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Antifeedant effects of *Ferulago longistylis* extracts from Erzincan on *Ephestia kuehniella*

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Abstract

Ferulago longistylis Boiss. is an endemic species naturally grown in Eastern Anatolia Region especially Erzincan and Malatya provinces. In this study, antifeedant effects of chloroform, hexane and water extracts of *F. longistylis* obtained by using soxhlet extractor, against *Ephestia kuehniella* Zeller (Lepidoptera: Prelidae) was investigated. Both our country and all around the world, insect pests living in the stored products cause a serious decline in the quality of products. One of them is *Ephestia kuehniella* which is well-known and called as a flour moth. The use of plant extracts against insect pests is becoming increasingly popular. Accordingly, in this study, consumption of wheat flour eaten by *Ephestia kuehniella* larvae were determined by following. Larvae were cultivated in sterile petri dishes including 50, 100, 250 and 500 ppm for each hexane, chloroform and water extracts for 24 hours and the average of the difference between the final and initial weight of the petri dishes was determined as the amount of consumption. According to the results, the highest antifeedant effect was observed in the petri including 50 ppm water extract. Hexan extract at 50 ppm and doses of water extracts at 250 and 500 ppm showed low antifeedant effect when compared to the control. All of chloroform extracts showed similar effects to the control.

Key words: antifeedant, endemic, *Ephestia kuehniella*, Erzincan, *Ferulago longistylis*

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Erzincan ilinden toplanan *Ferulago longistylis* özütlerinin *Ephestia kuehniella* üzerindeki antifeedant etkisinin belirlenmesi

Özet

Ferulago longistylis BOISS. Türkiye'de Doğu Anadolu Bölgesinde özellikle Erzincan ve Malatya illerinde yayılış göstermektedir ve endemik bir bitkidir. Bu çalışmada *F. longistylis* bitkisinden soxhlet yöntemiyle elde edilen kloroform, hekzan ve su özütlerinin *Ephestia kuehniella* Zeller (Lepidoptera: Prelidae)'ya karşı beslenmeyi durdurucu (antifeedant) etkisi araştırılmıştır. Hem ülkemizde hem de dünyada depolanan ürünlerde görülen zararlı böcekler ürünlerin kalitesinde ciddi düşüşlere neden olmaktadır. Bu böceklerin arasında un güvesi olarak da bilinen *Ephestia kuehniella* önemli yer tutar. Zararlı böceklere karşı bitkisel ekstraktların kullanımı üzerine yapılan araştırmalar giderek artmaktadır. Bu amaçla, *Ephestia kuehniella* larvaları, besinleri olan buğday ununu ve 50, 100, 250 ve 500 ppm'lik derişimlerdeki hekzan, kloroform ve su ekstraktlarını içeren petrilerde 24 saat bekletildikten sonra, başlangıç ve son ağırlıkları arasındaki farkların ortalamaları alınarak tüketim miktarları belirlenmiştir. Elde edilen sonuçlara göre en yüksek antifeedant etki, su özütünün 50 ppm'lik derişiminde tespit edilmiştir. Hekzan özütünün 500 ppm, su özütünün 250 ve 500 ppm'lik derişimleri kontrole göre düşük antifeedant etki göstermiştir. Kloroform özütünün tüm derişimleri kontrole göre benzer etki göstermiştir.

Anahtar kelimeler: antifeedant, endemik, *Ephestia kuehniella*, Erzincan, *Ferulago longistylis*

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1. Introduction

The flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Prelidae) is harmful on flour both our country and all around the world. This pest causes a serious decline in the quality of flour and stored products **(**Dabbaoğlu, 2004). Nowadays, protection of agricultural products from production to consumption with minimal losses is also very important. Usually, losses in stored products caused by animal organisms is considered average 10% an annual and this rate is over the 10% in different regions **(**Çanakçıoğlu, 2010). Generally, insecticides are used to control of insects in storage in our country **(**Ferizli and Emekçi, 2010). Insecticides are chemicals and they have several advantages and disadvantages**.** Especially, their harmful impacts on the environment and non-target species cannot be ignored (Igbedioh, 1991). Repeated application of insecticides causes insecticide resistance on the pest and pesticide residues in storages and several health problems on practitioners. For these reasons, use of plant extracts against insect pests is becoming increasingly popular **(**Isman, 1995; Yankanchi and Gonugade, 2009; Rajopadhye et al., 2016). There are many studies about the use of plant extracts from various families against to insect pest (Liu et al., 2011; Özger et al., 2013; Akumefula et al., 2014; Metin and Bürün, 2015). Also, Apiaceae family is among these families, it is a cosmopolite family and it is the eighth largest family in Turkey (Yılmaz and Koyuncu, 2015). Nowadays, impacts of endemic species are being wondered. Although 37 of endemic species are endangered, endemism rate this family is 33% in Turkey (Özhatay et al., 2009). *F. longistylis* in Ferulago genus in Apiaceae family is endemic species for Turkey. Endemic plants in different geographies may have a stronger effect on pest control. Because of this, the aim of this study was evaluate the antifeedant effects of extracts of *F. longistylis* against stored product pest, *Ephestia kuehniella* larvae.

2. Materials and methods

Stored product pest species, *E. kuehniella* Zeller. (Lepidoptera: Prelidae) larvae were brought from Ankara University, Department of Plant Protection for the antifeedant test. Larvae of flour moth are 4^{th} instars. An endemic plant, *F. longistylis* is collected from Erzincan during 2014 to 2015 in Turkey. Plant sample is dried in the shade in the laboratory with good air space. Dried aerial parts of the plant are used to extraction. *F. longistylis* extract, employing different solvent were obtained. Dried aerial parts of the plant were powdered. A portion (20 gm; 5 gm portions into 3 cartridges) of dried plant material was extracted with hexane in Soxhlet apparatus. Hexane phases were combined and evaporated down to dryness. Residual plant material was then extracted methanol for 4 h and solvent was evaporated. Methanol extract was further partitioned with chloroform and water mixture (1:1) to obtain more polar fractions. Two fractions were separated and chloroform was evaporated. Water phase was frozen at -80 °C and lyophilized (Christ Alpha 1-2 LD plus). The potential of the antifeedant effect of the extract against to *E. kuehniella* was determined by the antifeedant test. Wheat flour were used as test food. Hexane, chloroform and water extracts were prepared in 1000 ml and mixed with 1 gr of the wheat flour at a concentration of 50, 100, 250 and 500 ppm for the test. Each test group was set up with petri dishes (6 cm in diam.) each including 10 larvae (4^{th} instar). The prepared three different solvent (chloroform, water and hexane) to test groups was set up with 3 replicates for each test dose and replicated over 3 days. Larvae were weighed in petri dishes and saved. Then 1 g of wheat flour with different extracts and dose are added to the center of the petri dishes and weighed and saved. In addition, one control group was also set up for each test solution. In total, three control groups were set up for each repeat. All test groups placed in climatic chamber (Aralab-FITOKLIMA D1200PLH) (Temperature 25 ± 1 C°, Humidity ± 65% and 14/10 Light/Dark photoperiod) After 24 h, it was weighed and recorded together with the petri dish. *E. keuhniella* larvae were removed from petri dishes and then weighted and recorded. This process was repeated for each of the three treatment days. The amount of food consumed was calculated depending on the initial fresh weight of each petri dishes with larvae. The antifeedant index was calculated according to following formula (Sadek, 2003).

$$AFI=[(C-T)/(C+T)] \times 100$$

C: as the consumption of flour in control groups and T: the consumption of flour in treated groups. The food consumed by the 10 larvae were given control groups were averaged, and the means were used as C for the calculations of the AFI for each observed.

The antifeedant indices at different treatments were compared using an analysis of ANOVA followed by Duncan test for multiple-comparison where significant differences were observed. A Pearson correlation coefficient test was carried to determine the AFI-Day and AFI-Dose relations between the treatments. All of these analysis performed with SPSS version 21.0 for Windows (Yang, 2014).

3. Results

Antifeedant effects of hexane, choloroform and water extracts of *F. longistylis* against *E. kuehniella* larvae were evaluated in this study. Results were compared with the control (Table 1). According to the results, 50 ppm hexane extract of *F. longistylis* had negative antifeedant effect to the control. The increasing concentration of hexane extracts of *F. longistylis* reduced the amount of consumption (Figure 1). Therefore, the highest antifeedant effect was

observed at concentration of 250 and 500 ppm (amount of consumption: 0.249 g) and this concentrations of hexane extract of *F. longistylis* showed similar effects when compared to control. Average consumption amount of flour from other hexane extract concentration of this species (50 and 100 ppm) were calculated 0.282 g, 0.260 g respectively (Table 1).

Antifeedant effect of increasing concentration of chloroform extracts of *F. longistylis* was not different. All concentration of chloroform extracts of *F. longistylis* showed similar antifeedant effect with the control. Average consumption amount of flour from all chloroform extract concentration of this species (50, 100 and 250 ppm) were calculated 0.231 gr (Table 1).

The highest antifeedant effect was determined in the *F. longistylis* at the concentration of 50 ppm amongst the tested water extract concentrations. Only the 50 ppm of water extracts of *F. longistylis* have positive antifeedant effect due to their lowest amount of consumption (0.217 gr). Consumption amount of flour from other hexane extract concentration of *F. longistylis* (100, 250 and 500 ppm) were calculated 0.233 gr, 0.240 gr and 0.242 gr respectively (Table 1). The amount of consumption also increased with increasing concentration and therefore antifeedant effect was decreased.

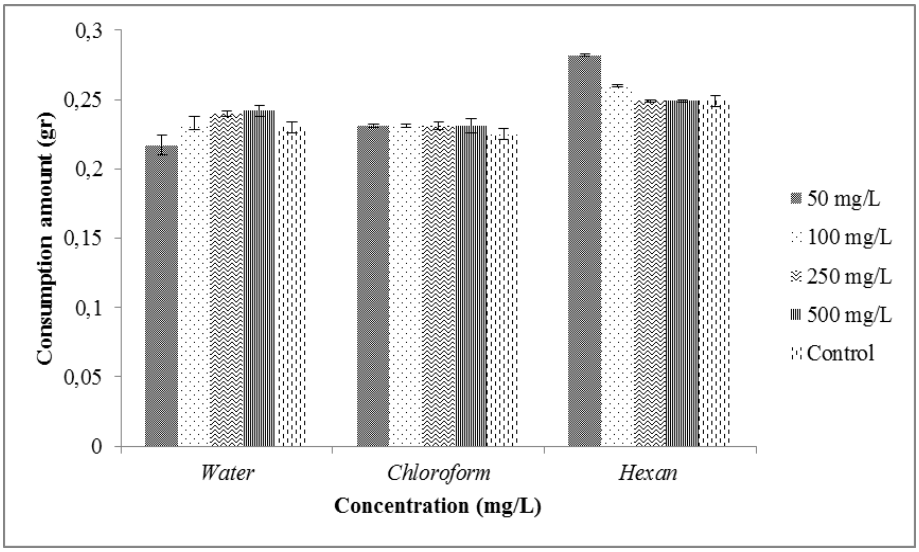

Figure 1. Consumption amounts of water, chloroform and hexan extracts (concentration of 50, 100, 250 and 500) of *F. longistylis.*

Finally, the highest antifeedant effect was observed at 50 ppm water extract. Doses of hexane extract at 50 ppm and water extracts at 250 and 500 ppm showed low antifeedant effects when compared to the control. All doses of chloroform extracts showed similar effects to the control. Increasing hexane extract concentrations of *F. longistylis* increases the antifeedant effect and increasing water extract concentrations declines the antifeedant effect.

Table 1. Consumption amounts of water, chloroform and hexane extracts of *F. longistylis*

	Concentration	Water	Chloroform	Hexane
F. longistylis	50 ppm	0,217±0,001a	0,231±0,003b	0,282±0,003c
	100 ppm	0,233±0,001b	0,231±0,004b	0,260±0,001b
	250 ppm	0,240±0,003c	0,231±0,002b	0,249±0,000a
	500 ppm	0,242±0,005c	0,231±0,001b	0,249±0,000a
	Control	0,230±0,004b	0,225±0,004a	0,249±0,007a

*Each values on concentrations are averages of three replicates. Similar letters in the same column are no different according to Duncan Multiple Comparison tests (P <0.005).

4. Conclusions and discussion

Antifeedant effects of hexane, chloroform and water extracts of *F. longistylis* against *Ephestia kuehniella* larvae were studied. Results were statistically variable and different from the control group. In the literature, there are not a study about antifeedant effect of this plant (*F. longistylis*). Therefore, the results compared with the results of obtained from different plant extracts. The toxic effects of ethanol extracts of several plant extracts (*Tamarix smyrnensis* Bunge, *Scorzonera mollis* Bieb, *Scorzonera tomentosa* L., *Reseda alba* L., *Linum bienne* Miller, *Artemisia santonicum* L., *Prunus laurocerasus* L. and *Laurus nobilis* L.) on the large diamondback moth, *Plutella xylostella* L. was investigated by Ertürk et al., (2004). They reported that ethanol extract of A*rtemisia santonicum* had antifeedant and toxic effects on *P. xylostella* but alcohol extracts of *T. smyrnensis*, *S.mollis*, *S. tomentosa*, *R. alba*, *L. bienne*, *P. laurocerasus* and *L. nobilis* did no toxic effects. In our study, especially 50 ppm concentration of water extract of *F.*

longistylis showed antifeedant effect on *E. kuehniella* larvae and the increasing concentration of hexane extracts of *F. longistylis* reduced the amount of consumption of food. Antifeedant and toxicity effects of some plant extracts belongs to different families (*Origanum vulgare* L., Family (Labiatae), *Buxus sempervirens* L., Family (Buxaceae), *Sambucus nigra* L., Family (Caprifoliaceae), *Aesculus hippocastanum* L, Family (Hippocastanaceae), *Hypericum perforatum* L., Family (Compositae), *Viscum album* L., Family (Loranthaceae), *Diospyros kaki* L., Family (Ebenaceae), *Ocimum basilicum* L., Family (Labiatae), *Alnus glutinosa* Goertn, Family (Betulaceae) and *Achilea biebersteinii* Willd., Family (Compositae) on *Lymantria dispar* L. (Lep: Lymantridae) on the 2-3rd instar larvae of the *L. dispar has* reported by Ertürk et al., 2006. In that study showed that *O. vulgare* and *A. hippocastanum* extracts have high antifeedant activity on the larvae of*L. dispar*andthe highest consumption of diet was observed with alcohol extract from *V. album* and the minimum consumption was also with alcohol extract from *B. sempervirens.* The other tested extracts showed similar activity in the same study. In our study, 4th instar larvae of E. *kuehniella* were used and the highest consumption was observed with water extract (50 ppm concentration) and the minimum consumption was with hexane extract (50 ppm concentration) from *F. longistylis.* Çakır and Kıvan, (2012) conducted a study to determine the effects of sodium chloride (NaCl) on the sunn pest, *Eurygaster integriceps* Put. (Heteroptera, Scutelleridae) feeding and using as an insecticide enhancer in the laboratory. Their results showed that NaCl had an arresting effect over the sunn pest. Similarly, increasing concentrations of hexane extracts of *F. longistylis* has antifeedant effect on *E. kuehniella* larvae. Özger et al., (2013) reported that the compounds derived from the neem tree are a type of bioinsecticide that can be used as an alternative to synthetic insecticides. Liang et al., (2003) studied application of agroneemt, ecozint and neemixt insecticide that are originated from neem on feeding of *Plutella xylostella* larvae. They reported that these insecticides have high antifeedant effect on *P. xylostella larvae.* The larvae that fed on leaves treated with neem extract turned out to be smaller than the larvae that fed on leaves treated with water. Similarly, in our study, *E. kuehniella* larvae that fed 50 ppm of water extracts of *F. longistylis* are smaller than larvae fed all concentrations of hexane and chloroform extract. Yorulmaz Salman et al., (2015) studied the contact toxicities of the extracts with hexane, ethanol and methanol of *Ocimum basilicum* L., *Thymus vulgaris* L., *Mentha spicata* L., *Melissa officinalis* L. and *Matricaria chamomilla* L. plants on adult, 3rd and 4th instar larvae of *Leptinotarsa decemlineata*. All of the plant extracts prepared with hexane, ethanol and methanol were found more effective in 3rd and 4th instar larvae of *L. decemlineata* than adult. The lowest effect was obtained from hexane extracts of *M. chamomilla* in their study, similarly the lowest effect was obtained from hexane extracts of *F. longistylis* in our study. Alkan and Gökçe, (2012) reported that hexane, ethyl acetate and methanol extracts of stem and flower of *Tanacetum abrotanifolium* caused reduction in feeding of both *Sitophilus oryzae* and *Sitophilus granarius* (Col.,Curculionidae), they known as granary weevil and rice weevil respectively. Karakoç ve Gökçe, (2013) indicated that antifeedant effects of 9 plant extracts on *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). They used aseton as a solvent and 4 of 9 plant extracts (*Delphinium consolida, Chrysanthemum segetum, Artemisia vulgaris, Tanacetum mucroniferium*) showed the strong antifeedant effect on *S. littoralis.* The highest antifeedant activity was seen on water extracts of 50 ppm concentration in our study. Antifeedant effect of *Salvia officinalis* and *Rosmarinus officinalis* extracts that they treated with imidakloprid, azadirachtin, *Bacillus thuringiensis* were tested on 4th instar larvae of *Leptinotarsa decemlineata* by Kara et al., (2014). The results indicate that bought plant extracts treated with azadirachtin have a potential in control of *L. decemlineata*.

Finally, antifeedant effects of concentration of 50, 100, 250 and 500 ppm of hexane, chloroform and water extracts of an endemic plant *F. longistylis* against *Ephestia kuehniella* larvae were different. Especially, concentration of 50 ppm water extracts of *F. longistylis* has strong antifeedant effect on *E. kuehniella* larvae. It can be used as an alternative control method. Otherwise, antifeedant effect of *F. longistylis* with the increase of the concentration of chloroform and hexane extracts increased. Therefore, in order to avoid loss of product during storage of agricultural products that consumed as food, plants which can be used for pest control and ıts concentrations should be determined instead of pesticides. Determination of the effective dose may occur expanding the use of herbal extracts at the same time scientifically important results and may reduce toxic effects.

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