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Original article (Orijinal araştırma)

Toxic efficacy of *Cuscuta campestris* Yunck. (Solanales: Convolvulaceae) and *Lupinus albus* L. (Fabales: Fabaceae) plant crude extracts against nymphs and adults of *Orosanga japonica* (Melichar, 1898) (Hemiptera: Ricaniidae) under laboratory conditions[1]

Orosanga japonica (Melichar, 1898) (Hemiptera: Ricaniidae)'nın nimf ve erginlerine karşı *Cuscuta campestris* Yunck. (Solanales: Convolvulaceae) ve *Lupinus albus* L. (Fabales: Fabaceae) bitki ham özütlerinin laboratuvar koşulları altında toksik etkinliği

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Abstract

The planthopper, *Orosanga japonica* (Melichar, 1898) (Hemiptera: Ricaniidae), is an important agricultural pest of grapevine, kiwifruit and tea in Asia and in some countries of Eastern Europe. The efficacy of the crude extracts of *Cuscuta campestris* Yunck. (Solanales: Convolvulaceae) and *Lupinus albus* L. (Fabales: Fabaceae) plants was evaluated under laboratory conditions for control of *O. japonica* nymphs and adults collected in 2019 from Rize (Turkey). Their toxic efficacies were investigated by two different methods. Fixed-dose death rates were used for LT_{50} calculation and dosage test results were used for LC_{50} calculation. Also, the phenolic constituents of active plant extracts were examined using HPLC-DAD. Generally, the LT_{50} values obtained using ethyl acetate extracts were lower than those with methanol extracts. LT_{50} values of adults were found lower than in nymphs. The test plants crude extracts had high activity at and below 2 g/L (LC_{90}) for two different plants. HPLC-DAD results showed that the high concentration of kaempferol and quercetin for each extract. Extracts of both plants gave promising results for use in *O. japonica* control, but more detailed studies on the active constituents of these candidate plants need to be undertaken.

Keywords: Biocontrol, field dodder, insecticidal activity, *Orosanga japonica,* white lupin

Öz

Yalancı kelebek, *Orosanga japonica* (Melichar, 1898) (Hemiptera: Ricaniidae), Asya'da ve bazı Doğu Avrupa ülkelerinde üzüm, çay ve kivide önemli bir tarımsal zararlıdır. 2019 yılında Rize (Türkiye)'den toplanan *O. japonica* nimfleri ve erginlerinin kontrolüne karşı *Cuscuta campestris* Yunck. (Solanales: Convolvulaceae) ve *Lupinus albus* L. (Fabales: Fabaceae)'un ham özütlerinin laboratuvar koşullarında etkinliği değerlendirilmiştir. Bunların toksik etkileri iki farklı yöntemle araştırılmıştır. Sabit doz ölüm oranları LT_{50} ve dozaj test sonuçları LC_{50} hesaplamaları için kullanılmıştır. Ayrıca aktif bitki özütlerinin fenolik bileşenleri HPLC-DAD kullanılarak incelenmiştir. Genellikle, etil asetat özütleri kullanılırken elde edilen LT_{50} değerleri, metil alkol özütlerinden düşük olmuştur. Erginlerin LT_{50} değerleri nimflere göre daha düşük bulunmuştur. Deneme bitkilerinin ham özütleri, iki farklı bitki için 2 g/L (LC_{90}) civarında ve altında yüksek aktivite göstermiştir. HPLC-DAD sonuçları, her bir özüt için yüksek kaempferol ve quercetin konsantrasyonunu göstermiştir. Her iki bitkinin özütleri, *O. japonica*'nın kontrolünde kullanımı için umut vericidir, ancak bu aday bitkilerin aktif bileşenleri üzerinde daha ayrıntılı çalışmaların yapılması gerekmektedir.

Anahtar sözcükler: Biyolojik mücadele, tarla küskütü, insektisidal aktivite, *Orosanga japonica,* beyaz acı bakla

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Introduction

Planthoppers are a large group of more than 9,000 species within the Fulgoromorpha suborder of the Hemiptera (O'Brien & Wilson, 1985). Most of the species are of little economic importance, but a few are major agricultural pests, including *Pochazia sublimate* (Schumacher, 1915), *Ricania speculum* (Walker, 1851) and *Scolypopa australis* (Walker, 1851) (Hemiptera: Ricaniidae), which can transmit plant pathogens by means of absorbent mouth parts or cause significant damage to plant tissues. They are among the most destructive pests of major agricultural products worldwide (Fletcher, 2008; Choi et al., 2012; Jeon et al., 2017). The family Ricaniidae is known as broad-winged planthoppers. Many species in this family have been recorded in tropical areas and some in the Palearctic regions (Demir, 2009). *Orosanga japonica* (syn. *Ricania japonica*) (Melichar, 1898) (Hemiptera: Ricaniidae), has become an important destructive species on agricultural products in the coastal regions of the Eastern Black Sea in Turkey (Demir, 2009; Ak et al., 2015; Akıner et al., 2019).

Orosanga japonica is a common species in Eastern Asian countries, including Korea, China and Japan. This species was first recorded in Russia, near Ukraine in the western Palearctic region. It is believed that during the transport of seedlings and plants purchased for botanical gardens in the 1900s, these insects were transported to Russia from their natural habitat. Then in the 1950s, this species was recorded in Georgia (EPPO, 2016). *Orosanga japonica* was first recorded in Turkey by Demir (2009). Since then, it has rapidly spread westward (Akcakoca/Düzce, Turkey) along the Black Sea coastline and in the European part of İstanbul (Demir, 2009, 2018; Öztemiz & Doğanlar, 2015; Arslangündoğdu & Hizal, 2019). The insect has become an economically important pest of some agricultural plants in the Eastern Black Sea Region, including bean, cucumber, fig, grapevine, hazelnut, kiwifruit, tea and tomato (Ak et al., 2015; Öztemiz & Doğanlar, 2015; Göktürk et al., 2017; Jeon et al., 2017; Akıner et al., 2019). This is particularly important as it reduces the production of nuts and tea, which comprise the livelihood of local people. In addition to the limited use of chemical fertilizers in the cultivation of agriculture products under natural conditions, the use of chemical pesticides is also restricted in this region by the General Directorate of Tea Enterprises in Turkey. For this purpose, different mechanical and biological control methods have been tested to reduce the population of *O. japonica*. However, neither of these types of control is sufficient for long-term population control (Güçlü et al., 2010; Ak et al., 2015; Öztemiz & Doğanlar, 2015; Göktürk et al., 2017).

Insect pest control using chemical insecticides can be a substantial problem due to insecticide resistance, effects on nontarget organisms and environmental pollution (Lichtfouse et al., 2009). Many plants produce biologically active metabolites, some of which are useful for insect control. Applying these natural products should decrease the use of chemical insecticides. The use of botanical based insecticides is becoming increasingly common, and the use of environmentally friendly pest control agents has become an international success (Lichtfouse et al., 2009). Plant-derived materials are nontoxic, biodegradable, safer for nontarget organisms such as humans or animals, and they are safer for the environment than chemical insecticides. Therefore, it would be ideal to use botanical insecticides for insect pest control. Three main classes of chemical compounds from plants with insecticidal activity are cited as having higher biological activity than others. These classes are terpenoids (37%), alkaloids (30%), and phenolic compounds (20%) (Boulogne et al., 2012). Although the effects of a large number of plant essential oil and crude extracts against different insect pest species have been investigated, few studies are available on the use of plant materials for the control of *Ricania* spp. (Singh & Upadhyay, 1993; Shin et al., 2010; Boulogne et al., 2012; Choi et al., 2012; Kim et al., 2013; Jeon et al., 2016; Göktürk et al., 2017; Jeon et al., 2017; Lee et al., 2018).

Turkey has a high diversity of natural aromatic and medicinal plant species. Previous studies for *Lupinus* and *Cuscuta* spp. have shown that phenolic and alkaloid contents of this plants are high. In addition, it has been observed that their potential to be natural alternative insecticides against many pests

has been investigated (Thackray et al., 2000; Torres et al., 2009; Shekarchi et al., 2014; Selvi et al., 2017; Karamac et al., 2018; Hassan et al., 2019). However, no study has found effectiveness of the same plants against *O. japonica*. Therefore, the main objective of this research was to determine the toxic efficacy of crude extracts from *Cuscuta campestris* Yunck. (Solanales: Convolvulaceae) and *Lupinus albus* L. (Fabales: Fabaceae) plants naturally growing in Turkey against nymphs and adults of *O. japonica* related to the high phenolic contents and possible bio insecticidal properties (Yorgancılar, 2013). The second objective of this study was to use HPLC-DAD to identify and quantify the phenolic compounds that are potentially the active compounds related to their insecticidal (on the nymph and adults) activities under laboratory conditions.

Materials and Methods

Chemicals and solvents

Phenolic standards (analytical grade) were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC (high pressure liquid chromatography) syringe filters (RC-membrane, 0.25 µm) were purchased from Sartorius Minisart RC 15, Sartorius (Goettingen, Germany). Other chemicals and solvents such as n-hexane, methanol and ethyl acetate were sourced from Merck (Darmstadt, Germany).

Plant materials

Cuscuta campestris was collected from Talas, Kayseri during the vegetative growing stage (1-3 August), and seeds of *L. albus* were collected from Doğanhisar, Konya 14-15 August 2018. Depending on the results of the preliminary laboratory studies, seed (*L. albus*) and vegetative growing stage (*C. campestris*) of the plants to be studied were determined. This choice is supported by the literature (Shekarchi et al., 2014; Hassan et al., 2019). The specimen identification was performed by Prof. Vagif Atamov from the Faculty of Science and Arts, Recep Tayyip Erdogan University in Rize, Turkey. A sample of each plant was prepared and deposited at mentioned above university in the Herbarium of the Biology Department, Rize, Turkey.

Biological material

Nymphs (third stage) and adults of *O. japonica* were collected with an electric aspirator around Rize City (Alipasa, 41º1’31.66” N, 40º28’58.09” E and Recep Tayyip Erdoğan University Campus, 41º2’11.28”’ N, 40º29’36.50” E) from early June to late August 2019. About 1000 *O. japonica* nymphs and adults collected from region were placed into the different cages (20 x 20 x 20 cm, maximum of 50 nymphs or 50 adults in each cage) and transferred to the laboratory. The samples brought to the insectarium were taken into cages of 50x50x50 cm for ease of feeding (maximum of 100 nymphs in each cage) during the acclimatization period. The cages were previously sterilized to avoid contamination. Samples were held at 25 ± 2ºC with 65 ± 10% RH and 12:12 h L:D photoperiod. Sufficient fresh blackberry branches were put into the cages for feeding and replaced daily for ensuring freshness. *Orosanga japonica* individuals were kept for 24 h in the insectarium to acclimatize them to the environment before testing.

Extraction of plant materials

Following specimen identification, the stems (*C. campestris*) and seeds (*L. albus*) were cleaned and thoroughly washed with distilled water and ethyl alcohol (1:1 v/v) to prevent fungal contamination. The fresh materials of *C. campestris* were then dried for 1 week at room temperature. Dried plant (*C. campestris*) and seed (*L. albus*) samples were ground with a blender and divided into 150 g portions for solvent extraction. Each sample was cleaned with 100 mL of chloroform at 30ºC for 30 min to remove waxy parts. Samples were extracted separately with ethyl acetate (3 x 50 mL) and methanol (3 x 50 mL) at room temperature for 1.5 h in an ultrasonic bath (Bandelin, Germany). The crude extracts were filtered with black ribbon filter paper (pore size 20-25 µm), evaporated to dryness and lyophilized at -80ºC and 0.5 kP to completely

remove the solvent used in the extraction (Freezone® 2.5 L Freeze Dry System, Labconco USA). The crude oil obtained was weighed. The amount of crude oil was from *C. campestris* was 2.35 g (ethyl acetate) and 5.76 g (methanol) per 100 g of plant tissue, and for *L. albus,* 3.86 and 3.46 g was obtained per 100 g of seed. After extraction, 25 mg/L of crude plant extract was reserved for HPLC-DAD analysis. A stock solution (0.1 g/mL) of each crude extract was prepared in dimethyl sulfoxide (DMSO) for the bioassays and stored at -4ºC until tested (Selvi et al., 2017).

Bioassays of plant extracts

Laboratory assays were conducted to assess the efficacy of the two plant extracts by two methods on *O. japonica* nymphs and adults. A leaf dipping bioassay (Güven et al., 2015) and spray bioassay with slight modifications (Choi et al., 2012) were used to evaluate the efficacy of treatments at different concentrations.

Residual bioassay with leaf dipping method

Blackberry branches with leaves without any insecticide application, collected from the field and washed with distilled water, were used. Branches (10 cm) with four to five leaves were selected. Branches were dipped in fixed concentrations of extract solution (in 0.1% v/v Tween 80) for 5 s and then kept at room temperature for 15-20 min to dry. Cotton wool moistened with 5 mL of distilled water was placed on the bottom of a glass jar to prevent drying, and the branches were placed on top. Tween 80 solution (0.1% v/v) was used as a negative control with the same procedure. Twenty acclimatized individuals (nymphs and adults, separately) were then placed in every glass jar for testing.

Residual bioassay with indirect spraying method

Filter paper (10 x 20 cm) was sprayed used for contact toxicity. DMSO impregnated filter papers were used as a negative control. The filter paper was placed on the inner surface of the 250 mL glass jar. Cotton wool moistened with 5 mL of distilled water was placed on the bottom of the glass jar. Fresh blackberry branches for feeding were placed on top of the cotton to prevent drying. Twenty acclimatized individuals (nymphs and adults, separately) were then placed in the glass jars for testing. Jars were covered with a cotton cloth held in place by a rubber band.

The bioassays were maintained at 25 ± 2ºC, 65 ± 10% RH and 12:12 h L:D photoperiod. All tests were done with three replicates over two consecutive weeks (3 x 2 replicates). Neem Azal® (0.5 g/L dose) was used as a positive control. Same application methods were used for two test type. A fixed dose was used for lethal time 50 (LT_{50}) calculation. Death rates were counted after 12, 24, 48 and 72 h for LT_{50}. 1 g/L was used as a highest dose for lethal concentrations 50 and 90 (LC_{50} and LC_{90}) calculations. Five-consecutive concentrations were used in the experiments (1, 0.5, 0.25, 0.125 and 0.0675 g/L). Death rates were determined after 24 h for LC_{50} and LC_{90} calculations.

Statistical analysis

The percent mortality was calculated and corrected using Abbott's formula (Abbott, 1925) with negative control results. Lethal concentrations and times (LC_{50}, LC_{90} and LT_{50}) for two tests condition were evaluated by probit analysis. Differences between groups were analyzed with an independent t test probit analysis and independent t-test were performed by using the IBM® SPSS® statistics program version 22.

HPLC-DAD procedure

This study measured 20 phenolic compounds: lapigenin, caffeic acid, chlorogenic acid, ellagic acid, ferulic acid, fisetin, gallic acid, isorhamnetin, kaempferol, myricetin, o-coumaric acid, p-coumaric acid, p-OH benzoic acid, paeonol, protocatechuic acid, quercetin, rutin, syringic acid, thymol and vanillic acid by HPLC-DAD.

HPLC-DAD analyses were performed using a Thermo Dionex Ultimate 3000 HPLC-DAD system. An Agilent reverse phase C18 column (150 mm x 4.6 mm i.d., 5 µm particle, 100 A°; Agilent) with a guard column (3 mm i.d., Macherey-Nagel, Düren, Germany) was used. The mobile phase was (A) 2% acetic acid in water and (B) 70:30 acetonitrile: water. The programmed solvent used began with a linear gradient held at 94.5% A:5.5% B for 3 min; decreasing to 77% A:23% B at 10 min; 69% A:31% B at 20 min; 44% A:56% B at 30 min; 15% A:85% B at 40 min; and finally, 9.45% A:90.55 % B at 60 min. The injection volume was 10 µL, the column temperature was 30°C, and the flow rate was 1.0 mL/min. Chromatograms were obtained at 254, 280, 315 and 370 nm (Selvi et al., 2017).

Determination of total phenolic content in extracts

Total phenolic compounds were analyzed with Folin-Ciocalteu phenol reagent (Singleton & Rossi, 1965). Gallic acid and quercetin were used to generate standard curves in a range from 0.015 and 0.5 mg/mL ($R^2_{\text{gallic acid}}$ = 0.999, $R^2_{\text{quercetin}}$ = 0.998). First, 20 µL of methanolic plant extract, 400 µL of 0.5 N Folin-Ciocalteu reagent, and 680 µL of distilled water were mixed. This mixture was well vortexed. Then 400 µL of Na_2CO_3 (10%) was added and incubated for 2 h at room temperature. The absorbance of the mixture was measured at 760 nm in HPLC (Thermo Dionex Ultimate 3000 HPLC-DAD). The concentration of total phenolic compounds was calculated as mg of gallic acid equivalent (GAE) per g dry weight (DW) and as mg of quercetin equivalent (QE) per g DW. All measurements were performed in triplicate.

Results and Discussion

Crude plant extracts are known to include complex mixtures of biologically active secondary metabolites. Two different crude extracts of *C. campestris* and *L. albus* were tested at a fixed concentration of 0.5 g/L with two different methods. Using the indirect spraying method, LT_{50} values varied between 12.2 h (*C. campestris* ethyl acetate extract on adults) and 24.5 h (*C. campestris* ethyl acetate extract on nymphs) (Table 1). Generally, the LT_{50} values of compounds extracted with ethyl acetate values were lower than those extracted with methanol in the same plant and life stage. The LT_{50} values of the adult stage were lower than for the nymphs. Statistical analysis indicated significant differences between LT_{50} values for nymphs and adults ($p < 0.05$, $F = 10.9$, p-value = 0.002). Although LT_{50} values against nymphs and adults for the different plant extracts varied widely, there was no significant difference in plant species efficacy ($p > 0.05$, $F = 0.709$, p-value = 0.403). Similarly, no difference was detected for extraction type ($p > 0.05$, $F = 0.009$, p-value = 0 926).

Using the leaf dipping method, LT_{50} values varied between 14.5 h (*C. campestris* methanol on adults) and 32.0 h (*C. campestris* methanol on nymphs). Results of the leaf dipping method were similar to the results found using the indirect spraying method. LT_{50} values calculated from using the leaf dipping method were higher than those found using the indirect spraying method, except for *C. campestris* ethyl acetate and *L. albus* methanol on nymphs. Although the leaf dipping method results were higher than those found using the indirect spraying method, no significant difference between test techniques was detected ($p > 0.05$, $F = 0.188$, p-value = 0.666). Similar results were found for Neem Azal® for two test techniques ($p > 0.05$, $F = 0.001$, p-value = 0.980).

LC_{50} and LC_{90} experiments were performed according to the promising LT_{50} results. Two different crude extracts of *C. campestris* and *L. albus* were tested at five-consecutive concentrations by with two different methods. The results are shown Table 2. LC_{50} results varied between 0.96 g/L (*C. campestris* ethyl acetate extraction against adult) and 1.32 g/L (*L. albus* ethyl acetate extraction against nymph) for residual indirect spraying method. LC_{50} results for leaf dipping method were varied between 1.05 g/L (*C. campestris* methanol extraction against adult) and 1.58 g/L (*L. albus* ethyl acetate extraction against nymph). The highest LC_{50} results for two plant species were found with ethyl acetate extraction against nymph except *C. campestris* methanol extraction for residual indirect spraying method. Although the results varied widely

between the two test and extraction methods, there is no significant differences between test and extraction method ($p < 0.05$, $F = 0.056$, p-value = 0.819 for test types, $F = 0.074$, p-value = 0.792 for extraction methods). Generally, nymph LC_{50} results were found higher than adults and showed significant differences between adults and nymphs ($p < 0.05$, $F = 9.77$, p-value = 0.003).

Table 1. LT_{50} values of *Orosanga japonica* nymphs and adults exposed to crude extracts (500 mg/L) of *Cuscuta campestris* (whole plant) and *Lupinus albus* (seed) using residual leaf dipping and residual indirect spraying methods*

Plant	Stage	Solvent	Residual Indirect spraying*					Residual leaf dipping				
			LT_{50}	LCL	UCL	χ^2	CM	LT_{50}	LCL	UCL	χ^2	CM
Cuscuta campestris	nymph	EtOAc	24.5	10.6	71.4	13.7	9.16	22.5	4.0	46.3	10.6	8.3
		MeOH	30.2	10.6	71.1	11.5	7.83	32.0	11.2	88.6	9.0	6.8
	adult	EtOAc	12.2	10.9	13.4	0.082	8.16	15.3	4.6	23.5	4.4	6.0
		MeOH	13.5	11.8	15.0	2.71	6.66	14.5	11.9	16.9	1.4	5.8
Lupinus albus	nymph	EtOAc	23.3	6.2	43.7	8.59	7.50	24.8	21.9	27.9	0.4	4.8
		MeOH	23.9	21.3	26.6	0.024	6.83	23.7	20.9	26.5	1.3	4.7
	adult	EtOAc	12.6	nd	nd	8.57	9.00	14.7	12.5	16.7	1.8	6.2
		MeOH	15.7	0.8	27.1	5.19	7.66	18.8	3.6	32.0	6.7	5.5
Neem Azal	nymph		40.9	27.4	56.3	4.08	3.50	40.1	14.4	70.3	8.1	2.7
	adult		54.7	nd	nd	14.7	3.66	53.9	nd	nd	14.0	2.8

* Each test methods include 120 individuals for each extraction solvents, plants and life stages. LT_{50}, lethal time 50; LCL, lower confidence limit (95% fiducial limit); UCL, upper confidence limit (95% fiducial limit); χ^2, chi-squared; CM, percent control mortality; EtOAc, ethyl acetate; MeOH, methanol; and nd, not determined.

LC_{50} and LC_{90} experiments were performed according to the promising LT_{50} results. Two different crude extracts of *C. campestris* and *L. albus* were tested at five-consecutive concentrations by with two different methods. The results are shown Table 2. LC_{50} results varied between 0.96 g/L (*C. campestris* ethyl acetate extraction against adult) and 1.32 g/L (*L. albus* ethyl acetate extraction against nymph) for residual indirect spraying method. LC_{50} results for leaf dipping method were varied between 1.05 g/L (*C. campestris* methanol extraction against adult) and 1.58 g/L (*L. albus* ethyl acetate extraction against nymph). The highest LC_{50} results for two plant species were found with ethyl acetate extraction against nymph except *C. campestris* methanol extraction for residual indirect spraying method. Although the results varied widely between the two test and extraction methods, there is no significant differences between test and extraction method ($p < 0.05$, $F = 0.056$, p-value = 0.819 for test types, $F = 0.074$, p-value = 0.792 for extraction methods). Generally, nymph LC_{50} results were found higher than adults and showed significant differences between adults and nymphs ($p < 0.05$, $F = 9.77$, p-value = 0.003).

In addition to the efficacy and LC_{50} results, HPLC-DAD analyses were performed for the determination of primary components of active extracts. As a result, the primary components of *L. albus* extracted by using ethyl acetate were kaempferol (15.0 mg/g), vanillic acid (8.58 mg/g), and *o*-coumaric acid (3.08 mg/g). The primary components in the methanol extract were almost the same, but their concentration was lower. Ferulic acid (2.59 mg/g) was the most abundant phenolic compound by using the methanol extract of the same plant. HPLC-DAD analysis of the *C. campestris* plant was done in the previous study (Selvi et al., 2017) and the primary component ratios included in this plant are given in Table 3 for comparison.

Table 2. LC_{50} and LC_{90} values (g/L) of *Orosanga japonica* nymphs and adults exposed to crude extracts of *Cuscuta campestris* (whole plant) and *Lupinus albus* (seed) using residual leaf dipping and residual indirect spraying methods

Plant	Stage	Solvent	Residual indirect spraying*						
			LC_{50}	LCL	UCL	LC_{90}	LCL	UCL	χ^2
Cuscuta campestris	nymph	EtOAc	1.11	0.75	1.86	1.84	1.36	3.76	19.30
		MeOH	1.26	1.02	1.71	1.90	1.52	2.87	8.91
	adult	EtOAc	0.96	0.61	1.75	1.69	1.21	3.99	20.00
		MeOH	1.01	0.61	1.76	1.97	1.41	4.24	17.80
Lupinus albus	nymph	EtOAc	1.32	0.96	1.98	2.10	1.60	2.87	15.60
		MeOH	1.27	0.81	2.22	2.15	1.55	4.62	23.00
	adult	EtOAc	1.22	1.02	1.52	1.90	1.58	2.54	5.95
		MeOH	1.12	0.74	1.92	1.92	1.40	4.02	19.70
Neem Azal	nymph		0.90	0.70	1.20	1.47	1.18	2.13	10.30
	adult		0.99	0.79	1.29	1.62	1.31	2.25	8.52
			Residual leaf dipping						
			LC_{50}	LCL	UCL	LC_{90}	LCL	UCL	χ^2
Cuscuta campestris	nymph	EtOAc	1.14	0.76	1.81	1.99	1.47	3.74	17.30
		MeOH	1.13	0.56	3.74	1.91	1.28	10.26	36.40
	adult	EtOAc	1.11	0.75	1.74	1.99	1.48	3.68	15.40
		MeOH	1.05	0.68	2.21	1.74	1.23	5.03	22.60
Lupinus albus	nymph	EtOAc	1.58	1.48	1.70	2.31	2.14	2.51	3.15
		MeOH	1.48	1.37	1.60	2.25	2.08	2.46	4.78
	adult	EtOAc	1.18	0.97	1.55	1.78	1.45	2.59	7.58
		MeOH	1.19	0.86	1.80	1.94	1.47	3.39	15.30
Neem Azal	nymph		0.86	0.65	1.20	1.46	1.14	2.24	11.60
	adult		1.05	0.87	1.29	1.71	1.43	2.23	5.61

* Each test methods include 120 individuals for each extraction solvents, plants and life stages. LC_{50}, lethal concentration 50; LC_{90}, lethal concentration 90; LCL, lower confidence limit (95% fiducial limit); UCL, upper confidence limit (95% fiducial limit); χ^2, ºhi-squared; EtOAc, ethyl acetate; and MeOH, methanol.

Table 3. Phenolic compounds present in *Cuscuta campestris* and *Lupinus albus* extracts after extraction with methanol and ethyl acetate identified by HPLC-DAD

Compounds	Retention Time (min)	*Lupinus albus*		*Cuscuta campestris* [a]	
		EtOAc*	MeOH	EtOAc[a]	MeOH[a]
p-OH Benzoic acid	11.8	nd	0.4	0.8	0.7
Vanillic acid	13.3	8.6	1.1	0.4	0.8
Rutin	14.8	nd	1.5	0.2	3.4
p-Coumaric acid	16.5	0.4	0.1	1.2	2.3
Ferulic acid	18.9	0.7	2.6	1.5	4.4
o-Coumaric acid	20.4	3.1	0.1	nd	nd
Quercetin	28.5	nd	0.4	5.6	12.5
Kaempferol	32.3	15.0	0.6	5.0	10.7
Isorhamnetin	33.1	-	-	6.6	17.0
Total		27.7	6.8	21.3	51.8
Total flavanol		15.0	2.5	17.4	43.6

* EtOAc, ethyl acetate; MeOH, methanol; nd, not detected. [a]HPLC-DAD analysis results of *C. campestris* in Selvi et al. (2017).

In the present study, the total phenolic content (TPC) determination of four extracts from two different plants and two extraction solvents was performed spectroscopically. Gallic acid and quercetin were used as standards for TPC with a linear calibration curve at $R^2_{\text{gallic acid}} = 0.999$ and $R^2_{\text{quercetin}} = 0.998$. The level of phenolic compounds ranged from 17.1 to 250 mg GAE/g, and from 8.00 to 140 mg QE/g, respectively. The highest TPC was obtained from the ethyl acetate extract of *C. campestris* while the lowest was obtained from the methanol extract of *L. albus* (Table 4).

Table 4. Total phenolic content (TPC) of *Cuscuta campestris* and *Lupinus albus* extracts

Sample	Extract	TPC	
		mgGAE/g*	mgQE/g*
Cuscuta campestris	MeOH	50.5 ± 0.03	38.4 ± 0.05
	EtOAc	250.5 ± 0.02	140.2 ± 0.01
Lupinus albus	MeOH	17.1 ± 0.02	8.0 ± 0.01
	EtOAc	20.4 ± 0.03	11.2 ± 0.02

* GAE, gallic acid equivalents; QE, quercetin equivalents; EtOAc: Ethyl acetate; MeOH: methanol.

Previous studies have reported that the differences in sensitivity of *Ricania* nymphs and adults to essential oils are related to variation in biological factors, such as detoxified glutathione S-transferase and hydrolase levels in nymphs and adults (Lee & Lee, 2015). In addition, several studies have reported the insecticidal activity of different plant essential oils on both nymphs and adults of a *Ricania* sp. and *O. japonica* (Choi et al., 2012; Lee et al., 2016, 2018; Jeon et al., 2016, 2017; Göktürk et al., 2017). Jeon et al. (2016) tested the possible activity of essential oils from seven different plants on nymphs and adults of *Ricania* sp. This study results showed that the high insecticidal toxicity of *Tagetes erecta* L. (Asterales: Asteraceae) essential oils, after exposure for 72 h. The primary constituents in the essential oil of this plant were identified as caryophyllene, terpinolene, (*E*)-ocimenone, ocimene, piperitenone, and limonene (Sefidkon et al., 2004). In another study, it was suggested that the essential oils of *Salvia officinalis* Spenn. (Lamiales: Lamiaceae) killed 74.1% of *O. japonica* insects after 96 h of exposure and could be used as an effective means of control (Göktürk et al., 2017). In contrast, our tested plants (*C. campestris* and *L. albus*) showed a higher efficacy against nymphs (highest LT_{50} value 32.0 h with methanol in *C. campestris)* and adults (highest LT_{50} value 18.8 h with methanol in *L. albus*) after 72 h of exposure. In a study by Jeon et al. (2017), results showed that high insecticidal toxicity of *Cinnamomum cassia* L. (Laurales: Lauraceae) (LC_{50} value of nymph was 37.7 mg/L and of adult 77.4 mg/L) and *Cinnamomum verum* J. Presl (Laurales: Lauraceae) (LC_{50} value of nymph 72.6 mg/L and on adult 135 mg/L) essential oils on a *Ricania* sp. The GC-MS analysis of the plants showed that cinnamaldehyde contained in *C. cassia* and *C. zeylanicum* oils (80.2% and 46.3%, respectively) were effective against a *Ricania* sp. nymphs and adults. LC_{50} values for nymphs and adults were 31.3 and 62.4 mg/L, respectively, in another study by the same group. Lee et al. (2018) tested the insecticidal effects of *Valeriana officinalis* L. (Dipsacales: Caprifoliaceae) essential oils extracted by steam distillation, solvent, and supercritical extraction on a *Ricania* sp. As a result, they observed that the extract obtained by steam distillation had the highest mortality for a *Ricania* sp. adults (1.04 µg/mL) and nymphs (2.37 µg/mL). Our results showed high mortality of nymphs and adults after 72 h of exposure to a fixed dose (500 ppm) of crude extracts and LC tests results showed high activity at and below 2 g/L (LC_{90}) crude extracts for both plants. Previous studies on *Ricania* or *Orosanga* species control have generally included essential oils from different genera and native plant species *a*. Our study included two plant species grown in Turkey using crude extracts. Therefore, more detailed study of different distillation methods and essential oils is needed.

In general, when plants exhibiting insecticidal activity are examined, the concentration of alkaloids, phenolic and terpenoids compounds is high. Therefore, in the second part of our study, the phenolic contents

of *C. campestris* and *L. albus*, which showed insecticidal activity, were determined by HPLC-DAD. In the previous study of Selvi et al. (2017) on *C. campestris* extracts extracted by using ethyl acetate and methanol, they reported isorhamnetin (6.61 and 17.0 mg/g), quercetin (5.60 and 12.5 mg/g), and kaempferol (5.00 and 10.7 mg/g) as major phenolic compounds (Table 2). Similarly, ferulic acid, p-coumaric acid, p-OH benzoic acid, rutin and vanillic acid have been detected in the two extracts of *C. campestris* (Selvi et al., 2017).

Our results were similar to the results for *Cuscuta* sp. and *Lupinus* sp. reported in the literature. Previous studies with methanol extracts of *Cuscuta* spp. have commonly identified astragaline, chlorogenic acid, hyperoside, isorhamnetin, kaempferol, quercetin, and rutin as phenolic compounds within the extracts (Ye et al., 2005). Król et al. (2018) reported that ferulic acid, p-coumaric acid and sinapic acid are present in *Cuscuta* sp. In another study, *p*-coumaric acid derivatives and apigenin-6,8-di-*C*-glucoside were detected in the same plant (Siger et al., 2012). In a study by Karamac et al. (2018), apigenin, coumarin derivatives, ferulic acid, gallic acid, hesperidin, kaempferol, quercetin and vanillic acid were detected in HPLC-DAD analysis of *Lupinus s*p. The importance of kaempferol, quercetin and their derivatives for insecticidal activity has been reported by other authors (Upasani et al., 2003; Mendki et al., 2005). Upasani et al. (2003) reported excellent insecticidal activity of aqueous leaf extracts of *Ricinus communis* L. (Malpighiales: Euphorbiaceae) against *Callosobruchus chinensis* (L., 1758) (Coleoptera: Bruchidae). They concluded that the quercetin and kaempferol constituents are important for insecticidal activity. Similarly, Mendki et al. (2005) reported that the alcoholic foliar extract of *Calotropis procera* Aiton (Gentianales: Apocyneceae) gave a mixture of flavonoids including quercetin-3-O-gal (hyperoside) and kaempferol-3-O-rha or quercetin-3-O-ara and exhibited excellent insecticidal activity on *C. chinensis*. Huang et al. (2013) reported that ferulic acid and derivatives have potential because of their insecticidal activities. Hussain et al. (2018) reported that different applications of coumarin affect agricultural pests and concluded that coumarin and its derivatives are highly phytotoxic but that their performance in controlling insects has shown very promising results. Our HPLC-DAD results show a high concentration of kaempferol and quercetin after using two different extraction solvents, as well as different concentrations of coumarin and ferulic acid. The high efficacy of crude extracts that we observed may be explained by these constituents.

This research is the first examination of the insecticidal activities of *C. campestris* and *L. albus* crude extracts against *O. japonica* nymphs and adults. The results showed that extracts from both plants may be candidates for use as botanical-based insecticides. This study is also important for its evaluation of bioactive compounds from naturally growing plants in long-term control, as other Ricaniidae family species are seen as invasive species in Western Palearctic.

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