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Differences in allelopathic effect of tree of heaven root extracts and isolated ailanthone on test-species

Razlike u alelopatskom učinku ekstrakata korijena pajasena i izoliranog ailantona na test-biljne vrste

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ABSTRACT

The objective of this paper was to analyse allelopathic effect of 3 different aqueous solutions from tree of heaven's (*Ailanthus altissima* (Mill.) Swingle) root extract and their dilutions on common wheat (*Triticum aestivum* L.), pigweed (*Amaranthus retroflexus* L.) and red bristlegrass (*Setaria pumila* L.). Investigated aqueous solutions obtained from tree of heaven's root extract were root aqueous solution, aqueous solution with isolated ailanthone and root aqueous solution without ailanthone. Each of these solutions was diluted with water in ratio 1:4 and 1:16 before application on seeds of 3 test-plant species. Concentrated root and ailanthone aqueous solution and dilutions were equivalent to concentration of 0.48 mg/mL ailanthone. High allelopathic effect on radicle and shoot length of all test-species was proven for all investigated aqueous solution and their dilutions. Inhibitory effect on initial growth of all test-species was proportional to the increase in concentration in all treatments. The effect on initial growth was stronger in relation to the effect on germination of test-species. Lower concentrations caused stimulation of red bristlegrass germination. Pigweed was the most sensitive and common wheat the least sensitive test-species. The least significant effect was measured in the aqueous solution of isolated ailanthone.

Keywords: allelochemicals, common wheat, dilutions, extrapolation, pigweed, red bristlegrass

SAŽETAK

Cilj ovoga rada bio je istražiti alelopatski učinak 3 različite vodene otopine iz ekstrakta korijena pajasena (*Ailanthus altissima* (Mill.) Swingle) i njihova razrjeđenja na pšenicu (*Triticum aestivum* L.), oštrodlakavi šćir (*Amaranthus retroflexus* L.) i zeleni muhar (*Setaria pumila* L.). Istraživane vodene otopine dobivene iz ekstrakta korijena pajasena su vodena otopina korijena pajasena, vodena otopina izoliranog ailantona i vodena otopina korijena bez ailantona. Svaka od tih otopina razrijeđena je s vodom u omjeru 1:4 i 1:16 prije primjene na sjeme 3 test-biljne vrste. Koncentrirana vodena otopina korijena i vodena otopina ailantona te razrjeđenja bili su ekvivalentni koncentraciji od 0.48 mg/mL ailantona. Utvrđen je visok alelopatski učinak na duljinu korijena i izdanka klice svih test-biljnih vrsta za sve istraživane vodene otopine i njihova razrjeđenja. Inhibitorni učinak na početni porast svih test-biljnih vrsta bio je proporcionalan povećanju koncentracije na svim tretmanima. Učinak na početni rast je jači u odnosu na klijavost test-biljnih vrsta. Niže koncentracije stimulirale su klijanje zelenog muhara. Oštrodlakavi šćir je najosjetljivija, a pšenica najmanje osjetljiva test-biljna vrsta. Vodena otopina izoliranog ailantona iskazala je najslabiji učinak.

Ključne riječi: alelokemikalije, ekstrapolacija, oštrodlakavi šćir, pšenica, razrjeđenja, zeleni muhar

INTRODUCTION

The over use of synthetic herbicides for weed control over more than five decades has resulted in growing public concern due to their impact on human health, environment and herbicide resistant weeds. Natural compounds from plants offer excellent potential for synthesis of new herbicidal solution, or lead to development of compounds for new herbicides (Moradi et al., 2013). Allelopathy is the common name for biochemical reaction between plants as a result of allelochemical secretion. Allelochemicals, depending on the plant species, can be secreted from leaves, flowers, seeds, stems and roots of living plant material or decaying material. After excretion from the plant, allelochemicals are involved in a number of metabolic and physicochemical processes (Rice, 1984). Their toxicity depends on the age and metabolic stage of the plant, climate, season and environmental factors, but the concentration of allelochemicals remains to be a major factor. Allelochemicals have a dual behaviour that is referred to as *hormesis* causing inhibition or stimulation (Duke et al., 2002). Numerous examples of allelopathic interactions between different plant species (cultivated and weeds) suggest that allelopathy is directly or indirectly involved in agricultural production. For example, aqueous extracts of ragweed (*Ambrosia artemisiifolia* L.) inhibited the growth of tomato seedlings by 50% (Vidotto et al., 2013). Leaf and stem aqueous extracts of common cocklebur (*Xanthium strumarium* L.) had an inhibitory effect on germination and early growth of corn seedlings (*Zea mays* L.) (Shajie and Saffari, 2007). Csiszár et al. (2013) found that aqueous extracts of tree of heaven (*Ailanthus altissima* (Mill.) Swingle), ragweed, indigobush (*Amorpha fruticosa* L.), and Japanese knotweed (*Reynoutria japonica* Houtt.) significantly inhibited germination and initial growth of white mustard (*Sinapis alba* L.). Cultivated plant species like barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.) and rice (*Oryza sativa* L.) also produce allelochemicals that can have phytotoxic effect on plant species and could be used for annual grass weed suppression (Dhima et al., 2006; Jabran, 2017). Weeds as well as invasive plant species, unlike cultivated species that were artificially selected, have been evolutionarily selected and are expected to have greater allelopathic potential from cultivated species (Novak et al., 2018).

Invasive alien species tree of heaven is one of the most frequently investigated species for the purpose of isolating and finding allelochemicals with herbicidal effect. It belongs to the Simaroubaceae family, which has high content of quassinoids, secondary metabolites that are responsible for a wide range of biological activities such as antitumor, antimalarial, antiviral, insecticide, herbicide, antiparasitic, etc. (Alvesa et al., 2014). Invasive plant species, including tree of heaven, directly or indirectly impact soil chemical properties (Nikolić et al., 2013). Because of their multiple effects on plant communities and ecosystem biodiversity, they are a logical choice in search for plants with high allelopathic potential (Sladonja et al., 2015).

The allelopathic effect of tree of heaven was initially observed by Mergen (1959) in testing the effect of aqueous extracts of tree of heaven's leaves on 46 tree species. Apart from proving the allelopathic potential of tree of heaven, numerous authors have also studied it with the aim of isolating allelochemicals that could be used to control weeds. Polonsky (1973, 1985, cit. Heisey, 1996) states that numerous quasinoid-based compounds have been isolated from plants belonging to the Simaroubaceae family, which also includes tree of heaven. De Feo et al. (2003) have isolated ailanthone, ailanthinone, chaparrine, and ailanthinol B (quassinoid derivatives) from aquatic extracts of tree of heaven's root. The same group of authors highlighted ailanthone as the most active compound and indicated the possibility of its use as an alternative herbicide. Raw (untreated) extracts of the tree of heaven's root bark can have high herbicidal effect on several plant species (Heisey, 1996).

Ailanthone is allelochemical belonging to quassinoids group, concentrated in root and bark of tree of heaven, polarized and easily displaced (Saxena, 2002). Heisey (1990a) was the first scientist to isolate phytotoxic compound from tree of heaven and identified it as ailanthone. Dichloromethane and ethyl-acetate have proven to be the most effective solvents for extracting ailanthone from the root bark (Heisey, 1990a; Pedersini et al., 2011). Heisey (1990a) has demonstrated that majority of tree of heaven's allelochemicals are concentrated in root and bark. The same author (Heisey, 1990b) also confirmed differences in seasonal toxicity of tree of heaven i.e. the highest concentration of ailanthone in roots were found in spring. Many other authors confirmed that ailanthone is mostly concentrated in root of tree of heaven (Saxena, 2002; Novak, 2017; Novak and Novak, 2019). Novak (2017) determined the concentration of ailanthone in tree of heaven's root aqueous solution by liquid chromatography (HPLC) from plant material collected in fall. It amounted to 0.35 mg/mL ailanthone which was the highest concentration compared to other tree of heaven's plant parts. However, Novak and Novak (2019), by using the same method of collecting and preparation of plant material, determined 0.48 mg/mL ailanthone in tree of heaven's root aqueous solution from plant material collected in spring. Because samples of plant material from both authors were collected at the same location, differences in variability between populations/species can be excluded, which is in line with research done by Heisey (1990b) concerning the seasonal toxicity of tree of heaven.

Majority of tree of heaven allelopathic studies relate to determination of its aqueous solutions effect on cultivated plants. Several authors (Heisey, 1996; 1997; 2003; Saxena 2002; Pedersini et al., 2011; Novak 2019; Novak and Novak, 2019) have investigated herbicidal effect of tree of heaven root extracts on weed species. Aqueous extracts of tree of heaven's root bark in various concentrations significantly reduced the growth of seedlings of pigweed, velvetleaf, red bristlegrass (*Setaria pumila* L.), barnyard grass (*Echinochloa crus-galli* L.) and maize (Heisey, 1997; Novak, 2019; Novak and Novak, 2019). All investigated test-plant species had a reduced degree of germination and growth. The broadleaf weed species, pigweed, was the most sensitive. The exception was velvetleaf, which proved to be extremely tolerant, followed by maize (Heisey, 1997). Heisey (1990b) proved that herbicidal effect was dose dependent i.e. higher amount of ailanthone significantly and permanently reduced seed germination, while germination was delayed and moderate at lower dosages. Saxena (2002) and Novak (2019) proved that broadleaf weeds are more sensitive to ailanthone compared to grass weeds and that plant species can absorb ailanthone before and after emergence.

The objective of this paper was to analyse allelopathic effect of root aqueous solution, ailanthone aqueous solution with the same amount of ailanthone as root aqueous solution and the root aqueous solution without ailanthone on common wheat (*Triticum aestivum* L.), pigweed (*Amaranthus retroflexus* L.) and red bristlegrass. The difference in effect between the investigated aqueous solutions and the claim whether ailanthone is indeed the most significant allelochemical in tree of heaven's root were also investigated.

MATERIAL AND METHODS

Plant extracts

Young shoots with roots of tree of heaven (donor species) were collected in May before flowering. Fresh material from donor species was collected on a non-agricultural surface (between abandoned buildings in Zagreb, N 45.804529, E 16.009786) to avoid possible herbicide residues and influence on results of the study. Representative sample was prepared from 10 plants of tree of heaven that were collected from the same site. Freshly collected roots were cut into small pieces (size 0.5-1 cm) and grained. In one litter of distilled water, 250 g of grained plant material was soaked at room temperature. After 24 hours, plant material was removed and extracts were filtered through filter paper (wrinkled 21/N, Munktell and Filtrak).

The seeds of red bristlegrass and pigweed were also collected on a non-agricultural area in the City of Zagreb (N 45.789477, E 15.991778) in October. The common wheat seeds were obtained from University of Zagreb Faculty of Agriculture, Department of Field Crops, Forage and Grassland in Croatia.

Collection of plant material, preparation of extracts and treatments were performed by adjusted method of

Kazinczi et al. (2004), Takács et al. (2004) and Kazinczi et al. (2013).

Chemical analyses

Identification and quantification of ailanthone from root aqueous solution was performed on HPLC. In tree of heaven's root aqueous solution concentration of ailanthone was 0.48 mg/mL. Confirmation of identity and quantification of ailanthone from the solution was determined by linear regression based on the calibration curve of the standard solution of the ailanthone (purity > 98%). After identification and quantification, isolation of ailanthone from one half of the root aqueous solution was performed with dichloromethane. The extraction of ailanthone was carried out 3 times in separating funnel and the sample was further purified with sodium sulphate. The sample was then evaporated in rotary evaporator at 50 °C to a dry residue (Novak, 2019).

Bioassays

When 1 L of tree of heaven root extract was available, after identification and quantification of ailanthone from root extract, 3 aqueous solutions were prepared: the root aqueous solution (RAS), ailanthone aqueous solution (AAS) with the same amount of ailanthone as RAS and the root aqueous solution without ailanthone (RASwA). RAS was the initial root solution from which ailanthone was isolated with dichloromethane. From concentrated solutions RAS, AAS and RASwA, 2 dilutions with distilled water were prepared in ratio 1:4 and 1:16 for each concentrated solution. RAS and AAS were equivalent to the determinate concentration of ailanthone and each dilution had 0.24 and 0.03 mg/mL ailanthone.

Pre-emergence effect of the aqueous solutions on the test-species was carried out in Petri dishes. In each sterilized Petri dish, 25 seeds of each test-species were placed on 2 filter paper layers in 4 replicates. Before placing seeds, 4 mL of each aqueous solution for red bristlegrass and pigweed and 8 mL for common wheat of each aqueous solution were added per Petri dish. Distilled water was used for the control treatment. Petri dishes were placed in darkness at 25-27 °C and relative humidity 70% in a climate chamber. Percentage of germination, radicle length and shoot length were determined for each variant. All investigated properties were determined after 7 days.

Data analysis

Multi factor analysis of variance (ANOVA) in 4 replicates for test-species * aqueous solutions * dilutions using the statistical software Statistica 10.0 (Stat Soft, Inc., USA) provided estimates of test-species, aqueous solutions and dilutions differences on germination percentage, shoot length and radicle length. Standard error was determined using Fisher LSD test with a 0.01 level of significance.

RESULTS

Based on data analysis, effects of all solutions in research are presented in Tables 1-4 for each investigated parameter separately.

Allelopathic effect on germination

Germination percentage was influenced by test-species, aqueous solutions and dilutions, as well as interaction of test-species, aqueous solutions and dilutions (Table 1). The lowest inhibition of pigweed's germination (2.4%) was determined in concentrated AAS treatment, while the highest inhibition was determined in treatments with concentrated RAS and RASwA (100% and 100%) (Table 1). Only concentrated RAS and RASwA caused the effect that statistically differed compared to control (Table 1). In treatment with red bristlegrass, all identified inhibitions on germination, except inhibition caused by RAS 1:4, were statistically significant. The lowest inhibition on germination (15.96%) was determined in treatment with RASwA at 1:16 dilution, while the highest inhibition was in treatment with concentrated RAS (93.1%) (Table 1). Stimulation of germination was recorded in treatment with 1:16 dilution of RAS (15.96%) and in the treatment with 1:4 and 1:16 dilutions of AAS by 18.62% and 23.94%, respectively (Table 1).

Table 1. The effect of aqueous solutions and dilutions on germination of test-species

Test-species	Aqueous solutions	Dilution	Germination (%)[4]	Deviation from control (%)
Pigweed (*Amaranthus retroflexus* L.)	RAS[1]	Concentrate	0a	-100.00
		1:4	89 ghij	7.20
		1:16	83 efgh	0.00
	AAS[2]	Concentrate	81 efgh	-2.40
		1:4	85 fghi	2.40
		1:16	83 efgh	0.00
	RASwA[3]	Concentrate	0a	-100.00
		1:4	84 efghi	1.20
		1:16	89 ghij	7.20
	Control		83efgh	
Red bristlegrass (*Setaria pumila* L.)	RAS[1]	Concentrate	5 ab	-93.10
		1:4	80 efg	6.65
		1:16	87 fghij	15.96
	AAS[2]	Concentrate	10 b	-86.45
		1:4	89 ghij	18.62
		1:16	93 ij	23.94
	RASwA[3]	Concentrate	40 c	-46.55
		1:4	38 c	-49.21
		1:16	63 d	-15.96
	Control		75e	
Common wheat (*Triticum aestivum* L.)	RAS[1]	Concentrate	79 ef	-12.21
		1:4	87 fghij	-3.33
		1:16	90 hij	0.00
	AAS[2]	Concentrate	81 efgh	-9.99
		1:4	93 ij	3.33
		1:16	95 fghij	5.55
	RASwA[3]	Concentrate	87 fghij	-3.33
		1:4	93 ij	3.33
		1:16	82 efgh	-8.88
	Control		90 hij	
P value				
Test-species			0.00	
Dilution			0.00	
Aqueous solution			0.00	
Test-species*dilution			0.00	
Test species*aqueous solution			0.00	
Dilution*aqueous solution			0.00	
Test-species*dilution*aqueous solution			0.00	

Values marked with different letter significantly differ according to (Fisher LSD) test at P≤0.01
ns = not significant (P>0.01)
[1] Root aqueous solution
[2] Ailanthone aqueous solution
[3] Root aqueous solution without ailanthone
[4] Percent of germinated seeds

In treatment with common wheat statistically significant inhibition on germination was only when treated with concentrated RAS (12.21%) (Table 1).

Allelopathic effect on radicle length

Radicle length was influenced by test-species and dilutions, as well as their interaction (Table 2). Radicle growth of all test-species was significantly inhibited at all concentrations for more than 47%, respectively. Inhibition of radicle length was proportional to the increase in concentrations of all solutions (Table 2). The highest inhibition of radicle length was recorded in red bristlegrass treated with concentrate and 1:4 dilution followed by pigweed treated with concentrate. The effect between these two dilutions did not differ statistically. The lowest inhibition was recorded in common wheat in treatment with 1:16 dilution. The highest reduction of radicle length was determined in all treatments on red bristlegrass, followed by amaranth. The lowest inhibition of radicle length in all treatments was determined on common wheat. In comparison to the effect of 1:16 dilution on red bristlegrass, even at concentrate it was inhibited by 2.2% (Table 2).

Table 2. The effect of investigated dilutions on radicle length of test-species

Test-species	Dilution	Radicle length (mm)	Deviation from control (%)
Pigweed (*Amaranthus retroflexus* L.)	Concentrate (RAS[1] + AAS[2] + RASwA[3])	0.71a	-94.40
	1:4 (RAS[1] + AAS[2] + RASwA[3])	2.29b	-81.95
	1:16 (RAS[1] + AAS[2] + RASwA[3])	2.92bc	-76.98
	Control	12.69f	
Red bristlegrass (*Setaria pumila* L.)	Concentrate (RAS[1] + AAS[2] + RASwA[3])	0.22a	-98.99
	1:4 (RAS[1] + AAS[2] + RASwA[3])	0.64a	-97.06
	1:16 (RAS[1] + AAS[2] + RASwA[3])	3.56c	-83.63
	Control	21.74h	
Common wheat (*Triticum aestivum* L.)	Concentrate (RAS[1] + AAS[2] + RASwA[3])	5.22d	-81.43
	1:4 (RAS[1] + AAS[2] + RASwA[3])	9.72e	-65.37
	1:16 (RAS[1] + AAS[2] + RASwA[3])	14.77g	-47.34
	Control	28.03i	
P value			
Test-species		0.00	
Aqueous solution		ns	
Dilution		0.00	
Test species*aqueous solution		ns	
Test-species*dilution		0.00	
Dilution*aqueous solution		ns	
Test-species*dilution*aqueous solution		ns	

Values marked with different letter significantly differ according to (Fisher LSD) test at P≤0.01
ns = not significant (P>0.01)
[1] Root aqueous solution
[2] Ailanthone aqueous solution
[3] Root aqueous solution without ailanthone

Allelopathic effect on shoot length

Shoot length was influenced by test-species and dilutions, as well as their interaction (Table 3). Shoot length of all test-species was significantly inhibited by all dilutions. Inhibition of pigweed and red bristlegrass shoot length was proportional to the increase in concentrations of all solutions, but not for common wheat in which 1:16 dilution exhibited stronger inhibition effect than the 1:4 dilution (Table 3). Inhibition of pigweed's shoot length was complete for concentrate and 1:4 dilution and less than 1 mm for 1:16 dilution, and these differences were not statistically significant. Applied dilutions in this research exhibited diverse effect to red bristlegrass shoot length. Complete inhibition of shoot growth occurred when concentrated solution was applied, followed by 1:4 dilution (93.97%) whereas 1:16 dilution had the lowest inhibition effect to red bristlegrass shoot growth in comparison to pigweed and common wheat. Common wheat was the only test-species at which concentrate did not completely inhibit shoot growth (Table 3).

Table 3. The effect of investigated dilutions on radicle length of test-species

Test-species	Dilution	Shoot length (mm)	Deviation from control (%)
Pigweed (*Amaranthus retroflexus* L.)	Concentrate (RAS[1] + AAS[2] + RASwA[3])	0.00a	-100.00
	1:4 (RAS[1] + AAS[2] + RASwA[3])	0.00a	-100.00
	1:16 (RAS[1] + AAS[2] + RASwA[3])	0.33a	-95.00
	Control	6.63d	
Red bristlegrass (*Setaria pumila* L.)	Concentrate (RAS[1] + AAS[2] + RASwA[3])	0.00a	-100.00
	1:4 (RAS[1] + AAS[2] + RASwA[3])	2.31bc	-93.97
	1:16 (RAS[1] + AAS[2] + RASwA[3])	10.88e	-72.11
	Control	39.16f	
Common wheat (*Triticum aestivum* L.)	Concentrate (RAS[1] + AAS[2] + RASwA[3])	1.67b	-71.99
	1:4 (RAS[1] + AAS[2] + RASwA[3])	3.38c	-43.29
	1:16 (RAS[1] + AAS[2] + RASwA[3])	2.89bc	-51.51
	Control	5.96d	
P value			
Test-species		0.00	
Aqueous solution		ns	
Dilution		0.00	
Test species*aqueous solution		ns	
Test-species*dilution		0.00	
Dilution*aqueous solution		ns	
Test-species*dilution*aqueous solution		ns	

Values marked with different letter significantly differ according to (Fisher LSD) test at P≤0.01
ns = not significant (P>0.01)
[1] Root aqueous solution
[2] Ailanthone aqueous solution
[3] Root aqueous solution without ailanthone

Differences in allelopathic effect between investigated solutions

The cumulative effect of concentrate and 2 dilutions from AAS was significantly lower on pigweed's germination compared to effect caused by RAS and RASwA (Table 4). Inhibition of red bristlegrass germination differ significantly between tested solutions. The highest inhibition of red bristlegrass germination was in treatment with RASwA. Germination of common wheat did not differ significantly between tested solutions. The inhibition of pigweed and red bristlegrass radicle length did not differ significantly between all investigated solutions. The cumulative effect of concentrate and 2 dilutions from AAS was significantly lower on shoot length of common wheat compared to the effect caused by RAS and RASwA (Table 4). There was no significant difference in the effect on shoot length of pigweed and common wheat between all investigated solutions. Significantly lower inhibition of red bristlegrass radicle length was recorded in treatment with ailanthone aqueous solution (Table 4).

DISCUSSION

The results confirmed frequently used research hypothesis which states that allelopathic effect on initial growth of seedlings is stronger in relation to the effect on germination of test-species (Kazinczi et al., 2013). The present study also found differences concerning germination. Tree of heaven concentrated root aqueous solution and concentrated root aqueous solution without ailanthone generally had significant inhibitory effect on germination of test-species. In treatment with common wheat, these effects were less expressed in comparison to weed test-species. Consistent with our results are results of many authors who have proved that different plant extracts had no significant impact on germination of test-species (Norby and Kozlowski, 1980; Đikić, 2005; Lovett et al., 2006; Novak, 2007; Novak et al., 2018). However, Vuković (2015) reported inhibition of test-species germination caused by tree of heaven extracts. It is evident that effect on germination can vary depending on donor species, test-species and concentration of applied aqueous solutions.

Table 4. The cumulative effect of dilutions per aqueous solutions on all variables and test-species

Test-species	Aqueous solutions	Germination (%)[4]	Radicle length (mm)	Shoot length (mm)
Pigweed (*Amaranthus retroflexus* L.)	RAS[1] (Concentrate+1:4+1:16)	64.00 ab	4.41a	1.67 a
	AAS[2] (Concentrate+1:4+1:16)	83.00 d	5.06a	1.66 a
	RASwA[3] (Concentrate+1:4+1:16)	64.00 ab	4.43a	1.88 a
Red bristlegrass (*Setaria pumila* L.)	RAS[1] (Concentrate+1:4+1:16)	61.75 a	6.41 b	12.41 c
	AAS[2] (Concentrate+1:4+1:16)	66.75 b	6.33 b	13.97 d
	RASwA[3] (Concentrate+1:4+1:16)	54.00 e	6.88 b	12.87 c
Common wheat (*Triticum aestivum* L.)	RAS[1] (Concentrate+1:4+1:16)	86.50 cd	13.72 c	3.29 b
	AAS[2] (Concentrate+1:4+1:16)	89.75 c	15.39 d	3.62 b
	RASwA[3] (Concentrate+1:4+1:16)	88.00 c	14.18 c	3.49 b

Values marked with different letter significantly differ according to (Fisher LSD) test at P≤0.01
[1] Root aqueous solution
[2] Ailanthone aqueous solution
[3] Root aqueous solution without ailanthone
[4] Percent of germinated seeds

Our results also confirm stimulation of red bristlegrass germination when treated with lower dilutions from root aqueous solution and ailanthone aqueous solution. Contrary to our results, for the same test-species only reduction in germination has been shown when treated with the same solutions (Novak, 2019). These different results can be attributed to a different location from which seeds were collected, i.e. to the difference in variability between populations of the studied species. The aqueous solution was from the same stock and both studies were conducted at the same time. The allelopathic effect on pigweed and common wheat was not significant, but led to an increase in germination. Consistent with our results, increase in pigweed and maize germination has been shown at lower concentrations of ailanthone when treated with the same aqueous solutions (Novak, 2019).

The present study also found that red bristlegrass was the most sensitive test-species when the effect on germination was investigated. Germination of red bristlegrass was significantly affected, either inhibitory or stimulatory, by all concentrations. Exception was the effect caused by dilution 1:4 from root aqueous solution. Conversely, significant reduction in germination of red bristlegrass was reported when treated with water extract of tree of heaven's root (Heisey, 1996; Saxena, 2002 and Heisey and Heisey, 2003). Isolated ailanthone has herbicidal effect on species from the genus bristlegrass (Setaria P. Beauv.) exclusively when applied to seeds (Saxena, 2002; Heisey and Heisey, 2003). Our results showed that common wheat was the least sensitive species in the study. Conversely, inhibitory effect on germination and growth of common wheat has been reported when treated with tree of heaven leaves extracts (Ullah et al., 2020). However, Novak (2019) confirmed significantly lower inhibitory effect on maize compared to weed test-species in treatment with tree of heaven's root and isolated ailanthone extracts. The same author also confirmed that reduction of maize germination was only shown in treatment with higher concentrations, which is in line with the present study.

Contrasting to the effect on germination, effects on initial growth, i.e. radicle and shoot growth, were significant and exclusively inhibitory when exposed to all solutions and all dilutions investigated in this study. Many plant extracts have herbicidal effect on different test-species which can be compared with the action of residual herbicides. Residual herbicides also control weeds soon after germination. Weed seedlings die in contact with "herbicidal film" in the soil, before or soon after emergence (Ostojić and Šilješ, 1982). Very strong inhibitory effect on radicle and shoot length of test-species was consistent with the results of De Feo et al. (2003) and Pisula and Meiners (2010). These authors also pointed to the tree of heaven as a plant with very strong allelopathic potential, among 10 studied plant species. Numerous studies confirmed inhibitory effect on initial growth of different test-species when treated with tree of heaven's different extracts and different dilutions. Consistent with our results, initial growth of pigweed has been completely reduced when treated with different concentrations of ailanthone isolated from the bark aqueous solution in pre-emergence application (Pedersini et al., 2011). The same inhibitory effect was confirmed for barnyard grass (Novak and Novak, 2019), pigweed and red bristlegrass (Novak, 2019). Our observation on greater reduction of initial growth in comparison with germination is consistent with Catalán et al. (2013) who also proved significant inhibitory effect on germination and radicle growth in 5 test-species when treated with tree of heaven's leaves extracts, whereas the effect on germination was not expressed. Inhibitory effect on initial growth of weed test-species was more expressed in regards to common wheat. Consistent with our results, red bristlegrass, pigweed, velvetleaf (*Abutilon theophrasti* Med.) have been more sensitive compared to maize in treatment with tree of heaven extracts (Saxena, 2002; Novak, 2019). Present study showed that inhibition of initial growth was very strong, greater than 70% in all treatments for weed test-species and greater than 47% for common wheat. Although the inhibitory effect on initial growth of common wheat was slightly weaker compared to weed test-species, no positive effect or stimulation was determined. Our results on inhibitory effect on initial growth of common wheat as the least sensitive species, are consistent with Heisey (1990b; 1997) who stated that aqueous extracts of tree of heaven have almost inhibitory effect on maize. In contrast to our results, stimulation of maize shoot length has been proven when treated with tree of heaven root extract and isolated ailanthone (Novak, 2019).

The inhibitory effect on radicle and shoot length for all 3 test-species was proportional to concentration increase in all treatments. The association of ailanthone concentration and allelopathic effect on susceptible test-species are consistent with many authors who state that different ailanthone content is directly related to the inhibitory effect on investigated properties of test-species (Heisey, 1996; Pisula and Meiners, 2010; Pedersini et al., 2011; Novak, 2017; Novak et al., 2018). Due to the different methods used, it is difficult to draw a parallel between the ailanthone concentrations used by each author and compare them with ailanthone concentrations used in our study. Our observation of correlation between the effect and concentration is consistent with Novak (2019) who also demonstrated that 6 different concentrations of ailanthone from the same aqueous solution and the inhibitory effect were in correlation.

Test-species differ in their sensitivity to all dilutions used (allelochemical complex from root, isolated ailanthone and allelochemical complex from root without ailanthone) when applied on seeds. Due to cumulative effect of all dilutions per solution it is clear that aqueous solution of isolated ailanthone had the lowest effect on germination of weed test-species, while no difference was determined in treatment with common wheat. Contrary to the effect on germination, no difference was found between the effects on radicle length of weed test-species when treated with different solutions. No difference was found in the effect on shoot length of pigweed and common wheat between investigated solutions. However, isolated ailanthone had the statistically least significant effect on radicle length of common wheat and shoot length of red bristlegrass. Even though the comparison of these results with results from other authors is difficult for the reasons already mentioned, but they are consistent with the theory presented by Heisey and Heisey (2003) and De Feo et al. (2003). These authors suggested that ailanthone is not the only allelochemical in plant material of tree of heaven, but certainly is a major one and allelopathic effect depends on concentration of ailanthone (Heisey, 1990a; Heisey and Heisey, 2003; Pedersini et al., 2011; Bostan et al., 2014). Our observations on similar effect between isolated ailanthone and complex of allelochemicals on majority of investigated variables are contrary to Heisey and Heisey (2003) who stated that ailanthone acts synergistic and has better effect on germination and initial growth of test plant species in combination with other allelochemicals. In addition to ailanthone, there are many other allelochemicals in the root of tree of heaven. However, not all allelochemicals from tree of heaven exhibit a phytotoxic effect (Heisey, 1999; De Fao et al., 2003). Only allelochemicals that have an oxymethylene ring reduce the growth of plant species. Except for ailanthone, in tree of heaven, these are holocanthone and chaparrinone (Dayan et al., 1999). These 3 allelochemicals are monoterpenes and their mode of action is not yet known (Duke and Oliva, 2005). Therefore, it is assumed that these allelochemicals act synergistically and, thus, enhance the allelopathic effect. We demonstrated that the concentration has a major effect, although exceptions were found where the effect was equally strong at all dilutions. However, a difference between the test-species was found. There are a number of factors that might affect the uptake of allelochemicals by acceptor species. One of the possible explanation for differences found between sensitivity of the test-species could be the mode of plant absorption, but also the differences in the size of the seeds. A review of the literature showed that the allelopathic effect of allelochemicals is most dependent on the test-species. One of the assumptions is that common wheat has significantly larger seeds compared to red bristlegrass and pigweed, which is supported with the results presented by Sladonja et al. (2014), who found that the test-species with larger seeds were less sensitive when the effect on initial growth was investigated. Another reason could be the difference in morphology, physiology, cytology and biochemistry of plants due to which a difference in sensitivity exist, i.e. the tolerance between test-species. Additionally, difference in the effect between the investigated aqueous solutions can be explained by the fact that plants assimilate different allelochemicals differently. Namely, after the uptake of allelochemicals, some plant species inactivate allelochemicals by metabolic processes faster than the other plants (Scognamiglio and Schneider, 2020). Also, the difference in the amount of hormone auxin, which is responsible for stimulating the initial growth, could explain the differences in sensitivity between the test-species. Monocots are known to respond differently to auxinic herbicides. In addition, the properties that distinguish monocots from dicots involve structures whose development is controlled by auxin. However, the molecular mechanisms controlling auxin biosynthesis, homeostasis, transport and signal transduction appear, so far, to be conserved between monocots and dicots, although there are differences in gene copy number and expression leading to diversification in function (McSteen, 2010). This also supports obtained results on greater sensitivity of pigweed compared to common wheat and red bristlegrass.

CONCLUSIONS

Generally, the allelopathic effect on germination is much lower than the effect on radicle and shoot length for all of the test-species. Lower concentrations caused stimulation of red bristlegrass germination. The effect on radicle and shoot growth was significant and exclusively inhibitory in all treatments. Inhibitory effect on initial growth of all test-species was proportional to the increase in concentration in all treatments. Pigweed was the most sensitive and common wheat the least sensitive test-species. The aqueous solution of isolated ailanthone had the least significant effect. Ailanthone has strong allelopathic potential but its inhibitory effect was increased when applied with other allelochemicals present in the roots of tree of heaven. Synergistic activity of allelochemicals from tree of heaven's root has distinct inhibitory effect, even when the most significant allelochemical ailanthone was removed from the complex.

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