

## Effects of Nitrogen Fertilizer on Productivity of Medicinal Pumpkin

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### Abstract

Medicinal pumpkin (*Cucurbita pepo* convar. *pepo* var. *styriaca*) is an important annual plant that belongs to Cucurbitaceae family. The seeds of this plant, which are used to cure the prostatic hypertrophy and urinary tract irritation, contain fatty oil,  $\beta$ -sitosterol and E-vitamin as active substances and Zn as important nutritional element. The effect of nitrogen levels on leaf N and chlorophyll (a, b, and total) content, seed yield, oil yield, and Zn content was examined. The nitrogen treatments were at five levels (0, 75, 150, 225, and 300 Kg $ha^{-1}$ ) that were applied at three different stages of the plant growth (seed sowing time, four leaf stage and flowering). The results indicated that nitrogen fertilization increased the amount of chlorophylls and N content of the leaves compared to the control. The highest rate of chlorophylls and N content of the leaves was obtained when nitrogen applied at 300 and 225 kg $ha^{-1}$  level, respectively. Also the highest seed dry weight and oil seed content were obtained at 75 kg $ha^{-1}$  of nitrogen. The highest  $\beta$ -sitosterol level showed at 150 kg $ha^{-1}$  of nitrogen. Zn content of the seeds decreased with nitrogen increasing, but the control indicated highest amount of the Zn.

### INTRODUCTION

Common pumpkin (*Cucurbita pepo* convar. *pepo* var. *styriaca*) is an important annual plant belonging to the Cucurbitaceae family. It has a sprawling prickly stem, about 300-500 cm long. The leaves are large, orange-yellow in color, male flowers appear in leaf axil and the female flowers on stalks. The fruits are green to orange. The root systems are fibrous (Hornok, 1990; Bernath, 1993; Robinson and Decker, 1997).

Pumpkin has been used as a vegetable and medicine since ancient times, but it has been cultivated as a medicinal plant only in recent decades. Nowadays, it is cultivated all over the world for with different kinds of usages. The seed oil is used as a local specialty, e.g. in Austria and Germany as salad dressings additive. Pumpkin seeds are used in bakeries for special breads and also as snacks (Kuhlman et al., 1999).

The seeds of pumpkins contain fatty oil,  $\beta$ -sitosterol and E-vitamin and is used as a raw material for certain pharmaceutical products including Peponen® Peponine® and Gronfing® capsules which are mainly used to cure the prostatic hypertrophy and urinary tract irritation (Horvath and Bedo, 1998; Bremness, 1994). Although it is still not fully clear which constituent is responsible for the cure of prostatic hypertrophy, a combination of several ingredients and particular content of sterols are generally considered to contribute to it (Kuhlman et al, 1999). According to popular experience in Hungary, the incidence of hypertension, arteriosclerosis and prostatic hypertrophy is lower in people regularly consuming pumpkin seed oil (Horvath and Bedo, 1988; Vas et al., 1988; Zambo, 1998).

Nitrogen is an important nutrient from the view point of yield, quality and environmental pollution. On the other hand, the natural products must be as pure as possible. Nitrogen application in excess of the crop demand is costly for several reasons; first it costs the grower money, second it causes pollution of the water system (Fontes et al, 1997) and pollution of the natural drug (Omidbaigi, 1995). To use efficiently, an accurate evaluation of plant N requirements is necessary. Total N and nitrate-N concentration of plant parts has proven a reliable indicator of current N status in a numerous

variety of crops, including vegetables (Jones et al., 1991). However, there are large genotype and environmental interactions for nitrogen accumulation by plants (Omidbaigi, 1995). Recently, a method that has attracted attention is based on measuring the chlorophyll content in the leaves as the chlorophyll content has been found to be directly related to N leaf concentration (Lopez-Cantarero et al., 1994). Several workers have utilized plant chlorophyll contents to assess the N status of crops, such as wheat and potato. Leaf chlorophyll may serve as a useful index for determining crop N requirements. However, the use of chlorophyll and total N concentration to assess the N status of medicinal pumpkin plants may not be practical without some kind of normalization procedure to account for variety, plant age, and location effects on the chlorophyll and total N levels. This study was undertaken to determine the chlorophyll and total N critical levels in medicinal pumpkin leaves at flowering stage. The dry weight of the seeds was determined after fruit harvesting, extraction and drying the seeds.

## **MATERIALS AND METHODS**

Medicinal pumpkin seeds, kakai cultivar, were sown in plastic pots that contained sandy loam soil in a controlled greenhouse and were kept moist until emergence. After this stage, the seedling was irrigated with half strength Hoaglands solution. The diameter of the plastic pots was 30 cm. The details of the physico-chemical properties of the soil are shown in table 1. Treatments consisted five N levels (0, 75, 150, 225, and 300 kg ha<sup>-1</sup> or 0, 25, 55, 85, and 110 g per plant respectively). The were pots arranged in RCBD with three replicates. Nitrogen was applied as NH<sub>4</sub>NO<sub>3</sub>. 1/3 of total fertilizer mixed with the soil before seed sowing and 2/3 of it was added at four leaf stage and flowering stage. Two wrapper leaves in each plant were collected at flowering stage. Ten leaf discs were detached from the leaves and used for chlorophyll determination following the Arnon (1949) procedure. The remainder leaves were rinsed and oven-dried at 70 °C in a forced-draft oven, and ground to pass a 0.03 mm (20 mesh) screen, digested in a mixtures of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), salicylic acid (C<sub>7</sub>H<sub>6</sub>O<sub>3</sub>) and selenium, and N determined by Kjeldahl method (Jones et al, 1991). After fruit ripening and extraction of the seeds, the fresh weight of the seeds determined and then the seeds were dried. Then dry matter of the seeds (%) determined. Oil was extracted from the seeds and then the Zn of seeds was determined with Atomic Emission Spectroscopy(AES) instrument. β-sitosterol of the oil seeds determined with Shimadzu UV\_2100 spectrometer instrument.

In each treatment there were 15 plants and five of them were sampled for analysis. Irrigation was given at regular intervals during the vegetative growth period. Other agronomic management practices were performed as and when needed.

To study the effect of nitrogen on leaf N and chlorophylls content, samples were taken from developing leaves at the flowering stage. The fruit picking was done in September at full maturity when the fruits become yellow-orange in color under all the treatments. Seeds were manually separated, cleaned, washed and dried. Oil was extracted from 20 g of powdered seed with 300 ml n-hexane in a Soxhlet apparatus at 60 °C.

Solutions were taken from the extract and were placed on a rotary evaporator. After removing the solvent the sample was dried in an oven at 80 °C. The difference between the beaker containing oil and the empty beaker determined the amount of oil.

Statistical data was analyzed for analysis of variance using the MSTATC software package and statistical differences were calculated according to Duncan's multiple range test.

## **RESULTS AND DISCUSSION**

### **Effect of Nitrogen on Leaf Chlorophylls**

The response of common pumpkin to nitrogen treatments differed and the concentration of treatment had different effect on the leaf chlorophyll content. The results show that the chlorophylls of the leaves were increased by N rates (Table 2). Leaf

chlorophyll was directly related to leaf N concentration at the flowering stage. It may be a useful index for pumpkin N status and thus could be an aid in predicting crop N requirements. Nitrogen enhancing chlorophyll contents in lettuce leaves (Fontes et al., 1997) was pointed out by Lopez-Cantarero and his co-workers

(1994), a non destructive hand-held meter is currently available to measure the green color intensity in crop leaves as a method that can be directly related to leaf chlorophyll content.

#### **Effect of Nitrogen on N Leaf Content**

Application of N increased ( $p < 0.05$ ) the total N concentration in the medicinal pumpkin plant in the flowering samples. The leaves showed highest level of nitrogen between other parts of the plant. Application of nitrogen at 150 and 225  $\text{kg ha}^{-1}$  levels resulted in highest accumulation of N in the leaves compared to control plants (Table 2).

#### **Relationship of Nitrogen Applied, Total Leaf Chlorophyll and N Leaf Content**

The results showed that the amount of total leaf chlorophyll in control plants was 56 % lower than plants that treated with 300  $\text{kg ha}^{-1}$  at flowering stage and highest leaf chlorophyll and nitrogen was obtained at 150  $\text{kg ha}^{-1}$  of nitrogen fertilizer (Fig. 1). A study on *Solanum melongena* (Lopez-Cantarero et al., 1994) and *Lactuca sativa* (Fontes et al., 1997) showed same results.

#### **Effect of Nitrogen on Seed DW**

Between nitrogen treatments the seed dry weight was highest at 75  $\text{kg ha}^{-1}$  and was lowest at 150  $\text{kg ha}^{-1}$  nitrogen level (Table 2). The results showed that oilseed pumpkin (Boguslawski and Taghizadeh, 1969), *Lycopersicon esculentum* (Adams, 1991), *Cucumis sativus* (Heuer, 1991), *Lycopersicon esculentum* (Bolarin et al, 1993) sweet pepper (Gomez et al., 1996) carrot (Inal et al., 1998) and *Solanum melongena* (Savvas and Lenz, 2000) had same response to nitrogen treatments.

#### **Effect of Nitrogen on Oil Seed Content**

There are significant differences between levels of nitrogen treatments ( $p = 5,1$ ). The highest level of the oil seed showed at 75  $\text{kg ha}^{-1}$  of nitrogen (Table 2). There were not any significant difference between control plants and the plants treated with 150  $\text{kg ha}^{-1}$  of nitrogen. The plants treated with 225 and 300  $\text{kg ha}^{-1}$  of nitrogen only showed vegetative growth without any fruit formation. Oil seed content of plants that received 75  $\text{kg ha}^{-1}$  of nitrogen was 8 % and 14 % higher than control plants and plants that received 150  $\text{kg ha}^{-1}$  of nitrogen. These results are similar to those reported on wheat (Masson et al, 1998) and soybean (Sugimoto et al, 1998).

#### **Effect of Nitrogen on Zn Seed Content**

In this study results showed that with increase rate of nitrogen the level of Zn seed content decreased (Table 2). Control plants had highest amount of Zn in its seeds. The reported on the dwarf tomato (Knight et al, 1992) and pepper (Cornillon and Palloix, 1997) obtained same results.

### **RECOMMENDATION**

The effect of nitrogen nutrition on common pumpkin are different. But using suitable concentration and suitable time of application have significant effect on the productivity (seed dry weight, oil seed content, and Zn level) of medicinal pumpkin. It seems applying of 75  $\text{kg ha}^{-1}$  of nitrogen treatment is most suitable for active substances of tested plant.

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## Tables

Table 1. Physio-chemical characteristics of the experimental soil.

Electrical conductivity ( $\text{dsm}^{-1}$ )	0.83
pH	7.52
Organic carbon (%)	0.83
Total N (%)	0.009
Available P (ppm)	2.97
Available K (ppm)	291
Texture	sandy loam

Table 2. Effect of Nitrogen on some characteristics of medicinal pumpkin.

Parameters	Nitrogen ( $\text{Kgha}^{-1}$ )				
	0	75	150	225	300
Leaf Chlorophyll (total) $\text{mgg}^{-1}\text{FW}$	1.166 e	1.408 d	1.651 c	2.408 b	2.711 a
N leaf content (%)	4.589 e	6.513 b	6.961 a	6.112 bc	6.084 bc
Seed DW (%)	59.6 b	66.7 a	62.8 ab	0 c	0 c
Oil seed content (%)	40.27 ab	47.56 a	34.33 b	0 c	0 c
Zn content of seed (ppm)	307.482 a	264.239 b	251.118 bc	0 d	0 d

\* Means followed by different letters in each rows indicate significant difference between treatments according to Duncan's Multiple Range Test ( $P=5$ ).

## Figures

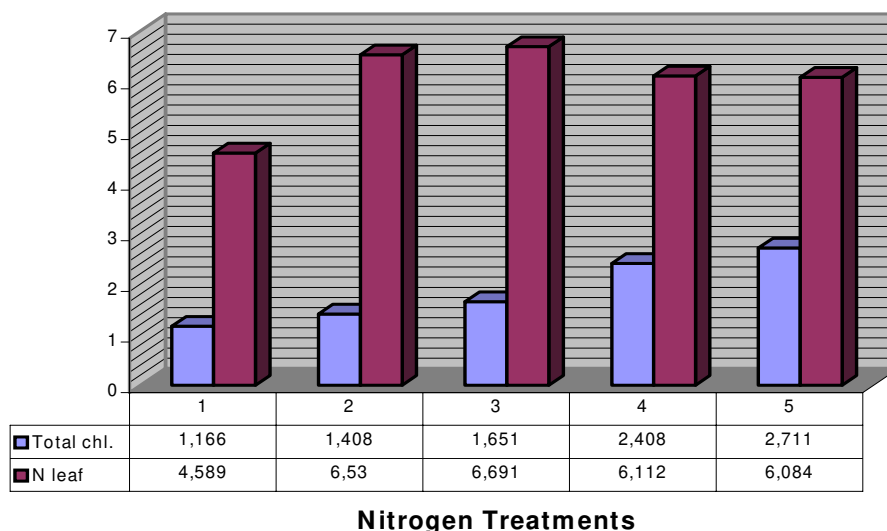


Fig. 1. Relationship between N leaf content and total chlorophylls of medicinal pumpkin under nitrogen treatments.