Effect of *Thymus vulgaris* L. and *Origanum vulgare* L. essential oils on toxicity, food consumption, and biochemical properties of lesser mulberry pyralid *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae)

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Abstract: The essential oils of *Thymus vulgaris* L. and *Origanum vulgare* L. were investigated for their toxicity and physiological aspects on the lesser mulberry pyralid *Glyphodes pyloalis* Walker in controlled condition (24±1°C, 75±5% RH and 16L : 8D). The leaf disc method was used to study acute toxicity, the effect of LC_{10}, LC_{30}, and LC_{50} on the feeding efficiency of 4th instar larva, and biochemical indices. The essential oil doses of LC_{10}, LC_{30} and LC_{50} were estimated to be 0.107%, 0.188% and 0.279% for *T. vulgaris,* and 0.67%, 1.173% and 1.728% for *O. vulgare,* respectively. The results showed that *T. vulgaris* was more toxic than *O. vulgare*. The essential oil sublethal dose LC_{30} affected the nutritional indices of 4th instar larvae of *G. pyloalis*. The essential oils reduced total protein, carbohydrate, and lipid. Some concentrations of essential oils changed the activity level of α-amylase, protease, lipase, general esterases, and glutathione S-transferase (GST) but others showed no effect on these enzymes. It was concluded that the used essential oil concentrations were both toxic to *G. pyloalis* and showed irreversible effects on key metabolic processes, therefore, the used essential oil concentrations may be considered as alternatives to the classic pest control agents.

Key words: *Glyphodes pyloalis,* lesser mulberry pyralid, *Origanum vulgare*, *Thymus vulgaris*

Introduction

On mulberry farm fields, the lesser mulberry pyralid is a monophagous and important pest. This pest was reported for the first time from Punjab, Pakistan in 1928 (Mathur 1980), and in 2002, from mulberry orchards in northern Iran (Jafari Khaljiri *et al*. 2006). The amount of food eaten by the 1st and 2nd instar larvae is negligible, but feeding increases in later instars. 4th and 5th instar larvae secrete fine threads which are able to fold the leaves. Then, these instars feed on the mesophyll inside the folds. The instars leave behind their black feces which cause loss of quality in mulberry leaves for silkworm rearing. 5th instar larvae feed on the whole leaf until only the ribs remain (Khosravi and Sendi 2010).

Currently, synthetic insecticides are most often used to control these pests. However, concerns over the development of resistance, toxicity, and environmental pollution associated with conventional synthetic insecticides compel us to look for new compounds (Coscolla 2004). There is an imperative need for safer, alternative, crop-protection agents, such as biopesticides and antifeedants (Sadek 2003). Among biopesticides, botanical pesticides have received a great deal of attention because of their favorable ecotoxicological properties, *e.g*. low human toxicity, rapid degradation, and reduced environmental impact. These properties make them suitable insecticides for organic agriculture (Shaaya *et al*. 1991, 1997; Regnault-Roger 1997). Natural products used as insecticides may have less of an environmental impact due to shorter latency, which means possible reduced resistance (Hardin *et al*. 2009). Certain plant families, particularly Meliaceae, Rutaceae, Labiateae, Asteraceae, Convolvulaceae, and Pedaliaceae are viewed as exceptionally promising sources of plant-based insecticides (Schmutterer 1990; Isman 2000). The extracts and essential oils have demonstrated antimicrobial and insecticidal activity (Deans and Svoboda 1989; Regnault-Roger *et al*. 2004; Pavela 2006).

Recently, essential oils used as an alternative pest control technology, have become popular because of their specificity to pests, their biodegradable nature, and their potential for commercial application (Liu *et al*. 2006). The insecticidal activity of several plant essential oils, powders, and other extracts has been evaluated against several insect pests of cereals and legumes. These oils and powders were found to have contact toxicity (Asawalam *et al*. 2006; Ogendo *et al*. 2008), repellence (Kéita *et al*. 2001; Rosman *et al*. 2007), fumigant toxicity (Lee *et al*. 2003; Rajendran and Muriladharan 2005), and antifeedant effects (Saxena *et al*. 1992).

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Secondary metabolites from plants are deleterious to insects and other herbivores in diverse ways, such as acute toxicity, enzyme inhibition, and interference with consumption and/or utilization of food (Lindroth 1991; Senthil Nathan *et al*. 2006). In many cases, however, the modes of action for these metabolites are unknown. In an effort to determine the effects of, and herbivore responses to dietary allelochemicals, their consumption of food is often quantified and various food utilization efficiencies are calculated (Waldbauer 1968; Slansky and Scriber 1985).

The objective of this study was to explore the toxic effects, and the sublethal effects if any, of the essential oils extracted from *Thymus vulgaris* L. and *Origanum vulgare* L. The nutritional indices, digestive and detoxifying enzymes of *Glyphodes pyloalis* Walker were taken into consideration.

Materials and Methods

Insect rearing and an evaluation of the insecticidal efficiency of the essential oils

The 5th instar larvae of the lesser mulberry pyralid were collected from infested mulberry orchards of the Iranian Silk Worm Research Center located in Pasikhan (Guilan province, northern Iran). These orchards produced organically-grown mulberries. The collected larvae were reared in a growth chamber in controlled conditions (24±1°C, 75±5% relative humidity – RH, and 16L : 8D h photoperiods) and reared on fresh mulberry leaves (Kenmochi variety). Adult moths were placed in transparent, 20 × 6 cm^2 jars. Adults were provided with fresh mulberry leaves for egg-laying. Cotton wool soaked in 10% honey was provided for feeding. Fourth larval instars were used in the subsequent experiments.

Preparation of the essential oils

T. vulgaris was collected from Masoule (northern Iran) and *O. vulgare* from Lahijan (northern Iran). The herbs were dried in the shade, and hand-ground to a powder. Dried herb powder (50 g) was briefly mixed with 750 ml distilled water, and after 24 h was transferred to the Clevenger-type apparatus according to the method recommended in British Pharmacopoeia. Distillation lasted about 2 h, and then the essential oil was obtained. This process was repeated several times in order to receive the required amount. The oil phase was isolated from the obtained solution. Sodium sulfate was used for dehydration.

Bioassay

Prior to the experimental phase, larvae were starved for 4 h. Preparatory tests were initially performed to find the effective dose ranges. Four concentrations (0.8%, 0.4%, 0.2%, and 0.1% for *T. vulgaris* and 1.8%, 1.5%, 1.2%, and 0.9% for *O. vulgare*) were determined to be the effective doses. This experiment was performed in 4 replications with 10 larvae of 4th instars (< 24 h) in each replication. The fresh leaf discs were cut from mulberry leaves (8 cm diameter) and were immersed in different concentrations of the essential oils for 10 sec and then air-dried. Each dish contained a disc. Ten larvae were released into each dish and allowed to feed. The control leaves were treated with methanol and air-dried. After 48 h the numbers of dead larvae were recorded. The values of $LC_{10,}$ LC_{30} and LC_{50} were estimated using Polo-PC software (LeOra 1987).

Efficiency measures of quantitative food utilization

To evaluate the effect of essential oil on the nutritional physiology in larvae of *G. pyloalis*, 8 cm diameter discs of mulberry 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 a LC_{30} concentration of essential oil for 10 sec. The control leaves were treated with methanol and air dried. A gravimetric method was used to determine weight gain, food utilization, and feces produced by the larvae. Nutritional indices were evaluated on the basis of dry weight. The newly molted 4th instar larvae were used for this experiment. The larvae were starved 4 h (10 larvae/concentration), and then 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 on. 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 newly 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 the consumed food. The dry weight of the consumed food was estimated on the basis of the dried weight of the total food provided to the insect. The feces produced each day was collected, and then oven dried and weighed to estimate the dry weight of the excreta. The weights of the larvae were recorded at the end of the day. A few larvae with similar biological and physiological conditions to the experimental insects, were weighed, and dried in the oven (45°C) for 48 h then reweighed for determining the dry weight of each larva. The duration of the experiment was three days, and the observations were recorded daily. Nutritional indices were calculated using the formula described by Waldbauer (1968):

- approximate digestibility:
$$AD = (E - F)/E \times 100\ (\%),$$
- efficiency of conversion of ingested food:
$$ECI = P/E \times 100\ (\%),$$
- efficiency of conversion of digested food:
$$ECD = P/(E - F) \times 100\ (\%),$$
- relative growth rate:
$$RGR = P/TA\ (mg/mg/day),$$
- relative consumption rate:
$$RCR = E/TA\ (mg/mg/day),$$

where: A – dry weight of the insect over unit time (mg), E – dry weight of food consumed (mg), F – dry weight of feces produced (mg), P – dry weight gain of insect (mg), T – the duration of the experimental period (day).

Preparation of samples for enzymatic assay

Leaf discs were initially treated with LC_{10}, LC_{30}, and LC_{50} concentrations of essential oil. In each experiment, 10 insects were tested with 4 replicates for each concentration. After 48 h, the live larvae were randomly selected and their guts were removed. Dissection was done under a stereomicroscope (Olympus, SZX12), in ice-cold buffer (Salin buffer). A certain number of larvae were placed in 2 ml of distilled water or buffer related to each test. Samples were then homogenized. The homogenates were centrifuged at 4°C for 10 min. The resulting supernatants were transferred into new micro-tubes and frozen at –20°C for further use.

Assay of α-amylase activity

The α-amylase activity was assayed by the dinitrosalicylic acid (DNS) procedure (Brenfeld 1955). As a substrate, 1% soluble starch (Merk, Darmstadt, Germany) was used. Ten microliters of the enzyme were incubated for 30 min at 35°C with 80 µl universal buffer (glycine, mes (2-[morpholino] ethansulphonic acid), succinate, NaOH, double 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. DNS 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 5 min.

Assay of lipase activity

The activity of lipase was estimated using the method of Tsujita *et al*. (1989). Ten µl of homogenate was mixed with 18 µl p-nitrophenyl butrate (50 mM) as the substrate, and mixed with 172 µl universal buffer (1 M) (pH = 7). This mixture was 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 the substrate (Garcia-Carreno and Haard 1993). Each gut was centrifuged in 10 µl distilled water, then 10 µl of supernatant and 15 µl of buffer (pH = 8) with 50 µl of substrate were reacted for 3 h at 37°C. Proteolysis was stopped by the addition of 150 µl of 10% trichloroacetic acid (TCA). The solution was transferred to 4°C in a refrigerator for 30 min, and the reaction mixture was centrifuged at 13,000 g for 10 min. One hundred µl of supernatant was mixed with 100 µl 1 N NaOH and the absorbance was read at 440 nm.

Assay of esterase activity

The activities of general esterases were determined according to the Van Asperen (1962) method. In this experiment, α-naphtylacetate (α-NA) and β-naphtylacetate (β--NA) were used as substrates. One gut was homogenized with 1,000 µl 0.1 M phosphate (pH = 7) containing Triton X-100 at a ratio of 0.01%, then the homogenized solution was centrifuged at 10,000 g for 10 min at 4°C. The supernatant was transferred to a 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. The absorbance was read at 630 nm.

Assay of glutathione S-transferase (GST)

The activity of GST was determined according to the method of Habing *et al*. (1974). As the substrate, 1-chloro-2,4-dinitrobenzene (CDNB) (20 mM) was used. Initially a larva was homogenized in 20 µl distilled water, then the homogenized solution was centrifuged at 12,500 g for 10 min at 4°C. Fifteen µl of supernatant was 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 (1994) was used for determining the protein total. First, each larva was homogenized in 350 µl of distilled water and samples were centrifuged at 10,000 rpm for 5 min at 4°C. Next, 10 µl of supernatant was mixed with 90 µl of distilled water and 2,500 µl of 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). This solution was brought to 100 ml with distilled water. Bovin serum albumin was used as the standard. The absorbance was read at 630 nm.

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 2%. Lipids and carbohydrates were extracted in 750 µl of chloroform : methanol (1 : 2). Individual tubes were centrifuged for 10 min at 8,000 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 a 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. The lipid total was calculated from the standard curve of cholesterol.

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

Statistical analysis

$LC_{10,}$ $LC_{30,}$ and LC_{50} of the toxicity bioassay were calculated 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

Bioassay

The LC_{10}, LC_{30}, and LC_{50} values of *T. vulgaris* and *O. vulgare* 48 h after treatment as per confident limits and the slope of line regression are shown in table 1. The corresponding LC_{50} values were respectively, 0.279% and 1.72% for *T. vulgaris* and *O. vulgare* 48 h after treatment.

The present study showed that essential oils of *T. vulgaris* and *O. vulgare* were effective against *G. pyloalis* larva. The lower LC_{50} (highest toxicity) belongs to *T. vulgaris*. The LC_{50} of *T. vulgaris* is 6.33 times more than *O. vulgare.*

Effect of *T. vulagris* and *O. vulgare* essential oils on nutritional indices

The feeding indices of 4th instar *G. pyloalis* larva feeding on leaves treated with *T. vulagris* and *O. vulgare* essential oils, were affected. Our experiment showed that *T. vulagris* and *O. vulgare* essential oils had an intense effect on the feeding behavior and growth of *G. pyloalis.* The results of the effect of LC_{30} of *T. vulgaris* and *O. vulgare* on the feeding efficiency of 4th instar larva of *G. pyloalis* are shown in table 2. AD ($F = 10.69$; $df = 2, 6$; $p = 0.0042$) was increased in larva treated with *O. vulgare* essential oil; with significant differences to that of the control. By using the essential oils, RGR ($F = 7.33$; $df = 2, 6$; $p = 0.012$), RCR ($F = 2.98$; $df = 2, 6$; $p = 0.101$), and ECI ($F = 5.13$; $df = 2, 6$; $p = 0.032$) were reduced. These indices were significantly reduced compared to the control. The RCR was lowest in the larvae treated with *T. vulgaris* essential oil (1.188 mg/mg/day) and was significantly different than the control. The amount of the ECD did not show any differences when compared with the control.

Effect of *T. vulagris* and *O. vulgare* essential oils on the total protein, the carbohydrates, and the lipid

The results of the total protein, lipid, and carbohydrates in fourth instar larvae of *G. pyloalis* after treatment with *T. vulagris* and *O. vulgare* essential oils are shown in figures 1–3. The amount of the total protein of the larva treated with LC_{30} ($F = 7.49$; $df = 2, 6$; $p = 0.0206$) and LC_{50} ($F = 46.65$; $df = 2, 6$; $p = 0.0002$) of *T. vulagris* and *O. vulgare* essential oils, showed differences in the treated larva in comparison with the control. All concentrations tested reduced the lipid value. The LC_{50} value of *T. vulagris* and *O. vulgare* essential oils reduced the amount of lipid, compared with the control ($F = 17.587$; $df = 2, 6$; $p < 0.0001$), and it was dose dependent. This reduction was more severe in the case of *O. vulgare* than *T. vulgaris*. The effect of *T. vulgaris* and *O. vulgare* on the carbohydrate activity was significant. Activity of this macromolecule in all of the treatments was concentration-dependent. The macromolecule activity declined to the lowest value with LC_{50} concentration (Fig. 3) ($F = 16.64$; $df = 2, 6$; $p < 0.0001$).

Table 1. Toxicity of *O. vulgare* and *T. vulgaris* to 4th instar larva of *G. pyloalis*

Plants	N	LC_{10} (95% CL)	LC_{30} (95% CL)	LC_{50} (95% CL)	Slope±SE	X^2 (df)
O. vulgare	200	0.67 (0.139–0.935)	1.17 (0.719–1.45)	1.72 (1.43–2.95)	3.11±0.204	0.43 (2)
T. vulgaris	200	0.107 (0.138–0.728)	0.188 (0.148–0.226)	0.279 (0.233–0.333)	3.08±0.405	0.58 (2)

CL (Confidence Limit) which has been calcutated with 95% confidence; essential oil activity is considered significantly different when the 95% CI (Confidence Interval) fails to overlap
N – the number of insects used in the bioassay

Table 2. Comparison of feeding efficiency in treated 5th instar larva the and control of *G. pyloalis* with LC_{30} concentration of *O. vulgare* and *T. vulgaris* essential oils

Plants	AD [%]	ECI [%]	ECD [%]	RCR [mg/mg/day]	RGR [mg/mg/day]
The control	76.62±0.6 b	5.20±0.68 a	4.87±0.89 a	1.45±0.07 a	0.066±0.003 a
T. vulgaris	80.66±1.21 ab	2.38±0.76 b	3.03±0.94 a	1.18±0.04 b	0.031±0.008 b
O. vulgare	83.87±1.37 a	2.92±0.17 b	2.96±0.15 a	1.3±0.08 ab	0.045±0.004 b

Within columns, means followed by the same letter do not differ significantly at $p < 0.05$
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

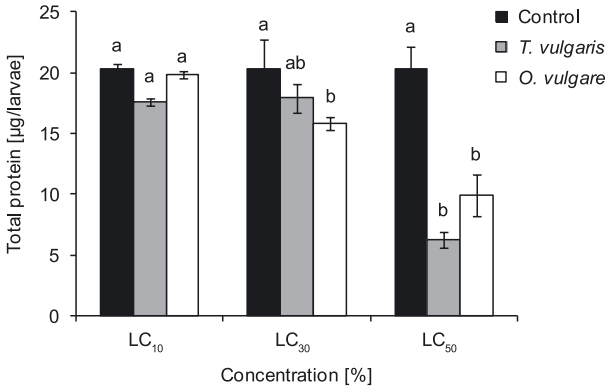

Fig. 1. Amount of total protein in 4th instar larvae of *G. pyloalis* after treatment with essential oils of *T. vulgaris* and *O. vulgare*. The small letters means significant at $p \leq 0.05$

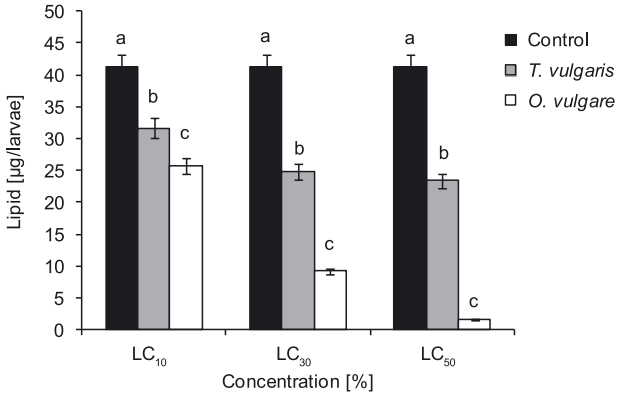

Fig. 2. Amount of total lipid in 4th instar larvae of *G. pyloalis* after treatment with essential oils of *T. vulgaris* and *O. vulgare*. The small letters means significant at $p \leq 0.05$

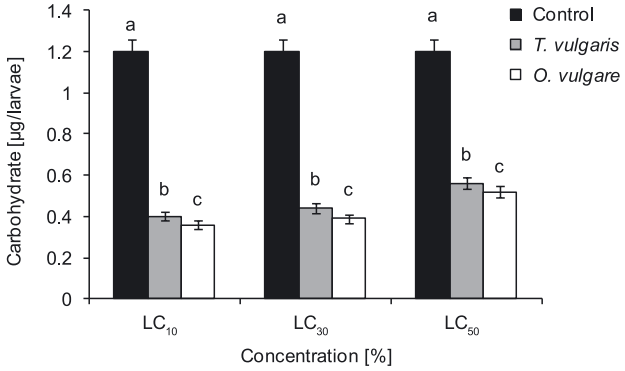

Fig. 3. Amount of carbohydrate in 4th instar larvae of *G. pyloalis* after treatment with essential oils of *T. vulgaris* and *O. vulgare.* The small letters means significant at $p \leq 0.05$

Effects of *T. vulgaris* and *O. vulgare* essential oils on digestive enzymes

Effects of *T. vulgaris* and *O. vulgare* essential oils on digestive enzymes after feeding on treated leaves with different concentrations ($LC_{10,}$ LC_{30} and LC_{50}) of both essential oils are shown in table 3. The results indicated that the specific activity of α-amylase sharply decreased in larvae treated with all the concentrations of *T. vulgaris* ($F = 27.27$; $df = 3, 8$; $p = 0.0002$) and *O. vulgare* ($F = 25.21$; $df = 3, 8$; $p = 0.0001$) essential oils. This reduction was more severe in the case of *O. vulgare* than *T. vulgaris*.

The activity of lipase in 4th instar larvae of *G. pyloalis* treated with different concentrations of *T. vulgaris* essential oil, did not show significant differences as compared to the control ($F = 3.22$, $df = 3, 8$, $p = 0.06$), whereas the LC_{50} concentration ($F = 3.22$; $df = 3, 8$; $p = 0.06$) of *O. vulgare* significantly increased the activity of this enzyme in the larvae of *G. pyloalis*. The activity of protease significantly increased in the larvae treated with LC_{30} and LC_{50} concentrations of *O. vulgare* ($F = 5.86$; $df = 3, 8$; $p = 0.0203$), but in the treatment using *T. vulgaris* essential oil, only larvae treated with the LC_{50} concentration differed statistically as compared to the control ($F = 9.77$; $df = 3, 8$; $p = 0.0047$).

Table 3. Activity of α-amylase, protease, lipase, esterase, and GST enzymes in 4th instar larva of the lesser mulberry pyralid *G. pyloalis* after treatment with LC_{10}, LC_{30} and LC_{50} concentrations of *O. vulgare* and *T. vulgaris* essential oils

Plants	Concentration	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]	
O. vulgare						*	**
	the control	$0.011\pm7\times10^{-4}$ a	$0.0036\pm4\times10^{-4}$ b	1.45±0.05 a	$0.00045\pm1\times10^{-4}$ c	$0.0051\pm1\times10^{-3}$ c	$0.024\pm4\times10^{-4}$ a
	LC_{10}	$0.0041\pm6\times10^{-4}$ b	$0.0057\pm2\times10^{-4}$ ab	1.35±0.4 a	$0.0013\pm1\times10^{-4}$ b	$0.012\pm5\times10^{-3}$ cb	$0.01\pm2\times10^{-3}$ ab
	LC_{30}	$0.0022\pm1\times10^{-3}$ b	$0.0062\pm2\times10^{-3}$ ab	5.74±0.4 b	$0.0018\pm6\times10^{-6}$ ab	$0.018\pm3\times10^{-3}$ ab	$0.012\pm3\times10^{-3}$ bc
	LC_{50}	$0.001\pm6\times10^{-4}$ b	$0.0097\pm1\times10^{-a}$	5.54±1.9 b	$0.0021\pm1\times10^{-4}$ a	$0.024\pm1\times10^{-2}$ a	$0.020\pm2\times10^{-3}$ c
T. vulgaris	the control	$0.038\pm7\times10^{-4}$ a	$0.003\pm4\times10^{-4}$ a	1.45±0.05 b	$0.00045\pm1\times10^{-4}$ b	$0.0051\pm1\times10^{-3}$ c	$0.0051\pm4\times10^{-4}$ c
	LC_{10}	$0.0119\pm1\times10^{-3}$ b	$0.0074\pm2\times10^{-4}$ a	1.94±0.02 ab	$0.00159\pm2\times10^{-4}$ a	$0.017\pm3\times10^{-3}$ ab	$0.0071\pm3\times10^{-4}$ c
	LC_{30}	$0.0112\pm4\times10^{-3}$ b	$0.0091\pm2\times10^{-3}$ a	1.35±0.2 b	$0.00152\pm2\times10^{-4}$ a	$0.0122\pm3\times10^{-3}$ b	$0.012\pm2\times10^{-4}$ b
	LC_{50}	$0.008\pm2\times10^{-3}$ b	$0.0094\pm1\times10^{-3}$ a	2.71±0.3 a	$0.0017\pm4\times10^{-6}$ a	$0.0183\pm1\times10^{-2}$ a	$0.018\pm1\times10^{-3}$ a

GST – glutathione S-transferase

* α-Naphtylacetate substrate; ** β-Naphtylacetate substrate

Within columns, means followed by the same letter do not differ significantly at $p < 0.05$

Effects of *T. vulgaris* and *O. vulgare* essential oils on detoxifying enzymes

General esterase with substrate α-naphtylacetate and β-naphtylacetate showed a significant increase compared with the control at the LC_{30} and LC_{50} ($F = 10.63$; df = 3, 8; $p = 0.0036$) concentrations of *O. vulgare* essential oil and at all the concentrations of *T. vulgaris* essential oil ($F = 52.56$; df = 3, 8; $p < 0.0001$). The activity of GST was increased by all the concentrations of both of the essential oils, and the activity was significant compared with the control ($F = = 9.15$; df = 3, 8; $p < 0.0001$).

Discussion

In this investigation, two essential oils were tested for their toxicity against larva of *G. pyloalis*. Our results clearly indicated that both essential oils possess insecticidal activity against lesser mulberry pyralid. The essential oil of *T. vulgaris* produced higher insecticidal activity against *G. pyloalis*. The mortality difference observed from using these oils could be due to their active volatiles; mostly monoterpenes, which are very active on insects (Liu and Ho 1999; Huang *et al*. 2000; Kouninki 2005). In a related study, Khosravi and Jalali (2013) stated that *T. vulgaris* and *Lavandula angustifolia* Mill. essential oils displayed strong toxicity on the elm leaf beetle *Xanthogaleruca luteola* Muller. Essential oil vapours from *O. vulgare* were toxic against the nymphs and adults of *Tetranychus urticae* Koch and the adults of *Bemisia tabaci* Gennandius (Calmasur *et al*. 2006).

The quality and quantity of food consumed may increasingly affect the growth, development, and the reproduction of insects (Scriber and Slansky 1981). The evaluation of the feeding indices under the LC_{30} concentration of *T. vulgaris* and *O. vulgare* essential oils showed that AD in larval feeding on treated leaves increased compared to the controls. Khosravi *et al*. (2010) reported similar results with *Artemisia annua* L. extract on *G. pyloalis*. In addition to decreases in ECI and ECD, those treatments with Thyme and Origanum essential oils also produced a significant decrease in the RCR and RGR. The results also showed that RCR and RGR were significantly lower among 4th instars larvae that were fed on a diet containing *T. vulgaris* and *O. vulgare* essential oils. We demonstrated that Thyme and Origanum oils significantly inhibited *G. pyloalis* larvae from feeding on mulberry leaves. *Thymus vulgaris* was more potent. The decreased RGR and RCR in treated larvae may indicate the toxic effects of plant allelochemicals on the peritrophic membrane and may indicate damage to the cellular surfaces of the midgut (Marie *et al*. 2009).

In physiological studies, the determination of the total protein and many chemical macromolecules, such as lipid and carbohydrate, is important. Proteins are major biochemical components necessary for an organism to develop, grow and perform its vital activities. The mean protein content values were determined in the 4th instars treated with LC_{10}, LC_{30} and LC_{50} concentrations of *T. vulgaris* and *O. vulgare* essential oils. From the data recorded in figure 1, it is clear that total protein was significantly decreased with LC_{30} and LC_{50} concentrations of *T. vulgaris* and *O. vulgare* essential oils. The protein content in an insect is dependent upon its synthesis, breakdown, water movement between tissues, and hemolymph. The reduction in protein content in the larvae was attributed to one or a combination of factors, like a reduction in the synthesis of proteins or an increase in the breakdown of proteins to detoxify the active principles present in the plant extracts or essential oils (Vijayaraghavan *et al*. 2010). Similar results were obtained by Schmidt *et al*. (1998) by using a methanolic extract of *Melia azedarach* L. on the hemolymph protein of *Spodoptera littoralis* (Boisduval) and *Agrotis ipsilon* (Hufnagel).

In this investigation, lipid reduction was significant in larvae treated with all the essential oil concentrations. Reduction of lipid levels in the larvae treated with plant essential oils may be due to their effect on the lipid me-

tabolism, and due to the utilization of lipid reserves for energy generation as a result of induced stress (Sancho *et al*. 1998; Olga *et al*. 2006).

The essential oils tested in the present study, considerably reduced the carbohydrate content of the lesser mulberry pyralid larvae. Under stress conditions, more sugars might be metabolized to meet the energy expenses. This could be the reason for the carbohydrate level depletion in the treated insects. Similar results were obtained by Seyoum *et al*. (2002) in desert locust, and by Khosravi *et al*. (2011) in *G. pyloalis* larva treated with *A. annua* extract.

The most important digestive enzymes of many insects that feed exclusively on plants during larval and/or adult life is α-amylase. When the action of the amylases is inhibited, nutrition of the organism is impaired causing a shortness in energy (Mehrabadi *et al*. 2010). The results of this study indicated that α-amylase activity in the midgut of larvae treated with *O. vulgare* and *T. vulgaris* essential oils was significantly reduced. The present results are consistent with other reports (Saleem and Shakori 1987; Lee *et al.* 1994; Shekari *et al*. 2008; Khosravi *et al*. 2011).

Proteases are important in digesting food and converting protein to amino acids needed for the body. Proteases play a crucial role in the food digestion of insects (Terra and Ferriera 2005). By treatments of LC_{30} and LC_{50} concentrations of *T. vulgaris* essential oil on 4th instar larvae of *G. pyloalis*, it was found that activity of this enzyme increased. But essential oil of *O. vulgare* could increase protease only at the highest concentration. Studies by Senthil Nathan *et al*. (2004 and 2006), Zibaee and Bandani (2010), and Khosravi and Jalali (2013) inferred that botanical insecticides may affect the construction of certain types of proteases.

Lipases are enzymes that preferentially hydrolyze the outer links of fat molecules, and have been studied in few insects. In our investigation, lipase activity was not significantly changed, but LC_{50} concentration of *O. vulgare* increased the activity of this enzyme. The increased activity of midgut lipase might account for a greater utilization of exogenous lipids and might result in the biomass production (Champagne *et al*. 1992; Desai and Desai 2000; Ahmad *et al*. 2006). Sujitha *et al*. (2010) reported that an extract of *Pedaliumm murex* L. increased lipase activity in *Spodoptera litura* (Fabricius).

Glutathione S-transferases are a major group of detoxification enzymes found in most organisms. 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, the activity of GST in larvae treated separately with the studied essential oils, was increased compared with the control. Our results are similar to the results obtained in other studies (Vanhaelen *et al*. 2001; Dugravot *et al*. 2004; Zibaee and Bandani 2010; Khosravi *et al*. 2011). General esterase activity increased in the *G. pylolais* larva treated separately with both the essential oils used in our study. These results show that GSTs and esterases play a role in the detoxification or in the metabolism of *T. vulgaris* and *O. vulgare* essential oils.

The present investigation indicated that *T. vulgaris* and *O. vulgare* essential oils possess antifeedant and toxic effects on *G. pyloalis*. We also found that *T. vulgaris* essential oil was more toxic than *O. vulgare* essential oil. In addition, compounds present in these essential oils affect nutritional indices and the activity of macromolecules, digestive enzymes as well as the detoxifying enzymes in this pest. However, further studies are needed to explore the toxic, antifeedant and growth regulatory substances in these plants.

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