Bulletin of Entomological Research (2017) **107**, 543–549
doi:10.1017/S0007485317000256

Insecticidal effects of extracts of *Humulus lupulus* (hops) L. cones and its principal component, xanthohumol

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Abstract

Insecticidal effects of the dichloromethane, ethyl acetate, acetone, ethanol and methanol extracts of *Humulus lupulus* (hops) L. cones and its principal components, xanthohumol was investigated on five stored pests, *Sitophilus granarius* (L.), *Sitophilus oryzae* (L.), *Acanthoscelides obtectus* (Say.), *Tribolium castaneum* (Herbst) and *Lasioderma serricorne* (F.). The mortality of adults of the insects treated with 2, 5, 5, 10 and 20 mg ml^{-1} concentrations of the extracts and xanthuhumol was counted after 24, 48, 72, 96 and 120 h. In order to determine the toxic effects of the substances tested against all tested insects, durations for 50% mortality of the adults, and LD_{50} values were also determined in the first 48 h by probit analysis. Our results also showed that xanthohumol was more toxic against the pests in comparison with the extracts applications. LD_{50} values for xanthohumol were found to be low dose as compared with the extracts. Xanthohumol was more toxic against *S. granarius* (L.) with 6.8 µg of LD_{50} value. Among the extracts, methanol extract was less effective than other extracts against all tested insects. The ethyl acetate extract of *H. lupulus* cones was the most effective extract against the tested pests. The quantitative amounts of xanthohumol in the extracts were determined using a high-performance liquid chromatography. The quantitative data indicated that amount of xanthohumol in the extracts increased with increase of polarity of the solvents used from methanol to dichloromethane. The methanol extract contained the high amount of xanthohumol with 5.74 g/100 g extract (0.46 g/100 g plant sample).

Keywords: *Humulus lupulus*, insecticidal effect, xanthohumol, stored pest

(Accepted 27 February 2017; First published online 23 March 2017)

Introduction

The *Humulus lupulus* L. (hops) is an important plant species belonging to the family *Cannabaceae* and it grows like grape. Hops cones involve high incidence of polyphenolic compounds and acyl phloroglucides (Kavalier *et al.*, 2011; Clark *et al.*, 2013; Gorjanović *et al.*, 2013; Masek *et al.*, 2014). At the present time it is a primary material in beer harsh-tasting and giving characteristic aroma (Chadwick *et al.*, 2006; Olas *et al.*, 2011; Arsene *et al.*, 2015; Taniguchi *et al.*, 2015). The hops preparations have sold in the America for anxiety and insomnia therapies and in pharmacies in the Europea (Anonymous, 2002; Chadwick *et al.*, 2006). The hops extract has antimicrobial, insecticidal, antiproliferative and detoxification activity in hepatoma and rat liver (Dietz *et al.*, 2005; Gokce *et al.*, 2006*a*; Arsene *et al.*, 2015; Onder *et al.*, 2016).

Principle component of the hops cones, xanthohumol was firstly characterized by Verzele *et al.* (1957) and its various biological activities are attracted notice (Liu *et al.*, 2015) (fig. 1). The xanthohumol has inhibition activity against some

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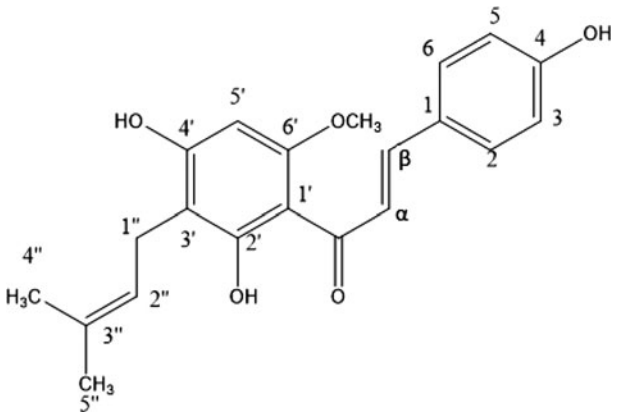

Fig. 1. Chemical structure of xanthohumol.

carcinogenesis (Zanoli & Zavatti, 2008; Sławinska-Brych *et al.*, 2015), anti-infective activity against Gram-positive bacteria, viruses, fungi and malarial protozoa (Gerhäuser, 2005; Zanoli & Zavatti, 2008). It has also antioxidant, antiviral and antitumoral activities, including liver, breast, colon, ovarian and prostate (Venè *et al.*, 2012; Yoshimaru *et al.*, 2014; Zhao *et al.*, 2016).

In the literature, insecticidal activities of the hops and its principle component xanthohumol against some insect species are reported (Gokce *et al.*, 2006*a*, *b*, *c*, 2007, 2012; Er *et al.*, 2009; Yanar *et al.*, 2011; Cam *et al.*, 2012; Karakoc & Gokce, 2012; Karaca & Gokce, 2014; Bedini *et al.*, 2015; Jackowski *et al.*, 2015; Stompor *et al.*, 2015). The methanolic extract of the hops showed the toxic effect against Colorado potato beetle larvae and adults (Gokce *et al.*, 2006*b*, 2007, 2012; Cam *et al.*, 2012; Alkan *et al.*, 2015). In an another study, it has been reported repellent activity of hops essential oils against *Rhyzopertha dominica* and *Sitophilus granarius* (L.) (Bedini *et al.*, 2015). The characteristic compound of the hops cone, xanthohumol exhibited potential antifeedant activity against the peach-potato aphid (*Myzus persicae*) (Stompor *et al.*, 2015). Xanthohumol and carbon dioxide spent hops extract showed antifeedant activity against adults of *S. granarius* (L.) and *T. castaneum* Duv. larvae (Jackowski *et al.*, 2015; Stompor *et al.*, 2015).

The stored pests are still a serious problem on grain and grain products through the harvest, transport and production process. Damages to grain products by the pests cause a serious economic losses in the world and herein, every year, thousands tons of the grain and grain products are damaged by storage pests. Therefore, the control of storage insects is extremely important issue to reduce their harmfull effects on the grain and grain products (Stejskal *et al.*, 2015). In recent years, there is an increasing attention on the control of storage pests by natural products, including plant extracts, essential oils and their pure components (Garcia *et al.*, 2005; Gokce *et al.*, 2006*a*, *b*, *c*, 2007, 2012; Kordali *et al.*, 2007, 2008; Tozlu *et al.*, 2011; Gokce *et al.*, 2012; Aydin *et al.*, 2014; Bedini *et al.*, 2015; Jackowski *et al.*, 2015; Stejskal *et al.*, 2015; Cakir *et al.*, 2016). Natural chemicals are less harmfull than synthetic chemicals to the environment and the health of livng organisms due to slow degradation of synthetic chemicals in the environment. In the current study, five common storage pests, *S. granarius* (L.), *Sitophilus oryzae* (L.), *Acanthoscelides obtectus* (Say.), *Tribolium castaneum* (Herbst) and *Lasioderma serricorne* (F.) were used to bioassay the insecticidal properties of *H. lupulus* cones. The *S. granarius* (L.) is especially known as 'grain weevil' or 'granary weevil' and a widespread pest worldwide (Yildirim, 2012). Herein, this insect cause serious economical losses in the world due to its harmfull effect (Bell, 2000). Other name of *S. oryzae* (L.) is 'rice weevil' and this pest is a seriously danger in the rice (Batta, 2004). *A. obtectus* (Say.) (Coleoptera: Chrysomelidae: Bruchinae) is a pest damaging to bean. It is a serious problem against bean *Phaseolus vulgaris* L. (Fabaceae) in the post-harvest and field pest (Masolwa & Nchimbi, 1991). *T. castaneum* (Herbst) (Coleoptera: Tenebrionidae), the red flour beetle is common especially in the tropical and humid regions and elevated temperature. The storage foods seriously damaged by the *T. castaneum* (Herbst) (Garcia *et al.*, 2005). The cigarette beetle, *L. serricorne* (Fabricius, Coleoptera: Anobiidae) is common and important a stored pest in the worldwide. It has damaged especially to stored cereals, tobacco, oilseeds and dried fruits (Ashworth, 1993; Ebadollahi *et al.*, 2010).

According to our litarature survey, there is no report on the toxicity of the extracts (dichloromethane, ethyl acetate, acetone, ethanol and methanol) and principle component, xanthohumol of hops cones against *S. granarius* (L.), *S. oryzae* (L.), *A. obtectus* (Say.), *T. castaneum* (Herbst) and *L. serricorne* (F.). For this reason, the aim of the present study was to evaluate the toxicity of the extracts and its principle component, xanthohumol of *H. lupulus* cones and malathion, an insect control reactive against adults of *S. granarius* (L.), *S. oryzae* (L.), *A. obtectus* (Say.), *T. castaneum* (Herbst) and *L. serricorne* (F.).

Materials and methods

Materials and chemicals

Hops cones were provided from the Pazaryeri District Directorate of Agriculture (Turkey) in August 2014. Plant sample was deposited in the Central Research and Application Laboratory, Agri Ibrahim Çeçen University. The pure chemicals were purchased from Fluka, Merck, Aldrich and Alfa. Vanillin was used as solid chemical substances. Malathion (Fermalathion 65 EC were purchased from Fertil Kimya (Turkey).

Extraction and isolation procedures of xanthohumol

Hop cones (1120 g) was dried in shadow at room temperature and powder using a blender. Afterwards, hop cones were extracted for 24 h with 5 L *n*-hexane. The same procedure was repeated five times. Extracts were filtered over filter paper and *n*-hexane was completely removed from the residual plant by evaporation. Residual plant was again extracted for 24 h with methanol (5×5 liter^2). Extracts were filtered over filter paper and methanol was evaporated using a rotary evaporator at low temperature and pressure. End of the evaporation of methanol, a brownish extract (128 g, yield % 11.43) was obtained. The methanol extract was solved in 500 ml of distilled water (60 °C) and then was filtered. The extract was divided into two portions, soluble (21.34 g) and insoluble (80 g).

The dried and powdered cones of hops (100 g) were individually extracted with dichloromethane (5×300 ml^2), ethyl acetate (5×300 ml^2), acetone (5×300 ml^2), ethanol (5×300 ml^2) and methanol (5×300 ml^2) for 24 h. The amounts of dichloromethane, ethyl acetate, acetone, ethanol and methanol

https://doi.org/10.1017/S0007485317000256 Published online by Cambridge University Press

extracts respectively were determined as 3.90, 3.47, 4.70, 6.20 and 8.00 g, respectively.

The insoluble part of methanol extract (80 g) was fractionated by CC (SiO_2 (600 g, 70–230 mesh); CH_2Cl_2/AcOEt 1 : 1). The fractions (50 ml each) were checked by TLC (silica gel 60 F-254, Merck, precoated plates; CH_2Cl_2, CH_2Cl_2/AcOEt, 1 : 1, AcOEt), and the fractions with the same R_f values were combined. Spots on the TLC plate were visualized by UV_{254} and UV_{365}, and spraying with 1% vanillin–H_2SO_4 followed by heating (105 °C). Two fractions, A (20.04 g) and B (22.50 g) were finally obtained. Fraction A completely dissolved in 100 ml of AcOEt and was extracted with 100 ml of 0.025 M NaOH. Thus, xanthohumol in the fraction A was transferred into aqueous phase. The extraction with NaOH solution was repeated for five times and afterward the water phase was neutralized to pH = 7.0 with acetic acid (1 M) using a pH meter. Xanthohumol in the aqueous phase was reextracted with AcOEt (3 × 100 ml^2). AcOEt phase was dried over anhydrous Na_2SO_4 and filtered. The organic solvent was evaporated under reduced pressure and temperature using a rotary evaporator. The AcOEt phase was checked with preparative silica gel TLC using $CHCl_3$–AcOEt (9 : 1) mobile phase. The extract (7 g) was subjected to silica gel CC (45 g, 200–400 mesh) using $CHCl_3$/AcOEt (9 : 1) mobile phase to yield xanthohumol (2.20 g). Nuclear magnetic resonance (NMR) spectra of xanthohumol were recorded on a Bruker 400 MHz spectrometer (^{1}H: 400 MHz and ^{13}C: 100 MHz) using $CDCl_3$. Chemical shifts are expressed in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard and coupling constants (*J*) reported in Hz. The infrared (IR) spectrum of xanthohumol was determined on a Perkin Elmer FTIR 1600 spectrophotometer (υ in cm^{-1}). Melting point of the xanthohumol was recorded using Thermo Scientific 9200 apparatus.

Spectral information for xanthohumol: ^{1}H-NMR (400 MHz, $CDCl_3$): (δ, ppm) 14.66 (*s*, 2× H, 2′ and 4′), 7.75 (*d*, *J* = 4.56 Hz, 2H, α and β), 7.47 (*d*, *J* = 8.40 Hz, 2× H, 3 and 5), 6.87 (*d*, *J* = 8.36 Hz, 2× H, 2 and 6), 5.95 (*s*, 5′), 5.28 (*t*, *J* = 7.00 Hz, 2″), 4.85 (*s*, H-4), 3.86 (*s*, OCH_3), 3.37 (*d*, *J* = 7.00 Hz, 1″), 1.81, (*s*, 3× H, 5″), 1.86 (*s*, 3× H, 4″). ^{13}C-NMR ($CDCl_3$): 193.0 (C═O), 165.1 (C3′), 161.8 (1′), 161.2 (6′), 158.3 (1), 142.5 (β), 130.3 (3 and 5), 127.9 (4), 125.0 (α), 122.0 (2″), 116.0 (2 and 6), 107.0 (4′), 106.1 (2′), 91.2 (5′), 55.7 ($O\underline{C}H_3$), 25.8 (5″), 21.6 (1″), 17.9 (4″).

Quantitavie analyses of the extracts of hops cones

Separations were performed using an high-performance liquid chromatography (HPLC) system (Shimadzu class LC) consisting of a FCV-10 ACVP pump, a DGU-20A5 degasser, a thermostated CTO-10VP column oven compartment and a SPD-20A prominence UV/VIS detector. A reverse-phase Kromasil 100-5 C18 (150 mm × 4.6 mm, 5 µm) column was used. The mobile phase consisted of solvents A (1% aqueous phosphoric acid (H_3PO_4)) and B (acetonitrile). The separation was performed using the following gradient conditions: 0–5 min, 40% B; 5–25 min, 85% B; 25–30 min, 95% B. The analysis duration was 20 min, and the flow rate was 1 ml min^{-1}. The injected volume was 20 µL and the column temperature was 35 °C. The wavelength used for the detection for all samples was 365 nm.

Insect materials and bioassays

In this series of the experiments, adults of *S. granarius* (L.), *S. oryzae* (L.), *A. obtectus* (Say.), *T. castaneum* (Herbst) and *L. serricorne* (F.). were used to test the toxicities of the extracts of hops cones and its charasteristic compound, xanthohumol. Adults of the insects were obtained from Bozok University, Faculty of Agriculture, Department of Plant Protection. In order to the cultivation of insects, same age adults of the insects were feed on wheat in the glass jars (5 liters) at 27 ± 2 °C, 64 ± 5% relative humidity and 12 h/12 h (L : D). The lids of the jars were covered with tulle through rubber band. The solutions (2.5, 5.0, 10.0 and 20.0 mg ml^{-1}) of the extracts and xanthohumol were prepared by suspending and/or dissolving in dimethyl sulfoxide (DMSO)/sterile water (1 : 10). A 2 µl aliquot of each solution of the extracts and xanthohumol were applied topically to the dorsal surface of one insect by a micro applicator (Hamilton, Bonaduz, GR, Switzerland). Thus, the applications corresponded to doses of 5, 10, 20 and 40 µg per insect. For each extract and dose, ten treated insects of mixed gender were placed individually into Petri dishes (9 × 1.5 cm^2) containing 10 g of wheat. Then, Petri dishes were closed with adhesive tape and placed in an incubator. After exposure, number of dead insects was counted after 24, 48, 72, 96 and 120 h. Three replicates were used for each dose and exposure time combination. Malathion solution, commercial insecticide (1 mg ml^{-1}) was used as positive control under the same conditions. Petri dishes applied with only DMSO/sterile water (1 : 10, v/v) were used as negative control groups. The toxicities of the extracts, xanthohumol and malathion were expressed as % mean mortality of the adults.

Table 1. Xanthohumol amounts of the extracts.

Extract	Xanthohumol amount (g/100 g extract)	Xanthohumol amount (g/100 g plant sample)
CH_2Cl_2	3.58	0.14
Ethyl acetate	3.73	0.13
Acetone	3.83	0.18
Ethanol	4.24	0.26
Methanol	5.74	0.46

Statistical analyses

For comparison of data, one-way variance analyses (ANOVA) using SPSS 10.0 software package was applied, and differences between means were tested through LSD, and values of $p < 0.05$ were considered significantly different. Doses causing 50% mortality (LD_{50}) were calculated for each treatment at 48 h by probit analysis.

Results

Quantitative amounts of xanthohumol in the extracts

Xanthohumol is a characteristic compound of the hop cones and it was isolated from the methanol extract by column and thin layer chromatographic methods as a yellowish powder (m.p. 157–159 °C). Its chemical structure was characterized by IR, ^{1}H-NMR, ^{13}C-NMR, one-dimensional (1D)- and 2D-NMR methods. Its chemical structure was also confirmed by comparing the previously reported spectral data (Zhao *et al.*, 2005; Chen *et al.*, 2012). Furthermore, the quantitative amounts of the xanthohumol in the extracts were analyzed by HPLC to explore the relationship with xanthohumol amount of the extracts and their insecticidal effects. According to the data presented, the amount of xanthohumol increase with polarity of the organic solvent (table 1).

https://doi.org/10.1017/S0007485317000256 Published online by Cambridge University Press

Table 2. The LD_{50} values of the extracts of *H. lupulus* cones and xanthohumol against the adults of the insects in the first 48 h.

Substance	LD_{50} (µg)				
	S. oryzae	*S. granarius*	*A. obtectus*	*T. castaneum*	*L. serricorne*
Dichloromethane extract	33.1 (28.5–39.5)	21.9 (12.7–37.6)	>40	32.4 (22.0–53.5)	33.2 (20.5–108.8)
Ethyl acetate extract	29.2 (18.0–74.2)	15.4 (8.7–28.3)	26.9 (13.5–66.9)	21.1 (5.7–78.9)	24.1 (12.2–91.2)
Acetone extract	>40	24.4 (11.8–84.0)	>40	29.6 (17.3–62.0)	30.7 (18.9–55.4)
Ethanol extract	>40	25.7 (14.8–60.1)	>40	>40	34.0 (23.0–57.6)
Methanol extract	>40	>40	>40	>40	>40
Xanthohumol	<10	<5	26.4 (15.5–48.6)	22.3 (9.5–54.8)	<5

The values in parentheses are the 95% confidence intervals.
(-): cannot be determined.

Quantitative amount of xanthohumol of CH_2Cl_2, ethyl acetate, acetone, ethanol and methanol extracts were 3.58, 3.73, 3.83, 4.24 and 5.74 g/100 g extract, respectively (table 1). However, the toxicity studies showed that insecticidal effects of the extracts increased with decrease of the organic solvent. These results showed that there are no relation with the amount of xanthohumol in the extracts and insecticidal activities of the extracts (table 1).

Insecticidal effects of the extracts and xanthohumol

The contact toxicities of dichloromethane, ethyl acetate, acetone, ethanol and methanol extracts and characteristic metabolite, xanthohumol of *H. lupulus* cones were evaluated against adults of five important stored pests, *S. granarius* (L.), *S. oryzae* (L.), *A. obtectus* (Say), *T. castaneum* (Herbst) and *L. serricorne* (F.). Four different doses, 5.0, 10.0, 20.0 and 40 µg per insect of the extracts and xanthohumol were tested, and their toxicities were compared with those of the commercial insecticide, malathion (2 µg). The mortalities of the adults of the insects were counted at 24, 48, 72, 96 and 120 h after exposure. The results were presented in Supplementary tables S1–S5. The results showed that toxicities of the extracts and xanthohumol increased with increase of doses of the substances and exposure times (Supplementary tables S1–S5). Among the tested substances, commercial insecticide, malathion was more active against the tested pests. The positive control (malathion) showed 100% mortality at a dose of 2 µg. However, mortality in the negative controls was always <10%. As can be seen from Supplementary tables S1–S5, malathion caused total mortality after 24 h of exposure. Furthermore, xanthohumol was more toxic against the all of the tested pests as compared with the toxic effects of the extracts. The 40 µg dose of xanthohumol showed higher toxicities than other concentration against all of the pests tested (Supplementary tables S1–S5). As shown in Supplementary table S1, xanthohumol showed the highest toxicity against *S. granaries* as well as *S. oryzae* (L.). Its 40 µg dose caused 100% mortality against the adults of *S. granaries* (L.) and *S. oryzae* (L.) after 48 h of exposure time (Supplementary tables S1 and S2). Xanthohumol was found to be less effective against *A. obtectus* (Say.) and *T. castaneum* (Herbst) adults (Supplementary tables S3 and S4). Moreover, the ethyl acetate extract of *H. lupulus* cones was the most effective extract. Among the tested extracts of hops cones, the ethyl acetate extract showed the highest toxic effects against the storage pests (Supplementary tables S1–S5). The toxic effects of dichloromethane and acetone extracts were found to be lower than the ethyl acetate extract but to be higher than ethanol and methanol extract. The 40 µg dose of ethyl acetate extract caused 100% mortality against the adults of *S. granarius* (L.) after 72 h of exposure (Supplementary table S1). Our results also indicated that polar extracts, ethanol and methanol extracts were less effective against the adults of the five storage pests.

The LD_{50} values of the extracts of *H. lupulus* cones and xanthohumol against adult insects after 48 h of exposure are shown in table 2. The LD_{50} values of the positive control, malathion were not calculated due to 100% mortality after first 24 h of exposure. Table 2 show that xanthohumol has low LD_{50} values as compared with those of the extracts. Its LD_{50} values were <5, <10, 22.3 and 26.4 µg doses. As can be seen from table 2, according to LD_{50} values, pure xanthohumol was classified as potent toxic insecticide (LD_{50} values respectively <10, <5 and <5 µg) against *S. oryzae* (L.), *S. granarius* (L.) and *L. serricorne* (F.) and as toxic insecticide (LD_{50} values 20–60 µg) against *A. obtectus* (Say.) and *T. castaneum* (Herbst) (table 2). Likewise, among the extracts contain xanthohumol, the ethyl acetate extract was classified as more toxic substance against *S. granarius* (L.) (LD_{50} = 15.4 µg) and as toxic substance against *S. oryzae* (L.) (LD_{50} = 29.2 µg), *A. obtectus* (Say.) (LD_{50} = 26.9 µg), *T. castaneum* (Herbst) (LD_{50} = 21.1 µg) and *L. serricorne* (F.) (LD_{50} = 24.1 µg). In the view of the results in table 2, the dichloromethane extract was also classified as toxic insecticide against all tested insects (LD_{50} values 21.9–33.2 or >40 µg). However, LD_{50} values were found to be high values for acetone, ethanol and methanol extracts as compared with those of xanthohumol and other extracts. These results show that the insecticidal effects of the extracts reduce with increase of polarity of the organic solvents. Many times, these extracts acted as medium insecticide reagents against the adults of some insect species such as *S. granarius* (L.) and *L. serricorna* (Ware, 1986).

The duration times were calculated by the probit analyses for the 50% mortality of the adults of the *S. granarius* (L.), *S. oryzae* (L.), *A. obtectus* (Say.), *T. castaneum* (Herbst) and *L. serricorne* (F.) exposed to different concentrations of the xanthohumol and the extracts (Supplementary table S6). Durations of the practices for 50% mortality of the adults of insects decreased with increasing of the concentration. According to the present data, the toxicities of the practices were concentration dependent. Between all of the tested substance and extracts, the xanthohumol was found to be the most toxic compound against all of the adults of the storage insects with short duration times of 10.9–107.6 h at the 10 µg dose (Supplementary table S6). Otherwise, according to the durations for 50% mortality of the insects, the ethyl acetate extract

https://doi.org/10.1017/S0007485317000256 Published online by Cambridge University Press

was more toxic than other extracts. The shortest duration time of ethyl acetate extract (43.3 h) for 50% mortality was determined for *S. garanarius* adults as compared with other insects. As can be seen from Supplementary table S6, the longest duration times were found for methanol extract.

Discussions

The insecticidal effects of various extracts of *H. lupulus* and its principal component, xanthohumol against adults and larvae of various insects species have been evaluated, indicating various toxic effects depending on the insect species, type of extract and dose or concentration (Gokce *et al.*, 2006*a*, *b*, *c*, 2007; Er *et al.*, 2009; Yanar *et al.*, 2011; Cam *et al.*, 2012; Gokce *et al.*, 2012; Karakoc & Gokce, 2012; Karaca & Gokce, 2014; Bedini *et al.*, 2015; Jackowski *et al.*, 2015). Jackowski *et al.* (2015) indicated that xanthohumol and supercritical carbon dioxide extract of spent hops posssess medium deterrent activity against three stored product pests, *S. granarius* (L.), *Tribolium confusum* and *Trogoderma granarium*. They also showed that the spent hops extract was more active than xanthohumol. On the other hand, our results indicate that xanthohumol and the nonpolar solvent extracts of hops was found to be active against *S. granarius* (L.) and the toxic effect of xanthohumol was higher than those of the extracts (Supplementary table S1). Recently, some previous reports have mostly focused on the insecticidal properties of *H. lupulus* against the larvae and adults of Colorado potato beetle (*Leptinotarsa decemlineata* L.) (Gokce *et al.*, 2006*b*, 2007, 2012; Cam *et al.*, 2012; Alkan *et al.*, 2015). Studies on the antifeedant activity of different extracts (hexane, ethyl acetate and methanol extracts) of hops showed that this plant extract significantly reduced the feeding of Colorado potato beetle when it was tested at 1% (w/v) concentration and their antifeedant activity could be attributed to their xanthohumol contents (Alkan *et al.*, 2015), confirming previous reports of the antifeedant action of hops extracts to this important pest (Gocke *et al.*, 2006*b*, 2007, 2012). *Thaumetopoea solitaria* is a serious pest of some cultivated plants, in particular of pistachio throughout the Mediterranian and the surrounding regions. Ingestion of a methanolic *H. lupulus* extract caused 83% mortality in *T. solitaria* after 48 h (Er *et al.*, 2009). Yanar *et al.* (2011) has studied the contact toxic effect of the methanolic extracts of different plant extracts besides *H. lupulus* against two-spotted spidermite, *Tetranychus urticae*, is a common pest of many plant species in greenhouses and field crops and they found that a methanol extract of *H. lupulus* caused 67.84% mortality after 24 h exposure time. In a recent study, the essential oils of *H. lupulus* cones were reported as a good insecticide and molluscicide against the invasive disease vectors *Aedes albopictus* and *Physella acuta* (Bedini *et al.*, 2016). In accordance with our findings (table 2 and Supplementary tables S1–S6), these results suggest that *H. lupulus* extracts appear to have a broad spectrum of activity against insect pests, which are harmful to stored and field products.

In conclusion, the currents results show that xanthohumol and ethyl acetate extract of hops cones were found to be toxic against all the tested insects species. Xanthohumol as well as the ethyl acetate extracts can be used as botanical insecticides. Xanthohumol, characteristic compound of hops cones can be used as an alternative insecticide against the storage insects, *S. granarius* (L.), *S. oryzae* (L.), *A. obtectus* (Say.), *T. castaneum* (Herbst) and *L. serricorne* (F.).

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S0007485317000256.

Acknowledgments

This study was financed by Ağrı İbrahim Çeçen University (BAP) (Project No: ECZF.14.004). Therefore, the authors were grateful to their universities for the financial support and to the Pazaryeri District Directorate of Agriculture for providing cones of *H. lupulus*. The authors were also grateful to Dr. Metin Acikyildiz (Kilis 7 Aralık University, Faculty of Science and Letter, Department of Chemistry, Kilis) for supporting on the statistical analyses.

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