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Allelopathic effect of aqueous extracts of Canadian goldenrod on germination and growth of radish

Alelopatski učinek vodnih izvlečkov kanadske zlate rozge na kalitev in rast redkvice

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Abstract: In this study we tested the effects of *Solidago canadensis* extracts on seed germination and early growth of radish (*Raphanus sativus*). The aqueous extracts of 2.5% (m/v) concentration were prepared from roots, rhizomes, stems, leaves and inflorescences and applied onto the filter paper in Petri dishes where radish seeds were sown. We determined the germination rate and seedling growth for four days. The extracts from leaves and inflorescences delayed germination, whereas extracts from stems and roots had no significant effect on seed germination. The extracts from rhizome had a slightly stimulatory effect on the seed germination and promoted shoot length of radish seedlings, while extracts from leaves inhibited root and shoot length and seedlings development.

Key words: *Solidago canadensis, Raphanus sativus*, germination, allelopathy, seedling growth

Izvleček: V raziskavi smo testirali učinke vodnih izvlečkov *S. canadensis* na kalitev in zgodnji razvoj redkvice (*Raphanus sativus*). Pripravili smo 2,5 % (m/v) vodne izvlečke iz korenin, korenik, stebel, listov in socvetij in jih dodali v petrijevke na filtrirni papir s semeni. Določili smo stopnjo kalivosti in štiri dni spremljali razvoj kalic. Listni in socvetni izvlečki so upočasnili kalivost semen, medtem ko stebelni in koreninski izvlečki niso vplivali na kalitev. Izvleček iz korenik je imel rahlo pozitiven učinek na kalitev semen in je pospešil rast poganjka kalic redkvice, medtem ko je listni izvleček zavrl rast korenin in poganjka ter upočasnil razvoj kalic redkvic.

Ključne besede: *Solidago canadensis, Raphanus sativus*, kalitev, alelopatija, razvoj kalic

Introduction

Canadian goldenrod (*Solidago canadensis* L.) is one of the most successful and widespread invasive plant species all over the Europe (Weber 1998). The plants form dense populations along roads, railways and other ruderal habitats (Strgulc Krajšek 2009). Diversity of native plants dramatically decreases, by almost 60% (De Groot et al. 2007) and the goldenrod cover of invaded areas can reach 90-100% (Moron et al. 2009). The plants can also limit the access of other organisms to light and water sources because of its height and high density (Chapuis-Lardy et al. 2006), and therefore strongly modify the ecosystem (Litt et al. 2014). In Slovenia, *S. canadensis* poses a serious threat to the diversity and abundance of native plants (Strgulc Krajšek 2009). It grows faster, has a larger plant size and produces more aboveground biomass than the native species in the invaded habitat. Regular mowing creates large quantities of biomass, which can be a source of biologically active substances.

There are many reports how *S. canadensis* affects natural environment. Many studies showed that the Canadian goldenrod decreases the growth and survival of natural plants (Abhilasha et al. 2008) and has a negative impact on many animals (Litt et al. 2014). However, small number of studies have shown that Canadian goldenrod can also have a positive impact on plants, microbes and animal communities (Litt et al. 2014, Dong et al. 2015). For example, Canadian goldenrod has a positive effect on native spider hunting success and increases spider abundance (Dudek et al. 2016). Additionally, Canadian goldenrod contains many biologically active compounds and has antioxidant, anti-inflammatory and antimicrobial activity (Deng et al. 2015, Paré et al. 2018). In our previous study, we confirmed antibacterial and antifungal activity of aqueous and organic extracts of *S. canadensis* (Anžlovar and Dolenc Koce 2014, Hladnik 2017). There are also reports about allelopathic activity of *S. canadensis* leaf extracts (Abhilasha et al. 2008), however, knowledge about allelopathic activity of other plant parts is limited.

The seed germination and seedling growth is generally considered as the critical process of the population recruitment and ecological expansion of plant species. Allelochemicals released by the invasive plants can have an influence on seed germination and seedling growth of the co-occuring native plant species (Grašič et al. 2016). They are produced as end- and by-products in stems, leaves, roots, flowers, inflorescences, seeds and fruits. A number of mechanisms have been studied for the release of allelochemicals from various tissues including volatilization or leaching from aerial parts, exudation from roots and decomposition of plant residues in soil (Latif et al. 2017). Aqueous plant extracts seem to be good technique to imitate the natural decomposition.

The present study focused on investigating the allelopathic activity of aqueous extracts prepared from different parts of *S. canadensis* plant, e. g. roots, rhizomes, stems, leaves and inflorescences on seed germination and early growth of the seedling of radish (*Raphanus sativus* L.) which is often used in ecotoxicological studies.

Material and methods

Plant material

Canadian goldenrod plants (*Solidago canadensis* L.) were collected in the flowering period in August 2018 in Ljubljana, Slovenia (N 46°4'19.86", E 14°25'52.25"). Leaves and inflorescences were separately removed from stems. Roots and rhizomes were also separated and washed in tap water. Rhizomes were then cut into 1 cm long pieces, frozen in liquid nitrogen, lyophilized (Christ Alpha 1-4LSC, Christ, Germany). All other parts were air dried at room temperature in the dark.

Seeds of *Raphanus sativus* cv. Saxa were obtained from Semenarna Ljubljana (Slovenia).

Preparation of aqueous extracts

Dry plant material was ground with the mill (IKA-Werke M20, IKA, Germany) and 2.5 g of this material was suspended in 100 ml of distilled water and put on a shaker (Laboshake 500, Gerhardt, Germany) under 100 rpm at room temperature for approximately 24 hours. The mixtures were filtered under pressure through filter paper (Whatman filter paper 520A, Ge Healthcare Life Sciences, US) to remove plant particles. The yield of extract (extractable component) expressed on dry weight basis of pulp was calculated from the following equation: Yield (%) = (W1 × 100)/W2, where W1 is the weight of the extract residue obtained after solvent removal and W2 is the weight of the dry plant before extraction.

Germination tests

To set the germination test, we used sterile Petri dishes (diameter, 9 cm) with one layer of autoclaved filter paper soaked with 4 ml of the extract or distilled water for a control. For each treatment we used 5 replicates each with 10 seeds in a 2x2 cm array (N = 50). Germination test took place in a growth chamber at 22ºC, 60% humidity and photoperiod of 16 h light / 8 h dark. The experiment lasted for four days.

Seeds were examined every day at roughly 24-hour intervals. A seed was considered germinated on the day of root emergence. We also registered the opening of cotyledons in every radish seedlings and measured root and shoot length on the last day of the experiment as markers of further seedling growth and development.

Statistical analysis

Mean values and standard errors were calculated for all treatments (Excel MS). The differences between treated and control samples were tested using the One-way ANOVA and Holm-Sidak post-hoc test. The level of significance was set at $p < 0.05$.

Results and discussion

Previous studies of allelopathic effects of *S. canadensis* aqueous extracts on seed germination showed that the effects are concentration dependent (Sun et al. 2006, Abhilasha et al. 2008). The 2% leaf extracts of *S. canadensis* significantly inhibited the germination of lettuce seeds (Wang et al. 2016, 2019), whereas 1% extracts exhibited positive and negative impact on germination, dependent of the target plant species (Sun et al. 2006, Wang et al. 2019). More concentrated 10% aqueous leaf extract completely inhibited seed germination of different seeds (Sun et al., 2006). Therefore, in preliminary experiments, we tested three different concentrations of aqueous extracts of *S. canadensis*: 10%, 5% and 2.5 %. The extracts were prepared from the whole plant and the first two concentrations completely inhibited germination of radish seeds (data not shown). Therefore, we used 2.5% extracts to observe the seedlings development not just seed germination.

Aqueous extracts from different parts of *S. canadensis* differently affected the germination of radish seeds. Table 1 shows that after 24 h, 86% of control seeds germinated, while the germination rate of the treated seeds was lower as follows: root extract (60%), rhizome extract (78%), stem extract (68%), leaf extracts (8%) and inflorescences extract (18%).

Additionally, aqueous extracts from stems and roots had a similar effect on germination rate of radish seeds during all four days. The germination rate was lower each day for about 10% compared to the control and on the last day reached 86% (Tab. 1). On the first two days, inhibition of germination of inflorescences extract was significant, then the germination increased and on the 4th day reached the germination rate comparable with root and stem extracts. Leaves extract significantly inhibited the germination on first three days when compared to the control, while on the 4th day the difference was not significant compared to the control and reached 76%. Germination rate of extract from rhizomes was comparable to control and on the 3rd day (92%) even exceeded the germination rate of seeds in control treatment (90%). The effects are similar to the reports for 0.1% aqueous extracts of *S. canadensis* which stimulated seed germination of rape and morning glory, however, at 1% concentration the inhibition of germination was detected (Sun et al. 2006).

Table 1: Germination rate of radish seeds treated with aqueous extracts of *S. canadensis* from roots, rhizomes, stems, leaves and inflorescences during 4 days. Data are means ±standard error (N = 50). Different letters indicate statistically significant differences ($P < 0.05$).

Tabela 1: Delež kalivosti semen redkvice tretiranih z vodnimi izvlečki *S. canadensis* iz korenin, korenik, stebel, listov in socvetij tekom 4 dni. Podatki so povprečja ± standardna napaka (n = 50). Različne črke prikazujejo statistično značilne razlike med tretmaji ($P < 0,05$).

	Germination rate (%)					
	Control	**Roots**	**Rhizomes**	**Stems**	**Leaves**	**Inflorescences**
Day 1	86 ± 7^{a}	60 ± 8^{a}	78 ± 4^{a}	68 ± 4^{a}	8 ± 4^{b}	18 ± 4^{b}
Day 2	90 ± 4^{a}	78 ± 7^{ab}	84 ± 3^{ac}	78 ± 7^{ab}	32 ± 10^{b}	56 ± 7^{bc}
Day 3	90 ± 4^{ab}	86 ± 7^{ab}	92 ± 2^{a}	80 ± 7^{ab}	70 ± 3^{b}	82 ± 4^{ab}
Day 4	90 ± 4^{a}	86 ± 7^{a}	92 ± 2^{a}	86 ± 4^{a}	76 ± 5^{a}	86 ± 4^{a}

Among the different parts tested in this study, leaves showed the highest allelopathic activity while rhizomes had no or even positive impact on final germination rate of radish seeds. Results of previous studies also confirm that 2% aqueous leaf extracts of *S. canadensis* significantly decreased germination rate (Wang et al. 2019), whereas 1% extracts exhibited positive or negative effects on germination, depending on target plant species (Sun et al. 2006, Wang et al. 2019). On the contrary, aqueous extracts from root, stem and leaf of *Croton bonplandianum* had no effect on seed germination of test plants regardless of concentration (Sisodia and Siddiqui, 2010). The observed difference in allelochemical effect of different parts of plant may be attributed to the presence of variable content of different allelopathic substances in different parts. Under natural conditions leaves are more easily degradable and therefore decomposition is quicker than the gradual decay of stems and rhizomes. In accordance, extracts from leaves contained more dry mass and had higher yield than of inflorescences, rhizomes and roots, while stem extract had the lowest yield (Tab. 2).

The early growth and development of the treated seedlings was slower than of the control. Figure 1 shows that 2.5% aqueous extracts of *S. canadensis* from inflorescences, leaves, roots, rhizomes and stems did significantly deterred the root growth compared to control. Sisodia and Siddiqui (2010) reported that stem extracts from *Croton bonplandianum* at low concentration (0.5% and 1%) generally promoted root growth, whereas 2% and 4% extracts significantly decreased root growth. Additionally, 1% *S. canadensis* leaf extract significantly increased root length, whereas 2% *S. canadensis* leaf extract significantly decreased root length of lettuce compared to the control (Wang et al. 2019). It seems that the main reason for positive or negative effect on root length is the concentration of extract and that the water extracts of *S. canadensis* had hormetic-like effects on the seedling root growth.

Among all studied *S. canadensis* extracts, only the ones from leaves significantly deterred the shoot growth compared to the control treatment, whereas rhizomes extract slightly promoted shoot growth (Fig. 1). On the contrary, *S. canadensis* leaf extracts, regardless to the concentration, had no effect on shoot height of lettuce (Wang et al. 2019). Similar tissue specific pattern was reported for aqueous extracts of *Croton bonplandianum*, whereas the leaf extract had inhibitory effect and the stem extract had a stimulatory effect on the shoot length of weed plants at all used concentrations (Sisodia and Siddiqui 2010). Overall, we can conclude that aqueous extracts of *S. canadensis* deter root growth more than shoot. Ye et al. (2019a) reported that growth of maize seedlings, estimated by shoot height and root length, was promoted at low concentrations (up to 12.5%) and decreased at high concentrations (12.5% to 25%) of *S. canadensis* aqueous extracts, with the remarkable stimulatory effects at 12.5%. It seems that different plant species are differently sensitive to *S. canadensis* extracts and effects on seedling growth are highly correlated to their concentration, but in general low concentrations promote and high inhibit growth of seedlings.

Table 2: Yield of goldenrod extracts. The yield was calculated as percentage of extract dry mass according to the starting material. Data are means (N = 3).

Tabela 2: Izkoristek izvlečkov zlate rozge. Izkoristek je delež suhe snovi, glede na maso začetnega materiala, izražen v odstotkih. Podatki so povprečja (N = 3).

Extract	Yield (%)
Roots	24.00
Rhizomes	24.00
Stems	13.32
Leaves	41.30
Inflorescences	29.32

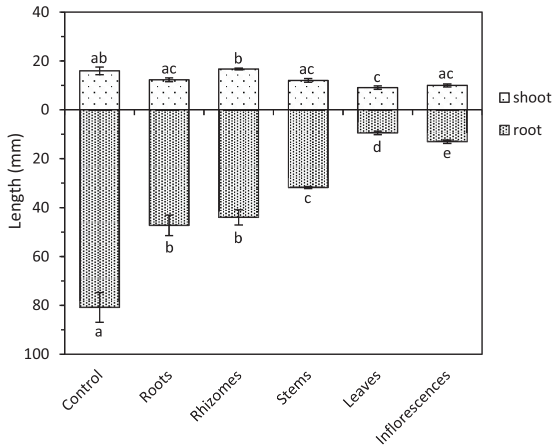

Figure 1: Shoot and root length of radish seedlings after 4 days of growth and treatment with aqueous extracts of *S. canadensis* prepared from roots, rhizomes, stems, leaves and inflorescences. Mean value ± SE is shown (N=50). Different letters indicate statistically significant difference among treatments (P <0.05).

Slika 1: Dolžina poganjka in korenine kalic redkvice po 4 dneh rasti in tretmaju z vodnimi izvlečki *S. canadensis* iz korenin, korenik, stebel, listov in socvetij. Prikazana so povprečja ± standardne napake (N = 50). Različne črke prikazujejo statistično značilne razlike (P <0,05).

We observed two crucial steps in seedlings development: the first phase was determined when the primary root emerged from the seed (germination), and the second phase was so called opening of cotyledons, when cotyledons spread horizontally and provide a broad surface for photosynthesis and are during the first few weeks only food-producing organs of the seedling (Vogel, 1980). The cotyledons opened on the 3rd and 4th day of the experiment. Results showed that leaf and stem extracts significantly extended the time of the opening of cotyledons in radish seedlings compared to the control (Fig. 2). In other extracts this was less evident. The delayed first phase of germination (root emergence) caused delayed development of shoots, which was reflected in the significantly lower number of cotyledons opened on the last day of the experiment. Stem extract also delayed the germination of radish seeds but did not influence the final germination rate (Tab.1). Also shoot height was not significantly affected (Fig. 1). It seems that only opening of cotyledons was delayed and if we would have monitored seedling growth for a few more days, the difference may no longer be significant. On the contrary, leaf extract significantly inhibited germination and seedling growth regarded to all parameters of the development (Tab.1, Fig. 1, Fig. 2).

Our data suggest that substances in different plant organs may contribute to the success of S. *canadensis* as an invader. All aqueous extracts delayed the germination of radish seeds. Leaf extract decreased and delayed the seedling development the most and can have an influence on competitive ability for soil nutrients and sunlight. Leaf extract contained more dry mass and had higher yield than other extracts. Additionally, rhizome extract can have a positive effect on the germination rate and growth of the aboveground part of radish seedlings. A possible explanation is that extracts from different parts of goldenrod contain different allelochemicals or just different amount of allelochemicals. Furthermore, *S. canadensis* plant had high content of macronutrients, which can also influence seedling development. Invasion of *S. canadensis* into grassland compared to native species revealed that its higher aboveground biomass together with greater litter production and faster litter decomposition results in higher concentration of macronutrient e.g., nitrogen, phosphorus, potassium (Ye et al. 2019b). Potassium is one of the most important nutrients for plant growth and plays fundamental roles in different plant functions (Armengaud et al. 2009). Furthermore, *S. canadensis* could utilize insoluble phosphorus (Wan et al. 2018), suggesting that root exudates

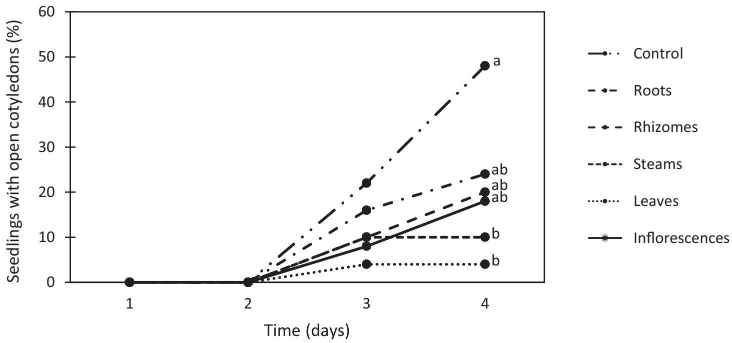

Figure 2: Development of shoots defined as opening of cotyledons of radish seedlings treated with aqueous extracts of *S. canadensis* from roots, rhizomes, stems, leaves and inflorescences during 4 days. Data are means (N = 50). Different letters indicate statistically significant differences (P <0.05).

Slika 2: Razvoj poganjkov redkvice, tretiranih z vodnimi izvlečki *S. canadensis* iz korenin, korenik, stebel, listov in socvetij glede na razprtje kličnih listov tekom 4 dni. Podatki so povprečja (N = 50). Različne črke prikazujejo statistično značilne razlike (P <0.05).

might be involved in releasing P and K from insoluble sources (Bais et al. 2006). Regular removing of *S. canadensis* from the ecosystem reduces negative effects on native species and provides biomass, which is through litter decomposition an important source of allelochemicals and nutrients. Different concentrations of allelochemicals and nutrients and their combinations could contribute to negative and positive effects on germination and growth of radish also in this study. Our results are valuable to future research in the potency of the separated parts of *S. canadensis*, especially the rhizomes, stems and inflorescences as a potential source for bioherbicides for agricultural and food production fields.

Conclusions

- All aqueous extracts delayed the germination of radish seeds.
- The extracts from rhizome had a slightly stimulatory effect on the seed germination and promoted shoot growth of radish seedlings.
- Leaf extract decreased and delayed the seedling development the most and can have an influence on competitive ability of Canadian goldenrod.

Povzetek

Kanadska zlata rozga je zelnata trajnica, ki izvira iz Severne Amerike. V Evropi je ena izmed najbolj razširjenih invazivnih vrst, ki tvori goste sestoje ter ogroža obstoj in pestrost domorodnih vrst. Že stoletja se uporablja kot zdravilna rastlina v tradiconalnem zdravilstvu, njeni izvlečki delujejo protimikrobno, protiglivno, antioksidativno in alelopatsko. Eden od ukrepov za preprečevanje njenega širjenja je redno odstranjevanje, s katerim dobimo veliko biomaso, ki jo lahko uporabimo za raziskave biološko aktivnih snovi.

V raziskavi smo testirali učinke vodnih izvlečkov *S. canadensis* na kalitev in zgodnji razvoj redkvice (*Raphanus sativus*). Pripravili smo 2,5 % vodne izvlečke iz korenin, korenik, stebel, listov in socvetij in jih dodali na filtrirni papir s semeni redkvice. Določili smo stopnjo kalivosti in spremljali razvoj kalic. Vodni izvlečki so različno vplivali na kalitev semen redkvice. Listni in socvetni izvlečki so upočasnili kalivost semen, medtem ko stebelni in koreninski izvlečki niso vplivali na kalitev. Izvleček iz korenik je imel rahlo pozitiven učinek na kalitev semen. Razvoj kalic redkvice smo spremljali z merjenjem višine poganjka in dolžine korenin, ter 4-dnevnim spremljanjem odpiranja kličnih listov. Vsi izvlečki so upočasnili rast korenin kalic rekvice, medtem ko je bila rast poganjkov manj prizadeta. Izvleček iz korenik je celo rahlo pospešil rast poganjkov.

Najbolj alelopatsko učinkovit je bil listni izvleček, ki je zavrl rast korenin in poganjka ter upočasnil razvoj kalic redkvic.

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