



## Alkaloids contents of *Hyoscyamus niger* L. at different organs in different growth stages

Elnaz Adibfar<sup>1</sup>, Kamalaldine Dilmaghani<sup>2\*</sup> and Hasan Hekmat Shoar<sup>2</sup>

1. Young Researchers Club, Marand Branch, Islamic Azad University, Marand, Iran

2. Department of Biology, Islamic Azad University, Marand Branch, Marand, Iran

### Abstract

*Hyoscyamus niger* L. is an important medicinal plant belonging to Solanaceae family. This plant is a rich source of medicinal substances including tropane alkaloids. In this investigation, we studied effects of different concentrations of nitrogen (0, 12.5, 27.5, 41, 55, 69, 83 mg in each pot) on growth and Tropane production. After the extraction of Tropanes, they were analyzed by TLC and HPLC. The results from TLC showed that roots had the highest content of hyoscyamine and scopolamine. In roots and leaves, scopolamine was the major alkaloid. Control plants had higher contents of hyoscyamine and scopolamine than treated plants. The plants treated with nitrogen had higher fresh weight and dry weight. On the other hand, increased nitrogen concentration increased root and leaf biomass. With HPLC analysis, scopolamine and hyoscyamine retention times obtained 9 and 11 min, respectively.

**Keywords:** *Hyoscyams niger*; Solanaceae; tropane alkaloids; hyoscyamin; scopolamine; HPLC; TLC

### Abbreviations:

HPLC: High Performance Liquid Chromatography; TLC: Thin Layer Chromatography

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### Introduction

Vascular plants produce a wide variety of secondary metabolites, which play an important role in the survival of plants in their ecosystems (Demeyer and Dejaegere, 1998). The synthesis of secondary metabolites is often induced by biological and non-biological stresses (Aniszewski,

2007). Alkaloids figure as a very prominent class of defense compounds show great variety in their botanical and biochemical origin, chemical structure and pharmacological action (Crozier et al., 2006). Solanaceous plants are rich sources of tropane alkaloids. The most important of tropane alkaloid producing plants belong to *Hyoscyamus*, *Atropa*, *Duboisia* (Crozier et al., 2006; Dewick, 2009). From a pharmacological point of view, tropane alkaloids show antimuscarinic activity (parasympatic inhibition), which at the peripheral level translates into antispasmodic effects on the gastrointestinal and genitourinary systems (Drager, 2002; Maldoni, 1991). Recent advances in molecular biology and plant transformation

\*Corresponding author. Tel: 091494139815

E-mail address: ko\_dil@marandiau.ac.ir

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techniques include attempts to enhance the productivity of useful secondary products by metabolic engineering. One way is to improve produced biomass and another way is to use genetic engineering (Doerk-Schemitz and Alfermann, 1993). So the objective of this study was to determine hyoscyamine and scopolamine levels at different organs and different growth stages in *H. niger* at different treatments of nitrogen in culture medium. For analysis of alkaloids, we used atropine sulfate and scopolamine hydrobromide as standards. Atropine with chemical formula  $C_{17}H_{23}NO_3$  is the racemic mixture of hyoscyamine (Ahmed and Fahmy, 1994).

## Materials and Method

The seeds of *Hyoscyamus niger* L. were gathered from Payam region, Marand, Azarbayjan, Iran. Similarly, soil was taken from the same region. The experiments included seven treatments each with four replicates. Nitrogen was added to pots as  $KNO_3$  with concentrations 0, 12.5, 27.5, 41, 55, 69 and 83  $mg \cdot Kg^{-1}$  soil where each pot weighed 1700 g. Plants were cultured in thermal condition  $25^\circ C - 29^\circ C$ , photoperiod 16 h light, light intensity 7600 lx at canopy and pots were pounding with 100 ml distil water daily. Treatment was started from second weeks after cultivation and harvest of plants was done in

three growth stages (6, 12 and 18 weeks after treatment). Roots and leaves of plants were dried separately in normal temperature of lab. For extraction of alkaloids, 5 ml ammonia (25%) and methanol was added over plant powder and was placed at gradual circulation for 12 hours until alkaloids entered alcoholic medium. Afterwards, methanol extraction was filtered through Whatman filter paper. The extraction was repeated for several times. Methanol was then evaporated by rotary evaporator and residual dry matter was washed with petroleum ether. Finally, lower phase was collected. This process was repeated for several times. Acidic solution was alkalized with ammonia (25%). Alkaline solution was washed with chloroform and chloroform lower phase was collected. This was repeated for several times. Chloroform solution was dehydrated with anhydrous sodium sulfate and filtrated with Whatman filter paper and methanol was added. Then methanol was evaporated by rotary evaporator. Residual matter was dissolved in absolute ethanol and then was used for quantification and qualification analysis. Plant extracts were analyzed by TLC and HPLC. For separation of alkaloids TLC plates (20×20 cm, silica gel 60) were used and mobile phase was a mixture of acetone - distilled water -ammonia (90:3:7, v:v:v). HPLC column (Knaver, Germany-Eurospher-100-CN, 406 ×250 mm) was calibrated with 5  $\mu m$ , T= 40 °C and UV= 210 nm and mobile phase was a mixture of SDS-

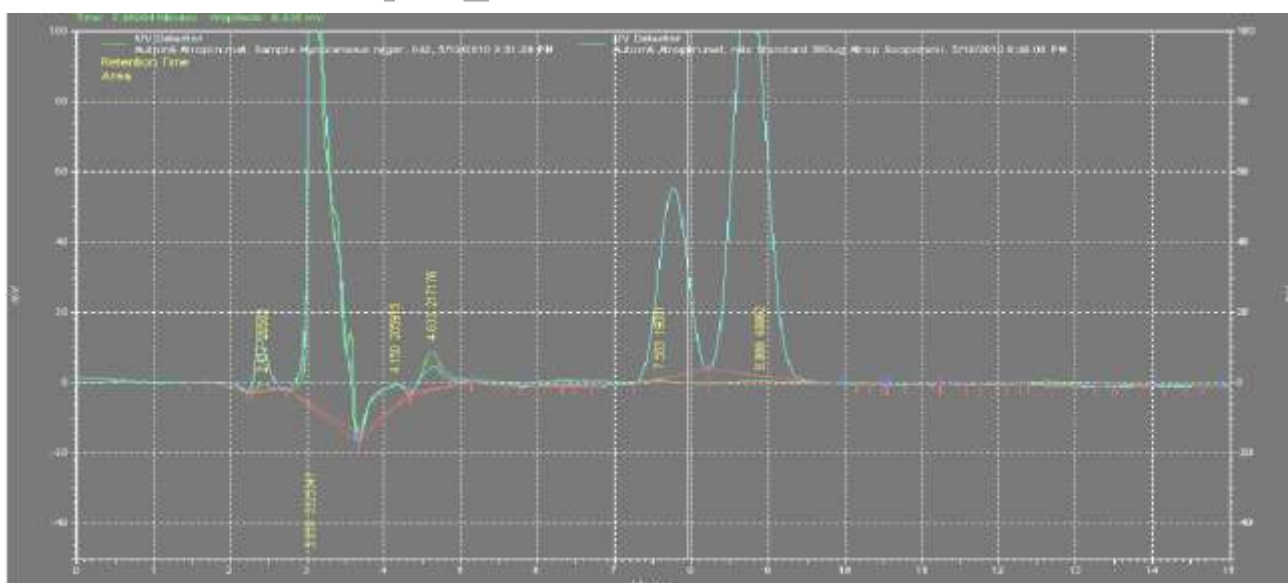


Fig. 1. Chromatogram of atropine sulfate and scopolamine hydrobromide (retention times for scopolamine hydrobromide and atropine sulfate were 9 min and 11 min respectively).

acetonitrile (70:30, v:v). Hyoscyamine and scopolamine retention times obtained were 11 and 9 min respectively (Fig. I). Results were analyzed by SPSS and Tokay test at possibility level  $P \leq 0.05$ . In this study, we analyzed scopolamine and hyoscyamine contents of root and shoot of treated plants in three growth stages also hyoscyamine content of root and leaf at 6 weeks after treatment was analyzed with SPSS (Version 14).

## Results

As diagram (Fig. I) shows, retention times of atropine (hyoscyamine) sulfate and scopolamine hydrobromide were 11 and 9 min respectively. The results showed that as N levels increased, the root and leaf scopolamine contents were decreased. But scopolamine amounts varied nonelderly with increased nitrogen concentration. It was the same for three growth phases. (Figs. II, III & IV).

Similarly, results showed that as N levels increased, the root and leaf hyoscyamine contents were decreased. However, hyoscyamine

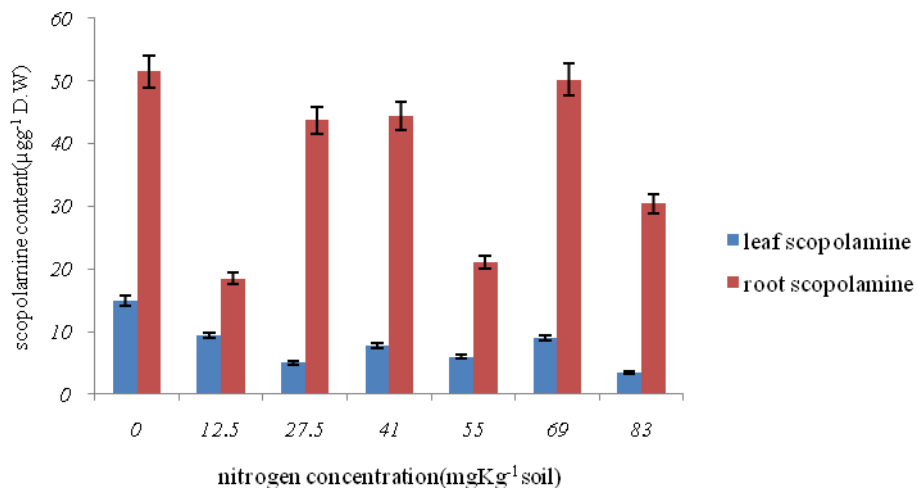


Fig. II. Scopolamine contents of root and leaf of *Hyoscyamus niger* plants after 6 weeks treatment

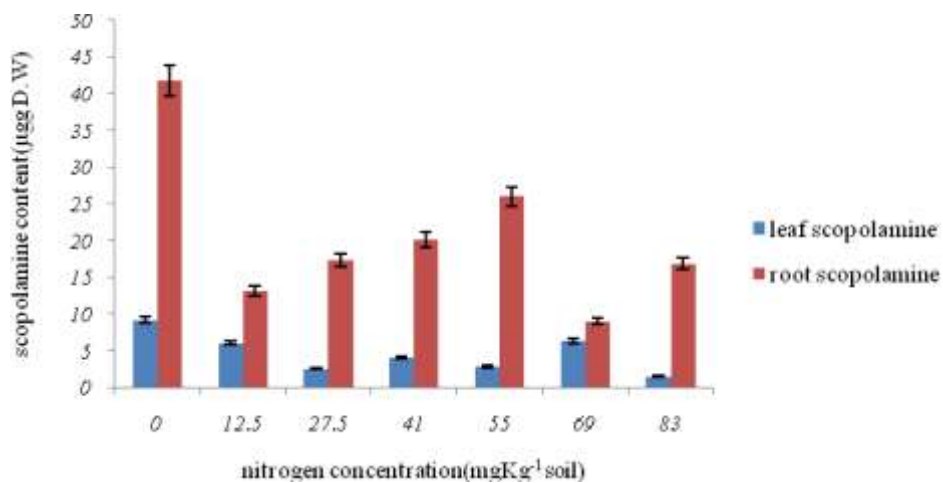


Fig. III. Scopolamine contents of root and leaf of *Hyoscyamus niger* plants after 12 weeks treatment

amounts varied nonelderly with increased nitrogen concentration and it was the same for three growth phases (Figs. V, VI & VII).

remained energy could be used for secondary metabolism (Demeyer and Dejaegere, 1998). On the other hand, increased concomitant cation  $K^+$ , decreases alkaloid precursor by decreasing ornithine decarboxylase and arginine

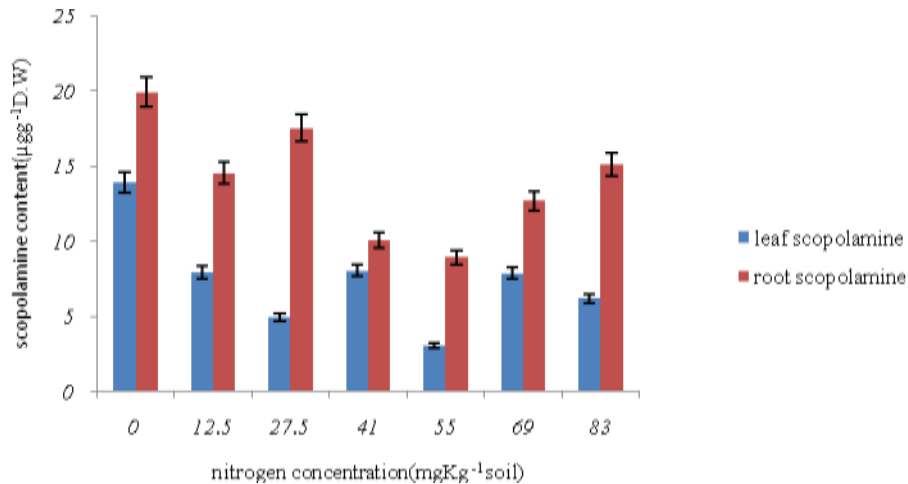


Fig. IV. Scopolamine contents of root and leaf of *Hyoscyamus niger* plants after 18 weeks treatment

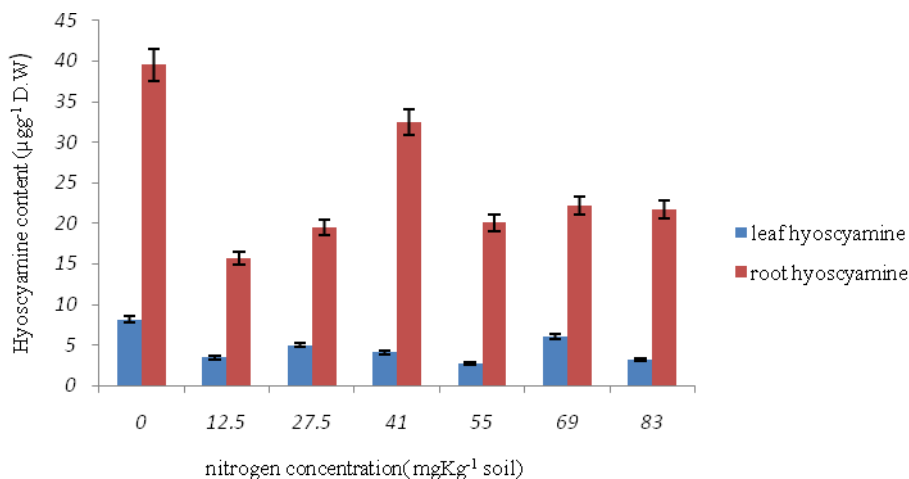


Fig. V. Hyoscyamine contents of root and leaf of *Hyoscyamus niger* plants after 6 weeks

## Discussion

Bensaddek et al. (2001) reported that reduced N concentration in *Atropa belladonna* hairy roots, increased alkaloid levels. Decline of scopolamine levels in roots as N concentrations increases may be due to excess requirement of juvenile plants for metabolic energy in order to  $\text{NO}_3^-$  uptake and reduction and/or participation in amino acids structure so that after supplying enough energy for metabolic processes, the

decarboxylase enzymes activity, responsible for putrescine production, and thereby decreases tropane alkaloids concentrations. Similarly,  $K^+$  directly regulates many enzymes activity, including enzymes related to alkaloids biosynthesis by affecting protein conformation (Demeyer and Dejaegere, 1998). Scopolamine levels were higher than hyoscyamine in both leaves and roots at 6, 12 and 18 weeks after treatment. This result is consistent with that reported by Shimomura et al. (1991) who

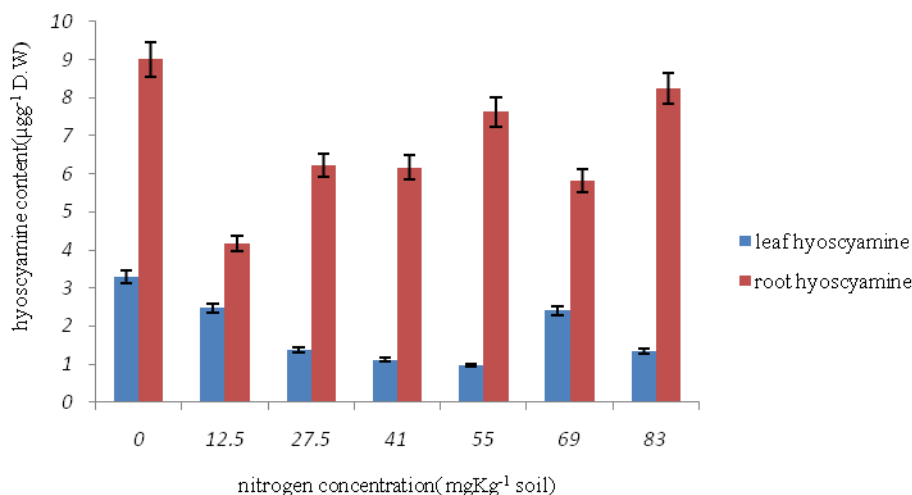


Fig. VI. Hyoscyamine contents of root and leaf of *Hyoscyamus niger* plants after 12 weeks treatment

observed that scopolamine content was higher in *H. niger* and *H. muticus* than hyoscyamine. Oksman-Caldenty et al. (1987) reported that the highest scopolamine levels in *H. muticus* were in leaves just before flowering. The results also

al., 2001; Crozier et al., 2006; Facchini, 2001; Fattorusso and Tagliabate-Scafati, 2008; Gryniewicz and Gadzikowska 2008).

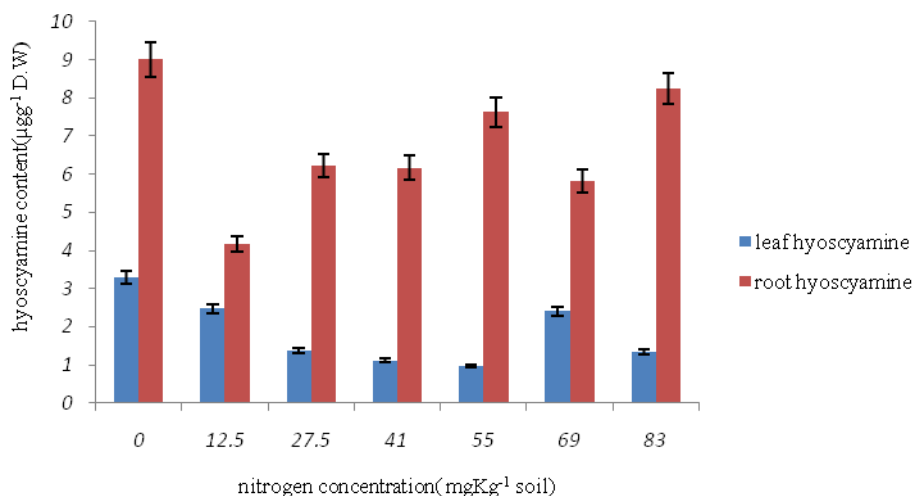


Fig. VII. Hyoscyamine contents of root and leaf of *Hyoscyamus niger* plants after 18 weeks treatment

show that at flowering stage, hyoscyamine / scopolamine ratio decreased in different plant organs. This ratio change may be result of flower development (due to plant development phase) on activity of enzymes responsible in converting hyoscyamine to scopolamine (Demeyer and Dejaegere, 1998). Roots had higher contents of both scopolamine and hyoscyamine than leaves. This difference is partly reflection of transport and storage effects and biosynthesis capacity (Bashir Khan and Harborne, 1991; Bensaddek et

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