

LOBELINE PRODUCTION OF IN VITRO PROPAGATED INDIAN TOBACCO (*LOBELIA INFLATA* L.) IN 2011-2012

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Abstract

Lobelia inflata L. is a medicinally important species of the *Lobeliaceae* family. It is native to North America and contains numerous piperidine alkaloids. The main alkaloid lobeline is used as a respiratory stimulant. The aim of our studies was to examine lobeline production of *L. inflata* in open field conditions. The nutrients were applied in the following methods and quantities in 2011 and in 2012: untreated (control), 50 kg/ha Nitrogen-, 100 kg/ha Nitrogen ground fertilizer, 50 kg/ha Mg- and 100 kg/ha Mg ground fertilizers. The experimental design was randomized blocks with 4 repetitions.

Keywords: *Lobelia inflata*, lobeline, in vitro, open field, production

1. INTRODUCTION

Lobelia inflata L. is a medicinally important species of the *Lobeliaceae* family. It is a native North American species that seems to be a useful medicinal plant to be introduced in Hungary. It is mainly an annual plant (Kelly 1992), but biennial populations can be found, too. The herb contains several piperidine skeleton alkaloids (Felpin & Lebreton 2004; Kursinszki, Ludányi & Szőke 2008). The main alkaloid lobeline is used as a respiratory stimulant. Since lobeline can act as a competitive nicotinic receptor antagonist, it is frequently used in antismoking preparations (Glover et al. 2010). Recently, significant amounts of polyacetylene compounds have been isolated from above ground organs of the plant (lobetylol, lobetolin and lobetyolin) (Bálványos 2002, Felpin & Lebreton 2004).

Recently, the lobeline has come into the limelight due to research on central nervous system (CNS), drug abuse and multidrug resistance (MDR) (Ma & Wink 2008; Beckmann et al. 2010). Dwoskin and Crooks (2002) described a novel mechanism of action and potential use of lobeline as a treatment for psychostimulant abuse.

It is important to increase the biomass and lobeline content of the plant by nitrogen and magnesium treatments in vitro (Bálványos 2002; Szőke & Máthé 2007; Takács-Hájos et al. 2007) and in open field (Vojnich, Máthé & Szőke 2012; Vojnich et al. 2012; Vojnich et al. 2013; Vojnich 2014). There was a favourable effect of NH_4^+ and NO_3^- on the biomass formation of in vitro cultures (Breteler & Siegerist 1984, Taya et al. 1989, Kino-Oka, Taya & Tone 1993) and aquatic cultures (Murray, Dennison & Kemp 1992; van der Eerden 1982). Britto and Kronzucker (2002) described the inhibitory effect of ammonia on growth in open field conditions.

Several previous experiments examined the influence of macroelements on growth and alkaloid production of hairy roots (Bálványos, Szőke & Kursinszki 1998; Bálványos, Szőke & Kursinszki 2003).

The aim of this research was to examine the effect of MgSO_4 and NH_4NO_3 fertilisation on biomass and on lobeline content of *in vitro* propagated *L. inflata*.

2. MATERIALS AND METHODS

The open field trials were carried out in 2011 and 2012 in Mosonmagyaróvár (University of West-Hungary). Nitrogen and Magnesium were applied in the form of ground fertilizers. The nutrients were applied in the following methods and quantities in 2011: untreated (control), 50 kg/ha N-, 100 kg/ha Nitrogen ground fertilizer, 50 kg/ha Magnesium- and 100 Mg ground fertilizers. The nutrients were

applied in the following methods and quantities in 2012: untreated (control), 50 kg/ha N-, and 100 kg/ha Nitrogen ground fertilizers, 50 kg/ha Mg- and 100 kg/ha Magnesium ground fertilizers. Soil analytical values in 2011: pH 7.12; humus 3.08 m/m%; Mg 310 mg/kg; NO₂-NO₃-N 20.1 mg/kg, K₂O 518 mg/kg, P₂O₅ 358 mg/kg. Soil analytical values in 2012: pH 7.38; 1.70 humus m/m%; Mg 177 mg/kg; NO₂-NO₃-N 12.3 mg/kg, K₂O 587 mg/kg, P₂O₅ 344 mg/kg. Table 1 and Table 2 summarize the specification of soil values. An extended soil analysis was carried out according to standard methods of UIS Ungarn laboratory (Hungary, Mosonmagyaróvár).

Appellation	Measure	Values
pH _{KCl}	-	7.12
salt%	m/m%	0.02
humus%	m/m%	3.08
CaCO ₃ %	m/m%	10.7
P ₂ O ₅	mg/kg	358
K ₂ O	mg/kg	518
Na	mg/kg	54.3
Mg	mg/kg	310
NO ₂ -NO ₃ -N	mg/kg	20.1
SO ₄	mg/kg	8.75
Cu	mg/kg	4.21
Mn	mg/kg	20.4
Zn	mg/kg	18.5

Table 1. Specification of soil values (2011)

Appellation	Measure	Values
pH _{KCl}	-	7.38
salt%	m/m%	0.02
humus%	m/m%	1.70
CaCO ₃ %	m/m%	18.6
P ₂ O ₅	mg/kg	344
K ₂ O	mg/kg	587
Na	mg/kg	72.8
Mg	mg/kg	177
NO ₂ -NO ₃ -N	mg/kg	12.3
SO ₄	mg/kg	25.0
Cu	mg/kg	3.30
Mn	mg/kg	17.1
Zn	mg/kg	5.09

Table 2. Specification of soil values (2012)

In the open field trials, Mg (2%) - and N (34%) fertilizers were spread onto the soil surface, one day prior to transplanting. Transplanting of *in vitro* *Lobelia inflata* plants into open field soil was carried out on 26th May 2011 and 4th June 2012. The number of plants per plot was 40. The experimental design was randomized blocks with 4 repetitions. During cultivation, mechanical weed control was applied. Plant heights (cm) were measured three times (22nd July, 29th July and 7th August) in 2011 and four times (31st July, 15th August, 22nd August and 30th August) in 2012. In each treatment group 8 plants were measured both in 2011 and in 2012 (dry biomass production, g/plant of *L. inflata* herb). The first harvesting was on 9-10th August 2011, and on 30th August 2012. During harvesting, the plants were flowering and the biomasses were recorded. After harvesting, the plants were dried in a shaded and well-ventilated glasshouse. The dry weight determination was carried out in early September. The flowering phenophase was observed in the period of July to September (Krochmal, Wilken & Chien 1970). The total alkaloid content was determined by a spectrophotometric method elaborated by Mahmoud and El-Masry (1980) and modified by Krajewska (1986). The statistical analysis was preformed with SPSS v19 software.

Alkaloid Extraction

The herb or roots of *L. inflata* (0.5000 g), dried and powdered, were extracted with 1 × 20 ml and 2 × 10 ml of 0.1 N HCl-methanol (1:1, v/v) by sonication (Braun Labsonic U, Melsungen, Germany) for 3 × 10 min. After centrifugation (6,000 rpm for 10 min, 2,500 g) and filtration, the methanol was evaporated and the remaining aqueous phase was diluted to a stock solution (to 25.00 ml) with 0.1 N HCl. Samples of this solution were purified with solid-phase extraction (SPE).

Quantitative determination of lobeline by HPLC

Determination of lobeline was performed with the external standard method. Standard solutions containing lobeline at 2.25, 5, 12.5, 20, 40, and 80 µg/ml were prepared in 0.1 N HCl. Each standard solution was injected (5 µl) to the LC column and the absorbance was recorded at 250 nm. Three repetitions were made with each solution. The calibration graph for lobeline was constructed by plotting the peak areas against the corresponding concentrations. The concentration of lobeline in samples was calculated from their peak areas using the calibration plot. Validation studies proved that both repeatability of the method and recovery were good (Kursinszki, Ludányi & Szőke 2008). The amounts of lobeline derivatives, i.e. norlobeline, norlobelanine and lobelidine were expressed in lobeline.

HPLC-MS/MS experiments

Analysis was performed in positive ion mode on an Agilent 6410 Triple Quad LC/MS system using electrospray ionization. Chromatographic conditions were the same as described earlier, except that 30 mM ammonium formate (pH 2.80) was used instead of 0.1% trifluoroacetic acid. The injection volume was 10 µl. By solvent splitting, 40 % eluent was allowed to flow into the mass spectrometer. The conditions of the LC-MS/MS analysis were as follows: nebulizer pressure 45.0 psi, drying gas flow rate 9 l/min, drying gas temperature 350 °C, capillary voltage 3500 V, scan range from m/z 50 to 700 at collision energy of 15 or 20 eV depending on molecular structure.

The lobeline content was determined by HPLC method designed by Yonemitsu et al. (1990) and modified by Bálványos, Kursinszki & Szőke (2001) and Kursinszki & Szőke (2015).

3. RESULTS AND DISCUSSION

Literature references on the mineral nutrition of *L. inflata* are scarce, although it is one of the basic factors of successful cultivation of this species. Our experiments were aimed at clearing the basic nutrient requirements. It could be established that in the form of ground fertilization, both nitrogen and magnesium had a favourable effect on the formation of biomass and lobeline content in open field conditions. Some information is available from *in vitro* hairy root experiments carried out by Bálványos (2002), according to whom the various nutrients he tried (Mg, Ca, Na, N), Mg had proved to be most effective in increasing both the dry biomass and lobeline content.

Treatments		Height of the plants (cm)		
		22 nd July	29 th July	7 th August
Control	Mean	24.75	33.75	40.25
	Number	8	8	8
	St. deviation	6.99	6.52	5.70
	Minimum	11	20	27
	Maximum	32	41	45
50 kg/ha N ground fertilizer	Mean	30.38	38.63	47.75
	Number	8	8	8
	St. deviation	12.59	11.02	7.50
	Minimum	14	23	36
	Maximum	50	56	60
100 kg/ha N ground fertilizer	Mean	29.25	37.88	45.38
	Number	8	8	8
	St. deviation	6.36	6.33	5.15
	Minimum	19	28	37
	Maximum	39	47	54
50 kg/ha Mg ground fertilizer	Mean	30.75	38.38	43.00
	Number	8	8	8
	St. deviation	6.16	6.05	6.97
	Minimum	22	29	33
	Maximum	39	47	54
100 kg/ha Mg ground fertilizer	Mean	26.25	36.50	45.13
	Number	8	8	8
	St. deviation	8.68	7.54	5.84
	Minimum	14	25	36
	Maximum	41	50	56

Table 3. Plant height (cm) of in vitro propagated *Lobelia inflata* in 2011

Table 3 and Table 4 summarize the effect of fertilizers on plant growth in 2011. As expected and shown by the analysis of variance, as well as Tukey test, the growth parameters show significantly different values for plant height.

Date of measurements	Treatments (A)	Treatments (B)	Mean Difference (A-B)	St. Error	Significance Level
22 nd July	Control	50 kg/ha N	-5.625	4.249	0.679 n.s.
		100 kg/ha N	-6.000	4.249	0.624 n.s.
		50 kg/ha Mg	-4.500	4.249	0.826 n.s.
		100 kg/ha Mg	-1.500	4.249	0.997 n.s.
29 th July	Control	50 kg/ha N	-4.875	3.856	0.714 n.s.
		100 kg/ha N	-4.625	3.856	0.752 n.s.
		50 kg/ha Mg	-4.125	3.856	0.821 n.s.
		100 kg/ha Mg	-2.750	3.856	0.952 n.s.
7 th August	Control	50 kg/ha N	-7.500	3.146	0.144 n.s.
		100 kg/ha N	-2.750	3.146	0.904 n.s.
		50 kg/ha Mg	-5.125	3.146	0.490 n.s.
		100 kg/ha Mg	-4.875	3.146	0.538 n.s.

* The mean difference is significant at the 0.1 level.

n.s. = not significant

Table 4. Tukey HSD test of Indian tobacco (*Lobelia inflata*). Values of the four treatments were measured three times in 2011. (Parameter: plant height).

Treatments		Height of the plants (cm)			
		31 st July	15 th August	22 nd August	30 th August
Control	Mean	12.50	35.50	40.63	42.00
	Number	8	8	8	8
	St. deviation	6.26	12.52	10.65	10.47
	Minimum	3	8	16	18
	Maximum	21	47	49	49
50 kg/ha N ground fertilizer	Mean	16.13	38.25	43.13	45.62
	Number	8	8	8	8
	St. deviation	9.06	11.98	10.32	8.80
	Minimum	4	17	27	34
	Maximum	30	56	60	60
100 kg/ha N ground fertilizer	Mean	17.88	40.75	46.00	47.88
	Number	8	8	8	8
	St. deviation	5.92	7.59	7.69	7.57

	Minimum	11	30	36	37
	Maximum	27	53	59	59
50 kg/ha Mg ground fertilizer	Mean	16.00	33.75	36.25	37.88
	Number	8	8	8	8
	St. deviation	10.09	18.63	19.72	19.51
	Minimum	2	3	3	4
	Maximum	27	50	52	53
100 kg/ha Mg ground fertilizer	Mean	20.63	41.00	43.75	44.25
	Number	8	8	8	8
	St. deviation	6.82	8.44	8.23	8.28
	Minimum	9	24	27	27
	Maximum	28	49	50	51

Table 5. Plant height (cm) of in vitro propagated *Lobelia inflata* in 2012

Table 5 and Table 6 illustrate the effect of fertilizers on plant growth in 2011. As expected and shown by the analysis of variance, as well as Tukey test, the growth parameters show significantly different values for plant height.

Date of measurements	Treatments (A)	Treatments (B)	Mean Difference (A-B)	St. Error	Significance Level
31 st July	Control	50 kg/ha N	-3.625	5.005	0.990 n.s.
		100 kg/ha N	-5.375	5.005	0.933 n.s.
		50 kg/ha Mg	-3.500	5.005	0.992 n.s.
		100 kg/ha Mg	-8.125	5.005	0.668 n.s.
15 th August	Control	50 kg/ha N	-2.750	6.973	1.000 n.s.
		100 kg/ha N	-5.250	6.973	0.988 n.s.
		50 kg/ha Mg	1.750	6.973	1.000 n.s.
		100 kg/ha Mg	-5.500	6.973	0.985 n.s.
22 nd August	Control	50 kg/ha N	-2.500	6.756	1.000 n.s.
		100 kg/ha N	-5.375	6.756	0.984 n.s.
		50 kg/ha Mg	4.375	6.756	0.995 n.s.
		100 kg/ha Mg	-3.125	6.756	0.999 n.s.
30 th August	Control	50 kg/ha N	-3.625	6.368	0.997 n.s.
		100 kg/ha N	-5.875	6.368	0.967 n.s.
		50 kg/ha Mg	4.125	6.368	0.995 n.s.
		100 kg/ha Mg	-2.250	6.368	1.000 n.s.

* The mean difference is significant at the 0.1 level.

n.s. = not significant

Table 6. Tukey HSD test of Indian tobacco (*Lobelia inflata*). Values of the four treatments were measured four times in 2012. (Parameter: plant height).

Figure 1 illustrates the dry biomass values recorded for herbs during growing seasons in 2011 and 2012. The highest values 11.2 g/plant (in 2011) and 11 g/plant (in 2012), were in the 100 kg/ha Magnesium treatment groups. The control values were 2.9 g/plant (2011) and 2.7 g/plant (2012). The 50 kg/ha Mg fertilizer values was 9.1 g/plant in 2011.

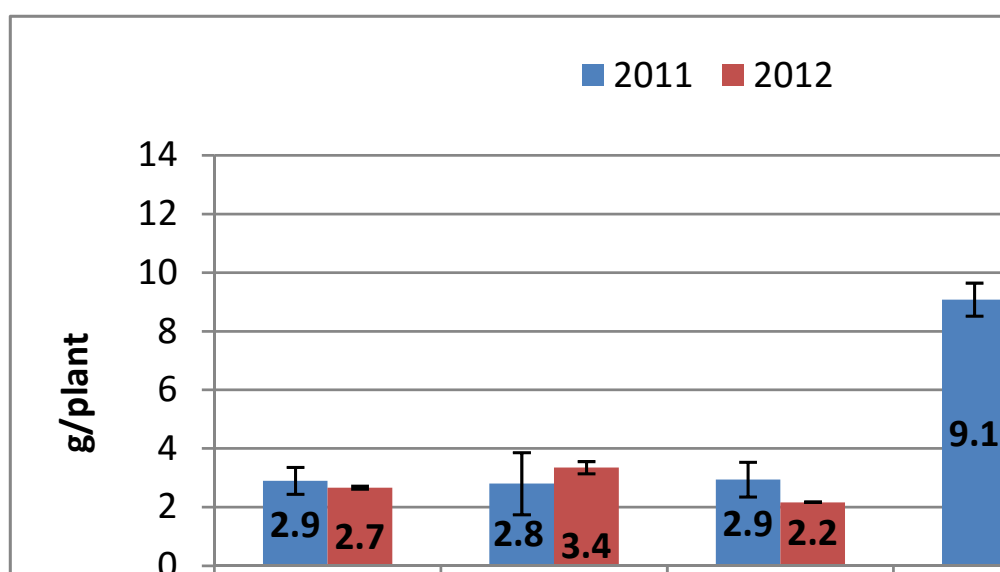


Fig. 1. Dry biomass production (g/plant) of *L. inflata* herb (2011-2012)

Figure 2 shows lobeline content of herbs in 2011 and 2012. The herb was also favourably influenced by the Magnesium fertilization. In 2011, the lowest values were recorded in the 100 kg/ha N ground fertilizer (336 µg/g) group. The highest value was in the 100 kg/ha Mg fertilizer treatment group (635 µg/g). In 2012, the highest lobeline content was measured in the non fertilizer control group (518 µg/g). The lobeline content in the fertilizer treatment groups were between 402 µg/g and 451 µg/g, the highest with the 50 kg/ha Mg ground treatment.

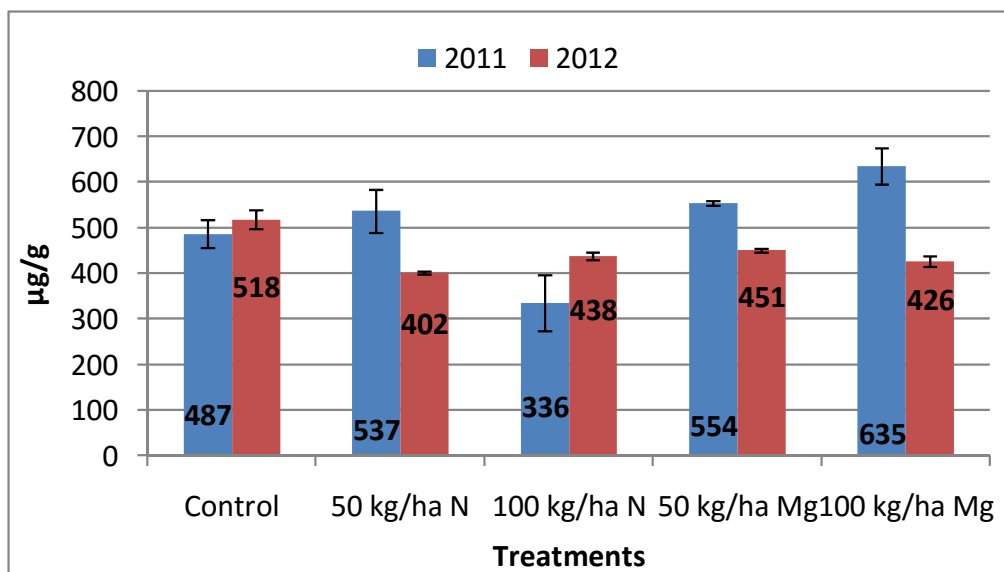


Fig. 2. Lobeline content (µg/g) of *L. inflata* herb (2011-2012)

Figure 3 summarizes the dry biomass values recorded for roots in 2012. The highest values were recorded in the non-fertilized control group (2.4 g). The lowest biomass value was measured in the 50 kg/ha Mg ground fertilizer treatment group (0.8 g).

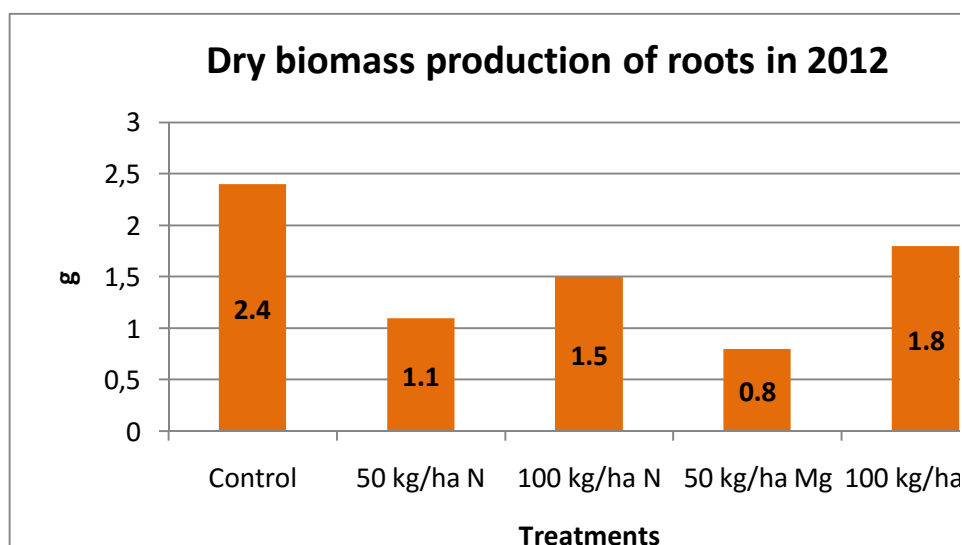


Fig. 3. Dry biomass production (g) of *L. inflata* roots in 2012

The lobeline content of roots was measured in 2012 (Fig. 4). The lowest value was measured in the 100 kg/ha Mg fertilizer group. As a result of fertilization, the highest lobeline content of the roots of *L. inflata* was in the 50 kg/ha N fertilizer group (692 µg/g). The 50 kg/ha Mg fertilizer group value was 636 µg/g.

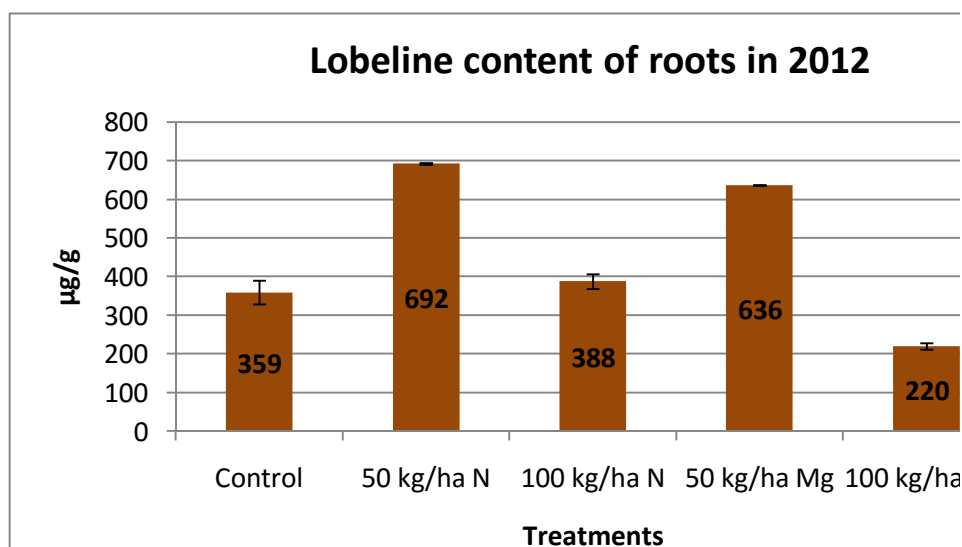


Fig. 4. Lobeline content (µg/g) of *L. inflata* roots in 2012

Table 7 illustrates calculated price for the lobeline content derived from open field cultivated *Lobelia inflata* plants of different treatment groups, based on yield and price in 2012. The *Lobelia* herb selling price was \$5.6 per 100 capsules (42.5 g) in 2012 (<http://www.scientific-publications.net>). 50 kg/ha Mg treatment means a yield of 648 kg of dried plant material per hectare. 1% of total plant material was the lobeline content. The 6.48 kg lobeline content price was \$ 853.8 per hectare.

Treatments	Herb dry mass (g)	Lobeline content (µg/g)	Plant/ m ²	Lobeline content/ha (g)	Lobeline price/ha (\$)
Control	2.7	518	18	251.74	33.17
50 kg/ha N	3.4	402	18	246.02	32.41
100 kg/ha N	2.2	438	18	173.44	22.85
50 kg/ha Mg	3.6	451	18	292.24	38.51
100 kg/ha Mg	11.0	426	18	843.48	111.14

Table 7. Calculated price for lobeline in 2012

4. CONCLUSIONS

The results in 2011 indicate that N and Mg fertilization increased growth (height in cm) to 47.75 cm (in the 50 kg/ha N fertilization group) compared to 40.25 cm of control. The value measured in the 50 kg/ha Mg treatment group was 43.0 cm. The highest dry biomass production (g/plant) of herb was 11.2 g/plant (in the 100 kg/ha Mg treatment group). The 50 kg/ha Mg ground fertilizer group's value was 9.1 g/plant. The control value was equal to the value measured in the 100 kg/ha N treatment group (2.9 g/plant). The highest lobeline content (µg/g) of herb was 635 µg/g (in the 100 kg/ha Mg treatment group). The value of the control was 487 µg/g. The 50 kg/ha Mg treatment resulted in 554 µg/g, and the 50 kg/ha N fertilizer resulted 537 µg/g lobeline.

In 2012, N and Mg fertilization resulted in plant growth (height in cm) between 47.88 cm (with 100 kg/ha N fertilization) and 37.88 cm (with 50kg/ha Mg fertilization). The average height of plants with 100 kg/ha Mg treatment was 44.25 cm. The control value was 42.0 cm. The average above ground dry

biomass production (g/plant) was 3.4 g/plant (in the 50 kg/ha N ground fertilizer treatment group). The 50 kg/ha Mg ground fertilizer resulted in 3.6 g/plant. The highest value was measured with 100 kg/ha Mg treatment (11.0 g/plant). The control plants weighed 2.7 g/plant. The highest lobeline content ($\mu\text{g/g}$) of above ground plant parts was 518 $\mu\text{g/g}$ (in the non-fertilized control), while the 50 kg/ha Mg treatment resulted in 451 $\mu\text{g/g}$. The lowest value was measured in the 50 kg/ha N fertilizer treatment group.

The roots production (biomass and lobeline content) were measured in 2012. Highest values were recorded in the non-fertilized control (2.4 g). Similar tendencies were observed in the 50 kg/ha N treatments and the 50 kg/ha Mg ground fertilizer of roots dry biomass compared to 100 kg/ha N fertilizer and the 100 kg/ha Mg treatments. As a result of fertilization, the highest lobeline content ($\mu\text{g/g}$) of the roots of *L. inflata* was an increased value by 92.7% (50 kg/ha N-treatments) compared to the control values. The lowest value was in the 100 kg/ha Mg fertilizer group (220 $\mu\text{g/g}$).

There were several economy experiments on lobeline content in the 1970s in the United States. 1% of the dry matter content was lobeline. In the 1970s, selling prices ranged from \$0.25 to \$0.80 per pound (1 pound = 453 g), which means that a yield of 1,700 pounds (770 kg) of dried plant material would gross \$425.00 to \$1,360.00 per acre (1 acre = 4,047 m²) (Krochmal, Wilken & Chien 1971).

The conclusions of the experiments is, that open field conditions the MgSO_4^+ and the NH_4NO_3 treatments were successful for the lobeline production of *Lobelia inflata*.

REFERENCES

- Bálványos, I 2002. Studies on the growth and secondary metabolite production of *Lobelia inflata* L. hairy root cultures. PhD theses, Semmelweis University, Budapest
- Bálványos, I, Kursinszki, L & Szőke, É 2001. The effect of plant growth regulators on biomass formation and lobeline production of *Lobelia inflata* L. hairy root cultures. Kluwer Academic Publishers, Printed in the Netherlands, Plant Growth Regulation, 34:339-345.
- Bálványos, I, Szőke, É & Kursinszki, L 1998. Effect of magnesium on the growth and alkaloid production of *Lobelia inflata* L. hairy root cultures. In: Kiss SA (ed) Magnesium and interaction of magnesium with trace elements. Hung Chem Soc, Budapest, pp. 358–361.
- Bálványos, I, Szőke, É & Kursinszki, L 2003. Effect of macroelements on the growth and lobeline production of *Lobelia inflata* L. hairy root cultures. Acta Horticult., 597:245–251.
- Beckmann, JS, Siripurapu, KB, Nickell, JR, Horton, DB, Denehy, ED, Vartak, A, Crooks, PA, Dwoskin, LP & Bardo, MT 2010. The novel pyrrolidine Nor-Lobelane analog UKCP-110 [cis-2,5-di-(2-phenetyl)-pyrrolidine hydrochloride] inhibits VMAT2 function, methamphetamine-evoked dopamine release, and methamphetamine self-administration in rats. J Pharmacol Exp Ther., 335:841–851.
- Breteler, H & Siegerist, M 1984. Effect of ammonium on nitrate utilization by roots of dwarf bean. Plant Physiol., 75:1099-1103.
- Britto, DT & Kronzucker, HJ 2002. NH_4^+ toxicity in higher plants: a critical review. J Plant Physiol., 159:567-584.
- Dwoskin, LP & Crooks, PA 2002. A novel mechanism of action and potential use for lobeline as a treatment for psychostimulant abuse. Biochem Pharmacol., 63:89-98.
- van der Eerden, LJM 1982. Toxicity of ammonia to plants. Agric Environ., 7:223-235.
- Felpin, F.-X & Lebreton, J 2004. History, chemistry and biology of alkaloids from *Lobelia inflata*. Tetrahedron (60) 10127–10153.

- Glover, ED, Rath, JM, Sharma, E, Glover, PN, Laflin, M, Tonnesen, P, Repsher, L & Quiring, J 2010. A Multicenter Phase 3 Trial of Lobeline Sulfate for Smoking Cessation. *Am J Health Behav.*, 34:101-109.
- Kelly, CA 1992. Reproductive phenologies in *Lobelia inflata* (*Lobeliaceae*) and their enviromental control. *American Journal of Botany*, vol. 79, no. 10, pp. 1126-1133.
- Kino-Oka, M, Taya, M & Tone, S 1993. Evaluation of inhibitory effect of ammonium ion on cultures of plant hairy roots. *J Chem Eng Jpn.*, 26:578-580.
- Krajewska, A 1986. The effect of new type of growth regulators on the *Lobelia inflata* L. tissue cultures (in Hungarian). PhD theses, Semmelweis University, Budapest
- Krochmal, A, Wilken, L & Chien, M 1970. Lobeline Content of *Lobelia inflata*: Structural, Environmental and Developmental Effects. U.S.D.A. Forest Service Research Paper NE- 178. Northeastern Forest Experiment Station, Upper Darby, PA. Forest Service, U.S. Department of Agriculture.
- Krochmal, A, Wilken, L & Chien, M 1971. Plant and Lobeline harvest of *Lobelia inflata* L. Respectively Principal Economic Botanist, Southeastern Forest Experimental Station, Forest Service, U.S.D.A., Auburn University, Auburn, Alabama. pp. 216-220.
- Kursinszki, L, Ludányi, K & Szőke, É 2008. LC-DAD and LC-MS-MS Analysis of Piperidine Alkaloids of *Lobelia inflata* L. (In Vitro and In Vivo). *Chromatographia*. (68) 27–33.
- Kursinszki, L & Szőke, É 2015. HPLC-ESI-MS/MS of brain neurotransmitter modulator lobeline and related piperidine alkaloid sin *Lobelia inflata* L. *J Mass Spectrom.*, 50:727-733.
- Ma, Y & Wink, M 2008. Lobeline, a piperidine alkaloid from *Lobelia* can reverse P-gp dependent multidrug resistance in tumor cells. *Phytomedicine*, vol. 15, no. 9, pp. 754-758.
- Mahmoud, ZF & El-Masry, S 1980. Colorimetric determination of lobeline and total alkaloids in *Lobelia* and its preparations. *Sci Pharm.*, 48:365-369.
- Murray, L, Dennison, WC & Kemp, WM 1992. Nitrogen versus phosphorus limitation for growth of an estuarine population of eelgrass (*Zostera marina* L.). *Aquat Bot.*, 44:83-100.
- Szőke, É & Máthé, Á 2007. GVOP 3.1.1.-2004-05-0309/3.0 report of research. NKTH, Budapest
- Takács-Hájos, M, Szabó, L, Rácz, I-né, Máthé, Á & Szőke, É 2007. The effect of Mg-leaf fertilization on Quality parameters of some horticultural species. *Cereal Res Commun.*, 35:1181-1184.
- Taya, M, Kino-Oka, M, Tone, S & Kobayashi, T 1989. A kinetic model of branching growth of plant hairy roots. *J Chem Eng Jpn.*, 22:698-700.
- Vojnich, VJ 2014. Study of the Indian tobacco (*Lobelia inflata* L.) productions, specifically the plant grows in Hungary. PhD theses, University of West-Hungary, Mosonmagyaróvár
- Vojnich, VJ, Máthé, Á & Szőke, É 2012. Comparison of the production of Indian tobacco (*Lobelia inflata* L.) propagated *in vitro* and *in vivo*. 34th Óvári Science Day, Mosonmagyaróvár, pp. 537-542.
- Vojnich, VJ, Máthé, Á, Szőke, É & Gaál, R 2012. Effect of Mg treatment on the production of Indian tobacco (*Lobelia inflata* L.). *Acta Hort.*, 955:125-128.
- Vojnich, VJ, Máthé, Á, Szőke, É, Bányai, P, Kajdi, F & Gaál, R 2013. Effect of nitrogen and magnesium nutrition on Indian tobacco (*Lobelia inflata* L.). *J Cent Eur Agric.*, 14:77-85.
- Yonemitsu, H, Shimomura, K, Satake, M, Mochida, S, Tanaka, M, Endo, T & Kaji, A 1990. Lobeline production by hairy root culture of *Lobelia inflata* L. *Plant Cell Rep.*, 9:307-310.
- http://www.amazon.com/Natures-Way-Lobelia-HerbCapsules/dp/B000I4AIDM/ref=cm_cr_pr_product_top (2012)