sub>25</sub> )	16	75.56 ± 0.27 ab	12	75.50 ± 0.34 a	40	75.54 ± 0.21 b
	Essential oil + Methanolic extract	12	75.17 ± 0.24 b	9	74.44 ± 0.41 b	35	74.86 ± 0.23 c
Adult longevity (d)	Control	22	18.41 ± 0.11 a	19	14.58 ± 0.18 a	47	16.63 ± 0.32 a
	Essential oil (LC <sub>25</sub> )	13	17.61 ± 0.11 b	9	13.93 ± 0.60 ab	36	16.16 ± 0.42 a
	Metanolic extract (LC <sub>25</sub> )	16	17.68 ± 0.12 b	12	13.87 ± 0.15 b	40	16.07 ± 0.37 a
	Essential oil + Methanolic extract	12	17.58 ± 0.15 b	9	13.67 ± 0.17 b	35	15.09 ± 0.45 b
Total developmental time (d)	Control	22	92.09 ± 0.35 d	19	88.53 ± 0.44 c	47	81.92 ± 2.93 a
	Essential oil (LC <sub>25</sub> )	13	93.69 ± 0.29 a	9	90.11 ± 0.51 a	36	58.90 ± 4.94 c
	Metanolic extract (LC <sub>25</sub> )	16	93.25 ± 0.35 b	12	89.42 ± 0.34 b	40	68.34 ± 4.42 b
	Essential oil + Methanolic extract	12	92.75 ± 0.28 c	9	88.11 ± 0.39 d	35	55.62 ± 4.94 c

Mean values in a column followed by different lowercase letters are significantly different (Tukey test,  $P < 0.05$ ).

<sup>a</sup>The *n* value shows the number of insects tested for each parameter.

The mean adult pre-oviposition period (APOP) of *O. lybicus* was 1.27 and 2.08 days for control and *M. pulegium* oil, respectively. Also, the mean total pre-oviposition period (TPOP) was 74.95 and 78.08 days for control and *M. pulegium* oil, respectively. The length of the reproductive period ranged from 13.31 to 16.09

days, with a mean of 51.69 and 79.18 nymphs for control and essential oil-treated insects, respectively.

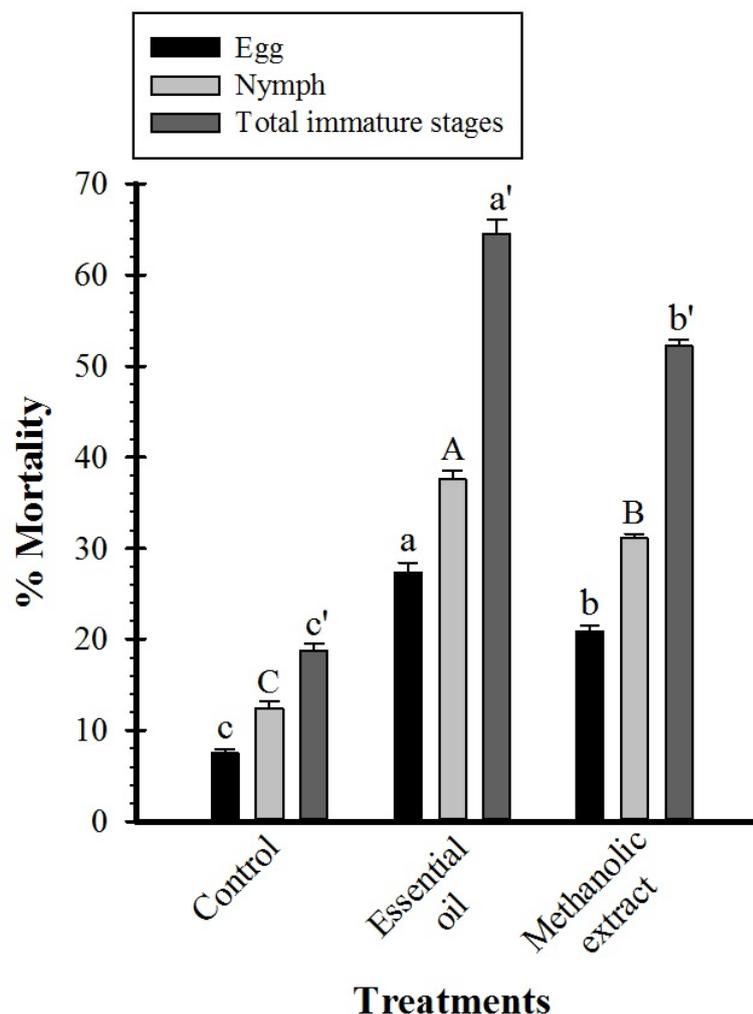
#### ***Sublethal effects of different treatments on life table parameters***

The sublethal effects of *M. pulegium* essential oil and methanolic extract on the life table parameters of *O. lybicus* are given in Table 4. Compared with control ( $34.84 \pm 5.59$  female/female), the net reproductive rate ( $R_0$ ) ( $F= 328.43$ ;  $df= 3, 699$ ;  $P < 0.0001$ ) showed significant differences after exposure to  $LC_{25}$  of essential oil or methanolic extract or both essential oil and methanolic extract ( $13.44 \pm 3.02$ ,  $16.96 \pm 3.50$  and  $13.36 \pm 3.81$  female/female, respectively). Also, the intrinsic rate of increase ( $r_m$ ) of treatments was significantly ( $F= 211.92$ ;  $df= 3, 699$ ;  $P < 0.0001$ ) lower than those of control *O. lybicus*. The individuals exposure to essential oil and both essential oil and methanolic extract had much lower  $r_m$  values ( $0.031 \pm 0.003$  and  $0.031 \pm 0.003$   $d^{-1}$ , respectively) than those on control ( $0.043 \pm 0.002$   $d^{-1}$ ). The finite rate of increase ( $\lambda$ ) followed the same pattern as intrinsic rate of increase ( $F= 213.89$ ;  $df= 3, 699$ ;  $P < 0.0001$ ). Furthermore, the generation time ( $T$ ) of the population were affected by the  $LC_{25}$  of essential oil or methanolic extract or both essential oil or methanolic extract ( $F= 523.23$ ;  $df= 3, 699$ ;  $P < 0.0001$ ). The population exposure to essential oil had a higher the generation time ( $T$ ) ( $84.17 \pm 0.30$  d) and those exposure to control had lowest  $T$  value ( $82.01 \pm 0.36$  d).

**Table 4.** Sublethal effects of essential oil ( $LC_{25}$ ) and methanolic extract ( $LD_{25}$ ) of *Mentha pulegium* on life table parameters of *Ommatissus lybicus*.

Life table parameters	Control	Essential oil ( $LC_{25}$ )	Methanolic extract ( $LD_{25}$ )	Essential oil ( $LC_{25}$ ) + Methanolic extract ( $LD_{25}$ )
Gross reproductive rate ( $GRR$ ) (female/female)	$50.69 \pm 6.70$ a	$31.70 \pm 5.42$ b	$31.99 \pm 4.94$ b	$34.56 \pm 5.92$ b
Net reproductive rate ( $R_0$ ) (female/female)	$34.84 \pm 5.59$ a	$13.44 \pm 3.02$ c	$16.96 \pm 3.50$ b	$13.36 \pm 3.81$ c
Intrinsic rate of increase ( $r_m$ ) ( $d^{-1}$ )	$0.043 \pm 0.002$ a	$0.031 \pm 0.003$ c	$0.034 \pm 0.002$ b	$0.031 \pm 0.003$ c
Finite rate of increase ( $\lambda$ ) ( $d^{-1}$ )	$1.044 \pm 0.002$ a	$1.031 \pm 0.003$ b	$1.034 \pm 0.003$ b	$1.031 \pm 0.003$ b
Generation time ( $T$ ) (d)	$82.01 \pm 0.36$ b	$84.17 \pm 0.30$ a	$83.86 \pm 0.31$ a	$83.83 \pm 0.34$ a

Mean values in a row followed by different lowercase letters are significantly different (Tukey test)



**Figure 1.** Mean ( $\pm$  SE) percentage mortality of *O. lybicus* exposed to essential oil and methanolic extract of *M. pulegium*. The means followed by different letters are significantly different (Tukey test,  $P < 0.05$ ).

### Discussion

The use of secondary organic compounds synthesized by plants, with known effects on insects, could be a useful complementary or alternative method to the heavy use of classical insecticides (Negahban et al. 2007). Commonly, these compounds can be ingested, inhaled or skin absorbed by immature and adult stages. Thus, plant secondary metabolites may show fumigant, contact, or stomach toxicity (Nouri-Ganbalani and Borzoui 2017). These compounds affect insects by being toxic causing a delay in developmental time (Huang et al. 1999). In the current study we analyzed the lethal and sublethal effects of *M. pulegium* essential oil and methanolic extract on *O. lybicus* adults. The tested compounds were shown here to possess fumigant and contact toxicity. Several studies have been reported previously concerning the activity of *M. pulegium* oil as fumigant on insect pests (Limiri et al. 2001; Esmaili et al. 2013; Barros et al. 2015; Zekri et al. 2016). In the present study, *M. pulegium* oil against *O. lybicus* adults may be accounted more toxic than *Callosobruchus maculatus* (F.) (Coleoptera:

Bruchidae) since its  $LC_{50}$  (9.68  $\mu\text{L/L}$  air) was at least ten times more potent than results obtained by Esmaili et al. (2013). Also, contact toxicity of the ethanolic extract has been reported from *M. pulegium* against *Sitophilus oryzae* L. (Coleoptera: Curculionidae) (Abou-Elnaga 2015) that the toxicity was lower than results obtained from the present study. The difference between our results with above-mentioned researcher may result from the differences in active volatiles of compounds and/or the differences in tested insect. The relative susceptibility of *O. lybicus* to a range of phytoinsecticides had been determined in previous assays under laboratory conditions (Mahmoudi et al. 2014; Al-dhamin 2015).

Joint toxicity of pesticides could be evaluated by combining compounds with either equitoxic ratio or equivalent concentrations, which are the two most commonly methods (Phyu et al. 2011; Stepic' et al. 2013). Few studies have been done about the interaction of essential oil and extract of plants for the management of pests. We report here the efficacy and effects of the application of essential oil and methanolic extract of *M. pulegium* against *O. lybicus* adults. The adult's exposure to sublethal values of essential oil ( $LC_{25}$ ) or methanolic extract ( $LD_{25}$ ) caused increased APOP and TPOP, and reduced oviposition period. However, exposure to both essential oil ( $LC_{25}$ ) and methanolic extract ( $LD_{25}$ ) did not result in an antagonism, additive or synergism interaction in terms of these parameters. The results regarding the interaction of tested compounds is not in agreement with those achieved by Borzoui et al. (2016), who reported positive interactions (additive and/or synergism) in larval *P. interpunctella* mortality that were exposed to combined pesticides.

The results regarding the incubation period, nymphal period and total lifespan of *O. lybicus* on the control closed with those achieved by Bagheri et al. (2016), who reported incubation period, nymphal period and adult longevity of this bugs on cultivar Bam 41.97, 42.81 and 110.21 days, respectively.

In our study, sub-lethal doses of *M. pulegium* essential oil ( $LC_{25}$ ) and methanolic extract ( $LD_{25}$ ) increased the immature stages period and reduced the survival of immature stages of *O. lybicus*. The increasing of the incubation period caused by different treatments may be due to the ability of these compounds to penetrate the chorion, and to act on the embryo and change its developmental rate (Croft 1990). These results are in agreement with Borzoui et al. (2016) which reported negative side effects of plant essential oils and extracts on the incubation period. The reduction in survival of nymphs caused by *M. pulegium* essential oil and methanolic extract may result from some combination of delayed toxicity following egg absorption and nymphal exposure to residues on surfaces following eclosion.

The results of this study show that *M. pulegium* essential oil and methanolic extract had a negative effect on the longevity and oviposition of *O. lybicus*. Also, we found that the longevity was reduced to a greater extent in the insects that exposed to both *M. pulegium* oil and extract. We hypothesize that this reduction results from the antifeedant activity of the methanolic extract as well as from a reduction in fitness parameters caused by the toxic effects of the essential oil. Mahmoudi et al. (2014) noted that after exposure of *O. lybicus* females to the sublethal values of kaolin and mineral oil, the adult longevity and fecundity were significantly reduced compared to the control.

In the present study, life table parameters data are similar to those reported from life table parameters of *O. lybicus* reared on cultivar Berhi under similar conditions (Bagheri et al. 2016). In agreement with previous research (Rumpf et al. 1998; Borzoui et al. 2016), the results of this study showed that different compounds had significant effects on *O. lybicus* life table parameters such as the intrinsic rate of increase ( $r_m$ ), the finite rate of increase ( $\lambda$ ), the growth reproduction rate ( $GRR$ ), mean generation time ( $T$ ), and the net reproduction rate ( $R_0$ ) (Table 4). All treatments reduced the  $R_0$ ,  $r_m$ ,  $\lambda$ , and  $GRR$  while those increased  $T$  of *O. lybicus* and prolonged the developmental time from egg to adult. In comparison to extract treatment,  $R_0$  and  $r_m$  parameters were reduced to a greater extent in insects exposed to both *M. pulegium* oil and extract, however, a statistically significant difference was not found when compared with essential oil treatment. The difference observed among the toxicity of *O. lybicus* caused by the tested compound may result from their active volatiles (which are differently toxic for insects) and mode of action of these insecticides (Tapondjou et al. 2002). Similar results have been obtained by Senthil Nathan et al. (2006) when they compared the individual and combined effects of bacterial toxins and botanical insecticides on the rice leaffolder.

In summary, the laboratory results presented here suggest that the essential oil and methanolic extract from *M. pulegium* could be an alternative to *O. lybicus* management. The observed mortality demonstrates that *M. pulegium* essential oil and methanolic extract are a source of biologically active compounds which may potentially prove to be efficient insecticides. Also, the present study demonstrates that the effect of essential oil of *M. pulegium* can not be enhanced by co-application with methanolic extract in *O. lybicus* adults. According to life table experiments, although, the tested treatments altered the parameters of life table, the average values of these parameters were positive, indicating that the population of *O. lybicus* was still able to increase. However, lower values of  $r_m$  are important because they represent smaller reproductive potential. Further work need to be conducted to test the efficacy of essential oils and their compatibility with natural enemies in the field.

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## اثرات زیر کشنده اسانس رازیانه و پونه روی پارامترهای زیستی زنجبرک خرما *Ommatissus lybicus*

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### چکیده

مدیریت تلفیقی آفات (IPM) یک رویکرد اکوسیستم برای تولید و حفاظت از محصول است که ترکیبی از استراتژی‌ها و شیوه‌های مختلف مدیریت برای تولید محصولات سالم و به حداقل رساندن استفاده از آفت‌کش‌ها می‌باشد. اسانس‌ها و عصاره‌ها در کشورهای در حال توسعه برای اهداف متعددی از جمله مدیریت حشرات آفت کشاورزی مورد استفاده قرار می‌گیرد. این مطالعه تأثیرات انفرادی و هم‌افزایی اسانس و عصاره‌ی متانولی *Mentha pulegium* را روی بالغین *Ommatissus lybicus* بررسی شد. مقادیر  $LD_{50}$  و  $LC_{50}$  برای *M. pulegium* به ترتیب  $9/68$  میکرولیتر بر لیتر هوا و  $36/97$  میلی‌گرم بر میلی‌لیتر می‌باشند. برای ارزیابی اثرات زیرکشندگی، سن‌های بالغ در معرض اسانس ( $LC_{25}$ )، عصاره متانولی ( $LD_{25}$ ) و ترکیب‌شان، و مرگ‌ومیر مراحل نابالغ و پارامترهای جدول زندگی *O. lybicus* زنده‌مانده مطالعه شد. بالاترین درصد مرگ‌ومیر تخم و پوره  $27/33$  درصد و  $37/60$  درصد بود که بعد از در معرض قرارگیری با  $LC_{25}$  اسانس *M. pulegium* مشاهده شد. تمامی تیمارها به‌طور معنی‌داری  $R_0$ ،  $r_m$ ،  $\lambda$  و  $GRR$  را کاهش دادند، درحالی‌که  $T$  برای *O. lybicus* افزایش یافت و زمان رشدونمو از تخم تا بلوغ طولانی‌تر شد. در مقایسه با تیمار عصاره، آماره‌های  $R_0$  و  $r_m$  در حشرات در معرض با هر دو اسانس و عصاره‌ی *M. pulegium* کاهش بیشتری داشت، هرچند، وقتی با تیمار اسانس مقایسه شد تفاوت آماری معنی‌داری یافت نشد. مطابق با این نتایج، هر دو اسانس و عصاره‌ی متانولی تست شده پتانسیل کاربرد برای مدیریت تلفیقی *O. lybicus* را دارند.

**واژه‌های کلیدی:** *Mentha pulegium*، تأثیر انفرادی، تأثیر ترکیبی، آماره‌های جدول زندگی، *Ommatissus lybicus*.

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