

## Allelopathic effect of Siberian iris (*Iris sibirica*) on the early growth of wild oat (*Avena fatua*) and Canada thistle (*Cirsium arvense*)

Zvonko PACANOSKI<sup>1</sup>, Arben MEHMETI<sup>2</sup> (✉)

<sup>1</sup> University Ss. Cyril and Methodius, Faculty for Agricultural Sciences and Food, 1000 Skopje, Republic of Macedonia

<sup>2</sup> University of Prishtina, Department of Plant Protection, Bill Clinton p.n., 10000 Prishtina, Republic of Kosovo

✉ Corresponding author: [arben.mehmeti@uni-pr.edu](mailto:arben.mehmeti@uni-pr.edu)

### ABSTRACT

During 2015 a laboratory and greenhouse experiments were carried out to explore the allelopathic potential of different plant parts of Siberian iris (*Iris sibirica*) on wild oat (*Avena fatua*) and Canada thistle (*Cirsium arvense*) early growth. The aqueous filtrates of Siberian iris rhizomes produced a strong inhibitory effect on germination, radicle and hypocotyl length and seedling weight of wild oat. Contrary, germination, radicle and hypocotyl length of wild oat was not significantly reduced by any Siberian iris leaves filtrate concentrations, but seedling weight of wild oat was significantly reduced at the 1/1 and 3/4 filtrate concentrations of Siberian iris leaves, relative to control. Similar, germination, hypocotyl length and seedling weight of Canada thistle, were significantly affected by the aqueous filtrates of Siberian iris rhizomes, and while same parameters were none significantly reduced by the aqueous leaves filtrates of Siberian iris in compare with the control. In green house experiment, all treatment with different Siberian iris rhizomes residues significantly affected density, height and fresh weight of wild oat plants. Opposite, no one treatment by Siberian iris leaf residues caused significant reduction of plants density, height and fresh weight of the wild oat plants in compare with the control. All Siberian iris mixtures with rhizomes residues highly significant decreased Canada thistle plants density in average for 60.1%, and fresh weight for 60.3% in compare with the control. No one treatment by Siberian iris leaf residues caused significant reduction of plants density and height of Canada thistle, but 1/1 and 1/2 residues mixtures of Siberian iris leaf significantly reduced its fresh weight.

**Keywords:** allelopathic effect, Canada thistle, early growth, Siberian iris, wild oat

### INTRODUCTION

Wild oat and Canada thistle are among the world's cropping systems' most common, noxious and economically damaging weeds (Donald, 1994; Holm et al., 1997; Owen and Powles, 2009). Globally, they are major weeds of cereal crops where they represent a serious economic threat to crop yields because of their competitiveness, aggressiveness, staggered germination, crop mimicry, seed shattering, ability of seed to persist in the soil seed bank, and the deep creeping roots, respectively (Medd and Pandey, 1990; Donald, 1994; Fogelfors and Lundkvist, 2008). In Republic of Macedonia

these weeds can be found in large numbers in many regions of the country, especially in soils rich in nutrients and moderately moist (Kostov, 2006). Siberian iris in Macedonia is mainly used as the ornamental plant and occurred in ruderal areas, but doesn't occur in arable lands.

In spite of the fact that accessible market herbicides may provide effective control of these weeds, environmental injuries occur (Zhu and Li, 2002). Herbicide resistance expansions among weeds (Heap, 2008) and health problems due to overuse and misuse of synthetic herbicides (Kudsk and Streibig, 2003) have led scientists

to target alternative weed management approaches (Jabran et al., 2008) and environmentally responsive methods, such as allelopathy (Ravlić et al., 2014). Allelopathy is defined as the direct or indirect harmful or positive effects of one plant on another through the production of chemical compounds (allelochemicals) that run off into the environment (Rice, 1984). Furthermore, many of the phytotoxic substances supposed of causing germination and growth inhibition have been identified from plant tissues and soils (Turk and Tawaha, 2003). Understanding allelochemicals' mode of action is of particular importance in a crop weed situation, wherein it may be possible to manipulate the system to the crop's advantage (Putnam and Duke, 1978).

Crude morphologic effects, such as a reduction in weed growth that result from allelochemical action, are general phenomena that may be caused by a variety of more specific effects acting at the cellular or molecular level at the target plant.

The allelopathic potential of plants of the genus *Iris*, comprising over 300 species (Ali and Mathew, 1993), has been intensively studied (Rigano et al., 2006; Nakai et al., 2010; Fu et al., 2012; Chen and Tang, 2014). Nevertheless, no study has demonstrated the allelopathic potential of an ornamental plant, Siberian iris, for weed suppression, particularly wild oat and Canada thistle. This species may act as a potential source of allelochemicals for the natural control of these weeds. Furthermore, Khan and Atter, (2006) reported that flavonoids, isoflavonoides and their glycosides, benzoquinones and terpenoids are mentioned as chemicals largely emerging in the genus *Iris*. Also, Kukula-Koch et al., (2015) reported that 122 chemical compounds were confirmed in *Iris* spp. The presence of terpenoids and phenols in rhizomes and leaflets was reported in studies of *Siberina iris* (Kaššák, 2014). Therefore, the present study's objective was to investigate the allelopathic effects (if any) of Siberian iris leaf and rhizome filtrate concentrations and residues on the noxious weeds of cereal crops wild oat and Canada thistle.

## MATERIALS AND METHODS

A laboratory and greenhouse experiments were conducted in the Weed Science laboratory and greenhouse at the Faculty of Agriculture Sciences and Food in Skopje during 2015, to explore the allelopathic potential of leaf and rhizome of Siberian iris on wild oat and Canada thistle germination and early growth. For that purpose, fresh plants of Siberian iris were uprooted at the at full vegetative growth stage (beginning of April) at the experimental field of the Faculty of Agriculture Sciences and Food in Sirkovo, Kavadarci region, while seeds of the wild oat and Canada thistle were collected at the ripening seed stage (end of July) from the cereal fields of Eastern Macedonia during 2013/2014 growing season.

### *Laboratory experiment*

From freshly uprooted Siberian iris plants, rhizome and leaf were separated, cut into small pieces and put in a grinder. After grinding, 100 g fresh biomass of rhizome and 100 g fresh biomass of leaf, respectively, was soaked each into 1000 mL distilled water (1:10 ratio w/v) for 24 h at room temperature. After 24 hours, plant material was removed and extracts were filtered through filter paper (MN 640w). The obtained aqueous filtrates were used in experiments without diluting (1/1) and diluting (3/4, 1/2 and 1/4) with distilled water. The experiment was carried out in Petri dishes. In each sterilized Petri dishes (150 mm x 25 mm) wetting of two filter paper layers, by 25 seeds of wild oat and 50 seeds of Canada thistle were "sowed" in three replicates. After "sowing", by 8 mL (for wild oat seeds) and 4 mL (for Canada thistle seeds) different aqueous filtrates (3/4, 1/2 and 1/4) were added per Petri dish to the end of the experiment period. For the control, only distilled water was used. The Petri dishes were kept at 24±2 °C in a seed germinator with 16 h and 8 h, day/night light regime. Germination percentage for both weed seeds was recorded at 7 days after sowing (DAS). At 10 days after sowing (DAS), root and shoot length, as well as fresh biomass of both weeds were noted.

### Greenhouse experiment

After separation, rhizome and leaf of Siberian iris were cut into small pieces and air-dried in the greenhouse condition for 7 days. 2 kg of dried rhizome and 2 kg of dried leaf, respectively, were mixed each in 20 kg soil (Fluvisol sandy loam with 10.50% coarse, 63.10% fine sand, 26.40% clay+silt, 2.66% organic matter and pH 6.7) + manure, in a ratio of 1:1, and were kept at greenhouse conditions with adding by 5 L water per week. After 90 days of decomposition, soil + manure mixtures containing rhizome and leaf residues of Siberian iris, respectively, were obtained. The obtained mixtures were used in the experiment without "diluting" (1/1) and "diluting" (3/4, 1/2 and 1/4) with soil + manure. The greenhouse experiment was carried out in plant growing containers (170 x 250 x 110 mm), which were filled with the different mixtures of soil + manure + plant residues (1/1, 3/4, 1/2 and 1/4). For the control, only soil + manure mixture (in a ratio of 1:1 and without Siberian iris residues) was used. In each plant growing containers, by 50 seeds of wild oat and Canada thistle were sowed in three replicates. After sowing, the plant growing containers were kept at greenhouse conditions and irrigated according needs during experiment period. Wild oat and Canada thistle plants were harvest 30 days after sowing (DAS). Data were recorded on the following parameters: plant density, height and fresh weight of wild oat and Canada thistle plants per container. Comparison of wild oat/Canada thistle parameters were made between control and different mixtures of soil + manure + plant residues of Siberian iris. The data were tested for homogeneity of variance and normality of distribution (Ramsey and Schafer 1997) and were log-transformed as needed to obtain roughly equal variances and better symmetry before ANOVA were performed. Data were transformed back to their original scale for presentation. Means were separated by using LSD test at 5% of probability.

## RESULTS AND DISCUSSION

### Laboratory experiment with wild oat and Canada thistle

A significant treatment by different Siberian iris aqueous filtrates interaction resulted in two distinct results for wild oat and Canada thistle, respectively in laboratory experiment.

Seven days after sowing (DAS), the aqueous filtrates of Siberian iris rhizomes produced a strong inhibitory effect on wild oat germination. After treatment with all filtrates from Siberian iris rhizomes, the wild oat seed germination rates decreased by between 22.2% and 38.9%, in comparison with the control. For the same period, the aqueous filtrates of Siberian iris leaves insignificantly increased the wild oat germination rates by between 5.5% and 7.9%, in comparison with the control (Table 1).

The methanolic extract of *Iris pseudopumila* caused a decrease in the percentage of *Raphanus sativus* seed germination (Rigano et al., 2006). Similarly, extracts from fresh black mustard plant leaves, stems, flowers, roots and mixture solutions showed inhibitory effects on wild oat seed germination.

The results by Turk and Tawaha, (2003) showed that degree of inhibition increased with the extract concentration. At the highest extract concentration (20 g\*kg<sup>-1</sup>), all aqueous extracts significantly reduced seed germination compared with the distilled water control. Results achieved by Jabran et al. (2010) showed that wild oat treatment with sorghum and barnyard grass water extracts improved the final germination percentage and germination energy compared with the control, but none of the wild oat seeds could germinate upon application of mulberry water extract.

The wild oat radicle length was significantly reduced only at the highest (1/1 and 3/4) Siberian iris rhizome filtrate concentrations (78.3 and 91.4 cm), relative to the control (112.9 mm). No significant reduction was found in

other lower (1/2 and 1/4) rhizome filtrate concentrations (108.8 and 102.1 mm, respectively). On the contrary, the wild oat radicle length was not significantly reduced by Siberian iris leaf filtrate concentrations, relative to the control (Table 1). In a study conducted by Turk and Tawaha (2003), all extracts of *B. nigra* caused obvious reduction in the radicle length of *A. fatua* L. seedlings.

Similar to the wild oat radicle length, the hypocotyl length was significantly affected by 1/1 and 3/4 Siberian iris rhizome filtrate concentrations. Conversely, an insignificantly decreasing wild oat hypocotyl length was recorded at all (1/1, 3/4, 1/2 and 1/4) Siberian iris leaf filtrate concentrations (70.1, 69.0, 71.0 and 73.7 mm, respectively) in comparison to the control (75.5 mm) (Table 1).

In Marwat and Khan's research (2006), the wild oat hypocotyl height was significantly affected by *Eucalyptus camaldulensis* leaf extracts. Wild oat root and shoot seedlings were significantly reduced by the different aqueous *Eucalyptus citriodora* fresh and dry leaf extracts (El-Rokiek and Eid, 2009). Wild oat treatment with sorghum and barnyard grass water extracts significantly increased the plumule length, while mulberry and winter cherry water extracts strongly reduced the wild oat plumule length (Jabran et al., 2010).

The wild oat seedling weight was significantly reduced with all filtrate concentrations of Siberian iris rhizomes and 1/1 and 3/4 filtrate concentrations of Siberian iris leaves, respectively (Table 1). However, no significant reduction was recorded in other lower (1/2 and 1/4) leaf filtrate concentrations (3.63 and 3.93 g, respectively), relative to the control (4.02 g). In Turk and Tawaha's study (2003), *B. nigra* leaf extracts reduced the hypocotyl dry weight significantly more than did extracts from other plant parts at all extract concentrations. In contrast, all *B. nigra* extracts caused a marked reduction in the radicle dry weight at all concentrations. The leaf extract was the most inhibitory at concentrations of 4, 8, 12, 16 and 20 g\*kg<sup>-1</sup> and reduced the radicle dry weight by 40.0%, 45.6%, 54.4%, 60.0% and 66.7%, respectively.

The germination of Canada thistle, measured 7 DAS, was significantly inhibited by all aqueous extracts of Siberian iris rhizomes between 50% and 77.5%. For the same period, the Siberian iris aqueous leaf filtrates insignificantly reduced the Canada thistle germination rates by between 2.6% and 23.7%, relative to the control (Table 1).

The hypocotyl length of Canada thistle was significantly affected by 1/1 and 3/4 Siberian iris rhizome filtrate concentrations, but not affected by rhizome filtrates at 1/2 and 1/4 concentrations, or leaf filtrates at 1/1, 3/4, 1/2 and 1/4 concentrations, relative to the control (Table 1). As a result of strongly inhibited germination, the Canada thistle seedling weight was significantly reduced with all filtrate concentrations of Siberian iris rhizomes, on average, by 70.0%. Conversely, insignificant degrees of the Canada thistle seedling weight, 11.6% on average, were recorded at 1/1, 3/4 and 1/2 Siberian iris leaf filtrate concentrations, while slightly increasing to 11.4% at the 1/4 leaf filtrate concentration, in comparison with the control (0.35 g) (Table 1).

The n-hexane-soluble, acetone and water-soluble fractions obtained from the extract of *Azadirachta indica* shoots inhibited the germination seeds of *Cirsium arvense* (Ashrafi et al., 2009). It is reported that buckwheat used as a cover crop suppressed or eradicated Canada thistle, with allelopathy as a possible mechanism (Robinson, 1980; Oplinger et al., 1989). A study by Dadkhah (2012) showed that the lowest (15%) extract concentrations of *Ephedra major* decreased the leaf area of *Cirsium arvense* plants by 50.8%, plant height by 39.2%, shoot dry weight by 55.9% and, root dry weight by 32.5%, respectively, whereas highest (45%) extract concentrations of *Ephedra major* decreased the leaf area of *Cirsium arvense* plants by 90.9%, plant height by 73.4%, shoot dry weight by 88.8% and root dry weight by 82%, respectively compared to the control plants.

**Table 1.** Effect of Siberian iris allelochemicals on the early growth of wild oat and Canada thistle in laboratory conditions

Treatments	Wild oat					Canada thistle								
	Siberian iris rhizoms filtrate		Siberian iris leaves filtrate			Siberian iris rhizoms filtrate		Siberian iris leaves filtrate						
	Germination (%) 7 DAS	Radicle length (mm)	Hypocotyl length (mm)	Seedling weight (g)	Germination (%) 7 DAS	Radicle length (mm)	Hypocotyl length (mm)	Seedling weight (g)	Germination (%) 7 DAS	Radicle length (mm)	Hypocotyl length (mm)	Germination (%) 7 DAS	Radicle length (mm)	Hypocotyl length (mm)
Control	72.0a	112.9a	75.5a	4.02a	72.0a	112.9a	75.5a	4.02a	48.0a	38.0a	0.350a	48.0a	38.0a	0.350a
Filtrate (1/1)	44.0c	78.3c	58.6c	1.93c	76.0a	103.7a	70.1a	3.46b	13.2c	27.0b	0.078c	53.2a	29.0a	0.322a
Filtrate (1/2)	52.0b	91.4bc	64.9bc	2.48b	77.3a	106.4a	69.0a	3.43b	10.8c	29.0b	0.072c	42.8a	37.0a	0.286a
Filtrate (3/4)	56.0b	108ab	70.5ab	2.60b	77.7a	107.2a	71.0a	3.63a	24.0b	42.0a	0.156b	48.0a	36.0a	0.320a
Filtrate (1/4)	54.7b	102ab	68.7ab	2.51b	76.0a	109.2a	73.7a	3.93a	18.8bc	42.0a	0.112bc	56.0a	37.0a	0.390a
LSD 0.05	7.31	14.30	9.22	0.77	5.81	14.84	8.31	0.43	9.07	6.81	0.07	9.61	9.83	0.20
Random effect														
Interactions														
Siberian iris filtrates x wild oat			*											
Siberian iris filtrates x Canada thistle			*											
Siberian iris filtrate x weeds			NS											

<sup>a</sup> Abbreviation: DAS-days after sowing

<sup>b</sup> Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at  $P < 0.05$  <sup>c</sup> NS, not significant; \* Significant level  $P < 0,05$

### **Greenhouse experiment with wild oat and Canada thistle**

A significant treatment by different Siberian iris aqueous filtrates interaction resulted in two distinct results for wild oat and Canada thistle, respectively in green house experiment, as well. Regarding the density of wild oat plants, statistical analysis of the data (Table 2) revealed that all treatment with different Siberian iris rhizome residues significantly affected their density, compared to the control. Generally, the number of wild oat plants per growing container filled with different mixtures of Siberian iris rhizome residues ranged between 17.0 (mixture with 1/1 rhizome residues) and 22.7 (mixture with 1/4 rhizome residues). Treatments with different Siberian iris leaf residues created a contrasting outcome. The highest wild oat density was recorded in containers with 3/4 leaf residues (28.3), followed by 1/4 leaf residues (26.8). The lowest wild oat density was recorded in containers filled with the mixture with 1/1 leaf residues (26.3) (Table 2).

The height of the wild oat plants was significantly reduced by 1/1 and 3/4 Siberian iris rhizome residue treatments (Table 2). The tallest wild oat plants in the mixtures with different Siberian iris rhizome residues (23.4 cm) were recorded in containers with 1/4, followed by 1/2 (21.7 cm), while the shortest wild oat plants (16.8 cm) were recorded in containers filled with the mixture with 1/1 Siberian iris rhizome residues. This was similar to the wild oat plant density: none of the treatments with different Siberian iris leaf residues caused a significant reduction of the height of the wild oat plants. The fresh weight of the wild oat plants depended on the previous parameters: density and height. Any of the Siberian iris rhizome residue treatments caused a highly significant ( $P < 0.01$ ) reduction in the fresh weight of wild oat plants

(Table 2). In comparison, insignificant degrees of the wild oat fresh weight, 7.2% on average, were recorded for 1/1, 3/4 and 1/2 Siberian iris rhizome residue treatments, while slightly increasing to 8.8% for the 1/4 rhizome residue treatment, compared with the control (6.35 g) (Table 2).

According to Turk and Tawaha (2003), the germination percentage, plant height and dry weight per *A. fatua* plant were all significantly lower with fresh-residue incorporation than with the control. However, similar results have been reported in previous studies Hegde and Miller (1990).

Regarding Canada thistle plants' density, statistical analysis of the data revealed (Table 2) that all Siberian iris mixtures with rhizome residues significantly decreased, 60.1% on average, in comparison with the control (16.3). Generally, the number of Canada thistle plants per growing container, compared to the rest of the treatments with different Siberian iris leaf residues, was insignificantly lower (14.0, 13.7 and 15.0), except for the 3/4 mixture of Siberian iris leaf residues, which was negligibly higher (17.0) compared with the control (16.3) (Table 2). Insignificant decreasing of the Canada thistle plant height, 15.7% on average, was recorded for 1/1, 3/4 and 1/4 Siberian iris rhizome residue treatments, while slightly increasing to 7.5% for the 1/2 rhizome residue treatment, compared with the control (5.3 cm). Similarly, no treatment with different Siberian iris leaf residues caused a significant reduction in the height of Canada thistle plants (Table 2). Fresh weight of the Canada thistle plants was significantly reduced at all mixtures of Siberian iris rhizome and 1/1 and 1/2 residues mixtures of Siberian iris leaves, compared with the control (0.46 g).

**Table 2.** Effect of Siberian iris residues on the wild oat and Canada thistle growth parameters in greenhouse conditions

Treatments	Wild oat						Canada thistle					
	Siberian iris rhizoms filtrate			Siberian iris leaves filtrate			Siberian iris rhizoms filtrate			Siberian iris leaves filtrate		
	Plant density	Height of the plants (cm)	Fresh weight of the plants/ container (g)	Plant density	Height of the plants (cm)	Fresh weight of the plants/ container (g)	Plant density	Height of the plants (cm)	Fresh weight of the plants/ container (g)	Plant density	Height of the plants (cm)	Fresh weight of the plants/ container (g)
Control	28.7 <sup>a</sup>	27.6 <sup>a</sup>	6.35 <sup>a</sup>	28.7 <sup>a</sup>	27.6 <sup>a</sup>	6.35 <sup>a</sup>	16.3 <sup>a</sup>	5.3 <sup>a</sup>	0.466 <sup>a</sup>	16.3a	5.3a	0.466 <sup>a</sup>
Filtrate (1/1)	17.0 <sup>c</sup>	16.8 <sup>c</sup>	3.68 <sup>d</sup>	26.3 <sup>a</sup>	24.9 <sup>a</sup>	5.79 <sup>a</sup>	4.7 <sup>d</sup>	3.9 <sup>a</sup>	0.102 <sup>e</sup>	14.0a	4.2a	0.389 <sup>b</sup>
Filtrate (1/2)	19.3 <sup>bc</sup>	18.5 <sup>bc</sup>	3.96 <sup>cd</sup>	26.7 <sup>a</sup>	28.3 <sup>a</sup>	5.88 <sup>a</sup>	5.0 <sup>cd</sup>	4.5 <sup>a</sup>	0.143 <sup>d</sup>	17.0a	4.8a	0.422 <sup>ab</sup>
Filtrate (3/4)	20.0 <sup>bc</sup>	21.7 <sup>abc</sup>	4.55 <sup>bc</sup>	27.0 <sup>a</sup>	25.5 <sup>a</sup>	6.01 <sup>a</sup>	7.3 <sup>b</sup>	5.7 <sup>a</sup>	0.225 <sup>c</sup>	13.7a	4.0a	0.387 <sup>b</sup>
Filtrate (1/4)	22.7 <sup>b</sup>	23.4 <sup>ab</sup>	5.11 <sup>b</sup>	29.3 <sup>a</sup>	26.8 <sup>a</sup>	6.91 <sup>a</sup>	9.0 <sup>b</sup>	5.0 <sup>a</sup>	0.271 <sup>b</sup>	15.0a	4.9a	0.414 <sup>ab</sup>
LSD 0.05	4.99	6.48	0.64	2.48	6.51	0.57	2.13	1.70	0.03	4.44	1.38	0.0 <sup>6</sup>
Random effect												
Interactions												
Siberian iris filtrates x wild oat			*									
Siberian iris filtrates x Canada thistle			*									
Siberian iris filtrate x weeds			NS									

<sup>a</sup> Abbreviation: DAS-days after sowing

<sup>b</sup> Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at P<0.05 <sup>c</sup> NS, not significant; \* Significant level P<0,05

## CONCLUSION

In conclusion, the results obtained demonstrate the allelopathic potential of the different plant parts of the Siberian iris for wild oat and Canada thistle early growth, suggesting that they may significantly affect some crops' parameters due to the inhibitory effects of allelochemicals present in the plant parts of Siberian iris. In most cases, their effects, particularly of rhizomes on the early growth of both weeds in laboratory and greenhouse conditions, were highly significant. Further investigations, under field conditions, are necessary to examine the application and behavior of different Siberian iris rhizome and leaf filtrate concentrations and residues in relation to wild oat and Canada thistle development.

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