Phytochemistry and antifeedant activity of root extracts from some *Vincetoxicum* taxa against *Leptinotarsa decemlineata* and *Spodoptera littoralis*

Sevda Guzel, Roman Pavela and Gamze Kokdil

**ABSTRACT**

Antifeedant activities of dichloromethane, dichloromethane: methanol (1:1), methanol and total ethanol extracts of five *Vincetoxicum* N.M. Wolf taxa were investigated using leaf disc no-choice method against third stadium larvae of *Spodoptera littoralis* Bois. and *Leptinotarsa decemlineata* Say.. Further, phytochemical constituents were also screened qualitatively. Among the 20 tested extracts, 7 extracts against *L. decemlineata* larvae and 3 extracts against *S. littoralis* larvae showed 100 % antifeedant activity after exposure maximal dose. The activity results indicated that roots of studied *Vincetoxicum* taxa were effective against both tested pests. The dichloromethane and dichloromethane: methanol (1:1) extracts of *V. fuscatum* subsp. *boissieri* (Kusn) Browicz indicated the highest effectiveness against *L. decemlineata* larvae (16 μg/cm² ED₅₀ value) and CH₂Cl₂ extract of *V. canescens* subsp. *canescens* (Willd.) Decne. showed the highest effectiveness against *S. littoralis* larvae (ED₅₀ 48 μg/cm² value). Furthermore the growth inhibition and chronic toxicity on *S. littoralis* larvae were determined. The CH₂Cl₂ extract of *V. fuscatum* subsp. *boissieri* and MeOH: CH₂Cl₂(1:1) extract of *V. canescens* subsp. *canescens* displayed the highest effectiveness causing growth inhibition with ED₅₀ 0.04 mg/g and 0.09 mg/g respectively. Also the same extract of *V. fuscatum* sub-species *boissieri* produced chronic toxicity with the highest effectiveness lethal dose (LD₅₀ 1.11 mg/g value). Phytochemical studies showed the presence of steroidal glycosides, sugars and starch. The root of *V. fuscatum* subsp. *boissieri* also contained saponins.

**Key words:** *Vincetoxicum*, antifeedant activity, growth inhibition, chronic toxicity, *Spodoptera littoralis*, *Leptinotarsa decemlineata*.

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**INTRODUCTION**

Pests cause loss of crops in the fields (Kordali et al., 2007) and various control methods are used to protect fields from pest destruction (Xu et al., 2009; Scapinello et al., 2014). *Leptinotarsa decemlineata* Say., is the major pest in the world feeding on the leaves of plants belonging to Solanaceae family such as potatoes, tomatoes and eggplants (Aydin et al., 2004; Kordali et al., 2007). Because of reaching higly feeding rates with consuming 40cm² of potato leaves for a larva, and 9.65cm² of foliage per day for an adult, during all development stages the pest can cause nearly complete damage in the fields. High fecundity is another problem because of the female beetles laying 300–800 eggs each. Therefore, this oligophagous pest is still classified in the most important potato pests (Pavela, 2010a). Addition to these the pest can easily develop resistance to every chemical used against it (Pavela, 2010a; Ghassemi-Kahrizeha and Aramideh, 2015) and the last findings indicated that the pest developed resistance against approximately 52 different compounds known as important pesticides (Aydin et al., 2004). Therefore there is no certain control techniques developed against *L. decemlineata* and finding out new pesticides to avoid problems mentioned above.
is the need of the hour (Aydin et al., 2004). Previously, the antifeedant properties of pyrrolizidine alkaloids, cucurbitacins, silphinens, limonoids (Aydin et al., 2004) steroidal glycosides, glycoalkaloids (Soule et al., 1999); monoterpenes (Kordali et al., 2007) triterpenes such as epilimonol and limonin diosphenol (Wheeler and Isman, 2001); sesquiterpenes such as polygodial (Prota et al., 2014) have been reported against L. decemlineata in different studies. One of the totally polyphagous representatives S. littoralis (Pavela, 2011) known as army worm is an important pest species that has heavily damaged several economically important crops (Ballesta-Acosta et al., 2008) including flax, maize, rice, soybeans, tea (Pavela, 2010a), cotton, tobacco, and vegetables (Ballesta-Acosta et al., 2008; Pavela, 2010a) (tomato, pepper (Ballesta-Acosta et al., 2008), brassica, phaseolus etc.) (Pavela, 2010a) covering 40 families and at least 90 plants species (Pavela, 2010a; Pavela, 2011). The pest is widely distributed throughout the world (Pavela, 2011) and because of polyphagous life style the larvae of S. littoralis causes destructive damage in the fields (Ballesta-Acosta et al., 2008). The neurotoxic insecticides such as chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids are generally preferred to protect fields from pest infestation or to bring pest under control (Pavela, 2011). But these methods did not succeed as the pest easily adapted itself to various plant chemicals and acquired resistance against synthetic pesticides (Pavela, 2010a). In previous studies the antifeedant properties of oxypeucedanin, xanthotoxin, isoimperatorin and prangol as coumarins, eriocephalin, salvianocin, aethiopinone and oxocandesalvone, abietane, labdane and rosane as diterpenes and flavones (Ballesta-Acosta et al., 2008) have been reported against S. littoralis. Synthetic pesticides are very effective (Scapinello et al., 2014) but increasing application rates leads to several ecological problems (Pavela et al., 2014) including development of pest resistance (Aydin et al., 2004; Xu et al., 2009), environmental pollution such as contamination of air, soil and water (Scapinello et al., 2014; Pavela et al., 2014) and low degradation rates (Scapinello et al., 2014). Hence, there is a need for development of safer and environment friendly biopesticides with natural origin (Xu et al., 2009; Baskar and Ignacimuthu, 2012; Julio et al., 2014; Sarwar et al., 2015).

Recently, there is a growing interest in developing new alternative pesticides (Kordali et al., 2007; Carlos et al., 2013) and plant extracts have been used against pests control with different applications (Pavela, 2009a). Biological pesticides obtained from plants and plant-derived products (Xu et al., 2009) such as extracts and secondary metabolites are safer, friendlier and more efficient alternatives to synthetic pesticides (Sahaf et al., 2007; Carlos et al., 2013). Botanical pesticides containing a mixture of biologically active constituents are generally isolated from medicinal plants using various methods (Pavela, 2010a; Pavela, 2013).

The genus Vincetoxicum N.M. Wolf (Apocynaceae : subfamily Asclepiadoideae) (Heywood et al., 2007) was used in European and Chinese traditional medicine for the treatment of malaria, rupture, fever, wounds, injuries (Ditommaso et al., 2004; Weston et al., 2005; Mansoor et al., 2011; Sliumpaite et al., 2013). Antibacterial and antifungal (Zaidi and Crow, 2005; Mogg et al., 2008), antileishmanial, antimalarial (Mansoor et al., 2011), cytotoxic (Staerk et al., 2000; Staerk et al., 2002) antifeedant and growth inhibition (Pavela et al., 2010b) effects of Vincetoxicum species were reported in the literature. The genus Vincetoxicum is represented by 8 species, 10 taxa of which, three are endemic to Turkey (Browicz, 1978; Tanker et al., 2004). There is no investigation on biological activities of Vincetoxicum species growing wild in Turkey. Therefore, the aim of the current study was to evaluate insect antifeedant/insecticide activities of roots of five taxa (V. fuscatum subsp. fuscatum (Hornem) Reichb., V. fuscatum subsp. boissieri (Kusn) Browicz (endemic), V. canescens subsp. canescens (Willd.) Decne.,
V. canescens subsp. pedunculata Browicz (endemic) and V. parviflorum Decne. (endemic) against destructive pests Leptinotarsa decemlineata Say. (Coleoptera: Chrysomelidae) and Spodoptera littoralis Boisd. (Lepidoptera: Noctuidae) larvae. The plant materials were also screened qualitatively for their phytochemical constituents.

**MATERIALS AND METHODS**

**Plant material**

All the plant samples (see the photographs below) were collected from different regions of Turkey during the summer of 2009 and identification of the samples were performed by one of the authors (S. Güzel) and confirmation was done by Dr. Ahmet İlçim, Department of Biology, Faculty of Sciences, Mustafa Kemal University (Antakya, Turkey). Voucher specimens were stored in the Herbarium of the Faculty of Science, Mustafa Kemal University (MKUH). The locality of plant materials were given in Table 1 and locality information was based on the Flora of Turkey grid system (Table 1).

**Chemicals**

Methanol and ethanol were purchased from Merck (Germany) and dichloromethane was purchased from Sigma Chemical Company (USA). All chemicals used in the study were analytical-reagent grade (≥99.0%) and all samples and solutions were prepared by using distilled water. All experiments were performed by using freshly prepared solutions.

**Extraction procedure**

Air-dried roots of the plants were powdered mechanically and macerated three times with 3L of CH\textsubscript{2}Cl\textsubscript{2} (100 g of plant powder in 600 mL of CH\textsubscript{2}Cl\textsubscript{2}) at room temperature (23°C). Then the residues were macerated three times with 3 L of MeOH: CH\textsubscript{2}Cl\textsubscript{2} (1:1) (100 g of plant powder in 600 mL of MeOH: CH\textsubscript{2}Cl\textsubscript{2} (1:1)) and then three times with 3 L of MeOH (100 g of plant powder in 600 mL of MeOH) to give crude extracts (Staerk et al., 2002). For the preparation of total ethanol extracts powdered plant materials were dispersed twice with 720 mL of 96% ethanol (100 g of plant powder in 720 mL of EtOH), sonicated for 30 min. and left overnight to shake at room temperature (23°C) (Mogg et al., 2008). All suspensions were separately filtered using Watman No: 1 filter paper. After filtration, filtrates were collected and evaporated to yield dry extracts under reduced pressure using a vacuum evaporator (Heidolph- Rotar TLR 1000) at 35-40 °C. The crude extracts were stored in the dark at 4 °C until further use (Fig. 1).

**Phytochemical screening**

Plants extracts were qualitatively screened by using standard procedures. Borntrager's test was used to show presence of anthraquinons and Mayer's and Dragendorff's tests were used for alkaloids (Evans, 2002; Tanker et al., 1986). Coumarins were detected with ammoniacal solutions due to giving violet fluorescens at ultraviolet light (Evans, 2002; Sener et al., 1985). Molisch's, Selivanoff's and Fehling's solution tests were used to determine presence of sugars (Evans, 2002; Tanker et al., 1986). Organoleptic characters such as odour and taste, and microscopic characters such as secretory organs were tested for essential oil (Evans, 2002; Tanker and Tanker, 2003). Gelatin, FeCl\textsubscript{3} (Evans, 2002), stiasny reagent (Cubukcu, 1992; Baytop, 1980) and...
bromine water (Cubukcu, 1992) were used for tannins. Keller-Kiliani, Baljet and Liebermann-Burchard tests were used to determine presence of cardiac glycosides and steroids (Bruneton, 1999). Cyanogenic glycosides were tested with picric acid/sodium carbonate (Bruneton, 1999; Tanker et al., 1986). Starches were examined in the presence of iodine with deep blue colour (Bruneton, 1999; Sener et al., 1985). Presence of saponins were determined by using foam value (Bruneton, 1999; Cubukcu, 1992). Cyanidin test (Bruneton, 1999), dilute NH₃, Pb(Ac)₂ and FeCl₃ (Tanker et al., 1986) were used to detect flavonoids. Oil stain test was used to determine presence of fixed oil. Some solutions such as dilute H₂SO₄, Pb(Ac)₂, amilalcol and NaOH with HCl were used to detect anthocyanins (Tanker et al., 1986).

Bioassay
Insects

Leptinotarsa decemlineata larvae were used for experiments selected from a renewed annually colony of wild adults from potato fields, fed on potato Solanum tuberosum, cv. Agria. The experiments and the all colonies were carried out in an environmental chamber with under a 16:8 h light:dark photoperiod, at 25±2 °C and 90 ±10% r.h. The third stadium larvae (L-3) of L. decemlineata were chosen for the study. Spodoptera littoralis larvae used for experiments were collected from laboratory colonies of S. littoralis population (The Crop Research Institute, Research Team-Secondary Plant Metabolites in Crop Protection, Czech Republic) and reared on an artificial insect diet (Stonefly Industries, Bryan, TX, USA). The experiments and all colonies were carried out at 25±1 °C and under a 16:8 h light:dark photoperiod and pre-weighed, newly-molted S. littoralis early third instar larvae (L-3) were chosen for the study.

Chronic toxicity

The different polarity extracts from the root were evaluated for their chronic toxicity against early third instar larvae of S. littoralis. The experiments were performed by measuring mortality after 5 days and oral applications were used. 10 doses of plant extracts including 20, 15, 10, 5, 2.5, 1.5, 1.0, 0.5, 0.25 and 0.1 mg/g were contaminated with diets and larvae of S. littoralis were administered to determine lethal doses by these prepared diets (Pavela and Vrchotova, 2013). The diet administered to larvae was prepared as 200 mg of an extract was stirred into 7.0 ml of water, and 3.0 g of dry artificial insect diet was added after the extract had dissolved for preparing 10 g of contaminated diet of maximum dose (20 mg/g). Then a stirrer (mechanical agitator, 300 RPM, stirring time 5 min) was used to get completely homogenized mixture. For the control larvae only a diet with water was carried out. The prepared diets were given to new third instar larvae of S. littoralis in ad libitum. Larval mortality was evaluated 5-days after the experiments were performed. Four replications of 20-larvae were studied for each dose. All larvae of each replicate were transferred into plastic boxes (10 cm × 10 cm × 7 cm). The boxes were set in a growth chamber under a L16:D8 photoperiod, at 25 °C for 5 days. Finally, death was noted when there was no movement of the larvae by prodding with forceps (Pavela et al., 2010b).

Effect on larval growth

For determining the efficiency of the tested plant extracts on larval growth diets
containing extracts in 10 doses (3, 1.5, 1.0, 0.5, 0.25, 0.15, 0.1, 0.05, 0.025 and 0.01 mg/g) were administered to *S. littoralis* larvae. The preparation of the diets was identically done according to the method described above. The weight of 3rd instar larvae emerged newly were measured and then inserted into Petri dishes individually (6 cm in diameter). The larvae were administered with the contaminated diets ad libitum for 5 days. Subsequently, the weight of the larvae were determined, and the growth inhibition index was calculated based on the determined weight increments according to the formula given below. Twenty new larvae of the 3rd instar were tested for all prepared doses. The experiments were performed in the growth room (L16: D8, 25 °C) and replicated 4 times (Pavela et al., 2010b).

The formula: GI (%) = 100−[(T/C) * 100] C- weight increments of the larvae that consumed the control diet T- weight increments of the larvae that consumed the contaminated diet (Pavela et al., 2010b).

**Antifeedant activity**

The antifeedant activity of tested plant extracts against larvae of *L. decemlineata* and *S. littoralis* were performed by using no-choice test which design most closely approximates a practical application (Pavela et al., 2010b). The tested larvae were left without food before the experiments, always for 3 hours. The experiment itself was carried out in Petri dishes (9 cm in diameter). Damp filter paper was laid on the bottom of the dishes, and 4 disks, 1.5 cm in diameter each and prepared using cork borer from tomato leaves, were always placed on the filter paper. An adequate quantity of stock solution was dissolved in acetone to get 5.0, 4.0, 3.0, 2.0, 1.5, 1.0, 0.5, 0.25, and 0.1% (w/w) solutions. Ten µL of the solution was uniformly applied to every disk using an automatic dosing device, corresponding approximately to doses of 500, 400, 300, 200, 150, 100, 50, 25 and 10 µg cm⁻². Only solvent applied disks were used as a control. After applications, the leaf disks were left at rest for nearly 10 min to allow the solvent to evaporate. Afterwards, 2

starved larvae of both tested pests were put into the center of every dish. The entire experiments were performed in 15 repetitions. The experiments were stopped when nearly 90% of the leaf disks were consumed by the control larvae (about 7 h, and 25 °C). Then the consumed areas of the leaf disks were measured and compared with control disks by using a screener software program (unpublished) to evaluate antifeedant activity of tested plant extracts (Pavela, 2010a). From test data feeding deterrence index were calculated by using the following formula.

The formula: (FDI) = 100 * [(C−T)/(C + T)], where C and T are the control and treated leaf consumed by the insect (Pavela, 2010a).

**Statistical analysis**

Effective doses leading to 50% (ED₅₀) feeding or growth inhibition, and lethal doses leading to 50% (LD₅₀) larval mortality, including corresponding values within a 95% confidence limit (CI₉₅), were determined by using probit analysis (Finney, 1971). Before undertaking the analysis, the arsin √(x/100) was used to transform percentages. The EPA Probit Analysis Program (Version 1.5) was used for statistical determination (Pavela, 2010a).

**RESULTS AND DISCUSSIONS**

Roots of *V. fuscatum* subsp. *boissieri* showed saponins. Ethanol was used to achieve extract yields ranging between 20-27% (w/w), which is significantly more than yields achieved using MeOH or CH₂Cl₂ (Fig. 1).

![Fig 1. Yields of extracts from roots of studied Vincetoxicum taxa (1: *V. fuscatum* subsp. *fuscatum*; 2: *V. fuscatum* subsp. *boissieri*; 3: *V. canescens* subsp. *canescens*; 4: *V. canescens* subsp. *pedunculata*; 5: *V. parviflorum*). When feed treated with extracts at 500 µg/cm² was given to *L. decemlineata* or *S. littoralis* larvae, the entire tested extracts showed](image-url)
antifeedant efficacy against both pests (Table 2). However, comparing the estimated ED50 values, we found significant differences (significant at P<0.05 level, Chi values are listed in the table) both among individual plant species and between both pests. In 20 tested extracts, 7 extracts exhibited 100 % feeding deterrence index against third instar larvae of *L. decemlineata*. All the tested CH2Cl2 extracts showed significant feeding deterrence between the range of 92.7-100 %. CH2Cl2 extracts obtained from all the tested plants caused the highest antifeedant activity except *V. canescens* subsp. *pedunculata*. When the effectiveness of all the tested plants on *L. decemlineata* larvae was compared, *V. fuscatum* subsp. *boissieri* was found to be the most effective of three extracts including CH2Cl2, MeOH:CH2Cl2 (1:1) and total EtOH extracts caused the highest activity with 100 % and methanol extract caused activity with 98.5 %. Addition to these results *V. fuscatum* subsp. *fuscatum* was more effective than other three plants consisting of *V. parviflorum* and two subspecies of *V. canescens*. It was observed that the antifeedant activity of CH2Cl2 and MeOH:CH2Cl2 (1:1) extracts were 100 % and total EtOH and MeOH extracts were 95.3 % and 89.9 % respectively for *V. fuscatum* subsp. *fuscatum*. The other 9 extracts showed antifeedant activity in the range of 98.5-75.7 % while 4 extracts manifested lower than 50 % antifeedant activity (Table 2).

Against 3rd instar larvae of *S. littoralis* 3 extracts including MeOH:CH2Cl2 (1:1) extract of *V. parviflorum* and both CH2Cl2 and MeOH:CH2Cl2 (1:1) extracts of *V. canescens* subsp. *canescens* showed the highest antifeedant activity (significant at P<0.05 level, chi values are listed in the table). Furthermore, 14 extracts demonstrated antifeedant activity in the range of 89.9-58.2 % while 3 extracts showed lesser antifeedant activity (significant at P<0.05 level, chi values are listed in the table) (Table 2). Effective doses leading to 50 % (ED50) toxicity against *L. decemlineata* and *S. littoralis* larvae were evaluated by using extracts (Table 2) and different ED50 values were observed for the tested plants. Both CH2Cl2 and MeOH:CH2Cl2 (1:1) extracts of *V. fuscatum* subsp. *boissieri* indicated the highest effectiveness against *L. decemlineata* larvae with 16 μg/cm2 ED50 value. MeOH:CH2Cl2 (1:1), total EtOH and CH2Cl2 extracts of *V. fuscatum* subsp. *fuscatum* established different ED50 values with 21, 36 and 37 μg/cm2, respectively. Also 35 μg/cm2 ED50 value for CH2Cl2 extract of *V. canescens* subsp. *canescens* and 43 μg/cm2 ED50 value for total EtOH extract of *V. fuscatum* subsp. *boissieri* and MeOH:CH2Cl2 (1:1) extract of *V. parviflorum* were observed. Against *S. littoralis* larvae, CH2Cl2 and MeOH:CH2Cl2 (1:1) extracts of *V. canescens* subsp. *canescens* showed similar effectiveness with 48 μg/cm2 and 52 μg/cm2 ED50 values respectively. While MeOH:CH2Cl2 (1:1) extract of *V. fuscatum* subsp. *boissieri* caused effectiveness with ED50 78 μg/cm2 value, the methanolic extract of *V. canescens* subsp. *canescens* showed efficacy with 92 μg/cm2 value of ED50.

Table 1. List of plant materials, their origins, collecting times and voucher numbers.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Locality</th>
<th>Collecting time</th>
<th>Voucher number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. fuscatum</em> subsp.</td>
<td></td>
<td></td>
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<tr>
<td><em>fuscatum</em></td>
<td>B6: Kayseri, Pınarbaşı, Hınızır Mountain, 1800 m.</td>
<td>10.07.2009</td>
<td>MKUH 1315</td>
</tr>
<tr>
<td><em>V. fuscatum</em> subsp.</td>
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</tr>
<tr>
<td><em>boissieri</em> <em>canescens</em></td>
<td>A5: Amasya, Ferhat Mountain, 460 m.</td>
<td>12.06.2009</td>
<td>MKUH 1316</td>
</tr>
<tr>
<td>V. <em>canescens</em> subsp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>canescens</em></td>
<td>C6: Kahramanmaraş, Engizek Mountain, Fallow fields, 1000 m.</td>
<td>25.06.2009</td>
<td>MKUH 1283</td>
</tr>
<tr>
<td>V. <em>canescens</em> subsp.</td>
<td></td>
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</tr>
<tr>
<td><em>pedunculata</em></td>
<td>B3: Afyon, Dinar; Kumalar Mountain, 1500-1600 m.</td>
<td>06.05.2009</td>
<td>MKUH 1284</td>
</tr>
<tr>
<td>V. <em>parviflorum</em></td>
<td>A7: Trabzon, 1200 m.</td>
<td>05.07.2009</td>
<td>MKUH 1334</td>
</tr>
</tbody>
</table>

*Endemic taxon, *Locality information is based on the Flora of Turkey grid system
Table 2. Antifeedant activities and effective doses of root extracts on larvae of *L. decemlineata* and *S. littoralis*.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Extract</th>
<th>Feeding deterrence index (%) (^{a})</th>
<th>Effective doses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>After exposure maximal dose</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td><em>L. decemlineata</em></td>
<td><em>S. littoralis</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>500 µg/cm(^2)</td>
<td>500 µg/cm(^2)</td>
</tr>
<tr>
<td><em>Vincetoxicum fuscatum</em> subsp. <em>fuscatum</em></td>
<td>CH(<em>{2})Cl(</em>{2})</td>
<td>100.0±0.0</td>
<td>72.1±3.2</td>
</tr>
<tr>
<td></td>
<td>MeOH:CH(<em>{2})Cl(</em>{2}) (1:1)</td>
<td>100.0±0.0</td>
<td>81.3±2.3</td>
</tr>
<tr>
<td></td>
<td>MeOH</td>
<td>89.9±5.1</td>
<td>89.9±5.1</td>
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<tr>
<td></td>
<td>Total EtOH</td>
<td>95.3±7.5</td>
<td>60.3±5.2</td>
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<tr>
<td></td>
<td>CH(<em>{2})Cl(</em>{2})</td>
<td>100.0±0.0</td>
<td>87.5±2.3</td>
</tr>
<tr>
<td><em>Vincetoxicum fuscatum</em> subsp. <em>boissieri</em></td>
<td>MeOH:CH(<em>{2})Cl(</em>{2}) (1:1)</td>
<td>100.0±0.0</td>
<td>86.3±2.4</td>
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<tr>
<td></td>
<td>MeOH</td>
<td>98.5±6.2</td>
<td>48.5±6.2</td>
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<td></td>
<td>Total EtOH</td>
<td>100.0±0.0</td>
<td>87.3±5.2</td>
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<td><em>Vincetoxicum canescens</em> subsp. <em>canescens</em></td>
<td>CH(<em>{2})Cl(</em>{2})</td>
<td>100.0±0.0</td>
<td>100.0±0.0</td>
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<tr>
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<td>MeOH:CH(<em>{2})Cl(</em>{2}) (1:1)</td>
<td>90.4±6.8</td>
<td>100.0±0.0</td>
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<td>MeOH</td>
<td>45.2±3.9</td>
<td>80.9±2.9</td>
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<td>Total EtOH</td>
<td>75.7±3.1</td>
<td>58.2±2.3</td>
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<td><em>Vincetoxicum canescens</em> subsp. <em>pedunculata</em></td>
<td>MeOH:CH(<em>{2})Cl(</em>{2}) (1:1)</td>
<td>31.4±2.5</td>
<td>38.4±2.5</td>
</tr>
<tr>
<td></td>
<td>MeOH</td>
<td>49.2±5.3</td>
<td>86.2±2.3</td>
</tr>
<tr>
<td></td>
<td>Total EtOH</td>
<td>81.7±2.6</td>
<td>46.7±3.5</td>
</tr>
<tr>
<td><em>Vincetoxicum parviflorum</em></td>
<td>CH(<em>{2})Cl(</em>{2})</td>
<td>100.0±0.0</td>
<td>79.2±5.1</td>
</tr>
<tr>
<td></td>
<td>MeOH:CH(<em>{2})Cl(</em>{2}) (1:1)</td>
<td>96.4±2.4</td>
<td>100.0±0.0</td>
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<tr>
<td></td>
<td>MeOH</td>
<td>42.6±2.3</td>
<td>82.6±3.3</td>
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<tr>
<td></td>
<td>Total EtOH</td>
<td>87.1±5.2</td>
<td>64.5±3.1</td>
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</tbody>
</table>

Mean FDI (±S.E.), numbers present the deterrent effect. FDI = [(C×T)/(C + T)]×100, where C and T are the control and treated leaf consumed by the insect. \(^{a}\)Effective doses (in µg/cm\(^2\)) causing 50% (ED\(_{50}\)) feeding deterrence of *Leptinotarsa decemlineata* or *Spodoptera littoralis* larvae relative to the control. CI\(_{95}\): 95% confidence intervals were given in parenthesis. \(^{b}\)Chi-square value, significant at P<0.05 level. ND: Not determined.
The CH$_2$Cl$_2$ extract of V. fuscatum subsp. boissieri and MeOH:CH$_2$Cl$_2$ (1:1) extract of V. canescens subsp. canescens produced the highest effectiveness causing growth inhibition with ED$_{50}$ 0.04 mg/g and 0.09 mg/g values respectively. The same extract of V. fuscatum subsp. boissieri showed chronic toxicity with the highest effectiveness lethal dose (LD$_{50}$ 1.11 mg/g value). The CH$_2$Cl$_2$ extracts of V. canescens subsp. canescens, V. parviflorum and V. canescens subsp.

### Table 3

The effect of crude extracts of five *Vincetoxicum* taxa incorporated into larval diet on growth inhibition and chronic mortality of *S. littoralis* larvae. The extracts were in the diet for 5 days.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Extract</th>
<th>Growth inhibition</th>
<th>Chronic toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ED$<em>{50}$ (CI$</em>{95}$)$^a$ mg/g</td>
<td>Chi$^c$</td>
</tr>
<tr>
<td>V. fuscatum subsp.</td>
<td>CH$_2$Cl$_2$</td>
<td>0.40 (0.31-0.47)</td>
<td>0.258</td>
</tr>
<tr>
<td>fuscatum</td>
<td>MeOH:CH$_2$Cl$_2$ (1:1)</td>
<td>0.35 (0.27-0.43)</td>
<td>0.553</td>
</tr>
<tr>
<td></td>
<td>MeOH</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>V. fuscatum subsp.</td>
<td>CH$_2$Cl$_2$</td>
<td>0.72 (0.63-0.81)</td>
<td>1.005</td>
</tr>
<tr>
<td>boissieri</td>
<td>MeOH:CH$_2$Cl$_2$ (1:1)</td>
<td>0.37 (0.31-0.43)</td>
<td>0.958</td>
</tr>
<tr>
<td></td>
<td>MeOH</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>V. canescens subsp.</td>
<td>CH$_2$Cl$_2$</td>
<td>0.51 (0.49-0.61)</td>
<td>0.458</td>
</tr>
<tr>
<td>canescens</td>
<td>MeOH:CH$_2$Cl$_2$ (1:1)</td>
<td>0.12 (0.07-0.17)</td>
<td>1.235</td>
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<tr>
<td></td>
<td>MeOH</td>
<td>1.18 (1.02-1.45)</td>
<td>0.245</td>
</tr>
<tr>
<td>V. canescens subsp.</td>
<td>CH$_2$Cl$_2$</td>
<td>0.42 (0.37-0.47)</td>
<td>0.695</td>
</tr>
<tr>
<td>pedunculata</td>
<td>MeOH:CH$_2$Cl$_2$ (1:1)</td>
<td>0.15 (0.09-0.21)</td>
<td>0.459</td>
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<tr>
<td></td>
<td>MeOH</td>
<td>0.17 (0.07-0.26)</td>
<td>0.445</td>
</tr>
<tr>
<td>V. parviflorum</td>
<td>CH$_2$Cl$_2$</td>
<td>0.47 (0.38-0.57)</td>
<td>0.689</td>
</tr>
<tr>
<td></td>
<td>Total EtOH</td>
<td>0.43 (0.34-0.48)</td>
<td>1.245</td>
</tr>
<tr>
<td></td>
<td>Total EtOH</td>
<td>0.15 (0.07-0.22)</td>
<td>1.228</td>
</tr>
<tr>
<td></td>
<td>CH$_2$Cl$_2$</td>
<td>0.21 (0.15-0.26)</td>
<td>1.067</td>
</tr>
<tr>
<td></td>
<td>MeOH</td>
<td>1.04 (0.93-1.19)</td>
<td>1.024</td>
</tr>
<tr>
<td></td>
<td>Total EtOH</td>
<td>0.67 (0.58-0.77)</td>
<td>2.133</td>
</tr>
</tbody>
</table>

$^a$Effective doses causing 50% (ED$_{50}$) growth inhibition of *S. littoralis* larvae relative to the control. $^b$The lethal dose (LD$_{50}$) causing 50% mortality of larvae compared with the control. CI$_{95}$: The corresponding 95% confidence intervals were given in parenthesis. ND: Not determined.

In the present study antifeedant, growth and toxic activities of four different polarity extracts obtained from the roots of five *Vincetoxicum* taxa were studied against *L. decemlineata* and *S. littoralis*. The activity results clearly showed that roots of studied *Vincetoxicum* taxa were effective against both tested pests.

The antifeedant and growth inhibition effects of V. rossicum (Kleopow) Borhidi] and V. hirundinaria Medik. were reported by some authors (Mogg et al., 2008; Pavela et al., 2010b). Mogg et al. (2008) reported antinsect activity of the extract obtained from the root of V. rossicum against *Allantus cinctus* (L.), *Drepana arcuata* Wlk. and *Ostrinia nubilalis* Hbn. (Mogg et al., 2008). Methanolic extract of aerial parts of V. hirundinaria showed larvacidal activity on *Culex quinguefasciatus* Say larvae at maximal
tested dose of 1.000 ppm (Pavela, 2009b) and methanolic extract from the stem of the same plant showed antifeedant activity on L. decemlineata and S. littoralis larvae at maximal tested dose of 500 μg/cm². Furthermore against S. littoralis larvae V. hirundinaria obtained effective doses (ED₅₀) 11 μg/cm² and (ED₉₀) 99 μg/cm² as the highest effectiveness (Pavela, 2010a). Previous studies showed presence of triterpenoids (Lavault et al., 1999; Nowak and Kisiel, 2000), phenanthroindolizidine alkaloids (Staerk et al., 2002; Staerk et al., 2005; Mogg et al., 2008; Gibson et al., 2011) and steroids (Nowak and Kisiel, 2000) in all parts of the Vincetoxicum species. Also alkanols reported from aerial parts (Nowak and Kisiel, 2000), and volatile compounds, acetophenone (Lavault et al., 1999), saponins (Tanker et al., 2004) and sugars reported from roots (Stöckel et al., 1969) of the species. Additionally flavonoids, saponins, phenolics (Zaidi and Crow, 2005; Pavela, 2010a; Shah et al., 2011) and steroidal glycosides (Nowak and Kisiel, 2000; Pavela, 2010a) reported as another secondary metabolites of these species. In the present work we found that roots of the tested plants contained steroidal glycosides, sugars and starch. Moreover only the roots of V. fuscatum subsp. boissieri contained saponins.

Several plants in different families like Meliaceae (Caballero et al., 2008; Scapinello et al., 2014), Labiatae (Pavela, 2004; Pavela et al., 2014), Scrophulariaceae (Kostic et al., 2007), Leguminosae (Xu et al., 2009) Asclepiadaceae (Mogg et al., 2008; Pavela, 2010a; Pavela et al., 2010b) and Rutaceae (Ramkumar et al., 2015) were tested as a source of natural pesticides (Caballero et al., 2008; Xu et al., 2009; Scapinello et al., 2014). Literature data indicated that several constituents of different plants including alkaloids, (Mogg et al., 2008; Pavela, 2010a; Pavunraj et al., 2011) tannins (Lingathurai et al., 2011); saponins (Pavela, 2010a; Lingathurai et al., 2011); terpenes (Caballero et al., 2001; Kordali et al., 2007; Prota et al., 2014), essential oils (Kordali et al., 2007; Pavela, 2011); coumarins (Ballesta-Acosta et al., 2008; Lingathurai et al., 2011); quinones (Lingathurai et al., 2011; Pavela, 2013); phenolics (Pavela, 2010a; Pavela, 2013), flavonoids, phenolic acids (Pavela, 2013; Usha Rani and Pratyusha, 2014); steroids, aliphatic molecules, glycoalkaloids, iridoid glycosides, steroidal glycosides (Soule et al., 1999) and sugars reported (Pavela, 2013) were tested for their different insect activities including antifeedant, insecticide, antiovipositional and growth inhibition. Among these secondary metabolites the antifeedant and growth inhibition effects of coumarins (Pavela, 2010b), phenolics (Pavela et al., 2010b), alkaloids, terpenes (Wheeler and Isman, 2001; Aydin et al., 2004; Kordali et al., 2007; Pavela, 2010a) (diterpene: clerodanes (Simmonds et al., 1996; Caballero et al., 2001; Ballesta-Acosta et al., 2008), phenols and polyphenols (Pavela, 2010a) were determined against both S. littoralis and L. decemlineata.

In our study, phytochemical screening results indicated that roots of all tested Vincetoxicum taxa contained steroidal glycosides, sugars and starches. Additionally, only roots of V. fuscatum subsp. boissieri contained saponins. Steroidal glycosides and sugars were reported as antifeedant constituents by some authors (Soule et al., 1999; Pavela, 2013). Also when compared with constituents of other tested plants, presence of saponins as a different constituent was determined in the root of V. fuscatum subsp. boissieri which showed the highest antifeedant activity against L. decemlineata with 3 different polarity extracts and growth inhibition and toxic activity against S. littoralis larvae with the same polarity extract (CH₂Cl₂ extract). In the literature various insect activities of saponins were reported (Pavela, 2010a; Lingathurai et al., 2011). Finally, it was thought to be that these constituents may be responsible from the effectiveness of the plants against both tested pests. Perhaps saponins make V. fuscatum subsp. boissieri more effective than other tested plants. Saponins are promising resource for novel biological pesticides but there is little knowledge about chemical structure-
activity relationships of saponins in the literature (Saha et al., 2010a; Saha et al., 2010b). Some authors reported that azadirachtin, two tetracyclic triterpene and saponins isolated from *Azadirachta indica* act as an antifeedant, and inhibits enzymes of digestive and nervous system of insects (Sami and Shakoori, 2014; Sarwar, 2015).

When we compared extracts yields we observed that the total EtOH extracts were much more than other tested extracts. It was considered that ethanol may be preferred as a solvent to make extract of *Vincetoxicum* species to get commercially available biological pesticides.

Based on our findings, further evaluation of the most effective plant extracts is needed to find constituents which are responsible for activities. Extracts obtained from plants contain complex mixtures of various secondary metabolites (Pavela, 2010a). Following this comparison of effectiveness of extracts, isolation, structure elucidation of constituents and mechanisms of action are needed to discover new, safer, biodegradable and environmentally friendly commercial pesticides using *Vincetoxicum* species as a botanical source.

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Phytochemistry and antifedant activity

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Sevda Guzel¹, Roman Pavela² and Gamze Kokdil¹*

¹Mersin University, Faculty of Pharmacy, Department of Pharmacognosy, Yenisehir Campus, 33169 Mersin, Turkey
²Crop Research Institute, Prague 161 06, Rusyne, Czech Republic
*Corresponding author:
E-mail: gkokdil@gmail.com

Sevda Guzel¹, Roman Pavela² and Gamze Kokdil¹*

¹Mersin University, Faculty of Pharmacy, Department of Pharmacognosy, Yenisehir Campus, 33169 Mersin, Turkey
²Crop Research Institute, Prague 161 06, Rusyne, Czech Republic
*Corresponding author:
E-mail: gkokdil@gmail.com