J. Entomol. Res. Soc., 23(3): 197-212, 2021 *Research Article*
Doi: 10.51963/jers.v23i3.1989 *Online ISSN:2651-3579*

Antifeeding and Insecticidal Activity of *Ailanthus altissima* and *Morus alba* Extracts Against Gipsy Moth (*Lymantria dispar* (L.), Lepidoptera, Lymantridae) Larvae Under Laboratory Conditions

Snežana TANASKOVIĆ[1] Sonja GVOZDENAC[2] Radenka KOLAROV[3]
Vojislava BURSIĆ[4] Bojan KONSTANTINOVIĆ[5] Dejan PRVULOVIĆ[6*]

[1]Faculty of Agronomy, University of Kragujevac, Cara Dušana 34, 32102 Čačak, SERBIA
[2]Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, SERBIA
[3,4,5,6]Faculty of Agriculture, University of Novi Sad,
trg Dositeja Obradovića 8, 21000 Novi Sad, SERBIA
e-mails: [1]stanasko@kg.ac.rs, [2]gvozdenacsonja@gmail.com,
[3]kolar.rada@gmail.com, [4]bursicv@polj.uns.ac.rs, [5]bojank@polj.uns.ac.rs,
[6*]dejanp@polj.uns.ac.rs
ORCID IDs: [1]0000-0002-7853-2328, [2]0000-0001-9366-5172, [3]0000-0003-1976-7618,
[4]0000-0001-8331-095X, [5]0000-0001-5071-1386, [6]0000-0002-1880-4934

ABSTRACT

Gypsy moth (*Lymantria dispar* (L.) is one of most important defoliating pests of deciduous trees. Due to increased environmental demands, the use of plant-based preparations is gaining in importance as a control option for this pest in forestry, agriculture and horticulture. The aim of this study was to evaluate antifeeding and insecticidal activity of 0.5, 1 and 2% extracts of *Ailanthus altissima* bark and leaves, and *Morus alba* leaves, against *L. dispar larvae under* laboratory conditions. Antioxidant capacity of plant extracts was determined, as well as the content of phenolic compounds by spectrophotometric and HPLC-DAD methods. Antifeeding and insecticidal effects were tested in a "no-choice" test. The highest content of all bioactive phenolic compounds was in *A. altissima* bark and *M. alba* leaf extracts. The lowest leaf consumption after 24 and 48 h was in *A. altissima* bark (5.03, 9.30%, respectively) and *M. alba* leaf (1.44, 3.22%, respectively) extracts. *A. altissima* bark and *M. alba* leaf extracts expressed strong antifeeding activity. After 24 h, all extracts expressed slight insecticidal effect (2.25-17.50% of mortality). The mortality increased after 48 h in treatments with *A. altissima* bark extract, at all applied concentrations (40.0-57.50%) and *M. alb*a leaves at 1 and 2% concentrations (30.0-62.50%). Our results indicate that extracts of *A. altissima* bark and *M. alba* leaves may act as effective low-cost natural protectants able to control the presence of gypsy moth in ecosystems. Extracts of *A. altissima* bark and *M. alba* leaves expressed strong antifeeding activity and significant insecticidal effect on gypsy moth larvae, at all applied concentrations.

Key words: Lymantria dispar (L.), botanical insecticides, feeding intensity, larval mortality, phenolic compounds.

Tanasković, S., Gvozdenac, S., Kolarov, R., Bursić, V., Konstantinović, B., & Prvulović, D. (2021). Antifeeding and insecticidal activity of *Ailanthus altissima* and *Morus alba* extracts against gipsy moth (*Lymantria dispar* [L.], Lepidoptera, Lymantridae) larvae under laboratory conditions. *Journal of the Entomological Research Society*, 23(3), 197-212.

INTRODUCTION

Gypsy moth *(Lymantria dispar* (L.), Lepidoptera; Lymantridae) is one of the most devastating defoliating pests of deciduous forests, but it is also very harmful in orchards and urban greenery (Kostić, Popović, Brkić & Milanović, 2008; Milanović et al, 2014; Cao et al, 2015). To prevent losses that gypsy moth larvae cause in forestry, agriculture and horticulture, it is necessary to harmonize pest control strategies with the principles of integrated pest management, using selective and less toxic insecticides, mechanical measures and biological insecticides (*Bacillus thuringiensis* var. *kurstaki* or botanical insecticides like azadirachtin) (Kostić et al, 2008; Singh, Cheema & Singh, 2020). Koul (2005) reported growing interest in use of botanical preparations (i.e. plant-based insecticides) for the control of gypsy moth in organic and sustainable agriculture due to increased environmental demands. Botanical insecticides based on secondary plant metabolites represent an important group of biopesticides (Drobnjaković et al, 2018).

Plants are a rich source of compounds that exhibit high biological activity against harmful insects, thus botanical preparations are good potential substitutes for synthetic insecticides in agricultural and forest pest control (Bohinc et al., 2020; Šućur et al, 2015; Hikal, Baeshen & Said-Al Ahl, 2017; Gvozdenac, Šućur, Manojlović, Prvulović & Malenčić, 2018). In recent years, antifeeding effects of various plants and plant extracts have been studied for gypsy moth larvae and several findings confirm the antifeedant or repellent activity of *Pinus taeda* L., *Juniperus virginiana* L., *Acer rubrum* L. (Keena & Richards, 2020), *Ocimum basilicum* L. (Kostić et al, 2008; Popović et al, 2013), *Morus alba* L. and *Aesculus hippocastanum* L. (Gvozdenac, Inđić, Vuković, Grahovac & Tanasković, 2012). Botanical insecticides have many advantages, primarily low toxicity and selectivity towards non-target, and high toxicity to target organisms and also the capacity to avoid the occurrence of insect resistance and biodegradability (Kostić et al, 2008; Krinski, Massaroli & Machado, 2014).

Ailanthus altissima (Mill.) Swingle, commonly known as the “tree of heaven”, is a perennial invasive species, native to China and introduced in Europe at the end of 18th century (Kowarik *&* Säumel, 2007; De Feo, Mancini, Voto, Curini & Digilio, 2009). The extracts and essential oils obtained from different parts of *A. altissima* have been reported to exhibit diverse biological activities, such as antioxidant (Aissani, Jabri, Mabrouk & Sebai, 2018), phytotoxic (Albouchi, Hassen, Casabianca & Hosni, 2013; Caser et al, 2020; Kozuharova, Benbassat, Berkov & Ionkova, 2020), antimicrobial (Albouchi et al, 2013; Aissani et al, 2018; Kozuharova et al, 2020), insecticidal (De Feo, Mancini, Voto, Curini & Digilio, 2009; Pavela, Zabka, Tylova & Kresinova, 2014), and also different pharmacological effects (Rahman, Rasool & Imran, 2019). *Morus alba* L., white mulberry, is native to eastern and central China, India and Japan (Kostić et al, 2013). The leaves of *M. alba* are the main source of food for silkworm, thus white mulberry is cultivated throughout the world, wherever silkworms are or were reared. In European countries it is grown also for fruit production (Sanghi & Mushtaq, 2017). The leaves of *M. alba* are traditionally used in Asian countries as a treatment for coughs, fever, sore and inflamed eyes, sore throats, headaches, dizziness and vertigo. Modern medicine proves the antidiabetic, antioxidant, anticancer, antimicrobial,

hepatoprotective and neuroprotective activity of *M. alba* extracts (Singh et al, 2013; Sanghi & Mushtaq, 2017; Thaipitakwong, Numhom & Aramwit, 2018). However, the reports on potential use of white mulberry extracts in pest management are scarce.

The aim of this study was to evaluate biological activity and assess effects of aqueous extracts of *A. altissima* leaves and bark, and *M. alba* leaves on feeding intensity (antifeeding activity) and mortality of *gypsy moth larvae in* laboratory conditions.

MATERIAL AND METHODS

Plant extracts

For bioassay, extracts of *Ailanthus altissima* (Mill.) Swingle bark and leaves, and *Morus alba* L. leaves were used. Plant material was collected on Suvobor (44.1339° N, 20.2195° E) and Jeljen (44.0007° N, 20.2589° E) mountain (Serbia). The plant species was identified by a botanist, and a voucher specimen has been deposited in the Herbarium (labelled as *A. altissima* - 17708 and *M. alba* - 17709) of Botanical Garden, Belgrade, Serbia.

Plant parts were previously dried at dark and windy place, at 20 °C. 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 h. Extracts were diluted in distilled water to concentrations of 0.5, 1 and 2%, and applied to oak leaf slices (30x30 mm) by soaking method for 5 sec. Leaf slices soaked in distilled water were untreated control. Leaves were air-dried for 20 sec after the extract application.

Chemical analysis of plant extracts

Total phenolic, total tannin and antioxidant capacity

Components of non-enzyme antioxidative system were determined using spectrophotometric methods with UV/VIS spectrophotometer (model Evolution 220, Thermo Fisher Scientific, USA).

Total phenol content (TP) was determined by colorimetric method using Folin-Ciocalteu reagent (Saha et al, 2013). Total tannin content (TT) was determined by the Folin-Ciocalteu procedure, after removal of tannins by their adsorption on insoluble matrix PVPP (polyvinylpolypyrrolidone). Calculated values were subtracted from total phenols content. Results were expressed as micrograms of quercetin equivalents per 1 mL of plant extracts (μg QE/mL).

Free radicals scavenging activity was tested in a DPPH (2,2-diphenyl-1-picrylhydrazyl) acetone solution (Lai & Lim, 2011). Ferric reducing antioxidant power (FRAP) assay was performed according to the method of Valeñtao et al (2002). The scavenging activity of the plant extracts on $ABTS^{+}$ radical (2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) was measured according to the method of Re et al (1999). The total antioxidant activity (TAA) of plant extracts were evaluated by phosphomolybdenum method as reported by Kalaskar & Surana (2014). The standard curve for antioxidant

capacity (activity) was plotted using ascorbic acid (AsA) solution. Superoxide dismutase (SOD) mimetic activity was assayed according to the method of Mandal, Mitra & Mallick (2008) slightly modified by measuring ability of plant extracts to inhibit photochemical reduction of nitro blue tetrazolium (NBT) chloride. One unit of the SOD mimetic activity was defined as the amount of enzymes required to inhibit reduction of NBT by 50%. The activity was expressed as IU per 1 mL of plant extracts.

HPLC-DAD analysis of phenolic compounds

All solvents used were of chromatography grade and obtained from J.T. Baker (Deventer, Netherlands). The analytical standards (manufactured by Sigma-Aldrich) used in the research are: trans-cinnamic acid (99.0%), 2-hydroxy cinnamic acid (97.0%), caffeic acid (98.0%), p-coumaric acid (98.0%), chlorogenic acid (95.0%), quercetin (98.0%), and kaempferol (97.0%). The stock standard solutions were prepared by dissolving an analytical standard in methanol while the working solution i.e. the mixture of the studied phenol compounds was obtained by mixing and diluting the stock standards with mobile phase resulting in the final mass concentration of 100 μg/mL. The composite mixtures of all phenol compounds at appropriate concentrations were used to spike samples in validation data settings. Acetic acid was of “pure for analysis” grade (J.T. Baker, Deventer, Netherlands).

The chromatographic separation of phenolic compounds was achieved using the Agilent 1100 (Agilent Technologies, USA) HPLC system with a binary pump and diode array detector - DAD. The phenolic acids were separated on a ZORBAX SB-Aq (5 μm particle size: 4.6 x 250 mm, Agilent) column. The extracts were filtered through 0.45 μm syringe filters and directly injected through a 30 μl fixed loop into the column. The mobile phase was acetonitrile with 2.0% acetic acid (solvent A) and Milli-Q water with 2.0% acetic acid (solvent B) in gradient mode, with the flow rate of 1.0 mL/min, equipped with a ZORBAX SB-Aq column. The gradient was as follows: 92% A at 0 min, 80% A at 18 min, 60% A at 25 min, 55% A at 30 min, 35% A at 40 min and 20% A at 42 min. Stop time was 2.5 min.

The detector linearity response was checked by preparing the blank plant extract sample (bark and leaves separately) and after the extraction the residue was diluted in 1.5 mL of the phenol compounds mixture standard in mass concentrations of 10.0, 25.0, 50.0 and 100.0 μg/mL.

The extracts were filtered through 0.45 μm syringe filters and directly injected into the HPLC-DAD. The repeatability of the method was determined by analyzing the sample of the same mass concentration level (10.0 μg/mL) in six replicates and shown as the relative standard deviation - RSD (%). The detection limit (LOD) was defined as the amount of phenolic compounds which produces the signal three times the noise signal. The quantification limit (LOQ) is the amount of phenolic compounds produces a signal ten times the noise signal. The LODs were determined by adding 100 μL of phenols mixture standard to the concentration of 1.0 μg/mL, in 0.5 g of the sample in six replicates and the LOQs was calculated.

Bioassay

Insects collecting and rearing

Egg masses of gypsy moth field population were collected from oak trees (Apatin, northern Serbia) during the winter 2018/19 and kept in a refrigerator (3 ºC) until the beginning of the experiment. Eggs were separated from mass and placed in glass tubes (Ø 1cm, height 16cm), previously filled with water up to ¼ of volume, on a layer of cotton wool (which does not have the contact with water). Tubes were closed with cotton wool cover to prevent the larvae from leaving the tube after hatching. Tubes with eggs were incubated at 22-25 ºC and a normal light regime for 3-5 days. After hatching, L_1 larvae were isolated from the tubes with a soft brush, placed in Petri dishes and fed daily with fresh *Quercus robur* leaves, until the stage L_2/ L_3.

Feeding intensity and insecticidal effects

Feeding intensity of extracts was assessed in a “no-choice” test. Ten larvae (L_2/ L_3 ratio 1:1) per replication were introduced in Petri dishes (Ø14) containing two oak leaf slices (30x30mm) previously treated with an extract, or distilled water in the control variant. “No choice” test was carried out at room temperature (22-25 ºC) and usual light regime (16:8). Feeding intensity, expressed as consumed leaf area (mm^2), was measured after 24 and 48 h and for further analysis the obtained values were transformed in relative values (%).

Antifeeding activity of extracts was assessed using Antifeeding index (AFI) and calculated according to Farrar, Barbour & Kennedy (1989):

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

C- Consumed area in the control variant (%); T- consumed area in the treatment (%)

The criterion according to Liu, Goh & Ho (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 (+++)

Insecticidal effect was also assessed in the “No-choice” test. Dead and paralyzed larvae (determined by “palpation method”) were counted after 24 and 48 h and the values were transformed into %.

All experiments were set up in four replications.

Statistical analysis

The data on the consumed leaf area (%) were subjected to Two-way analysis of variance (ANOVA) to evaluate the influence of two factors and their interaction. The first factor was the plant species and the second was the concentration of extracts. Duncan`s multiple range test was used to assess the significance of differences

between treatments, only for the factor that had significant influence on feeding intensity and mortality. All tests were performed at the level of significance 95% in software STATISTICA.10.

RESULTS

Chemical composition of plant extracts

Spectrophotometric methods are widely used for a rapid determination of different phe-nolic compounds and antioxidant capacity of plant samples. The content of extracted phenolic and antioxidant compounds differed on the plant species and selected plant part (Table 1). The yield of total phenols in extracts, expressed as quercetin equivalents (QE), varied between 2.74 (M. alba leaves extract) and 163.25 (*A. altisima* bark extract) mg/g of dry plant material. The content of total tannins and antioxidant capacity of extracts of selected plants, measured by five different assays, followed the same pattern as the total phenolic content.

Table 1. Phenolic content and antioxidant capacity of *A. altissima* and *M. alba* extracts.

Parameter	Extract			F value
	A. altissima bark	*A. altissima* leaves	*M. alba* leaves	
TP (mg QE/g)	163.25 ± 3.42 a	17.90 ± 0.55 b	2.74 ± 0.07 d	8324.07**
TT (mg QE/g)	141.51 ± 1.73 a	14.93 ± 0.15 b	1.96 ± 0.03 d	18931.69**
DPPH (mg AsA/g)	41.07 ± 1.66 a	3.76 ± 0.09 b	0.19 ± 0.02 d	7495.9**
FRAP (mg AsA/g)	15.79 ± 2.37 a	5.27 ± 0.36 b	0.19 ± 0.01 c	295.6**
ABTS (mg AsA/g)	84.52 ± 7.47 a	13.19 ± 1.26 b	1.21 ± 0.05 c	354.70**
TAA (mg AsA/g)	16.67 ± 1.47 a	2.64 ± 0.11 b	0.29 ± 0.02 d	340.49**
SOD mimetic (IU/g)	1324.27 ± 126 a	365.55 ± 32.2 b	38.10 ± 3.73 d	469.49**

DPPH - 2,2-diphenyl-1-picrylhydrazyl; FRAP - Ferric reducing antioxidant power; ABTS - 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); TAA - total antioxidant activity; SOD - superoxid dismutase; AsA - ascorbic acid; TP - total phenolics; TT - total tannins; QE- quercetin equivalents

The method for HPLC-DAD analysis of phenolic compounds was evaluated in terms of linearity and repeatability, LOD and LOQ for trans-cinnamic, 2-hydroxy cinnamic, caffeic, p-coumaric and chlorogenic acid, quercetin and kaempferol. The obtained LODs for all investigated phenolic compounds were 0.01 µg/mL with the LOQs of 0.03 µg/mL. The HPLC analysis detected the presence of phenolic compounds in *A. altissima* bark, leaves and *M. alba* leaves (Table 2).

The dominant compound in *A. altissima* bark extract was kaempferol (450.14 µg/g) and 2 hidroxycinnamic acid (434.49 µg/g) and dominant compound in *A. altissima* leaf extract was also kaempferol followed by quercetin and 2 hidroxycinnamic acid. The most prevailing compound in *M. alba* leaves extract was kaempferol, followed by 2 hydroxycinnamic acid, quercetin and caffeic acid.

Table 2. HPLC-DAD detected and quantified phenolic compounds in *A. altissima* and *M. alba* extracts.

Phenolic compounds (µg/g)	Extract		
	A.altisima bark	*A.altisima* leaves	*M.alba* leaves
caffeic acid (hydro cinnamic)	55.91	<LOQ	6.8
trans cinnamic acid	35.94	<LOQ	<LOQ
2 hidroxycinnamic acid	434.49	3.96	12.84
chlorogenic acid	71.83	<LOQ	<LOQ
p-coumaric acid	42.16	<LOQ	<LOQ
Kaempferol	450.14	19.22	32.0
Quercetin	8.86	5.96	6.95

LOQ - Limit of quantification.

Bioassay

Feeding intensity of gypsy moth larvae

Two-way ANOVA results indicate that after 24 h only plant species, and the interaction of plant species x concentration, had statistically significant influence on the feeding intensity (consumed leaf area) of gypsy moth larvae (Table 3). However, after 48 h, both factors and their interaction had significant influence of the feeding intensity of gypsy moth larvae.

Table 3. The influence of different factors and their interaction on feeding intensity of gypsy moth larvae.

Factors (sources of variation)	SS	DF	MS	F value
24 h				
extract	136345.5	5	27269.1	2005.05**
concentration	38.5	2	19.2	1.41 ns
extract x concentration	468.9	10	46.9	3.45**
48 h				
extract	145626.0	5	29125.2	4207.09**
concentration	76.9	2	38.4	5.55**
extract x concentration	394.3	10	39.4	5.70**

ns - non significant differences ($p>0.05$), * - significant differences ($p<0.05$); ** - highly significant differences ($p<0.01$); SS-sum of squares; df-degrees of freedom; MS-median

Feeding intensity of gypsy moth larvae was assessed based on the average value of consumed leaf area (%) (Table 4). After 24 h the lowest average feeding intensity was registered in treatments with extracts of *M. alba* leaves (1.44%) and *A. altissima* bark (5.03%). The increase of concentration significantly reduced the feeding intensity

in treatments with *A. altissima* bark. The difference between the average consumed leaf area was highly significant (F=21604.43**, p<0.05). After 48 h, the lowest feeding intensity was in treatments with *M. alba* leaves (3.22%) and *A. altissima* bark (9.30%) and it was concentration-dependant, namely the feeding intensity decreased with the increase of concentration. The differences between consumed leaf area are highly significant (F=76689.33**, p<0.01).

Table 4. Feeding intensity of gypsy moth larvae.

Extract	% of consumed leaf area			Average (%)	F value
	0.5%	1%	2%		
24 h					
A. altissima (bark)	6.72 ± 0.81 aB	5.12 ± 1.03 aB	3.06 ± 0.44 bB	5.03 c	4.25*
A. altissima (leaves)	91.31 ± 2.11 aA	90.22 ± 1.22 aA	89.10 ± 2.17 aA	90.21 a	2.19ns
M. alba (leaves)	2.04 ± 0.15 aC	1.07± 0.11 aC	1.20 ± 0.09 aB	1.44 d	4.33 ns
Control	90.33 ± 2.41 aA	90.47 ± 1.22 aA	82.11 ± 3.65 aA	87.64 b	0.85 ns
F value	28253.89**	27622.35**	28824.55**	21604.43**	
48 h					
A. altissima (bark)	11.97 ± 4.23 aB	11.27 ± 1.3 aB	4.67 ± 2.02 bB	9.30 b	621.97**
A. altissima (leaves)	96.92 ± 0.67 aA	98.51 ± 2.15 aA	95.87 ± 2.11 aA	97.10 a	4.11ns
M. alba (leaves)	6.55 ± 0.11 aC	1.52 ± 0.16 bC	1.12 ± 0.11 bC	3.22 c	47.60**
Control	97.55 ± 1.34 aA	97.40 ± 1.75 aA	97.11 ± 0.63 aA	97.35 a	1.33ns
F value	79379.91**	28824.96**	96485.43**	76689.33**	

Values with the same lowercase letters are at the same level of significance in columns- between concentrations (α=0.05); Values with the same uppercase letters are at the same level of significance in rows- between plant species (α=0.05); ns - non significant differences (p>0.05), * - significant differences (p<0.05); ** - highly significant differences (p<0.01).

Based on antifeeding activity index (AFI values), after 24 and 48 h, the extracts of *A. altissima* bark and *M. alba* leaves expressed strong antifeeding activity on gypsy moth larvae regardless on the concentration (Table 5). However, the extracts of *A. altissima* leaves did not cause antifeeding effect regardless on the applied concentration (AFI ranged from 0.20 to 3.55 after 24h and 0.32 to 0.87 after 48 h).

The mortality of gypsy moth larvae increased after 48 h in treatments with *A. altissima* bark and leaf extracts, at all applied concentrations (40.0 - 57.50%) as presented in Table 6.

CONCLUSIONS AND DISCUSSION

The content of phenolic compounds in *M. alba* leaf extracts is greatly influenced by a growing region (Radojković, Zeković, Vidović, Kočar & Mašković, 2012; Kim

et al, 2014; Polumackanycz, Sledzinski, Goyke, Wesolowski & Viapiana, 2019), cultivar (Lee & Choi, 2012; Sánchez-Salcedo, Mena, García-Viguera, Hernández & Martínez, 2015; Pothinuch & Tongchitpakdee, 2019), harvest period (Lee & Choi, 2012; Pothinuch & Tongchitpakdee, 2019), heat processing (Lee & Choi, 2012), extraction solvent (Polumackanycz et al, 2019), and processing conditions (Przeor et al, 2020).

Table 5. Antifeeding activity of tested plant extracts on gypsy moth larvae.

Extracts	Antifeeding activity			
	24 h		48 h	
	AFI	activity	AFI	activity
A. altissima (bark) 0.5%	91.34	+++	77.43	+++
A. altissima (bark) 1%	95.05	+++	87.50	+++
A. altissima (bark) 2%	93.52	+++	79.33	+++
A.altissima (leaves) 0.5%	3.55	-	0.32	-
A. altissima (leaves) 1%	2.32	-	0.36	-
A. altissima (leaves) 2%	0.20	-	0.87	-
M. alba (leaves) 0.5%	85.54	+++	92.08	+++
M. alba (leaves) 1%	89.11	+++	95.15	+++
M. alba (leaves) 2%	96.44	+++	93.43	+++

- no antifeeding activity; +++ - strong antifeeding activity.

Table 6. Mortality (%) of gypsy moth larvae in treatments with *A. altissima* and *M. alba* extracts.

Extracts	Mortality (%)			
	24 h	F value	48 h	F value
A. altissima (bark) 0.5%	13.50 ± 0.50 ab	36.76**	40.00 ± 0.00 bc	556.47**
A. altissima (bark) 1%	10.00 ± 1.00 b		43.33 ± 0.51 b	
A. altissima (bark) 2%	16.50 ± 1.03 a		57.50 ± 1.50 a	
A.altissima (leaves) 0.5%	2.25 ± 0.25 d	83.31*	45.00 ± 0.00 b	649.23**
A. altissima (leaves) 1%	3.75 ± 0.20 d		30.00 ± 1.00 c	
A. altissima (leaves) 2%	4.77 ± 0.17 cd		47.50 ± 2.50 b	
M. alba (leaves) 0.5%	6.50 ± 0.50c	129.99**	8.21 ± 0.60 d	10107.71**
M. alba (leaves) 1%	10.00 ± 1.00 b		30.00 ± 0.40 c	
M. alba (leaves) 2%	17.50 ± 0.25 a		62.50 ± 0.50 a	
Control	2.50 ± 0.10 d	/	3.75 ± 0.30 e	/
F value	646.06**	/	2324.11**	/

Values with the same lowercase letters are at the same level of significance in columns- between concentrations and plant species (α=0.05); ns - non significant differences (p>0.05), * - significant differences (p<0.05); ** - highly significant differences (p<0.01)

Various factors affect the content of phenolic compounds and antioxidant activity in *A. altissima* extracts: selected tissue or organ (Luis, Gil, Amaral, Domingues & Duarte, 2012; Aissani et al, 2018), growing conditions (Vidović, Morina, Milić & Veljović Jovanović, 2015), extraction solvent (Luis et al, 2012; Poljuha et al, 2017; Aissani et al, 2018), and processing conditions (Poljuha et al, 2017). According to several authors (Luis et al, 2012; Albouchi et al, 2013; Poljuha et al, 2017; Aissani et al, 2018) leaves and bark of *A. altissima* are rich sources of polyphenolic compounds and possess very strong antioxidant activity. Presented work is in agreement with the results obtained in our study that reveal that the bark extract possessed ten times higher content of total phenolics and total tannins compared to leaf extracts. Bark extract also manifested higher antioxidant capacity from three times (measured by FRAP assay) up to 11 times (measured by DPPH assay) comparing to *A. altissima* leaves extract.

Several authors reported that *M. alba* leaf extracts contain relatively high content of polyphenolic compounds and strong antioxidant activity (Kim *et al*., 2014; Polumackanycz et al, 2019; Lee & Choi, 2012; Sánchez-Salcedo et al, 2015; Przeor et al, 2020). However, due to different extraction procedures and presentation of the results, it is hard to compare the results. Polumackanycz et al (2019) and Przeor et al (2020) obtained results similar to those reported in our study.

Several studies reveal more detailed chemical composition of *A. altissima* bark. Kowarik & Säumel (2007) report that the bark contains oleoresin, resin, some mucilage, ceryl alcohol, ailanthon, 'quassiin', calcium oxalate crystals, and isoquercetin (quercitin 3-glycoside), tannin, phlobaphene, ceryl palmitate, saponin, quassin and neoquassin. Ailanthone was identified as the most effective phytotoxic component and may potentially be used as a broad spectrum herbicide (Lin et al, 1995; Heisey, 1996) and may potentially be used as a broad spectrum herbicide (Heisey & Heisey, 2003). Besides ailanthone, which showed the greatest inhibitory activity, De Feo, Mancini, Voto, Curini & Digilio (2009) isolated ailanthinone, chaparrine, and ailanthinol B (quassinoid derivatives), while Okunade et al (2003) isolated the quassinoids ailanthone and 6-alpha-tigloyloxychaparrinone and revealed antiplasmodial activity of these compounds and Tamura et al (2003) and Tamura, Fukamiya, Okano & Koike (2006) detected new quassinoids: ailantinol E, F, G, and H. From the bark, Hwang et al (2005) isolated five coumarin derivatives.

Aissani et al (2018) identified quinic and syringic acids as dominant component in *A. altissima* leaf extract followed by caffeic and p-coumaric acids. The composition of *A. altissima* leaves differed from that of the bark. In bark extract, all tested phenolic acids (caffeic acid (hydro cinnamic, trans cinnamic acid, 2 hidroxycinnamic acid, chlorogenic acid, p-coumaric acid, kaempferol, quercetin) were detected in different amounts. The content of the majority of phenols investigated was many-fold lower in leaves (kaempferol, quercetin and 2 hydroxycinnamic acid), which is partially in accordance with the reports of other authors. In several studies (Luis et al, 2012; Albouchi et al, 2013; Vidović et al, 2015) quercetin was detected as one of the dominant phenolic compounds in extracts of *A. altissima* leaves, while our results point kaempherol and 2 hidroxycinnamic acid as the dominant components.

The obtained results for the most prevailing compound in *M. alba* leaves (kaempferol, followed by 2 hydroxycinnamic acid, quercetin and caffeic acid) are partially in agreement with previous findings. Flaczyk et al (2013) and Przeor et al (2020) detected chlorogenic and caffeic acids as a dominant components followed by p-coumaric acid, kaempferol and quercetin. Radojković et al (2012) identified ferulic acid, rutin, gallic acid and chlorogenic acid, among other compounds, in *M. alba* samples. Sánchez-Salcedo et al (2015) have reported quercetin derivatives, chlorogenic and caffeoylquinic acid as major components, but also kaempferol derivates.

Flavonoids, quercetin, chlorogenic acid and rutin are the most frequently found phenolic compounds among diverse crop species and represent a base of plants resistance (Rojht, Kosir & Trdan, 2012; Martens, Preuss & Matern, 2010; Hichri et al, 2011). They affect herbivore larval growth and development mainly by feeding inhibition (Treutter, 2006; Page, Sultana, Paszkiewicz, Florance & Smirnoff, 2012). The results of our work is in compliance with these reports. The highest amount of all tested phenolic compounds including quercetin, chlorogenic acid and kaempferol detected by HPLC analysis was in the extract of *A. altissima* bark (8.86, 71.83, and 450.14 µg/g, respectively) which expressed the strongest antifeeding activity against gypsy moth larvae in this work. Onyilagha, Lazorko, Gruber, Soroka & Erlandson (2004) also reported the detrimental effect of kaempferol from *Brasica napus* L. leaves to another lepidopterian pest, *Mamestra configurata* Walker. Beninger et al (2004) found that chlorogenic acid (100 and 1000 ppm) when added in artificial diet significantly reduced growth of *L. dispar* and *Trichloplusia ni* (Hübner) larvae, and according to Simmonds & Stevenson (2001) and Simmonds (2003), the same effects were caused to *Helicoverpa armigera* (Hübner) (50 ppm).

The extract of *M. alba* leaves exhibited strong antifeeding effect, which is in accordance with the results of previous research of Gvozdenac et al (2012). Antifeeding activity can be attributed to kaemferol, 2 hidroxycinnamic and caffeic acid and quercetin detected in the extract. Stamp, Temple, Traugott & Wilkens (1994) proved negative effects of caffeic acid on early stages of *Manduca sexta* (L.), which was also detected as the dominant phenolic compound in leaf extract of *M. alba*. Additionally, according to Pelletier (1996) over 250 different glycosides can be isolated from the leaves of *M. alba*, which are most probably responsible for feeding inhibition.

Previous studies prove that secondary metabolites released from roots and leaves of *A. altissima* have pronounced insecticidal effect (Heisey, 1996; Tsao, Romanchuk, Peterson & Coats 2002), which is in accordance with the results obtained in this study. According to De Feo et al (2009), extract isolated from *A. altissima* plant was successfully used to control *Acyrtosiphon pisum* (Harris). Pavela (2011) reported insecticidal effects and anteefidant activity (FDI 41.6%) of *A. altissima* leaf fractions on 4th instar *Leptinotarsa decemlineta* (Say) larvae and acute and chronic toxicity, antifeedant efficacy and larval growth inhibition of leaf extract on *Spodoptera littoralis* (Bois.) (Pavela et al 2014). *A. altissima* leaf extract expressed oviposition deterrence of *Spodoptera frugiperda* (Smith) in a concentration-dependent manner, as reported

by Wagner & Card (2020). In this work, *A. altissima* bark extract expressed stronger insecticidal activity, compared to leaf extract. This is in compliance with reports of Lu & Wu (2010) who tested essential oil of *A. altissima* bark but on different insect pests. The essential oil exhibited strong contact toxicity to *Sitophilus oryzae* (L.) adults (76.5% mortality after 72 h of exposure), fumigant activity (99,3% and 81.9% mortality within 24 h) for *Oryzaephilus surinamensis* (L.) and *S. oryzae* respectively, and repellency (class IV) for adults of *Tribolium castaneum* (Herbst), *O. surinamensis, S. oryzae* and *Liposcelis paeta* (Pearman & J.V.). However, there is no available research data on insecticidal effects of *A. altissima* bark extracts on lepidopteran larvae. Therefore, this work presents the first report on the bioactivity of *A. altissima* bark extract on gypsy moth larvae, emphasizing its high potential as a moth control agent. Extract of *M. alb*a leaves expressed significant insecticidal effect at 1 and 2% concentrations (30.0 - 62.50% mortality). These results comply with the reports from laboratory feeding tests of *L. dispar* larvae performed by Miller & Hanson (1994). The authors 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. Also, as mentioned, *M. alba* leaves contain nine glycoside compounds belong to the group of deoxynojirimycin which expressed inhibitory effect on phytophagous (Lepidoptera) larvae of *Spodoptera frugiperda* (Smith) (Pelletier, 1996).

Insect pests represent a growing economic and environmental problem worldwide. Due to their high availability and low toxicity, plant extracts may represent excellent repellents and insecticides to be used in different ecosystems. Our results indicate that extracts of *A. altissima* bark and leaves and *M. alba* leaves may act as an effective low-cost natural protectants able to control the presence of gypsy moth in ecosystems. Extracts of *M. alba* leaves and *A. altissima* bark at all applied concentrations expressed strong antifeeding activity and significant insecticidal effect on gypsy moth larvae.

ACKNOWLEDGEMENTS

This research was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant numbers: 451-03-68/2020-14/200117 and 451-03-68/2020-14/200032). The authors are thankful to the Dr Duško Brković, Department of Biology, Industrial Microbiology and Plant Protection, University of Kragujevac for the formal identification of the plant material used in study i.e. providing necessary vauchers for used plant material.

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Received: December 25, 2020 *Accepted: October 05, 2021*