ACTA TECHNICA CORVINIENSIS
– Bulletin of Engineering
Tome IX [2016], Fascicule 4 [October – December]
ISSN: 2067 – 3809

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BIOMASS AND LOBELINE PRODUCTION OF IN VITRO PROPAGATED INDIAN TOBACCO

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Abstract: *Lobelia inflata* L. is a medicinally important species of the *Lobeliaceae* family. It is native to North America, it contains numerous piperidine alkaloids. The main alkaloid lobeline has been used as a respiratory stimulant. Recently, it has been come into the limelight due to research on CNS, drug abuse and multidrug resistance. It has been found that the plant can be successfully introduced (cultivated) and due to its favourable active principle production it can qualify for utilization. The outlined experiments have verified that N- and Mg-fertilization exerts a positive effect on plant production. The aim of this project was to examine the effect of magnesium and nitrogen fertilisation on the biomass and on the lobeline production of *in vitro* propagated *Lobelia inflata* in Hungary.
Keywords: *Lobelia inflata* (Indian tobacco), lobeline, biomass production, in vitro

INTRODUCTION

Indian tobacco (*Lobelia inflata*) is a native North American species (Canada and US. east countries) [1]. 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].

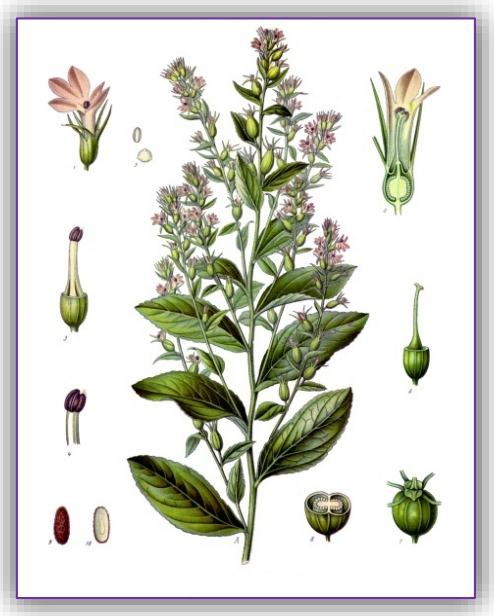

Figure 1. Indian tobacco (*Lobelia inflata*)

The *Lobelia inflata* synthesize important medicinal materials. The herb contains several piperidine skeleton alkaloids [4]. 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 [5]. Recently, it has been come into the limelight due to research on CNS, drug abuse and multidrug resistance [6,7].

Figure 2. Indian tobacco (*Lobelia inflata*) habitus

Another important active agent in the plant is an antidepressant known β-amirin-palmitate. 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.

It is important to increase the biomass and lobeline content of the plant by nitrogen and magnesium treatments in vitro [8,9,10] and in vivo in open field [11,12,13,14]. There was a favourable effect of NH_4^+ and NO_3^- on the biomass formation of in vitro cultures [15,16,17], of in vivo in open field [18,19] and aquatic cultures [20,21]. Britto and

Kronzucker [22] 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 [23,24].
The aim of this project was to examine the effect of magnesium and nitrogen fertilisation on the biomass and on the lobeline production of *in vitro* propagated *Lobelia inflata* in Hungary.

MATERIAL AND METHODS

The open field trials were carried out in 2011 in Mosonmagyaróvár, University of West-Hungary (Széchenyi István University). 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. 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.
An extended soil analysis was carried out according to standard methods of UIS Ungarn laboratory (Hungary, Mosonmagyaróvár).
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. 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. In each treatment group 8 plants were measured both in 2011 (dry biomass production, g/plant of L. inflata herb).
The first harvesting was on 9-10th August 2011. 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 [25]. The total alkaloid content was determined by a spectrophotometric method elaborated by Mahmoud and El-Masry [26] and modified by Krajewska [27]. The statistical analysis was preformed with SPSS v19 software [28].
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.

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. The analysis of dry biomass production (g/plant) also underlined the favourable effect of Magnesium.
Dry biomass production was highest of the 100 kg/ha Mg-treatment of above-ground plant parts, as compared to the untreated control and N-application (Figure 3). The lobeline content was the highest *in vitro* culture (Figure 4) of the 100 kg/ha Mg-treatment (635 µg/g).

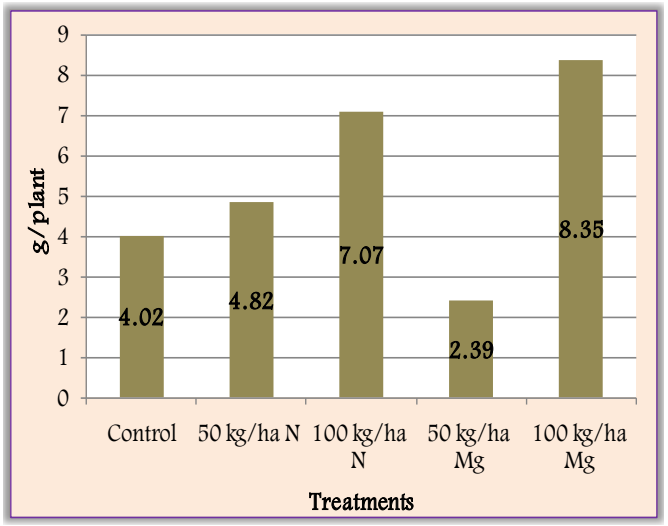

Figure 3. Dry biomass (g/plant) production of *in vitro Lobelia inflata* herb (2011)

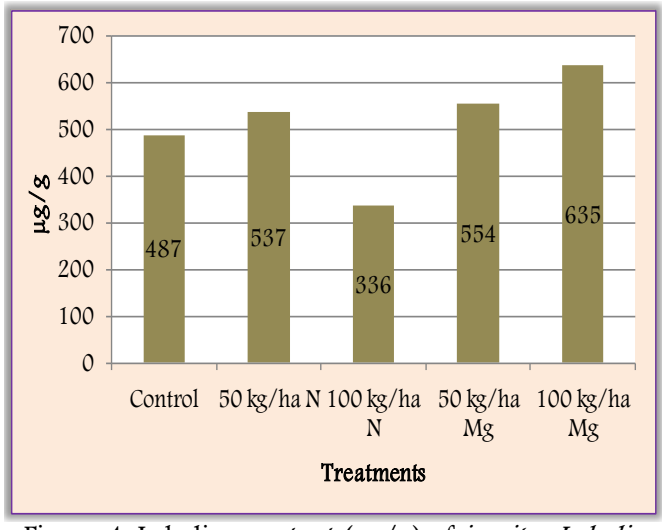

Figure 4. Lobeline content (µg/g) of *in vitro Lobelia inflata* herb (2011)

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) [29].

CONCLUSIONS AND RECOMMENDATIONS

The results indicate the favourable effect of Mg-fertilization and are in harmony with our previous *in vitro* experiments. Lobeline content (µg/g) determination by HPLC.

With respect to the lobeline content determined by HPLC it can be stated that values of plants treated with Magnesium (dry biomass production) and Mg 100 kg/ha treatments (lobeline content) were the highest.

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ACTA Technica CORVINIENSIS
BULLETIN OF ENGINEERING

ISSN:2067-3809