



Effects of *Tanacetum cadmeum* ssp. *orientale* (Compositae)' EtOAc Extract Fractions on Feeding Behaviour of *Spodoptera littoralis* (Lepidoptera: Noctuidae)*

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Abstract: The antifeedant activity of *Tanacetum cadmeum* ssp. *orientale* (Compositae) against third instars larvae of *Spodoptera littoralis* Boisd. (Lep: Noctuidae) was evaluated using lettuce leaf disk bioassay. In the experiments, ethyl acetate (EtOAc) extract fractions of aerial parts of *T. cadmeum* ssp. *orientale* showed limited antifeedant activity with 24 h values of 17.64, 23.62, 6.97, 5.69 and 7.8% for fractions 1, 2+3, 4, 5 and 7, respectively. Any fraction did not deter feeding by the larvae. Similarly, no statistical differences were observed on body weight between the treated and control larvae after the experiment. In two cases, there was no significant decrease in growth with the short-term dipping of the fractions to the lettuce disks.

Key Words: *Tanacetum*, Compositae, *Spodoptera littoralis*, antifeedant activity

Tanacetum cadmeum ssp. *orientale* (Compositae)'nin EtOAc Ekstrakt Fraksiyonlarının *Spodoptera littoralis* (Lepidoptera: Noctuidae)'in Beslenme Davranışı Üzerindeki Etkileri

Özet: *Tanacetum cadmeum* ssp. *orientale* (Compositae)'nin beslenme engelleyici etkisi *Spodoptera littoralis* Boisd. (Lep: Noctuidae)'in 3. dönem larvalarına karşı marul yaprak disk bioassay yöntemi kullanılarak değerlendirilmiştir. Denemelerde 24 saat sonunda *T. cadmeum* ssp. *orientale*'nin toprak üstü aksamının etil asetat (EtOAc) fraksiyonları olan 1, 2+3, 4, 5 ve 7 sırasıyla % 17.64, % 23.62, % 6.97, % 5.69 ve % 7.8 ile sınırlı düzeyde beslenme engelleyici etkiler göstermiştir. Hiçbir fraksiyon larvaların beslenmesinde engelleyici etki göstermemiştir. Benzer şekilde, deneme sonrası uygulama ve kontrol larvaları arasında vücut ağırlıkları açısından istatistiksel farklılıklar gözlenmemiştir. Her iki durumda da, marul disklerine fraksiyonların kısa süreli daldırılması ile büyümede önemli azalma olmamıştır.

Anahtar Kelimeler: *Tanacetum*, Compositae, *Spodoptera littoralis*, beslenme engelleyici etki

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Introduction

Plants have been suggested as alternative sources of materials for insect control because they contain a range of bioactive chemicals (Wink, 1993) and are potentially suitable for use in integrated pest management (Schmutterer, 1992). Members of the genus *Tanacetum* L. (Asteraceae) comprise several medicinally important taxa. This genus is represented in Turkey by 44 species and altogether 59 taxa Gören et al. (2001). *Tanacetum* species have been used in insect control for centuries (Duke, 1985). Essential oils and extracts of members of the genus *Tanacetum* exhibit insecticidal and antifeedant effects (Panasiuk, 1984; Suomi et al., 1986; Nottingham et al., 1991; Hough-Golstein and Hahn, 1992; Susurluk et al., 2007). The genus is found to contain acetylenes and sesquiterpene lactones (Mahmood 2002; Susurluk et al. 2007) which are thought to be associated with the biological activity of *Tanacetum*. Recently, we demonstrated the antifeedant effects of *Tanacetum cadmeum* ssp. *cadmeum*, *T. parthenium*, *T. corymbosum* ssp. *cinereum*, *T. chiliophyllum* var. *chiliophyllum*, *T. kotschyi* and *T. cadmeum* ssp. *orientale* extracts on larvae of *S. littoralis*. Among the extracts tested, *T. cadmeum* ssp. *cadmeum*' total methanol extract and *T. corymbosum* ssp. *cinereum*' total methanol extracts showed significant antifeedant effects against larvae of *Spodoptera littoralis* (Susurluk et al. 2007). This paper is a part of the study which was carried out by Susurluk et al. (2007) that described with a laboratory study that assessed the antifeedant effects on *S. littoralis* larvae of the fractions of *T. cadmeum* ssp. *orientale* total EtOAc extract.

Material and Methods

Plant material and preparation of the extracts

Aerial parts of *T. cadmeum* ssp. *orientale* were collected and identified in the previous study (Susurluk et al. 2007). The fractions of the total EtOAc extract of the aerial parts of the plant were prepared and then tested at 1000 ppm concentrations. The preparing of the concentration is well documented in Susurluk et al. (2007).

Insects

Pupae of *S. littoralis* were brought from Israel Volcani Center. Cultures of *S. littoralis* were maintained at $25\pm 3^{\circ}\text{C}$, $65\pm 5\%$ RH, and a light regime of 16 h L: 8 h D on lettuce leaves in plastic cages in a culture room. All assays were conducted under the same conditions.

Antifeedant Activity

The fractions of the total EtOAc extract of *T. cadmeum* ssp. *orientale* were assayed by using lettuce leaf disks (3.8 cm in diameter) for antifeedant activities. Laboratory bioassays were performed using the third instar larvae of *S. littoralis* with the concentration of 1000 ppm of the extracts. The disks were dipped into the 1000 ppm of the extract solution for 5 seconds then left to dry for 1 h. Assays were performed in a plastic assay tray. The leaf disks and third instars *S. littoralis* larvae were randomly placed in the holes of the plastic tray. They were allowed to feed for 24 h. After this period, larvae were removed from the disks. Then the area of the leaf disks consumed by larvae was assessed and compared with control disks by using a LM 2000 software programme to determine antifeedant activity.

No mortality was observed in both treatment and control groups. The antifeedant activity was calculated as $[1 - (\% \text{ of treated food consumed} / \% \text{ of non-treated food consumed})] \times 100$ (Schmidt et al., 1997). For each fraction there were sixteen replicates each with 1 larva. In addition to this work, weights of control and treated larvae were measured to support to this experiment results after the experiment.

Statistical Analysis

Analysis of variance and comparison of the remaining leaf disk area means after being fed (24 h) were calculated by ANOVA (Breakdown and one-way Anova) and followed by Least Significant Difference (LSD) test ($P < 0.05$) as post hoc comparison of means of the remaining leaf area (Statistica, 1991), considering each larva as a replicate. Each experiment was carried out at different times. As a result, means were compared with only its control.

Results and Discussion

In order to assess their potential as natural pesticides against insects, we tested *T. cadmeum* ssp. *orientale* EtOAc extract' fractions. Antifeedant effects in bioassays of *T. cadmeum* ssp. *orientale* EtOAc fractions on *S. littoralis* larvae are given in Table 1. In the previous study, the total EtOAc extract of *T. cadmeum* ssp. *orientale* aerial parts showed antifeedant activity 19.12% (Table 1, Susurluk et al. 2007). But, its fractions **1**, **2+3**, **4**, **5** and **7** showed antifeedant activity 17.64, 23.62, 6.97, 5.69 and 7.8%, respectively (Table 1). Among the extracts, the mixture of fractions **2+3** (23.62% AA) was more active than the total EtOAc extract. But, no fractions of EtOAc extract of *T. cadmeum* ssp. *orientale* aerial parts affected leaf consumption ($P > 0.05$). These results support the results of larval weight measurement (Table 2). Thus, the all fractions of *T. cadmeum* ssp. *orientale* were not statistically different from its control (Table 1).

Table 1. Antifeedant activity against *S. littoralis* of the EtOAc extract and fractions isolated from *T. cadmeum* ssp. *orientale* aerial parts. Antifeedant activity $[1 - (\% \text{ of treated food consumed} / \% \text{ of non treated food consumed}) \times 100]$ of the extracts obtained from *T. cadmeum* ssp. *orientale* (n=16)

Extract (1000 ppm)	The remaining leaf area			AA (%)
	Fractions	Treated (mm ² ±S.E.)	Control (mm ² ±S.E.)	
<i>T. cadmeum</i> ssp. <i>orientale</i> ²	Total ¹	1347±60 a	1094.8±81 a	19.12
	1 ¹	1614.4± 66 a	1442.4± 67 a	17.64
	(2+3) ¹	1496.5± 88 a	1336.5± 74 a	23.62
	4 ¹	1640.3± 60 a	1583± 74 a	6.97
	5 ¹	1555.5± 83 a	1550.4± 63 a	5.69
	7 ¹	1553.4± 65 a	1509.4± 80 a	7.8

AA: Antifeedant Activity. Each extract was statistically compared with its control.

¹ Ethyl acetate extract; ² Aerial Parts; * ($P < 0.05$)

Table 2. Average body weights of control and treated *S. littoralis* larvae after the experiment

Extract (1000 ppm)	Average body weight of larvae		
	Fractions	Treated (g)	Control (g)
<i>T. cadmium</i> ssp. <i>orientale</i> ²	Total ¹	0.043±0.002 a	0.044±0.0032 a
	1 ¹	0.040± 0.00275 a	0.035±0.003 a
	(2+3) ¹	0.036± 0.00325 a	0.038±0.004 a
	4 ¹	0.036± 0.00275 a	0.036±0.0035 a
	5 ¹	0.031± 0.00225 a	0.032± 0.0035 a
	7 ¹	0.026± 0.00186 a	0.026± 0.0026 a

Each extract was statistically compared with its control.

¹Ethyl acetate extract; ²Aerial Parts; * ($P<0.05$)

Previous chemical investigations on different species of *Tanacetum* have shown the presence of acetylenes (Bohlman et al. 1965), flavonoides (Harborne et al. 1970), sesquiterpene lactones (Rustaiyan et al. 1990) and essential oils (Rustaiyan et al. 1999; Monfared et al. 2002; Majed-Jabari et al. 2002). In our previous study, the MeOH extract of *T. cadmeum* ssp. *cadmeum* aerial parts (62% AA) and the MeOH extract of *T. corymbosum* ssp. *cinereum* flowers (55.05% AA) showed antifeedant activity against *S. littoralis* larvae by lowering the consumption of the treated lettuce leaf disks. Artesin, taurin, artemin, tavulin, tanachin, tamirin, scopoletin, tanetin (6-hydroxykaempferol 3,7,4-trimethylether), 6-hydroxykaempferol 3,6-dimethylether were isolated from *T. cadmeum* ssp. *cadmeum* (Susurluk et al. 2007). The results showed that this group of compounds is responsible for the observed antifeedant effects. On the contrary, in this work, because the fractions of *T. cadmeum* ssp. *orientale* have low rates of antifeedant activity levels at concentration of 1000 ppm, chemical analysis of the fractions were not made.

The results of this study do not suggest promising potential for the use of *T. cadmeum* ssp. *orientale*' the fractions as antifeedants against third instar larvae of *S. littoralis*.

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