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Chemical composition, toxicity and physiological effects of essential oil of *Rosemarinus officinalis* on lesser mulberry pyralid, *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae)

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Abstract: Mulberry pyralid, *Glyphodes pyloalis* Walker is an important pest on mulberry. The essential oil of Rosemary *Rosemarinus officinalis* L. (Lamiales: Lamiaceae) has been investigated on toxicity and physiological characteristics of this moth under controlled conditions. The major compounds of the oil were analyzed as 1, 8 Cineol (20.021%), Borneol (7.17%), Camphor (6.541%), Geraniol (6.281%), Camphene (5.623%), Linalool (4.993%) Alpha fenchyl acetate (4.222%) and Verbenone (4.147%). Lethal and sublethal concentrations (LC_{10}, LC_{30} and LC_{50}) were estimated 0.77%, 1.18% and 1.59% (v/v) respectively. The essential oil affected the nutritional indices of fourth instar larvae of *G. pyloalis*. efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD), relative consumption rate (RCR) and relative growth rate (RGR) were reduced in larvae treated with *R. officinalis* essential oil while approximate digestibility (AD) in treated larvae was significantly increased compared with the control. The essential oil affected the insect's some key metabolic compounds like; lipid, protein and carbohydrates. Similarly this effect was also significant in the activities of certain key enzymes like; alpha- amylase, lipase, protease, glutathione-s-transferase and esterases.

Keywords: *Glyphodes pyloalis*, *Rosmarinus officinalis*, GC-MS; nutritional indices

Introduction

The lesser mulberry moth, *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae) is an important pest on mulberry and has been reported from Iran (Guilan province) since 2002 (Jafari *et al.,* 2006). Mulberry trees are important for silkworm (*Bombyx mori* L.) nutrition and are also used as shade trees in urban areas (Kumar *et al.,* 2002). Fourth and 5th instar larvae of *G. pyloalis* secrete fine threads to fold the leaf and feed on the mesophyl inside the folds, and 5th instar larvae feed on the whole leaf until only ribs remain (Khosravi and Jalali, 2010).

Chemicals that are largely used as pesticides in crop protection have undesirable effects such as ozone depletion, environmental pollution, toxicity to non-target organisms, pest resistance, pesticide residues and direct toxicity to users (Isman, 2006). With heightened concern for environmental problems and human health, the search for readily biodegradable and environmentally friendly insecticides is of interest among scientists (Shaaya *et al.,* 1997; Isman, 2000). Natural products are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment (Khani and Asghari, 2012). Many plant essential oils show a broad spectrum of activity against pest insects and plant pathogenic

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fungi ranging from insecticidal, antifeedant, repellent, oviposition deterrent, growth regulatory and antivector activities. These green pesticides can also prove effective in agricultural situations, particularly for organic food production. Ultimately it is in developing countries which are rich in endemic plant biodiversity that these pesticides may have their greatest impact in future integrated pest management (IPM) programmers due to their safety to non-target organisms and the environment (Koul, 2008).

Plant essential oils are produced commercially from several botanical sources, many of which are members of the mint family (Lamiaceae) (Isman, 2000). The Rosemary, *Rosmarinus officinalis* L. (Lamiales: Lamiaceae) is an aromatic evergreen shrubby herb highly distributed in the Mediterranean region. It is a well-known and greatly valued medicinal herb that is widely used in pharmaceutical products and folk medicine as a digestive, tonic, diuretic, diaphoretic and useful for urinary ailments (Chang *et al.,* 1977; Aqel, 1991; Leung *et al.,* 1996; Haloui *et al.,* 2000). Essential oils are natural products that contain natural flavors and fragrances grouped as monoterpenes, sesquiterpens and aliphatic compounds that provide characteristic odors (Mahdi *et al.,* 2011). Among the essential oil compounds the monoterpenoids have drawn the greatest attention for insecticidal activity against insect pests (Ogendo *et al.,* 2008). The mechanisms of toxicity of essential oils have not been fully identified. According to Lee *et al.,* (2003), the monoterpenes that may be volatiles and lipophylic, can penetrate through breathing and quickly intervene in physiological functions of insects. These compounds can also act directly as neurotoxic compounds, affecting acetylcholinesterase activity or octopamine receptors (Isman, 2000). Compounds extracted from plants or their derivatives have been shown to affect insect biology and physiology (Shekari *et al.,* 2008). Secondary metabolites from plants are deleterious to insect and other herbivores in diverse ways, such as through acute toxicity, enzyme inhibition, and interference with the consumption and/or utilization of food (Lindroth, 1991; Senthil Nathan, 2006; Motazedian *et al.,* 2012). In many cases, however, the mode of action for these metabolites is unknown. In an effort to determine the effects of, and herbivore responses to, dietary allelochemicals, their consumption and utilization of food is often quantified and various food utilization efficiencies are calculated (Waldbauer, 1968; Slansky and Scriber, 1985). In insects the complex food molecules are utilized after they have been broken down into simpler molecules through the action of digestive enzymes in the gut of the larva, thus the enzyme system in the insect plays a vital role in food digestion (Lokesh *et al.,* 2006). Currently, one of the most important aspects of pest control is the selective inhibition of digestive enzymes of many insect pests. These inhibitors are insecticidal because they form complexes with digestive enzymes which are stable and dissociate slowly. Inactivation of digestive enzymes by inhibitors results in blocking of gut hydrolases, such as proteinases and carbohydrases, which leads to poor nutrient utilization, retarded development and death by starvation (Jongsma and Bolter, 1997; Gatehouse and Gatehouse, 1999). The metabolic enzymes are also important as they metabolize toxic compounds (Jakoby and Habig, 1980). In the present study we have tried to elucidate the effect of essential oil of *R. officinalis* on toxicity, nutritional indices and different digestive and detoxifying enzymes of lesser mulberry pyralid.

Materials and Methods

Plant material and essential oil extraction

Rosmarinus officinalis L. foliage was collected from university campus Rasht in north of Iran, near the point referenced 49°36′ E longitude, 37°16′ N latitude and 7m altitude. Rosemary`s foliages were collected, dried away from sunlight, cut in pieces and distilled. The extraction of essential oil was carried out in a Clevenger type apparatus during 2h. The oil phase was separated from obtained solution. Sodium sulfate was used for dehydration.

Analysis of essential oil

One microliter of the prepared essential oil was injected to GC-Ms (HP Agilent 6800N/(61530N) with CPSil5CB column (Chrompack, 100% dimethyl polysiloxane 60

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m, 0.25 mm (ID), film thickness 0.25 micron). The analysis was performed under temperature programming from 100 °C (3 min) to 250 °C (5 min) with the rate of 3 °C/min. Injector temperature was 230 °C. Identification of spectra was carried out by study of their fragmentation and also by comparison with standard spectra. Area normalization was used for determination of composition percentage.

Insect rearing and evaluation of insecticidal efficiency of essential oil

The fifth instar larvae of lesser mulberry pyralid (*G. pyloalis*) were collected from infested mulberry plantation in Guilan province, northern Iran. They were reared in growth chamber in controlled condition (24 ± 2 °C, 75 ± 10% RH and a photoperiod of 16: 8 (L: D) h) on fresh mulberry leaves (Kenmochi variety). Adult moths were placed in transparent jars of 20 × 6 cm^2 and provided with fresh mulberry leaves for egg laying and cotton wool soaked in 10% honey for feeding. Fourth larval instars were used in the subsequent experiments.

Before bioassay tests, larvae were starved for 4 hours. Initially, preliminary tests were performed to find the effective dose ranges. Four concentrations of the essential oil (2%, 1.6%, 1.3% and 1%) were prepared by methanol (v/v) as a solvent for bioassays. This experiment was performed in 4 replications with 10 larvae of 4^{th} instar (< 24 h) in each replication. The fresh leaf discs were cut from mulberry leaves (8cm diameter) and were immersed in different concentrations of essential oil for 10 seconds and air-dried. Ten larvae were deposited into each disc and allowed to feed. Control leaves were treated with methanol and air-dried. After 48 hours the numbers of dead larvae were recorded. $LC_{10,}$ LC_{30} and LC_{50} values were estimated using Polo-PC software (LeOra, 1987).

Quantitative food utilization efficiency measures

To evaluate the effect of essential oil on the nutritional physiology in larvae of *G. pyloalis*, 8cm diameter discs of mulberry leaf were prepared. All weights were measured using a monopan balance accurate to 0.1 mg (Sartorius GMBH, Type: A 120 S). Leaf discs were dipped in LC_{30} concentration of essential oil for 10 seconds. Control leaves were treated with methanol and air dried. To determine weight gain, food utilization and feces produced by the larvae, a gravimetric method was used. Nutritional indices were evaluated on the basis of dry weight. The newly molted fourth instar larvae were used for this experiment. The larvae were starved 4 h (10 larvae/ concentration) and allowed to feed on weighed quantities of treated and untreated leaves. Four replicates were carried out (n = 40). Leaves were weighed individually and placed in petri-dishes (8 cm diameter) for larvae to feed upon. After measuring the initial weight of the larvae, they were individually introduced into separate containers. After 24 h the remains of leaves were replaced by new treated leaves. The remaining leaves were weighed at the end of 24 h and placed in an oven (45 °C) for 48 h and reweighed in order to calculate the dry weight of consumed food. The feces produced each day were collected, oven dried and weighed to estimate the dry weight of excreta. The weights of larvae were recorded at the end of the day. The duration of the experiment was three days and the observations were recorded each day. Nutritional indices were calculated using formulae described by Waldbauer (1968): approximate digestibility (AD) = 100 (E-F)/E; efficiency of conversion of ingested food (ECI) = 100 P/E; Efficiency of conversion of digested food (ECD) = 100 P/ (E-F); Relative growth rate (RGR) = P/TA and Relative consumption rate (RCR) = E/TA. Where A = mean dry weight of insect over unit time, E = dry weight of food consumed, F = dry weight of feces produced, P = dry weight gain of insect and, T = duration of experimental period.

Preparation of sample for enzymatic assay

Initially, leaf discs were treated with LC_{10}, LC_{30} and LC_{50} concentrations of the essential oil. In each experiment, 10 insects were tested with 4 replicates for each concentration. After 48 h of feeding, the live larvae were randomly selected and their guts were removed by dissection under a stereomicroscope (Olympus, SZX12) in ice-cold buffer (Salin buffer). Certain numbers of

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larvae guts were placed in 2 ml of distilled water or buffer related to each test and then samples were homogenized. The homogenates were centrifuged at 4 °C for 10 minutes. The resulting supernatants were transferred into new micro tubes and frozen at-20 °C until further use.

Assay of α-amylase activity

The α-amylase activity was assayed by dinitrosalicylic acid (DNS) procedure (Bernfeld 1955). One percent soluble starch (Merck, Darmstadt, Germany) was used as the substrate. Ten micro liters of the enzyme were incubated for 30min at 35 °C with 80 µL universal buffer (Glycine, Mes (2-[morpholino] ethansulphonic acid), Succinate, NaOH, Distilled water) and 20 µL soluble starch. In order to stop the reaction, 90 µL DNS was added and the mixture was heated in boiling water for 10 min. Dinitrosalicylic acid is a color reagent and the reducing groups released from starch by α-amylase action were measured by the reduction of 3, 5 Dinitrosalicylic acid. A standard curve of α-amylase absorbance against the amount of released maltose was constructed to enable the calculation of the amount of maltose released during the α-amylase assay. All assays were performed in four replicates. Absorbance was measured at 540 nm after cooling in ice for five minutes.

Assay of lipase activity

The activity of lipase was estimated by the method of Tsujita *et al.,* (1989). Ten microlters of homogenate were mixed with 18µL P-nitrophenyl Butyrate (50mM) as substrate and mixed with 172 µL universal buffer (1M) (pH = 7) and incubated at 37 °C. The absorbance was read at 405 nm.

Assay of protease activity

The protease activity of larval guts was determined using azocasein 1% as substrate (Garcia-Carreno and Haard, 1993). Each gut was centrifuged in 10 µl distilled water, then 10 µl of supernatant and 15 µL buffer (pH = 8) with 50 µL substrate were reacted for 3 h at 37 °C. Proteolysis was stopped by the addition of 150 µLof 10% trichloroacetic acid (TCA). The solution was transferred to 4 °C in a refrigerator for 30 min, and the reaction mixture was centrifuged at 13000 g for 10 min. One hundred microliters of supernatant were mixed with 100 µL 1N NaOH and the absorbance was read at 440 nm.

Assay of esterase activity

The activities of general esterases were determined according to Van Asperen (1962) method. In this experiment, α-naphtlyacetate (α-NA) and β-naphtylacetate (β-NA) were used as substrates. One gut was homogenized with 1000 µL 0.1 M phosphate (pH = 7) containing Triton x-100 at the ratio of 0.01%, then the homogenized solution was centrifuged at 10000 g for 10 minutes at 4 °C. The supernatant was transferred to new micro tube and was diluted with phosphate buffer. This solution reacted with the substrate and by using dye indicator (Fast Blue RR salt) (1 mM) a colored solution was formed and the absorbance was read at 630 nm.

Assay of glutathione S-transferase

The activity of glutathione S-transferase was determined according to method of Habing *et al.,* (1974). 1-chloro-2, 4-dinitrobenzene (CDNB) (20 mM) was used as the substrate. Initially a larva was homogenized in 20 µL distilled water, then the homogenized solution was centrifuged at 12500 g for 10 minutes at 4°C. Fifteen microliters of supernatant were mixed with 135 µL of phosphate buffer (pH = 7), 50 µL of CDNB and 100 µL of GST. The absorbance was read at 340 nm.

Estimation of protein

The method of Bradford (Bradford, 1976) was used for determining total protein. Each larva was homogenized in 350 µL of distilled water and samples were centrifuged at 10000 rpm for 5 min at 4 °C. Then 10 µL of supernatant was mixed with 90 µL of distilled water and 2500 µL dye (10 mg powder of Coomassie Brilliant Blue (Bio-Red, Munchen, Germany) in 5 ml ethanol 96% and 10 ml phosphoric acid 85% (w/w) then solution brought to 100 ml with distilled water). Bovin serum albumin was used as the standard. The absorbance was read at 630 nm.

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Estimation of lipid and carbohydrate

Determination of lipids and carbohydrates was performed according to Yual *et al.,* (1994). Larvae were homogenized individually in 100 µL of % Na_2SO_4. 2Lipids and carbohydrates were extracted in 750 µL of chloroform: methanol (1: 2). Individual tubes were centrifuged for 10 min at 8000 rpm at 4˚C. After that 500 µL were taken from each tube and dried at 40˚C. Samples were then dissolved in 500 µL H_2SO_4 and incubated for 10 min at 90 ˚C in water bath. Samples of 30 µL were placed into wells on Elisa plates; together with 270 µL of vanillin reagent (600 mg vanillin dissolved in 100 ml distilled water and 400 ml 85% H_3PO_4). After 30 min the absorbance was read at 530 nm. Total lipid was calculated from standard curve of cholesterol.

To determine the amount of carbohydrate in each larva, 150 µL was taken from the chloroform: methanol extract, then 100 µL distilled water was added along with 500 µL of anthrone (500 mg of anthrone dissolved in 500 ml H_2SO_4). The samples were placed at 90 °C for reaction to take place, and after that 250 µL of sample were placed in the Elisa plates. The absorbance was read at 630 nm and carbohydrate level was calculated by a standard curve.

Statistical analyses

$LC_{10,}$ LC_{30} and LC_{50} values were estimated with Polo-PC (LEORA 1987). Data from nutritional indices and enzymes activity were compared by one-way analysis of variance (ANOVA). Differences between the various treatments were determined at 5% by Tukey`s multiple range tests using SAS software (SAS, 1997).

Results

Chemical composition of essential oil

The chemical composition of Rosemary was studied by gas chromatography mass spectrometry (GC-MS). About 33 compounds were identified of which the major compounds were: 1, 8 Cineol (20.021%), Borneoll (7.17%), L. Camphor (6.541%), Geraniol (6.281%), Camphene (5.623%), Linalool (4.993%) Alpha fenchyl acetate (4.222%), Verbenone (4.147%), Alpha pinene (3.487%), Alpha terpineol (3.148%) and Beta caryophyllene (2.702%). These components are shown in Table 1.

Table 1 Chemical constituents of the essential oil of *R. officinalis.*

Compound	Retention time (min)	Composition (%)
Tricyclene	4.754	0.381
Camphene	5.401	5.623
Verbenene	5.48	1.615
Beta- Pinene	5.94	3.487
3-Octanone (CAS)	6.147	0.591
Myrcene	6.27	2.535
Alpha-Phellandrene	6.508	0.28
Alpha-Terpinene	6.781	0.446
Para-Cymene	6.956	0.725
1,8- Cineole	7.237	20.021
Gamma-Terpinene	7.709	0.681
Terpinolene	8.344	1.133
Linalool	8.685	4.993
Chrysanthenone	9.137	0.596
L-Camphor	9.601	6.541
Bicyclo[3.1.1] heptan.3.one,2,6,6.trimethyl	9.887	0.658
Pinocarvone	9.93	0.39
Borneoll	10.121	7.17
Iso-pinocamphone	10.216	1.525
Terpinen-4-ol	10.287	2.047
Alpha-Terpineol	10.529	0.792
Alpha-Terpineol	10.593	0.721
Alpha-Terpineol	10.68	3.148
Myrtenol (Bicyclo)	10.815	0.893
Verbenone	10.974	4.147
Verbenone	11.077	6.761
Citronellol	11.319	0.476
Camphene	11.481	0.522
Delta,3-carene	11.608	0.93
Geraniol	11.945	6.028
Geranial	12.175	0.456
Alpha-Fenchyl acetate	12.497	4.222
Methyl eugenol	14.678	0.466
Beta-Caryophyllene	14.999	2.702
Beta-Selinene	15.578	0.534
Caryophyllene oxide	17.823	0.962
Caryophylla-3,8 [13]-dien-5.beta-ol	19.013	0.311

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Bioassays

The LC_{10}, LC_{30} and LC_{50} values for *R. officinalis* essential oil after 48h are shown in Table 2. Bioassay results showed that the mortality of larvae was dose-dependent. There were significant differences between control and treatments. The $LC_{10,}$ LC_{30} and LC_{50} of *Rofficinalis* were 0.777%, 1.18% and 1.59% (v/v) respectively.

The effect of *R. officinalis* essential oil on nutritional indices.

The results of the effect of LC_{30} concentration of *R. officinalis* essential oil on nutritional indices are shown in Table 3. ECI, ECD, RCR and RGR have been reduced in larvae treated with *R. officinalis* essential oil while AD in treated larvae showed significant increase compared with the control. The reduction of ECD and RGR was significant compared with the control.

Table 2 LC_{10}, LC_{30} and LC_{50} of *Rosmarinus officinalis* essential oil on 4th instar larva of *G. pyloalis.*

LC^*_{10} (0.77% v/v)	LC_{30} (1.18% v/v)	LC_{50} (1.59% v/v)	χ^2 (df)	Slope ± SE
0.777 (0.41-0.985)	1.18 (0.905-1.35)	1.59 (1.4-1.89)	0.738 (2)	1.038 ± 0.46

*CL: Confidence Limit which has been calculated with 95% confidence.

Table 3 Comparisons of feeding efficiency in fourth instar larvae of *G. pyloalis* treated with LC_{30} concentration of *R. officinalis* essential oil and control.

	AD%	ECI%	ECD%	RCR (mg/mg/Day)	RGR (mg/mg/Day)
Control	76.62 ± 0.6	5.2 ± 0.68	4.87 ± 0.89	1.45 ± 0.07	0.066 ± 0.003
R. officinalis	80.66 ± 0.97*	4.13 ± 0.27	4.22 ± 0.27*	1.36 ± 0.1	0.043 ± 0.008*

Means in a column followed by * are significant at $P < 0.05$ (student t-test).
AD: Approximate Digestibility.
ECI: Efficiency of Conversion of Ingested food.
ECD: Efficiency of Conversion of Digested food.
RGR: Relative Growth Rate.
RCR: Relative Consumption Rate.

The effect of *R. officinalis* essential oil on macromolecules

Amount of total protein in the treated larvae with *R. officinalis* essential oil showed a significant decrease compared with the control larvae. It was highest in the larvae treated with LC_{10} ($F = 85.88$, df = 3, $p < 0.0001$) concentration and the lowest in larvae treated with LC_{50} concentration and had significant differences compared with the control (Fig. 2). The amount of lipid and carbohydrate was decreased in the larvae treated with different concentrations, level of carbohydrate and lipid was also decreased as essential oil concentrations increased (Figs. 2, 3).

The effect of *R. officinalis* essential oil on digestive and detoxifying enzymes

Activity level of α-amylase enzyme was decreased at all concentrations and its lowest level is seen in larvae treated with LC_{10} ($F = 1.04$, df = 3, $P = 0.427$) concentration of Rosemary essential oil (Table 4). The level of protease in treated 4th instar larvae of *G. pyloalis* with various concentrations was increased, this increase was significant at LC_{10} ($F = 147.4$, df = 3, $P < 0.0001$). LC_{30} and LC_{50} of *R. officinalis* increased the activity level of protease but this increase was not significant compared with the control. The level of lipase was enhanced in treated larvae where this increase was significant at LC_{50} concentration

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(F = 0.82, df = 3, P = 0.0471) of *R. officinalis*. Results showed that Rosemary essential oil increased activity of glutathion S-transferase in larvae fed on treated leaves (Table 4). The esterases were increased by all the concentrations of essential oil when α-naphtyl acetate was used as a substrate (F = 132.96, df = 3, P < 0.0001). By using β- naphtyl acetate indicated that the treated larvae by LC_{10}, LC_{30} and LC_{50} concentrations of *R. officinalis* essential oil, esterases activity was increased significantly compared with the control (F = 12.44, df = 3, P = 0.0023). In the present investigation it was shown that the activity of esterases in 4th instar larvae of *G. pyloalis* was increased 48h after treatment (Table 4).

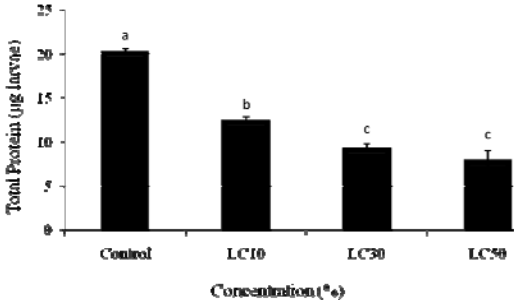

Figure 1 Amount of Total protein in fourth instar larvae of *G. pyloalis* after treatment with essential oil of *R. officinalis*. Different letters indicate differences at P < 0.05 by Tukey`s multiple range tests.

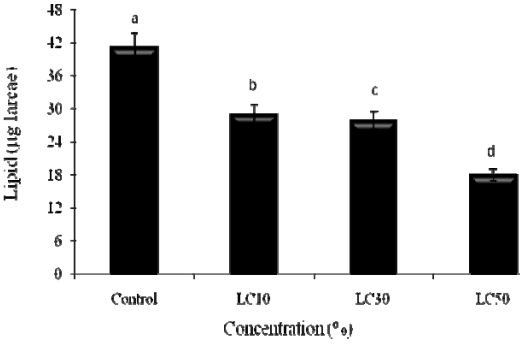

Figure 2 Amount of Lipid in fourth instar larvae of *G. pyloalis* after treatment with essential oil of *R. officinalis*. Different letters indicate differences at P < 0.05 by Tukey`s multiple range tests.

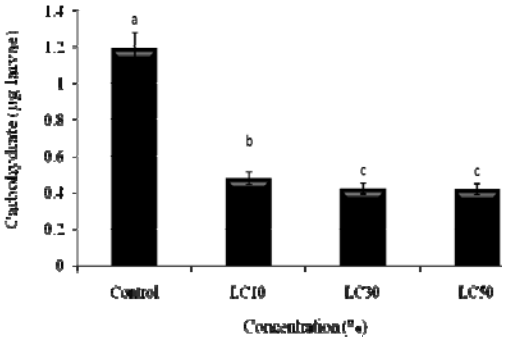

Figure 3 Amount of Carbohydrate in fourth instar larvae of *G. pyloalis* after treatment with essential oil of *R. officinalis*. Different letters indicate differences at P < 0.05 by Tukey`s multiple range tests.

Table 4 Activity of alpha-amylase, protease, lipase, esterase and glutathione S-transferase enzymes in fourth instar larvae of lesser mulberry pyralid *G. pyloalis* after treatment with LC_{10}, LC_{30} and LC_{50} concentrations of *R. officinalis* essential oil.

Treatments	α-Amylase (nmol/min/mg protein)	Lipase (µmol/min/mg protein)	Protease (OD/min/mg protein)	GST (µmol/min/mg protein)	Esterase (nmol/min/mg protein)	
Control	$0.0193 \pm 7 \times 10^{-4}$ a	$0.003 \pm 4 \times 10^{-4}$ b	1.45 ± 0.05 b	$0.0004 \pm 1 \times 10^{-4}$ b	$0.02 \pm 1 \times 10^{-3}$ d*	$0.005 \pm 4 \times 10^{-4}$ b**
LC_{10}	$0.013 \pm 1 \times 10^{-3}$ a	$0.006 \pm 3 \times 10^{-4}$ b	5.05 ± 0.9 a	$0.0014 \pm 1 \times 10^{-4}$ a	$0.041 \pm 4 \times 10^{-3}$ c	$0.013 \pm 1 \times 10^{-3}$ a
LC_{30}	$0.017 \pm 4 \times 10^{-4}$ a	$0.004 \pm 1 \times 10^{-3}$ b	2.47 ± 0.16 b	$0.002 \pm 9 \times 10^{-6}$ b	$0.07 \pm 2 \times 10^{-3}$ b	$0.016 \pm 2 \times 10^{-3}$ a
LC_{50}	$0.017 \pm 6 \times 10^{-3}$ a	$0.013 \pm 1 \times 10^{-3}$ a	2.24 ± 0.1 b	$0.021 \pm 3 \times 10^{-3}$ a	$0.1 \pm 3 \times 10^{-3}$ a	$0.014 \pm 1 \times 10^{-3}$ a

Within columns, means followed by the same letter do not differ significantly at P < 0.05.
* α-Naphtyl Acetate substrate
** β-Naphtyl Acetate substrate.

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Discussion

Essential oils are very complex natural mixtures which contain about 20-60 components at quite different concentrations. Chemical analysis of essential oil of *R. officinalis* revealed 33 components in which 1, 8 Cineol (20.021%), Borneoll (7.17%), L. Camphor (6.541%), Geraniol (6.281%), Camphen (5.623%), Linalool (4.993%), Alpha fenchyl acetate (4.222%), Verbenone (4.147%). and some other compounds were the major constituents. Multiple studies have been reported on the chemical composition of the essential oils of *R. officinalis* belonging to different regions in the world (Khorshidi *et al.,* 2009). Furthermore, several extracts, essential oils and chemical constituents isolated from this species demonstrated a number of interesting biological activities such as antioxidant (Inatani, 1983; Houlihan *et al.,* 1985; Aruoma *et al.,* 1992; Aruoma *et al.,* 1996; Haraguchi *et al.,* 1995; Curelier *et al.,* 1996; Frankel *et al.,* 1996; Dorman *et al.,* 2003) antiulcerogenic (Dias, 2000), and anticarcinogenic (Offord, 1995). Najafian *et al.,* (2012) reported that the major components of the essential oil form *R. officinalis* in full flowering stage which were cultivated in Shiraz, Iran were α-pinene (13.2%), β-pinene (2.0%), 1,8-cineole (7.1%), Camphor (9.5%), Borneol (8.7%), Linalool (3.7%) and Verbenon (12.1%). The chemical composition of a plant product depends on the plant species, the plant part, the season (temperature, photoperiod and hygrometry), the method of harvesting, the geographical zone, pedological conditions and the method used to isolate the plant product. Therefore, the extract of the same species from different geographical areas and various plant parts can be different in chemical composition (Iskber *et al*., 2006). Generally, these major components determine the biological properties of the essential oils.

Plant secondary metabolites play a major role in defense against herbivore, insects as growth regulators or by acting as antifeedant and the function through toxicity (Isman, 2006). Our findings showed that the Rosemary essential oil was toxic to *G. Pyloalis* larvae and acted as antifeedant at LC_{30} concentration by interfering with key metabolic pathways. Essential oil of *R. officinalis* had repellent effect on *P. interpuntella* adults (Rafiei-Kahroodi *et al.,* 2011) and fumigant toxicity on adults of *T. castaneum*, *S. granaries*, *C. maculates*, *P. interpunctella* (Mahmoudvand *et al.,* 2011) and fumigant toxicity, repellency and oviposition deterrent activity on *C. maculatus* and *T. castaneum* (Mirkazemi *et al.,* 2010). The findings of the present investigation indicate larvicidal activity in the essential oil of *R. officinalis* against lesser mulberry pyralid.

The nutritional indices particularly AD and RGR values of *G. pyloalis* on LC_{30} concentration of essential oil were significantly different. Analysis of nutritional indices can lead to understanding of the behavioral and physiological basis of insect response to host plant (Lazarvic and Peric-Mataruga, 2003). Broadly, these indices define or describe the efficiencies of digestion or utilization of diets or diet components that is how easily the insect can convert foods or their own biomass. The effects of rosemary essential oil on insect growth (nutritional indices) observed in the present study can be compared to effects observed in other insects (Adel *et al.,* 2000; Aerts and Mordue, 1997; Koul and Isman, 1991; Koul *et al.,* 2004; Senthil-Nathan and Kalaivani, 2005).

The AD takes into account the amount of food consumed in a given period of time and the amount of fecal matter (egested material) that is eliminated and that is associated with the feeding cycle of the insect being tested. The estimates of AD are more reflective of true digestibility of the foods. Clearly, AD measures a very important aspect of diets- their digestive availability to the insects (Cohen, 2005). The AD in the present study has been increased in the treated insects, which is indicative of low consumption rate hence, the insect tries to compensate and this enhance digestibility to overcome the lacunae of food. Similar studies on the effects of extract and essential oil from plants on food consumption (Wheeler *et al.,* 2001; Senthil-Nathan *et al.,* 2005;

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Senthil- Nathan *et al.,* 2006) have been previously conducted. The second index that is widely used to evaluate insect`s responses to diets and diet ingredients is the efficiency of conversion of ingested food to insect biomass (ECI). It is also known as the growth efficiency index. The ECI is a robust index for showing the overall ability of the insect to use the food in question for building biomass (Cohen, 2005). ECD is also known as metabolic efficiency because it takes into account already digested food (the weight of the food ingested minus the weight of feces). The measurement of ECD provides a resolution to the question of the food`s overall nutritional value once the non-digestible materials are eliminated. ECI and ECD indices decreased in treated larvae with essential oil of Rosemary. A drop in ECI indicates that more food is being metabolized for energy and less is being converted to body mass. Reduction in ECD value is likely to result from a reduction in the efficiency to convert foodstuffs into growth, perhaps by a diversion of energy from production of biomass into detoxification of savory essential oil (Silveira Ramos *et al.,* 2009). Bradway and Duffy (1988) and Haung *et al.*, (2004) considered the dependency of ECI and ECD on the activity of digestive enzymes. It may be inferred from the previous studies that the decreased larval growth coupled with lower RGR, which is more likely due to longer retention of food in the gut for maximization of AD to meet the increased demand of nutrients (Senthil-Nathan *et al.,* 2005; Senthil-Nathan and Sehoon, 2006). The results of this study revealed that although the treated larvae were capable of maintaining the AD (increased during treatment), they failed to maintain the RGR during larval development. The percentage reduction in ECI and ECD results from a food conversion deficiency, which reduces growth perhaps through a diversion of energy from biomass production into detoxification (Wheeler *et al.,* 2001). The decreased RGR and RCR in treated larvae may indicate the toxic effects of plants' allelochemicals on peritrophic membrane and damage to cellular surfaces of the midgut (Marie *et al.,* 2009).

In physiological studies, determination of total protein and many of chemical macromolecules such as lipids and carbohydrates are very important. The present study also showed that the total protein content of treated larvae was decreased compared with the control. Shekari *et al.,* (2008) reported lower protein and glucose in larvae of *Xanthogaleruca luteola* treated with *A. annua* extract. Mukherjee *et al.,* (1993) also showed that higher concentrations of azadirachtin affected the amount of protein in the hemolynph of *Tribolium castaneum*.

Lipids are an important source of energy for reproduction, embryonic growth, cell maintenance, and ecdysis. They are reserved in fat bodies (Chapman, 1998). In this investigation reduction of lipid is dose dependent. Essential oils of Jojoba and Sesame caused reduction in lipid of *S. littoralis* larvae (Marie *et al.,* 2009).

In this research amount of carbohydrates of treated larvae with rosemary essential oil was reduced compared with the controls. This was consistent with the reports of Etebari *et al.,* (2006), Shekari *et al.,* (2008) and Khosravi *et al.,* (2010). Starvation may reduce biochemical components in the midgut of lesser mulberry pyralid.

Digestive enzymes play a major role in insect physiology by converting complex food materials into micro molecules necessary to provide energy and metabolites for growth, development and other vital functions (Erturk and Turki., 2006). The results of this study indicated that there is considerable variation in midgut amylase, lipase and protease activity between the insects treated with the different concentrations of essential oil. The present results demonstrated the increased protease activity after essential oil treatments. The increased activity of midgut lipase in all concentrations of essential oil treatments might account for a greater utilization of exogenous lipids that result in the biomass production (Champagne *et al.,* 1992; Babu and Kavitha, 1997; Desai *et al.,* 2000; Ahmad *et al.,* 2006).

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Alpha amylase is an enzyme that hydrolyses the alpha bond of large alpha-linked polysaccharides, such as starch and glycogen, yielding glucose and maltose. The present study clearly depicts the enzyme`s lower activity following treatment with essential oil, which is consistent with other reports on various chemical treatments (Nath, 2003; Zibaee *et al.,* 2008; Hasheminia *et al.,* 2011; Khosravi *et al.,* 2011, Khosravi and Jalali Sendi, 2013). The reduction of α-amylase activity by plant essential oils and plant extracts could be due to the plant`s defense compounds, including inhibitors that act on insect gut hydrolases such as α-amylase and proteinases (Franco *et al.,* 2002). The reduction of α-amylase activity could also be due to a cytotoxic effect of different plant compounds on epithelial cells of the midgut that synthesize α-amylase (Jbilou *et al.,* 2008).

Lipases are enzymes that preferentially hydrolyze the outer links of fat molecules and have been studied in few insects. These enzymes are also the basic components in many physiological processes like growth, reproduction and defense against pathogens. The level of lipase was enhanced in treated larvae and this increase was significant at LC_{50} concentration of *R. officinalis*. Khosravi *et al.*, (2011) found similar results when larvae of *G. pyloalis* were fed on food treated with *A. annua* extract. Senthil- Nathan *et al.,* (2006) showed that treating *Cnaphalocrocis medinalis* (Guenee), with BtK. NSKE and VNLE (azadirachtin and neem components) decreased the activity of lipase in the midgut.

Proteases have a crucial role in food digestion by insects that hydrolyze peptide bonds in protein and convert them into their respective amino acids (Terra and Ferriera, 2005). In the present study it was shown that protease activity was increased with the use of rosemary essential oi.l. Perhaps in high concentration (LC_{50}), the activity of protease increased to degrade secondary metabolites. Studies by Johnson *et al.,* (1990) Senthil-Nathan *et al.* (2006) and Zibaee and Bandani (2010) concluded that botanical insecticides may interfere with the production of certain types of proteases and disable them to digest ingested proteins. Khosravi *et al.* (2011) showed that protease activity was reduced using 0.013% and 0.026% concentrations of methanol extract of *A. annua* compared with the controls but increased with 0. 107% concentration.

The metabolic enzymes have a role in metabolizing toxic materials. Four types of detoxifying enzymes have been found that react against botanical insecticides. They include general esterases, glutathione s-transferase and phosphatases. Glutathione s-transferases are a major family of detoxification enzymes found in most organisms. GSTs play an important role in insecticide resistance (Zibaee *et al.,* 2009). They help to protect cells from oxidative stress and chemical toxicants by aiding the excretion of electrophilic and lipophilic compounds from the cell (Hayes and Pulford, 1995). In the present study, it was shown that activity of GST in the fourth instar larvae of *G. pyloalis* treated with *R. officinalis* essential oil was increased compared with the control. Vanhaelen *et al.* (2001) showed that Brassicacea secondary metabolites induced GST activity in *Myzus persicae* and several Lepidopteran species such as *Heliothis virescens* Fabricius, *Trichoplusia ni* Hubner and *Anticarsia gemmatalis* Hubner.

In our study, the essential oil of *R. officinalis* caused increase in esterase activity in the midgut of *G. pyloalis* and this increase was dose-dependent. Khosravi *et al*. (2011) reported that *A. annua* extract does not have an inhibitory effect on detoxification enzyme of *G. pyloalis* after the consumption of *A. annua* extract. LIU *et al*. (2008) reported that GST and general esterase activity didn`t change in the larvae of *Ostrinia furnacalis* after feeding on fraxinellone treated food. This shows that GST and esterase play a role in detoxification or in the metabolism of *R. officinalis* essential oil.

Conclusion

Essential oil of *R. officinalis* possesses the property of larvicidal activity and antifeedant effect against *G. pyloalis*. Moreover, compounds present in the essential oil of *R. officinalis* affected the activity of

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chemical macromolecules, digestive enzymes and detoxification enzymes. However, further investigation is needed to study the effect of individual components of this essential oil which are responsible for inhibiting the various activities of the lesser mulberry pyralid.

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References

Adel, M. M., Sehnal, F. and Juryzsta, M. 2000. Effect of alfalfa saponins on the moth *Spodoptera litoralis*. Journal of Chemical Ecology, 26: 1065-1078.

Aerts, R. J. and Mordue, A. L. 1997. Feeding deterrence and toxicity of neem triterpenoids. Journal of Chemical Ecology, 23: 2117-1231.

Ahmad, M., Emam, S. S., Naidia, Y. and Megally, G. 2006. Furocouarin and quinoline alkaloid with larvicidal and antifeedant activities isolated from *Ruta chalepensis* Leaves. Journal of Natural Products, 2: 10-22.

Aqel, M. B. 1991. Relaxant effect of the volatile oil of *Rosmarinus officinalis* on tracheal smooth muscle. Journal of Ethnopharmacology, 33: 57-62.

Aruoma, O. I., Halliwell, B., Aeschbach, R. and Loligers, J. 1992. Antioxidants and pro-oxidant properties of active Rosemary constituents: carnosol and carnosic acid. Xenobiotica, 22: 257-268.

Aruoma, O. I., Spencer, J. P. E., Ross R., Aesefhbach, R., Khan A., Mahmood, N., Munor, A., Murcia, A., Butler, J. and Halliwell, B. 1996. An evaluation of the antioxidant and antiviral action of extracts of Rosemary Provencal herbs. Food and Chemical Toxicology, 34: 449-456.

Babu, R. and Kavitha, R. 1997. Impact of Azadirachtin on quantitative protein and lipid profiles during gonodotrophic period of *Atractomorphha crenutata* Fab. Indian Journal of Experimental Biology, 35: 998-1001.

Bernfeld, P. 1955. α-Amylases. Methods in Enzymology, 1: 149-151.

Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Analytical Biochemistry, 72: 248.

Broadway, R. M. and Duffey S. S. 1988. The effect of plant protein quality on insect digestive physiology and the toxicity of plant proteinase inhibitors. Journal Insect Physiology, 34: 1111-1117.

Chang, S. S., Ostric-Monjasevic, C. B., Hsie, O. L. and Huang, C. L. 1977. Natural antioxidant from Rosemary and sage. Journal of Food Science, 42: 1102-1106.

Champagne, D. E., Koul, O., Isman, M. B., Scudder, G. G. E. and Towers, G. H. N. 1992. Biological activity of limonoids from Rutales. Phytochemistry, 31: 377-394.

Chapman, R. F. 1998. The Insects Structure and Function, 4th edition, Cambridge University Press, Cambridge. pp. 782.

Cohen, A. C. 2005. Insect diets: Science and Technology. CRC Press. 168-171.

Curelier, M. E., Richard, H. and Berset C. 1996. Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. Journal of the American Oil Chemists' Society, 73: 645-652.

Desai, D. E. and Desai, B. D. 2000. Studies on insecticides properties of some plants. Shaspha, 71:85-88.

Dorman, H. J. D., Peltoketo, A., Ailtunen, R. and Tilkkanen, M. J. 2003. Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. Food Chemistry, 83, 255-262.

Dias, P. C., Foglia, M. A., Possenti, A. and De Carvalho, J. E. 2000. Antiulcerogenic activity of crude hydroalcoholic extracts of *Rosmarinus officinalis* L. Journal of Ethnopharmacology, 69: 57-62.

Erturk, O. 2006. Antifeedant and toxicity effects of some plant extracts on *Thaumetpoae*

[DOR: 20.1001.1.22519041.2013.2.4.3.7]

solitaria Frey. (Lep: Thaumetopoeitidae). Turkish Journal of Biology, 30: 51-57.

Etebari, K., Bizhannia, A. R., Sorati, R. and Matindoost, L. 2006. Biochemical changes in haemolymph of silkworm larvae due to pyriproxyphen residue. Pesticide Biochemistry and Physiology, 88: 14-19.

Franco, O. L., Rigden, D. J., Melo, F. R. and Grossi-De-Sa, M. F. 2002. Plant α-amylase inhibitors and their interaction with insect α-amylase: structure, function and potential for crop production. European Journal of Biochemistry, 269: 397-412.

Frankel, E. N., Huang, S., Prior, E. and Aeschbach, R. 1996. Evaluation of antioxidant activity of Rosemary extracts, carnosol and carnosic acid in bulk vegetable oils and fish oil and their emulsions. Journal of the Science of Food and Agriculture, 72: 201-208.

Garcia-Carreno, F. L. and Haard, N. 1993. Characterization of protease classes in langostilla *Pleuroncodes planipes* and crayfish *Pacifastacus astacus* extracts. Journal of Food Biochemistry, 17: 97-113.

Gatehouse, J. A. and Gatehouse, A. M. R. 1999. Genetic engineering of plants for insect resistance. In: Rechcigl, J. E., Rechcigl, N. A (Eds.), Biological and Biotechnological Control of Insect Pests. CRC Press, Boca Raton. FL. pp. 211-241.

Habing, W. H., Pabst, M. J. and Jakoby W. B. 1974. Glutathione S-transferases. The first step in mercapturic acid formation. Journal of Biological Chemistry, 249: 7130-7139.

Haloui, M., Loudec, L., Michel, J. B. and Lyoussi, B. 2000. Experimental diuretic effects of *Rosmarinus officinalis* and *Centaurium erythraea*. Journal of Ethnopharmacology, 71: 465-472.

Haraguchi, H., Saito, T., Okamura, N. and Yagi, A. 1995. Inhibition of lipid peroxidation and superoxide generation by diterpenoids from *Rosmarinus officinalis*. Planta Medicinal, 61: 333-336.

Hasheminia, S. M., Jalali Sendi, J., Talebi Jahromi, Kh. and Moharamipour, S. 2011. The effects of *Artemisia annua* L. and *Achillea millefolium* L. crude leaf extracts on the toxicity, development, feeding efficiency and chemical activities of small cabbage *Pieris rapae* L. (Lepidoptera: Pieridae). Pesticide Biochemistry and Physiology, 99: 244-249.

Hayese, J. D. and Pulford, D. J. 1995. The glutathione s-transferase supergene family regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Critical Reviwes in Biochemistry and Molecular Biology, 30: 445-660.

Houlihanc, M., HO, C. M. and Chang, S. S. 1985. The structure of rosemariquinone- A new antioxidant isolated from *Rosmarinus officinalis* L. Journal of the American Oil Chemists` Society, 62: 96-98.

Huang, Z., SHI, P., Dai, J. and Du, J. 2004. Protein metabolism in *Spodoptera litura* (F.) is influenced by the botanical insecticide azadirachtin. Pesticide Biochemistry and Physiology, 80: 85-93.

Inatani, R., Nakatami, N. and Fuwa, H. 1983. Antioxidant effect of constituents of Rosemary (*Rosmarinus officinalis*) and their derivatives. Agricaltural Biology and Chemistry, 47: 521-528.

Iskber A. A., Alma, M. H., Kanat, M. and Karci, A. 2006. Fumigant toxicity of essential oils from *Laurus nobilis* and *Rosmarinus officinalis* against all life stages of *Tribolium confusum*. Phytoparasitica, 34: 167-177.

Isman, M. B. 2000. Plant essential oils for pest and disease management. Crop Protection, 19: 603-608.

Isman, M. B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Reviw Entomology, 51: 45-66.

Jafari, Y., Rezaee, V. and Zargarpour, P. 2006. Study on biology of Glyphodes pyloalis Walker (Lepidoptera: Pyralidae), a new pest of mulberry in Guilan province, Iran. The 17 th Iranian plant protection congress, Karaj, Iran, p. 257.

Jakoby, W. B. and Habig, W. H. 1980. An Enzyme Basis of Detoxification (Edited by

[DOR: 20.1001.1.22519041.2013.2.4.3.7]

JAKOBY W. B), New York: Academic Press. 2: 63-94.

Jbilou, R., Mmri, H., Bouayad, N., Ghailani, N., Ennabili, A. and Sayal, F. 2008. Insecticidal effects of extract of seven plant species on larval development α-amylase activity and off spring production of *Tribolium castaneum* (Herbs) (Insecta: Coleopteran: Tenebrionidae). Bioresource Tecnology, 99: 959-964.

Johnson, D. E., Brookhart, G. L., Kramer, K. J., Barnett, B. D. and Mc Gaughey, W. H. 1990. Resistance to *Bacillus thuringiensis* by the Indian meal moth *Plodoa interpuntella*: Comparison of midgut proteinase from susceptible and resistance larvae. Journal of Invertebrate Pathology, 55: 235-244.

Jonsma, M. A. and Bolter, C. 1997. The adaption of insect to plant protease inhibitors. Journal of Insect Physiology, 43: 885-896.

Khani, A. and Asghari, J. 2012. Insecticide activity of essential oils of *Mentha longifolia*, *Pulicaria gnaphalodes* and *Achillea wilhelmsii* against tow stored product pests, the flour beetle, *Tribolium castaneum* and the cowpea weevil, *Calsobruchus maculatus*. Journal of Insect Science, 12: 1-10.

Khorshidi, J., Mohammadi, R., Fakhr, M. T. and Nourbakhsh, H. 2009. Influence of drying methods, extraction time and organ type on essential oil content of Rosemary (*Rosmarinus officinalis* L.). Nature Science, 7: 42-44.

Khosravi, R., Jalali Sendi, J. 2010. Biology and demography of *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae) on mulberry. Journal of Asia-Pacific Entomology, 13: 273-276.

Khosravi, R., Jalali Sendi, J. and Ghadamyari, M. 2010. Effect of *Artemisia annua* L. on deterrence and nutritional efficiency of lesser mulberry pyralid (*Glyphodes pyloalis* Walker) (Lepidoptera: Pyralidae). Journal of Plant Protection Research, 50: 423-428.

Khosravi, R., Jalali Sendi, J., Ghadamyari, M. and Yazdani, E. 2011. Effect of sweet wormwood *Artemisia annua* crude leaf extracts on some biological and physiological characteristics of the lesser mulberry pyralid, *Glyphodes pyloalis*. Journal of Insect Science, 11: 156.

Khosravi, R. and Jalali Sendi, J. 2013. Toxicity, development and physiological effect of *Thymus vulgaris* and *Lavandula angustifolia* essential oils on *Xanthogaleruca luteola* (Coleoptera: Chrysomelidae). Journal of King Saud University Science, In Press. http://dx.doi.org/10.1016/j.jksus.2013.01.002.

Koul, O. and Isman, M. B. 1991. Effects of azadirachtin on the dietary utilization and development of the variegated cutworm, *Peridroma sacucia*. Journal of Insect Physiology, 37: 591-598.

Koul, O., Singh, R., Singh, J., Singh, W., Daniewski, M. and Berlozecki, S. 2004. Bioefficacy and mode of action of some limonoids of salannin group from *Azadirachta indica* and their role in a multicomponent system against Lepidopteran larvae. Journal of Bioscience, 29: 409-416.

Koul O. 2008. Phytochemicals and insect control: an antifeedant approach. Critical Reviwes in Plant Science, 27: 1-24.

Kumar, V., Kumar, V., Rajaadurai, S., Babu, A. M., Katiyar, R. L. Kariappa, B. K., Thiagarajan, V. and Jayawal, K. P., 2002. The chronic architecture and sell structure of Diaphania pulverulentalis (Hampson) (Lepidoptera: Pyralidae). Russian Entomology Journal, 11: 307-310.

Lazarvic, J. and Peric-Mataruga, V. 2003. Nutritive stress effects on growth and digestive physiology of *Lymantaria dispar* Larvae. Tugo Medicinal Biochemistry, 22: 33-39.

Lee, S., Peterson, C. J. and Coats, J. R. 2003. Fumigation toxicity of monoterpenoids to several stored product insects. Journal of Stored Products Research, 39: 77-85.

Leora Software. 1987. Polo-Pc: A users guide to probit or logit analysis. LeOra Software, Barkely California.

Leung, A. Y. and Foster, S. 1996. Encyclopedia of common natural ingredients used in food, drugs and cosmetics. Wiley, New York, pp. 339-342.

[DOR: 20.1001.1.22519041.2013.2.4.3.7]

Lindroth, L. R. 1991. Differential toxicity of plant allelochemicals to insects: roles of enzymatic detoxication systems, in: E. A. Bernays (Ed.), Insect-plant interactions. CRC Press, Boca Raton, FL, pp. 1-33.

Liu, Z. L., Ho, S. H. , and Goh, S. H. 2008. Effect of fraxinellone on growth and digestive physiology of Asian corn borer, *Ostrinia furnacalis* Guenee. Pesticide Biochemistry and Physiology, 91: 122-127.

Lokesh, G., Narayanaswamy, M. and Ananthanarayana, S. R. 2006. The effect of chemical mutagen on haemolymph proteins of silkworm, *Bombyx mori* L. (Lepidoptera: Pyralidae) in F_1 stage. Journal of Applied Science and Environmental Management, 10: 21-25.

Mahdi, S. K., Sasan, J. and Sara, K. 2011. Contact toxicities of oxygenated monoterpenes to different populations of Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). Journal of Plant Protection Research, 51: 225-233.

Mahmoudvand, M., Abbasipour, H., Hosseinpour, M. H., Rastegar, F. and Basij, A. 2011. Using some plant essential oils as natural fumiganst against adults of *Callosobruchus maculates* (F) (Colepotera: Bruchidae). Munis Entomology and Zoology Journal, 6: 150-154.

Marie, S. S., Amr, E. M. and Salem, N. Y. 2009. Effect of some plant oils on biological, physiological and biochemical aspects of *Spodoptera littoralis* (Boisd.). Research Journal of Agriculture and Biological Sciences, 5: 103-107.

Mirkazemi, F., Bandani, A. R. and Sahabi, G. A. 2010. Fumigant toxicity of essential oil from five officinal plants against two stored product insects: cowpea weevil, *Cllosobruchus maculates* (Fabricus) and red flour beetle *Tribolium castaneum* (Herbst). Plant Protection, 32: 37-53.

Mukherjee, S. N., Rawal, S. K., Ghumare, S. S. and Sharma, P. N. 1993. Hormetic concentration of azadirachtin and isoesterase profiles in *Tribolium castaneum* (Herbst) (Colepotera: Tenebrionidae). Experientia, 94: 557-560.

Najafian, Sh., Rowshan, V. and Tarakemeh, A. 2012. Comparing essential oil composition and essential oil yield of *Rosmarinus officinalis* and *Lavandula angustifolia* before and full flowering stages. International Journal of Applied Biology and Technology, 3: 212-218.

Nath, B. S. 2003. Shifts in glycogen metabolism in hemolymph and fat body of the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae) in response to organophosphorus insecticide toxicity. Pesticide Biochemistry and Physiology, 74: 75-84.

Offord, E. A., Mace, K., Ruffieux, A. and Pfeifer, A. MA. 1995. Rosemary components inhibit benzopyrene induced genotoxicity in human bronchial cells. Carcinogenesis, 16: 2057-2062.

Ogendo, J. O., Kostyukovsky, M., Ravid, U., Matasyoh, J. C. Deng, A. L. 2008. Bioactivity of *Ocimum gratissimum* L. oil and two of its constituents against five insect pests attacking stored food products. Journal of Stored Products Research, 44: 328-334.

Rafiei-Kahroodi, Z., Moharamipour, S., Farazmand, H. and Karimzadeh-Esfahani, J. 2011. Insecticidal effect of six native medicinal plants essential oil on Indian meal moth, Plodia *interpunctella Hubner* (Lepidoptera: Pyralidae). Munis Entomology and Zoology Journal, 6: 339-345.

SAS Institute. 1997. SAS/STAT User`s Guide for Personal Computers, SAS Institute, Cary, NC.

Senthil- Nathan, S. and Kalaivani, K. 2005. Efficacy of nucleopolyhydrovirus and azadirachtin on Spodoptera litura Fabricius (Lepidoptera: Noctuidae). Biological control, 34: 93-98.

Senthil- Nathan, S., Kalaivani, K. and Chung, P.G. 2005. The effect of azadirachtin and nucleopolyhydrovirus on midgut enzymatic profile of *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). Pesticide Biochemistry and Physiology, 83: 46-57.

Senthil-Nathan, S. 2006. Effects of *Melia azadarach* on nutritional physiology and

[DOR: 20.1001.1.22519041.2013.2.4.3.7]

enzyme activities of the rice leaffolder *Cnaphalocrocis medinalis* (Guene) (Lepidoptera: Pyralidae). Pesticide Biochemistry and Physiology, 84: 98-108.

Senthil-Nathan, S., Chung, P.G. and Murugan, K. 2006. Combined effect of biopesticides on the digestive enzymatic profiles of Cnaphalocrocis medinalis (Guenee) (the rice leaffolder) (Insecta: Lepidoptera: Pyralidae). Ecotoxicology and Environmental Safety, 64: 382-389.

Senthil- Nathan, S. and Sehoon, K. 2006. Effect of *Melia azadarach* L. extract on the teak defoliator *Hyblaea puera* Cramar (Lep: Hyblaeidae). Crop Protection, 25: 287-291.

Shaaya, E., Kostyukovski, M., Eilberg, J. and Sukprakarn, C. 1997. Plant oils as fumigants and contact insecticides for the control of stored-product insects. Journal of Stored Product Research, 33: 7-15.

Shekari, M., Jalali, Sendi, J., Etebari, K., Zibaee, A. and Shadparvar, A. 2008. Effect of *Artemisia annua* L. (Asteracea) on nutritional physiology and enzyme activities of elm leaf beetle, *Xanthogaleruca luteola* Mull. (Colepotera: Chrysomellidae). Pesticide Biochemistry and Physiology, 91: 66-74.

Silveira Ramo, V., Gracas Machado Freir, M., Roberto Postali Parra, J. and Ligia Rodrigues Macedo, M. 2009. Regulatory effects of an inhibitor from *Plathymenia foliolosa* seeds on the larval development of *Anagasta kuehniella* (Lepidoptera). Comparative Biochemistry and Physiology, 152: 255-261.

Slansky, F. and Sciber, J. M. 1985. Food consumption and utilization, in: Kerkut, G. A., Gilbert, L. I (Eds.), Comprehensive Insect Biochemistry and Physiology, Vol. 4, Pergamon Press, New York, pp. 87-163.

Terra, W. R., Ferriera, C. 2005. Biochemistry of digestion. In: Comprehensive molecular insect science by Lawrence I. Gilbert, Kostas Iatrou, and Sarjeet S. Gill, volum 3. Elsevier. pp. 171-224.

Tsujita, T., Ninomiya, H. and Okuda, H. 1989. P-nitrophenyl 865. buyrate hydrolyzing activity of Hormone-senditive lipase 866 from bovine adipose tissue. Journal of Lipid Research, 30: 867-997.

Van Asperen, K. 1962. A study of housefly esterases by mean of sensitive colorimetric method. Journal of Insect Physiology, 8: 401-416.

Vanhaelen, N., Haubruge, E., Lognay, G. and Francis, F. 2001. Hoverfly Glutathione *S*-Transferases and Effect of Brassicaceae Secondary Metabolites. Pesticide Biochemistry and Physiology, 71: 170-177.

Waldbauer, G. P. 1968. The consumption and utilization of foods by insects. Advances in Insect Physiology, 5: 229-288.

Wheeler, D., Isman, M. B., Sanchez-Vindas, P. E. and Arnason, J. 2001. Screening of Costa Rican Trichilia species for biological activity against the larvae of *Spodoptera litura* (Lepidoptera: Noctuidae). Biochemical Systematic and Ecology, 29: 347-358.

Yuval, B., Holliday, Hanson, M. and Washino, R. K. 1994. Energy budget of swarming male mosquitoes. Ecological Entomology, 19: 74-78.

Zibaee, A., Bandani, A. R., Kafil, M. and Ramzi, S. 2008. Characterization of α-amylase in the midgut and salivary glands of rice stiped stem borer, *Chilo supressalis* Walker (Lepidoptera: Pyralidae). Journal of Asia Pacific Entomology, 11: 201-205.

Zibaee, A. and Bandani, A. R. 2009. Effects of *Artemisia annua* L. (Asteracea) on the digestive enzymatic profiles and the cellular immune reactions of the sun pest, *Eurygaster integriceps* (Heteroptera: Scutellaridae), against *Beauvaria bassiana*. Bulletin of Entomological Research, 12: 1-11.

Zibaee, A., Jalali Sendi, J., Alinia, F., Ghadamyari, M. and Etebari, K. 2009. Diazinon resistance in different selected strains of *Chilo supresalis* Walker (Lepidoptera: Pyralidae) rice striped stem borer, in the north of Iran. Ecological Entomology, 102: 1189-1196.

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ترکیبات شیمیایی، سمیت و اثرات فیزیولوژیکی اسانس *Rosemarinus officinalis* روی پروانه برگ‌خوار توت *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae)

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چکیده: پروانه برگ‌خوار توت *Glyphodes pyloalis* Walker یکی از مهم‌ترین آفات درخت توت است. سمیت اسانس رزماری (*Rosemarinus officinalis* L.(Lamiales: Lamiaceae و اثر آن روی ویژگی‌های فیزیولوژیکی این پروانه تحت شرایط کنترل شده مورد بررسی قرار گرفت. ترکیبات شیمیایی عمده اسانس شامل، ۱و ۸ سینئول (۲۰/۰۲۱٪)، بورنئول (۷/۱۷٪)، ال-کامفور (۶/۵۴۱٪)، گرانیول (۶/۲۸۱٪)، کامفن (۵/۶۲۳٪)، لینالول (۴/۹۹۳٪)، آلفا فنچیل استات (۴/۲۲۲٪) و وربنون (۴/۱۴۷٪) بود. غلظت-های کشنده و زیرکشنده (LC_{10}، LC_{30} و LC_{50}) به‌ترتیب ۰/۷۷۷٪، ۱/۱۸٪ و ۱/۵۹٪ (نسبت حجم به حجم) محاسبه شدند. اسانس رزماری، شاخص‌های تغذیه لاروهای سن چهارم *G. pyloalis* را تحت تأثیر قرار داد. کارایی تبدیل غذای خورده شده (ECI)، کارایی تبدیل غذای هضم شده (ECD)، نرخ مصرف نسبی (RCR) و نرخ رشد نسبی (RGR) در لاروهای تیمار شده با اسانس *R. officinalis* کاهش یافت درحالی‌که قابلیت هضم نسبی (AD) در لاروهای تیمار شده افزایش معنی‌داری را در مقایسه با شاهد نشان داد. اسانس رزماری برخی ترکیبات متابولیکی کلیدی مانند لیپید، پروتئین و کربوهیدرات‌ها را نیز تحت تأثیر قرار داد. به‌طور مشابه روی فعالیت آنزیم‌های کلیدی معین مانند آلفا آمیلاز، لیپاز، پروتئاز، گلوتاتیون اس ترانسفراز و استرازها نیز تأثیر معنی‌داری داشت.

واژگان کلیدی: *Glyphodes pyloalis*، *Rosemarinus officinalis*، GC-MS و شاخص‌های تغذیه

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