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# Effect of Urea Fertilizer on the Weight of Different Parts of *Datura stramonium* and their Alkaloidal Contents at Different Developmental Stages

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## Abstract

Plants of *Datura stramonium* var. *stramonium* were cultivated in pots at different nitrogen levels (60,120, 180, 240, and 300 kg/ha) using urea fertilizer as the nitrogen source. At various developmental stages, the weights of different parts of the plants which recieved 60-240 kg N/ha were significantly increased as compared with the relevant parts of untreated (control) plants. The 240 kg N/ha usually caused the highest increases in the weights of different parts of the plant.

Atropine and scopolamine contents of each dried part of the plant were determined by a previously published TLC-spectrophotometric method. The stem of 80 days old pretreated plants with 180 kg N/ha accumulated significantly the highest percentages of atropine and scopolamine. However, the 120 kg N/ha level caused the highest enhancement in the yield of atropine (65.24 %) and scopolamine (40.38%) in the whole plant during the reproductive (80 days old) stage as compared with the yield of these alkaloids in the relevant controls.

## Introduction

The two well known tropane alkaloids, atropine and scopolamine, have long been used medicinally and are of sufficient commercial importance to warrant investigation into the potential production of them by field-cultivated plants. It is generally assumed that increasing the nitrogen suppply to a plant will increase alkaloid production (1). Interest has been shown in providing different types of nitrogen fertilizers to *Datura* plants with the expectation of higher yield of atropine and scopolamine (2-5). However, there are no reports indicating the use of urea as a source of nitrogen fertilizer to *Datura* plants. Urea is cheap , suitable for use in most types of soils, and is the most commonly used nitrogen fertilizer in I.R. Iran. The present study has analyzed the effects of different levels of urea nitrogen on the weight of root, stem, and leaves of *Datura stramonium* as well as the relationship between the levels of used nitrogen and the production of atropine and scopolamine in various parts of the plant during different developmental stages.

## Materials and Methods

**Plant Material**: Seeds of *Datura stramonium* L. var. *stramonium* were sown in pots filled with soil and prepared according to the experimental design. The pots were devided into six groups. The first group of the pots to which no fertilizer was added acted as the control, while to the second, third, forth, fifth and sixth groups of pots the amounts of 60, 120, 180, 240 and 300 kg nitrogen per a hectare were added respectively when the plants were 45 days old. The pots were arranged in raws 80 cm apart, and 45 cm distance was left between them in each raw at the Agricultural Experimental Station of Isfahan University, Isfahan, I.R. Iran. They were uniformly irrigated once a day at the beginning, while, at the end of development, they were irrigated every three days. Fully developed plants were identified in the Botany Department, faculty of Sciences, University of Isfahan.

Three plant samlpes were collected at random at 9 am when they were 60, 80, and 100 days old. The collected plants were washed and weighed. Then root, stem, and leaves of each plant were separated and weighed. Using bed dryer , the samples were dried uniformly at 45°C, then weighed again and powdered separately and sifted.

**Extraction and Determination of alkaloids:** Three different samples of each part of the plant (obtained from three different plants) were analyzed separately at eachstage of development by a previously published method [6].

## Results and Discussion

Table 1 shows the weights of dried parts of the plant at different developmental stages (i.e.: when it was 60 days old or at the pre-reproductive stage, 80 days old or at the reproductive stage, and 100 days old or at the post-reproductive stage). The weights belong to different parts of the control or untreated plant (0 kg N/ha) and different parts of the treated plant with different levels of nitrogen fertilizer (i.e:60, 120,180, 240 and 300 kg N/ha). It is notable that, at all developmental stages, the weights of different parts of the plants previously treated with 60,120, 180, and 240 kg N/ha show significant increases ($p < 0.01$) if compared with the weights of the relevant parts of the untreated plants. The results also indicate that the 240 kg N/ha treatment usually causes the highest increases in the weights of different parts of the plant. Increase of the weights of dried leaves of *Datura stramonium* and *Atropa belladonna* (3) and the total biomass of Atropa acuminata (7) after treatment with other nitrogen fertilizers was reported by other investigators.

The 300 kg N/ha treatment usually causes significant decreases in the weights of different parts of treated plants (Table 1). Ammonia, which is produced either through the absorption and metabolic decomposition of urea fertilizer (8) or by energy-dependent nitrogen fixation in the plant (9) is, in fact, toxic to most plants when it is available in more than trace amounts (8,10) .

Table 2 shows the percentages of atropine and scopolamine compositions in different parts of the untreated and treated plants with urea fertilizer. It is notable that the amounts of the alkaloids in all plant parts have some fluctuations at different developmental stages. This phenomenon was also noted in *D. metel* (6) and *D. innoxia* (11,12) when their alkaloidal contents were determined.

The highest percentage of atropine accumulation in the roots of both the untreated and treated plants was after 60 days. However, percentage of the alkaloid in the roots of 60 days old plants treated previously with 120, 180, and 240 kg N / ha was significantly higher ($p < 0.05$) if compared with the relevant controls.

The highest accumulaiton of scopolamine in the root was after 80 days, and the 120 kg N/ha treatment caused the highest enhancement in the production and accumulaiton of the alkaloid in this organ.

Stem of the 80 days old plant treated with 180 kg N/ha showed significantly the highest ($p< 0.05$) percentages of atropine and scopolamine if compared with the relevant controls.

In the leaves, the highest accumulation of both atropine and scopolamine was in the 80 days old treated plants with 120 kg N/ha.

As a conclusion, if the total lyield of each alkaloid in the whole plant is considered, we can say that the treatment of *D.stramonium* with 120 kg of urea nitrogen/ha causes the highest enhancement in the yield of atropine (65.24%) and scopolamine (40.38 %) in the whole plant during the reproductive stage (80 days old) if compared with the yield of the alkaloids in the relevant controls. Increase of the total alkaloid yield in *Datura stramonium* (2,3,4,13), *Datura innoxia* (5), *Atropa belladonna* (3), and *Atropa acuminata* (7), as a result of using other nitrogen fertilizers was reported by other investigators. High nitrogen fertilization of *Datura stramonium* increases the plant contents of free ammonia, asparagine, and non-protein amino acids. These changes in plant composition seem to result from the discrepancy in rates of photosynthesis and nitrogen uptake by the plants (14).

Higher amounts of nitrogen (specially 300 kg/ha) , mostly have no effect or even negative effect on the biosynthesis and accumulation of the alkaloids in the plant (Table 2).

**Table 1: Weights of dried parts of *Datura stramonium* untreated (0 kg N/ha) and treated with different levels of urea nitrogen.**

| Part of plant | Age of plant (days) | Weight of dried part ( gm ) Nitrogen levels ( kg / ha ) 0 | 60 | 120 | 180 | 240 | 300 |
|---|---|---|---|---|---|---|---|
| ROOT | 60 | 0.510 ±1.00 | 0.703 ±0.30 | 0.715 ±0.30 | 0.718 ±0.20 | 0.719 ±0.50 | 0.493 ±0.30 |
| | 80 | 1.280 ±3.00 | 1.410 ±0.20 | 1.570 ±0.30 | 1.520 ±0.30 | 1.550 ±0.10 | 1.030 ±0.30 |
| | 100 | 7.350 ±1.00 | 7.420 ±0.25 | 7.410 ±1.00 | 7.050 ±0.45 | 7.220 ±0.31 | 7.380 ±0.64 |
| STEM | 60 | 0.432 ±0.30 | 0.510 ±0.50 | 0.519 ±0.40 | 0.594 ±0.30 | 0.604 ±0.35 | 0.325 ±0.50 |
| | 80 | 2.221 ±1.01 | 2.851 ±1.00 | 2.890 ±0.40 | 3.016 ±1.53 | 3.571 ±0.15 | 2.060 ±0.25 |
| | 100 | 9.840 ±0.30 | 10.02 ±0.18 | 11.41 ±2.00 | 11.78 ±0.45 | 12.16 ±0.31 | 9.660 ±0.74 |
| LEAVES | 60 | 0.955 ±0.20 | 1.020 ±0.20 | 1.030 ±6.14 | 1.300 ±0.40 | 1.250 ±1.00 | 0.759 ±0.30 |
| | 80 | 2.230 ±1.00 | 2.346 ±0.72 | 2.370 ±1.00 | 2.531 ±0.30 | 2.810 ±1.00 | 2.110 ±0.30 |
| | 100 | 7.860 ±2.00 | 7.980 ±3.00 | 8.510 ±0.36 | 8.629 ±0.15 | 8.659 ±0.42 | 7.950 ±1.15 |

-Each value is a mean of three detreminations belong to three different plants ± S.D x 100.

-All weights of each part of treated plants with 60-300 kg N/ha are significantly different ($p < 0.01$) from weights of the relevant controls (0 kg N/ha).

**Table 2: Percentages of atropine and scopolamine in different parts of *Datura stramonium* untreated (0 kg N/ha) and treated with different levels of urea nitrogen.**

| Part of plant | Age of plant (days) | % ATROPINE Nitrogen level ( kg / ha ) 0 | 60 | 120 | 180 | 240 | 300 | % SCOPOLAMINE Nitrogen level ( kg / ha ) 0 | 60 | 120 | 180 | 240 | 300 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ROOT | 60 | 0.360 ±1.58 | 0.369 ±3.90 | 0.436* ±3.90 | 0.470* ±3.80 | 0.425* ±4.10 | 0.325 ±2.80 | 0.034 ±3.30 | 0.088* ±4.00 | 0.086* ±2.10 | 0.070* ±1.50 | 0.051* ±1.90 | 0.032 ±2.00 |
| | 80 | 0.221 ±2.90 | 0.225 ±3.80 | 0.236 ±3.90 | 0.249* ±2.80 | 0.268* ±4.80 | 0.112* ±3.90 | 0.291 ±2.90 | 0.296 ±4.42 | 0.350* ±2.90 | 0.297 ±4.09 | 0.255 ±3.00 | 0.343* ±3.29 |
| | 100 | 0.141 ±4.80 | 0.110* ±4.70 | 0.176* ±1.90 | 0.191* ±4.90 | 0.184* ±4.80 | 0.129 ±3.80 | 0.002 ±0.30 | 0.073* ±7.20 | 0.072* ±5.70 | 0.074* ±3.60 | 0.065* ±4.58 | 0.069* ±4.60 |
| STEM | 60 | 0.128 ±1.70 | 0.137 ±2.30 | 0.177* ±3.10 | 0.180* ±2.20 | 0.152* ±3.80 | 0.112* ±3.90 | 0.137 ±4.12 | 0.144 ±3.90 | 0.159* ±3.80 | 0.169* ±2.90 | 0.148 ±2.80 | 0.128 ±2.50 |
| | 80 | 0.396 ±3.90 | 0.415* ±4.90 | 0.610* ±3.80 | 0.628* ±4.80 | 0.588* ±4.90 | 0.372 ±3.80 | 0.356 ±3.80 | 0.363 ±4.10 | 0.389* ±3.07 | 0.394* ±4.35 | 0.381 ±3.77 | 0.300* ±4.30 |
| | 100 | 0.300 ±4.10 | 0.304 ±5.10 | 0.381* ±4.00 | 0.322 ±3.70 | 0.307 ±5.80 | 0.315 ±4.70 | 0.060 ±8.60 | 0.064 ±4.50 | 0.012* ±4.30 | 0.141* ±3.05 | 0.094* ±6.10 | 0.051* ±3.60 |
| LEAVES | 60 | 0.092 ±2.20 | 0.094 ±3.90 | 0.095 ±3.70 | 0.139* ±4.90 | 0.156* ±5.40 | 0.227* ±3.50 | 0.126 ±4.93 | 0.139 ±2.11 | 0.149* ±3.41 | 0.162* ±2.20 | 0.182* ±2.35 | 0.220* ±1.14 |
| | 80 | 0.255 ±4.40 | 0.251 ±5.40 | 0.301* ±3.70 | 0.261* ±4.90 | 0.275 ±3.80 | 0.241 ±4.70 | 0.293 ±2.51 | 0.297 ±5.05 | 0.368* ±5.21 | 0.311 ±4.90 | 0.359* ±3.10 | 0.348* ±2.71 |
| | 100 | 0.251 ±3.80 | 0.296* ±5.70 | 0.262* ±4.70 | 0.256 ±3.70 | 0.251 ±4.80 | 0.260 ±2.00 | 0.069 ±3.80 | 0.105* ±4.80 | 0.121* ±3.50 | 0.098* ±2.70 | 0.081 ±2.99 | 0.061 ±2.60 |

-Percentages of alkaloid contents were calculated on the basis of dried plant material.

-Each value is a mean of three determintions belong to three different plants ± S.D x 100.

(*) = Values of parts of treated plants with nitrogen fertilizer which are significantly different ($p < 0.05$) from values of the relevant controls (0 kg N/ha).

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