Allelopathic activity of extracts from *Impatiens* species

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ABSTRACT

We have tested the effect of water, methanol and dichloromethane extracts from the leaves of several species of *Impatiens* (*I. noli-tangere*, *I. parviflora*, *I. glandulifera*) on germination of seeds *Leucosinapis alba* and *Brassica napus*. All of the tested extracts had inhibitory effects to seeds of all studied plants (except the dichloromethane extracts). The highest activity revealed methanol extract and extract from *I. glandulifera*.

Keywords: *Impatiens*; allelopathy; inhibitory effect

Genus *Impatiens* belongs to the Balsaminaceae family. It is distributed over the whole north hemisphere and tropical zone. In the Central Europe three species occur: *Impatiens noli-tangere* L., *I. parviflora* DC. and *I. glandulifera* Royle (Kubát et al. 2002). They are perennial plants which grow on humid soil. *I noli-tangere* (Touch-me-not Balsam) is original species settled in leaf forests and bushes. *I. parviflora* (Small Balsam) looks like *I. noli-tangere* but it came from Asia. It grows on similar localities. *I. glandulifera* (Himalayan Balsam) originates from the Himalayan mountains. It differs from the other mentioned species in its height (1–3 m) and red-violet flowers. It grows along the river banks and in the same way it invaded new territories (Pyšek and Prach 1994).

While the content of chemical compounds in *I. balsamina* is well known, the content and biological activity of phenolic compounds from *I. glandulifera*, *I. noli-tangere*, *I. parviflora* was only little studied. In the aboveground parts of *I. glandulifera*, *I. noli-tangere*, *I. parviflora* naphthoquinones (Lobstein et al. 2001, Šerá et al. 2005), derivatives of quercetin (glucosides) and the derivatives of caffeic acid (Šerá et al. 2005) were identified. It was mentioned that naphthoquinones (e.g. 2-methoxy-1,4-naphthoquinone) have allelochemical and pesticide-like effects (www.ars-rin.gov/duke).

At the present time, various naphthoquinones of plant origin are pharmacologically tested. They revealed cytotoxic effect on the cancer cells in vitro (Babula et al. 2006a,b). An interesting biological effect was also observed in flavonols and derivatives of caffeic acid (www.ars-rin.gov/duke).

The aim of our study was testing the extracts from the above mentioned *Impatiens* species on germination of seeds and to prove their phytotoxic (allelopathic) effect.

MATERIAL AND METHODS

We prepared nine types of extracts (solvents: water, methanol, dichloromethane) from the leaves of *Impatiens* species (species: *I. glandulifera*, *I. noli-tangere*, *I. parviflora*). The dried and pulverized plant material (3.5 g) was extracted by 80 mL of the above mentioned solvent for two hours. Consequently the extracts were filtered through glass filters, sediments were washed twice with 15 mL of the solvent used for extraction. The extracts were filtered, joined and filled up to 100 mL.

The test of germination was performed on the glass Petri dishes 90 mm in diameter. Each dish contains three filter papers. On the papers 6 mL of tested extract were applied (in the control 6 mL of extracting solvent). The Petri dishes were left to evaporate the solvents (methanol and dichloromethane) at laboratory temperature. When the solvents were evaporated we applied 6 mL of

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distilled water. In this way we excluded the effect of methanol and dichloromethane on the seed germination. After it we put 30 seeds of *Leucosinapis alba* or *Brassica napus* on each Petri dish. For every combination of the solvent and species fine dishes with 30 seeds of both tested sorbs were used.

Than the dishes were transferred into thermostat and incubated at 22°C for 48 h. The germinated seeds were counted and the lengths of radicle and hypocotyls were manually measured.

The number of germinated seeds were recalculated on percents and for 'normalisation' we used arcsin transformation. The logarithmic transformation of the obtained morphometric data (lengths of radicles and hypocotyls) were done for the same purpose. For the calculation of morphometric characteristics we calculated the means of obtained data and then we took only these means for the following data processing.

The statistical data processing was performed according to the Statistica programme (1999). The difference between data from all three solvents were computed with relevant control by the Student's *t*-test. The aim of this calculation was to find if an individual of all combinations of the solvent and *Impatiens* species differs from the relevant control. For evaluation of influence of the extracts on germination and growth of seedlings the two-way ANOVA test with repetition was used (balanced design, firm factor: solvents water, methanol, dichloromethane, accidental factor: species: *I. glandulifera*, *I. noli-tangere*, *I. parviflora*). The dependent variances were the percentage of germinated seeds, lengths of radicles and hypocotyls. The detailed testing of experimental variances between each other was done using the one-way ANOVA test and then by the Tukey HSD test for

Table 1. Percentage of germinated seeds and radicle and hypocotyl lengths in germinated seeds of *Leucosinapis alba* (A) and *Brassica napus* (B) after various extract treatments (three *Impatiens* species and three solvents were used)

Tested seed	Extract		Germination (%)			Radicle (mm)			Hypocotyl (mm)		
			mean	SD	*P*-level	mean	SD	*P*-level	mean	SD	*P*-level
A	H_2O	*I. parviflora*	20	11	**0.000**	2.25	0.64	**0.000**	0.60	0.89	**0.000**
		I. noli-tangere	3	2	**0.000**	–	–	–	–	–	–
		I. glandulifera	1	2	**0.000**	–	–	–	–	–	–
		control	96	5	–	17.06	1.90	–	5.56	1.04	–
	MeOH	*I. parviflora*	38	8	**0.000**	1.08	0.07	**0.000**	0.00	0.00	**0.000**
		I. noli-tangere	16	9	**0.000**	1.00	0.00	**0.000**	0.00	0.00	**0.000**
		I. glandulifera	2	4	**0.000**	–	–	–	–	–	–
		control	96	1	–	16.90	1.28	–	3.73	0.34	–
	DCM	*I. parviflora*	93	5	0.681	5.12	0.32	**0.000**	2.28	0.34	**0.000**
		I. noli-tangere	83	10	0.065	6.05	2.76	**0.000**	1.59	0.73	**0.000**
		I. glandulifera	72	14	**0.011**	6.62	2.61	**0.000**	1.80	1.43	**0.002**
		control	94	4	–	18.70	3.96	–	4.88	0.46	–
B	H_2O	*I. parviflora*	92	5	0.394	2.42	0.51	**0.000**	1.49	0.69	**0.001**
		I. noli-tangere	89	8	0.793	2.64	0.62	**0.000**	0.94	0.21	**0.000**
		I. glandulifera	73	11	**0.048**	2.30	0.67	**0.000**	0.22	0.19	0.087
		control	88	9	–	16.55	1.63	–	7.00	0.41	–
	MeOH	*I. parviflora*	87	4	0.068	1.94	0.25	**0.000**	0.46	0.31	**0.000**
		I. noli-tangere	83	8	**0.040**	1.15	0.10	**0.000**	0.01	0.02	**0.000**
		I. glandulifera	11	13	**0.003**	–	–	–	–	–	–
		control	93	4	–	21.75	2.25	–	6.48	0.57	–
	DCM	*I. parviflora*	93	3	0.832	10.05	0.60	**0.000**	4.08	0.37	**0.001**
		I. noli-tangere	89	7	0.335	15.21	1.28	**0.008**	4.19	0.70	**0.004**
		I. glandulifera	95	4	0.529	6.86	2.29	**0.000**	3.57	0.96	0.266
		control	93	5	–	17.88	1.07	–	2.89	0.32	–

Differences between single extracts and corresponding control were analysed with *t*-test. Significant differences are in bold. Solvents: H_2O – water; MeOH – methanol; DCM – dichloromethane, – not countable

multiple comparison. All the statistical tests were done on the level of 0.05 significance.

RESULTS AND DISCUSSION

The obtained results showed strong phytotoxicity of the substances present in the extracts on germination of seeds of *Leucosinapis alba* (White Mustard) and *Brassica napus* (Oilseed Rape) (Tables 1–3).

In the tests with water and methanol extracts from *Impatiens* species the most of seeds did not germinat (Table 1). Also the lengths of radicles were intangible when compared with the control samples. The hypocotyls were measurable only in the test with water extract from *I. parviflora*. Both extracts contain highly toxic substances which inhibit germination. The extracts from leaves of *Impatiens* species contain mainly flavonols and derivatives of caffeic acid (Šerá et al. 2005).

In the test with dichloromethane extracts more than 70% of seeds germinated, but the lengths of radicles and hypocotyls were several times shorter than in controls (Table 1). Dichloromethane extracts contain non-polar, water insoluble or poorly soluble compounds. We suppose that these substances penetrated only in small amount in the seeds or the concentration of biologically active substrates in the dichloromethane extracts is not sufficient.

The effect of extracts on the germination testing seeds differs, as it is seen in Table 1. It is evident that the seeds of *Leucosinapis alba* are more sensitive than seeds of *Brassica napus*. An important phytotoxic effect on germination, growing of roots and hypocotyls in the seedlings of *Leucosinapis alba* had factor solvent (Table 2). A significant inhibitory effect on germination and growth of *Brassica napus* seeds were observed at species and factor interaction solvent × species (Table 2). The most important solvent factor on growth and germination of both seed species was observed in methanol (Table 3). On the other hand, practically very small effect was found in dichloromethane. The highest inhibitory effect from all the tested *Impatiens* species on both sorts of seeds was obtained by *I. glandulifera* (Tables 3).

The similar tests on the *Leucosinapis alba* seeds were carried out on extracts from *Reynoutria* sp. (Japanes, Giant and Bohemian knotweeds) (Šerá et al. 2008, Vrchotová and Šerá 2008). The comparison of the results demonstrated that the *Impatiens* extracts are much more toxic. The *Impatiens* extracts hardly influenced the seed germination and also length of radicles and hypocotyls of *Leucosinapis alba*.

Table 2. Relationship between extracts (from various extract solvents and *Impatiens* species) and percentage number of germinated seeds and radicle and hypocotyl lengths in seeds of *Leucosinapis alba* (A) and *Brassica napus* (B)

Tested seed	Variable	Factor	*df*	MS (factor)	*F*	*P*
A	germination	solvent	2	8.187	8.964	**0.033**
		species	2	3.761	35.234	**0.000**
		solvent × species	4	0.913	8.557	**0.000**
	radicle	solvent	2	1.521	52.880	**0.001**
		species	2	0.031	1.397	0.264
		solvent × species	4	0.029	1.295	0.296
	hypocotyle	solvent	2	362.283	43.544	**0.002**
		species	2	13.793	1.533	0.234
		solvent × species	4	8.320	0.925	0.464
B	germination	solvent	2	0.758	1.120	0.386
		species	2	0.810	18.906	**0.000**
		solvent × species	4	0.676	15.785	**0.000**
	radicle	solvent	2	2.134	6.295	**0.034**
		species	2	2.257	343.510	**0.000**
		solvent × species	4	0.339	51.574	**0.000**
	hypocotyle	solvent	2	91.074	3.383	0.104
		species	2	47.618	28.166	**0.000**
		solvent × species	4	26.923	15.925	**0.000**

Two-way ANOVA was used. Significant results are in bold. More details are in the Methods

Table 3. Relationship among all types of extracts in relation to percentage number of germinated seeds, radicle and hypocotyl lengths in seeds of *Leucosinapis alba* (A) and *Brassica napus* (B)

		Germination			Radicle			Hypocotyle		
		IP	INT	IG	IP	INT	IG	IP	INT	IG
A	H_2O	a	b	b	a	a	a	a	a	a
	MeOH	a	a	b	a	a	a	a	a	a
	DCM	a	ac	bc	a	a	a	a	a	a
		H_2O	MeOH	DCM	H_2O	MeOH	DCM	H_2O	MeOH	DCM
	IP	a	b	c	a	b	c	a	a	b
	INT	a	b	c	a	a	b	a	a	a
	IG	a	a	b	a	a	b	a	a	a
B		IP	INT	IG	IP	INT	IG	IP	INT	IG
	H_2O	a	a	b	a	a	b	a	a	a
	MeOH	a	a	a	a	b	b	a	b	c
	DCM	a	a	a	a	b	b	a	a	a
		H_2O	MeOH	DCM	H_2O	MeOH	DCM	H_2O	MeOH	DCM
	IP	a	a	a	a	a	a	a	b	a
	INT	a	a	b	a	b	c	a	b	c
	IG	a	b	c	a	b	a	a	b	a

One-way ANOVA was used, results of Tukey HSD test are presented, no differences are given in the same letters (in row). Species: IP – *Impatiens parviflora*; INT – *I. noli-tangere*; IG – *I. glandulifera*. Solvents: H_2O – water; MeOH – methanol; DCM – dichloromethane. More details are in the Methods

The following experiments with the seeds of other plants can respond the question of practical usage of the substances extracted from Impatiens species for protection of cultural plants. The subject of our next study will be to discover, which compounds are responsible for their toxicity.

REFERENCES

Babula P., Mikelová R., Adam V., Kizek R., Havel L., Sladký Z. (2006a): Naphthoquinones – biosynthesis, occurrence and metabolism in plants. Czech and Slovak Pharmacy, *55*: 151–159.

Babula P., Mikelová R., Adam V., Potěšil D., Zehnálek J., Kizek R., Havel L., Sladký Z. (2006b): Chromatographic analysis of naphthoquinones in plants. Chemické Listy, *100*: 271–276.

Kubát K., Hrouda L., Chrtek J. jun., Kaplan Z., Kirschner J., Štěpánek J. (2002): Key to the Flora of the Czech Republic. Academia, Prague.

Lobstein A., Brenne X., Feist E., Metz N., Weniger B., Anton R. (2001): Quantitative determination of naphthoquinones of *Impatiens* species. Phytochemical Analysis, *12*: 202–205.

Pyšek P., Prach K. (1994): How important are rivers for supporting plant invasions? In: de Waal L.C., Child L.E., Wade P.M., Brock J.H. (eds): Ecology and Management of Invasive Plants. John Wiley and Sons Ltd, New York, 19–26.

Šerá B., Vrchotová N., Tříska J. (2005): Phenolic compounds in the leaves of alien and native Impatiens plants. In: Alford D.V., Backhaus G.F. (eds): Plant Protection and Plant Health in Europe: Introduction and Spread of Invasive Species. British Crop Production Council, Alton, 281–282.

Šerá B., Vrchotová N., Cvrčková K., Krejčová J. (2008): On phytotoxic effect of *Fallopia taxa*. Bulletin of the Czech Botanical Society, Praha, *43*: 141–150.

Statistica (1999): Statistica for Windows (Volume 1): General Conventions and Statistics I. StatSoft, Inc., Tulsa.

Vrchotová N., Šerá B. (2008): Allelopathic properties of knotweed rhizome extracts. Plant, Soil and Environment, *54*: 301–303.

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