EFFECT OF NITROGEN AND MAGNESIUM TREATMENTS ON THE PRODUCTION OF INDIAN TOBACCO (*LOBELIA INFLATA* L.)

Vojnich V.J.[1], Máthé Á.[1], Szőke É.[2], Csapó Z.[1]

[1]Department of Botany, Faculty of Agriculture and Food Sciences, University of West Hungary, Vár 2, 9200 Mosonmagyaróvár, Hungary

[2]Department of Pharmacognosy, Faculty of Pharmacy, Semmelweis University, Üllői út 26, 1085 Budapest, Hungary

E-mail: vojnichv@mtk.nyme.hu

ABSTRACT

The aim of this project was to examine the effect of magnesium and nitrogen fertilisation on the biomass and on the alkaloid production of *Lobelia inflata* in Hungary. Indian tobacco (*Lobelia inflata L.)* is a native North American species domesticated at the University of West Hungary, Department of Botany in 2010 and 2011. The establishment of plants, propagated by *in vitro* and *in vivo* methods, was studied. Both the *in vitro* experiments and the examination of active substances were carried out at the University Semmelweis, Department of Pharmacognosy in Budapest. When propagated by seed *L. inflata* needs several months to reach the transplant stage required for planting into the open-field. The *in vitro* propagation method, as an alternative for large-scale production, was examined. Our observations indicated that plants propagated and acclimatised *in vitro* have the same characteristics as those grown from seeds.

Key words: lobeline, alkaloid, biomass production, fertilizer

Acknowledgments: This work was supported by Semmelweis University, Department of Pharmacognosy in Budapest. Thanks for the laboratory research worker Lenke Tóth. The support of GVOP 3.1.1.-2004-05-0309/3.0 is gratefully acknowledged.

INTRODUCTION

Indian tobacco (*Lobelia inflata* L., Lobeliaceae) 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 (Kelly, 1992), but biennial populations can be found, too.

The herb contains more than 20 piperidine skeleton alkaloids (Kursinszki et al., 2008). Its main alkaloid is lobeline, used in cases of gas- and narcotic poisoning for its stimulating effect on the respiratory system (Dwoskin and Crooks, 2002). It is also used in anti-smoking preparations (Szőke and Máthé, 2007). Recently, significant amounts of polyacetylene compounds have been isolated from above ground organs of the plant (lobetyol, lobetolin and lobetyolin) (Bálványos 2002, Felpin and Lebreton 2004).

The aim of this project was to examine the effect of magnesium and nitrogen fertilisation on the biomass and on the alkaloid production as well as on the total alkaloid content in *L. inflata* in Hungary.

MATERIAL AND METHODS

The open field trials were carried out in 2010 and 2011 at the University of West-Hungary, 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 2010: untreated (control), 50 kg/ha N-, 100 kg/ha Nitrogen and 50 kg/ha Magnesium fertilizer. The nutrients were applied in the following methods and quantities in 2011: 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 7.12; humus 3.08 m/m%; Mg 310 mg/kg; NO_2-NO_3-N 20.1 mg/kg, K_2O 518 mg/kg, P_2O_5 358 mg/kg. An extended soil analysis was carried out according to standard methods of UIS Ungarn laboratory (Hungary, Mosonmagyaróvár).

Plants were sown in glasshouse of January in 2010 and 2011. Seedlings were transplanted to multi-cellular transplant raising trays, between end of April and early of May.

Mg (2%) - and N (34%) fertilizers were spread onto the soil surface, one day prior to transplanting (15. June, 2010) with 27 plants per plot. Transplanting in 2011 two days (26-27. May) with 40 plants per plot. The experimental design was a randomized blocks with 4 repetitions. Mechanical weed control was applied.

Plant height (cm) was measured in 2010 four times (8. July, 17 July, 24 July, 1. August) and in 2011 three times (22. July, 29. July, 7. August). In each treatment 7 plants (2010) and 8 plants (2011) were measured. The first harvest took place on 5th of August 2010, respectively on 9-10th of August 2011, when the biomass was recorded. Following harvest, the plants were dried in a shaded and well-ventilated glasshouse. The dry weight determination was 30th of August 2010 and

1th September of 2011. The flowering phenophase was observed in the period July between Septembers (Vojnich et al., 2011). 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 accomplished with SPSS v19 software.

RESULTS AND DISCUSSION

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.

It could be established that in the form of ground fertilization, both nitrogen and magnesium had a favourable effect on the formation of biomass. Table 1 and Table 2 summarize the effect of fertilizers on plant growth (in 2010). As expected and shown by the analysis of variance, as well as Tukey test, the growth parameters show significantly different values for plant height. Figure 1 illustrates the dry biomass values recorded (in 2010) for above ground plant parts, at the flowering phenophase that was highest in the 50 kg/ha N-treatment followed by the 50 kg/ha Mg-, 100 kg/ha N-treatments. The lowest values were recorded for the control.

Total alkaloid content (in 2010) of above ground plant parts (Figure 2) is 490 mg/100 g (50 kg/ha Mg-treatment), this value is 8.9% higher than the control. The 50 kg/ha N-treatment value is 8.4% higher than the control. The value of the 100 kg/ha N ground fertilizer is 13.7% lower than the control. Similar results were obtained by Szőke et al. (1994) and Takács-Hájos et al. (2007), in experiments where the root formation of in vitro organized cultures was studied.

Tab.1. Influence of N- and Mg-fertilization on the growth (height in cm) of Indian tobacco (Lobelia inflata) in the course of the vegetation period, in 2010

Treatments		Height of the plants (cm)			
		8 July	17 July	24 July	1 August
Control	**Mean**	**6.6**	**11.0**	**19.3**	**30.6**
	N	*7*	*7*	*7*	*7*
	Std. Deviation	*3.59*	*6.56*	*9.52*	*7.89*
	Minimum	*3.0*	*3.0*	*4.0*	*16.0*
	Maximum	*13.0*	*21.0*	*31.0*	*39.0*
50 kg/ha N	**Mean**	**14.0**	**22.4**	**32.4**	**40.3**
	N	*7*	*7*	*7*	*7*
	Std. Deviation	*7.95*	*10.84*	*12.60*	*11.75*
	Minimum	*3.0*	*4.0*	*7.0*	*15.0*
	Maximum	*28.0*	*38.0*	*44.0*	*49.0*
100 kg/ha N	**Mean**	**3.4**	**4.6**	**6.4**	**16.0**
	N	*7*	*7*	*7*	*7*
	Std. Deviation	*0.53*	*0.97*	*1.81*	*5.94*
	Minimum	*3.0*	*3.0*	*5.0*	*5.0*
	Maximum	*4.0*	*6.0*	*10.0*	*25.0*
50 kg/ha Mg	**Mean**	**3.6**	**4.7**	**9.0**	**23.1**
	N	*7*	*7*	*7*	*7*
	Std. Deviation	*0.47*	*0.75*	*2.94*	*6.44*
	Minimum	*3.0*	*4.0*	*5.0*	*10.0*
	Maximum	*4.0*	*6.0*	*12.0*	*28.0*

Tab.2. Tukey HSD test of Indian tobacco (Lobelia inflata) measured four times in 2010 in relation to four fertilising treatments (Parameter: plant height)

Date of mesasuremens	Treatments (A)	Treatments (B)	Mean Difference (A-B)	Std. Error	Sig. level
8 July	Control	*50 kg/ha N*	*-7.4286*	*2.3421*	*0.020 **
		100 kg/ha N	*3.1429*	*2.3421*	*0.546 n.s.*
		50 kg/ha Mg	*2.9286*	*2.3421*	*0.602 n.s.*
17 July	Control	*50 kg/ha N*	*-11.4286*	*3.4032*	*0.013 **
		100 kg/ha N	*6.4286*	*3.4032*	*0.259 n.s.*
		50 kg/ha Mg	*6.2857*	*3.4032*	*0.277 n.s.*
24 July	Control	*50 kg/ha N*	*-13.1429*	*4.3217*	*0.027 **
		100 kg/ha N	*12.8571*	*4.3217*	*0.031 **
		50 kg/ha Mg	*10.2857*	*4.3217*	*0.108 n.s.*
1 August	Control	*50 kg/ha N*	*-9.7143*	*4.4508*	*0.157 n.s.*
		100 kg/ha N	*14.5714*	*4.4508*	*0.016 **
		50 kg/ha Mg	*7.4286*	*4.4508*	*0.361 n.s.*

**The mean difference is significant at the 0.1 level.*

n.s. = not significant

Fig.1. Dry biomass production (g/plant) of above ground plant parts of Indian tobacco (Lobelia inflata), at the flowering phenophase in 2010

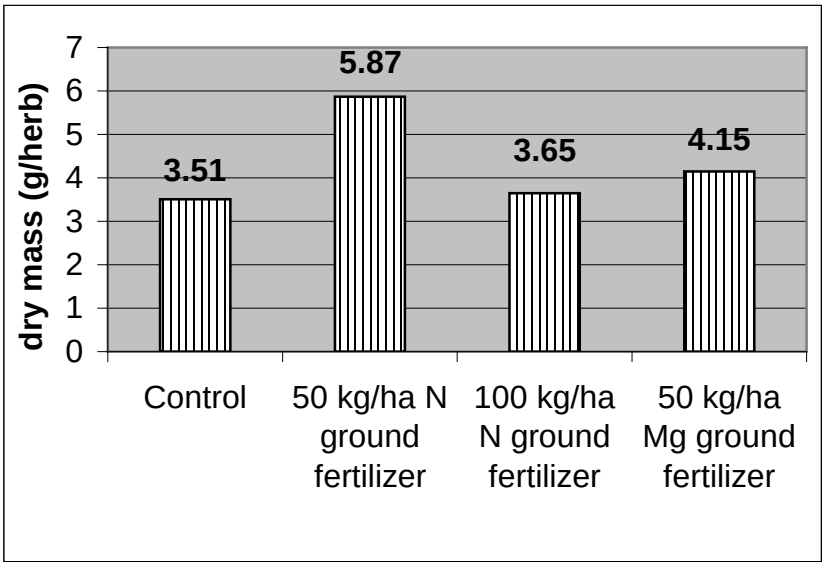
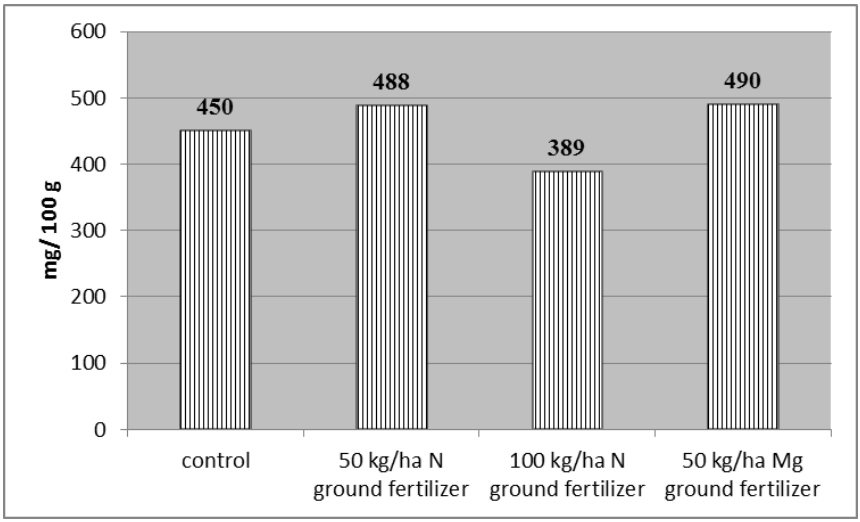


Fig.2. Total alkaloid content (mg/100g) of above ground plant parts of Indian tobacco (Lobelia inflata) in 2010



Tables 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. Figure 3 illustrates the dry biomass values recorded (in 2011) for above ground plant parts, at the flowering phenophase that was highest in the 100 kg/ha N-treatment followed by the 50 kg/ha Mg-, 100 kg/ha Mg-treatments and control. The lowest values were recorded for the 50 kg/ha N-treatment.

Total alkaloid content (in 2011) of above ground plant parts (Figure 4) is 439 mg/100 g (100 kg/ha N-treatment), this value is 6.4% higher than the control. In the 50 kg/ha Mg-treatment, its value is 5.6% higher than the control. The application of 100 kg/ha Mg ground fertilizer resulted 5.7% higher total alkaloid content than the unfertilized control. The value for the 50 kg/ha N-treatment is 2.2% lower than the control.

Tab.3. Influence of N- and Mg-fertilization on the growth (height in cm) of Indian tobacco (Lobelia inflata) in the course of the vegetation period, in 2011

Treatments		Height of the plants (cm)		
		22 July	29 July	7 August
Control	**Mean**	**25.0**	**33.75**	**40.25**
	N	*8*	*8*	*8*
	Std. Deviation	*6.44*	*6.52*	*5.7*
	Minimum	*13.0*	*20.0*	*27.0*
	Maximum	*32.0*	*41.0*	*45.0*
50 kg/ha N	**Mean**	**30.38**	**38.63**	**47.75**
	N	*8*	*8*	*8*
	Std. Deviation	*12.59*	*11.02*	*7.5*
	Minimum	*14.0*	*23.0*	*36.0*
	Maximum	*50.0*	*56.0*	*60.0*
100 kg/ha N	**Mean**	**29.25**	**37.88**	**45.38**
	N	*8*	*8*	*8*
	Std. Deviation	*6.36*	*6.33*	*5.15*
	Minimum	*19.0*	*28.0*	*37.0*
	Maximum	*39.0*	*47.0*	*54.0*
50 kg/ha Mg	**Mean**	**30.75**	**38.38**	**43.0**
	N	*8*	*8*	*8*
	Std. Deviation	*6.16*	*6.05*	*6.97*
	Minimum	*22.0*	*29.0*	*33.0*
	Maximum	*39.0*	*47.0*	*54.0*
100 kg/ha Mg	**Mean**	**26.25**	**36.5**	**45.13**
	N	*8*	*8*	*8*
	Std. Deviation	*8.68*	*7.54*	*5.84*
	Minimum	*14.0*	*25.0*	*36.0*
	Maximum	*41.0*	*50.0*	*56.0*

Tab.4. Tukey HSD test of Indian tobacco (Lobelia inflata) measured three times in 2011 in relation to four fertilising treatments (Parameter: plant height)

Date of mesasuremens	Treatments (A)	Treatments (B)	Mean difference (A-B)	Std. Error	Sig. level
22 July	Control	50 N	*-5.375*	*4.20576*	*0.706 n.s.*
		100 N	*-4.250*	*4.20576*	*0.849 n.s.*
		50 Mg	*-5.750*	*4.20576*	*0.652 n.s.*
		100 Mg	*-1.250*	*4.20576*	*0.998 n.s.*
29 July	Control	50 N	*-4.875*	*3.85646*	*0.714 n.s.*
		100 N	*-4.125*	*3.85646*	*0.821 n.s.*
		50 Mg	*-4.625*	*3.85646*	*0.752 n.s.*
		100 Mg	*-2.750*	*3.85646*	*0.952 n.s.*
7 August	Control	50 N	*-7.500*	*3.14614*	*0.144 n.s.*
		100 N	*-5.125*	*3.14614*	*0.490 n.s.*
		50 Mg	*-2.750*	*3.14614*	*0.904 n.s.*
		100 Mg	*-4.875*	*3.14614*	*0.538 n.s.*

**The mean difference is significant at the 0.05 level.*

n.s. = not significant

Fig.3. Dry biomass production (g/plant) of above ground plant parts of Indian tobacco (Lobelia inflata), at the flowering phenophase in 2011

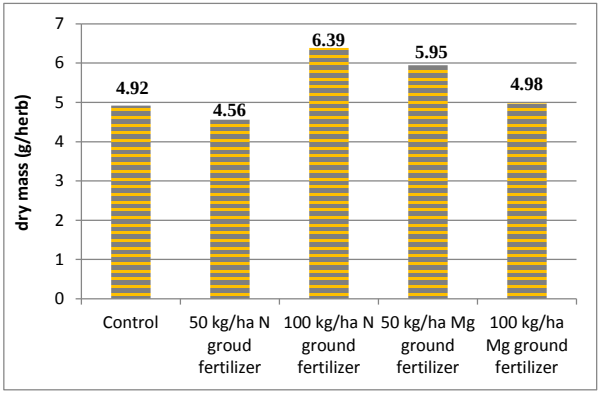
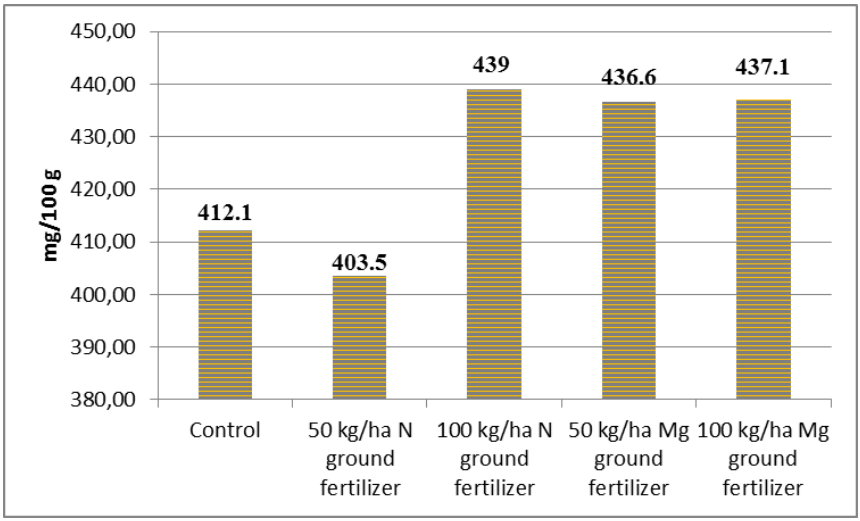


Fig.4. Total alkaloid content (mg/100g) of above ground plant parts of Indian tobacco (Lobelia inflata) in 2011



CONCLUSIONS

In the field trials of *L. inflata* we have established the favourable effect of fertilization. As a result, in 2010, N- and Mg-fertilization increased plant growth (height in cm) by 40.3 and 16.0 cm, respectively (50 kg/ha N-fertilization 40.3 cm). The average height of plants in the 50 kg/ha Mg-treatment is 23.1 cm. The average above ground dry biomass production (g/plant) is 5.87 g/plant (50 kg/ha N ground fertilizer treatment). The 50 kg/ha Mg ground fertilizer value is 4.15 g/plant. The lobeline content (mg/100g) of above ground plant parts is 490 mg/100g (50 kg/ha Mg-treatment), while in the 50 kg/ha N-treatment is 488 mg/100g.

The results in 2011 indicate that N- and Mg-fertilization increased growth (height in cm) by 47.75-40.25 cm (50 kg/ha N-fertilization 47.75 cm). The value of the 100 kg/ha Mg-treatment is 45.13 cm. The dry biomass production (g/plant) of above ground plant parts is 6.39 g/plant (100 kg/ha N-treatment). The 50 kg/ha Mg ground fertilizer value is 5.95 g/plant. The lobeline content (mg/100g) of above ground plant parts is 439 mg/100g (100 kg/ha N-treatment). The value of the 100 kg/ha Mg-treatment is 437.1 mg/100g.

Based on our results, it seems possible to make the right choice of Mg- and N-fertilization with a favourable effect on both biomass and alkaloid production of *Lobelia inflata*.

REFERENCES

Bálványos I. (2002): Studies on the growth and secondary metabolite production of *Lobelia inflata* L. hairy root cultures. PhD thesis, Budapest.

Dwoskin L. P., Crooks P. A. (2002): A novel mechanism of action and potential use for lobeline as a treatment for psychostimulant abuse. Biochemical Pharmacology, 63: 89-98.

Felpin F.-X., Lebreton J. (2004): History, chemistry and biology of alkaloids from *Lobelia inflata*. Tetrahedron, 60(2004):10127-10153.

Kelly C.A. (1992): Reproductive phenology in *Lobelia inflata* L. (Lobeliaceae) and their environmental control. American Journal of Botany, 79(10):1126-1133.

Krajewska A. (1986): The effect of new type of growth regulators on the *Lobelia inflata* L. tissue cultures in Hungarian. PhD thesis, Budapest.

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.

Mahmoud Z. F., El-Masry S. (1980): Colorimetric determination of lobeline and total alkaloids in *Lobelia* and its preparations. Scie. Pharm., 48:365-369.

Szőke É., Kiss A. S., Kursinszki L., Petri G. (1994): Inhibition of aluminium toxicity by magnesium and citric acid in tobacco tissue culture. Magnesium Research, 5:83-87.

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 Research Communications, 35(2): 1181-1184.

Vojnich V. J., Máthé Á., Gaál R., Tüű Sz. (2011): Botanical and chemical variability of Indian tobacco (*Lobelia inflata* L.). Acta Agronomica Óváriensis, 53(2): 37-48.