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

ANDRÁS PALKOVICS[1], VIKTOR JÓZSEF VOJNICH[2]
[1] ***Kecskemét College, Kecskemét, Hungary, palkovics.andras@kfk.kefo.hu***
[2] ***University of West Hungary, Mosonmagyaróvár, Hungary, vojnichvj@gmail.com***

Abstract: *Lobelia inflata, native to North America, seems to possess various pharmaceutically significant properties. It contains several piperidine alkaloids. Interest in Lobelia alkaloids, and in particular lobeline, the most active of them, has increased in recent years due to their activity on the central nervous system. As lobeline can ast as a competitive nicotinic receptor antagonist, it is frequently used in antismoking preparations. Our experiments implemented in randomized blocks with 4 repetitions indicate the favourable effects of Mg-fertilization and are in harmony with our previous in vitro and in vivo experiments. Based on our results, it can be estimated that under the influence of Mg treatment an established population of L. inflata can produce some 3.2 kg/ha total alkaloids.*

Keywords*: Indian tobacco (Lobelia inflata L.), Nitrogen and Magnesium treatment, lobeline yield, alkaloid*

INTRODUCTION

Indian tobacco, a native North American species is a useful medicinal plant that can be introduced in Hungary. *Lobelia inflata* L. belongs to the order Campanulales, to the family Lobeliaceae. Indian tobacco is an annual plant (Kelly, 1992) but biennial populations can be found, too. The herb contains several piperidine skeleton alkaloids (Kursinszki et al., 2008). Its main alkaloid is the lobeline that due to its stimulating effect on the respiratory centre is used in cases of gas- and narcotic poisoning. (Glover, 2010). It is also used in anti-smoking preparations because it is a competitive nicotinic antagonist (Szőke and Máthé, 2007; Takács-Hájos et al., 2007). The significance of lobeline has increased in recent years due to its activity on the central nervous system. Recently, significant amounts of polyacetylene compounds have also been isolated from the plant (lobetyol, lobetolin and lobetyolin) (Felpin and Lebreton, 2004). The aim of this project was to examine the effect of magnesium and nitrogen fertilisation on both biomass and alkaloid production of *L. inflata* in Hungary.

MATERIALS AND METHODS

The open field trials were carried at the University of West-Hungary, Faculty of Agriculture and Food Sciences. N- and Mg- fertilizers were applied in the form of ground fertilizers, as follows: untreated (control), 50 kg/ha N -, 100 kg/ha N - and 50 kg/ha Mg ground fertilizer. No other fertilizers were applied. 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). Experimental plants were propagated by seeding and subsequent transplant raising in glasshouse controlled by a fully automatic energy-umbrella. Seeding took place in glasshouse on 15th of January 2010. Seedlings were transplanted to multi-cellular transplant raising trays, between 1st of May and 5th of May and grown for one and a half month. Mg (2%) - and N (34%) fertilizers were spread onto the soil surface, one day prior to transplanting. Date of transplanting: 15th June, 2010. The number of plants per plot was 27. The measurement of plot was 1.2 m^2. The experimental design was a randomized blocks with 4 repetitions. It should be noted that due the significant damages caused by Spanish slug (Arion vulgaris) in the first two days, major

problems were observed in the care of the plantation. As a result, several plants had to be substituted. Mechanical weed control was applied in terms of plant care. No chemicals or herbicides were applied. Plant height (cm) leaf length and width were four times: 8 July, 17 July, 24 July and 1st of August, and fresh biomass (g) on 5th August. In each treatment 7 plants were measured. The first harvest took place on 5-6th of August, when the biomass was recorded. Following harvest, the plants were dried in a shaded and well-ventilated greenhouse. The dry weight determination was 30th of August. 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. The total alkaloid content was determined by a spectrophotometric method elaborated by Mahmoud and El-Masry (1980) and modified by Krajewska (1986). The lobeline content was determined by HPLC method by Bálványos, et al. (2001) and modified by Kursinszki, et al. (2008).

For the lobeline content were several economically experiments in 1970th in US. The 1970 selling prices ranged from $0.25 to $0.80 per puond, which means that a yield of 1,700 pounds of dried plant material would gross $425.00 to $1,360.00 per acre (Krochmal et al., 1971).

RESEARCH RESULTS

References in the special literature on the mineral nutrition of *Lobelia inflata* L. are scarce, although it is one of the basic factors for the successful production of this species. Some information is available from in vitro hairy root experiments by Bálványos (2002), according to whom among the various nutrients tried (Mg, Ca, Na, N), Mg has proved to be most effective in increasing both the dry biomass and lobeline content. In order to control the validity of these observations for the open field conditions, our nutrition investigations were aimed at the study of the effect of Mg, N-nutrition.

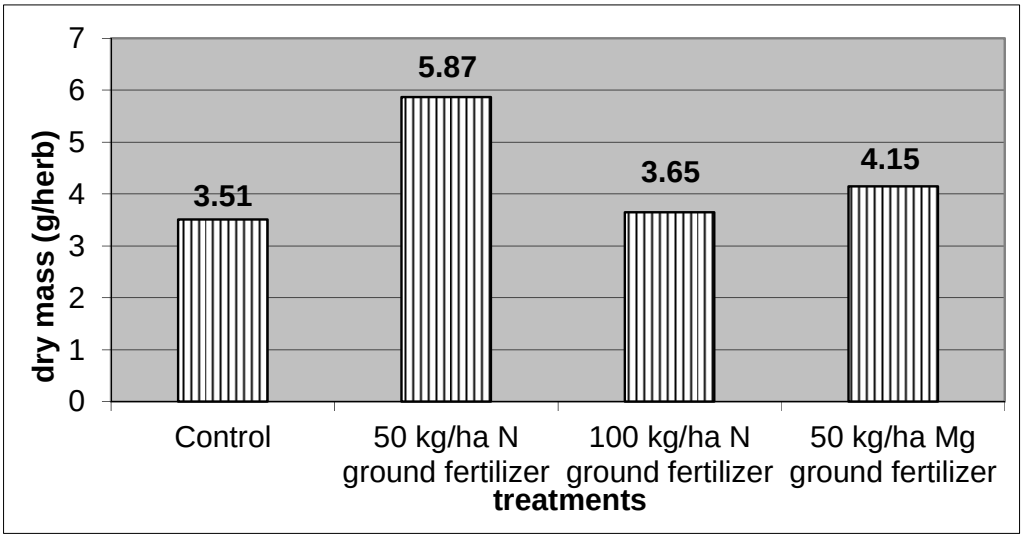

Figure 1 Dry biomass production (g/plant) of above ground plant parts of *Lobelia inflata* at the flowering phenophase in 2010.

Figure 1 illustrates the dry biomass values recorded 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. Remarkably, nitrogen had a positive influence also on root development, but in a slightly different way. The two N-treatments gave the highest root dry biomass values (Figure 2), however remarkably, in this case the highest mean values were not resulted by the 50 kg/ha, but the 100 kg/ha N treatment.

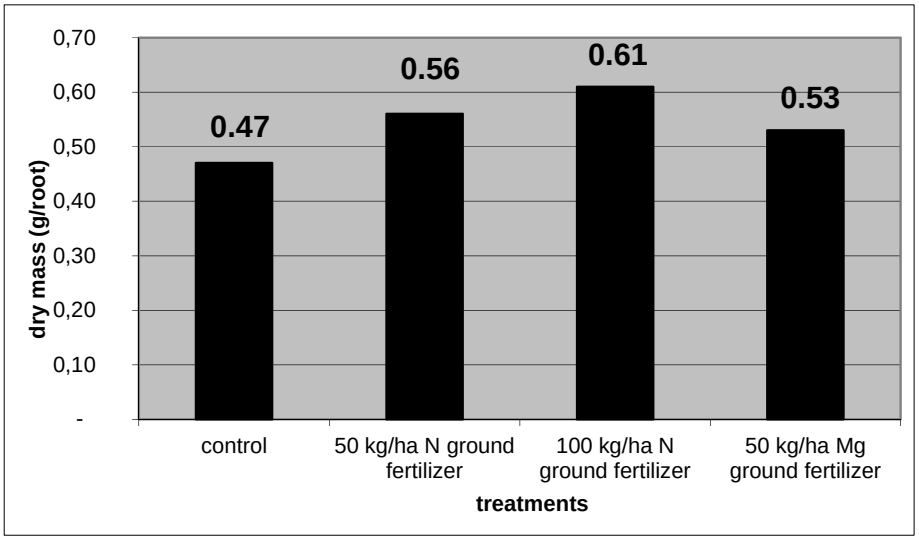

Figure 2 Dry biomass production of roots (g/plant) of *Lobelia inflata* at the flowering phenophase in 2010.

The alkaloid production of above ground organs was also favourably influenced by the Mg-fertilization. As shown in Figure 3 the lobeline content of above ground plant parts varied between 234 µg/g and 294.6 µg/g. Lowest values were recorded in the non-fertilized control (234 µg/g), whereas a 20.1 % increment was observed in the 50 kg/ha Mg ground fertilizer treatment: 281.2 µg/g. As regards the effect of N-fertilization, the response of alkaloid production was similarly favourable. In the 50 kg/ha N ground fertilizer treatment: 294.6 µg/g alkaloid production was recorded.Remarkably the application of 100 kg/ha N ground fertilizer produced lower valueswith a mean alkaloid content of 255.4 µg/g (9.1%). The lobeline content of aboveground plant parts in the 50 kg/ha Mg ground fertilizer treatment was lower than in the 50 kg/ha N ground fertilizer treatment. The total alkaloid content of plants in the 50 kg/ha Mg ground fertilizer treatment was (0.4%) higher than in the 50 kg/ha ground fertilizer treatment.

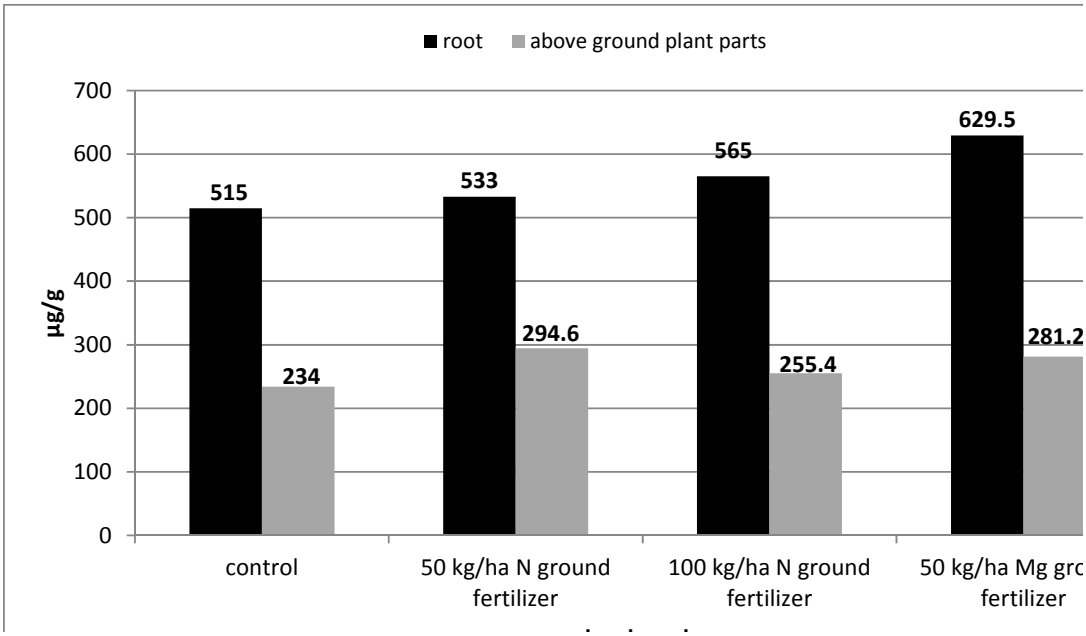

Figure 3 Lobeline content (µg/g) of root and above ground plant parts in 2010.

The lobeline content of roots was increased by Mg-application (22.2%) from 515 µg/g to 629.5 µg/g (Figure 3). Similar values for the 50 kg/ha N ground fertilizer reatment were: 533 µg/g, 100 kg/ha N ground fertilizer treatment: 565 µg/g. The lobeline content at the phenophase of bud set: control 185.3 µg/g, 50 kg/ha N ground fertilizer: 213.4 µg/g, 100 kg/ha N ground fertilizer: 297.6 µg/g, 50 kg/ha Mg ground fertilizer: 302 µg/g. The lobeline content at the phenophase of flowering: control 218.2 µg/g, 50 kg/ha N ground fertilizer: 300.4 µg/g, 100 kg/ha N ground fertilizer: 364 µg/g, 50 kg/ha Mg ground fertilizer: 355.5 µg/g (Figure 4).

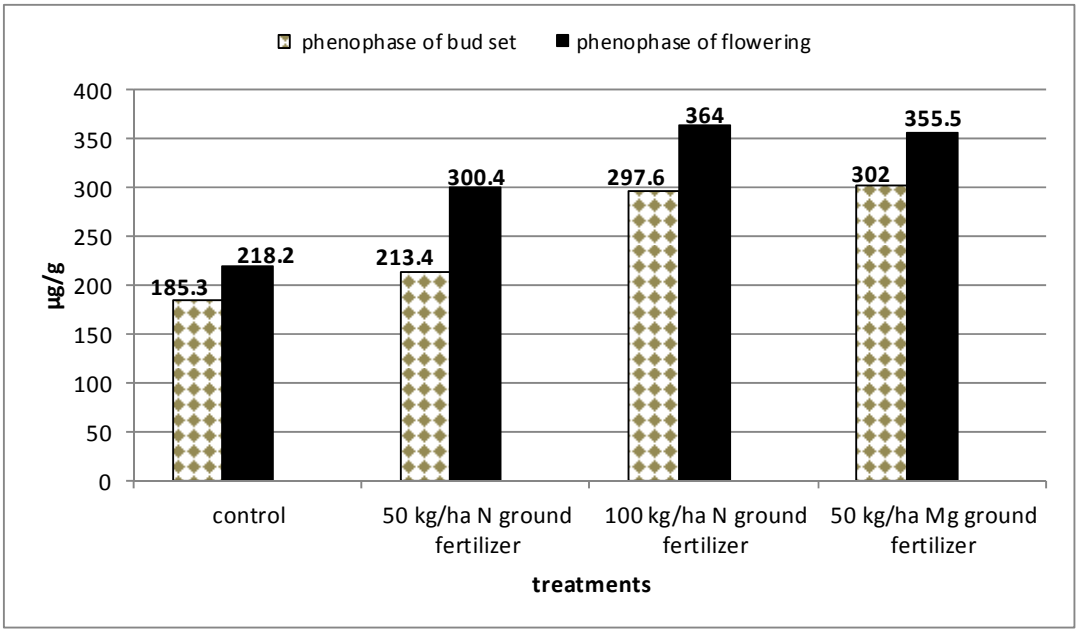

Figure 4 Lobeline content (µg/g) at the phenophase of bud set and flowering phenophase in 2010.

Table 1

Lobeline price

Treatments	herb dry mass [g]	lobelin content of phenophase of flowering [µg/g]	plant / 1 m^2	lobeline content / 1 ha [g]	lobeline price / 1 ha [$]
Control	3.51	218	18	137.7	18.14
50 kg/ha N	5.87	300	18	317	41.76
100 kg/ha N	3.65	364	18	239.1	31.5
50 kg/ha Mg	4.15	355	18	265.1	34.9

Table 1 illustrates for the lobeline content. The lobelia herb selling prices $5.6 per 100 capsules (42.5 g) in 2012 (***). The 50 kg/ha Mg treatment means that a yield of 747 kg of dried plant material would gross $26,070.3 per hectare.

CONCLUSIONS

In the field trials of *L. inflata* we have established the favourable effect of fertilization. The best results were obtained under the influence of 50 kg/ha Magnesium, applied as ground fertilizer. Based on our results, the right choice of Mg-fertilization can have a favourable effect on both biomass production and alkaloid production, and we estimate that an established population of *L. inflata* can produce some 3.2 kg/ha total alkaloids under the influence of Mg treatment.

As a result, the highest lobeline content of the roots of *Lobelia inflata* L. increased by 3.5-22.2 % (629.5 µg/g - 50 kg/ha Mg ground fertilizer treatment) and exceeding by 3.5% the control values. The lobeline content of above ground plant parts is 281.1 µg/g (50 kg/ha Mg ground fertilizer treatment) and is 20.1% higher than that of the control.

The lobeline content of above ground plant parts in the 50 kg/ha Mg ground fertilizer treatment was lower than in the 50 kg/ha N ground fertilizer treatment, although the total alkaloid content in the 50 kg ha Mg ground fertilizer treatment exceeded (0.4%) that of the 50 kg/ha N ground fertilizer treatment. The highest lobeline content at the phenophase of bud set was 302 µg/g (50 kg/ha Mg ground fertilizer treatment) which is 61.3% higher than the control. The highest lobeline content of phenophase of flowering was 364 µg/g (100 kg/ha N ground fertilizer) which is 60% higher than the control.

The results indicate the favourable effect of Mg-fertilization and are in harmony with our previous experiments. The lobeline content values of plants treated with Mg, determined by HPLC, were the highest in all treatments but, the 50 kg/ha N-treatment (above ground plant parts).

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