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

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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 /cm^2 ED_{50} value) and CH_2Cl_2 extract of *V. canescens* subsp. *canescens* (Willd.) Decne. showed the highest effectiveness against *S. littoralis* larvae (ED_{50} 48 µg /cm^2 value). Furthermore the growth inhibition and chronic toxicity on *S. littoralis* larvae were determined. The CH_2Cl_2 extract of *V. fuscatum* subsp. *boissieri* and MeOH: CH_2Cl_2(1:1) extract of *V. canescens* subsp. *canescens* displayed the highest effectiveness causing growth inhibition with ED_{50} 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 letal dose (LD_{50} 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.

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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 40cm^2 of potato leaves for a larvae, and 9.65cm^2 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 oliphagous 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 famlies 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, salviacoccin, 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 bio-pesticides 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 bellow) 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_2Cl_2 (100 g of plant powder in 600 mL of CH_2Cl_2) at room temperature (23°C). Then the residues were macerated three times with 3 L of MeOH: CH_2Cl_2 (1:1) (100 g of plant powder in 600 mL of MeOH: CH_2Cl_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 precence 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 precence 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, $FeCl_3$ (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 precence 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 precence of iodine with deep blue colour (Bruneton, 1999; Sener *et al*., 1985). Precence of saponins were determined by using foam value (Bruneton, 1999; Cubukcu, 1992). Cyanidin test (Bruneton, 1999), dilue NH_3, $Pb(Ac)_2$ and $FeCl_3$ (Tanker *et al*., 1986) were used to detect flavonoids. Oil stain test was used to determine precence of fixed oil. Some solutions such as dilue H_2SO_4, $Pb(Ac)_2$, 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.

Chronical 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, 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% (ED_{50}) feeding or growth inhibition, and lethal doses leading to 50% (LD_{50}) larval mortality, including corresponding values within a 95% confidence limit (CI_{95}), 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 CH_2Cl_2 (Fig. 1).

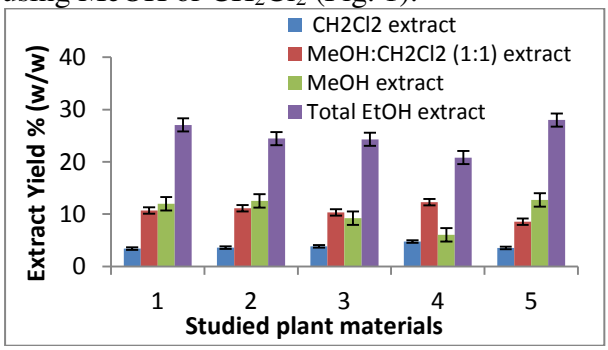

Fig 1. Yields of extracts from roots of studied *Vincetoxicum* taxa (1: *V. fuscatum* subsp. *fuscatum*; 2: *V. fuscatum* subsp. *boissieri*; 3: *V. canescens* subsp. *canescens*; 4: *V. cancescens* subsp. *pedunculata*; 5: *V. parviflorum.*

When feed treated with extracts at 500 µg/cm^2 was given to *L. decemlineata* or *S. littoralis* larvae, the entire tested extracts showed

antifeedant efficacy against both pests (Table 2). However, comparing the estimated ED_{50} 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 CH_2Cl_2 extracts showed significant feeding deterrence between the range of 92.7-100 %. CH_2Cl_2 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 CH_2Cl_2, MeOH:CH_2Cl_2 (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 CH_2Cl_2 and MeOH:CH_2Cl_2 (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:CH_2Cl_2 (1:1) extract of *V. parviflorum* and both CH_2Cl_2 and MeOH:CH_2Cl_2 (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 % (ED_{50}) toxicity against *L. decemlineata* and *S. littoralis* larvae were evaluated by using extracts (Table 2) and different ED_{50} values were observed for the tested plants. Both CH_2Cl_2 and MeOH:CH_2Cl_2 (1:1) extracts of *V. fuscatum* subsp. *boissieri* indicated the highest effectiveness against *L. decemlineata* larvae with 16 µg/cm^2 ED_{50} value. MeOH:CH_2Cl_2 (1:1), total EtOH and CH_2Cl_2 extracts of *V. fuscatum* subsp. *fuscatum* established different ED_{50} values with 21, 36 and 37 µg/cm^2, respectively. Also 35 µg/cm^2 ED_{50} value for CH_2Cl_2 extract of *V. canescens* subsp. *canescens* and 43 µg/cm^2 ED_{50} value for total EtOH extract of *V. fuscatum* subsp. *boissieri* and MeOH:CH_2Cl_2 (1:1) extract of *V. parviflorum* were observed. Against *S. littoralis* larvae, CH_2Cl_2 and MeOH:CH_2Cl_2 (1:1) extracts of *V. canescens* subsp. *canescens* showed similar effectiveness with 48 µg/cm^2 and 52 µg/cm^2 ED_{50} values respectively. While MeOH:CH_2Cl_2 (1:1) extract of *V. fuscatum* subsp. *boissieri* caused effectiveness with ED_{50} 78 µg/cm^2 value, the methanolic extract of *V. canescens* subsp. c*anescens* showed efficacy with 92 µg/cm^2 value of ED_{50} .

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

Taxon	Locality [a]	Collecting time	Voucher number
. *fuscatum* subsp. *fuscatum*	B6: Kayseri, Pınarbası, Hınzır Mountain, 1800 m.	10.07.2009	MKUH 1315
V. fuscatum subsp. *boissieri* *	A5: Amasya, Ferhat Mountain, 460 m.	12.06.2009	MKUH 1316
V. canescens subsp. *canescens*	C6: Kahramanmaraş, Engizek Mountain, Fallow fields, 1000 m.	25.06.2009	MKUH 1283
V. cancescens subsp. *pedunculata* *	B3: Afyon, Dinar; Kumalar Mountain, 1500-1600 m.	06.05.2009	MKUH 1284
V. parviflorum *	A7: Trabzon, 1200 m.	05.07.2009	MKUH 1334

*Endemic taxon, [a]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.*

Taxon	Extract	Feeding deterrence index (%)[a] After exposure maximal dose		Effective doses			
		L. decemlineata	*S. littoralis*	*L. decemlineata*		*S. littoralis*	
		500 µg/cm^2	500 µg/cm^2	ED_{50} (CI_{95})[b]	Chi[c]	ED_{50} (CI_{95})[b]	Chi[c]
Vincetoxicum fuscatum subsp. *fuscatum*	CH_2Cl_2	100.0±0.0	72.1±3.2	37(29-44)	3.289	123(110-132)	0.516
	MeOH:CH_2Cl_2 (1:1)	100.0±0.0	81.3±2.3	21(17-25)	4.216	269(258-278)	0.129
	MeOH	89.9±5.1	89.9±5.1	111(98-125)	1.926	112(102-122)	2.189
	Total EtOH	95.3±7.5	60.3±.5.2	36(30-42)	0.097	435(359-469)	0.256
Vincetoxicum fuscatum subsp. *boissieri*	CH_2Cl_2	100.0±0.0	87.5±2.3	16(12-19)	0.073	235(218-249)	0.151
	MeOH:CH_2Cl_2 (1:1)	100.0±0.0	86.3±2.4	16(9-21)	0.853	78(69-81)	1.358
	MeOH	98.5±6.2	48.5±6.2	66(60-71)	0.124	ND	-
	Total EtOH	100.0±0.0	87.3±5.2	43(36-50)	0.011	129(115-148)	0.312
Vincetoxicum canescens subsp. *canescens*	CH_2Cl_2	100.0±0.0	100.0±0.0	35(32-38)	0.125	48(42-49)	0.028
	MeOH:CH_2Cl_2 (1:1)	90.4±6.8	100.0±0.0	135(124-148)	3.181	52(48-61)	2.128
	MeOH	45.2±30.9	80.9±2.9	ND	-	92(87-101)	0.212
	Total EtOH	75.7±3.1	58.2±2.3	194(177-213)	1.532	258(195-295)	1.225
Vincetoxicum canescens subsp. *pedunculata*	CH_2Cl_2	92.7±5.2	59.7±3.2	158(144-173)	0.177	418(399-425)	2.115
	MeOH:CH_2Cl_2 (1:1)	31.4±2.5	38.4±2.5	ND	-	ND	-
	MeOH	49.2±5.3	86.2±2.3	ND	-	233(219-239)	1.125
	Total EtOH	81.7±2.6	46.7±3.5	167(128-202)	0.058	ND	-
Vincetoxicum parviflorum	CH_2Cl_2	100.0±0.0	79.2±5.1	95(70-113)	0.391	110(102-123)	2.119
	MeOH:CH_2Cl_2 (1:1)	96.3±2.4	100.0±0.0	43(40-51)	2.836	128(119-138)	0.989
	MeOH	42.6±2.3	82.6±3.3	ND	-	262(251-273)	0.215
	Total EtOH	87.1±5.2	64.5±3.1	222(195-250)	0.679	428(398-456)	0.785

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. [b]Effective doses (in µg/cm^2) causing 50% (ED_{50}) feeding deterrence of *Leptinotarsa decemlineata* or *Spodoptera littoralis* larvae relative to the control. CI95: 95% confidence intervals were given in parenthesis. [c]Chi-square value, significant at $P<0.05$ level. ND: Not determined.

he CH_2Cl_2 extract of *V. fuscatum* subsp. *boissieri* and MeOH:CH_2Cl_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 letal dose (LD_{50} 1.11 mg/g value). The CH_2Cl_2 extracts of *V. canescens* subsp. *canescens*, *V. parviflorum* and *V. canescens* subsp. *pedunculata* indicated significant effective doses between the range of 0.12-0.15 mg/g ED_{50} values. Moreover the dichloromethane and methanolic extracts obtained from *V. canescens* subsp. *canescens* were lead to chronic toxicity with LD_{50} 1.59 and 5.22 mg/g values respectively (Table 3). The MeOH:CH_2Cl_2 (1:1) extract of *V. fuscatum* subsp. *boissieri* caused chronic toxicity with 2.01 mg/g value of LD_{50}.

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

Taxon	Extract	Growth inhibition		Chronic toxicity	
		ED_{50} (CI_{95})[a] mg/g	Chi[c]	LD_{50} (CI_{95})[b] mg/g	Chi[c]
V. fuscatum subsp. *fuscatum*	CH_2Cl_2	0.40 (0.31-0.47)	0.258	2.93 (2.06-3.94)	1.025
	MeOH:CH_2Cl_2 (1:1)	0.35 (0.27-0.43)	0.553	4.81 (3.85-5.53)	0.256
	MeOH	ND		ND	
	Total EtOH	0.72 (0.63-0.81)	1.005	2.46 (1.93-2.54)	1.256
V. fuscatum subsp. *boissieri*	CH_2Cl_2	0.04 (0.01-0.08)	1.243	1.11 (1.09-1.99)	0.265
	MeOH:CH_2Cl_2 (1:1)	0.37 (0.31-0.43)	0.958	2.01 (1.53-2.97)	1.223
	MeOH	ND		ND	
	Total EtOH	0.51 (0.49-0.61)	0.458	3.01 (2.59-4.71)	0.699
V. canescens subsp. *canescens*	CH_2Cl_2	0.12 (0.07-0.17)	1.235	1.59 (1.09-3.11)	0.915
	MeOH:CH_2Cl_2 (1:1)	0.09 (0.03-0.11)	2.053	2.14 (1.29-3.94)	0.158
	MeOH	1.18 (1.02-1.45)	0.245	5.22 (4.49-6.27)	0.112
	Total EtOH	0.42 (0.37-0.47)	0.695	1.79 (1.42-2.17)	0.205
V. canescens subsp. *pedunculata*	CH_2Cl_2	0.15 (0.09-0.21)	0.459	2.34 (1.84-3.35)	0.861
	MeOH:CH_2Cl_2 (1:1)	0.17 (0.07-0.26)	0.445	5.01 (4.32-6.28)	1.055
	MeOH	0.47 (0.38-0.57)	0.689	5.54 (4.33-6.75)	0.521
	Total EtOH	0.43 (0.34-0.48)	1.245	2.08 (1.56-2.38)	0.896
V. parviflorum	CH_2Cl_2	0.15 (0.07-0.22)	1.228	5.63 (4.71-6.91)	1.213
	MeOH:CH_2Cl_2 (1:1)	0.21 (0.15-0.26)	1.067	2.06 (1.42-3.72)	2.504
	MeOH	1.04 (0.93-1.19)	1.024	3.12 (2.44-4.53)	0.231
	Total EtOH	0.67 (0.58-0.77)	2.133	2.96 (2.17-3.86)	0.331

[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* (Kleo.) Barb. [syn: *Cynanchum rossicum* (Kleopow) Borhidi] and *V. hirundinaria* Medik. were reported by some authours (Mogg *et al.,* 2008; Pavela *et al.*, 2010b). Mogg *et al*. (2008) reported anti-insect 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/cm^2. Furthermore against *S. littoralis* larvae *V. hirundinaria* obtained effective doses (ED_{50}) 11 μg/cm^2 and (ED_{90}) 99 μg/cm^2 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 (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, 2010a), flavonoids (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 (CH_2Cl_2 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 enviromentally friendly commercial pesticides using *Vincetoxicum* species as a botanical source.

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