Journal of Applied Botany and Food Quality 86, 198 - 204 (2013), DOI:10.5073/JABFQ.2013.086.027

[1]Atatürk University, Faculty of Agriculture, Department of Plant Protection, Erzurum, Turkey
[2]Karamanoğlu Mehmetbey University, Kamil Özdağ Faculty of Science, Department of Biology, Karaman, Turkey

Insecticidal effects of monoterpenes on *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

E. Yildirim[1*], B. Emsen[2], S. Kordali[1]

(Received April 2, 2013)

Summary

Twenty eight monoterpenes including monoterpene hydrocarbons and oxygenated monoterpenes (borneol, borynl acetate, camphene, camphor, 3-carene, carvone, 1,8-cineole, citronellal, β-citronellene, β-citronellol, dihydrocarvone, fenchol, fenchone, geranyl acetate, isomenthol, limonene, limonene oxide, linalool, linalyl acetate, menthol, menthone, myrcene, nerol, neryl acetate, α-pinene, β-pinene, terpinen-4-ol, α-terpineol), the active compounds of essential oils obtained from different plant species were tested against adults of *Sitophilus zeamais* Motschulsky under laboratory conditions. The monoterpenes were applied at contents of 10, 20 and 30 µl for liquid compounds and 10, 20 and 30 µg for solid compounds. The results show that most of the monoterpenes have significantly insecticidal effect on the tested insects. Insecticidal effects of monoterpene hydrocarbons were found to be lower than those of oxygenated monoterpenes. The ketone and aldehyde and epoxide derivatives of oxygenated monoterpenes were also found to be more toxic as compared with their other derivatives. Mortality percentage of *S. zeamais* adults, after 96th h of exposure at the maximum dose (30 µl/µg) of oxygenated monoterpenes including borneol, fenchol, linalool, menthol, terpinen-4-ol, α-terpineol (alcohols group); 1,8-cineole, limonene oxide (epoxides group); camphor, carvone, citronellal, dihydrocarvone, fenchone, menthone (ketones and aldehydes group) and neryl acetate (esters group) attained 100 %. Concurrently, 3-carene from monoterpene hydrocarbons showed 100 % mortality after 96th h of exposure at the maximum dose (30 µl). Carvone, dihydrocarvone, fenchone, limonene oxide, menthone and terpinen-4-ol from these compounds showed 100 % insecticidal effect after 48th h of exposure. Among the monoterpenes tested, carvone, dihydrocarvone, menthone and terpinen-4-ol showed the strongest insecticidal activities with 100 % of mortality at all doses (96 h after treatment) and then 1,8-cineole, fenchone, linalool and limonene oxide showed stronger insecticidal activities in comparison with other monoterpenes with lethal doses (LD_{50}) values of 1.989, 2.445, 2.445 and 3.235 µl (96 h after treatment) against the test insects, respectively. Mortality rate of *S. zeamais* adults increased significantly ($p < 0.01$), as the dosage level and/or exposure time increased. Based on the present results, it can be concluded that the oxygenated monoterpenes may have a potential action for control of *S. zeamais* adults.

Introduction

The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is an insect that causes yield losses in storage products in Turkey (Yildirim, 2012).

S. zeamais is present in corn storage and can destroy the entire corn harvest. It may attack the corn and makes useless the product (Yildirim et al., 2012a). Several insecticides have been tried for years to prevent this damage. Chemical/synthetic insecticides constitute the first part of the trials. Synthetic insecticides demonstrate a high degree of impact of insecticide. At the same time, they cause many negative results such as death of non-target animals, residue problems and resistance against insecticides (Isman, 2006). These disadvantage sides of synthetic pesticides have created a viewpoint for the introduction of alternative pesticides. Hence, in recent years research showed that natural products can be preferred by farmers to protect stored grains from insect invasion (Tapondjou et al., 2005). Among the natural products plant extracts, plant secondary metabolites, plant essential oils and monoterpenes are shown as botanical pesticides. One of the most important features of botanical pesticides is to perform different effects on some insects. These pesticides can be used as insect antifeedants and repellants (Isman, 2006).

Monoterpenes, the chemical constituents of essential oils found in plants, are known biologically active compounds. These major constituents are generally isolated from essential oils in Asteraceae (Leonardi et al., 2013; Umpierrez et al., 2013), Hypericaceae (Rouis et al., 2013), Lauraceae (Chang and Chu, 2011), Myrtaceae (Shelton et al., 2004; Webb et al., 2013), Pinaceae (Sadeghi et al., 2013), Rubiaceae (Del Terra et al., 2013), Rutaceae (Shimada et al., 2004) and Solanaceae (Murungi et al., 2013) families. They constitute the main feature of many plants and keep away bacteria and fungi from plants. Also, plants become attractive for insects by these metabolites. Thereby some essential oils have bactericidal, fungicidal, antiparasitical and insecticidal properties (Bakkali et al., 2008). At the same time, essential oils and their constituents demonstrate fumigant and topical toxicity as well as antifeedant and repellent effects (Regnault-Roger, 1997; Shaaya et al., 1997). Fumigation is a very important technique in insect pest elimination in stored products. Fumigants penetrate homogenously to endpoints because of their diffusion property and they can be applied to a large amount in a short time (Zettler and Arthur, 2000). Contact and fumigant insecticidal activities of plant essential oils and monoterpenes against stored product pests have been demonstrated (Tripathi et al., 2000; Lee et al., 2003; Yildirim et al., 2005, 2011; Abdelgaleil, 2009; Wang, 2009; Kordali et al., 2012; Kumar et al., 2012). However, to the best of our knowledge, studies have not yet been conducted to evaluate the insecticidal activities of monoterpenes towards *S. zeamais*. Therefore, the present research was therefore undertaken to investigate the bioactivity of the some monoterpenes against adults of *S. zeamais*, important stored-product insects in grain storage in Turkey.

Materials and methods

Insects rearing conditions

In this study, *S. zeamais* was obtained from storage house in Tokat region, Turkey. Corn grains were purchased from local market and stored in a freezer at -20 °C to maintain freshness. After the corn grains were removed from the freezer, in order to prevent pre-infestation they were washed by tap water, dried and heated before using it in

* Corresponding author

the experiments. *S. zeamais* adults were reared on whole corn in the laboratory at 12-13 % moisture content in plastic box (diameter 25 cm, height 30 cm) at 64 ± 5 relative humidity, 25 ± 1 °C and L:D = 12 h:12 h in the Department of Plant Protection, Faculty of Agriculture, Atatürk University, Erzurum-Turkey. Adults obtained from laboratory culture were stored in separate insect cages provided with corn. Tests were also carried out under the same laboratory conditions.

Determination of adults' age

Four to six day-old *S. zeamais* adults were used as the test material. In order to get adults at the same age, few grains of corn that included larvae and pupae were placed separately in Petri dishes. After adult emergence, the same age adults were collected and used.

Individual monoterpenoids

The compounds tested were borneol (Fluka), bornyl acetate (Sigma), camphene (Fluka), camphor (Fluka), 3-carene (Aldrich), carvone (Fluka), 1,8-cineole (Sigma), citronellal (Sigma), β-citronellene (Fluka), β-citronellol (Fluka), dihydrocarvone (Alfa), fenchol (Fluka), fenchone (Fluka), geranyl acetate (Alfa), isomenthol (Alfa), limonene (Fluka), limonene oxide (Aldrich), linalool (Fluka), linalyl acetate (Fluka), menthol (Fluka), menthone (Fluka), myrcene (Aldrich), nerol (Sigma), neryl acetate (Alfa), α-pinene (Fluka), β-pinene (Fluka), terpinen-4-ol (Aldrich), α-terpineol (Merck) and DDVP (2, 2-dichlorovinyl dimethyl phosphate) (Erzurum, Turkey).

Bioassays

In order to test the toxicity of the monoterpenes against *S. zeamais* adults, 33 individuals with 33 grains of corn were placed into Petri dishes (9 cm diameter). 10, 20 and 30 µl/Petri dish contents of liquid compounds and 10, 20 and 30 µg/Petri dish contents of solid compounds were applied with an automatic pipette on a filter paper (2 x 2 cm) attached to the upside of the Petri dish. Solid compounds were dissolved in ethanol (1:1, w/v). Filter papers were impregnated with the appropriate amounts of the solutions (10, 20 and 30 µg/Petri dish), placed on the lid of Petri dishes and then kept to evaporate the ethanol. Petri dishes were closed with an adhesive tape to prevent escaping of volatile compounds and kept at (23 ± 2) °C on a laboratory bench. Mortality rate of the adults was determined after an exposure for 24^{th}, 48^{th}, 72^{th} and 96^{th} h. Grains were divided into two and in this way insects inside the grains became visible. At the end of each trial period, it was touched to the insects with forceps and observed movements of them. It was decided that completely inactive insects were dead. Petri dishes applied with sterile water and ethanol (waited for evaporating of ethanol) served as control and petri dishes applied with DDVP served as positive control. The treatments were arranged in a completely randomized design with three replications including controls. Insecticidal action of monoterpenes was expressed as % mean mortality of the adults.

Statistical analyses

Differences among the insecticidal activities of the monoterpenes tested were determined according to analysis of variance (ANOVA) test by using Statistical Package for Social Sciences (SPSS®, version 15.0). Mortality was expressed as mean (percentage) ± standard error. Differences between means were tested through Duncan test and values with $p < 0.01$ were considered significantly different. LD_{50} and LD_{90} values at 96 h were calculated with regression analysis by probit using SPSS. Probit analysis of dose-mortality data was conducted to estimate the LD_{50} and LD_{90} values and associated 95 % confidence limits for each treatment.

Results and discussion

In the present study, insecticidal effects of 28 commercial monoterpenes (borneol, borynl acetate, camphene, camphor, 3-carene, carvone, 1,8-cineole, citronellal, β-citronellene, β-citronellol, dihydrocarvone, fenchol, fenchone, geranyl acetate, isomenthol, limonene, limonene oxide, linalool, linalyl acetate, menthol, menthone, myrcene, nerol, neryl acetate, α-pinene, β-pinene, terpinen-4-ol, α-terpineol) including monoterpene hydrocarbons and oxygenated monoterpenes were tested against adults of *S. zeamais*. The present results showed that the tested compounds have insecticidal effects on adults of *S. zeamais* in comparison with control groups (Tab. 1).

Analysis of variance demonstrated that the effects of these monoterpenes on the mortality rate of *S. zeamais* were highly significant on the basis of both dosage rate and exposure time ($p < 0.01$).

Higher doses and longer exposure times scored maximum toxicities on *S. zeamais* (Fig. 1 and 2). Generally, oxygenated monoterpenes had higher insecticidal effect on *S. zeamais* as compared with monoterpene hydrocarbons. Some oxygenated monoterpenes were strong toxic compounds and among them, borneol, fenchol, linalool, menthol, terpinen-4-ol (alcohols group); 1,8-cineole, limonene oxide (epoxides group); camphor, carvone, citronellal, dihydrocarvone, fenchone, menthone (ketones and aldehydes group) and neryl acetate (esters group) attained 100 % insecticidal effect. The emergence of early effects of insecticides is very important. Carvone, dihydrocarvone, fenchone and menthone from ketones and aldehydes group of oxygenated monoterpenes attained 100 % of mortality at 30 µl dose after 24 h. In the monoterpene hydrocarbons, compounds that brought out the highest mortality results were 3-carene and β-citronellene. After 96^{th} h of treatments, at 30 µl dose, 3-carene and β-citronellene achieved 100 and 90.91 % mortality, respectively. However, it was determined that there was no statistically ($p < 0.01$) significant difference between the 24 h results of control groups and the highest doses of five monoterpenes (bornyl acetate, myrcene, nerol, α-pinene, β-pinene) (Tab. 1).

As shown in Fig. 1 and 2, there are positive correlations between the treatment dose and insecticidal effect; elapsed time and insecticidal effect.

The highest levels of mortality percentages were achieved after 96^{th} h of treatments at all of 10, 20 and 30 µl doses of the essential oils of carvone, dihydrocarvone, menthone (oxygenated monoterpenes-ketones and aldehydes) and terpinen-4-ol (oxygenated monoterpenes-alcohols) (100 %), at 20 and 30 µl/µg doses of fenchol, linalool, menthol (oxygenated monoterpenes-alcohols), 1,8-cineole, limonene oxide (oxygenated monoterpenes-epoxides) and camphor, fenchone (oxygenated monoterpenes-ketones and aldehydes) (100 %), at 30 µl/µg doses of 3-carene (monoterpene hydrocarbon), borneol, α-terpineol (oxygenated monoterpenes-alcohols), citronellal (oxygenated monoterpenes-ketones and aldehydes) and neryl acetate (oxygenated monoterpenes-esters) (100 %) (Tab. 1, Fig. 1 and 2).

The present results showed that borneol, borynl acetate, camphene, camphor, 3-carene, carvone, 1,8-cineole, citronellal, β-citronellene, β-citronellol, dihydrocarvone, fenchol, fenchone, geranyl acetate, isomenthol, limonene, limonene oxide, linalool, linalyl acetate, menthol, menthone, myrcene, nerol, neryl acetate, α-pinene, β-pinene, terpinen-4-ol and α-terpineol had varying degrees of insecticidal activity against adults of *S. zeamais* as the insecticidal activity increased with increasing dose and exposure times (Fig. 1 and 2).

Tab. 1: Percent mortality effects of twenty eight monoterpenes to adults of *Sitophilus zeamais*

Treatments	Dose	Mean mortality (%)[a]			
		24[b]	48[b]	72[b]	96[b]
Control 1 (Ethanol)	10 µl	0.00 ± 0.00 a	0.00 ± 0.00 a	2.02 ± 0.33 a	5.05 ± 0.33 a
Control 2 (Sterile water)	10 µl	0.00 ± 0.00 a	0.00 ± 0.00 a	2.02 ± 0.67 a	5.05 ± 0.67 a
DDVP (Positive control)	10 µl	100.00 ± 0.00 w	100.00 ± 0.00 γ	100.00 ± 0.00 z	100.00 ± 0.00 y
Monoterpene hydrocarbons					
Camphene	10 µg	0.00 ± 0.00 a	0.00 ± 0.00 a	4.04 ± 0.33 ab	6.06 ± 0.58 ab
	20 µg	5.05 ± 0.67 abcd	7.07 ± 0.33 abcdef	13.13 ± 0.33 abcdefg	30.30 ± 1.15 defgh
	30 µg	11.11 ± 1.20 cdefghi	17.17 ± 0.33 fghij	28.28 ± 0.88 hijk	51.52 ± 3.61 jklmn
3-Carene	10 µl	12.12 ± 0.58 defghij	22.22 ± 0.67 hijkl	47.47 ± 2.03 mno	86.87 ± 1.86 tuvwxy
	20 µl	57.58 ± 0.58 r	62.63 ± 0.67 rst	84.85 ± 1.15 tuvwx	97.98 ± 0.67 y
	30 µl	74.75 ± 1.20 s	78.79 ± 1.53 vwxy	96.97 ± 0.58 yz	100.00 ± 0.00 y
β-Citronellene	10 µl	0.00 ± 0.00 a	5.05 ± 0.67 abcde	29.29 ± 0.33 ijkl	54.55 ± 1.53 klmn
	20 µl	3.03 ± 0.58 ab	20.20 ± 0.33 ghijk	48.48 ± 0.58 mnop	75.76 ± 1.73 pqrstu
	30 µl	16.16 ± 1.2 hijk	32.32 ± 0.33 lmno	65.66 ± 1.20 qrs	90.91 ± 1.15 vwxy
Limonene	10 µl	0.00 ± 0.00 a	4.04 ± 0.33 abcde	6.06 ± 0.00 abc	34.34 ± 0.88 ghi
	20 µl	10.1 ± 0.33 bcdefgh	15.15 ± 1.53 efghij	15.15 ± 1.53 bcdefg	58.59 ± 1.33 lmno
	30 µl	27.27 ± 0.58 mno	34.34 ± 1.86 mno	27.27 ± 1.53 hijk	78.79 ± 1.00 qrstuv
Myrcene	10 µl	0.00 ± 0.00 a	0.00 ± 0.00 a	4.04 ± 0.33 ab	10.10 ± 0.33 abc
	20 µl	1.01 ± 0.33 a	1.01 ± 0.33 ab	7.07 ± 0.33 abcd	22.22 ± 0.67 cdefg
	30 µl	3.79 ± 0.73 abc	9.09 ± 1.00 abcdefg	22.22 ± 0.67 ghij	33.33 ± 0.58 fgh
α-Pinene	10 µl	0.00 ± 0.00 a	0.00 ± 0.00 a	4.04 ± 0.33 ab	16.16 ± 0.88 abc
	20 µl	0.00 ± 0.00 a	0.00 ± 0.00 a	7.07 ± 0.33 abcd	31.31 ± 0.88 efgh
	30 µl	2.02 ± 0.33 a	6.06 ± 0.58 abcdef	17.17 ± 0.33 cdefgh	49.49 ± 0.88 jklm
β-Pinene	10 µl	0.00 ± 0.00 a	0.00 ± 0.00 a	3.03 ± 0.00 a	17.17 ± 0.88 abcd
	20 µl	0.00 ± 0.00 a	2.02 ± 0.33 abc	8.08 ± 0.67 abcd	41.41 ± 0.88 hijk
	30 µl	3.03 ± 0.00 ab	7.07 ± 0.33 abcdef	18.18 ± 1.15 defghi	53.54 ± 0.88 jklmn
Alcohols (Oxygenated monoterpenes)					
Borneol	10 µg	3.03 ± 0.58 ab	10.10 ± 0.67 abcdefg	30.3 ± 0.58 jkl	58.59 ± 1.76 lmno
	20 µg	18.18 ± 1.00 ijkl	25.25 ± 0.88 jklm	52.53 ± 0.88 nop	89.90 ± 0.33 uvwxy
	30 µg	33.33 ± 1.53 o	41.41 ± 0.88 op	73.74 ± 1.20 st	100.00 ± 0.00 y
β-Citronellol	10 µl	0.00 ± 0.00 a	0.00 ± 0.00 a	5.05 ± 0.33 ab	35.35 ± 1.67 ghi
	20 µl	3.03 ± 0.00 ab	5.05 ± 0.33 abcde	9.09 ± 0.00 abcde	50.51 ± 1.33 jklm
	30 µl	10.1 ± 0.33 bcdefgh	13.13 ± 0.88 cdefghi	18.18 ± 0.58 defghi	63.64 ± 1.53 mnop
Fenchol	10 µg	2.02 ± 0.33 a	20.20 ± 0.67 ghijk	51.52 ± 0.58 nop	80.81 ± 1.33 rstuvw
	20 µg	16.16 ± 0.33 hijk	56.57 ± 0.88 qr	89.90 ± 0.33 vwxyz	100.00 ± 0.00 y
	30 µg	30.3 ± 0.58 no	71.72 ± 0.88 tuv	100.00 ± 0.00 z	100.00 ± 0.00 y
Isomenthol	10 µg	0.00 ± 0.00 a	14.14 ± 0.67 defghi	44.44 ± 0.33 mn	65.66 ± 1.20 nopq
	20 µg	15.15 ± 0.58 ghijk	25.25 ± 0.88 jklm	59.60 ± 0.88 pqr	82.83 ± 1.76 rstuvwx
	30 µg	29.29 ± 0.33 no	38.38 ± 1.20 nop	75.76 ± 1.73 stu	94.95 ± 1.20 wxy
Linalool	10 µl	2.02 ± 0.67 a	55.56 ± 0.88 qr	76.77 ± 0.33 stu	95.96 ± 0.88 xy
	20 µl	6.06 ± 0.00 abcde	71.72 ± 0.67 tuv	91.92 ± 0.33 vwxyz	100.00 ± 0.00 y
	30 µl	15.15 ± 0.58 ghijk	88.89 ± 0.88 yzαβγ	100.00 ± 0.00 z	100.00 ± 0.00 y
Menthol	10 µg	1.01 ± 0.33 a	9.09 ± 0.00 abcdefg	56.57 ± 0.67 opq	79.80 ± 0.88 qrstuv
	20 µg	10.10 ± 0.67 bcdefgh	59.60 ± 0.33 rs	88.89 ± 0.33 vwxyz	100.00 ± 0.00 y
	30 µg	18.18 ± 0.58 ijkl	72.73 ± 0.58 tuvw	100.00 ± 0.00 z	100.00 ± 0.00 y
Nerol	10 µl	0.00 ± 0.00 a	0.00 ± 0.00 a	3.03 ± 0.58 a	20.20 ± 0.88 cdef
	20 µl	2.02 ± 0.33 a	3.03 ± 0.00 abcd	8.08 ± 0.33 abcd	39.39 ± 0.58 hij
	30 µl	8.08 ± 0.33 abcdefg	12.12 ± 0.58 bcdefgh	20.20 ± 0.33 efghij	51.52 ± 0.58 jklmn

Tab. 1 (continued)

Treatments	Dose	Mean mortality (%)[a]			
		24[b]	48[b]	72[b]	96[b]
Terpinen-4-ol	10 µl	43.43 ± 0.33 pq	83.84 ± 0.88 xyzα	96.97 ± 0.58 yz	100.00 ± 0.00 y
	20 µl	83.84 ± 0.88 t	97.98 ± 0.33 βγ	100.00 ± 0.00 z	100.00 ± 0.00 y
	30 µl	96.97 ± 0.58 vw	100.00 ± 0.00 γ	100.00 ± 0.00 z	100.00 ± 0.00 y
α-Terpineol	10 µg	19.19 ± 0.33 jkl	25.25 ± 0.33 jklm	45.45 ± 0.58 mno	69.7 ± 1.53 opqr
	20 µg	40.40 ± 0.88 p	46.46 ± 1.33 pq	76.77 ± 1.76 stu	95.96 ± 0.88 xy
	30 µg	54.55 ± 1.15 r	60.61 ± 1.00 rs	90.91 ± 1.15 vwxyz	100.00 ± 0.00 y
Epoxides (Oxygenated monoterpenes)					
1,8-Cineole	10 µl	13.13 ± 0.33 efghij	75.76 ± 2.52 uvwx	89.90 ± 0.88 vwxyz	98.99 ± 0.33 y
	20 µl	24.24 ± 1.15 lmn	89.90 ± 1.45 zαβγ	98.99 ± 0.33 z	100.00 ± 0.00 y
	30 µl	40.40 ± 1.20 p	98.99 ± 0.33 γ	100.00 ± 0.00 z	100.00 ± 0.00 y
Limonene oxide	10 µl	84.85 ± 1.00 t	87.88 ± 1.00 yzαβ	91.92 ± 1.20 vwxyz	96.97 ± 1.00 xy
	20 µl	89.90 ± 0.33 tuv	100.00 ± 0.00 γ	100.00 ± 0.00 z	100.00 ± 0.00 y
	30 µl	98.99 ± 0.33 w	100.00 ± 0.00 γ	100.00 ± 0.00 z	100.00 ± 0.00 y
Ketones and aldehydes (Oxygenated monoterpenes)					
Camphor	10 µg	13.13 ± 0.33 efghij	37.37 ± 1.45 nop	75.76 ± 1.73 stu	91.92 ± 1.45 vwxy
	20 µg	32.32 ± 0.33 o	67.68 ± 1.45 stu	94.95 ± 0.33 wxyz	100.00 ± 0.00 y
	30 µg	47.47 ± 0.33 q	80.81 ± 1.20 vwxyz	100.00 ± 0.00 z	100.00 ± 0.00 y
Carvone	10 µl	89.9 ± 0.33 tuv	98.99 ± 0.33 γ	100.00 ± 0.00 z	100.00 ± 0.00 y
	20 µl	93.94 ± 0.58 uvw	100.00 ± 0.00 γ	100.00 ± 0.00 z	100.00 ± 0.00 y
	30 µl	100.00 ± 0.00 w	100.00 ± 0.00 γ	100.00 ± 0.00 z	100.00 ± 0.00 y
Citronellal	10 µl	0.00 ± 0.00 a	13.13 ± 0.33 cdefghi	53.54 ± 1.86 nop	70.71 ± 1.67 opqrs
	20 µl	10.1 ± 0.88 bcdefgh	28.28 ± 0.67 klmn	83.84 ± 0.88 tuvw	98.99 ± 0.33 y
	30 µl	19.19 ± 0.88 jkl	41.41 ± 0.88 op	95.96 ± 0.88 xyz	100.00 ± 0.00 y
Dihydrocarvone	10 µl	92.93 ± 0.67 uvw	98.99 ± 0.33 γ	100.00 ± 0.00 z	100.00 ± 0.00 y
	20 µl	95.96 ± 0.88 vw	100.00 ± 0.00 γ	100.00 ± 0.00 z	100.00 ± 0.00 y
	30 µl	100.00 ± 0.00 w	100.00 ± 0.00 γ	100.00 ± 0.00 z	100.00 ± 0.00 y
Fenchone	10 µl	72.73 ± 1.15 s	82.83 ± 0.33 wxyzα	88.89 ± 0.67 vwxyz	95.96 ± 1.33 xy
	20 µl	96.97 ± 0.58 vw	100.00 ± 0.00 γ	100.00 ± 0.00 z	100.00 ± 0.00 y
	30 µl	100.00 ± 0.00 w	100.00 ± 0.00 γ	100.00 ± 0.00 z	100.00 ± 0.00 y
Menthone	10 µl	87.88 ± 0.58 tu	91.92 ± 1.2 αβγ	98.99 ± 0.33 z	100.00 ± 0.00 y
	20 µl	97.98 ± 0.33 w	100.00 ± 0.00 γ	100.00 ± 0.00 z	100.00 ± 0.00 y
	30 µl	100.00 ± 0.00 w	100.00 ± 0.00 γ	100.00 ± 0.00 z	100.00 ± 0.00 y
Esters (Oxygenated monoterpenes)					
Bornyl acetate	10 µl	0.00 ± 0.00 a	3.03 ± 0.58 abcd	37.37 ± 1.45 klm	54.55 ± 2.08 klmn
	20 µl	1.01 ± 0.33 a	6.06 ± 0.58 abcdef	67.68 ± 3.38 rs	84.85 ± 2.89 stuvwxy
	30 µl	7.07 ± 0.33 abcdef	15.15 ± 0.58 efghij	86.87 ± 2.03 uvwxy	94.95 ± 1.67 wxy
Geranyl acetate	10 µl	2.02 ± 0.33 a	3.03 ± 0.00 abcd	12.12 ± 2.00 abcdefg	43.43 ± 2.33 hijk
	20 µl	7.07 ± 0.88 abcdef	8.08 ± 0.67 abcdef	39.39 ± 0.58 lm	70.71 ± 2.91 opqrs
	30 µl	16.16 ± 0.88 hijk	23.23 ± 0.88 ijkl	53.54 ± 0.88 nop	88.89 ± 1.76 uvwxy
Linalyl acetate	10 µl	0.00 ± 0.00 a	0.00 ± 0.00 a	9.09 ± 1.00 abcde	19.19 ± 0.67 bcde
	20 µl	5.05 ± 0.33 abcd	7.07 ± 0.33 abcdef	10.1 ± 0.67 abcdef	32.32 ± 1.76 efgh
	30 µl	13.13 ± 0.67 efghij	20.2 ± 0.33 ghijk	21.21 ± 0.58 fghij	47.47 ± 2.33 ijkl
Neryl acetate	10 µl	0.00 ± 0.00 a	14.14 ± 0.33 defghi	55.56 ± 1.20 nopq	72.73 ± 0.58 opqrst
	20 µl	14.14 ± 0.33 fghij	41.41 ± 2.73 op	81.82 ± 1.53 tuv	96.97 ± 0.58 xy
	30 µl	22.22 ± 0.67 klm	55.56 ± 2.85 qr	92.93 ± 1.20 vwxyz	100.00 ± 0.00 y

[a] Mean ± SE of three replicates, each set-up with 33 adults

[b] Exposure time (h)

Values followed by different letters in the same column differ significantly at $p < 0.01$

Tab. 2: The 96 h LD_{50} and LD_{90} values of twenty eight monoterpenes to adults of *Sitophilus zeamais*

Treatments	LD_{50} (Limits)	LD_{90} (Limits)	Slope ± SE
Borneol	8.992 (7.401-10.241)	18.143 (16.120-21.441)	4.204 ± 0.587
Bornyl acetate	9.161 (7.098-10.775)	23.493 (20.212-29.522)	3.133 ± 0.461
Camphene	28.865 (25.517-34.504)	69.510 (52.436-113.778)	3.358 ± 0.489
Camphor	4.867 (0.035-7.145)	9.365 (3.753-11.235)	4.508 ± 1.985
3-Carene	4.815 (1.665-6.821)	11.159 (8.737-13.468)	3.511 ± 0.950
Carvone	[a]	[a]	0.000 ± 0.000
1,8-Cineole	1.989 ([b])	4.808 ([b])	3.343 ± 4.259
Citronellal	8.086 (6.374-9.103)	13.240 (11.953-15.908)	5.984 ± 1.255
β-Citronellene	9.144 (6.397-11.192)	31.359 (25.303-46.204)	2.394 ± 0.419
β-Citronellol	21.939 (19.778-24.668)	51.737 (41.793-72.794)	3.440 ± 0.439
Dihydrocarvone	[a]	[a]	0.000 ± 0.000
Fenchol	7.395 (3.015-8.649)	11.481 (10.427-16.326)	6.708 ± 2.494
Fenchone	2.445 ([b])	6.815 ([b])	2.879 ± 1.536
Geranyl acetate	11.831 (9.669-13.617)	33.658 (27.754-46.197)	2.822 ± 0.411
Isomenthol	6.903 (3.994-9.039)	24.293 (20.022-34.169)	2.345 ± 0.455
Limonene	15.062 (12.671-17.293)	49.265 (37.635-79.876)	2.490 ± 0.391
Limonene oxide	3.235 ([b])	6.957 ([b])	3.854 ± 2.795
Linalool	2.445 ([b])	6.815 ([b])	2.879 ± 1.536
Linalyl acetate	35.024 (26.940-64.394)	206.010 (94.329-1766.180)	1.665 ± 0.402
Menthol	7.521 (3.403-8.722)	11.599 (10.546-16.492)	6.812 ± 2.482
Menthone	[a]	[a]	0.000 ± 0.000
Myrcene	53.801 (37.031-151.354)	286.547 (115.998-4201.509)	1.764 ± 0.451
Nerol	28.207 (23.108-40.568)	136.787 (75.125-569.351)	1.869 ± 0.397
Neryl acetate	7.364 (5.416-8.670)	14.099 (12.524-16.709)	4.543 ± 0.838
α-Pinene	32.400 (26.251-48.564)	139.764 (78.071-531.672)	2.019 ± 0.413
β-Pinene	26.365 (22.376-34.051)	99.263 (63.331-254.050)	2.220 ± 0.406
Terpinen-4-ol	[a]	[a]	0.000 ± 0.000
α-Terpineol	7.680 (5.858-8.958)	14.869 (13.220-17.589)	4.467 ± 0.764

[a] For this monoterpene no LD values are computed because the ratios of response counts to subject counts are the same, i.e. the slope is zero
[b] Slope is not significantly different from zero. LD fiducial limits cannot be computed

The most effective ones among twenty eight monoterpenes were carvone, dihydrocarvone, menthone and terpinen-4-ol with 100 % of mortality at all doses (96 h after treatment). Other compounds that had strong fumigant insecticidal efficacy were 1,8-cineole, fenchone, linalool and limonene oxide according to LD values. LD_{50} and LD_{90} values at 96th h belong to 1,8-cineole were 1.989 and 4.808 µl; values belong to fenchone were 2.445 and 6.815 µl; values belong to linalool were 2.445 and 6.815 µl; values belong to limonene oxide were 3.235 and 6.957 µl, respectively (Tab. 2).

Some of the previous studies demonstrated that in general, the toxicity of essential oils isolated from plant samples against stored pests was mainly related to their major components. These compounds are generally described as monoterpenes (Banchio et al., 2005; Kordali et al., 2006; López et al., 2011; Kumar et al., 2012) and secondary metabolites (Emsen et al., 2012a, 2012b; Yildirim et al., 2012a, 2012b).

The results in this study suggested that the monoterpenes obtained from different essential oils might have different toxicity ratios and these differences originate from chemical constituents which is unique to them.

Increasing use of natural insecticides will help to decrease the negative effects like toxicity to non-target animals, residue problems (environmental pollution) and insecticide resistance of synthetic or chemical insecticides. In this context, bio-insecticides may be also effective, bio-degradable, selective and associated with little advancement of resistance in the insect population and as a result, more safe to the environment. In this study, 96th h of exposure to the maximum dose (30 µl/µg) of borneol, camphor, 3-carene, carvone, 1,8-cineole, citronellal, dihydrocarvone, fenchol, fenchone, limonene oxide, linalool, menthol, menthone, neryl acetate, terpinen-4-ol, *α*-terpineol was determined to cause the highest mortality rate in *S. zeamais* adults. These compounds may be suggested to be potential insecticidal agents for controlling the adults of *S. zeamais* in stored food products.

Obtained results and those reported earlier clearly indicated the variations in the effects of monoterpenes in regard to the stage, the

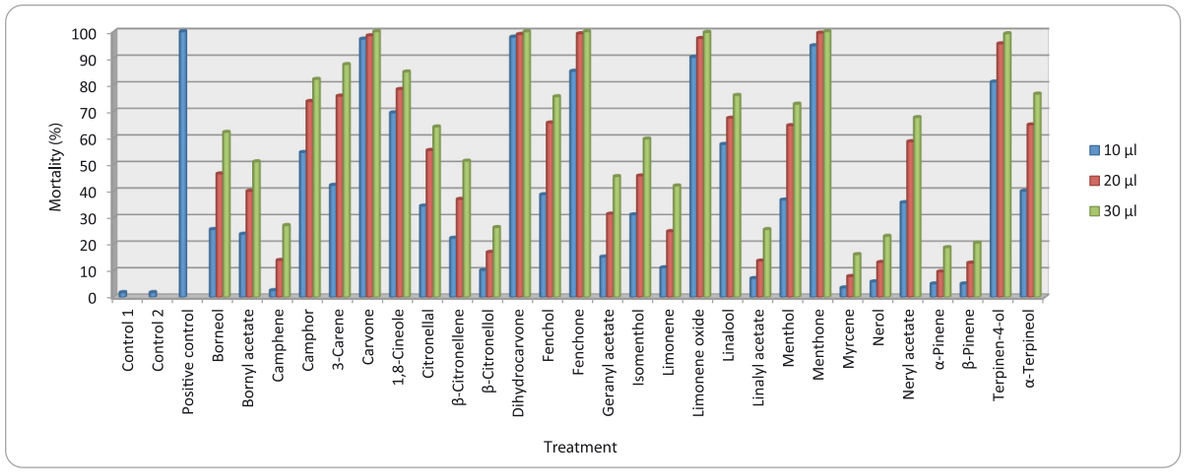

Fig. 1: Total mortality rates in adults of *Sitophilus zeamais* exposed to twenty eight monoterpenes at different doses

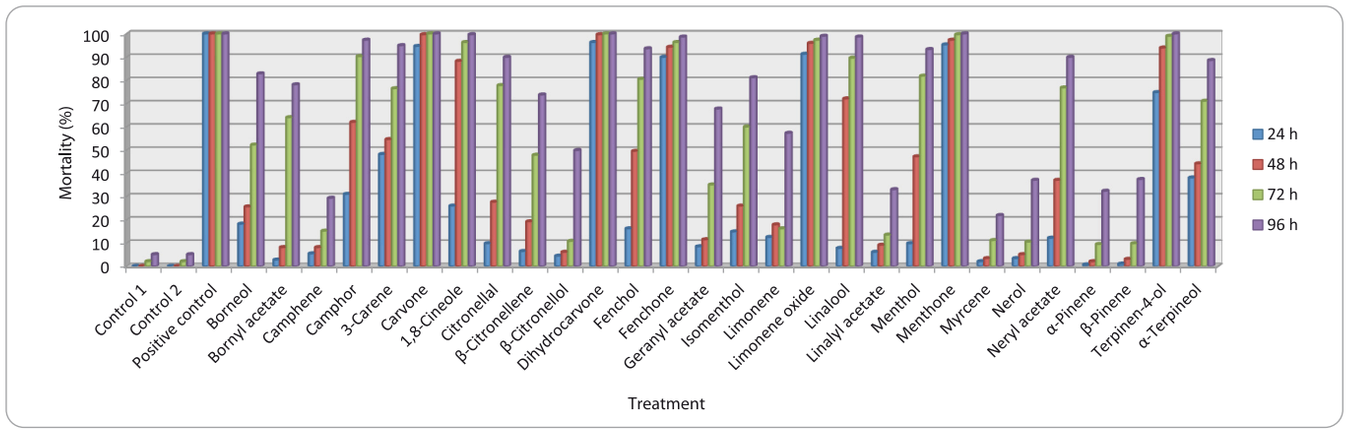

Fig. 2: Total mortality of adults of *Sitophilus zeamais* according to treatment times of twenty eight monoterpenes

species of insect. Otherwise, DDVP which is an effective chemical pesticide was used in this study and 10 µl DDVP with a standard of pesticide applications showed 100 % insecticidal activity on *S. zeamais* after 24 h. However, in recent years negative effects of chemical-containing pesticides as DDVP were investigated. Excessive usage of such pesticides causes environmental pollution (TORTELLI et al., 2006; KOPECKA-PILARCZYK, 2012). Moreover, it was reported that using of DDVP increased the human cancer risk (MAELE-FABRY and WILLEMS, 2004; KOUTROS et al., 2008). Therefore, tested essential oils proved to be promising as control alternatives against stored product insects especially, *S. zeamais*. Additionally, there is no statistically ($p < 0.01$) significant difference between the 24 h results of DDVP and six monoterpenes (carvone, dihydrocarvone, fenchone, limonene oxide, menthone and terpinen-4-ol) (Tab. 1). When considered from this point of view, it can be considered using botanical insecticides instead of chemical insecticides which have adverse effects on animals and/or humans. Furthermore, one of the reasons to prefer botanical insecticides is cheaper compared to chemical insecticides.

The present study demonstrates the possibility of using the test monoterpenes particularly carvone, dihydrocarvone, menthone and terpinen-4-ol, as insecticides against *S. zeamais*. The effect of these monoterpenes at low concentrations might reduce the chemical residues and environmental pollution. The diverse activities of the test monoterpenes warrant further research into their potential development as compounds for the control of maize weevils.

References

ABDELGALEIL, S.A.M., MOHAMED, M.I.E., BADAWY, M.E.I., EL-ARAMI, S.A.A., 2009: Fumigant and contact toxicities of monoterpenes to *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) and their inhibitory effects on acetylcholinesterase activity. J. Chem. Ecol. 35, 518-525.

BAKKALI, F., AVERBECK, S., AVERBECK, D., IDAOMAR, M., 2008: Biological effects of essential oils e a review. Food Chem. Toxicol. 46, 446-475.

BANCHIO, E., ZYGADLO, J., VALLADARES, G.R., 2005: Quantitative variations in the essential oil of *Minthostachys mollis* (Kunth.) Griseb. in response to insects with different feeding habits. J. Agr. Food Chem. 53, 6903-6906.

CHANG, Y.T., CHU, F.H., 2011: Molecular cloning and characterization of monoterpene synthases from *Litsea cubeba* (Lour.) Persoon. Tree Genet. Genomes. 7, 835-844.

DEL TERRA, L., LONZARICH, V., ASQUINI, E., NAVARINI, L., GRAZIOSI, G., LIVERANI, F.S., PALLAVICINI, A., 2013: Functional characterization of three *Coffea arabica* L. monoterpene synthases: Insights into the enzymatic machinery of coffee aroma. Phytochemistry 89, 6-14.

EMSEN, B., BULAK, Y., YILDIRIM, E., ASLAN, A., ERCISLI, S., 2012a: Activities of two major lichen compounds, diffractaic acid and usnic acid against *Leptinotarsa decemlineata* Say, 1824 (Coleoptera: Chrysomelidae).

Egypt. J. Biol. Pest Co. 22, 5-10.

Emsen, B., Yildirim, E., Aslan, A., Anar, M., Ercisli, S., 2012b: Insecticidal effect of the extracts of *Cladonia foliacea* (Huds.) Willd. and *Flavoparmelia caperata* (L.) Hale against adults of the grain weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Egypt. J. Biol. Pest Co. 22, 145-149.

Isman, M.B., 2006: Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol. 51, 45-66.

Kopecka-Pilarczyk, J., 2010: The effect of pesticides and metals on acetylcholinesterase (AChE) in various tissues of blue mussel (*Mytilus trossulus* L.) in short-term in vivo exposures at different temperatures. J. Environ. Sci. Heal. B 45, 336-346.

Kordali, S., Cakir, A., Sutay, S., 2006: Inhibitory effects of monoterpenes on seed germination and seedling growth. Z. Naturforsch. C 62, 207-214.

Kordali, S., Yildirim, E., Yazici, G., Emsen, B., Kabaagac, G., Ercisli, S., 2012: Fumigant toxicity of essential oils of nine plant species from Asteraceae and Clusiaceae against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Egypt. J. Biol. Pest Co. 22, 11-14.

Koutros, S., Mahajan, R., Zheng, T., Hoppin, J.A., Ma, X., Lynch, C.F., Blair, A., Alavanja, M.C.R., 2008: Dichlorvos exposure and human cancer risk: results from the Agricultural Health Study. Cancer Cause. Control 19, 59-65.

Kumar, P., Mishra, S., Malik, A., Satya, S., 2012: Insecticidal evaluation of essential oils of *Citrus sinensis* L. (Myrtales: Myrtaceae) against housefly, *Musca domestica* L. (Diptera: Muscidae). Parasitol. Res. 110, 1929-1936.

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

Leonardi, M., Ambryszewska, K.E., Melai, B., Flamini, G., Cioni, P.L., Parri, F., Pistelli, L., 2013: Essential-Oil Composition of *Helichrysum italicum* (Roth) G.DON ssp. *italicum* from Elba Island (Tuscany, Italy). Chem. Biodivers. 10, 343-355.

López, S.B., López, M.L., Aragon, L.M., Tereschuk, M.L., Slanis, A.C., Feresin, G.E., Zygadlo, J.A., Tapia, A.A., 2011: Composition and anti-insect activity of essential oils from *Tagetes* L. species (Asteraceae, Helenieae) on *Ceratitis capitata* Wiedemann and *Triatoma infestans* Klug. J. Agr. Food Chem. 59, 5286-5292.

Maele-Fabry, G.V., Willems, J.L., 2004: Prostate cancer among pesticide applicators: a meta-analysis. Int. Arch. Occ. Env. Hea. 77, 559-570.

Murungi, L.K., Kirwa, H., Torto, B., 2013: Differences in essential oil content of berries and leaves of *Solanum sarrachoides* (Solanaceae) and the effects on oviposition of the tomato spider mite (*Tetranychus evansi*). Ind. Crop. Prod. 46, 73-79.

Regnault-Roger, C., 1997: The potential of botanical essential oils for insect pest control. Integ. Pest. Man. Rev. 2, 25-34.

Rouis, Z., Laamari, A., Abid, N., Elaissi, A., Cioni, P.L., Flamini, G., Aouni, M., 2013: Chemical composition and larvicidal activity of several essential oils from *Hypericum* species from Tunisia. Parasitol. Res. 112, 699-705.

Sadeghi, H., Tahery, Y., Moradi, S., 2013: Intra- and inter-specific variation of turpentine composition in Eldar pine (*Pinus eldarica* Medw.) and black pine (*Pinus nigra* Arnold). Biochem. Syst. Ecol. 48, 189-193.

Shaaya, E., Kostjukovski, M., Eilberg, J., Sukprakarn, C., 1997: Plant oils as fumigant and contact insecticides for the control of stored product insects. J. Stored Prod. Res. 33, 7-15.

Shelton, D., Zabaras, D., Chohan, S., Wyllie, S.G., Baverstock, P., Leach, D., Henry, R., 2004: Isolation and partial characterisation of a putative monoterpene synthase from *Melaleuca alternifolia*. Plant Physiol. Bioch. 42, 875-882.

Shimada, T., Endo, T., Fujii, H., Hara, M., Ueda, T., Kita, M., Omura, M., 2004: Molecular cloning and functional characterization of four monoterpene synthase genes from *Citrus unshiu* Marc.. Plant Sci. 166, 49-58.

Tapondjou, A.L., Adler, C., Fontem, D.A., Bouda, H., Reichmuth, C., 2005: Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* du Val. J. Stored Prod. Res. 41, 91-102.

Tortelli, V., Colares, E.P., Robaldo, R.B., Nery, L.E.M., Pinho, G.L.L., Bianchini, A., Monserrat, J.M., 2006: Importance of cholinesterase kinetic parameters in environmental monitoring using estuarine fish. Chemosphere 65, 560-566.

Tripathi, A.K., Prajapati, V., Aggarwal, K.K., Khanuja, S.P.S., Kumar, S., 2000: Repellency and toxicity of oil from *Artemisia annua* to certain stored product beetles. J. Econ. Entomol. 93, 43-47.

Umpierrez, M.L., Lagreca, M.E., Cabrera, R., Grille, G., Rossini, C., 2013: Essential oils from Asteraceae as potential biocontrol tools for tomato pests and diseases. Phytochem. Rev. 11, 339-350.

Wang, J.L., Li, Y., Lei, C.L., 2009: Evaluation of monoterpenes for the control of *Tribolium castaneum* (Herbst) and *Sitophilus zeamaise* Motschulsky. Nat. Prod. Res. 23, 1080-1088.

Webb, H., Lanfear, R., Hamill, J., Foley, W.J., Kulheim, C., 2013: The yield of essential oils in *Melaleuca alternifolia* (Myrtaceae) is regulated through transcript abundance of genes in the MEP pathway. Plos One 8, e60631.

Yildirim, E., Kesdek, M., Aslan, I., Calmasur, O., Sahin, F., 2005: The effects of essential oils from eight plant species on two pests of stored product insects. Fresen. Environ. Bull. 14, 23-27.

Yildirim, E., Kordali, S., Yazici, G., 2011: Insecticidal effects of essential oils of eleven plant species from Lamiaceae on *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Rom. Biotech. Lett. 16, 6702-6709.

Yildirim, E., 2012: Pests of stored products and their control methods. (3th ed.). Erzurum: Atatürk University, Agriculture Faculty Press, No: 191.

Yildirim, E., Emsen, B., Aslan, A., Bulak, Y., Ercisli, S., 2012a: Insecticidal activity of lichens against the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Egypt. J. Biol. Pest Co. 22, 151-156.

Yildirim, E., Aslan, A., Emsen, B., Cakir, A., Ercisli, S., 2012b: Insecticidal effects of *Usnea longissima* (Parmeliaceae) Extract against *Sitophilus granarius* (Coleoptera: Curculionidae). Int. J. Agric. Biol. 14, 303-306.

Zettler, J.L., Arthur, F.H., 2000: Chemical control of stored product insects with fumigants and residual treatments. Crop Prot. 19, 577-582.

Address of the authors:
Dr. Erol Yildirim (corresponding author) and Dr. Saban Kordali, Atatürk University, Faculty of Agriculture, Department of Plant Protection, 25240, Erzurum, Turkey
Bugrahan Emsen, Karamanoğlu Mehmetbey University, Kamil Özdağ Faculty of Science, Department of Biology, 70200, Karaman, Turkey