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ORIGINAL RESEARCH ARTICLE

Insecticidal effects of some essential oils against box tree moth (*Cydalima perspectalis* Walker (Lepidoptera: Crambidae))

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Abstract

The box tree moth *Cydalima perspectalis* (Walker) (Lepidoptera: Crambidae) is one of the most alien insects found in the *Buxus* areas of Georgia and World. Many methods have been used to control this pest up to now. But, the problem is still going on. In this study, insecticidal effects of the essential oils obtained from plants *Artemisia absinthium* L., *Seriphidium santonicum* (L.) Sojak, *Seriphidium spicigerum* (K.Koch) Poljakov, *Cuminum cyminum* L., *Mentha pulegium* L., *Origanum majorana* L., *Origanum onites* L., *Origanum syriacum* L., *Origanum vulgare* L., and *Satureja hortensis* L. on *C.perspectalis* were tested in laboratory conditions. In this context, larvae of the 2nd and 5th instars of *C.perspectalis* were exposed to essential oils at doses of 10, 15 and 20 μl/petri for 24, 48, 72 and 96 h. All of the essential oils used in the study caused mortality at different rates; the highest effect on 2nd and 5th instar larvae of *C.perspectalis* was obtained with the essential oil from *O.onites* with a mortality rate of 80.0–71.6%. The oils from *O. onites* (73.3–65.0%), *O.syriacum* (73.3–63.3%), *O.majorana* (71.6–66.6%), *A.absinthium* (68.3–61.6%), *S.santonicum* (68.3–60.0%), *S.spicigerum* (66.6–60.0%), *S.hortensis* (66.6–61.1%), *C.cyminum* (58.3–53.3%) and *M.pulegium* (51.6–45.0%) followed this in this order. As a results of the dose effect tests conducted in the second part of the study, the most toxic plant essential oils were determined to be from *O.vulgare* and the lowest toxic effect from *M.pulegium* based on LD_{50} and LD_{90}. The results obtained show that the essential oils from *O.vulgare* can be used in the control against *C.perspectalis.*

Keywords Cydalima perspectalis · Essential oils · Insecticide

Introduction

Alien species are a great ecological and economic threat, with a multitude of negative impacts on biodiversity (Kenis et al. 2007) and causing enormous damage to ecosystems and economies (Kenis and Branco 2010). In the Republic of Georgia, insects are one of the groups with the most alien species which cause economic impacts. The box tree moth *Cydalima perspectalis* (Walker) (Lepidoptera: Crambidae) is originated from the East Asia and it is an alien species for Republic of Georgia (Matsiakh et al. 2018). It spread rapidly across Europe and it is now present at least 16 European countries, in which it has become a serious pest of ornamental box trees (*Buxus* spp.) in forest, parks and gardens (Safian and Horvath 2011; Budashkin 2016; Bury et al. 2017). The pest was added in the alert list of the European Plant Protection Organization (EPPO) in 2007 (EPPO 2011). *C.perspectalis* causes widespread damage in Georgia, Imereti (Zestaponi, Kutaisi, Tkibuli), Samegrelo-Zemo Svaneti (Zugdidi, Tsalenjikha, Martvili), Guria (Lanchkhuti, Ozurgeti, Chokhatauri), Autonomous Republic of Adjara (Khelvachauri, Batumi).

Buxus semperivens L. which is endemic species of Caucasian flora is an evergreen Tertiary-period relict plant on the IUCN Red List of Threatened Species. *B.semperivens* has been also included on the 'Red List' of the Republic of Georgia in the category VU since

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2006, due to the tendency of areal fragmentation and habitat loss (Matsiakh et al. 2018). The fact that *C.perspectalis* has two-to-five generations (She and Feng 2006) and six larval instar in a year depending on the climate conditions increases the damage it causes. The pest is reported to have 2–3 generations in Georgia. The larvae feed on leaves and shoots, caused serious damages, defoliating box trees, causing economic, social and environment problems in Georgia since 2015 (Matsiakh et al. 2016). The damage, *Buxus* plants infested by young larvae of *C.perspectalis* can feed in the lower surfaces of the leaves only and leave the upper epidermis intact, whereas older larval instar feed all leaves also attack the bark, causing defoliation and even death of the affected plants (Leuthardt and Baur 2013).

In order to minimize this damage, every method to be used in Integrated Pest Management (IPM) is of great significance. Box trees can be protected by chemical insecticides (pyrethroid) (Zhou et al. 2005), the ones based on *Bacillus thuringiensis* var. *kurstaki* (Dipel DF®) (Lacey et al. 2015), baculovirus *Anagrapha falcifera* nucleopolyhedrovirus (AnfaNPV) (Rose et al. 2013) or nematodes (*Steinernema carpocapsae*) (Lee et al. 1996) to the larvae in April and October. Pheromon trap (WitaTrap® Funnel trap system, and Delta sticky trap with pheromone CYDAWIT® (Witasek, Pflanzenschutz, GmbH, Austria) can using for adults Kim and Park 2013). Among these methods, chemical insecticide has been the most widely used in the world. However, since chemical insecticides cause environmental problems and have adverse effects on non-target organisms, the use of plant-based insecticides has become more attractive (Isman 2006). Nowadays essential oils have been used for bactericidal, virucidal, fungicidal, antiparasitical, insecticidal, medicinal and cosmetic applications. Another using way for essential oils are applicable in the control of harmful insects. Recently, there has been a growing interest in research concerning the possible use of plant extracts as alternatives to synthetic insecticides. Insecticidal activity of many plant products against various insect pests has been demonstrated by many researchers (Isman 2006; Tripathi et al. 2009; Regnault Roger et al. 2012).

In this study, insecticidal effects of the essential oils obtained from plant species *Artemisia absinthium* L., *Seriphidium santonicum* (L.) Sojak, *Seriphidium spicigerum* (K.Koch) Poljakov, *Cuminum cyminum* L., *Mentha pulegium* L., *Origanum majorana* L., *Origanum onites* L., *Origanum syriacum* L., *Origanum vulgare* L., and *Satureja hortensis* L. applied in the laboratory environment on the 2nd and 5th instar larvae of *C.perspectalis* collected from the Municipality of Khelvachauri (Batumi-Adjara-Georgia) and surroundings were attempted to be determined.

Materials and methods

Test insects

In this study, the 2nd and 5th instar larvae of *C.perspectalis*, which fed substantially on *Buxus semperivens* leaves in Municipality of Khelvachauri of Georgia during the months of April and October in 2019, were collected and placed in the growing jars in the laboratory environment. The larvae were subjected to experiments in the laboratory at 26.7 °C, at 70% relative humidity, and at lighting conditions of 16:8 h (light, dark).

Plant material and isolation of essential oils The plants used in the study, *Artemisia absinthium* (L.) (Pelin otu) (wormwood) (Asteraceae) *Seriphidium santonicum* (L.) Sojak, (Deniz Yavşanı) (Salt steppe wormwood) (Asteraceae), *Seriphidium spicigerum* (K.Koch) Poljakov (Yavşan otu) (wormwood) (Asteraceae), *Cuminum cyminum* L. (Kimyon) Cumin (Apiaceae), *Mentha pulegium* L. (Yarpuz) pennyroyal (Lamiaceae), *Origanum majorana* L. (Sweet marjoram) (Mercanköşk), *Origanum onites L.* (İzmir kekiği) (Turkish oregano) *Origanum syriacum* L. (Güve otu) (Syrian oregano), *Origanum vulgare* L. (Keklikotu), oregano (Lamiaceae), *Satureja hortensis* (Lamiaceae) were collected from different regions of Turkey in June and July in 2018–2019.

The plants protected in the herbarium of Muğla Sıtkı Kocman University Fethiye Agriculture Faculty, Department of Plant Protection were dried in a cool and shaded place and then the plants dried with the help of a grinder were ground. 500 g dry plant material was placed in Neo-Clevenger equipment and subjected to hydrodistillation for 4 h. 500 g of ground dry plant material and 1000 ml of water were placed in glass flask, placed on Neo-Clevenger equipment and subjected to hydrodistillation for 3–4 h. *A.absinthium, S.santonicum, S.spicigerum, C.cyminum*, *M.pulegium, O.majorana, O.onites, O.syriacum, O.vulgare* and *S.hortensis* were 0.6%, 0.8%, 0.5%, 2.4%, 1.31%, 0.98%, 3.7%, 4.0%, 3.6% and 1.49% (*w*/w, dry weight basis), respectively. The oils were dried over anhydrous Na_2SO_4 and stored under N_2 in a sealed vial at 4 °C until used for toxicity bioassays.

Bioassays using essential oils

After exposure, the mortality of the adults was recorded at 24, 48, 72 and 96 h. Sterile water and ethanol were used as a control under same conditions. Each sample was replicated for three times at each dose. Glass petri dishes (12x12x1.5 cm) were used to test the toxicity of essential oils from six plants against the 2nd and 5th instar larvae of *C.perspectalis.* The oils were dissolved in ethanol-water solution (10%, *v*/v) in order to determine their contact toxicity. The

concentrations of 10, 15 and 20 μL/petri were preferred for implementations. The bottom of the petri dishes was laid with filter paper; *Buxus semperivens* leaf and 20 *C.perspectalis* larvae were placed on it. Prepared doses were sprayed onto the larvae. The operated petri dishes were stored at 25 ± 1 °C, 64 ± 5 humidity, and light: dark (16:8) cycle and inspected for 4 days. Neemazal® (10, 15 and 20 μL/petri) was used as a positive control in the study. Inspections were done at 24, 48, 72 and 96 h after the application; dead and healthy individuals were counted. A mixture of sterile water and ethanol was also used as a control. The trials were carried out in 3 replicates.

Major constituents of the essential oils of test plants has been previously reported by, Kordali et al. (2006); Tozlu et al. (2011); Carroll et al. (2017); Duran and Kaya (2018); Amor et al. (2019); Vieira et al. (2019); Montenegro et al. (2020); Paiano et al. (2020). A list of the constituents and grouped components of this essential oils are presented in Table 1.

Table 1 Major constituents of the essential oils of test plants

Test Plants	Major constituents	Relative percent (%)	Literature
A.absinthium	Chamazulene	17.8	Kordali et al. (2006)
	Nuciferol butanoate	8.2	
	Nuciferol propionate	5.1	
	Caryophyllene oxide	4.3	
S.santonicum	Camphor	18.2	Kordali et al. (2006)
	1,8-Cineole	7.5	
	β-Eudesmol	7.2	
	Cubenol	4.2	
S.spicigerum	Camphor	34.9	Kordali et al. (2006)
	1,8-Cineole	9.5	
	Borneol	5.1	
	Spathulenol	3.7	
C.cyminum	Cuminaldehyde	32.66	Vieira et al. (2019)
	γ-terpinene	19.87	
	β-pinene	15.22	
	o-cymene	14.00	
M.pulegium	Menthol	28.79	Montenegro et al. (2020)
	Menthone	20.48	
	Isopulegol	9.75	
	Menthyl acetate	8.35	
O.majorana	Terpinen-4-ol	34.1	Amor et al. (2019)
	α- terpinene	19.2	
	endo-Fenchyl-acetate	9.8	
	Terpineol	8.9	
O.onites	Carvacrol	75.70	Carroll et al. (2017)
	Linalool	9.0	
	p-Cymene	4.33	
	Thymol	1.9	
O.syriacum	Thymol	42.18	Duran and Kaya (2018)
	Carvacrol	33.95	
	Cymene	8.87	
	γ- terpinene	8.21	
O.vulgare	Carvacrol	72.12	Paiano et al. (2020)
	γ –Terpinene	4.81	
	p-Cymene	4.81	
	Linalool	3.03	
S.hortensis	Carvacrol	54.74	Tozlu et al. (2011)
	γ –Terpinene	20.94	
	p-Cymene	12.30	
	α- Pinene	1.76	

GC-MS analysis

The analyses of the essential oils performed with a Thermofinnigan Trace GC/Trace DSQ/A1300 (E.I. Quadrapole) equipped with a SGE-BPX5 MS fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). For GC–MS detection, an electron impact ionization system with ionization energy of 70 eV was used. Carrier gas was helium at a flow rate of 1 mL/min. Diluted samples (1/100, *v*/v, in methylene chloride) of 1.0 μL were injected in the split-less mode. Injector and MS transfer line temperatures were set at 220 °C and 290 °C, respectively. The oven temperature was programmed from 50 °C to 150 °C at 3 °C/min, then held isothermal for 10 min and finally raised to 250 °C at 10 °C/min. The components were identified based on the comparison of their relative retention time and mass spectra with those of standards, Wiley 7 N, TRLIB library data of the GC-MS system and literature data. The results were also confirmed by the comparison of the compounds elution order with their relative retention indices on non-polar phases reported in the literature.

Statistical analysis

The data analyses were carried out by one-way ANOVA followed by comparison of mean values using post hoc Duncan test at $p \leq 0.05$. All the statistical analysis was performed using SPSS software ver. 17.0. Lethal dose and Lethal concentration (LD_{50} and LD_{90}) values after 96 h were calculated using the Finney method (Finney 1971). To determine LD values at 95% confidence limits EPA Probit Analysis Program was used. The results showed significant differences at $P \leq 0.05$ levels.

Period of study

This study was carried out in April–December in 2018–2019.

Results and discussion

As a result of the trials of insecticidal effects of essential oils of different plant species in this study, it was determined that all plant oils the resulted in mortalities of different rates and that there are statistical differences between them. All essential oils obtained from *A.absinthium, S.santonicum, S.spicigerum, C.cyminum*, *M.pulegium, O.majorana, O.onites, O.syriacum, O.vulgare* and *S.hortensis* were displayed toxicity against on 2nd and 5th instar larvae of *C.perspectalis* in comparison to control, but the effects of these essential oils varied among each plant species. Furthermore, the mortality rates increased with increasing doses and exposure times for essential oils of tested plant species The effects of different concentrations of essential oils on 2nd and 5th instar larvae *C.perspectalis* are given in Tables 2 and 3 and Figs. 1 and 2.

When the mortality rates caused by plant essential oils at the end of 24 h were compared, statistically significant differences were found between the treatments. When the efficacy rates 2nd instar larvae of *C.perspectalis* were examined at 10–15-20 μL/petri doses and at the end of 24, 48, 72 and 96 h, the highest effect was observed in *O. vulgare* and the lowest effect was seen in *M.pulegium* essential oil. When the mortality rates 2nd instar larvae of *C.perspectalis* caused by plant essential oils were compared, the highest effect was observed in *O.vulgare* essential oil (48.3–70.0-80.0%) at 10–15-20 μL/petri doses at the end of 96 h. Other essential oil the mortality rates 2nd instar larvae of *C.perspectalis* is *A.absinthium* (21.6–61.6-68.3%), *S.santonicum* (25.0–56.6-68.3%), *S.spicigerum* (26.6–56.6-66.6%), *C.cyminum* (23.3–38.3-58.3%), *O.majorana* (45.0–58.3-71.6%), *O.onites* (41.6–56.6-73.3%), *O.syriacum* (40.0–60.0-73.3%), *S.hortensis* (36.6–50.0-66.6%) at 10–15-20 μL/petri doses at the end of 96 h. When the mortality rates 2nd instar larvae of *C.perspectalis* caused by plant essential oils at the end of 96 h were compared, the lowest effect was observed in *M.pulegium* (15.0–35.0-51.6%) essential oil. The effects of the *Origanum* species essential oils were found close to each other.

In this study, the highest effect 26.6% at 24 h, 60.0% at 48 h, 93.3% at 72 h and 100% were observed at the end of 96 h at the maximum dose of Neemazal (20 μL/petri), which was used as positive control (Table 2). In an experiment; *Artemisia absinthium* essential oil 10, 15 and 20 μL / petri doses on *Taumetopoea pityocamp*a 1st, 2nd, 3rd, 4th and 5th instars larvae at the 12, 24, 36 and 48 h reported that it caused death 6.66–100% between (Usanmaz Bozhuyuk et al. 2018). In another study, *Artemisia absinthium*, *Seriphidium santonicum*, *Seriphidium spicigerum* and *Achillea santolinoides* different doses of essential oils of while the mortalities were recorded between 23 and 100% for *T.urticae*, they were between 45 and 100% for *A.obtectus* (Usanmaz Bozhüyük et al. 2020). It is seen that the results of the studies in the literature are similar to our study findings.

According to the results of dose-response studies on 2nd instar larvae of *C.perspectalis*, the According to the results of dose-response studies on 2nd instar larvae of *C.perspectalis*, the most toxic plant essential oils were determined to be from *O.vulgare* based on LD_{50} and LD_{90}. The lowest toxic effect was found to be of essential oils from *M.pulegium* based on LD_{50} and LD_{90}. All *Artemisia* and *Seriphidium* species showed similar toxicity on LD_{50} and LD_{90} (Table 2). The insecticidal activity increased with increasing doses and exposure times. Most of the essential oils caused significant mortality (Fig. 1).

When the efficacy rates 5th instar larvae of *C.perspectalis* were examined at 10–15-20 μL/petri doses, the highest effect was observed in *O. vulgare* and the lowest effect was seen in *M.pulegium* essential oil.

Table 2 The results of multiple comparison with mean (M) and std. error (SE) of exposure time and dose of essential oil of ten plant species on 2nd instar larvae of *C.perspectalis*

Treatment essential oils	Dose (μL/petri)	Exposure time – Mortality (%)			
		24 h	48 h	72 h	96 h
	10	5.0 ± 0.0 ef	11.6 ± 1.7 jkl	18.3 ± 3.3 opr	21.6 ± 1.7 rs
A.absinthium	15	10.0 ± 0.0 cde	25.0 ± 0.0 defg	41.6 ± 1.7 efghı	61.6 ± 1.7 efgh
	20	11.6 ± 1.7 cd	26.6 ± 1.7 def	45.0 ± 2.9 efgh	68.3 ± 1.7 def
	10	1.66 ± 1.7 fg	8.33 ± 1.7 kl	15.0 ± 2.9 prs	25.0 ± 2.9 r
S.santonicum	15	6.66 ± 1.7 e	21.6 ± 1.7 fghı	38.3 ± 1.7 ghıj	56.6 ± 1.7 hıjk
	20	11.6 ± 1.7 cd	26.6 ± 1.7 def	46.6 ± 1.7 efg	68.3 ± 1.7 def
	10	1.66 ± 1.7 fg	8.33 ± 3.3 kl	15.0 ± 2.9 prs	26.6 ± 4.4 r
S.spicigerum	15	10.0 ± 0.0 cde	25.0 ± 0.0 defg	40.0 ± 0.0 fghıj	56.6 ± 1.7 hıjk
	20	10.0 ± 0.0 cde	25.0 ± 0.0 defg	43.3 ± 1.7 efgh	66.6 ± 1.7 defg
	10	0.0 ± 0.0 g	5.0 ± 0.0 lm	13.3 ± 1.7 rs	23.3 ± 1.7 r
C.cyminum	15	5.0 ± 0.0 ef	11.6 ± 1.7 jkl	23.3 ± 1.7 mnop	38.3 ± 1.7 op
	20	6.66 ± 1.7 e	21.6 ± 1.7 fghı	36.6 ± 1.7 hıjk	58.3 ± 1.7 ghıj
	10	0.0 ± 0.0 g	1.66 ± 1.7 m	6.66 ± 1.7 s	15.0 ± 2.9 st
M.pulegium	15	5.0 ± 0.0 ef	11.6 ± 1.7 jkl	21.6 ± 1.7 nopr	35.0 ± 0.0 p
	20	8.33 ± 1.7 de	18.3 ± 1.7 ghıj	33.3 ± 1.7 ıjkl	51.6 ± 4.4 ıjkl
	10	5.0 ± 0.0 ef	13.3 ± 1.7 jk	28.3 ± 1.7 klmn	45.0 ± 0.0 lmno
O.majorana	15	10.0 ± 0.0 cde	23.3 ± 1.7 efgh	41.6 ± 1.7 efghı	58.3 ± 1.7 ghıj
	20	10.0 ± 0.0 cde	25.0 ± 0.0 defg	41.6 ± 1.7 efghı	71.6 ± 1.7 cd
	10	5.0 ± 0.0 ef	16.6 ± 1.7 hıj	28.3 ± 3.3 klmn	41.6 ± 4.4 mnop
O.onites	15	6.66 ± 1.7 e	21.6 ± 1.7 fghı	38.3 ± 1.7 ghıj	56.6 ± 1.7 hıjk
	20	12.1 ± 1.7 cd	30.0 ± 2.9 de	50.0 ± 2.9 de	73.3 ± 1.7 cd
	10	5.0 ± 0.0 ef	15.0 ± 0.0 ıjk	26.6 ± 1.7 lmno	40.0 ± 2.9 nop
O.syriacum	15	10.0 ± 0.0 cde	25.0 ± 0.0 defg	36.6 ± 3.3 hıjk	60.0 ± 0.0 fghı
	20	11.6 ± 1.67 cd	30.0 ± 0.0 de	48.3 ± 1.7 def	73.3 ± 1.7 cd
	10	6.66 ± 1.7 e	16.6 ± 1.7 hıj	31.6 ± 1.7 jklm	48.3 ± 1.7 klmn
O.vulgare	15	10.0 ± 0.0 cde	25.0 ± 0.0 defg	48.3 ± 1.7 def	70.0 ± 0.0 de
	20	13.6 ± 1.7 c	31.6 ± 1.7 cd	56.6 ± 1.7 cd	80.0 ± 0.0 bc
	10	5.0 ± 0.0ef	13.3 ± 1.7 jk	23.3 ± 1.7 mnop	36.6 ± 1.7 op
S.hortensis	15	5.0 ± 0.0 ef	16.6 ± 1.7 hıj	31.6 ± 1.7 jklm	50.0 ± 0.0 jklm
	20	10.0 ± 0.0 cde	25.0 ± 0.0 defg	43.3 ± 1.7 efgh	66.6 ± 1.7 defg
Pozitive Control (Neemazal)	10	11.6 ± 1.7 cd	36.6 ± 1.7 c	60.0 ± 2.9 c	85.0 ± 2.9 b
	15	21.6 ± 1.7 b	50.0 ± 2.9 b	76.6 ± 3.3 b	95.0 ± 0.0 a
	20	26.6 ± 1.7 a	60.0 ± 2.9 a	93.3 ± 1.7 a	100 ± 0.0 a
Control (Ethanol+Sterile water mix)	20	0.0 ± 0.0 g	0.0 ± 0.0 m	6.67 ± 1.7 s	10.0 ± 2.9 t

Values followed by different letters in the same column differ significantly at $P \leq 0.05$ according to Duncan Multiple test

Mean ± SE of three replicates, each set up with 20 larvae

The highest effect was seen in the *O.onites* essential oil at 24 h at 20 μL/petri dose; it was determined at 48 and 72 h, 26.6% and 48.3% respectively. When the mortality rates 5th instar larvae of *C.perspectalis* caused by plant essential oils were compared, the highest effect was observed in *O. vulgare* essential oil (43.3–60.0-71.6) at 10–15-20 μL/petri doses at the end of 96 h. Other essential oil the mortality rates 5th instar larvae of *C.perspectalis* is *A.absinthium* (16.6–43.3-61.6%) *S.santonicum* (18.3–48.3-60.0%), *S.spicigerum* (18.3–50.0-60.0%), *C.cyminum* (18.3–33.3-53.3%), *O.majorana* (40.0–53.3-66.6%), *O.onites* (35.0–51.6-65.0%), *O.syriacum* (33.3–50.0-63.3%), *S.hortensis* (31.6–45.0-61.6%) at 10–15-20 μL/petri doses at the end of 96 h. When the mortality rates 5th instar larvae of *C.perspectalis* caused by plant essential oils at the end of 96 h were compared, the lowest

Table 3 The LD values of essential oils obtained from ten plants against 2nd instar larvae of *C.perspectalis*

Treatment essential oils	LD_{50}[b]	LD_{90}[c]	X^{2d}	Df[d]	P[f]	Slope ± SE[e]
A. absinthium	2.131	7.365	2.033	7	0.95	2.697 ± 0.525
S. santonicum	2.248	7.499	1.402	7	0.90	2.450 ± 0.519
S. spicigerum	2.215	7.356	0.933	7	0.98	2.459 ± 0.518
C. cyminum	2.914	12.352	1.031	7	0.98	2.043 ± 0.523
M. pulegium	3.349	13.330	1.513	7	0.96	2.421 ± 0.572
O. majorana	1.577	10.044	0.500	7	0.95	1.397 ± 0.484
O. onites	1.783	11.359	0.662	7	0.98	1.593 ± 0.487
O. syriacum	1.899	12.049	0.370	7	0.99	1.597 ± 0.488
O.vulgare	1.308	5.969	0.349	7	0.96	1.533 ± 0.487
S. hortensis	2.113	7.736	0.752	7	0.97	1.577 ± 0.489

[a] The lethal concentration causing 50% mortality after 96 h

[b] The lethal concentration causing 90% mortality after 96 h

[c] Chi square value $P \leq 0.01$

[d] Slope of the concentration-mortality regression line ± standard error

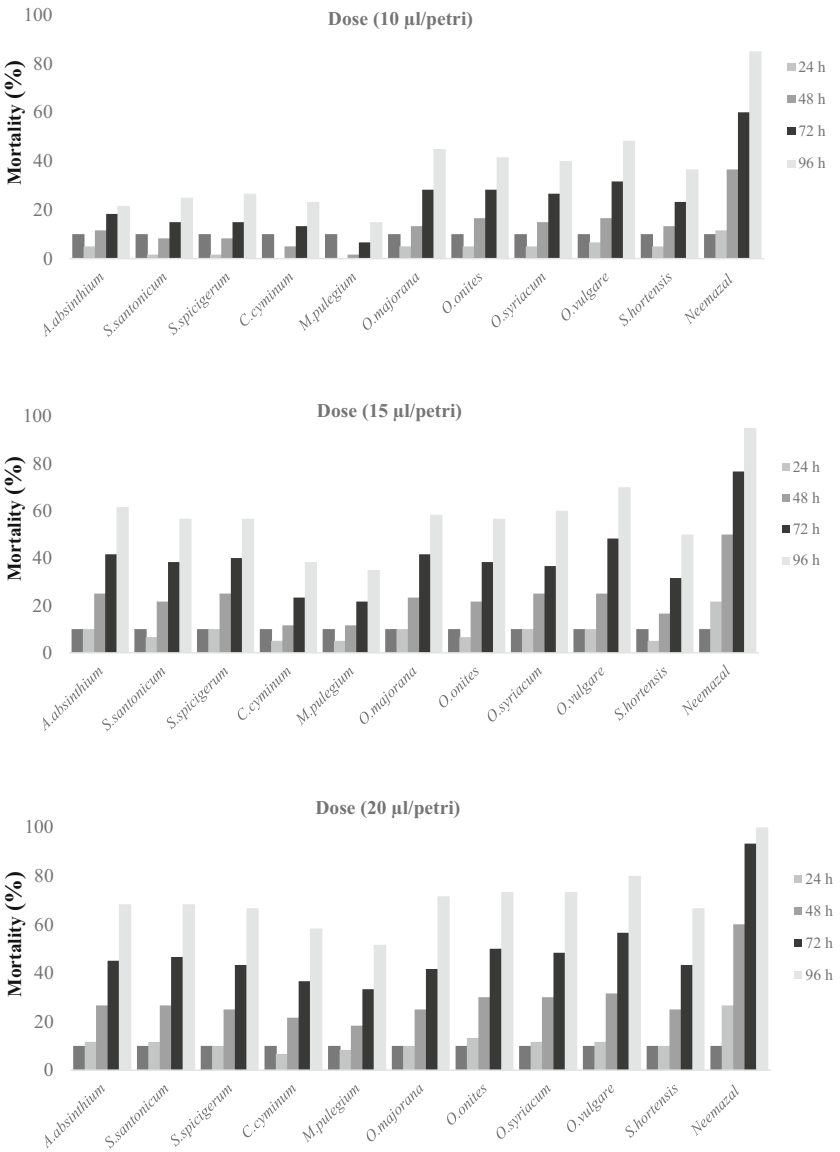

Fig. 1 Percent mortality of 2nd instar larvae of *C.perspectalis* after treatment with 10, 15, 20 µL/petri doses ten plant essential oils and treatment times

effect was observed in *M.pulegium* (10.0–30.0-45.0%) essential oil. The highest effect (90%) was observed at the end of 96 h at the maximum dose of Neemazal (20 µL/ petri), which was used as positive control (Table 4). Also, the highest effect 21.6% at 24 h, 50.0% at 48 h, 81.6% at 72 h were observed at the maximum dose of Neemazal

Table 4 The results of multiple comparison with mean (M) and std. error (SE) of exposure time and dose of essential oil of ten plant species on 5th instar larvae of *C.perspectalis*

Treatment essential oils	Dose (µL/ petri)	Exposure time – Mortality (%)			
		24 h	48 h	72 h	96 h
	10	0.0 ± 0.0 f	6.66 ± 1.7 ıj	13.3 ± 3.3 nop	16.6 ± 1.66 l
A.absinthium	15	5.0 ± 0.0 cde	20.0 ± 0.0 defg	35.0 ± 0.0 efgh	43.3 ± 1.7 ef
	20	6.66 ± 1.7 cd	21.6 ± 1.7 def	38.3 ± 1.7 efg	61.6 ± 1.7 d
	10	0.0 ± 0.0 f	3.33 ± 1.7 jk	10.0 ± 2.9 opr	18.3 ± 3.3 l
S.santonicum	15	1.66 ± 1.7 ef	16.6 ± 1.7 fgh	38.3 ± 1.7 efg	48.3 ± 1.7 fgh
	20	6.66 ± 1.7 cd	21.6 ± 1.7 def	41.6 ± 1.7 def	60.0 ± 1.7 de
	10	0.0 ± 0.0 f	3.33 ± 3.3 jk	10.0 ± 2.9 opr	18.3 ± 1.7 l
S.spicigerum	15	5.0 ± 0.0 cde	20.0 ± 0.0 defg	35.0 ± 0.0 efgh	50.0 ± 0.0 fgh
	20	5.0 ± 0.0 cde	20.0 ± 0.0 defg	38.3 ± 1.7 efg	60.0 ± 0.0 de
	10	0.0 ± 0.0 f	0.0 ± 0.0 k	8.33 ± 1.7 pr	18.3 ± 1.7 l
C. cyminum	15	0.0 ± 0.0 f	6.66 ± 1.7 ıj	18.3 ± 1.7 lmno	33.3 ± 1.7 jk
	20	1.66 ± 1.7 ef	16.6 ± 1.7 fgh	31.6 ± 1.7 ghıj	53.3 ± 1.7 ef
	10	0.0 ± 0.0 f	0.0 ± 0.0 k	1.66 ± 1.7 mnop	10.0 ± 2.9 m
M.pulegium	15	0.0 ± 0.0 f	6.66 ± 1.7 ıj	16.6 ± 1.7 mnop	30.0 ± 0.0 k
	20	3.33 ± 1.7 def	13.3 ± 1.7 ghı	28.3 ± 1.7 hıjk	45.0 ± 2.9 ghı
	10	0.0 ± 0.0 a	8.33 ± 1.7 ıj	21.6 ± 1.7 klmn	40.0 ± 0.0 ıj
O.majorana	15	5.0 ± 0.0 cde	18.3 ± 1.7 efg	36.6 ± 1.7 efg	53.3 ± 1.7 ef
	20	5.0 ± 0.0 cde	20.0 ± 0.0 defg	36.6 ± 1.7 efg	66.6 ± 1.7 cd
	10	0.0 ± 0.0 f	11.6 ± 1.7 hı	23.3 ± 3.3 jklm	35.0 ± 2.9jk
O.onites	15	1.66 ± 1.7 ef	16.6 ± 1.7 fgh	33.3 ± 1.7 fghı	51.6 ± 1.7 fg
	20	8.33 ± 1.7 c	25.0 ± 2.9 de	43.3 ± 1.7 cde	65.0 ± 0.0 d
	10	0.0 ± 0.0 f	10.0 ± 0.0 ıj	21.6 ± 1.7 klmn	33.3 ± 1.7 jk
O.syriacum	15	5.0 ± 0.0 cde	20.0 ± 0.0 defg	33.3 ± 3.3 fghı	50.0 ± 0.0 fgh
	20	6.66 ± 1.7 cd	25.0 ± 0.0 de	43.3 ± 1.7 cde	63.3 ± 1.7 d
	10	1.66 ± 1.7 ef	10.0 ± 0.0 ıj	26.6 ± 1.7 ıjkl	43.3 ± 1.7 hı
O. vulgare	15	5.0 ± 0.0 cde	20.0 ± 0.0 defg	43.3 ± 1.7 cde	60.0 ± 0.0 de
	20	6.66 ± 1.7 cd	26.6 ± 1.7 cd	48.3 ± 1.7 cd	71.6 ± 1.7 bc
	10	0.0 ± 0.0 f	8.33 ± 1.7 ıj	18.3 ± 1.7 lmno	31.6 ± 1.7 k
S.hortensis	15	0.0 ± 0.0 f	11.6 ± 1.7 hı	26.6 ± 1.7 ıjkl	45.0 ± 0.0 ghı
	20	5.0 ± 0.0 cde	20.0 ± 0.0 defg	38.3 ± 1.7 efg	61.6 ± 1.7 d
Pozitive Control	10	6.66 ± 1.7 cd	31.6 ± 1.7 c	51.6 ± 4.4 c	75.0 ± 2.9 b
(Neemazal)	15	16.6 ± 1.7 b	40.0 ± 2.9 b	66.6 ± 3.3 b	85.0 ± 0.0 a
	20	21.6 ± 1.7 a	50.0 ± 2.9 a	81.6 ± 1.7 a	90.0 ± 0.0 a
Control (Ethanol+Sterile water mix)	20	0.0 ± 0.0 f	0.0 ± 0.0 k	1.66 ± 1.7 r	3.33 ± 1.7 n

Values followed by different letters in the same column differ significantly at $P \leq 0.05$ according to Duncan Multiple test

Mean ± SE of three replicates, each set up with 20 larvae

(20 μL/petri), which was used as positive control (Table 4). In another study on essential oils; *Achillea gypsicola*, *Achillea wilhelmsii*, *Achillea millefolium*, *Achillea biebersteinii*, *Achillea biserrata*, *Artemisia absinthium*, *Artemisia santonicum*, *Artemisia spicigera*, *Origanum onites*, *Origanum acutidens*, *Origanum syriacum*, *Origanum vulgare subsp. hirtum*, *Thymus sipyleus* and *Thymus fallax* essential oils of *Leptinotarsa decemlineata* on adults 24, 48, 72 and 96 h the toxicity degrees were found to be variable ranging from 2.22 to 100% mortality (Kesdek et al. 2015). It also caused 77.7–100% death of the positive control (Izoldesis) chemical. After 96 h of exposure, *Sitophilus zeamais* on adults at the maximum concentration (20 μL/L essential oil) of *A.biserrata, A.coarctata, A.gypsicola, A.santonicum, H.perforatum, M.officinalis, O.onites, O.rotundifolium, S.hortensis, S.spicigera, T.agrophyllum* recorded 100% mortality, while *O.syriacum, O.acutidens, A.wilhemsii* and *S.nemorosa* attained 99–76.77 mortality (Kordali et al. 2013). Although the applied insect groups are different; plant essential oils have been shown to have similar effects and and essential oils have been found to have an insecticidal effect.

Furthermore, according to LD values (LD_{50} and LD_{90}), the most toxic plant essential oils LD values on 5th instar larvae of *C.perspectalis*, was recorded for the essential oils of *O.vulgare* whereas the essential oils of *M.pulegium* had the lowest toxicity. All *Artemisia* and *Seriphidium* species showed similar toxicity on LD_{50} and LD_{90} (Table 5). The insecticidal activity increased with increasing doses and exposure times. Most of the essential oils caused significant mortality (Fig. 2).

The demand for effective insecticides in pest control with low toxicity to the environmental persistence and mammalian toxicity is increasing steadily. One of them good alternative for synthetic insecticides is natural compounds, including essential oils. Essential oils have been largely employed for their properties already observed in nature. Thus, it was shown that essential oils might constitute new alternatives to currently used insecticides not only against stored product pests but also against such as aphids, moth or others (Aslan et al. 2004).

Conclusions

As a result, the study showed the insecticidal potential of *Artemisia absinthium*, *Seriphidium santonicum*, *Seriphidium spicigerum*, *Cuminum cyminum*, *Mentha pulegium*, *Origanum majorana*, *Origanum onites*, *Origanum syriacum*, *Origanum vulgare* and *Satureja hortensis* essential oils. Such studies can contribute to a greater understanding of the format of action of natural products with insecticidal potential. And, we suggest that the effects of these essential oils must be field-tested in the Batumi of Georgia under all circumstances, and results must be compared with those obtained in the laboratory. The essential oil activity in creased with the increasing of the dose and exposure times. The essential oils caused significant mortality at 2nd and 5th instar larvae of *C.perspectalis*. Essential oils can be applied more environmentally. According to the results presented in this study, not all the essential oils tested showed satisfactory activity, but the essential oils of *O.onites* proved to be promising as

Table 5 The LD values of essential oils obtained from ten plants against 5th instar larvae of *C.perspectalis*

Treatment essential oils	LD_{50}[b]	LD_{90}[c]	X^{2}[d]	Df[d]	P[f]	Slope ± SE[e]
A. absinthium	2.131	7.365	2.033	7	0.92	2.697 ± 0.525
S .santonicum	2.248	7.499	1.402	7	0.93	2.450 ± 0.519
S. spicigerum	2.215	7.356	0.933	7	0.99	2.459 ± 0.518
C. cyminum	2.914	12.352	1.031	7	0.99	2.043 ± 0.523
M. pulegium	3.349	13.330	1.513	7	0.97	2.421 ± 0.572
O. majorana	1.577	12.044	0.500	7	0.93	1.397 ± 0.484
O. onites	1.783	11.359	0.662	7	0.98	1.593 ± 0.487
O. syriacum	1.899	12.049	0.370	7	0.99	1.597 ± 0.488
O.vulgare	1.308	6.969	0.349	7	0.96	1.533 ± 0.487
S. hortensis	2.113	12.736	0.752	7	0.98	1.577 ± 0.489

[a] The lethal concentration causing 50% mortality after 96 h

[b] The lethal concentration causing 90% mortality after 96 h

[c] Chi square value $P \leq 0.01$

[d] Slope of the concentration-mortality regression line ± standard error

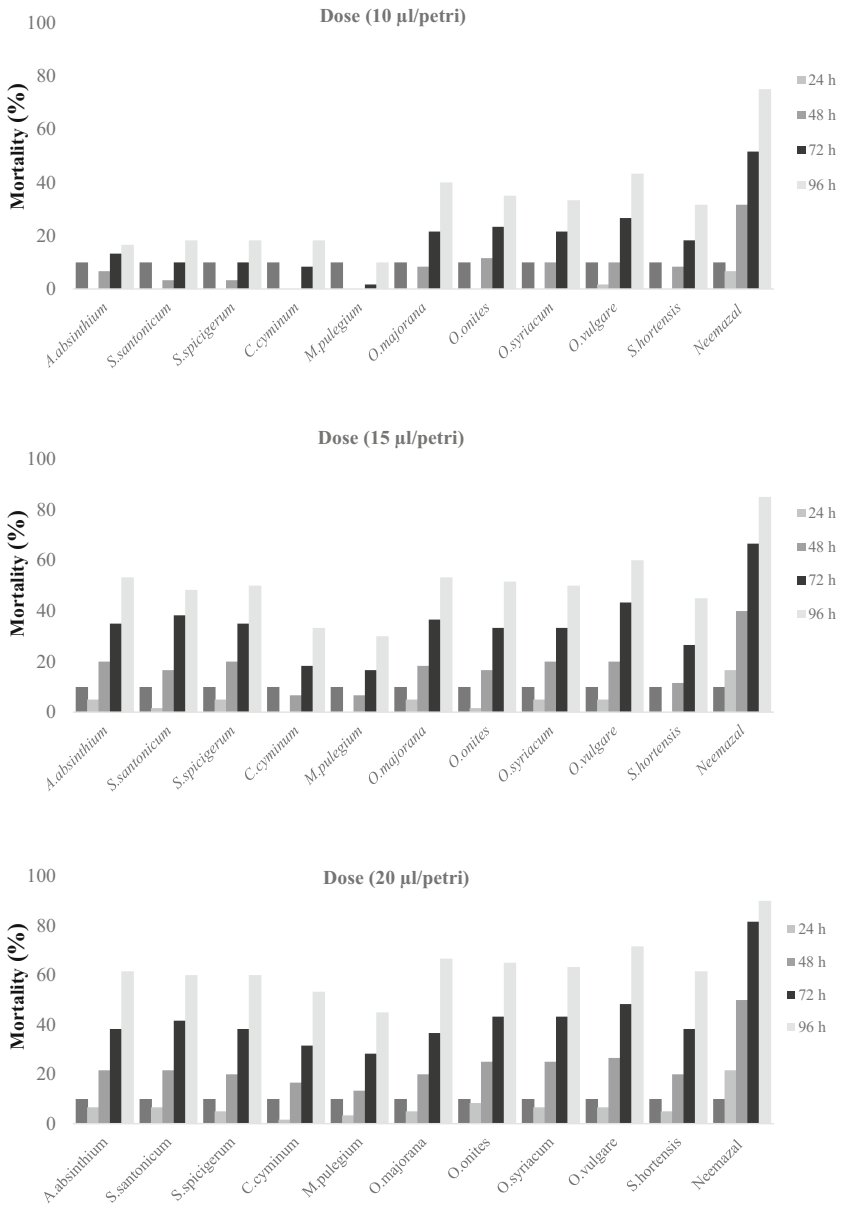

Fig. 2 Percent mortality of 5th instar larvae of *C.perspectalis* after treatment with 10, 15, 20 µL/petri doses ten plant essential oils and treatment times

a control agent against the on 2nd and 5th instar larvae of *C.perspectalis.*

Compliance with ethical standards

Conflict of interest Authors; Temel Gokturk, Nunu Chachkhiani-Anasashvili, Saban Kordali, Guguli Dumbadze and Ayse Usanmaz Bozhuyuk declares that they have no conflict of interest.

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