LOBELINE PRODUCTION OF IN VITRO LOBELIA INFLATA HERB

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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 has been used as a respiratory stimulant. The aim of our studies was to follow the 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-, 50 kg/ha Mg ground fertilizers and 100 kg/ha Mg ground fertilizer. The experimental design was a randomized blocks with 4 repetitions.*

Key words*: Lobelia inflata, lobeline, in vitro, open field, production*

INTRODUCTION

Lobelia inflata L. is a medicinally important species of the *Lobeliaceae* family [1]. Is a native North American species that seems to be a useful medicinal plant that can be introduced in Hungary. It is mainly an annual plant [2], but biennial populations can be found, too. Lobelia is named after Flemish Botanist Matthias de L'Obel (1538-1616) [3]. The *Lobelia inflata* synthesize important medicinal materials. The herb contains several piperidine skeleton alkaloids [4]. Its main alkaloid is the lobeline that has a stimulating effect on the respiratory centre [5]. Recently, it has been come into light due to a research on CNS, drug abuse and multidrug resistance [6, 7]. To satisfy the market needs, it is important to increase the content values and the biomass of the plant [8, 9], for which a great opportunity arises through the nutrient supply of the plant. The aim of our studies was to compare the lobeline and biomass production of in vitro *L. inflata* in open field conditions.

MATERIALS AND METHODS

The open field trials were carried out in 2011 and 2012 at the Széchenyi István University, Faculty of Agricultural and Food Sciences. N- and Mg- 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, 50 kg/ha Mg- and 100 kg/ha Mg Magnesium ground fertilizer. The nutrients were applied in the following methods and quantities in 2012: untreated (control), 50 kg/ha N-, 100 kg/ha Nitrogen ground fertilizers, 50 kg/ha Mg- and 100 kg/ha Magnesium ground fertilizers. Soil analytical values: pH 7.38; humus 1.70 m/m%; Mg 177 mg/kg; NO_2-NO_3-N 12.3 mg/kg, K_2O 587 mg/kg, P_2O_5 344 mg/kg. An extended soil analysis was carried out according to standard methods of UIS Ungarn laboratory (Hungary, Mosonmagyaróvár).

Experimental plants were propagated in glasshouse controlled by a fully automatic energyumbrella. Table 1 summarises the *Lobelia inflata* date collection (2011-2012).

Table 1

Dates of *Lobelia inflata* data collection (2010–2012)

	2011	2012
***in vitro* seedlings transferred into the glasshouse**	12th April 2011	24th April 2012
transplanting into open field soil	26-27th May 2011.	4-5th June 2012.
dates of measurement (plant height)	22th July 2011. 29th July 2011. 7th August 2011.	31th July 2012. 15th August 2012. 22th August 2012. 30th August 2012.
dates of harvest	9-10th August 2011.	30th August 2012.

Mg (2%) - and N (34%) fertilizers were spread onto the soil surface, one day prior to transplanting. Date of transplanting: 26-27. May in 2011; 4-5. June in 2012. The number of plants per plot was 40. The experimental design was a randomized blocks with 4 repetitions.

Plant height (cm) leaf length and width were tree times in 2011 and four times in 2012. In each treatment 8 plants were measured. The first harvest took place on 9-10th of August on 2011, and 30th August on 2012, when the biomass was recorded. Following harvest, the plants were dried in the greenhouse. The dry weight determination. The flowering phenophase was observed in the period July between September.

Alkaloid Extraction: *Lobelia inflata* L. (1 g), dried and powdered, was extracted with 1x20 ml, and 2x15 ml of 0.1 N HCl-methanol (1:1, v/v) by sonication for 3x10 minute. After centrifugation and filtration the methanol was evaporated off and the remaining aqueous phase was made up to a stock solution with 0.1 N HCl. Samples of this solution were purified by solid-phase extraction (SPE) for the quantitative HPLC (High Performance Liquid Chromatography) determinations.

Alkaloid Extraction

The herb or roots of L. inflata (0.5000 g), dried and powdered, were extracted with 1 × 20, 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 off and the remaining aqueous phase was made up to a stock solution (25.00 mL) with 0.1 N HCl. Samples of this solution were purified by solid-phase extraction (SPE).

The lobeline content was determined by HPLC method [10].

RESEARCH RESULTS

References in the literature on the mineral nutrition of *L. inflata* are scarce, although it is one of the basic factors for the successful production of this species. With the goal of introducing *L. inflata* into cultivation in Hungary, our experiments were aimed at clarifying the basic nutrient requirements. Table 2 and Table 3 summarises the in vitro propagated *Lobelia inflata* height (cm) in 2011 and in 2012. Figure 1 and Figure 2 illustrates the lobeline content (µg/g) of in vitro *L. inflata* in 2011 and in 2012.

Table 2

***In vitro* propagated *Lobelia inflata* height (cm) in 2011**

Treatments		Plant height (cm) in 2011		
		22th July	29th July	7th August
Control	**Mean**	**24.75**	**33.75**	**40.25**
	Number	8	8	8
	St. error	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. error	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. error	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. error	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. error	8.68	7.54	5.84
	Minimum	14	25	36
	Maximum	41	50	56

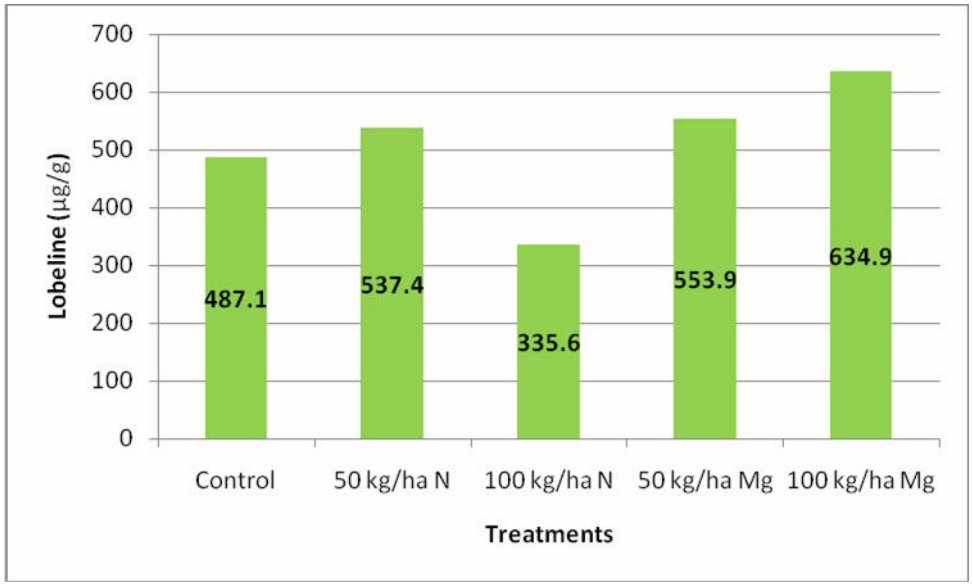

Figure 1. Lobeline content of in vitro *L. inflata* in 2011

Table 3

***In vitro* propagated *Lobelia inflata* height (cm) in 2012**

Treatments		Plant height (cm) in 2012			
		31th July	15th August	22th August	30th August
Control	**Mean**	**12.50**	**35.50**	**40.63**	**42.00**
	Number	8	8	8	8
	St. error	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. error	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. error	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. error	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. error	6.82	8.44	8.23	8.28
	Minimum	9	24	27	27
	Maximum	28	49	50	51

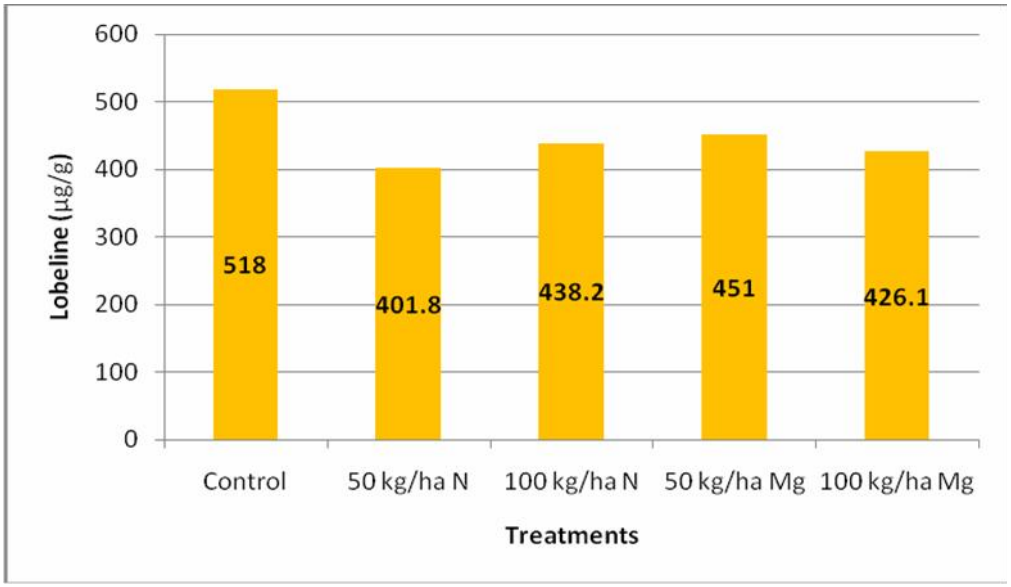

Figure 2. Lobeline content of in vitro *L. inflata* in 2012

CONCLUSIONS

In the field trials of *L. inflata* we have evaluated the favourable effect of fertilization. As a result, in 2011, N- and Mg-fertilization increased the lobeline content (µg/g) of above ground plant parts is 553.9 µg/g (50 kg/ha Mg-treatment), while in the 50 kg/ha N-treatment is 537.4 µg/g. The control was 487.1 µg/g.

The highest lobeline content is 634.9 µg/g (100 kg/ha Mg-treatment). The average above ground plant height is 47.75 cm (50 kg/ha N ground fertilizer treatment). The 50 kg/ha Mg ground fertilizer value is 43.0 cm. The control was 40.25 cm.

The results in 2012 indicate that N- and Mg fertilization not increased the lobeline content µg/g. The highest lobeline value is a control 518 µg/g.

The lowest lobeline value was the 50 kg/ha N-treatment (401.8 µg/g). The 50 kg/ha Mg ground fertilizer value is 451 µg/g. The plant height value of the 50 kg/ha N-treatment is 45.62 cm. The 100 kg/ha Mg ground fertilizer value is 44.25 cm. The control was 42.0 cm. The highest plant height value was the 100 kg/ha N-treatment (47.88 cm).

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