



Allelopathic potential of the invasive species *Aster lanceolatus* Willd.

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Abstract

Background and Purpose: *Aster lanceolatus* Willd. is a highly invasive plant in Serbia. However, mechanisms that allow *A. lanceolatus* to be so competitive and to become dominant in riverine plant communities are not clear. Several potentially allelopathic compounds have been identified in *A. lanceolatus* tissues and those substances could possibly contribute to the rapid spread of this species.

Materials and Methods: In this paper, the allelopathic potential of *Aster lanceolatus* Willd. was studied. *Lactuca sativa* L. and *Sinapis alba* L. were selected as the bioassay species. Under laboratory conditions, different concentrations of aqueous extracts of *A. lanceolatus* were applied to determine their effect on seed germination and seedling growth of the test species. Influences of various aqueous extracts on the germination of bioassay species were tested using one-way analysis of variance (ANOVA). In addition, radicle and hypocotyl lengths and dry weight of seedlings were expressed as a percentage of growth inhibition of the control.

Results and Conclusion: The bioassay present in this paper has shown that the aqueous extracts obtained from different vegetative organs of *A. lanceolatus* have an inhibitory effect on seed germination and seedling growth of the test species and that those potentially allelopathic compounds could contribute to the competitive ability of *A. lanceolatus* against native plants.

INTRODUCTION

Many plant communities and their diversity are widely influenced and threatened by invasive plants. Once they are in a new environment, some of the non-native plants have the ability to alter the conditions of ecosystems and thus encourage their competition in relation to other native plants. Some invasive species have biochemicals that may give them an advantage over the native ones, which are facing novel biochemicals (1). Allelochemicals found in different parts of invasive plants (stems, leaves, roots, rhizomes, flowers, pollen and seeds) can have a negative impact on other species. The impacts can be direct, when allelochemicals present in the exudates inhibit germination and seedling development and hinder the formation of stable populations, or indirect, when these substances affect soil organisms (2). Some invasive plants produce allelochemicals that influence various primary and secondary physiological processes in the native species and soil microorganisms, thereby affecting biodiversity (3).

There is a large volume of published studies describing the role of allelochemicals in invasion success. Ridenour and Callaway (4) found

that *Centaurea maculosa* Lam. has an allelopathic effect on native *Festuca idahoensis* Elmer. Allelochemicals extracted via *C. maculosa* root exudates have a large impact on native species while the impact on other non-native species is not as pronounced. However, Callaway *et al.* (5) found that some individuals of the native species that had survived *C. maculosa* invasion and were cloned and grown from seeds showed greater resistance to *C. maculosa* allelochemicals than conspecific individuals that had never experienced invasion. The authors suggested that it is possible that native plants are capable of adapting to the impacts of new allelochemicals. The leaf aqueous extract of some invasive plants reduced the germination and seedling growth of test species in a laboratory bioassay (8, 9, 10). Several studies have documented that invasive plant allelochemicals can affect mycorrhizae of native plants (6, 7) and the activity of pathogenic organisms in the soil (11).

Despite the large number of studies investigating plant allelopathy, little research has been conducted under natural conditions since it is difficult to separate the impact of allelopathic substances from others influences. Laboratory bioassays are widely used to assess potential impacts of allelopathic compounds. Biological tests carried out in laboratory conditions allow researchers to eliminate other alternative interferences through the controlled condition in which the research takes place (12).

Plant habitats and communities along the river are one of the most vulnerable habitats to invasion (13). *Aster lanceolatus* Willd. is one of the most invasive plants in Serbia along river banks and on the forest edge in wet habitats (14). However, mechanisms that allow *A. lanceolatus* to be so competitive and to become dominant in riverine plant communities are not clear. Several potentially allelopathic compounds have been identified in *A. lanceolatus* tissues (15) and it is possible that those substances could contribute to the rapid spread of this species. The objective of this research is to determine the allelopathic potential of aqueous extracts of fresh and dry biomass of *Aster lanceolatus* Willd. on the germination and seedling growth of bioassay species.

MATERIALS AND METHODS

Plant material

To determine the allelopathic potential of *A. lanceolatus* under laboratory conditions, samples of *A. lanceolatus* were collected from Ada Medica, an island on the Sava river in Belgrade. All plants were between 25 and 30 cm high. Collected plant samples were rinsed with tap water and separated into rhizomes, stems and leaves.

Lettuce (*Lactuca sativa* L.) and mustard (*Sinapis alba* L.) seeds were used as the bioassay species. *L. sativa* seeds were purchased from Semenarnacoop (Petrovaradin, Ser-

bia) and *S. alba* seeds were purchased from the Institute of Medicinal Plants Research „Dr Josif Pančić“ (Belgrade, Serbia).

Preparation of aqueous extracts from the fresh tissue of *A. lanceolatus*

Aqueous extracts were prepared from fresh rhizomes, stems and leaves. Fifty grams of each type of explants were cut into small pieces and soaked in 250 ml of distilled water for 24 hours at 20 °C (16). Each aqueous extract was then filtered through four layers of cheesecloth and then through filter paper. The filtrates were diluted to 25% and 50% extracts or used as a 100% extract (16).

Preparation of aqueous extracts from the dry tissue of *A. lanceolatus*

Fifty grams of fresh rhizomes, stems and leaves were cut into small pieces and oven-dried at 40 °C for 48 hours (17). The dried tissue was milled to a powder and added to 250 ml of distilled water to soak for 24 hours. Each aqueous extract was then filtered through filter paper. The filtrates were diluted to 25% and 50% extracts or used as a 100% extract (16).

Bioassays

In a growth chamber, 15 seeds of *Lactuca sativa* or *Sinapis alba* were germinated in 9 cm Petri dishes on filter paper at 20 °C (± 2) and a 16h photoperiod was applied. The effect of *A. lanceolatus* was investigated in the presence of 25%, 50% and 100% aqueous extracts from its rhizomes, stems and leaves. Treatments were applied in a 5 ml volume per dish. Distilled water (pH 6.81), in the same volume, was used for the control. Treatments were replicated three times. Percentage of seed germination, length of radicles and hypocotyls, and dry seedling weight of bioassay species were recorded after 7 days. Because the rate of seed deterioration after radicle emergence was high, cotyledon expansion was established as a criterion for germination. The number of deteriorated seeds was also recorded. Seedlings were oven-dried at 70 °C and after 48 hours seedling were weighed.

Data analysis

Influences of various concentrations of aqueous extracts on the germination of bioassay species were tested using one-way analysis of variance (ANOVA) followed by Fisher's LSD test ($P < 0.05$). Radicle and hypocotyl lengths and dry weight of seedlings were expressed as % of growth inhibition of the control, based on the formula (17):

$$\% \text{ growth inhibition} = 100 (pc - pt) / pc$$

where pc and pt are the radicle or hypocotyl length or dry weight of seedlings of the control and the treated sample, respectively.

RESULTS

Effects of *A. lanceolatus* extracts on the seed germination of bioassay species

The aqueous extract of fresh plant tissue had an effect on the germination of both test species (Table 1). This was especially pronounced in the germination of *S. alba*. The extract of the stem significantly reduced the germination of *S. alba* in all concentrations. In addition, the germination of *S. alba* was reduced significantly by 50% and 25% rhizome extracts and 100% and 25% leaf extracts, while in the presence of a 100% rhizome extract and a 50% leaf extract germination was reduced but not significantly. Extracts made of fresh vegetative parts had similar effects on the seed germination of *L. sativa*, but those effects were not so pronounced compared to *S. alba*. However, the 50% stem extract and the 50% rhizome extract significantly reduced the germination of target species.

Extracts made from dry vegetative parts of *A. lanceolatus* significantly reduced germination in all concentrations, except in treatments with the lowest concentration of extracts where the germination of the test species was without a significant decrease (Table 1). A decrease in the germination percentage was particularly pronounced in treatments with the stem extract in the highest concentration, and in treatments with the rhizome extract in the highest and the 50% concentration. The germination of *L. sativa* was also affected by extracts of all vegetative parts in all concentrations, but the most prominent effects were produced by extracts of the rhizome (100%, 50%), following the extracts of the stem (50%, 25%) and leaf (25%).

During the experiment, seed deterioration of bioassay species was observed (Table 2).

After the third day of the germination experiment, many seeds with a sprouted radicle stopped developing. This was especially noticeable for *S. alba* in all three treatments with extracts made from fresh vegetative parts. *Sinapis* seeds also deteriorated after the treatment with the extract of dry vegetative parts of *A. lanceolatus*, especially in treatments with the stem extract in all concentrations. *L. sativa* seeds deteriorated in treatments with 50% stem and rhizome extracts and a 100% leaf extract made from fresh tissue and 50% rhizome and 50% and 25% leaf extracts from dry tissue. There was no seed deterioration in control treatments.

Effects of *A. lanceolatus* extracts on the seedling growth of bioassay species

A. lanceolatus extracts affected the dry weight of seedlings of both bioassay species (Table 3). All treatments led to the reduction in biomass of *S. alba* seedlings compared to the control, except for treatments with dry leaf and stem extracts in the lowest concentrations. The reduction in *S. alba* seedlings biomass was highest in the presence of 100% stem and rhizome extracts and a 50% dried rhizome extract. Extracts obtained from fresh and dry vegetative parts reduced the biomass of *L. sativa* seedlings by more than 75% compared to control treatments, except for the lowest concentration of the fresh stem extract, which reduced biomass by 15.08%.

A. lanceolatus fresh and dry tissue extracts inhibited the growth of the radicle of *S. alba* in all concentrations (Table 4).

Table 1. Effects of *A. lanceolatus* extracts on the seed germination of bioassay species

Vegetative part used for extract preparation	Extract conc. (%)	Germination percentage (%)			
		Aqueous extracts from fresh vegetative parts		Aqueous extracts from dry vegetative parts	
		<i>S. alba</i>	<i>L. sativa</i>	<i>S. alba</i>	<i>L. sativa</i>
control	0	84.47 ^a ±2.233	80 ^a ±7.679	84.47 ^a ±2.233	80 ^a ±7.679
	100	42.23 ^d ±12.38	80 ^a ±3.868	20 ^d ±3.868	68.9 ^{ab} ±5.877
stem	50	55.57 ^{cd} ±12.37	48.9 ^b ±8.02	66.67 ^{bc} ±3.839	57.8 ^{bcd} ±5.877
	25	60 ^{bcd} ±7.679	66.7 ^{ab} ±11.547	82.2 ^a ±5.877	55.5 ^{bcd} ±2.233
	100	77.8 ^{ab} ±5.877	71.1 ^{ab} ±11.1	13.37 ^d ±6.667	51.1 ^{bcd} ±5.877
rhizome	50	60 ^{bcd} ±3.868	55.6 ^b ±5.884	13.33 ^d ±3.839	42.2 ^d ±7.997
	25	62.23 ^{bcd} ±7.997	55.5 ^b ±9.678	73.3 ^{abc} ±0	62.2 ^{abc} ±5.877
	100	51.1 ^d ±2.2	66.7 ^{ab} ±3.839	62.23 ^c ±9.678	64.5 ^{ab} ±2.233
leaf	50	73.33 ^{abc} ±7.708	57.8 ^{ab} ±5.877	66.63 ^{bc} ±6.667	64.4 ^{ab} ±5.884
	25	53.3 ^{cd} ±0	57.8 ^{ab} ±5.877	80 ^{ab} ±0	44.4 ^{cd} ±8.02

Values with a common letter within column are not significantly different at <0.05. Values are means ±s.e. of three replicates.

Table 2. Seed deterioration of the bioassay species. Percentages of vital seeds in treatments on the first (I), third (III) and seventh (VII) day of the experiment are shown.

Vegetative part used for extract preparation	Extract conc. (%)	Aqueous extracts from fresh vegetative parts						Aqueous extracts from dry vegetative parts					
		<i>S. alba</i>			<i>L. sativa</i>			<i>S. alba</i>			<i>L. sativa</i>		
		I	III	VII	I	III	VII	I	III	VII	I	III	VII
control	0	44.44	80.00	84.47	2.22	62.22	80.00	44.44	80.00	84.47	2.22	62.22	80.00
	100	2.22	66.67	42.22	0.00	75.56	80.00	2.22	55.56	20.0	0.00	46.67	55.5
	50	11.11	80.00	55.55	4.44	51.11	48.89	24.44	53.33	13.4	4.44	48.89	51.1
stem	25	35.56	82.22	60.00	2.22	62.22	66.67	60.00	84.44	62.2	13.33	53.33	64.5
	100	26.67	80.00	77.80	2.22	68.89	71.13	11.11	53.33	66.7	4.44	53.33	68.9
	50	24.44	62.22	60.00	13.33	64.44	55.55	40.00	57.78	13.3	2.22	51.11	42.2
rhizome	25	15.56	82.22	62.22	2.22	53.33	55.55	57.78	82.22	66.6	4.44	64.44	64.4
	100	0.00	66.67	51.11	0.00	71.11	66.67	0.00	53.33	82.2	0.00	51.11	57.8
	50	6.67	86.67	73.33	6.67	53.33	57.78	17.78	68.89	73.3	0.00	68.89	62.2
leaf	25	6.67	68.89	53.33	0.00	57.78	57.78	35.56	86.67	80.0	4.44	53.33	44.4

Table 3. Dry seedlings biomass reduction in the treatments compared to the control (%).

Vegetative part used for extract preparation	Extract conc. (%)	Aqueous extracts from fresh vegetative parts		Aqueous extracts from dry vegetative parts	
		<i>S. alba</i>	<i>L. sativa</i>	<i>S. alba</i>	<i>L. sativa</i>
		control	0	0.00	0.00
stem	100	53.15	76.66	86.53	79.51
	50	26.94	85.94	18.46	76.20
	25	20.44	15.08	-1.82	77.48
rhizome	100	15.06	77.48	90.31	85.48
	50	35.43	80.52	86.07	86.49
	25	30.21	85.85	7.18	79.41
leaf	100	43.67	78.49	25.89	75.65
	50	6.13	82.91	24.30	75.37
	25	37.84	81.99	-7.02	78.31

Table 4. Inhibition of radicle growth of seedlings in the treatments compared to the control (%).

Vegetative part used for extract preparation	Extract conc. (%)	Aqueous extracts from fresh vegetative parts		Aqueous extracts from dry vegetative parts	
		<i>S. alba</i>	<i>L. sativa</i>	<i>S. alba</i>	<i>L. sativa</i>
		control	0	0.00	0.00
stem	100	91.32	28.35	97.16	73.00
	50	72.19	-11.88	87.04	29.49
	25	71.77	6.76	15.63	14.72
rhizome	100	45.77	-10.13	97.62	89.57
	50	66.60	0.42	98.50	77.67
	25	57.39	17.79	96.59	82.45
leaf	100	90.77	28.29	77.53	53.34
	50	63.79	26.18	64.15	40.05
	25	42.58	15.44	37.87	36.25

The percentage of inhibition ranged from 15.63% to 98.50%. The reduction was especially pronounced in the fresh stem extract in all concentrations and in the 100% and 50% dry stem extracts. In addition, a decrease in radicle growth was noticed in treatments with dry rhizome extracts in all concentrations, and with fresh and dry leaf extracts in the highest concentration (Table 4). Likewise, hypocotyl length was reduced as a result of the treatments (Table 5). The highest reduction was in the treatment with the dry stem extract (83.79%) and in treatments with 100% and 50% dry rhizome extracts (86.77% and 87.12%, respectively).

Extracts of fresh vegetative parts had a lower impact on hypocotyl growth of *S. alba*. The lowest concentration of these extracts encouraged hypocotyl elongation, compared to control treatments. Extracts of fresh stem, in 50% concentration and 100% rhizome extract promoted *L. sativa* radicle elongation while other extracts of fresh vegetative parts inhibited radicle growth compared to control treatments. Extracts of dry vegetative parts showed inhibitory effects on radicle growth of test species and the percentage of inhibition ranged from 14.72% to 89.57%. Hypocotyl length of test species *L. sativa* was higher in the treatment with extract of fresh vegetative parts than in control treatments. In addition, 50% and 25% extracts of dried stem and leaf had a similar effect on the length of the hypocotyl, while the 25% extract of dry rhizomes had the highest inhibitory effect on hypocotyl length of *L. sativa*.

DISCUSSION

Bioassay presented in this paper has shown that the extracts obtained from different vegetative parts of *A. lanceolatus*, negatively affect seed germination and seedling growth of test species in laboratory conditions. The present findings are consistent with research of Dias *et al.* (18) which found that aromatic water of *A. lanceolatus*

inhibited germination and hypocotyl growth of *Lactuca sativa*. Inhibited or delayed germination and lower seedling development are secondary indicators of primary influence of allelochemicals on metabolic processes (19). Both types of test species have shown sensitivity to extracts. In addition, reduction in seed germination was significant and inhibition of the seedling growth was by more than 75% compared to control treatments, in some cases. However, it should be noted that the biological tests carried out under controlled conditions, such as Petri dishes, probably overestimate the effect of allelopathy since soil can significantly neutralize effects of secondary metabolites (20, 21).

Although Rice (22) defined allelopathy as positive or negative impacts of a plant on another plant and some extracts of *A. lanceolatus* stimulated elongation of the hypocotyl and the radicle of the test species, this cannot be rated as a positive effect, since seedling biomass was lower compared to the control. The phytotoxic effect of the applied extracts was corroborated by the decay of seeds caused after the radicle ruptured the seed coat.

Plant secondary metabolites can alter ecosystem components, which can influence the processes and relationships in an ecosystem and, consequently, induce changes within plant communities (21, 23). Sharma and Raghubanshi (24) suggested that allelopathic activity of invasive *Lantana camara* L. affects the structure of the forest by changing the soil pH. Low floristic diversity in a community with one or two dominant species allows one species to „take over” in controlling the biochemical processes of the soil, which can greatly affect other plants in the community (21). Therefore, the influence of allelopathic compounds can be more pronounced in communities that are characterized by a low floristic diversity, compared to communities comprised of a large number of species (21). *A. lanceolatus* occupies new habitats via sexual reproduction followed by seed dispersal, and continues to spread

Table 5. Inhibition of the hypocotyl growth of the seedlings in the treatment compared to the control (%)

Vegetative part used for extract preparation	Extract conc. (%)	Aqueous extracts from fresh vegetative parts		Aqueous extracts from dry vegetative parts	
		<i>S. alba</i>	<i>L. sativa</i>	<i>S. alba</i>	<i>L. sativa</i>
control	0	0.00	0.00	0.00	0.00
	100	39.97	-37.06	83.79	16.40
stem	50	2.62	-56.16	33.46	-31.14
	25	-7.13	-34.92	6.98	-44.93
	100	9.27	-65.05	86.77	61.00
	50	8.54	-150.70	87.12	2.78
rhizome	25	-6.48	-56.24	45.87	16.40
	100	55.56	-65.42	44.62	7.14
	50	13.83	-117.49	10.11	-16.29
leaf	25	-18.63	-64.11	20.93	-19.27

via rhizomes forming monodominant stands. Taking into account that *A. lanceolatus* is perennial and perennials release exudates into the soil over several growing seasons, the results of this study could explain the high number of individuals of this invasive species.

Non-native species can affect the biodiversity of an ecosystem by altering the dynamics of succession (25). By changing environmental conditions in an ecosystem, some non-native plants ensure their survival and hinder or prevent the development of plants that occur in the later stages of succession (26). In addition, some allelochemicals lead to poor accessibility of nutrients by inhibiting the development of nitrifying plants and organisms and thus alter the floristic composition and succession of communities (27). Obratov-Petković *et al.* (28) reported that *A. lanceolatus* is the edifier in the *Asteretum lanceolati* community. The community consists of 107 species (28), of which 16% are invasive. It is known that coexisting species are adapted to each other's metabolites produced during evolution (29, 30). When a non-native species with allelopathic ability enters a new community, it can become more competitive compared to native plants, which are vulnerable to new allelochemicals. It is possible that phytotoxic compounds of *A. lanceolatus* inhibit the development of indigenous flora, which creates empty space on the site and causes increased nutrient availability. Increase in the amount of accessible resources in an ecosystem increases the susceptibility of ecosystems to invasion (31). Thus, the allelopathic ability of edifier species could explain the existence of a large number of invasive species in the *Asteretum lanceolati* community.

The biological test described in this paper showed that extracts have an impact on tested species and provides justification for further analysis in order to determine active substances and their role in other physiological processes. However, various components of an ecosystem are closely interlinked and factors that change one component often have an indirect impact on other components. Therefore, the results of this study should be confirmed and supplemented by additional experiments in natural conditions in order to determine the exact role of allelochemicals in the spread of this invasive species. This is especially important because different plant species can exhibit different reactions to same treatments. In addition, potential effect on co-occurring natives should preferably be tested, in order to make any conclusions on the role of allelopathy in a specific community.

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