https://doi.org/10.26651/allelo.j/2022-56-2-1395 0971-4693/94 Euro 20.00
Allelopathy Journal 56 (2): 159-172 (July, 2022) *International Allelopathy Foundation* 2022
Table: 4, Figs : 2

Phytotoxicity of velvetleaf (*Abutilon theophrasti* Medik.), common ragweed (*Ambrosia artemisiifolia* L.) and common cocklebur (*Xanthium strumarium* L.) to lettuce and tomato

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(Received in revised form: May 24, 2022)

ABSTRACT

We studied the main phenolic components of velvetleaf (*Abutilon theophrasti*), common ragweed (*Ambrosia artemisiifolia*) and common cocklebur (*Xanthium strumarium*) aqueous extracts and evaluated their phytotoxicity to germinating seeds and seedling growth of lettuce and tomato. A total of 13 phenolic compounds were identified in aqueous extracts. The highest total phenolic content was found in velvetleaf leaves, followed by common ragweed leaves and common cocklebur stems. Caffeoyl quinic acids were the most abundant phenolics in these three weeds, while common ragweed and velvetleaf leaves were also rich in *p*-coumaric acid and rutin, respectively. Other phenolic compounds were present in moderate quantities in the stems and leaves of the tested weeds. The weed extracts at 50 % concentration or higher significantly inhibited the seed germination and early seedling growth of lettuce and tomato. The leaf aqueous extracts were more phytotoxic to lettuce and tomato than stem aqueous extracts. The leaf aqueous extract of common ragweed had the strongest inhibitory effects on lettuce, while the leaf aqueous extract of velvetleaf was most inhibitory to tomato. The aqueous extracts of common cocklebur were less inhibitory to lettuce and tomato.

Key words: *Abutilon theophrasti*, *Ambrosia artemisiifolia*, Aqueous extracts, common cocklebur, common ragweed, lettuce, phytochemical screening, phytotoxicity, tomato, velvetleaf, *Xanthium strumarium.*

INTRODUCTION

Velvetleaf (*Abutilon theophrasti* Medik.), common ragweed (*Ambrosia artemisiifolia* L.) and common cocklebur (*Xanthium strumarium* L.) are common noxious spring invasive weeds in Serbia (41). Common ragweed and common cocklebur are native to America, and velvetleaf is native to China and India, but all these weeds are distributed worldwide, including Europe and the Balkan Peninsula (6,14). In Serbia, these weeds infest field crops (maize, soybean, sugar beet, sunflower), vegetable crops, and orchards. They are also found along roadsides, in urban empty lots, and in other disturbed habitats (2,41). Additionally, common ragweed is one of the most harmful plants in Europe due to its high production of allergenic pollen (11). These species are highly competitive for light, soil, water and nutrients, hence, reduces crop yields and biodiversity. Velvetleaf, common ragweed and common cocklebur cause heavy yield losses in different crops (10,16,21,35,43,45,48).

The term "allelopathy" denotes complex effects exerted by one plant species (donor) to another one (acceptor) by releasing a variety of chemical substances, termed

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*Correspondence author, [1]Faculty of Agriculture, University of Zagreb, Svetosimunska cesta No 25, 10 000 Zagreb, Republic of Croatia allelochemicals, into the environment (34). Allelochemicals affects the vital physiological processes in plants, such as respiration, photosynthesis, cell division and elongation, membrane fluidity, protein biosynthesis and the activity of many enzymes, and they might also affect tissue water status (13,18,19). Allelochemicals belong to many chemical families, such as phenols, flavonoids, terpenoids, glucosinolates, benzoquinones, and cyanogenic compounds (9,20,24,26). Several phenolic allelochemicals are present in plant stems, leaves, roots, flowers, pollen and fruits (22,26,32,39, 48). Phenolic compounds (*p*-coumaric acid, ferulic acid, rutin, etc.) are phytotoxic and found in many plants, including weeds (1,19,24,25,29,30,33). Nine flavonoid compounds have been isolated and identified from velvetleaf (3,27), while the phenolic composition of common ragweed includes isorhamnetin, quercetin, and kaempferol (28). Chemical constituents of common cocklebur include phenolic compounds such as gallic acid, protocatechuic acid, quercetin, chlorogenic acid, caffeic acid, rutin and ferulic acid (36,38).

Allelopathy plays a significant role in agroecosystems. Allelopathic weeds directly or indirectly affect the germination, growth, establishment and yield of various crops (37). About 240 weed species, including velvetleaf, common ragweed and common cocklebur, have been reported as allelopathic and interfering with the growth and production of crops (31). After crop harvest, light and other environmental resources become readily available in the field under such conditions weeds, including velvetleaf, ragweed and cocklebur, grow rapidly, forming large above-ground biomass. The biomass of these weeds is ploughed into soil when preparing a field for sowing the next crops in crop rotation.

This study aimed (i) to evaluate *in-vitro* the potential phytotoxic effects of aqueous leaf and stem extracts of velvetleaf, common ragweed and common cocklebur on seed germination and seedling length of lettuce and tomato as succeeding crops in a common crop rotation in Serbia, and (ii) to chemically characterize these weeds aqueous extracts.

MATERIAL AND METHODS

I. Plant material and aqueous extract preparation

Leaves and stems of velvetleaf (*Abutilon theophrasti* Medik.), common ragweed (*Ambrosia artemisiifolia* L.), and common cocklebur (*Xanthium strumarium* L.) were collected in Zemun Polje (Belgrade, Serbia, 44°52′05.3″ N 20°19′07.1″ E) in July 2019. Seeds of lettuce (Attraktion, Semenarna, Ljubljana, Slovenia) and tomato (Mondial F_1, Enza Zaden, The Netherlands) were used in the laboratory experiment. All experiments were conducted during July and August 2019.

Aqueous extracts were made from the whole fresh aerial plant parts (leaves and stems). Fresh plant samples were cleaned several times with tap water and stems and leaves were chopped into 1 cm long fragments, ground, soaked and kept in deionized water for 24 h at a 1:4 ratio (0.25 g/ml; 250 g fresh material in 1000 ml water). The extracts were filtered through two-layer filter paper # 1 to remove plant debris and centrifuged for 10 min at 1500 rpm. The supernatant was stored at 4 °C in dark until used. The initial aqueous extract (0.25 g/ml) represented 100 % concentration and was further diluted to 10 %, 25 %, 50 % and 75 % concentrations. Deionized water was used as a control.

II. Phytochemical analyses of aqueous extracts

Standards and reagents: Reference standards of phenolic compounds were obtained from Sigma-Aldrich Chem (Steinheim, Germany), Fluka Chemie gmbh (Buchs, Switzerland) or Chromadex (Santa Ana, USA). Gradient grade methanol for HPLC was purchased from J.T. Baker (Deventer, The Netherlands) and p.a. grade formic acid and DMSO from Merck (Darmstadt, Germany).

HPLC–MS-MS analysis: Aqueous extracts were filtered through 0.45 µm filters (KX Syringe Filter: Nylon 25mm 0.45µm, Kinesis ESF-NY-25-045-D). Fifteen working standards, ranging from 1.53 ng/ml to 48.8 ng/ml, were prepared by 1:1 serial dilutions of standard mixture with solvents A (0.05 % aqueous formic acid) and B (methanol) (1:1). Samples and standards were analyzed using Agilent Technologies 1260 Series high-performance liquid chromatography coupled with Agilent Technologies 6460A Triple Quad tandem mass spectrometer with an electrospray ion source and controlled by Agilent Technologies MassHunter Workstation software – Data Acquisition (ver. B.06.00). Five microlitres were injected into the system and compounds were separated on Zorbax Eclipse XDB-C18 (50 mm * 4.6 mm, 1.8 µm) rapid resolution column held at 40 ˚C. The mobile phase was delivered at a 0.4 ml/min flow rate in a gradient mode (0 min 30 % methanol, 6 min 70 % methanol, 9 min 100 % methanol, 12 min 100 % methanol, re-equilibration time 5 min). Eluted components were detected by MS, using the ion source parameters as follows: nebulization gas (N2) pressure 40 psi, drying gas (N2) flow 11 l/min and temperature 350 ˚C, negative polarity. Data were acquired in dynamic MRM mode, using the optimized compound-specific parameters (retention time, precursor ion, production, fragment or

Table 1. Optimized dynamic multiple reaction monitoring parameters

Compound	Precursor m/z	Product m/z	$V_{fragmentor}$ (V)	$V_{collision}$ (V)	t_R (min)
Apigenin	269	117	130	25	4.81
Apigenin 7-O-glucoside	431	268	135	41	2.83
Luteolin 7-O-glucoside	447	285	230	30	2.23
Chrysoeriol	299	284	125	20	4.80
Apiin	563	269	250	36	2.77
Quercetin 3-O-glucoside	463	300	210	30	2.34
Kaempferol 3-O-glucosid	447	284	190	30	2.21
Rutin	609	300	135	42	2.32
Daidzein	253	208	145	31	3.54
Naringenin	271	151	130	16	3.98
Matairesinol	357	122	130	24	3.70
Amentoflavone	537	375	220	35	6.10
Vanillic acid	167	109	100	15	1.55
Ferulic acid	193	134	90	11	1.78
p-Coumaric acid	163	119	90	9	1.57
Quinic acid	191	85	150	20	0.51
Scopoletin	191	176	80	8	1.56
Umbelliferone	161	133	120	19	1.57

voltage, collision voltage) given in Table 1. For all compounds, concentrations were determined using the Agilent MassHunter Workstation Software - Quantitative Analysis (ver. B.06.00). Calibration curves were plotted and sample concentrations were calculated using the Quant Report Microsoft Excel 2013. Data were expressed as ng of phenolic compound per g of fresh plant material (ng/g f.p).

III. Seeds Bioassay

The effects of aqueous extracts of three donor test weeds on seed germination and seedlings growth of lettuce and tomato were tested. The experiment was done under controlled conditions, in an incubator (Binder CE) at 25±1 °C in dark. Seeds were sterilized with 5 % sodium hypochlorite solution (NaOCl) for 2 min and then rinsed thrice with distilled water to prevent inhibitory effects of fungal or bacterial toxins on seed germination. Twenty-five disinfected seeds of lettuce or tomato were placed into each sterilized Petri dish (9 cm dia). Ten ml of each concentration of aqueous extracts were added to each dish as per treatment and the dishes were then placed in an incubator. All dishes were sealed with parafilm to avoid evaporation. After 8 days, germination (%) was calculated and seedling length was measured. Inhibition (%) of germination and seedling length was calculated as under:

$$\% \text{ Inhibition} = [(Xc-Xt)/Xc]\times 100$$

Where Xc : % Germination and seedling length in control;

Xt : % Germination and seedling length in treatment with aqueous extracts

The experimental treatments were replicated 4 times in a randomized complete block design and the experiment was repeated twice and data combined for analysis.

Statistical Analysis: Data are expressed as means ± standard deviation (SD). Normality distribution and homogeneity of variances were checked for all data using the Kolmogorov-Smirnov and Levene tests. Data were analyzed by a two-factorial analysis of variance (ANOVA) using the STATISTICA 8.0. software package. When F-values were statistically significant (p <0.05) treatments were compared by Fisher's Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

Phytochemical analysis of aqueous extracts

The tested extracts contained 13 phenolic compounds belonging to 6 chemical classes (Table 2). The highest total phenolic content was found in velvetleaf leaves (15459.50 ng/g f.p) and it was 3-folds higher than the phenolic content in velvetleaf stems (4969.94 ng/g f.p.). The main phenolic constituents were quercetin 3-O-glucoside, rutin, and caffeoyl quinic acids. In common ragweed, total phenolic compounds content was also notably high in leaves (10166.15 ng/g f.p.) and nearly twice the content in stem (5178.77 ng/g f.p.), with *p*-coumaric acid as the main constituent, followed by caffeoyl quinic acids. In common cocklebur, the total amount of phenolic compounds was higher in stems (7632.48 ng/g f.p.) than in leaves (2910.57 ng/g f.p.) and caffeoyl quinic acid was the most abundant phenolic acid (Table 2).

Table 2. Quantification of phenols in aqueous extracts of tested weeds

Chemical class	Compound	Velvetleaf		Common ragweed		Common cocklebur	
		Stem	**Leaves**	**Stem**	**Leaves**	**Stem**	**Leaves**
		Content of Phenolic Compounds (ng/g FW of plant)					
Flavones	Apigenin	27.02±0.54^{a}	18.23±0.22^{e}	24.85±0.50^{b}	20.76±0.31^{d}	25.02±0.63^{b}	23.42±0.47^{c}
	Apigenin 7-O-glucoside	11.77±0.14^{a}	11.96±0.18^{a}	13.26±0.19^{a}	n.d.^{b}	n.d.^{b}	11.76±0.15^{a}
	Luteolin 7-O-glucoside	184.61±4.62^{c}	187.70±3.75^{c}	186.09±3.72^{c}	186.17±3.35^{c}	199.55±4.39^{b}	217.57±4.57^{a}
	Chrysoeriol	69.80±1.40^{b}	68.81±1.24^{b}	68.67±1.44^{b}	77.05±1.70^{a}	68.63±1.37^{b}	67.88±1.36^{b}
	Apiin	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}
Flavonols	Quercetin 3-O-glucoside	171.33±3.60^{c}	1155.45±28.89^{a}	171.88±3.78^{c}	174.65±3.32^{c}	383.62±7.67^{b}	n.d.^{d}
	Kaempferol 3-O-glucoside	186.38±3.73^{a}	192.24±4.42^{a}	187.43±3.75^{a}	186.84±3.74^{a}	186.45±3.73^{a}	n.d.^{b}
	Rutin	168.57±3.37^{b}	5411.48±64.94^{a}	167.59±3.35^{b}	173.76±2.61^{b}	166.40±4.16^{b}	168.82±3.38^{b}
Isoflavones	Daidzein	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}
Flavanons	Naringenin	67.33±1.01^{ab}	66.88±0.80^{b}	66.29±0.93^{b}	69.89±1.05^{a}	66.68±1.00^{b}	67.09±1.07^{b}
Lignan	Matairesinol	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}
Biflavonoid	Amentoflavone	81.85±1.64^{cd}	86.23±1.47^{c}	193.52±4.06^{a}	82.79±1.66^{cd}	79.21±1.66^{d}	102.52±2.05^{b}
Hydroxybenzoic acids	Vanillic acid	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}
Hydroxycinnamic acids	Ferulic acid	n.d.^{d}	125.49±3.01^{b}	n.d.^{d}	204.89±5.12^{a}	36.62±0.92^{c}	n.d.^{d}
	p-Coumaric acid	1139.58±30.77^{b}	509.86±9.18b^{c}	336.96±6.47b^{cd}	6765.70±196.21^{a}	225.10±4.50^{de}	33.49±0.67^{e}
Quinic acid derivatives	Caffeoyl quinic acids	2841.16±51.14^{d}	7612.33±152.25^{a}	3660.38±62.23^{c}	2126.25±38.27^{e}	5630.58±123.87^{b}	2104.51±29.46^{e}
Coumarins	Scopoletin	20.53±0.31^{e}	12.85±0.15^{e}	101.86±1.22^{c}	97.43±1.46^{d}	564.62±8.47^{a}	113.52±1.36^{b}
	Umbelliferone	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}
	Total	4969.94	15459.50	5178.77	10166.15	7632.48	2910.57

n.d.: Not detected (below the limit of detection of 2,44 ng/mL). Data are reported as mean ± standard deviation. Differences between different plant materials were assessed for significance using a one-way analysis of variance. Values with different lowercase letters (a–e) within a row differ significantly based on Fisher`s LSD test at $p< 0.05$.

The results of this study indicated that the above-ground parts of velvetleaf, common ragweed and common cocklebur are definitely rich sources of phenolic compounds. Variations in the accumulation and concentration of these compounds in different plant parts is closely related to their functions in the plant lifecycle and growth phase. The leavers of velvetleaf and common ragweed, were richer in phenols (quercetin 3-O-glucoside, rutin and p-coumaric acid), which are well-known for their phytotoxicity and probably the most important allelochemicals of invasive species. In common cocklebur, stem has higher amount of phenols, and the main compond was caffeoyl quinic acid. This is possibly related to the transportation of quinic acid, the precursor of aromatic rings of phenolic compounds, through the conducting systems of the stem. The phenols are well-known weed inhibitory agents, variations in their composition in different plant extracts could be responsible for differences in their allelopathic activities.

A. Seeds germination

(a). Lettuce: Lettuce and tomato seed germination are shown in Figures 1 and 2, respectively. Lettuce seed germination was significantly inhibited by leaf aqueous extracts at 50 % concentration (p <0.05) and completely inhibited at higher concentrations (Fig. 1a). The stem aqueous extract of common ragweed at 75 % and 100 % concentrations had the highest inhibitory effects on lettuce seed germination (Fig. 1b). The same concentrations of velvetleaf aqueous extract inhibited germination by 58 % and 78 %, respectively, while cocklebur at 100 % concentration caused significant inhibition. Statistical analysis confirmed that stem aqueous extracts had weaker impact on lettuce seed germination than leaf aqueous extracts.

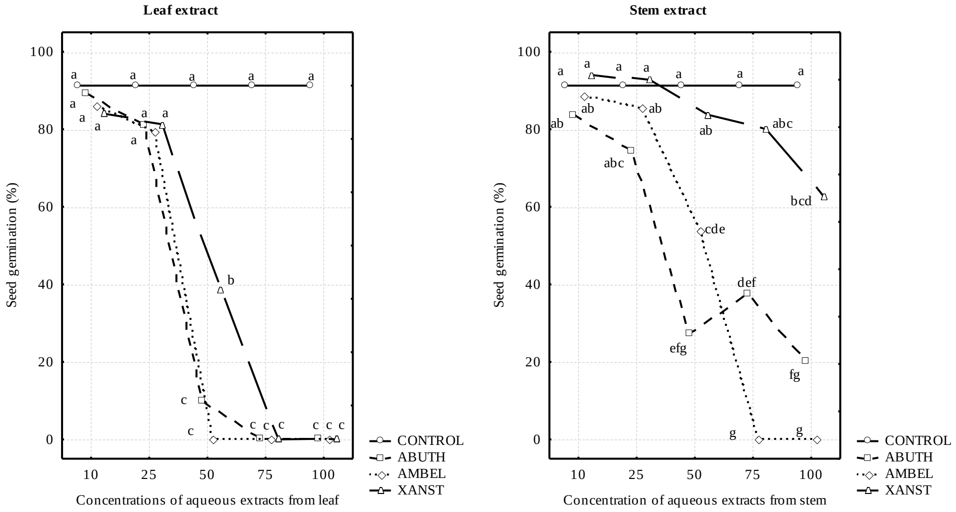

Figure 1. Effects of aqueous extracts from leaf and stem of weeds on lettuce seed germination. ABUTH – velvetleaf (*Abutilon theophrasti*); AMBEL – common ragweed (*Ambrosia artemisiifolia*); XANST – common cocklebur (*Xanthium strumarium*); a-e - Fisher's LSD test, p <0.05.

(b). Tomato: Germination of tomato seeds was inhibited by the velvetleaf leaf extract at concentrations > 50 %, and inhibition ranged from 83 % to 100 %. Common ragweed at these concentrations caused 22 % to 68 % inhibition (Fig. 2a). The aqueous extract of common cocklebur leaves at 100 % concentration caused significant inhibition (24 %) of tomato seeds germination. On the other hand, the lowest concentration (10 %) of all three aqueous extracts was found to stimulate tomato seed germination. The 10 % and 25 % concentrations of aqueous extracts of velvetleaf and common ragweed stems were either even less inhibitory (4 %) or stimulatory (2 %-7 %) to tomato seed germination (Fig. 2b). Significant inhibition (46 %-100 %) was recorded at concentrations ≥50 %. The two highest concentrations (75 % and 100 %) of aqueous extracts of common cocklebur stems significantly inhibited (56 %-60 %) tomato seed germination, while concentration ≤50 % stimulated the seed germination of tomato.

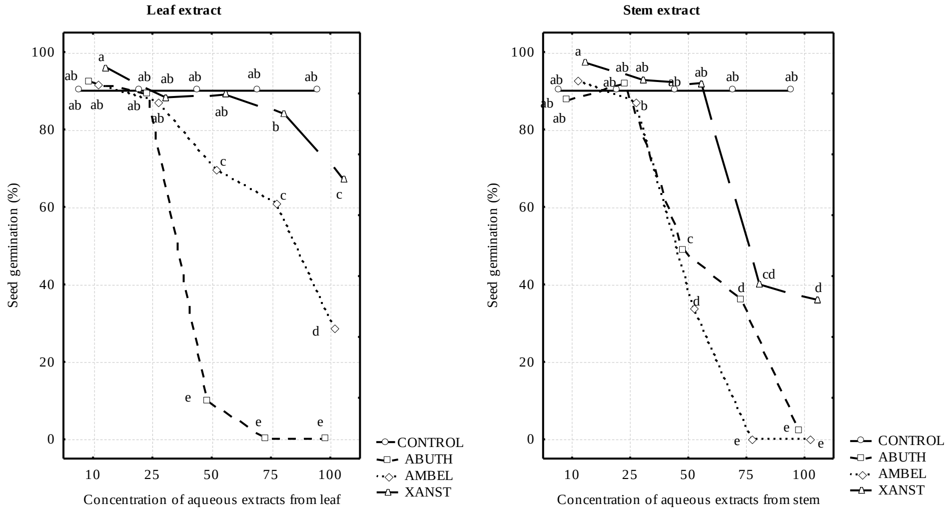

Figure 2. Effects of aqueous extracts from leaf and stem of weeds on tomato seed germination. ABUTH – velvetleaf (*Abutilon theophrasti*); AMBEL – common ragweed (*Ambrosia artemisiifolia*); XANST – common cocklebur (*Xanthium strumarium*); a-e - Fisher's LSD test, p <0.05.

B. Seedling growth

(a). Lettuce: All aqueous extracts of leaves and stems at concentrations > 50 % significantly inhibited the early seedling growth of lettuce and tomato (Tables 3 and 4). The observed inhibition of lettuce seedling growth by the two highest concentrations (75 % and 100 %) of aqueous extracts from leaves of all species was 100 % (Table 3). The 50 % concentration significantly influenced lettuce seedling length, while the two lowest concentrations (10 % and 25 %) caused 17 % inhibition of lettuce seedling growth.

Table 3. Effects of aqueous extracts from leaf and stem of weeds on lettuce seedling growth

Aqueous extract (%)	Velvetleaf	Common ragweed	Common cocklebur
0	2.45±0.50 a	2.45±0.50 a	2.45±0.50 a
	Leaf		
10	2.45±0.26 a	2.22±0.31 a	2.51±0.53 a
25	2.31±0.24 a	2.04±0.36 a	2.43±0.41 a
50	0.31±0.03 bc	0.00±0.00 c	0.45±0.05 b
75	0.00±0.00 c	0.00±0.00 c	0.00±0.00 c
100	0.00±0.00 c	0.00±0.00 c	0.00±0.00 c
	Stem		
10	2.37±0.47 a	2.47±0.67 a	3.03±0.51 a
25	2.31±0.54 a	2.31±0.43 a	2.91±0.69 a
50	1.00±0.42 bc	1.42±0.58 b	2.93±0.55 a
75	0.74±0.45 bcd	0.00±0.00 d	1.47±0.68 b
100	0.28±0.02 cd	0.00±0.00 d	0.79±0.10 bcd

a-d - Fisher's LSD test (p<0.05)

The aqueous extracts from velvetleaf and common ragweed stem at ≥50 % concentrations significantly inhibited lettuce seedling length by 59 % to 89 % and 42 % to 100 %, respectively. The aqueous extract from common cocklebur stem at 75 % and 100 % concentrations caused inhibition of 40 % and 68 %, respectively. Contrarily, the aqueous extract of common cocklebur stems at concentrations of 10 % to 50 % stimulated lettuce seedling length by 20 %. The two lowest concentrations (10 % and 25 %) of velvetleaf and common ragweed aqueous extracts inhibited or stimulated the lettuce seedling growth < 6 % (Table 3).

(b). Tomato : Aqueous extracts from leaves of all test weed species at concentrations > 50 % significantly impacted (*p* <0.05) tomato seedling length (Table 4). Velvetleaf concentrations > 50 % caused significant inhibition (96 %-100 %) in tomato seedling length, while, the common ragweed and common cocklebur extracts caused 80 % to 94 % and 24 % to 79 % inhibirion, respectively. The two lowest concentrations (10 % and 25 %) of all leaf aqueous extracts inhibited (< 26 %) the growth of tomato seedlings.

The aqueous stem extracts of all three weeds at concentrations ≥ 50 % inhibited tomato seedling length over control (Table 4). The two highest concentrations (75 % and 100 %) of test weeds stem extracts caused 62 % to 100 % inhibition, while the 50 % concentration caused 21 % - 79 % inhibition in seedling length. The remaining two concentrations (10 % and 25 %) did not significantly impact the tomato seedling growth (Table 4).

Table 4. Effects of aqueous extracts from leaf and stem of weeds on tomato seedling growth

Aqueous extract (%)	Velvetleaf	Common ragweed	Common cocklebur
0	2.93±0.48 a	2.93±0.48 a	2.93±0.48 a
	Leaf		
10	2.26±0.27 ab	2.68±0.33 a	2.94±0.37 a
25	2.18±0.21 b	2.56±0.25 a	2.86±0.23 a
50	0.13±0.00 e	0.59±0.03 d	2.22±0.32 b
75	0.00±0.00 e	0.25±0.01 de	1.23±0.12 c
100	0.00±0.00 e	0.17±0.02 e	0.62±0.10 d
	Stem		
10	2.97±0.24 a	2.81±0.31 a	3.01±0.28 a
25	2.39±0.22 ab	2.84±0.27 a	2.89±0.25 a
50	0.60±0.05 d	1.08±0.13 c	2.32±0.11 b
75	0.51±0.02 de	0.00±0.00 f	1.10±0.16 c
100	0.08±0.00 ef	0.00±0.00 f	0.72±0.08 cd

a-f - Fisher's LSD test (p<0.05)

The reduction in seed germination and seedling length of lettuce and tomato after the application of stem and leaf aqueous extracts of velvetleaf, common ragweed and common cocklebur may be attributed to phenolic allelochemicals in extract because they inhibit cell division and alter the ultrastructure of cells (26). The reductions in seedling growth might be attributable to the involvement of phenols in suppressing protein and nucleic acids synthesis and inactivation of several enzymes in growing plants (8). Bojović (5) found that common nettle (*Urtica dioica* L.) leachate was rich in total phenolics and flavonoids which drastically inhibited the germination and seedling growth of cereals (wheat, barley and oat) and vegetables (lettuce, spinach and radish). Li *et al.* (24) found that phenolic acids reduced the seed germination of watermelon in a concentration-dependent manner. Similarly, phenolic acids isolated from *Ambrosia trifida* L. significantly inhibited the germination and growth of wheat and maize crops (42) as they did with lettuce and tomato in our study. Allelopathic effects of invasive weeds (4,15,17,23,32,42) were found more pronounced, which is consistent with results in our study, where the three tested invasive weeds (*Abutilon theophrasti, Ambrosia artemisiifolia* and *Xanthium strumarium*) were used as donor plants. Vidotto *et al.* (40) found that tomato was the most sensitive indicator crop species to common ragweed allelochemicals, as its growth was reduced by > 50 % in both laboratory and greenhouse experiments and the effects were higher than other tested species (alfalfa, barley, maize, lettuce and wheat). In the same experiment, lettuce root and shoot growth were also inhibited but only when common ragweed residues, and not root exudates, were added to the substrate. Also, Lehoczky *et al.* (23) investigated the allelopathic effects of aqueous extracts from shoots and seeds of common ragweed on the germination and growth of maize, winter wheat, rye and oat, and found that both extracts were inhibitory to all these test plants. Shoot extracts had stronger inhibitory impact on tested crops, while maize was the most sensitive plant, followed by rye, wheat and oat. Young seedlings in the genus *Xanthium* release toxic allelochemicals, which proves fatal to neighboring plants (15), reducing germination, seedling growth and chlorophyll content in lentils due to the allelopathic activity of common cocklebur aqueous extract (4). Consistently, our results confirmed a significant impact of leaf and stem aqueous extracts of common cocklebur on the germination and early growth of tomatoes. Lettuce showed less sensitivity to common cocklebur extracts isolated from stems. It was also found that stem extracts had a stimulating effect on lettuce early growth.

The obtained results from bioassay tests in this study showed that higher phytotoxicity in terms of both seed germination and seedling growth of lettuce and tomato was obtained with leaf extracts, compared to stem extracts. The highest inhibitory effects on both seed germination and seedling growth of lettuce were obtained when ragweed leaf extract was applied, while, the highest phytotoxic effects on seed germination and seedling growth of tomato were observed when the extract of velvetleaf leaf was used. For both plants, extracts of common cocklebur had the lowest inhibitory effects on germination and seedling growth. These differences in allelopathic activity could be precisely due to the different phenolic compositions of extracts. Common ragweed and velvetleaf leaf extracts were rich in *p*-coumaric acid and rutin, respectively, and those phenols are phytotoxic. Yang *et al.* (44) found that *p*-coumaric acid severely retarded the growth of rice seedlings, while Bundit *et al.* (7) reported that *trans-p*-coumaric acids significantly inhibited early seedling growth of several weeds (*Ageratum conyzoides* L., *Bidens pilosa* L., *Echinochloa crus-galli* L., and *Lactuca sativa* L.) and Zheng *et al.* (47) revealed that *o*-coumaric acid is phytotoxic and probably the most important allelochemical in the invasive species *Ageratina adenophora* (Spreng) King & H.Rob. As for rutin, Hussain and Reigosa (19) showed that higher concentrations of this phenol decreased the contents of leaf proteins and photosynthetic pigments in *Arabidopsis* plants. Also, high concentrations of rutin in aqueous solution strongly inhibited the seed germination and seedling growth of rice (29). This finding was also supported by Fahmy *et al.* (12), who showed that rutin significantly reduced the seedling growth of *Corchorus olitorius* (L.). Caffeoyl quinic acids were abundant in all tested weeds but they do not have a significant allelopathic potential. Ragweed and common cocklebur revealed their highest contents in stems, while velvetleaf had them in leaves. A study has demonstrated *in-vitro* antifungal activity for quinic acid derivatives (46), but the data are insufficient to prove their influence on seed germination and/or seedling growth. Although there are numerous reports of higher or lower phytotoxic effects of individual phenols, it is also very likely that those individual components could be additive in mixtures of phenolic compounds, i.e. there is a high probability of synergistic activity occurring among different phenolic compounds, which may be an additional explanation of the results obtained in this present study. Further research is still needed to find evidence for this synergism.

CONCLUSIONS

The above-ground parts of velvetleaf, common ragweed and common cocklebur are rich in phenolic compounds and we identified a total of 13 phenolic compounds in aqueous extracts. The highest amount of phenols was found in velvetleaf leaves, followed by common ragweed leaves and common cocklebur stems. These 3- invasive weeds species exerted the allelopathic effects on some crops e.g. lettuce or tomato especially when they are less competitive. The ragweed leaf extract was most inhibitory to both seed germination and seedling growth of lettuce. While the velvetleaf leaf extract was most phytotoxic to seed germination and seedling growth of tomato. Therefore, sowing of lettuce or tomato should be avoided in fields where these weeds are dominant. Further, pot assays and field studies are needed to fully, determine the impact of these invasive weed species on lettuce and tomato in successive crop systems.

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, (grant No. 451-03-9/2022-14/ 200214).

DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

REFERENCES

1. Al Harun, M.A.Y., Johnson, J., Uddin, M.N. and Robinson, R.W. (2015). Identification and phytotoxicity assessment of phenolic compounds in *Chrysanthemoides monilifera* subsp. *monilifera* (Boneseed). *PLoS ONE* **10:** e0139992.
2. Anđelković, A., Živković, M., Cvijanović, D., Novković, M., Marisavljević, D., Pavlović, D. and Radulović, S. (2016). Riparian areas as invasion corridors of *Xanthium strumarium* in Serbia. *Acta Herbologica* **25:** 45-55.
3. Balah, M.A. and Mahmuod, I.N. (2011). Allelopathic constituents from *Abutilon theophrasti* aerial parts to other weeds. *Research Journal of Agriculture and Biological Sciences* **7:** 243-250.
4. Benyas, E., Hassanpouraghdam, M.B., Zehtab Salmasi, S. and Khatamian Oskooei, O.S. (2010). Allelopathic effects of *Xanthium strumarium* L. shoot aqueous extract on germination, seedling growth and chlorophyll content of lentil (*Lens culinaris* Medic.). *Romanian Biotechnological Letters* **15:** 5223-5228.
5. Bojović, B.M., Jakovljević, D.Z., Ćurčić S.S. and Stanković, M.S. (2018). Phytotoxic potential of common nettle (*Urtica dioica* L.) on germination and early growth of cereals and vegetables. *Allelopathy Journal* **43:** 175-186.
6. Boršić, I., Milović, M., Dujmović, I., Bogdanović, S., Cigić, P., Resetnik, I., Nikolić, T. and Mitić, B. (2008). Preliminary checklist of invasive alien plant species (IAS) in Croatia. *Natura Croatica* **17:** 55-71.
7. Bundit, A., Yamada, K., Shigemori, H., Laosripaiboon, W., Datta A. and Pornprom, T. (2019). Potential of *trans-p*-coumaric acid released from *Rottboellia cochinchinensis* for weed control in vegetable fields. *Allelopathy Journal* **46:** 185-194.
8. Chou, C.H. (2006). Introduction to allelopathy. In: *Allelopathy: A Physiological Process with Ecological Implications (*Eds., M.J. Reigosa, N. Pedrol and L. Gonzalez), pp.1-9. Springer, the Netherlands.

9. Chung, I.M., Kim, K.H., Ahn, J.K., Chun, S.C., Kim, C.S., Kim, J.T. and Kim, S.H. (2002). Screening of allelochemicals on barnyard grass (*Echinochloa crus-galli*) and identification of potentially allelopathic compounds from rice (*Oryza sativa*) variety hull extracts. *Crop Protection* **21:** 913-920.
10. Cowbrough, M.J., Brown, R.B. and Tardif, F.J. (2003). Impact of common ragweed (*Ambrosia artemisiifolia*) aggregation on economic thresholds in soybean. *Weed Science* **51:** 947-954.
11. Essl, F., Biró, K., Brandes, D., Broennimann, O., Bullock, J.M., Chapman, D.S. and Karrer, G. (2015**)**. Biological flora of the British Isles: *Ambrosia artemisiifolia*. *Journal of Ecology* **103:** 1069-1098.
12. Fahmy, G.M., Al-Sawaf, N.A., Turki, H. and Ali, H.I. (2012). Allelopathic potential of *Pluchea dioscoridis* (L.) DC. *Journal of Applied Science Research* **8:** 3129-3142.
13. Field, B., Jordan, F. and Osbourn, A. (2006). First encounters–deployment of defense-related natural products by plants. *New Phytologist* **172:** 193–207.
14. Follak, S., Aldrian, U. and Schwarz, M. (2014). Spread dynamics of *Abutilon theophrasti* in Central Europe. *Plant Protection Science* **50:** 157-163.
15. Ghayal, N., Biware, M. and Gharpure, P. (2018). Phytotoxic effects of leaf leachates of invasive weeds *Cosmos sulphureus* and *Xanthium strumarium* on agricultural crops. *Biosciences Biotechnology Research Asia* **15:** 821-832.
16. Gladieux, P., Giraud, T., Kiss, L., Genton, B.J., Jonot, O. and Shykoff, J.A. (2011). Distinct invasion sources of common ragweed (*Ambrosia artemisiifolia*) in Eastern and Western Europe. *Biological Invasions* **13**: 933-944.
17. Horzawa, M. and Nawata, E. (2020). Allelopathic effects of leaf litter leachates of *Ulex europaeus* on other species and its own seed germination. *Allelopathy Journal* **49**: 217-228.
18. Hussain, M.I. and Reigosa, M.J. (2011). Allelochemical stress inhibits growth, leaf water relations, PSII photochemistry, non-photochemical fluorescence quenching and heat energy dissipation in three C3 perennial species. *Journal of Experimental Botany* **62:** 4533-4545.
19. Hussain, M.I and Reigosa, M.J. (2016). Plant secondary metabolite rutin affects the photosynthesis and excitation energy flux responses in *Arabidopsis thaliana. Allelopathy Journal* **38:** 215-228.
20. Hussain, M.I., El-Sheikh, M.A. and Reigosa, M.J. (2020). Allelopathic potential of aqueous extract from *Acacia melanoxylon* R. Br. on *Lactuca sativa*. *Plants* **9:** 1228.
21. Hussain, Z., Marwat, K.B., Cardina, J. and Khan, I.A. (2014). *Xanthium strumarium* L. impact on corn yield and yield components. *Turkish Journal of Agriculture and Forestry* **38**: 39-46.
22. John, J. and Sarada, S. (2012). Role of phenolic in allelopathic interactions. *Allelopathy Journal* **29:** 215-230.
23. Lehoczky, É., Gólya, G., Szabó, R. and Szalai, A. (2011). Allelopathic effects of ragweed (*Ambrosia artemisiifolia* L.) on cultivated plants. *Communications in Agricultural and Applied Biological Sciences* **76:** 545-549.
24. Li, M., Ma, L., Song, Y., Yan, X., Lei, Q. and Zhang, X. (2020). Allelopathic effects of phenolic acids on germination of watermelon (*Citrullus lanatus* Thunb): Dose-effect relationship and quantitative structure-activity relationship. *Allelopathy Journal* **51**: 165-176.
25. Li, P., Junwei, S. and Cheng, Z. (2016). Effect of rutin in *Amaranthus spinosus* L. on antioxidative metabolism for rice (*Oryza sativa* L.). *Advance Journal of Food Science and Technology* **12:** 562-567.
26. Li, Z.H., Wang, Q., Ruan, X., Pan, C.D. and Jiang, D.A. (2010). Phenolics and plant allelopathy. *Molecules* **15:** 8933-8952.
27. Matławska, I. and Sikorska, M. (2005). Flavonoids from *Abutilon theophrasti* flowers. *Acta Poloniae Pharmaceutica* **62:** 135-139.
28. Mihajlović, L., Radosavljević, J., Burazer, L., Smiljanić, K. and Ćirković Veličković, T. (2015). Composition of polyphenol and polyamide compounds in common ragweed (*Ambrosia artemisiifolia* L.) pollen and sub-pollen particles. *Phytochemistry* **109**: 125–132.
29. Pengcheng, L., Junwei, S. and Cheng, Z. (2016). Effects of rutin in *Amaranthus spinosus* L. on antioxidative metabolism for rice (*Oryza sativa* L.). *Advance Journal of Food Science and Technology* **12:** 562-567.
30. Puig, C.G., Reigosa, M.J., Valentão, P., Andrade, P.B. and Pedrol, N. (2018). Unraveling the bioherbicide potential of *Eucalyptus globulus* Labill: Biochemistry and effects of its aqueous extract. *PLoS ONE* **13:** e0192872.
31. Qasem, J.R. and Foy, C.L. (2001). Weed allelopathy, its ecological impacts and future prospects: A review. *Journal of Crop Production* **4**: 43-119.
32. Radivojević, Lj., Sarić-Krsmanović, M., Gajić Umiljendić, J. and Šantrić, Lj. (2019). Allelopathic effects of invasive weed species *Abutilon theophrasti* Medik., *Ambrosia artemisiifolia* L., *Datura stramonium* L. and *Xanthium strumarium* L. on tomato. *Pesticides & Phytomedicine* **34:** 181-191.

33. Razavi, S.M., Zahri, S., Zarrini, G., Nazemiyeh, H. and Mohammadi, S. (2009). Biological activity of quercetin-3-O-glucoside, a known plant flavonoid. *Russian Journal of Bioorganic Chemistry* **35**: 376-378.
34. Rice, E.L. (1984). *Allelopathy*, 2nd Ed. Academic Press, New York, USA, 422 pp.
35. Saeed, A., Hussain, A., Khan, M.I., Arif, M., Maqbool, M.M., Mehmood, H., Iqbal, M., Alkahtani, J. and Elshikh, M.S. (2020). The influence of environmental factors on seed germination of *Xanthium strumarium* L.: Implications for management. *PLoS ONE* **15**: e0241601.
36. Scherer, R. and Godoy, H.T. (2014). Effects of extraction methods of phenolic compounds from *Xanthium strumarium* L. and their antioxidant activity. *Revista Brasileira de Plantas Medicinais* **16:** 41-46.
37. Singh, H.P., Batish, D.R. and Kohli, R.K. (2003). Allelopathic interactions and allelochemicals: New possibilities for sustainable weed management. *Critical Reviews in Plant Sciences* **22:** 239-311.
38. Srinivas, P.V., Rao, R.U., Venkateshwarulu, E.L. and Kumar, A.C. (2011). Phytochemical screening and *in-vitro* antimicrobial investigation of the methanolic extract of *Xanthium strumarium* leaf. *International Journal of Drug Development & Research* **3:** 286–293.
39. Subtain, M.U., Hussain, M., Ahmad, M., Tabassam, R., Akbar, M., Ali, M. and Mubushar, M. (2014). Role of allelopathy in the growth promotion of plants. *Scientia Agriculturae* **2:** 141-145.
40. Vidotto, F., Tesio, F. and Ferrero, A. (2013). Allelopathic effects of *Ambrosia artemisiifolia* L. in the invasive process. *Crop Protection* **54:** 161-167.
41. Vrbničanin, S., Malidža, G. and Gavrić, M. (2015). Criteria, methods and results of mapping of alien invasive weeds in the region of Serbia. In: *Invasive weeds: Invasion, Processes, Ecological and Genetic Potential, Introduction, Prediction, Risks, Spread, Damage and Mapping* (Ed., S. Vrbničanin), pp. 233-306. Weed Science Society of Serbia, Belgrade, Serbia. (Serbian)
42. Wang, P., Liang, W., Kong, C. and Jiang, Y. (2005). Allelopathic potential of volatile allelochemicals of *Ambrosia trifida* L. on other plants. *Allelopathy Journal* **15:** 131-136.
43. Werner, E.I., Curran, W.S., Harper, J.K., Roth, G.W. and Knievel, D.P. (2004). Velvetleaf (*Abutilon theophrasti*) interference and seed production in corn silage and grain. *Weed Technology* **18:** 779-783.
44. Yang, C.M., Chang, I.F., Lin, S.J. and Chou, C.H. (2004). Effects of three allelopathic phenolics on chlorophyll accumulation of rice (*Oryza sativa*) seedlings: II. Stimulation of consumption orientation. *Botanical Bulletin of Academia Sinica* **45:** 119-125.
45. Zanin, G. and Sattin, M. (2006). Threshold level and seed production of velvetleaf (*Abutilon theophrasti* Medicus) in maize. *Weed Research* **28:** 347-352.
46. Zhang, M., Liu, W.X., Zheng, M.F., Xu, Q.L., Wan, F.H., Wang, J., Lei, T., Zhou, Z.Y. and Tan, J.W. (2013). Bioactive quinic acid derivatives from *Ageratina adenophora. Molecules* **18:** 14096-14104.
47. Zheng, G.W., Jia, Y.X., Zhao, X., Zhang, F.J., Luo, S.H., Li, S.H. and Li, W.Q. (2012). *O*-coumaric acid from invasive *Eupatorium adenophorum* is a potent phytotoxin. *Chemoecology* **22:** 131-138.
48. Zohaib, A., Abbas, T. and Tabassum, T. (2016). Weeds cause losses in field crops through allelopathy. *Notulae Scientia Biologicae* **8:** 47-56.

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