https://doi.org/10.26651/allelo.j/2020-49-2-1263 0971-4693/94 Euro 20.00
Allelopathy Journal 49 (2): 177-188 (Mar, 2020) *International Allelopathy Foundation* 2020
Table: 4, Figs : 2

Allelopathic potential of *Juglans nigra* L. to control the invasive tree-of-heaven (*Ailanthus altissima* (Mill.) Swingle)

P. Ferus*, K. Menčik[1] and J. Konôpková
Mlyňany Arboretum, Institute of Forest Ecology,
Slovak Academy of Sciences, Vieska nad Žitavou 178,
95152 Slepčany, Slovakia
E. Mail: peter.ferus@savba.sk, pferus23@gmail.com

(Received in revised form: February 3, 2020)

ABSTRACT

Tree-of-heaven (*Ailanthus altissima* (Mill.) Swingle), native to China, is one of the worst invasive tree in Europe due to its large impact on ecosystem functioning and an enormous eradication cost. However, limited information is available about the allelopathy-based inhibitory plant against this tree species. Therefore, effect of 1 % leaf aqueous extracts from black walnut (*Juglans nigra* L.), common hackberry (*Celtis occidentalis* L.), false indigo bush (*Amorpha fruticosa* L.) or Chinese toon (*Cedrela sinensis* Juss.) on its seed germination and seedling growth were examined. Black walnut extract caused maximum 44 % reduction in its germination, but other 3 species caused only 25-30 % decrease. The extract from black walnut and Chinese toon adversely affected the leaf dry matter of the *A. altissima* seedlings. However, the black walnut and common hackberry extracts significantly increased their root-to-shoot ratio. Black walnut extract increased the leaf soluble sugars (37 %), but reduce the leaf hydrogen peroxide (-27 %) and leaf chlorophyll a+b contents (-20-25%). Thus, the black walnut extract strongly allelopathically suppressed the tree-of-heaven by reducing the germination and seedlings growth. However, the pure juglone (an allelopathically active black walnut flavonoid) at the same concentration that found in leaf extract markedly suppressed the seed germination (-70 %), decreased the leaf hydrogen peroxide concentration (-50 %) but stimulated the seedling roots growth (25 %). The effects of juglone were lower than black walnut extracts on young tree-of heaven plants and synergy with other components of extract has been suggested.

Key words: Allelopathy, germination, growth, juglone, metabolism, tree-of-heaven

INTRODUCTION

Tree-of-heaven (*Ailanthus altissima* (Mill.) Swingle), is 20-30 m tall Chinese tree. It was introduced in Europe in 1740, and was widely planted in city parks, along roads and in forests up to second half of twentieth century. It is dangerous invasive plant in all continents (except Antarctica), from temperate to Mediterranean zone, occupying built-up areas, transportation corridors, river banks and forests (22). Nowadays, it is listed in 100 worst invasive organisms in Europe (Delivering Alien Invasive Species Inventories for Europe, DAISIE) and appeared on the black list in many countries, including Slovakia (27). As per Regulation no. 158/2014 of the Ministry of Environment, land owners in Slovakia are obliged to eradicate it from their land and prevent its further spread.

Allelopathy, defined as intra- and inter-specific plant-to-plant interactions due to allelochemicals, playing a great role in agriculture and forestry (41), could provide clues for its efficient eradication. However, till now only herbicidal activity of tree-of-heaven residues/extracts are known. Heisey and Heisey (17) described the phytotoxic effect of

*Correspondence author, [1]Department of Botany and Genetics, University of Constantine the Philosopher, Nábrežie mládeže 91, 94974 Nitra, Slovakia.

methanol extract of its bark on 17 weeds and crop plant species, where only 0.3 kg ha^{-1} ailanthone equivalent caused mortality exceeding 50 % in 9 species and significantly reduced the shoot biomass in 13 species. In another study, impact of co-habitation of tree-of-heaven in temperate forests of the north-eastern United States on seedlings emergence, survival and growth of red maple (*Acer rubrum* L.), sugar maple (*Acer saccharum* Marshall) and red oak (*Quercus rubra* L.) were analysed (16). Tree-of-heaven had neutral or slightly positive influence on the red maple growth, but it adversely affected the sugar maple and red oak. The indirect effect of tree-of-heaven root exudates on the red oak growth was mainly due to decrease in ectomycorrhizal fungi colonization and shift in the ectomycorrhizal community (3). The tree-of-heaven reduced the growth of United States-native herb yellow crownbeard (*Verbesina occidentalis* L.) but had negligible effects on the invasive weed wild teasel (*Dipsacus fullonum* L.), when grown for 6 weeks in tree-of-heaven plantation (36).

This study aimed to determine the plant species suppressive to tree-of-heaven. The selection of test species was based on the study of Csiszár *et al.* (7), where inhibitory effects of numerous invasive woody plant species was compared to that of 1 mM juglone produced mostly by black walnut (*Juglans nigra* L.). Apart from black walnut, those species were used, which recorded inhibitory effects exceeding the juglone equivalent index, namely common hackberry (*Celtis occidentalis* L.), false indigo bush (*Amorpha fruticosa* L.). All are native to the North America, except for south-east Asian Chinese toon (*Cedrela sinensis* Juss.), a favourite leaf vegetable tree with strong smell, substantially contributed by *cis* and *trans* isomers of 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (26), which had a strong suppressive effect on weeds (21). Besides seed germination and production parameters of tree-of-heaven seedlings treated by aqueous extracts from the above mentioned woody plant species, we also focussed on basic metabolites outlining target metabolisms for allelochemicals produced by tested species - soluble sugars [providing a view into carbon assimilate source-sink relations (14)], leaf chlorophyll concentration [(representing an indirect measure of plant nitrogen supply (34)] and hydrogen peroxide level [reflecting an oxidative damage caused by allelochemicals (15)].

MATERIAL AND METHODS

I. Petri plate bioassays

Fruits collection : Fruits of tree-of-heaven (*Ailanthus altissima* (Mill.) Swingle) were collected in early spring 2017 from a 35 years old individual tree in Mlyňany Arboretum IFE SAS (GPS: N 48.3194°, E 18.3656°, height: 194 m a.s.l., climatic characteristics (1971-2000): Mean annual temperature: 10.6 °C, Mean annual precipitation: 541 mm). After hydration (1 day), fruits of tree-of-heaven were washed thrice under tap water and stored at 4 °C till testing.

(i). **Aqueous leachates :** One hundred fruits (containing 1 seed each) without wings were placed into glass Petri dishes (25 cm diameter) lined with 2 mm thick paper tissue (i.e. 100 seeds were placed equidistance in each Petri dish). Leaves and fine twigs of the donor plants collected from trees with southern exposition in Mlyňany Arboretum in August 2016 were air- and then oven-dried (40 °C), and stored in tightly closed dark plastic bags. Just before their use, these were cut into 0.5 cm pieces and soaked in distilled water at the lab temperature for 24 h (Table 1). Thereafter, 100 ml of distilled water (control) or 1 % aqueous extract (w/v) prepared from leaves and twigs of black walnut (*Juglans nigra* L.), common hackberry (*Celtis occidentalis* L.), false indigo bush (*Amorpha fruticosa* L.) or Chinese toon (*Cedrela sinensis* Juss.) from the arboretum was added per Petri dish, to keep the paper tissue completely soaked, but without excess of water/extract. Three Petri dishes per treatment were positioned into growth chamber (Lovibond, Tintometer GmbH, Germany) and incubated at 25 °C in dark. After fourteen days of cultivation, relative germination was determined as under :

$$RG = (AG / CG) * 100 \quad (\%)$$

Where: RG : Relative germination, AG : Absolute germination (%), CG : Mean germination (%) in Control.

Table 1. Physico-chemical properties of the aqueous extracts prepared from leaves and twigs of black walnut, common hackberry, false indigo bush and Chinese toon, deionised water (control) pH of 6.79 and EC of 0.70 µS.cm^{-1}.

Parameter	Black walnut	Common hackberry	False indigo bush	Chinese toon
pH	5.93	6.21	6.10	5.82
Electric conductivity (mS.cm^{-1})	0.44	0.71	0.51	0.74
Osmotic potential (-MPa)	0.007	0.004	0.002	0.015
Phenolic acids (µg GAE.ml^{-1})	255.6	240.7	262.8	347.4
Flavonoids (µg QE.ml^{-1})	15.92	22.03	13.08	16.36
Tannins (µg TAE.ml^{-1})	61.6	46.3	62.4	122.2

GAE - gallic acid equivalent, QE - quercetin equivalent, TAE - tannic acid equivalent.

(ii). **Juglone :** The effects of different juglone (5-hydroxy-1,4-naphthoquinone) concentrations on tree-of-heaven fruits germination were tested in 2019. One hundred wings-bare fruits (containing 1 seed each), collected in spring were placed equidistant into glass Petri dishes (25 cm diameter lined with 2 mm thick paper tissue) and 100 ml juglone solution (dissolved in water bath at 40 °C for 2-5 days) of 0, 2.5, 5, 10 or 15 µg.ml^{-1} concentrations) were added to them. Three Petri dishes per treatment were kept in growth chamber (Lovibond, Tintometer GmbH, Germany) in dark at 25 ^{o}C. The seeds germination was recorded 14 days after sowing.

Pot Experiments

(i). **Aqueous leachates :** Tree-of-heaven fruits from the same individual tree were sown in spring 2017 into soil substrate compost-peat-gravel (≤ 4 mm) in the ratio 5:3:1. In early June, seedlings grown in half-shadow stand (maximal photosynthetically active radiation of ca. 600 µmol.m^{-2}.s^{-1}), were first transplanted into plastic pots (9 cm side, 10 cm depth) containing the above soil substrate. In early July, the seedlings were transferred with the root ball into plastic pots of 17 cm dia, 13 cm depth, containing the same soil substrate. These pots were irrigated and also sprayed on leaves with 100 ml 1 % aqueous leaf extracts of donor woody plants added with 3 drops of Tween 20 detergent or with 100 ml distilled water (control) twice a week for 6- weeks (6 plants for each treatment). Leaves and twigs for these leachates were collected in July 2017. Before and after beginning of the treatments, growth parameters (leaf, stem and root dry matter) leaf metabolites (soluble sugar, chlorophyll and hydrogen peroxide concentration) were determined

(ii). **Juglone :** In 2019, the effects of juglone concentrations (of 0, 2.5, 5, 10 and 15 $\mu g.ml^{-1}$) on the seedling growth and metabolism of tree-of-heaven, were studied. Tree-of-heaven fruits (collected from the same individual in spring 2019) were sown into pots with identical soil substrate used in the first pot experiment in end of April and placed into half-shadow external conditions like in the first experiment. After 60 days of cultivation (in end of June) seedlings were transplanted into plastic pots (9 cm dia, 10 cm depth) with the same soil substrate. In early July, seedlings were treated with 25 ml juglone solution of 0, 2.5, 5, 10 and 15 $\mu g.ml^{-1}$ concentration with 3 drops of Tween 20 (sprayed on leaves and soil) twice a week for six weeks.

Chemical Analyses of Aqueous extracts

I. pH and electric conductivity : These were determined by pH/conductivity meter Jenway Enterprise 430 (Jenway, UK). Osmotic potential was measured using a refractometer. Refractive indexes of extracts were determined with manual refractometer PZO RR11 (PZO, Poland) and obtained data was recalculated into the osmotic potential using a regression built for different glucose concentrations (35).

II. Total phenols : These were determined using the method of Nguyen and Niemeyer (32). To 50 µl aqueous extract, 450 µl of distilled water, 250 µl of Folin-Ciocalteau reagent and 1.25 ml of 20 % sodium carbornate were added and mixed together. After 20 min lab incubation, reaction mixture absorbance was measured at 735 nm (Jasco-V600, Jasco Inc., Japan).

III. Total flavonoids : 0.4 ml water extract was added to 0.2 ml of 10 % (w/v) aluminium nitrate and 0.2 ml of 1M potassium acetate and 8 ml of 80 % ethanol. After 40 min incubation at lab temperature, absorbance of the reaction mixture was determined at 415 nm (Jasco-V600, Jasco Inc., Japan) (33).

IV. Total tannins : One ml water extract was mixed with 5 ml distilled water, 1 ml of 0.35 % (w/v) brown ammonium iron (III) citrate and 1 ml of 0.8% (v/v) ammonia. After 10 min incubation at the lab temperature, absorbance of the reaction mixture was measured at 525 nm (Jasco-V600 (Jasco Inc., Japan) (37).

V. Total soluble sugars : Fifty mg leaf tissue was homogenized with 6 ml deionised water, the homogenate was transferred into a test tube and centrifuged at 6000 rpm for 15 min. Thereafter, 30 µl of supernatant and 2.5 ml of 96 % sulphuric acid were added to 0.5 ml of 4 % (w/v) phenol. These were thoroughly mixed and incubated for 15 min in dark. Finally, reaction mixture absorbance was determined at 490 nm (Jasco-V600, Jasco Inc., Japan) (13).

VI. Chlorophyll a+b content : Leaves were homogenized using mortar and pestle with sea sand, $MgCO_3$ and cold acetone. After its evaporation, powder was quantitatively transferred into 80 % acetone, filtered using vacuum pump and filtrate absorbance was determined 647 and 663 nm (Jasco-V600, Jasco Inc., Japan). Chlorophyll concentration was calculated according to Lichtenthaler (25).

VII. Hydrogen peroxide : 0.1 g leaves was homogenized in 6 ml cold acetone, quantitatively transferred into a test tube and centrifuged at 6000 rpm for 5 min. To 2 ml supernatant, 0.8 ml of titanium reagent [0.04% (w/v) Ti^{4+} in the form of $Ti(SO_4)_2$] and 1 ml of concentrated (30 %) ammonia were added. Then the reaction mixture was mixed and centrifuged at 6000 rpm for 10 min. Upper layer of the supernatant was discarded and lower one - containing Ti-H_2O_2 crystals - was mixed with 2 ml 2M sulphuric acid. After syringe filtration (0.2 µm teflon membrane), absorbance was measured at 415 nm (Jasco-V600 (Jasco Inc., Japan) (modified method of Mukherjee and Choudhuri (29)).

Statistical Analysis

The data of statistical effects of aqueous extracts/different juglone concentration on the growth and leaf metabolic parameters were analysed by one-way ANOVA. Comparison among means was done using Duncan´s multiple range test. Letters indicate significant differences between the treatments at confidence level $P \leqslant 0.05$. Thereafter, seed germination, seedling growth and metabolic parameters were submitted to the regression analysis with each other as well as respective woody plants extracts features/juglone concentration and *P*-values of the linear regression, were determined.

RESULTS

Tree-of-heaven seed germination was strongly influenced by the 1% aqueous extracts of donor woody plants species (Figure 1). Compared to the control, common hackberry, false indigo bush and Chinese toon caused 25-30% reductions in the relative germination, but the water extract from black walnut was more detrimental to tree-of-heaven seed germination (reduction by ca. 44%). Juglone at 5 $\mu g.ml^{-1}$ concentration significantly influenced (negatively) the germination (Figure 2). However, two or three-fold higher concentration didn't cause further decrease in the relative germination percentage.

Table 2. Effects of 1 % aqueous extract from black walnut, common hackberry, false indigo bush or Chinese toon on the dry matter of tree-of-heaven seedlings.

Extract	Leaf DW (g)	Stem DW (g)	Root DW (g)	Total plant DW (g)	Root:shoot ratio
CC	0.46±0.03 a	0.11±0.01 a	0.23±0.03 a	0.81±0.05 a	0.38±0.02 a
Distilled water	3.15±0.19 c	1.07±0.05 b	2.71±0.14 b	7.04±0.49 b	0.60±0.04 b
Black walnut	2.54±0.10 b	1.00±0.06 b	2.63±0.15 b	6.46±0.31 b	0.77±0.06 c
Common hackberry	2.69±0.14 bc	1.09±0.07 b	2.70±0.16 b	6.60±0.29 b	0.80±0.05 c
False indigo bush	2.88±0.20 bc	1.17±0.08 b	2.85±0.14 b	7.08±0.38 b	0.68±0.02 bc
Chinese toon	2.64±0.14 b	1.25±0.15 b	2.60±0.11 b	6.67±0.13 b	0.64±0.02 b

CC – control at the beginning of the experiment. Results are means of six replicates and a,b,c letters indicate significant differences between treatments at $P \leqslant 0.05$.

Seedlings of tree-of-heaven, treated for 6-weeks both on leaf and soil by the same collection of donor woody plant extracts, significantly decreased (-20%) the leaf dry matter only in the case of black walnut and Chinese toon (Table 2). Stem dry matter, root dry matter as well as total dry matter per plant in treated individuals did not show any differences compared to the control. On the other hand, root:shoot ratio increased after the application of extracts - in black walnut and common hackberry significantly (by ca. 30%) and slightly (13%) in false indigo bush. Chinese toon extract did not affect this growth parameter.

From leaf metabolites, significant changes (almost 37% increases) in soluble sugars concentration (Figure 1) were observed only in seedlings treated by black walnut extract. The rest extracts also only slightly increased the soluble sugars. Chlorophyll a+b content in the tree-of-heaven leaves was affected (by 20-25 %) only by black walnut and common hackberry extracts. However, slight decrease in chlorophyll content was caused by false indigo bush and Chinese toon extract. Hydrogen peroxide concentration was markedly decreased (27%) only by the black walnut extract.

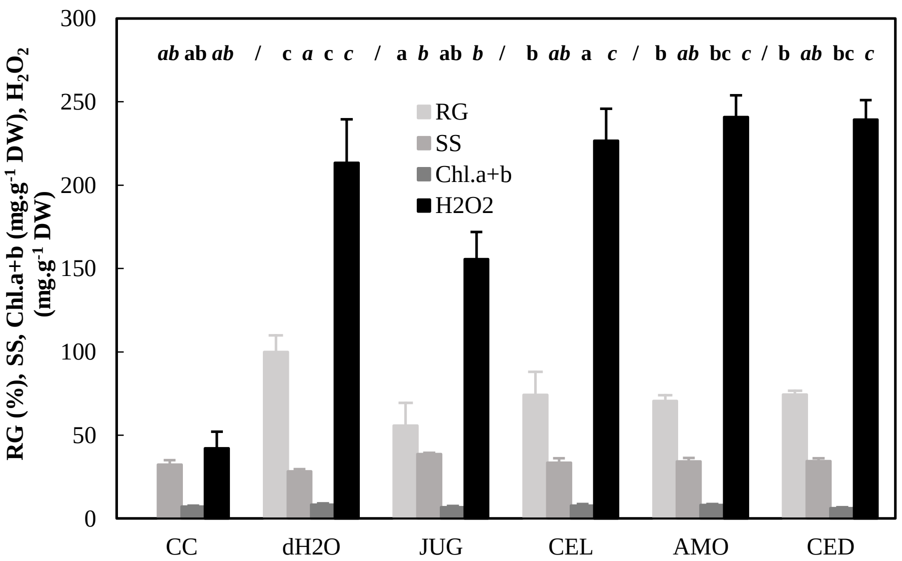

Figure 1. Relative germination of tree-of-heaven seeds after two weeks (Petri plate bioassay) as well as soluble sugar, chlorophyll a+b and hydrogen peroxide concentrations in the top expanded leaves of tree-of-heaven seedlings sprayed and watered with 1% aqueous extracts of donor trees twice a week for six weeks: black walnut (JUG), common hackberry (CEL), false indigo bush (AMO) or Chinese toon (CED) (pot experiment). Results are means ± SE of three replicates (germination tests, each of 100 seeds) or six replicates (pot experiments, each plant represents one replicate) and letters indicate significant differences between the treatments at $P \leqslant 0.05$. RG - relative germination, SS - leaf concentration of soluble sugars, Chl.a+b - leaf concentration of chlorophyll a and b, H_2O_2 - leaf concentration of hydrogen peroxide, CC - the first control (before start of the juglone treatment), dH_2O_2 - distilled water.

The increasing juglone concentration didn´t influence the total leaf dry matter production than control, however, a slight increase up to 10 $\mu g.ml^{-1}$ followed by decrease at 15 $\mu g.ml^{-1}$ concentration than control (Table 3). The juglone at 10 $\mu g.ml^{-1}$ concentration significantly increased (ca. 32%) the stem dry weight but the root dry matter was increased more (ca. 25 %) in all juglone treatments. However, total dry matter per plant as well as root: shoot ratio in treated plants didn't showed any statistical difference from the control.

The leaf concentration of soluble sugars showed a slight increase only in seedlings treated by juglone solution of 10 μg.ml^{-1} and was slightly reduced at 15 μg.ml^{-1} juglone treatment (Figure 2). Chlorophyll a+b content remained stable across the juglone concentrations (ca. 7 mg.g^{-1} DW), although it was slightly decreased at 15 μg.ml^{-1}. The lowest juglone concentration stimulated hydrogen peroxide production (difference of ca. 100 μg.g^{-1} DW) but higher ones decreased it to the half values.

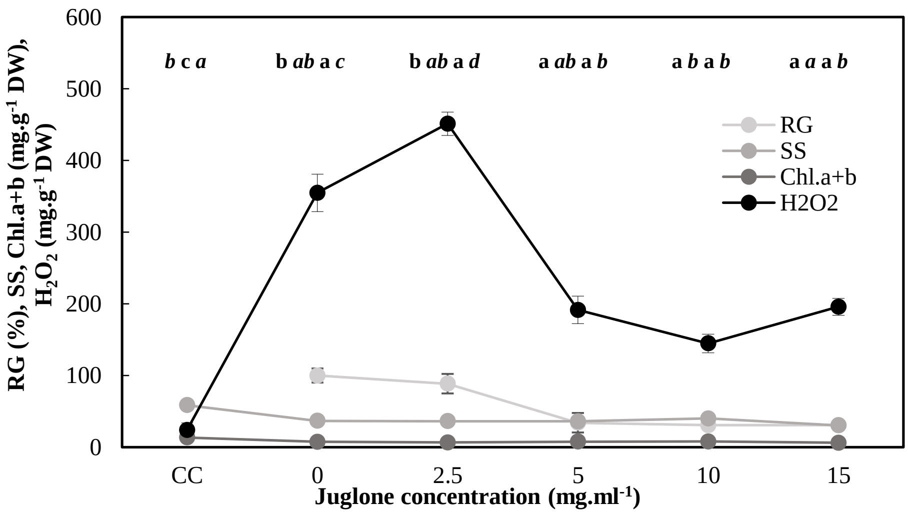

Figure 2. Relative germination of tree-of-heaven seeds after two weeks (Petri plate bioassay) as well as soluble sugar, chlorophyll a+b and hydrogen peroxide concentrations in the top expanded leaves of tree-of-heaven seedlings, as affected by different concentration of juglone (pot experiment). Results are means ± SE of three replicates (germination tests, each of 100 seeds) or six replicates (pot experiments, each plant represents one replicate) and letters indicate significant differences between treatments at the confidence level of $P \leqslant 0.05$. RG - relative germination, SS - leaf concentration of soluble sugars, Chl.a+b - leaf concentration of chlorophyll a and b, H_2O_2 - leaf concentration of hydrogen peroxide, CC - the first control (before the juglone treatment).

Table 3. Effects of different concentrations of juglone on the dry matter production in tree-of-heaven seedlings.

Juglone (μg.ml^{-1})	**Leaf DW (g)**	**Stem DW (g)**	**Root DW (g)**	**Total plant DW (g)**	**Root:shoot ratio**
CC	0.14±0.01 a	0.04±0.001 a	0.08±0.003 a	0.25±0.01 a	0.42±0.01 a
0	1.36±0.06 b	0.63±0.05 b	0.80±0.02 b	2.86±0.14 b	0.45±0.02 a
2.5	1.40±0.13 b	0.62±0.04 b	0.97±0.08 c	3.05±0.14 b	0.54±0.06 a
5	1.54±0.09 b	0.78±0.07 bc	1.03±0.07 c	3.36±0.19 b	0.49±0.06 a
10	1.57±0.11 b	0.83±0.05 c	1.10±0.03 c	3.46±0.17 b	0.44±0.03 a
15	1.33±0.18 b	0.70±0.09 bc	1.01±0.05 c	3.13±0.35 b	0.52±0.04 a

CC : Control at the beginning of the experiment. Results are means of six replicates and letters a,b,c indicate significant differences between treatments at the confidence level of $P \leqslant 0.05$.

The linear regression analysis for experiments, where the leaf/twig aqueous extracts were applied, revealed strong ($P \leqslant 0.01$) positive relationship (Table 4) between extract pH and relative germination of the tree-of-heaven seeds (P=0.005) as well as between total flavonoid level in the extracts and the root:shoot ratio (P=0.007). Strong negative relationships were found only between concentration of phenolic acids/flavonoids in the extracts and seed relative germination (P=0.002 and 0.006, respectively), and then between extract flavonoids and leaf chlorophyll a+b content (P=0.001). Moreover, there were weaker ($P \leqslant 0.05$) relationships, as well (pH-leaf dry matter, pH-leaf soluble sugars, Electric conductivity-root:shoot, Electric conductivity-H_2O_2, Flavonoids-leaf dry matter and Flavonoids-leaf soluble sugars). Analysis of relationships between seedling metabolic and growth parameters showed the only one stronger negative correlation between the leaf chlorophyll a+b content and root:shoot ratio (P=0.003). In experiments with the application of different juglone concentration, stronger negative relationships between juglone content and seed relative germination (P=0.003) as well as leaf hydrogen peroxide level in seedlings (P=0.000), were observed. The strong negative correlation between leaf chlorophyll a+b content and root:shoot ratio was found again (P=0.000). Moreover, there was a weaker relationship between total leaf dry matter and chlorophyll a+b concentration.

Table 4. *P*-values of linear regressions between physico-chemical characteristics of applied water extracts or concentration of juglone and relative seed germination, seedling growth and leaf metabolites of tree-of heaven, then those of linear regressions between the metabolic and growth parameters.

Parameter	RG	W_L	R:S	SS	Chl.a+b	H_2O_2
Aqueous extracts						
pH	0.005**	0.012*	0.167	0.025*	0.145	0.919
Electric conductivity	0.028*	0.529	0.211	0.109	0.502	0.037*
Osmotic potential	0.143	0.053	0.934	0.217	0.744	0.755
Phenolic acids	0.002**	0.018*	0.184	0.050	0.133	0.580
Flavonoids	0.006**	0.016*	0.007**	0.035*	0.001***	0.890
Tannins	0.030*	0.048*	0.816	0.155	0.715	0.399
	SS	**Chl.a+b**	**H_2O_2**			
Leaf dry matter	0.213	0.190	0.911			
Root:Shoot	0.078	0.003**	0.536			
	RG	**W_L**	**R:S**	**SS**	**Chl.a+b**	**H_2O_2**
Juglone						
Juglone	0.003**	0.954	0.949	0.205	0.433	0.000***
	SS	**Chl.a+b**	**H_2O_2**			
Leaf dry matter	0.835	0.015*	0.462			
Root:Shoot	0.259	0.000***	0.148			

EC - electrical conductivity, OP - osmotic potential, RG - relative germination, W_L - leaf dry matter, R:S ratio - root-to-shoot dry matter ratio, SS - soluble sugars concentration, Chl.a+b - chlorophyll a+b concentration. * - significant difference at the confidence level $P \leqslant 0.05$, ** - significant difference at the confidence level $P \leqslant 0.01$, *** - significant difference at the confidence level $P \leqslant 0.001$.

DISCUSSION

Little information is available about allelopathic potential of Chinese toon (*Cedrela chinensis* Juss.). Wu *et al*. (40) reported insignificant effect of 2.5 % aqueous extract of its leaves on wheat germination. Marked decrease in the germination was observed at 7.5 % extract concentration. On the other hand, wheat shoot elongation was slightly stimulated by 2.5 % extract and significant decrease occurred in response to 5 % concentration treatment. Root system was much more sensitive to Chinese toon leachate (affected from the 2.5 % concentration). Ko *et al.* (21) studied herbicidal effects of growing concentration of the Chinese toon extract on numerous grass and broadleaf weed species and found varaiability but high sensitivity of weeds (even at lowest extract concentration of 1,250 $\mu g.ml^{-1}$). Following analysis of extract revealed that four quassinoids in the ethyl acetate fraction were responsible for the herbicidal effect of this tree species. However, limonoid-type triterpenoids (9,28) and flavonoids (5,38) could also act in these allelopathic effects. In our research, 1 % of aqueous extract significantly impaired the tree-of-heaven germination and reduced the leaf growth (Figure 1, Table 2), causing insignificantly larger accumulation of soluble sugars (Figure 1).

A few studies concerned with the false indigo bush (*Amorpha fruticosa* L.) plant-plant interactions, as well. Liang *et al*. (24) studied the suitability of forest soil with residues for cultivation of medicinal plants. They found that the presence of the false indigo bush negatively affected the integrated principal component analysis value, indicating inhibition of *Arisaema heterophyllum* Blume, *Astragalus membranaceus* (Huangqi), and *Isatis tinctoria* L. Nevertheless, compared to residues from other tested shrub species, it had relatively weak or even stimulatory effect on their germination, shoot height and root dry weight. The *A. heterophyllum* strongly decreased the shoot dry weight and it impaired chlorophyll content in *I. tinctoria*. Malondialdehyde, referring to the lipid membrane oxidative damage, decreased or remained at the same level. Hovanet *et al*. (19) investigated the effect of different concentrations of pure false indigo bush leaf extract on wheat root elongation for 5- days. Only 0.6 $\mu g.ml^{-1}$ caused 50% reduction in the root length. This shrub produces the rotenoids (e.g. 8,23) and specific phenolic compounds (e.g. 30). However in our study, false indigo bush suppressed the tree-of-heaven seed germination but did not affect the seedling growth (Figure 1 and Table 2).

Common hackberry extract significantly affected the germination, increased the root:shoot ratio (Table 2) and decreased the leaf chlorophyll content in tree-of-heaven seedlings (Figure 1). Defects in nitrogen assimilation and/or metabolism, associated with lower nodulation level, root and shoot growth as well as leaf nitrogen metabolite concentrations, were identified in black locust (*Robinia pseudoacacia* L.) seedlings treated by the same extract (12). Castells (6) described the indirect effect of phenolics on target plant performance through changes in the soil nitrogen availability caused by soil microflora. Recently, two flavonoids, namely 2''-*O*-β-D-galactopyranosylvitexin and 4'''-α-rhamnopyranosyl-2''-*O*-β-D-galactopyranosylvitexin, were found in the leaves of common hackberry (10).

Black walnut, producing very strong and widely studied allelochemical, juglone (5-hydroxy-1,4-naphtoquinone) (39), significantly influenced the tree-of-heaven germination, growth as well as metabolic parameters. Reduction of the leaf growth, caused jump of the root:shoot ratio (Table 2), leading to the increase in leaf soluble sugar content (Figure 1) due to limited assimilate sink (14). On the other hand, juglone inhibits the net photosynthesis, transpiration and stomatal conductance (20). Leaf growth, similarly to the root growth, could be affected by the disruption of plasma membrane H^+-ATPase function and mitochondrial respiration (18). However, decrease of hydrogen peroxide level in our study (Figure 1) implies slower cell elongation process probably due to down-regulated cell wall-bound peroxidases favouring lignin synthesis (4,15). Chlorophyll concentration, as an indicator of the total nitrogen content (34), was significantly lower than that in the control plants (Figure 1). Juglone blocks the nitrogenase activity in actinorrhizal symbiotic European black alder (*Alnus glutinosa* (L.) Gaertn.) (31). It also had negative impact on strawberry nutrients (N, K, Ca, Mg, Fe, Mn, Zn, B) uptake (11). Interestingly, in the review of Willis (39) tree-of-heaven is listed as a species insensitive to juglone. However, juglone is not the only one substance active in walnut allelopathic interactions. The presence of phenolic acids, flavonoids, amines, alkaloids and terpenes (39), cannot be omitted. Our results further confirmed this perception. Since juglone itself in concentration similar to that found in the leaf extract of black walnut had suppressive effect on the seed germination (-70%, Figure 2) but not on the leaves and stem or even stimulatory on seedlings roots (+25%) (Table 3), despite the reduced (ca. -50%, Figure 2) leaf hydrogen peroxide level, synergy with other components of extract needs to be considered.

There were strong relationship between total flavonoid level in the extracts and relative seed germination, root:shoot ratio and chlorophylls level in tree-of-heaven seedlings (Table 4). Similarly, relative germination was strongly related to the phenolic acids concentration and pH of extracts. Thus, the extract/leachate reaction can also be a part of the complex plant-plant interaction (1). Then, juglone itself strongly influenced the relative seed germination and leaf hydrogen peroxide level in tree-of-heaven seedlings.

CONCLUSIONS

The tree-of-heaven was very sensitive to the black walnut extract with inhibition in seeds germination, leaf growth and plant N metabolism. The common hackberry extract, was less inhibitory to tree-of-heaven germination and leaf growth (by N metabolism) and than those from false indigo bush and Chinese toon reducing only its germination.

ACKNOWLEDGEMENTS

Special thanks to Dr. Dominika Bošiaková for the technical support.

REFERENCES

1. Bais, H.P., Vepachedu, R., Gilroy, S., Callaway, R.M. and Vivanco, J.M. (2003). Allelopathy and exotic plant invasion: from molecules and genes to species interaction. *Science* **301**: 1377-1380.
2. Ballero, M., Ariu, A., Falagiani, P. and Piu, G. (2003). Allergy to *Ailanthus altissima* pollen. *AllergyNet* 532-533.

3. Bauman, J.M., Byrne, C. and Hiremath, S. (2013). *Ailanthus altissima* interfers with beneficial symbionts and negatively impacts oak regeneration. *Journal of American Society of Mining and Reclamation* **2(1)**: 1-16.
4. Böhm, P.A.F., Zanardo, F.M.L., Ferrarese, M.L.L. and Ferrarese-Filho, O. (2006). Peroxidase activity and lignification in soybean root growth-inhibition by juglone. *Biologia Plantarum* **50**: 315-317.
5. Cao, J., Chen, W., Zhang, Y., Zhang, Y. and Zhao, X. (2010). Content of selected flavonoids in 100 edible vegetables and fruits. *Food Science and Technology Research* **16(5)**: 395-402.
6. Castells, E. (2008). Indirect effect of phenolics on plant performance by altering nitrogen cycling: another mechanism of plant-plant negative interactions. In: *Allelopathy in sustainable agriculture and forestry* (Eds. R.S. Zeng et al.) pp. 137-156. Springer, New York, USA.
7. Cziszár, A. (2009). Allelopathic effects of invasive woody plant species in Hungary. *Acta Silvatica et Lignaria Hungarica* **5**: 9-17.
8. Dat, N.T., Lee, J.H., Lee, K., Hong, Y.S., Kim, Y.H. and Lee, J.J. (2008). Phenolic constituents of *Amorpha fruticosa* that inhibit NF-κB activation and related gene expression. *Journal of Natural Products* **71(10)**: 1696-1700.
9. Dong, X.J., Zhu, Y.F., Bao, G.H., Hu, F.L. and Quin, G.W. (2013). New limnoids and dihydrobenzofuran norlignan from the roots of *Toona sinensis*. *Molecules* **18**: 2840-2850.
10. El-Alfy, N., El-Gohary, H.M.A., Sokkar, N.M., Hosny, M. and Al-Mahdy, D.A. (2011). A new flavonoid C-glycoside from *Celtis australis* L. and *Celtis occidentalis* L. leaves and potential antioxidant and cytotoxic activities. *Scientia Pharmaceutica* **79**: 963-975.
11. Ercisli, S. and Turkkal, C. (2005). Allelopathic effects of juglone and walnut leaf extracts on growth, fruit yield and plant tissue composition in strawberry cvs. 'Camarosa' and 'Sweet Charlie'. *Journal of Horticultural Science and Biotechnology* **80**: 39-42.
12. Ferus, P. Bošiaková, D., Konôpková, J., Hoťka, P., Kósa, G., Melnykova, N. and Kots, S. (2019) Allelopathic interactions of invasive black locust (*Robinia pseudoacacia* L.) with secondary aliens: the physiological background. *Acta Physiologiae Plantarum* **41**: 182.
13. Fournier, E. (2001). Colorimetric quantification of carbohydrates. *Current Protocols in Food Analytical Chemistry* E1.1.1-E1.1.8
14. Franck, N., Vaast, P., Génard, M. and Dauzat, J. (2006). Soluble sugars mediated sink feedback down-regulation of leaf photosynthesis in field-grown *Coffea arabica*. *Tree Physiology* **26**: 517-525.
15. Gniazdowska, A., Krasuska, U., Andrzejczak, O. and Soltys, D. (2015). Allelopathic compounds as oxidative stress agents: Yes or No. In: *Reactive Oxygen Signaling and Communication in Plants. Signalling and Communication in Plants 23* (Eds. K.J. Gupta and A.U. Igamberdiev) pp. 155-176. Springer, Switzerland.
16. Gomez-Aparacio, L. and Canham, C.D. (2008). Neighbourhood analyses of the allelopathic effects of the invasive tree *Ailanthus altissima* in temperate forests. *Journal of Ecology* **96**: 447-458.
17. Heisey, R.M. and Heisey, T.K. (2003). Herbicidal effects under field conditions of *Ailanthus altissima* bark extract, which contains ailanthone. *Plant and Soil* **256(1)**: 85-99.
18. Hejl, A.M. and Kosner, K.L. (2004). Juglone disrupts root plasma membrane H^+-ATPase activity and impairs water uptake, root respiration, and growth in soybean (*Glycine max*) and corn (*Zea mays*). *Journal of Chemical Ecology* **30(2)**: 453-471.
19. Hovanet, M.V., Marinas, I.C., Dinu, M., Oprea, E., Chifuriuc, M.C., Stavropoulou, E. and Lazar, V. (2015). The phytotoxicity and antimicrobial activity of *Amorpha fruticosa* L. leaves extract. *Romanian Biotechnological Letters* **20(4)**: 10670-10678.
20. Jose, S. and Gillespie, A.R. (1998). Allelopathy in black walnut (*Juglans nigra* L.) alley cropping. II. Effects of juglone on hydroponically grown corn (*Zea mays* L.) and soybean (*Glycine max* L. Merr.) growth and physiology. *Plant and Soil* **203**: 199-205.
21. Ko, Y.K., Choi, J.S., Kim, Y.S., Kim, J.D. and Park, K.W. (2016). Herbicidal characteristics of crude extracts of *Cedrela sinensis* for a potential biocontrol agent. In: *7th International Weed Science Congress*, pp. 471.
22. Kowarik, I. and Säumel, I. (2007). Biological flora of Central Europe: *Ailanthus altissima* (Mill.) Swingle. *Perspectives in Plant Ecology, Evolution and Systematics* **8**: 207-237.
23. Lee, H.J., Lee, H.J., Kwon, Y.H., Choi, D.H. and Paik, K.H. (2003). Extractives from fruits of *Amorpha fruticosa* (I). *Journal of Korean Wood Science and Technology* **31(6)**: 8-14.
24. Liang, X., Liu, X.B., Zhang, X.X, Liu, Z.W., Gaudel, G. and Zhang, J.Z. (2016). Allelopathic effects of humus forest soil of *Hippophae rhamnoides*, *Caragana korshinskii* and *Amorpha fruticosa* on medicinal plants. *Emirates Journal of Food and Agriculture* **28(7)**: 468-476.
25. Lichtenthaler, H.K. (1987). Chlorophylls and carothenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology* **148**: 350-382.

26. Liu, C., Zhou, Z., Hua, Z., Wan, H., Xie, Y., Wang, Z. and Deng, L. (2013). Analysis of volatile compounds and identification of characteristic aroma component of *Toona sinensis* (A. Juss.) Roem. using GC-MS and GC-O. *Food and Nutrition Sciences* **4**: 305-314.
27. Medvecká J., Kliment J., Májeková J., Halada Ľ., Zaliberová M., Gojdičová E., Feráková V. and Jarolímek I. (2012). Inventory of the alien flora of Slovakia. *Preslia* **84**: 257-309.
28. Meng, Q.Q., Peng, X.R., Lu, S.Y., Wan, L.S., Wang, X., Dong, J.-R., Chu, R., Zhou, L., Li, X.N. and Qiu, M.H. (2016). Lactam triterpenoids from the bark of *Toona sinensis*. *Natural Products and Bioprospecting* **6**: 239-245.
29. Mukherjee S.P. and Choudhuri M.A. (1983). Implications of water stress-induced changes in the level of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Physiologia Plantarum* **58**: 166-170.
30. Muharini, R., Díaz, A., Ebrahim, W., Mándi, A., Kurtán, T., Rehberg, N., Kalscheuer, R., Hartmann, R., Orfali, R.S., Lin, E., Liu, Z. and Proksch, P. (2017). Antibacterial and cytotoxic phenolic metabolites from the fruits of *Amorpha fruticosa*. *Journal of Natural Products* **80(1)**: 169-180.
31. Neave, I.A. and Dawson, J.O. (1989). Juglone reduces growth, nitrogenase activity, and root respiration of actinorhizal black alder seedlings. *Journal of Chemical Ecology* **15(6)**: 1823-1836.
32. Nguyen P.M. and Niemeyer E.D. (2008). Effects of nitrogen fertilization on the phenolic composition and antioxidant properties of basil (*Ocimum basilicum* L.). *Journal of Agricultural and Food Chemistry* **56**: 8685-8691.
33. Park, Y.K., Koo, M.H., Ikegati, M. and Contado, J.L. (1997). Comparison of the flavonoid aglycone contents of *Apis mellifera* propolis from various regions of Brazil. *Arquivos de Biologia e Tecnologia* **40**: 97-106.
34. Peng, S., García, F.V., Laza, R.C. and Cassman, K.G. (1993). Adjustment for specific leaf weight improves chlorophyll meter´s estimate of rice leaf nitrogen concentration. *Agronomy Journal* **85**: 987-990.
35. Slavík B (1965). Metody studia vodního provozu rostlin. [Methods in the Plant Relations Study.] Nakladatelství ČSAV, Praha, Czechoslovakia.
36. Small, C.J., White, D.C. and Hargbol, B. (2010). Allelopathic influences of the invasive *Ailanthus altissima* on a native and non-native herb. *Journal of the Torrey Botanical Society* **137(4)**: 366-372.
37. ÚKZÚZ (2013). Stanovení obsahu taninů v čiroku spektrofotometricky. [Spectrophotometric determination of tannins in sorghum.] ÚKZÚZ, Praha, Czech Republic, 50321.1.
38. Wang, K.J., Yang, C.R. and Zhang, Y.J. (2007). Phenolic antioxidants from Chinese toon (fresh young leaves and shoots of *Toona sinensis*). *Food Chemistry* **101(1)**: 365-371.
39. Willis, R.J. (2000). *Juglans* spp., juglone and allelopathy. *Allelopathy Journal* **7(1)**: 1-55.
40. Wu, Y., Cheng, X., Cai, Q. and Lin, C. (2012). Allelopathic effects of twelve hedgerow plant species on seed germination and seedling growth of wheat (*Triticum aestivum* L.). *Advanced Materials Research* **356-360**: 2767-2773.
41. Zeng, R.S., Mallik, A.U. and Luo, S.M. (2008). *Allelopathy in Sustainable Agriculture and Forestry*, Springer, New York, USA.