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Scientific paper

Antifeeding Activity of Several Plant Extracts Against *Lymantria dispar* L. (Lepidoptera: Lymantriidae) Larvae

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SUMMARY

Lymantria dispar L. is the most devastating polyphagous pest of deciduous forests, orchards and urban greenery. To prevent damages that *L. dispar* larvae cause in forestry, agriculture and horticulture, mechanical measures and the use of biological insecticides are the most frequently applied practices. However, the use of conventional insecticides is inevitable in crop protection and forest management on smaller areas, especially in gradation years. However, inadequate use of these chemicals has led to disturbance of biocoenotic balance, outbreaks of some previously less harmful organisms and pesticide residues in soil and watercourses in some regions. To mitigate these consequences it is necessary to harmonize *L. dispar* control with integrated management principles by applying selective and less toxic insecticides. Therefore, the potential of botanical insecticides and antifeeding substances is gaining in importance. The aim of this study was to assess the influence of ethanol extracts (1, 2 and 5%) of *Ambrosia artemisiifolia* L., *Erigeron canadensis* L., *Daucus carota* L., *Morus alba* L. and *Aesculus hippocastanum* L. on the feeding intensity of *L. dispar* larvae, i.e. to evaluate their antifeeding activity under the conditions of "no-choice" test. Ten larvae per repetition were placed in Petri dishes and offered oak leaf slices (2 x 9 cm^2/repetition) previously dipped in plant extract or ethanol (1, 2, and 5%) for the control. Feeding intensity, expressed as a percentage of consumed leaf area (%), was measured after 48 h. For assessing the antifeeding activity of plant extracts AFI was calculated and the extracts were classified according to scale: no antifeeding activity, slight antifeeding activity, moderate antifeeding activity and strong antifeeding activity. Data were analyzed using a two-way ANOVA and Duncan`s multiple range test. The results indicate that plant species, i.e. the origin of extracts, had a significant influence on the feeding intensity of *L. dispar* larvae, while concentration and interaction (plant species x concentration) were not factors of influence. *Ae. hippocastanum* and *M. alba* extracts significantly reduced the consumed leaf area (6.24, 18.93%, respectively), compared to the control (97.59%), while the extract of *D. carota* had a phagostimulative effect (98.88%). Based on AFI values, *Ae. hippocastanum* extract (87.10-89.05%) had a strong antifeeding activity, and *M. alba* medium-to-strong (64.33-71.37%).

Keywords: Gypsy moth; Plant extracts; Antifeedants; Insecticides

INTRODUCTION

Gypsy moth *(Lymantria dispar* L.) belongs to a group of economically most important pests of deciduous forests, but it is also very harmful in orchards and urban greenery, causing defoliation, especially in gradation years and when protection measures are not adequate (Almaši et al., 2004; Mihajlović, 2004, 2008; Tabaković-Tošić, 2006; Kostić et al., 2008; McEwan and Rieske, 2009).

To prevent losses that *L. dispar* larvae cause in forestry, agriculture and horticulture, mechanical measures and the use of biological insecticides (*Bacillus thuringiensis* var *kurstakii*) are the most frequently applied practices. However, the use of conventional insecticides is inevitable in crop protection and forest management on smaller areas, especially in gradation years. According to Kamaraj et al. (2008) and Kaushik et al. (2009), inadequate use of these chemicals has resulted in disturbed biocoenotic balance, outbreaks of some previously less harmful organisms and presence of pesticide residues in soil and watercourses in some regions. To mitigate these consequences, it is necessary to harmonize pest control strategies with the principles of integrated pest management, using selective and less toxic insecticides (Kostić et al., 2008). The Ministries of Agriculture and National Environmental Protection Agencies in most EU countries have developed programmes for reducing the use of conventional insecticides in which preference is given to non-chemical and biological preparations in agriculture, forestry and communal hygiene (Bočarov-Stančić, 2002; Vuković et al., 2002). Also, due to increased environmental demands, the promotion of pest control agents of botanical origin (plant-based insecticides) is gaining in importance regarding the control of *L. dispar* in organic and sustainable agriculture. Plant secondary metabolites (terpenoids, alkaloids, saponins, polyphenols and phenolic glycosides) and their derivatives are known to be effective repellents for a number of harmful insects (Milanović, 2006; Erturk et al., 2006; Shields et al., 2006). For *L. dispar*, repellent activity is especially common by plants with high alkaloid contents (Dosktotch, 1980). In recent years, antifeeding effects of various plant extracts have been studied by several authors (Zabel et al., 2002; Milanović, 2006; Kostić, 2008; Gvozdenac et al., 2010, 2011; Pavela, 2010). According to Simmonds (2000), more than 6,250 plants have been tested for different insecticidal activities since 1985, while Parkash and Rao (1997) confirmed that about 870 herbal products with insecticidal or repellent antifeeding effect were used in agriculture. Botanical insecticides have many advantages, primarily low toxicity and selectivity towards non-target, and high toxicity to target organisms (Etrurk et al., 2006; Kostić et al., 2008). Also, unlike synthetic insecticides, plant-based preparations contain many biologically active compounds, which make it unlikely that insects would acquire resistance to the entire biologically active complex. Some previously reported findings indicate the antifeedant or repellent activity of the following extracts against *L. dispar* larvae: *Malaleuca leucadendron* (Doskotch, 1980), *Robinia pseudoacacia* L. (Barbosa and Krischik, 1987), *Fraxinus pennsylvanica* Marsh, which is effective only under a slight increase in population abundance (Marković et al., 1996); root of *Jeffersonia dubia* (Park et al., 1997); the essential oil of *Myristica fragrans* Houtte with a strong antifeeding but no toxic effect on larvae (Zabel et al., 2000); *Origanum vulgare* L., *Buxus sempriverans* L. and *Sambucus nigra* L. (Erturk et al., 2006a), *Ocimum basilicum* (Kostić et al., 2008), *Morus alba* L. and *Aesculus hippocastanum* L. (Gvozdenac et al., 2010, 2011).

The aim of this study was to assess the effect of ethanol extracts of three cultivated plants (*Daucus carota* L., *Morus alba* L. and *Aesculus hippocastanum* L.) and two weeds (*Ambrosia artemisiifolia* L. and *Erigeron canadensis* L.) on the feeding intensity of *L. dispar* larvae, i.e. to evaluate their antifeeding activity. The plants for bioassay were chosen based on their registered antifeeding or phagostimulative activity to insects that belong to the order Lepidoptera. A basic assumption was that similar effects would be achieved for this pest insect as well.

MATERIAL AND METHODS

Extracts preparation

In this work, ethanol extracts of *Ambrosia artemisiifolia* L., *Erigeron canadensis* L., *Daucus carota* L., *Morus alba* L. and *Aesculus hippocastanum* L. were used. Plants were collected during the vegetation period in 2009. Only leaves were used for extracts of *M. alba* and *Ae. hippocastanum*, while whole plants were used for the other three species. Plant material (10.0 g) was extracted with 70% ethanol (100.0 ml) as a solvent. The extraction was carried out using ultrasonic bath at room temperature for 1 hour. After filtration, 5 ml of liquid

extract was used for extraction yield determination. The solvent was removed by rotary evaporator under vacuum, and dried at 60°C until constant mass. Dry extracts were stored for the analysis in glass bottles at 4°C to prevent oxidative damage (Anonymous,1984).

Prior to application, the extracts were diluted in distilled water to concentrations of 1, 2 and 5%, and then applied to oak leaf slices (30x30 mm) by soaking for 5 sec. Ethanol (1, 2 and 5%) was used for the control variant. Leaves were then air-dried for 20 sec.

L. dispar rearing

Egg hatches of a field population of *L. dispar* were collected from oak trees (Kupinovo site) during the winter of 2009/10 and were kept refrigerated at around 3°C until the experiment was set. The eggs were separated from hatch and placed in glass tubes (Ø 1 cm, height 16 cm) (previously filled with water up to ¼ of volume) on a layer of cotton wool (making no contact with water), and than closed with cotton wool cover to prevent the larvae from leaving the tube after hatching. The tubes with eggs were incubated at 22-25°C under standard light regime for 3-5 days. After hatching, L_1 larvae were isolated from tubes with a soft brush, placed in Petri dishes, and daily fed with fresh *Q. robur* leaves until the stage L_2/ L_3.

"No-choice" test

The effect of extracts on the feeding intensity of *L. dispar* larvae was assessed in a "no-choice" test in the laboratory. Ten larvae (L_2/L_3 ratio 1:1) per replication were placed into Petri dishes (Ø14) containing two oak leaf slices (30x30 mm) previously treated with plant extracts or ethanol (1, 2, 5%) for the control. The „no-choice" test was carried out at room temperature (22-25°C) and under the standard light regime of 16:8 h (L:D). Feeding intensity, expressed as a percentage of consumed leaf area (%) was measured after 48 h. The experiment was set up in four replications.

For assessing the antifeeding activity of plant extracts, AFI (antifeeding index) was calculated according to Farrar et al. (1989):

AFI = (C–T) / (C + T) x 100

AFI- Antifeeding index; C- consumed area in the control variant (%); T- consumed area in treatments (%)

The criterion according to Liu et al. (2007) was applied to categorize the plants:

AFI <20% - no antifeeding activity (–)
50%> AFI ≥ 20% - slightly antifeeding activity (+)
70%> AFI ≥ 50% - medium antifeeding activity (++)
AFI ≥ 70% - strong antifeeding activity (+++)

Statistical analysis

The data for consumed leaf area (%) were subjected to a two-way analysis of variance (ANOVA) to evaluate the effect of two factors (plant species and concentration) and their interaction. Duncan`s multiple range test was used to assess the significance of differences between treatments, but only for the factor that had significant influence on feeding intensity. All tests were performed at the level of significance of 95% (STATISTICA.10).

RESULTS

Two-way ANOVA

The results of the two-way ANOVA (Table 1) indicate that only plant species, i.e. the origin of extract, had a statistically significant effect (source of variation) on the percentage of consumed leaf area (F=334,967**, P< 0.01). Concentration and interaction of the two factors (plant species x concentration) were not factors of influence (F=0.707 and 0.731, respectively, P>0.05).

Table 1. Two-way ANOVA for the effect of different plant species, concentrations and their interaction on feeding intensity of *L. dispar* larvae

Source of variation	SS	df	MS	F
Plant species	**109056**	**5**	**21811.2**	**334.967****
Concentration	92.1	2	46	0.707 NS
Plant species x concentration	475.9	10	47.6	0.731 NS
Error	3581.3	55	65.1	

NS-P>0.05, *P<0.05; ** P<0.01; SS-sum of sqares; df-degrees of freedom; MS-median;

The effect of plant extracts on consumed leaf area (%)

The area of oak leaf slices consumed by *L. dispar* larvae differed significantly depending on plant species (Table 2). Regardless of extract concentration, the highest percentage of consumed leaf area after 48 h was recorded in the variants with leaves treated with *D. carota* extract (98.88%), control (97.59%), *E. canadensis* (96.89%) and *A. artemisiifolia* (90.05%), and differences were not statistically significant. However, larval feeding in treatments with oak leaf slices soaked in the extracts of *M. alba* and *Ae. hippocastanum* was highly significantly reduced, regardless of concentration. Larvae consumed only 18.93% of leaf area in the treatment with *M. alba* extract and 6.24% in the treatment with *Ae. hippocastanum*, and the difference between these two treatments was also highly significant. The results indicate that *M. alba* and *Ae. hippocastanum* extracts, regardless of their concentration, reduced the feeding of *L. dispar* larvae as expressed by the percentage of consumed leaf area in the „no-choice" test.

Antifeeding activity of plant extracts

According to AFI values (Table 3), the extract of *Ae. hippocastanum* (87.1-89.05) had strong (+++) antifeeding activity against *L. dispar* larvae, regardless of concentration. The antifeeding effect of *M. alba* extract applied at concentrations of 1% (64.33%) and 2% (66.97%) was medium (++), while it was strong (+++) at 5% concentration (71.37%). The extracts of *A. artemisiifolia*, *E. canadensis* and *D. carota* were not found to demonstrate antifeeding activity at any applied concentration (AFI ranged from 0.34 to 7.21%).

Table 3. Antifeeding activity of the tested ethanol extracts

Plant species	AFI			Antifeeding activity (1; 2; 5% conc)
	1%	2%	5%	
A. artemisifolia	7.21	4.22	0.80	– – –
E. canadensis	1.20	3.16	0.78	– – –
D. carotta	0.50	1.14	0.34	– – –
Ae. hippocastanum	87.10	87.78	89.05	+++ +++ +++
M. alba	64.33	66.97	71.37	++ ++ +++

DISCUSSION

Polyphagous herbivorous insects are usually exposed to a wide range of different potential hosts. The selection of a suitable host plant depends on its physical and chemical characteristics. Unfavoured species possess quantitative defense mechanisms, which include anatomical, morphological and chemical characteristics such as low nutrient content (Lazarević et al., 2002) and presence of antifeeding components with repellent or phagodeterrent activity (Marković et al., 1996). A plant-derived compound that possesses antifeeding activity can be used to control target pest insects (Mansson, 2005).

The most favorable composition of nutrients, secondary metabolites and other biochemical compounds is certainly contained in the primary host. According to Shields et al. (2006), *L. dispar* best develops on species belonging to the genus *Quercus* (Janković, 1958), especially *Q. robur*, *Q. cerris* (Milanović, 2006) and/or *Q. rubra*. Previous research by Gvozdenac et al. (2010, 2011) has also indicated that *L. dispar* larvae feed most intensively on *Q. robur* as their primary host, which was confirmed in this work.

Table 2. The effect of plant extracts on feeding intensity (consumed leaf area - %) of *L. dispar* larvae after 48 h

Plant extract	Consumed leaf area (%)			Average	F value
	Concentration				
	1%	2%	5%		
A. artemisifolia	84.46 ± 17.68 aA	89.67 ± 12.23 aA	96.03 ± 4.28 aA	90.05 A	0.839 NS
E. canadensis	99.96 ± 0.04 aA	91.60 ± 4.14 bA	99.11 ± 1.56 aA	96.89 A	12.996**
D. carotta	98.56 ± 2.83 aA	99.84 ± 2.83 aA	98.25 ± 3.06 aA	98.88 A	0.016 NS
Ae. hippocastanum	6.73 ± 5.88 aC	6.35 ± 6.85 aC	5.65 ± 1.08 aC	6.24 C	0.043 NS
M. alba	21.18 ± 14.1 aB	19.30 ± 4.78 aB	6.30 ± 14.08 a B	18.93 B	0.173 NS
*Control (5 % ethanol)**	97.58 ± 3.18 aA	97.59 ± 3.52 aA	97.60 ± 2.14 aA	97.59 A	1.084 NS
Average (%)	68.03 a	67.39 a	68.82 a		
F value	76.92 **	164. 81 **	205.35 **		

The results are Means±SD; Mean values with the same lowercase letters indicate the same level of significance in rows-between concentrations (α=0.05); Mean values with the same uppercase letters indicate the same level of significance in columns-between plant species (α=0.05); NS P>0.05, *P<0.05; ** P<0.01;

*The results are presented only for the 5 % ethanol control since there were no significant differences in consumed leaf area between treatments with 1, 2 and 5 % ethanol

The reduced feeding intensity of *L. dispar* larvae on leaves treated with the extracts of *Ae. hippocastanum* in our work is in consistence with data reported by Erturk et al. (2006), who pointed out a strong antifeeding activity of this species on *L. dispar* larvae, and by Gvozdenac et al. (2010, 2011). According to Marković et al. (1996), antifeeding effect is due to the presence of esculin, which is also a main component of *Fraxinus ornus*, another well known antifeedant for *L. dispar* larvae.

The ethanol extract of *M. alba* also expressed antifeeding activity in our bioassay. These results are consistent with reports from laboratory feeding tests on the development of *L. dispar* larvae (Miller and Hanson, 1994), which suggested that plants from the genus *Morus* were unsuitable for larval development and even indicated a high mortality of younger larvae and reduced feeding intensity by fifth instars' caterpillars. Antifeeding activity, whether repellent or phagodeterrent, is probably based on a high content of glycosides. According to Pelletier (1996) over 250 glycosides have been isolated from *M. alba* leaves, out of which nine belong to the group of deoxynojirimycin that was confirmed to have inhibitory effect on phytophagous larvae of *Spodpotera frugiperda*, also a member of the order Lepidoptera.

L. dispar larvae in our study expressed a feeding affinity towards oak leaves treated with *A. artemisiifolia* extract, especially at 5% concentration. Similar results were presented by Pavela and Chermenskaya (2004) in an experiment with 18 plant species, showing an absence of antifeeding effect of *A. artemisiifolia* on *S. littoralis* larvae. The active components detected in *A. artemisiifolia* leaves are triterpene derivatives (ALPHA-amyrin acetate and BETA-amyrin acetate) and caffeic acid derivatives, i.e. chlorogenic acid and 3.5-dicaffeoylquinic acid (Tamura, 2004), while Rugutt et al. (2001) detected the presence of sesquiterpenic lactones ambrosin, isabelin, psilostachyin, cumanin and peruvin with confirmed biological activity.

Extracts of *E. canadensis* are known as effective attractants due to their high contents of secondary metabolites, primarily sesquiterpenoid lactones. The results of this work showed that the extract of *E. canadensis* increased the attractiveness of primary host leaves, although the same extract was found by Tvedten (2006) to be repellent for *Popillia japonica* Newman. Also, Cao et al. (2004) pointed out a negative effect on the survival and eclosion of *Diaeretiella rapae* McIntosh adults. In this work, more intensive larval feeding was recorded in treatment with *D. carota* extract than in the control. Due to a high content of sesquiterpens in it, this plant is known as an attractant for a number of beneficial insects of the family Chripsohidae (Miller and Hanson, 1994), lacewings, ladybugs, hoverflies, mini-wasps, (Pirate/Damsel/Big-eyed Bugs), assassin bugs, as well as pests such as *Papilio machaon* L. (Hagen, 2003).

Since medicinal herbs contain many biologically active substances and complexes, it is difficult to define which one has antifeeding activity without a detailed chemical analysis (Pavela, 2010). In this paper we assessed only the antifeeding activity (repellent or phagodeterrent) of several chosen plant species, while further studies are required to clarify the biochemical basis of these effects.

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Antifiding aktivnost nekoliko biljnih ekstrakata za gusenice *Lymantria dispar* L. (Lepidoptera: Lymantriidae)

REZIME

Lymantria dispar L. je ekonomski najznačajnija polifagna štetočina listopadnih šuma, voćnjaka i urbanog zelenila. Mehaničke mere i upotreba bioloških insekticida su najčešće primenjivane mere zaštite s ciljem smanjenja šteta koje prouzrokuju gusenice svojom pojavom. Međutim, na manjim površinama, a posebno u godinama gradacije, neizostavna je upotreba konvencionalnih insekticida u zaštiti bilja i šumarstvu. Višegodišnja neodgovarajuća primena ove grupe insekticida je dovela do narušavanja ravnoteže u biocenozi, prenamonženja ranije manje štetnih organizama, pojave rezistentnosti štetnih vrsta na insekticide, kao i prisustva rezidua pesticida u zemljištu i vodotokovima. U cilju smanjenja štetnih posledica neophodno je uskladiti suzbijanje sa principima integralnog pristupa smanjenju brojnosti gubara, primenom selektivih i manje toksičnih insekticida. Naime, sve više se istražuje potencijal botaničkih insekticida i antifiding sredstava. Cilj ovog rada je bio da se proceni uticaj etanolnih ekstraktata *Ambrosia artemisiifolia* L., *Erigeron canadensis* L., *Daucus carota* L., *Morus alba* L. i *Aesculus hippocastanum* L. na intenzitet ishrane gusenica *L. dispar*, kao i njihove antifiding aktivnosti u uslovima "no-choice" testa. Ogled je realizovan u laboratorijskim uslovima. Po 10 larvi po ponavljanju je stavljeno u petri-posude i ponuđena su im po dva isečka lista hrasta (2 x 9 cm^2/ponavljanju), potopljena u biljne ekstrakte, odnoso u 1, 2 i 5% rastvor etanola u kontroli. Intenzitet ishrane, iskazan preko procenta konzumirane površine, je meren nakon 48 h. Za procenu antifiding aktivnosti, izračunat je AFI (antifeeding index), a biljni ekstrakti su kategorizovani prema već utvrđenoj skali: nema, slabo, srednje i jako antifiding dejstvo. Podaci su statistički obrađeni dvofaktorijalnom analizom varijanse, uz primenu Dankanovog testa višestrukih poređenja. Rezultati rada ukazuju da je značajan uticaj na konzumiranu površinu lista imalo poreklo biljnog ekstrakta, dok koncentracija i interakcija dva faktora (biljna vrsta x koncentracija), nisu statistički značajno uticale na vrednost konzumirane površine. Ekstrakti *Ae. hippocastanum* i *M. alba* statistički su značajno smanjili procenat konzumirane površine (6.24, 18.93%, respektivno) u poređenju sa kontrolom (97.59%), dok je ekstrakt *D. carota* ispoljio fagostimulativni efekat (98.88%). Na osnovu AFI vrednosti, ekstrakt *Ae. hippocastanum* (87.10-89.05%) je ispoljio jaku antifiding aktivnost, a *M. alba* srednju do jaku (64.33-71.37%).

Ključne reči: Gubar; biljni ekstrakti; antifiding; insekticidi