Journal of King Saud University – Science (2013) 25, 349–355

King Saud University
Journal of King Saud University – Science
www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Toxicity, development and physiological effect of *Thymus vulgaris* and *Lavandula angustifolia* essential oils on *Xanthogaleruca luteola* (Coleoptera: Chrysomelidae)

Roya Khosravi [a], **Jalal Jalali Sendi** [b,*]

[a] *Young Researchers Club, Islamic Azad University, Arak Branch, Arak, Iran*
[b] *University of Guilan, Faculty of Agriculture, Department of Plant Protection, 41635-1314 Rasht, Iran*

Received 19 December 2012; accepted 16 January 2013
Available online 30 January 2013

KEYWORDS
Essential oil;
Elm leaf beetle;
Insecticide;
Digestive enzyme

Abstract Effects of essential oils from garden thyme *Thymus vulgaris* L. and lavander *Lavandula angustifolia* L. on mortality of third instar larvae, development of treated larvae, adult emergence and activity of digestive enzymes of elm leaf beetle *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae), were determined under laboratory conditions. The LC_{50} values on third instar larvae were estimated at 0.3% and 0.63 %, respectively. Greater mortality was observed with increasing concentration of essential oils. Both essential oils disrupted the development of treated larvae, significantly decreased adult emergence as compared to untreated larvae. Present results also indicated that when larvae fed on treated leaves, activity level of digestive enzyme decreased in their midguts. Based on this study, essential oils derived from *T. vulgaris* possessed the greatest effect on larval development and activity level of digestive enzymes.

1. Introduction

Elm leaf beetle, *Xanthogaleruca luteola* Müller is one of the serious pests of ornamental elms throughout Iran. Both adult and larvae feed on the parenchyma of leaves and cause them to die, affect the beauty of trees, can cause economic problems. Adult feeding causes irregular holes on the elm leaf surface. Repeated injuries may reduce tree vigor and increase susceptibility to more serious pests such as the smaller European elm bark beetle which is responsible for spreading the Dutch elm disease fungus. Defoliation also reduces tree shade in summer and the aesthetical values of elms (Arbab et al., 2001). Due to the presence of this tree in the urban environment, chemical control of this pest will follow environmental contamination.

Chemical control is an effective approach used extensively in daily life. One of the key problems in agriculture is development of resistance of pests to synthetic insecticides. Harmful effect of chemical pesticides on non target organisms, farm workers and consumer of agriculture products is considerable. Therefore development of safe, more environmentally friendly and efficient alternative crop protectant is required (Akhtar and Isman, 2004).

In order to resolve the replacement of chemical pesticides, essential oils extracted from aromatic plants have been highly

* Corresponding author. Tel.: +98 66 908 17.
E-mail address: jjalali@guilan.ac.ir (J.J. Sendi).
Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

1018-3647 © 2013 Production and hosting by Elsevier B.V. on behalf of King Saud University.
http://dx.doi.org/10.1016/j.jksus.2013.01.002

considered (Isman, 2000). Aromatic hydrocarbon mixtures are obtained or isolated as a result of steam distillation or supercritical fluid extraction from aerial plant parts (Isman 2000; Pavela et al., 2009). Plant essential oils do not accumulate in the environment and do not cause pollution, reduce the risk of developing resistant strains and have low toxicity for natural enemies and some are selective (Singh and Upadhyay, 1993; Isman, 2006; Pavela, 2007). There are different modes of action of EO against insects such as toxicity, repellent and antifeedant activities, interference in enzyme activity, and changes in insect growth (Isman, 2000; Enan, 2001; Akhtar and Isman, 2004).

Recently growth inhibitory and insecticidal activities of some plants against elm leaf beetle have been reported. Ethanolic extract of *Daphne gnidium* L. caused reduction of larval survival, weight gain and adult emergence, and appearance of malformations in elm leaf beetle (Maistrello et al., 2005). Aqueous and ethanolic extracts of *Schinus molle* L. were effective against *X. luteola* and caused mortalities greater than 97% with the ethanolic extract and near 27% with the water (Huerta et al., 2010).

Thymus vulgaris L. and *Lavandula angustifolia* Mill belong to the Lamiaceae family. The lavender *L. angustifolia* is an evergreen bushy shrub with straight, woody branches, the lower parts are leafless. It is often cultivated as ornamental plants for garden use and extraction of essential oil. Lavender is native to southern Europe and the Mediterranean, southwest Asia to southeast India (Shawl et al., 2000). Thymus is a herbaceous plant, shrub with small aromatic leaf, with a tiny white flower that grows in a single umbrella. It is native to southern Europe (Morales, 2002).

This study was undertaken to investigate insecticidal and growth inhibitory activity of *T. vulgaris* and *L. angustifolia* essential oils against elm leaf beetle *X. luteola*. Further objectives were to investigate effects of garden thyme and lavender treatment on feeding and digestive enzyme activity of this pest.

2. Materials and methods

2.1. Mass rearing of insects

Eggs of *X. luteola* were collected from elm trees in Rasht, and were maintained in the laboratory in a rearing chamber at conditions of constant temperature (25 ± 1 °C), relative humidity (75 ± 5%) and photoperiod (16:8 h light: dark). Larvae were placed in plastic jars 10 × 20 cm and provided with fresh elm leaves. Adults were reared as larvae and eggs were used to create the culture.

2.2. Extraction of essential oils

Plant leaves from thyme garden and lavender were collected from growing locations [(*T. vulgaris* from Masoule (North of Iran), *L. angustifolia* from Astara (North of Iran)]. Leaves of plants were dried in the shade, and then powdered by hand grinding. The dried samples were subjected to hydro distillation for 2 h using a Clevenger-type apparatus. After distillation, the oil obtained was isolated from water and dried over anhydrous Na_2SO_4.

2.3. Bioassays

In this experiment newly ecdysed third instar larvae of *X. luteola*, concentrations of 1%, 0.5%, 0.25% and 0.125% of *T. vulgaris* and 1.6%, 0.8%, 0.4% and 0.2% of *L. angustifolia* were used by methanol as solvent. Forty larvae per concentration were used for all the experiments, and each experiment was replicated four times. The elm leaf disks (6 cm in diameter) were dipped for 10 s in the desired concentrations and control leaves were treated with methanol alone. The leaves were allowed to dry at room temperature and then placed over a humid filter paper to avoid dryness. Mortality was recorded 24 h after the beginning of experiment and LC_{25}, LC_{50} and LC_{75} and 95% confidence limit were estimated using probit regressions by Polo-PC software.

The newly ecdysed third instar larvae were obtained from the stock culture and starved for 2 h prior to exposure to treated leaf disks. Elm leaf disks were dipped in essential oil solution in concentrations of 0.5%, 0.25% and 0.125% of *T. vulgaris* and concentrations of 0.8%, 0.4% and 0.2% of *L. angustifolia* for 10 s. Control leaves were treated with methanol alone in the same way. Larvae (40 per concentration) were placed in Petri dishes; each treatment was replicated four times. The surviving larvae were fed with fresh elm leaves and their development was followed and percent of pupation and emergence to adults were recorded.

2.4. Preparation for enzyme activity

Digestive enzyme activity was determined in surviving larvae obtained after feeding of treated leaves with essential oils for 24 h. Larval midguts were removed by dissection under a stereomicroscope in ice-cold saline buffer (0.15 M NaCl). For each sample, 10 midguts were pooled, rinsed in ice cold saline buffer, placed in a pre-cooled homogenizer and ground in 1 mL of universal buffer and then centrifuged at 10,000*g* for 10 min at 4 °C. The supernatants were stored at −20 °C until measuring the activity of digestive enzymes.

2.5. Assay of α-amylase activity

Measuring of α-Amylase activity was performed by the dinitrosalicylic acid (DNS) reagent (Bernfeld, 1955), using 1% soluble starch (Merck, Darmstadt, Germany) as substrate. Twenty micro liters of the enzyme was added to 100 µl universal buffer and 40 µl soluble starch in microtubes, then incubated for 30 min at 35 °C. After incubation in water bath, to stop the reaction 100 µl DNS was added, and heated in boiling water for 10 min. The boiling water stops α- amylase activity and catalyzes the reaction between DNS and the reducing groups of starch. Absorbance was then read at 540 nm using microplate reader (Stat Fax 3200, Awareness Technology, USA). One unit of α-amylase activity was defined as the amount of enzyme that produced 1 mg maltose in 30 min at 35 °C. A blank sample without substrate with α-amylase extract and a negative control containing no α-amylase extract with substrate were run simultaneously. All assays were performed in duplicate and each assay was repeated three times.

2.6. Assay of α- and β-glucosidase activity

Activities of α- and β-glucosidases were assayed with pNαG and pNβG as substrates, respectively. 10 µl of homogenate was incubated at 37 °C with 45 µl of substrate (25 mM) and 115 µl of 40 mM glycine–phosphate–acetic–citric buffer. After 30 min incubation of mixture, the reaction was stopped by addition of 600 µl of NaOH (0.25 M). Absorbance was then read at 405 nm after 10 min. A standard curve of absorbance against the amount of *p*-nitrophenol released was constructed to enable calculation of the amount of *p*-nitrophenol released during the α- and β-glucosidase assays.

2.7. Assay of Lipase activity

The activity of lipase was determined by the method of Tsujita et al. (1989). *p*-nitrophenyl butyrate (50 mM) was used as substrate. 10 µL of midgut homogenate was mixed with 18 µL *p*-nitrophenyl butyrate, and 172 µL of universal buffer solution (1 M) (pH = 7) and this mixture incubated at 37 °C. The optical density was read at 405 nm. One unit of enzyme releases 1.0 nmol of *p*-nitrophenyl per minute at pH 7.2 at 37 °C using *p*-nitrophenyl butyrate as the substrate.

2.8. Assay of protease activity

General protease activity in midguts of third instar larvae treated with essential oils was measured by azocasein as substrate (Elpidina et al., 2001). The midgut homogenate (30 µl) was added to 80 µL of 2 % azocasein solution in 40 mM universal buffer of specified pH. The reaction mixture was incubated at 37 °C for 60 min. Proteolysis was stopped by addition of 300 µL of 10% trichloroacetic acid (TCA). Precipitation was achieved by cooling at 4 °C for 120 min and the reaction mixture was centrifuged at 16,000 rpm for 10 min. Then 100 µl of 1 N NaOH was added to the 100 µL supernatant and the absorbance was recorded at 440 nm.

2.9. Protein determination

The method of Bradford (1976) was used for determining total protein, using bovine serum albumin (Bio-Rad, Munchen, Germany) as the standard.

2.10. Statistical analysis

The mortality and lethal concentration were obtained by using Probit analysis and Polo-Pc software (LeOra Software, 1987). Data obtained from the experiments were subjected to analysis of variance (ANOVA) ($P < 0.05$). Treatment means were compared by Tukey's studentized range test, accepting significant differences at $P = 0.05$ (SAS Institute, 1997).

3. Results

The susceptibility of the third instars to essential oils of *T. vulgarsi* and *L. angustifolia* was analyzed by leaf dip bioassay (Table 1). The corresponding LC_{50} values were respectively 0.3% and 0.63% for *T. vulgaris* and *L. angustifolia* 24 h after treatment.

Results of insecticidal activity of *T. vulgaris* and *L. angustifolia* L. are presented in Table 2. Treatment of third instar larvae of *X. lutola* with plant essential oils affected the development of this pest. Larval duration increased in all treatments with *T. vulgaris* except for one concentration as compared with controls. *T. vulgaris* and *L. angustifolia* essential oils significantly decreased pupation in 0.5% concentrations, whereas in the treatment of essential oil pupation decreased only in 1.6% concentration as compared to 100% pupation in control. Pupation did not show a significant difference in

Table 1 Toxicity of *Thymus vulgaris* L. and *Lavandula angustifolia* L. to third instar of *Xanthogaleruca luteola* after 24 h.

Plants	*N*.	LC_{25} (95% CI)	LC_{50} (95% CI)	LC_{90} (95% CI)	Slope ± S.E.	X^2 (df)
Thymus vulgaris	200	0.12 (0.05–0.55)	0.3 (0.21–0.4)	1.85 (1.08–5.74)	1.63 ± 0.32	0.21 (2)
Lavandula angustifolia	200	0.23 (0.11–0.34)	0.63 (0.46–0.89)	4.1 (2.22–15.94)	1.57 ± 0.32	0.2 (2)

LC_{25}, LC_{50} and LC_{90} values based on % and CI 95% Confidence intervals, essential oil activity is considered significantly different when the 95% CI fail to overlap.
N is the number of insects that is used in bioassay.

Table 2 Effects of *T. vulgaris* and *L. angustifolia* essential oils on third instar larval duration, pupation, pupal duration and adult emergence (means ± SE).

Plant	Concentration	Larval duration (days)	Pupation (%) ± SE	Pupal duration (days)	Adult emergence (%)
T. vulgaris					
	0.5	7.97 ± 0.53a	95.5 ± 2.27a	6.5 ± 0.64a	25 ± 3.16dc
	0.25	6.17 ± 0.72b	100 ± 0.00a	5.73 ± 0.6ab	37.5 ± 3.09c
	0.125	4.59 ± 0.4c	100 ± 0.00a	6.11 ± 0.37b	77.5 ± 3.54b
	Control	4.1 ± 0.3c	100 ± 0.00a	6.07 ± 0.3b	98.75 ± 1.58 a
L. angustifolia					
	0.8	7.12 ± 0.5a	95.25 ± 2.34a	7.57 ± 0.71a	37.5 ± 3.54c
	0.4	4.75 ± 0.64b	98.25 ± 1.87a	6.31 ± 0.48b	47.5 ± 2.23c
	0.2	4.39 ± 0.32b	100 ± 0.00a	5.83 ± 0.6b	85 ± 2.4b
	Control	4.1 ± 0.3b	100 ± 0.00a	6.07 ± 0.3b	98.75 ± 1.58 a

Within columns, means followed by the same letter do not differ significantly (Tukey's test, $P \leqslant 0.05$).

all groups treated with both essential oils. Pupal life duration shows a significant difference only in larvae treated with 0.5% concentration of *T. vulgaris* essential oil. In the case of *L. angustifolia* higher concentration (0.8%) resulted in an increase of pupal duration as compared to control. Both essential oils significantly affected adult emergence as compared to control (Table 2).

Effects of *T. vulgaris* and *L. angustifolia* were evaluated on the development and survival of elm leaf beetles feeding on leaves treated with thyme and lavander essential oils. Lengthening of the larval period was observed in treatments of 0.25% and 0.5% of thyme and 0.8% of lavander essential oils as compared to the control.

Effects of *T. vulgaris* and *L. angustifolia* essential oils on digestive enzymes after feeding on treated leaf with different concentrations of both essential oils were examined and results are presented in Figs. 1–4. The results declared indicated that, the specific activity of α-amylase sharply decreased in treated larvae with *T. vulgaris* essential oil so that lowest activity was observed in concentration of 1% (Fig. 1) ($F = 187.17$, df = 4, $p \leqslant 0.001$). Concentrations of 0.8%, 0.4% and 0.2% of *L. angustifolia* essential oil significantly decreased the activ-

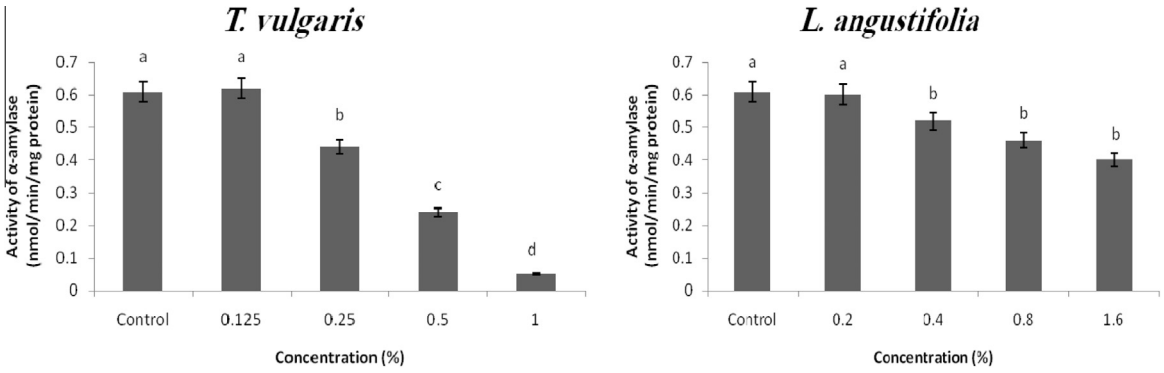

Figure 1 Effect of different concentrations of *T. vulgaris* and *L. angustifolia* essential oils on α-amylase activity in the midgut of third instar larvae of *X. luteola*.

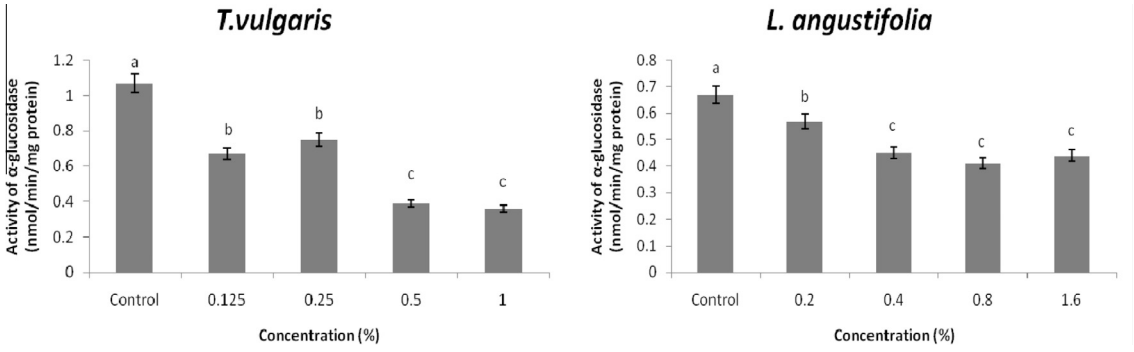

Figure 2 Effect of different concentrations of *T. vulgaris* and *L. angustifolia* essential oils on α-glucosidase activity in midgut of third instar larvae of *X. luteola*.

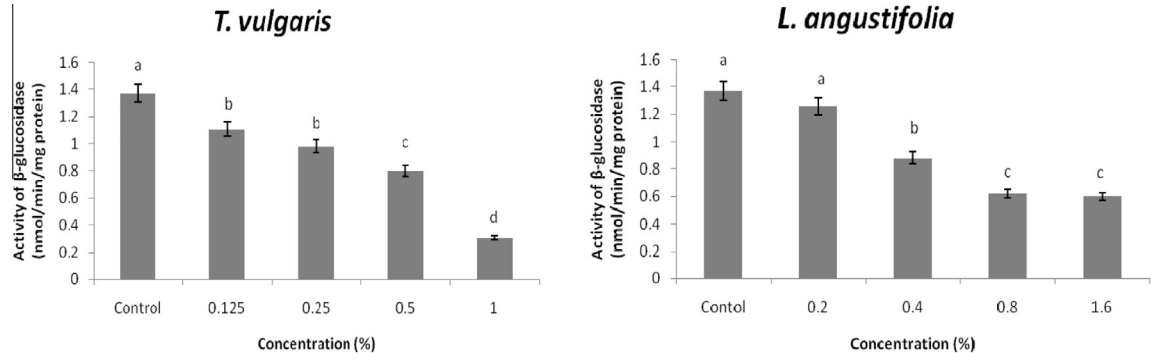

Figure 3 Effect of different concentrations of *T. vulgaris* and *L. angustifolia* essential oils on β-glucosidase activity in the midgut of third instar larvae of *X. luteola*.

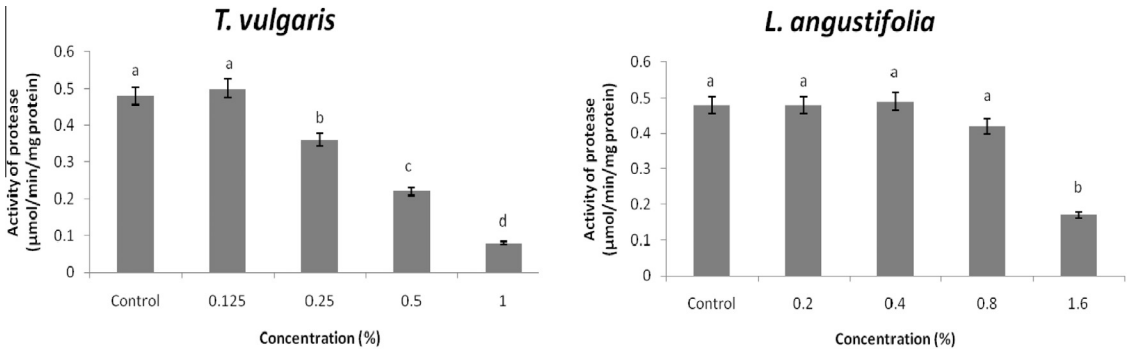

Figure 4 Effect of different concentrations of *T. vulgaris* and *L. angustifolia* essential oils on protease activity in midgut of third instar larvae of *X. luteola*.

ity of α-amylase in third instar larvae of *X. luteola* (Fig. 1) ($F = 34.77$, df = 4, $p \leqslant 0.001$). The results showed that the highest values of the α-glucosidase activity were found in the untreated larvae and the activity was gradually decreased with the increase of both essential oil concentrations (Fig. 2) ($F = 230.86$, df = 4, $p \leqslant 0.001$). The effect of *T. vulgaris* on β-glucosidase activity was significant. Activity of this enzyme in all treatments was concentration dependent and its activity declined to the lowest value with high concentration (1%) (Fig. 3) ($F = 141.92$, df = 4, $p \leqslant 0.001$). The results indicated that the activity of β-glucosidase significantly decreased in all treatments of *L. angustifolia* except for concentration of 0.2 % as compared to the control (Fig. 3) ($F = 65.29$, df = 4, $p \leqslant 0.001$). The activity of protease significantly decreased in larvae treated with 0.25, 0.5 and 1% concentrations of *T. vulgaris* ($F = 50.30$, df = 4, $p \leqslant 0.001$), but in the treatment of *L. angustifolia* essential oil, only larvae treated with 1.6% concentration differed statistically as compared to the control (Fig. 4) ($F = 9.65$, df = 4, $p = 0.001$). Different concentrations of *T. vulgaris* essential oils significantly decreased the activity of lipase in third instar larvae of *X. luteola* ($F = 20.87$, df = 4, $p \leqslant 0.001$), whereas activity of this enzyme in larvae treated with different concentrations of *L. angustifolia* did not show significant differences as compared to the control ($F = 3.22$, df = 4, $p = 0.06$) (Fig. 5).

4. Discussion

The present studies were performed to increase our knowledge on the effect of *T. vulgaris* and *L. angustifolia* essential oils on the growth, development and digestive enzyme activity of elm leaf beetle *X. luteola*. Our results clearly confirm that both essential oils possess insecticidal activity against *X. luteola*. Essential oils from thyme produced highest insecticidal activity against elm leaf beetle. Pavela (2011) studied insecticidal activity of essential oil from nine aromatic plants on *Meligethes aeneus* adults and demonstrated that essential oils obtained from *Carum carvi* and *T. vulgaris* were most efficient where LD_{50} was estimated as 197 and 250 µg cm^{-2}, respectively. Maistrello et al. (2005) showed mortality of larvae of *X. luteola* with ethanol extracts from leaves of *Daphne gnidium* obtained with concentrations of 0.1%, 0.2% and 0.3% were 27%, 70% and 73%, respectively. Shekari et al. (2008) studied on the effect of methanolic extract of *Artemisia annua* on third instar larvae of elm leaf beetle. The LC_{50} was estimated as 48% and 43.77% of a methanolic leaf extract at 24 and 48 h, respectively. LC_{50} concentrations of both essential oils obtained in our study were smaller than those of methanolic extract of *A. annua*, so indicating that both essential oils in our study would be much more effective on larvae of *X. luteola*.

Inhibition of larval development in terms of reduction in survival was observed also in both essential oil treatments. Growth inhibitory activity of *D. gnidium* on *X. luteola* was reported by Maistrello et al. (2005), they showed that the presence of *D. gnidium* leaf extract in the diet induced an increase on larval duration of at least 4–6 days. Valladares et al. (1997) demonstrated that none of the third instar larvae of *X. luteola* that fed on elm leaves sprayed with 2%, 5% or 10% aqueous extract of *Melia azedarach* were able to survive after 14 days, whereas 60% of the control larvae had already pupated and successfully emerged. Ethanolic extract of *Mun-*

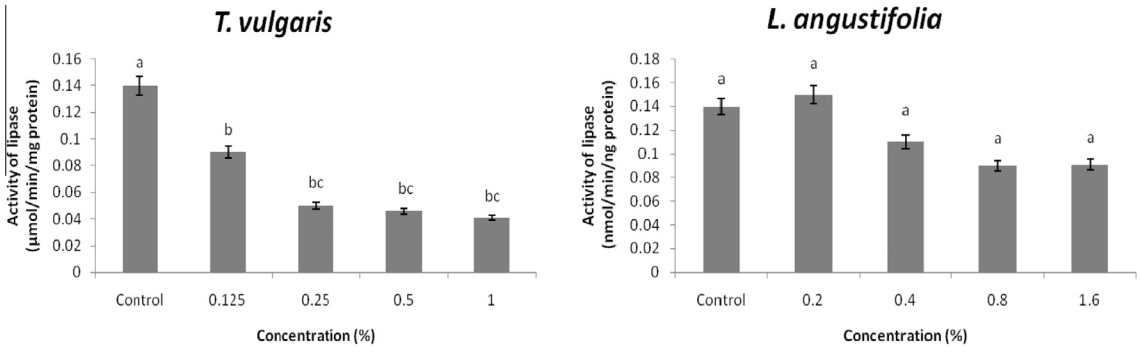

Figure 5 Effect of different concentrations of *T. vulgaris* and *L. angustifolia* essential oils on lipase activity in midgut of third instar larvae of *X. luteola*.

tingia calabura increased larval duration of *Plutella xylostella* larvae (Neto Bandeira et al., 2013). Senthil-Nathan et al. (2005), Senthil-Nathan and Sehoon (2006) and Shekari et al. (2008) showed the development inhibitory effects of plant extracts on insect species studied. These authors reported that larval and pupal duration was extended and adult longevity was reduced. The development inhibitory effects exhibited by *T. vulgaris* and *L. angustifolia* essential oils may be due to the presence of some allelochemicals on the leaf of thyme and lavander. It is possible that some of the constituents of thyme and lavander acted as antifeedants for phytophagous insects, as it has been demonstrated for many plant-derived compounds such as limonoid, terpenoids, quinines and others (Isman, 2000). In addition, allelochemicals may act as stress factors to increase the susceptibility of insects to other toxins (Murray et al., 1993).

In the present study effect of *T. vulgaris* and *L. angustifolia* essential oils on the activity of digestive enzymes was evaluated in surviving larvae after feeding on different concentrations. Digestive enzymes have a major role in the body of insects because of converting complex food materials into smaller molecules that are necessary to provide energy and metabolites (Wigglesworth, 1984). Our results showed that the activity level of α-amylase decreased in both treatments; however *T. vulgaris* essential oil showed inhibitory activity more than *L. angustifolia* essential oil. Similar results were also found by the treatment of *X. luteola* with *A. annua* methanolic extract which decreased the activity level of this enzyme 24 h after treatment and sharply increased at 48 h after treatment (Shekari et al., 2008). Khosravi et al. (2011) also reported reduction in α-amylase activity 24 h after the treatment with methanolic extracts of *A. annua* on *X. luteola*. The reduction of this enzyme activity could be due to a cytotoxic effect of different extracts on epithelial cells of midgut that synthesize α-amylase (Jbilou and Sayah, 2008).

The glycosidases catalyze the hydrolysis of terminal, non-reducing 1,2-linked alpha-D-glucose residues with releasing of alpha-D-glucose. In the present study, we found that treatments of third instar larvae of *X. luteola* with thyme and lavander essential oil concentrations showed reduction in the activity level of α- and β-glucosidase in response to increasing plant essential oils. Hemmingi and Lindroth (1999, 2000) determined the effect of phenolic components on gypsy moth and forest tent caterpillar, finding reduction of glucosidase activity in both treated larvae.

Proteases have a critical role in food digestion by insects. By treating *T. vulgaris* essential oil on third instar larvae of *X. luteola* it was found that activity of this enzyme decreased, but essential oil of *L. angustifolia L.* could decrease protease only at the highest concentration. This may be due to a drop in the consumption rates and leveling off or decline in food conversion. Senthil Nathan et al. (2004) showed that botanical insecticides may affect the construction of certain types of proteases and inhibit them to digest ingested proteins.

Lipases play a major role in storage and lipid mobilization. These enzymes are also the basic components in many physiological processes like, reproduction, growth and defense against pathogens. Our results showed that *T. vulgaris* essential oil decreased the activity of lipase in all concentrations, whereas no significant differences were observed between all treatments of *L. angustifolia L.* as compared to control. Lipase activity could be due to a greater insect effort for use from storage lipids. Zibaee and Bandani (2009) reported low lipase activity in *Eurygaster integriceps* treated by *A. annua* methanolic extract.

5. Conclusion

Plant allelochemicals may be quite useful in increasing the efficacy of biological control agents, because plants produce a large variety of compounds that increase their resistance to insect attacks. The present results showed that essential oils from *T. vulgaris* and *L. angustifolia* have toxic effects on elm leaf beetle *X. luteola* and caused delay in larval and pupal development and malformed adults. In addition, compounds present in these essential oils affect the activity of digestive enzyme in this pest.

References

Akhtar, Y., Isman, M.B., 2004. Comparative growth inhibitory and antifeedant effects of plant extracts and pure allelochemicals on four phytophagous insect species. J. Appl. Entomol. 128, 32–38.

Arbab, A., Jalali, J., Sahragard, A., 2001. On the biology of elm leaf beetle *Xonthogaleruca luteola* (Coleoptera: Chrysomellidae) in laboratory conditions. J. Entomol. Soc. Iran 21, 73–85.

Bernfeld, P., 1955. α-Amylases. Meth. Enzymol. 1, 149–151.

Bradford, M.M., 1976. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Elpidina, E.N., Vinokurov, K.S., Gromenko, V.A., Rudenskaya, Y.A., Dunaevsky, Y.E., Zhuzhikov, D.P., 2001. Compartmentalization of proteinases and amylases in *Nauphoeta cinerea* midgut. Arch. Insect Biochem. Physiol. 48, 206–216.

Enan, E., 2001. Insecticidal activity of essential oils: octopaminergic sites of action. Comp. Biochem. Physiol. 130, 325–337.

Hemmingi, J.D.C., Lindroth, R.L., 1999. Effects of light and nutrient availability on aspen: growth, phytochemistry and insect performance. J. Chem. Ecol. 26, 1687–1714.

Hemmingi, J.D.C., Lindroth, R.L., 2000. Effects of phenolic glycosides and protein on gypsy moth (Lepidoptera: Lymantriidae) and Forest tent caterpillar (Lepidoptera: Lasiocampidae) performance and detoxication activities. Environ. Entomol. 29, 1108–1115.

Huerta, A., Chiffelle, I., Puga, K., Azua, F., Araya, J.E., 2010. Toxicity and repellence of aqueous and ethanolic extracts from *Schinus molle* on elm leaf beetle *Xanthogaleruca luteola*. Crop Protec. 29, 1118–1123.

Isman, M.B., 2000. Plant essential oils for pest and disease management. Crop Protec. 19, 603–608.

Isman, M.B., 2006. Botanical insecticides, deterrent, and repellent in modern agriculture and increasingly regulated world. Annu. Rev. Entomol. 51, 45–66.

Jbilou, R., Sayah, F., 2008. Effects of *Peganum harmala* (Zygophyllaceae) seed extracts on the development of *Tribolium castaneum* (Coleoptera: Tenebrionidae). Inter. J. Trop. Insect Sci. 27, 199–209.

Khosravi, R., Sendi, J.J., Ghadamyari, M., Yezdani, E., 2011. Effect of sweet wormwood *Artemisia annua* crude leaf extracts on some biological and physiological characteristics of the lesser mulberry pyralid. *Glyphodes Pyloalis*. J. Insect Sci. 11, 156.

Maistrello, L., Lpez, M.A., Soria, F.J., Ocete, R., 2005. Growth inhibitory activity of *Daphne gnidium* L. (Thymelaeaceae) extracts on the elm leaf beetle (Col., Chrysomelidae). J. Appl. Entomol. 129, 418–424.

Morales, R., 2002. In: Stahl-Biskup, E., Saez, F. (Eds.), . In: Medicinal and Aromatic Plants – Industrial Profiles, vol. 24. Taylor &Francis, p. 16.

Murray, K.D., Alford, A.R., Groden, E., Drummond, F.A., Storch, R.H., Bentley, M.D., Sugathapala, P.M., 1993. Interactive effects of an antifeedant used with *Bacillus thuringiensis* var. *san diego* delta endotoxin on Colorado potato beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 86, 1793–1801.

Neto Bandeira, G., Augusto Gomes da Camara, C., Martins de Moraes, M., R. Barros, R., Muhammad, S., Akhtar, Y., 2013. Insecticidal activity of Muntingia calabura extracts against larvae and pupae of diamondback Plutella xylostella (Lepidoptera, Plutellidae). J. King Saud Univer. Sci. 25, 83–89.

Pavela, R., 2007. Larvicidal effects of various Euro-Asiatic plants against *C. quinquefasciatus* Say larvae (Diptera: Culicidae). Parasitol. Res. 36, 821–823.

Pavela, R., 2011. Insecticidal and repellent activity of selected essential oils against of the pollen beetle, *Meligethes aeneus* (Fabricius) adults. Indust. Crops Produc. 34, 888–892.

Pavela, R., Sajfrtova, M., Sovova, H., Karban, J., Barnet, M., 2009. The effects of extracts obtained by supercritical fluid extraction and traditional extraction techniques on larvae *Leptinotarsa decemlineata* S. J. Essen. Oil Res. 21, 367–373.

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

Senthil Nathan, S., Gene, Chung.P., Murugan, K., 2004. Effect of botanical insecticides and bacterial toxins on the gut enzyme of the rice leaffolder *Cnaphalocrocis medinalis*. Phytoparasitica 32 (5), 433–443.

Senthil-Nathan, S., Sehoon, K., 2006. Effects of *Melia azedarach* L. extract on the teak defoliator *Hyblaea puera* Cramer (Lepidoptera:Hyblaeidae). Crop Protec. 25, 287–291.

Senthil-Nathan, S., Kalaivani, K., Murugan, K., Chung, P.G., 2005. Efficacy of neem limonoids on *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae) the rice leaf folder. Crop Protec. 24, 760–763.

Shawl, A.S., Kumar, S., Med, J., 2000. Potential of *Lavender* oil industry in Kashmir. Arom. Plant. Sci. 22, 319–321.

Shekari, M., Jalali Sendi, J., Etebari, K., Zibaee, A., Shadparvar, A., 2008. Effects of Artemisia annua L. (Asteracea) on nutritional physiology and enzyme activities of elm leaf beetle, Xanthogaleruca luteola Mull. (Coleoptera: Chrysomellidae). Pestic. Biochem. Physiol. 91, 66–74.

Singh, G., Upadhyay, R.K., 1993. Essential oils a potent source of natural pesticides. J. Sci. Indust. Res. 52, 676–683.

Software, LeOra, 1987. Polo-Pc: A User Guide to Probit or Logit Analysis. LeOra Software, Berkeley, CA.

Tsujita, T., Ninomiya, H., Okuda, H., 1989. *P*nitrophenyl 865 butyrate hydrolyzing activity of hormone–sensitive lipase 866 from bovine adipose tissue. J. Lipid Res. 30, 997–1004.

Valladares, G., Defago, M.T., Palacios, S., Carpinelia, M.C., 1997. Laboratory evaluation of *Melia azedarach* (Meliaceae) extracts against the elm leaf beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 90, 747–750.

Wigglesworth, V.B., 1984. Insect Physiology, 8th ed. Chapman and Hall, London.

Zibaee, A., 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 *Beauveria bassiana*. Bull. Entomol. Res. 12, 1–11.