https://doi.org/10.26651/allelo.j./2018-45-1-1174 0971-4693/94 Euro 20.00
Allelopathy Journal 45 (1): 45-54 (September, 2018) *International Allelopathy Foundation* 2018
Table: 1, Figs : 5

Allelopathic activity of two invasive *Impatiens* species in temperate climate of Lithuania

L. Baležentienė
Institute of Environment and Ecology,
Aleksandras Stulginskis University, Akademija,
Kaunas Distr. LT-53361, Lithuania
E. Mail: ligitaba@gmail.com

(Received in revised form: July 30, 2018)

ABSTRACT

This study determined the total phenolics content in various plant parts of invasive *Impatiens* species and evaluate their phytotoxicity on germination and seedling growth of wheat (*Triticum aestium* L.) 'Hamlet'(monocot) and rapeseed (*Brasica rapus* L.) 'Cult'(dicot). In 2016, the allelopathic activity of two invasive *Impatiens* species (Balsaminaceae) viz., balsam [*I. parviflora* (origin C. Asia)] and Himalayan balsam [*I. glandulifera* (origin Himalayas)] was assessed on seed germination of wheat and rapeseed *ex situ*. In *Impatiens* extracts phenolics content ranged between 0.001 and 0.008 mg g^{-1} and these significantly inhibited the seed germination and seedling growth of both recipient species. However, germination inhibition differed for each recipient specie. Both *Impatiens* spp. extracts drastically inhibited the rapeseed germination (4.5-81%) than wheat germination (62-86.5%) possibly due to different seed coat anatomy and thus, its permeability. Therefore the most drastic inhibition (90% and 86.5%) was recorded in rapeseed germination (4.5% and 11.5%) in 0.2% flower and fruit+seed extract of *I. parviflora* and *I. glandulifera*, respectively. Consequently, recruitment and regeneration of native species might be negatively affected by the invasive *Impatiens* species in invaded habitats.

Key words: Allelopathy, *Brassica napus*, germination, *Impatiens glandulifera*, *Impatiens parviflora*, inhibition, phenolics, rapeseed, seedling growth, *Triticum aestium*, wheat.

INTRODUCTION

Invasive alien plants affect numerous natural habitats and pose major threat to integrity and biodiversity of native systems. The knowledge how invasive alien species spread in a new environment, might be useful for their management. Biochemical interactions underlies the novel weapon hypothesis, thus presenting one of numerous explanations for species invasiveness. Spread of invasive alien species (IAS) is considered a second environmental problem after habitat threat, which changes the ecosystem functioning and the ecosystem biodiversity (8). Invasive alien species outcompete native species, thus changing the ecosystems diversity, pushing autochthones species from native habitats, decreasing biodiversity and transforming the landscape (26). in Europe, about 6000 species are considered alien plants in Europe (9,18) and ca.1 800 species are currently naturalized, a number that may increase by 6 species every year.

In Lithuania, 548 non-native vascular plant have been recorded since 16th century. Between them 46 alien plant species are considered invasive and 60 species are potentially invasive. To prevent and manage the dispersion of invasive species, the new National checklist of IAS (approved in 2016) includes two *Impatiens* species (14).The *Impatiens* spp. possess high invasive potential and thus successfully spread across the world.

Impatiens comprises more than 1000 species and is one of the largest genera of flowering plants, which recently attracted the interest due to their wide subcosmopolitan distribution (8-10). Amongst *Impatiens, I. parviflora* (*I parv*) and *I. glandulifera* (*I glan*) native to Asia and Himalaya are remarkably successful invaders worldwide (27), which also spread in Lithuania. *I. parviflora* is one of the most widespread invasive plants occurring in temperate and northern Europe (1). Genus *Impatiens* includes a number of cultivated ornamental species popular in horticulture, i.e. *I. glandulifera, I. walleriana, etc.* Among them are several widespread invasive species : *Impatiens* species (*I. glandulifera, etc.*) (4).

The invasions pathways must be identified, prioritized and managed to prevent the introduction, to control or eradicate IAS (6,7). Particular habitats strongly vary in their susceptibility to invasion and consistent knowledge about invasive species establishment and dispersion is quite patchy (3). Recently the phenomenon of allelopathy becomes acknowledged alternative explanation for the establishment and spread of invasive species in undisturbed communities, achieved through release of phytochemicals by the invader in the invaded ecosystem (2,16,21). Fraenkel (1959) emphasized the ecological significance of secondary metabolites for the species biochemical interactions in ecosystems (11). Phenolic compounds is large group among secondary metabolites in plants and thus play major role in plant communities (13,14). Significant content of phenolic acids accumulate in leaves, flowers and roots of different *Impatiens* spp. (25).

Though *Impatiens* biological traits of good adaption to local conditions are associated with the ability to naturalize in new territories, however allelopathic potential might contribute to their spread beyond the natural range. This study aimed to assess the total phenols content of both *I. parviflora* and *I. glandulifera* and evaluate their allelopathic impact on seed germination and seedling growth *ex situ* in spring wheat (monocot) and winter rapeseed (dicot).

MATERIALS AND METHODS

I. Site and plant material

Lithuania lies in Centre of Europe on Baltic Sea and with temperate climate and 660 mm precipitation, 17°C and 4°C summer and winter mean temperature respectively (15). A plant data set was pre-selected to screen the central part of Lithuania, near busy traffic highway Via Baltica, an invasion corridor. The plants data set comprised the *I. parviflora* (54°54'01.3"N 23°50'07.2"E) and *I. glandulifera* (54°54'12.2"N 23°51'29.5"E) as an invasive species of EPPO list (2006) also present in Lithuania. These species are enlisted in the Lithuanian National list of exterminate plants (14).

I parv is a herb that form large pure populations and widely spread by seeds in European forests. It was identified first time in Lithuania in 1934. It prefers shaded and moist nutrient-rich soils of neutral-acidic conditions. It thrives in beech and alder forests, but can also be found in parks, along hedgerows, on forest edges, in waste grounds and ruderal habitats. Seeds of *I parv* were unpicked and TPC was unmeasured due to its autochory spread.

I glan was introduced as ornamental plant in end of 19th century and become highly invasive in Central and Western Europe due to high seed germination, seedling emergence and frost resistance (23). Its spread can be attributed to autochory and accidental zoochory or anthropochory (16). This species was found along banks of Nemunas and Marvele rivers.

Allelopathic activity of these two invasive *Impatiens* species (*Balsaminaceae*), namely *I. parviflora* and *I. glandulifera* was assessed *ex situ* in 2016.

II. Germination bioassay

Fast germinating and high germination energy winter oil rapeseed (*Brassica napus* L., Dicot) cv. *Cult* and spring wheat (*Triticum aestivum* L., Monocot) cv. *Hamlet* were chosen as recipient plants. One hundred seeds were placed on filter paper in each Petri dish (6-cm dia). Five ml aqueous extracts prepared from air-dried and grinded biomass of *Impatiens* spp. (concentrations 0, 0.02, 0.05, 0.1 and 0.2 % w/v) were put in Petri dish as per treatments. Treatments were replicated four times. Petri dishes were kept at 26 °C for 16 h. Seeds sown in distilled water served as control. Seed was considered germinated, when >1cm radicle emerged from the seed coat. The germination was recorded as seed germination >50% (G_{50}) in distilled water (control). Thereafter the G_{50} rate was equated to 100% (19). This method enabled us to assess not only the inhibitory, but also the stimulatory effects of extracts.

III. Phenolic compounds

Total phenolics content (TPC) was determined in extract samples using Singleton and Rossi's (1965) colorimetric method, which is based on colorimetric reaction and direct measurement of photo absorption in the ultraviolet (22). In determining the TPC, the standard curve with chlorogenic acid (Sigma, Aldrich, Germany) was used. One ml of extract was mixed with 45 ml distilled water. One ml of Folin-Ciocalteu reagent (Merck, Darmstadt, Germany) was added and mixed thoroughly. After 3 min 3 ml of Na_2CO_3 was added then the mixture was allowed to stand for 2 h. The absorbance was measured at 760 nm. Samples were analysed in two replications. Identification and quantification of individual target polyphenolic compounds was carried out by UV-V is spectrophotometry (Bechman DU-40, Germany). To evaluate the effects of selected chemicals, a standard equivalent of the total phenolics content in *Impatiens* spp. was estimated via the standard curve of chlorogenic acid. Equivalent value was calculated by multiplying the absorbance of each sample by a single value of equivalent chemical weight per absorbance unit determined under the same condition. In crude extracts of each fraction, TPC of *Impatiens* spp. was expressed as a fresh weight basis in mg per g chlorogenic acid equivalent (CAE).

IV. Statistical analysis

Significant differences among the means were determined using Kruskal-Wallis test, KW-H. Correlation coefficient between extracts and seedlings parameters was calculated in order to evaluate their interaction. The results of allelopathic effects were statistically evaluated by using the statistical package STATISTICA. The results regarding phenols concentration, germination, and seedlings parameters are presented as mean ± SE of 4 independent analyses at the $p<0.05$ significance level.

RESULTS AND DISCUSSION

Germination

The aqueous extracts had allelopathic effects on both germination and seedling growth of recipient plant spp. Allelochemicals accumulated in both *Impatience* extracts possibly inhibited the germination of both recipient species (26). Extracts of both *Impatiens* species showed significant phytotoxicity by inhibiting the recipients germination, which was strongly correlated with TPC ($r_{rapeseed}$ =-0.7 and r_{wheat}=-0.7, p=0,00) for *I. glandulifera* and $r_{rapeseed}$= -0.6 and r_{wheat}=-0.9, p=0,00 for *I. parviflora*) (Figs. 1,2, Table 1). However, germination inhibition differed in each tested recipient due to their different structures of the seed coat (Testa). The extracts of both *Impatiens* species caused drastic inhibition in rapeseed germination than in wheat, possibly due to different seed coat anatomy and thus, its permeability (17). The thick, lignified seed coats of wheat grain were little impermeable to extracts, thereby maintaining their ability to reduce the phenols inflow to embryo. Consequently, wheat grains had higher mean germination than rapeseed in extracts of both *Impatiens* species. *I glan* strongly inhibited the rapeseed (mean G_{50}=42.9%) than wheat germination (mean G_{50}=19.9%). The germination results suggested that invasive *Impatiens* species may spread to new territories through inhibitory effects on neighbouring species germination.

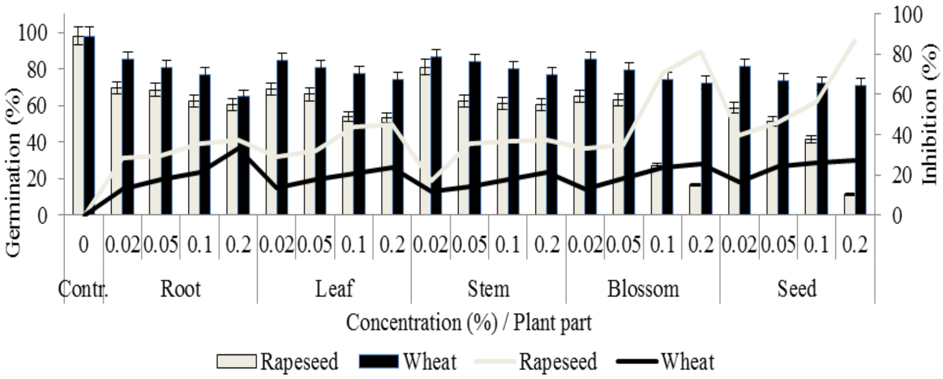

Figure 1. Germination response to *I. glandulifera* extracts (mean±SE). Rapeseed *Cult* and wheat *Hamlet* seeds are recipients.

The comparative *Impatiens* species' research revealed stronger mean inhibition of *I. parviflora* than *I. glandulifera* of recipient rapeseed and wheat germination by 8 and 4%, respectively (Fig. 2). Stronger germination inhibition *ex situ* of *I. parv* than *I. glan* indicated higher allelopathic potential and invasive aggressiveness and thus might be suggested as important ecological constraint that effectively suppresses the regeneration potential of species in natural microhabitats. Moreover, invasive *I parv* thrives in varying environmental conditions (5). However, species evidence for allelopathic effects should not be restricted to *ex situ* assessment, but also based on research in fields in natural environment.

Seedlings growth

I. parv exhibited the stronger inhibitory effects on seedlings morphological parameters than *I. glan* (Figs. 3,4). The highest suppression from *I. parv* extracts was observed on growth of both recipients hypocotyls than on radicles. Mean radicle length of wheat (14.93 and 14.20 mm) and rapeseed (3.92 and 4.4 mm) was greater than hypocotyls length in wheat (3.72 and 4.4 mm) and rapeseed (1.17 and 1.4 mm) in *I. parv* and *I. glan*

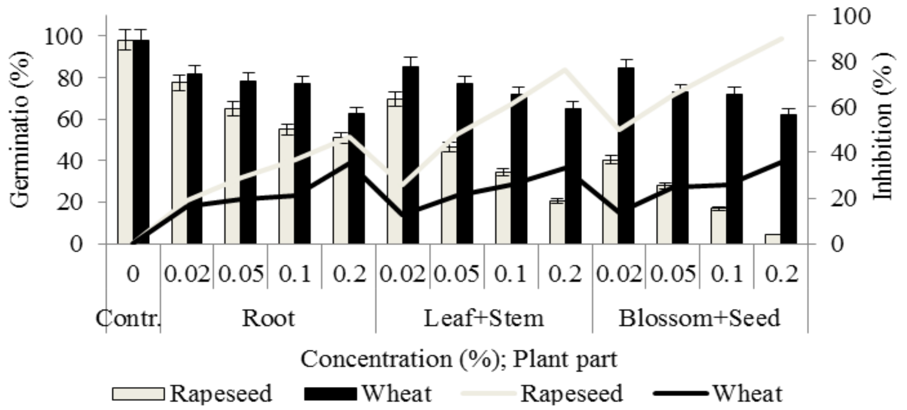

Figure 2. Germination response to *I. parviflora* extracts (mean±SE). Rapeseed *Cult* and wheat *Hamlet* seeds are recipients.

extracts, respectively. Hypocotyl growth was most sensitive variable affected by the both *I. parv* and *I. glan* extracts in the current study. The effects of allelopathy on the germination and growth of plants may occur through various of mechanisms including the reduction of mitotic activity in roots and hypocotyls (19). The allelochemical compounds may be accumulated in the hypocotyl thus its growth probably decreased due to the inhibitory effects of the extract on cell division in plant tissues by hindering the arrangement of microtubule during cell division. Moreover, Rice (20) reported that the allelopathic effects on the germination and growth of plants may occur through many mechanisms [decline of mitotic activity in the roots and hypocotyls, suppression of hormone activity, reduction of the ion uptake rate, inhibition of photosynthesis and respiration, inhibition of protein formation, decreased protein formation or reduced permeability of cell membranes and inhibition of enzyme action]. Thick seed coat of wheat contributed to its considerable buffering capacity for donor extracts thus better

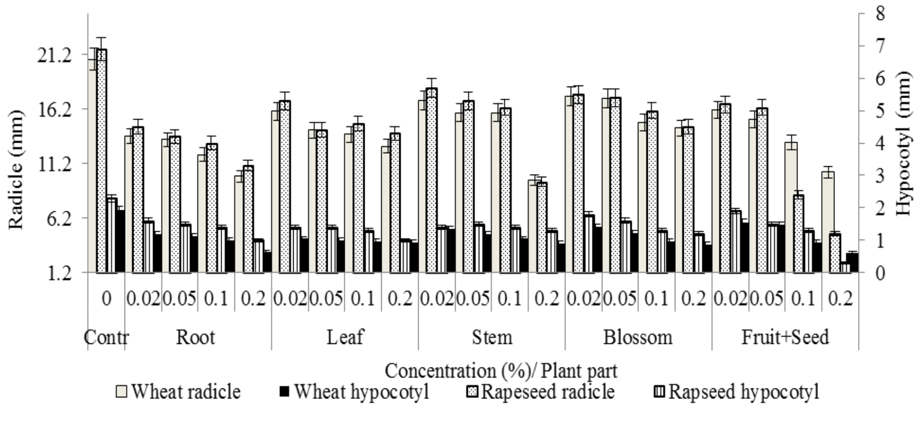

Figure 3. Wheat and rapeseed seedlings' response to *I. glandulifera* extracts (mean±SE)

germination and seedling growth than rapeseed. The radicle emerged first and started to grow, thus its length was longer than hypocotyl in extracts of both donors. The seedling parameters correlated with donor part (p = 0.0260 for W) and extract concentration (p = 0.0094 for R) due to different TPC (Table 1). Significant negative correlation r between the TPC and recipient seedling variables ranged from -0.4 to -0.9 (p = 0.00) and from -0.5 to -0.7 (p = 0.00) in *I. parv* and *I. glan* extracts, respectively. The correlation values confirmed the inhibitory impact of phenolics on recipients seedlings parameters.

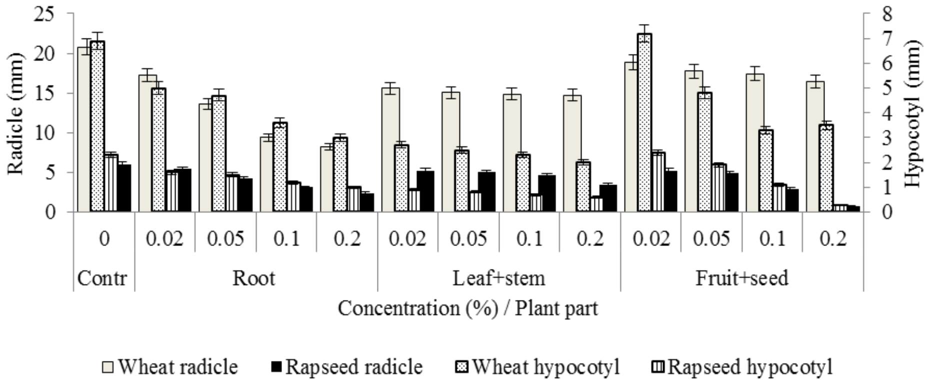

Figure 4. Wheat and rapeseed seedling' response to *I. parviflora* extracts (mean ±SE)

Total phenolics content (TPC)

Though it is referred about high content polyphenols (12,23,26), especially flavonoids in *Impatience* species, nonetheless low TPC (0.320 - 7.567 mg g^{-1}) was recorded in assessed extracts, which was also tested on species, concentration (r=0.9) and plant part (r=0.2) (Fig. 5, Table 1). Low TPC might be related to favourable conditions, as lacking pests in invaded habitats since these secondary metabolites generally are produced in response to environmental factors (light, temperature and pollution) or damages in plants (10,23).

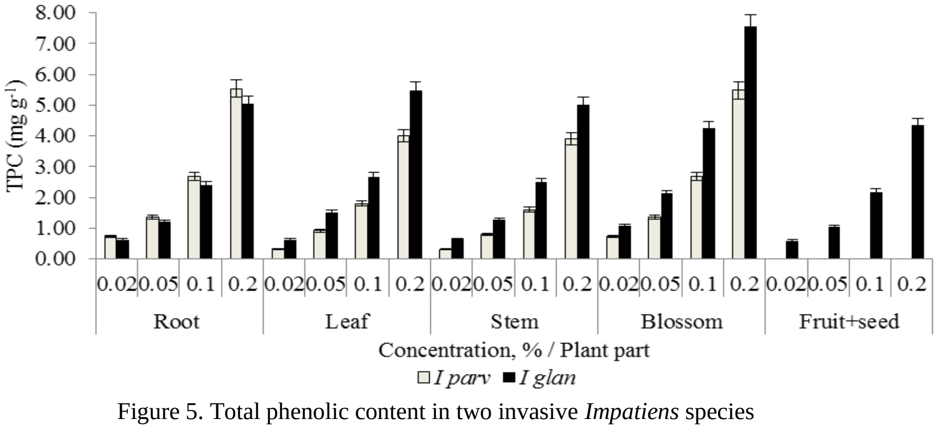

Figure 5. Total phenolic content in two invasive *Impatiens* species

The quantitative analyse of phenolic presence in extracts revealed significantly higher yield (p<0.03) of mean TPC in *I glan* (2.60 mg g^{-1}) than in *I parv* (2.30 mg g^{-1}). This result was supported by results of Szewczyk and Olech (24) who reported that different phenolic acids prevail in separate *Impatiens* species and their parts. They indicated that the greatest quantities and most abundant characteristic compounds for *Impatiens* species are protocatechuic, 4-hydroxybenzoic, vanillic, trans-pcoumaric, trans-ferulic and 3-hydroxycinnamic acids.

Table 1. Kruskal-Wallis and ANOVA tests for differences in the effects of *I. parvflora* and *I. glandulifera* on recipient parameters across different plant parts and concentrations of donor extracts.

Parameter	*I. parvflora*		*I. glandulifera*	
	Plant part	Conc. (%)	Plant part	Conc. (%)
TPC (mg g-^{1})	F(3;9) = 0,5692; p = 0,6491; KW-H(3;13) = 3; p = 0,3916	F(4;8) = 34,6035; p = 0,00004; KW-H(4;13) = 11,5679; p = 0,0209	F(5;15) = 0,5907; p = 0,7074; KW-H(5;21) = 3,8629; p = 0,569	F(4;16) = 30,2191; p = 0,00000; KW-H(4;21) = 18,7238; p = 0,0009
Wheat germination (%)	F(3;9) = 2,3112; p = 0,1447; KW-H(3;13) = 2,9088; p = 0,4059	F(4;8) = 50,5099; p = 0,00001; KW-H(4;13) = 11,0387; p = 0,0261	F(5;15) = 3,0496; p = 0,0427; KW-H(5;21) = 6,1751; p = 0,2895	F(4;16) = 16,7609; p = 0,00001; KW-H(4;21) = 15,5521; p = 0,0037
Rapseed germination (%)	F(3;9) = 7,4223; p = 0,0083; KW-H(3;13) = 8,1429; p = 0,0431	F(4;8) = 3,1239; p = 0,0798; KW-H(4;13) = 6,1099; p = 0,1911	F(5;15) = 3,4216; p = 0,0292; KW-H(5;21) = 9,5059; p = 0,0905	F(4;16) = 4,51; p = 0,0125; KW-H(4;21) = 11,7061; p = 0,0197
Wheat radicle (mm)	F(3;9) = 5,1046; p = 0,0247; KW-H(3;13) = 8,5385; p = 0,0361	F(4;8) = 1,4437; p = 0,3045; KW-H(4;13) = 5,2308; p = 0,2644	F(5;15) = 2,8721; p = 0,0516; KW-H(5;21) = 8,512; p = 0,1302	F(4;16) = 9,2465; p = 0,0005; KW-H(4;21) = 12,0988; p = 0,0166
Wheat hypocotyl (mm)	F(3;9) = 5,0026; p = 0,0260; KW-H(3;13) = 8,456; p = 0,0375	F(4;8) = 2,2937; p = 0,1477; KW-H(4;13) = 4,7033; p = 0,3191	F(5;15) = 2,1855; p = 0,1105; KW-H(5;21) = 3,4951; p = 0,6241	F(4;16) = 15,0065; p = 0,00003; KW-H(4;21) = 16,7166; p = 0,0022
Rapseed radicle (mm)	F(3;9) = 0,9915; p = 0,4397; KW-H(3;13) = 3,3471; p = 0,3411	F(4;8) = 7,1478; p = 0,0094; KW-H(4;13) = 10,4463; p = 0,0335	F(5;15) = 1,9952; p = 0,1378; KW-H(5;21) = 8,0026; p = 0,1561	F(4;16) = 5,095; p = 0,0077; KW-H(4;21) = 11,6409; p = 0,0202
Rapseed hypocotyl (mm)	F(3;9) = 2,3865; p = 0,1367; KW-H(3;13) = 5,5549; p = 0,1354	F(4;8) = 2,7553; p = 0,1038; KW-H(4;13) = 6,2857; p = 0,1788	F(5;15) = 1,5173; p = 0,2431; KW-H(5;21) = 3,8863; p = 0,5659	F(4;16) = 9,8028; p = 0,0003; KW-H(4;21) = 16,2713; p = 0,0027

Notes: F; ANOVA test. KW-H ;Kruskal-Wallis test. Recipient: W- wheat and R- rapeseed

The recorded TPC was different and related on plant part and extract concentration. Significantly the highest (7.567 mg g^{-1}) and the lowest TPC (0.586 mg g^{-1}) accumulated *I glan* in 0.2% blossom and 0.02% fruit + seed extracts, respectively. Szewczyk and Olech (24) reported that the roots and flowers of *I glan* accumulated the highest amounts of phenolic acids. Root, leaf and stem of *I glan* accumulated the similar TPC. *I parv* accumulated lowest TPC (0.320 mg g^{-1}) in leaf 0.02% extract than in other tested plant parts.

The quantitative analyse of phenolic occurrence in extracts revealed that both invasive donor species contains phenolics that might be phytotoxic to recipient germination and supress seedlings growth.

CONCLUSIONS

The allelopathic research has shown that the allelochemicals activity is potential ecological factor driving the plant invasions. The previous studies had indicated that *Impatiens* spp. accumulate numerous allelochemicals (23,25), but our findings revealed that both these test invasive species contains phenolics that are phytotoxic, inhibitory to germination and suppressed the seedlings growth of recipient. Thus phytotxicity of *I. parviflora* and *I. glandulifera* might impact the germination and regeneration or recruitment of neighbouring species in microhabitats. The revealed allelochemical phytotoxicity *ex situ* of *Impatiens* spp. should be considered as partial explanation of their high aggressiveness, and thus may help to improve our knowledge of species invasiveness. However, species allelopathic evidence should not be restricted to assessment in lab, but also must be based on research in fields in natural environment.

ACKNOWLEDGEMENTS

Author would like to thank the Insitute of Ecology and Environment for the lab space and for technical support and assistance.

REFERENCES

1. Acharya, K.P., de Frenne, P., Brunet, J., Chabrerie, O. and Graae, B.J. (2017). Latitudinal variation of life-history traits of an exotic and a native impatiens species in Europe. *Acta Oecologica* **81**:40-47.
2. Callaway, R.M. and Ridenour, W.M. (2004). Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers of Ecology and Environment* **2**:436-443.
3. Chytry, M., Pysek, P., Wild, J., Pino, J., Maskell, L.C. and Vilà, M. (2009). European map of alien plant invasions based on the quantitative assessment across habitats. *Diversity and Distributions* **15**: 98-107.
4. Čuda, J., Skálová, H., Janovský, Z., and Pyšek, P. (2016). Juvenile biological traits of *Impatiens* species are more strongly associated with naturalization in temperate climate than their adult traits. *Perspectives in Plant Ecology, Evolution and Systematics* **20**: 1-10.
5. Elemans, M. (2004). Light, nutrients and the growth of herbaceous forest species. *Acta Oecologica* **26**: 197-202.
6. EU. (2011). *The EU Biodiversity Strategy to 2020*. Strasbourg, France. http://ec.europa.eu/environment /nature/biodiversity/comm2006/2020.htm
7. EU. (2014). Regulation No 1143/2014 on Invasive Alien Species. *Official Journal of the European Union* **1143:** 317-335.
8. Hulme, P.E. (2007). Biological invasions in Europe: Drivers, pressures, states, impacts and responses. In: *Biodiversity under Threat* (Eds., R. Hester, R. M. Harrison), pp. 56-78. Royal Society of Chemistry, Cambridge, UK.
9. Janssens, S.B., Knox, E.B., Huysmans, S., Smets, E.F. and Merckx V.S.F.T. (2009). Rapid radiation of *Impatiens* (Balsaminaceae) during Pliocene and Pleistocene: Result of a global climate change. *Molecular Phylogenetics and Evolution* **52**: 806-824.
10. Jarcuskasca, B., Slezák, M., Hrivnákc, R., and Senko, D. (2016). Invasibility of alien *Impatiens parviflora* in temperate forest understories. *Flora* **224**: 14-23.

11. Jason, G.R., Dicke, M. and Hartley, S.E. (2012). *The Ecology of Plant Secondary Metabolities. From Genes to Global Process*. University press, Cambridge, United Kingdom. 335 pp.
12. Li, A.N., Li, S., Li, H.B., Xu, D.P., Xu, X.R. and Chen, F. (2014) . Total phenolic contents and antioxidant capacities of 51 edible and wild flowers. *Journal of Functional Foods* **6:** 319-330.
13. Li, Z., Wang, Q., Ruan, X., Pan, C.D. and Jiang, D.A. (2010). Phenolics and plant allelopathy. *Molecules* **15**: 8933-8952.
14. *List of Invasive Species in Lithuania*. (2016). D1-726/2012-09-10.
15. *Lithuanian Climate.* (2007). (Eds, A. Galvonaitė, M. Misiūnienė, D. Valiukas, M. S. Buitkuvienė). Lithuanian Hydrometeorology Service, Vilnius, Lithuania. 180 pp.
16. Malíková, L. and Prach, K. (2010). Spread of alien *Impatiens glandulifera* along rivers invaded at different times. *Ecohydrology and Hydrobiology* **10**: 81-85.
17. Moise, J.A., Han, S., Gudynaite-Savitch, L., Johnson, D.A. and Miki, B.L.A. (2005). Seed coats: Structure, development, composition and biotechnology. *In vitro* cellular deviation biology. *Planta* **41**: 620-644.
18. Nentwig, W. (Ed.) (2007). *Biological Invasions*. Springer-Verlag, Berlin-Heidelberg, Germany. 444 pp.
19. Politycka, B. (2007). The effects of allelopathy on the germination and growth of plants may occur through a variety of mechanisms including the reduction of mitotic activity in roots and hypocotyls. *Plant Physiology* **5**: 59-69.
20. Rice, E.L. (1974). *Allelopathy*. Academic Press, New York, USA.
21. Ridenour, W.M. and Callaway, R.M. (2011). The relative importance of allelopathy in interference: The effects of an invasive weed on a native bunchgrass. *Oecologia* **126**: 444-450.
22. Singleton, V.L. and Rossi, J.A.Jr. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Viticulture* **16**:144-158.
23. Skálová, H., Moravcová, L. and Pyšek, P. (2011). Germination dynamics and seedling frost resistance of invasive and native *Impatiens* species reflect local climatic conditions. *Perspectives in Plant Ecology, Evolution and Systematics* **13**: 173-180.
24. Szewczyk. K. and Olech, M. (2017). Optimization of extraction method for LC-MS based determination of phenolic acid profiles in different *Impatiens* species. *Phytochemistry Letters* **20:** 322-330.
25. Theoharides, K.A. and Dukes, J.S. (2007). Plant invasion across space and time: Factors affecting non-indigenous species success during four stages of invasion. *New Phytologist* **176:** 256-273.
26. Vrchotová, N., Será, B. and Krejcová, J. (2011). Allelopathic activity of extracts from *Impatiens* species. *Plant, Soil and Environment* **57**: 57-60.
27. Weber, E. (2003). *Invasive Plant Species of the World. A Reference Guide to Environmental Weeds*. CABI Publishing, Oxon, UK.