The effect of differential nitrogen fertilization on morphological and physiological traits of Aloe vera plants

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ABSTRACT: Aloe barbadensis Miller, commonly known as Aloe Vera is extensively used as a base element in preparation of medicine, cosmetics and food supplements. The objective of this study was to find out the effect of different levels of nitrogen on morphological and physiological characteristic of Aloe vera plants. Treatments included four levels of nitrogen (0, 500, 1000 and 1500 mg pot\(^{-1}\)). After 12 months growing morphological and physiological treats such as plant height, leaf number, leaf length, leaf diameter, leaf wide, leaf weight, gel fresh weight, fresh weight of leaf peel, gel to peel ratio were measured. The results showed that the application of nitrogen increased the growth factors such as leaf number, leaf thickness, offset numbers and leaf. The highest levels of aloin concentration and chlorophyll content were obtained in N1500 mg. Increasing N supply positively on increasing aloe vera yield, growth and aloin concentration.

Keywords: Aloe barbadensis, Aloin, Growth, Nitrogen, Yield

Introduction

Aloe barbadensis Miller is an important medicinal plant from Liliaceae family with African origin. About 300 species have been described in the genus Aloe. Among them, Aloe vera is the most commonly grown in the world and it is considered as an important medicinal plant in many countries (Hasanuzzaman et al., 2008; Reynolds, 2004). The two major liquid sources of Aloe vera are yellow latex and clear gel, which are obtained from the large parenchymatic cells of the leaf (Ni et al., 2004). It’s bright green gelatinous delicate leaves contain a very small quantity of viscous yellow fluid known as aloe juice or aloe latex, which is embedded in the pericyclic cells of the vascular bundles of aloe vera leaves. The main constituents of the latex are anthraquinones including the hydroxyathracene derivatives, aloin A and B, barbaloin, isobarbaloin and aloe amedin (Bradley, 1992). The dried latex known as aloin is primarily used as a laxative or cathartic agent in traditional medicines of western countries (Grindlay and Reynolds, 1986). A. vera possesses different biological and physiological activities, Such as wound healing, anti-inflammatory, antibiotic, anti-bacterial, anti-viral, anti-fungal, anti-diabetic and anti-neoplastic against some diseases (diabetes, cancer and allergy) (Eshun and He, 2005; Hamman, 2008; Reynolds, 2004). Aloe vera is a succulent plant and therefore it is more responsive to nutrients in comparison to the other plants. Nevertheless, the excess doses of chemical nutrients are improper sources and can show negative effect on its quality (Hasanuzzaman et al., 2008).

Fertilizers are sources of plant nutrient that can be added to the soil to supply its natural productivity. There is usually a dramatic improvement in both quantity and quality of plant growth when appropriate fertilizers are added (Sakakibara et al., 2006). Fertility management in Aloe vera under greenhouse conditions may be one of the strategies for increasing the yield of Aloe vera (Hasanuzzaman et al., 2008). It has been reported that the application of N fertilizer enhanced the growth and yields of Aloe vera (Khandelwal et al., 2009; Van Schaik et al., 1997). Inoculation with mycorrhizal fungi stimulated growth
parameters and aloin concentration of Aloe vera (Tawaraya et al., 2007) which might be due to the increase in nitrogen supply (Ji-Dong et al., 2006).

The objective of this study was to determine the effects of different levels of nitrogen on the growth, yield and aloin concentration of Aloe vera plants under greenhouse conditions.

**Materials and Methods**

**Plant material and growth conditions**

Pot experiment was carried out during the successive years 2009-2010 under greenhouse conditions. When, offsets of Aloe vera had 18-20 cm length and their weight was 100-125 gr, these seedlings were left to grow in each pot (12 inches in thickness and of 12 inches in height) which contained 20 kg soil. The experiment was arranged in on complete randomized block design (RCBD) with four replications. Treatments included four levels of Nitrogen from Urea source (N1: control, N2:500, N3:1000 and N4:1500 mg pot\(^{-1}\)) the fertilizers were added every three months during the 12 month experiment. The air temperature in the greenhouse was regulated at a maximum of 28°C for the day and a minimum 22°C for night during the growth period. The used soil was homogenous and the results of its analysis are presented in Table 1. Also plants were irrigated according field capacity during the period of the experiments. After one year of growth, five plants per treatment were randomly selected and four leaves per plant were harvested.

**Data collection**

The data recorded at harvest on growth parameters included leaf length (cm), leaf thickness (cm), leaf width (cm), leaf number, leaf weight (g), fresh weight of gel and peel, leaf volume (cm\(^3\)), and number of offsets. Leaf volume was calculated using the leaf length (L), leaf width (W) and leaf thickness (T) (Hernandez-Cruz et al., 2002) as shown below:

\[ V = \frac{(L/12)^3}{1.14} WT. \]

**The aloin concentration assay**

The aloe juice was collected from leaf samples and freeze dried for 48 h. Then, aloin content was determined using an HPLC system (Model, 2487 Waters, USA) (Guliaa et al., 2009). An aloin stock solution (5000 ppm) was made up in a 1:1 of methanol/water. The solvents were selected 500, 100, 80, 50 and 25 ppm. The accuracy of the calibration curves for aloin was tested using reference samples with known concentration of the compounds. Aloe powder (20 mg) was dissolved in 2 ml methanol and water (1:1) passed through a C18 cartridge to selectively extract only the phenol fraction. Injection volume is true 20 \(\mu\)l. The chromatography was obtained by using HPLC equipped with a C18 column (4.6x250mm, dp 10\(\mu\)m). A diode – array detector with two channels was used (channel A set at 275nm, chanal B set at 365 nm) (Waller et al. 2004).

**Determination of chlorophyll content**

Determination of chlorophyll content was performed according to the method of Arnon (1949). Two hundred milligrams of fresh leaf samples was ground with 10 ml of 80% acetone at 4°C and centrifuged at 2500 rpm for 10 minutes at 4°C. Three milliliters aliquots of the extract were transferred to a cuvette and the absorbance was read at 645, 663 and 480 nm with spectrophotometer.

**Statistical analysis**

Data were statistically analyzed using two way analysis of variance (SAS Institute, 9.1.3). The significance of differences among treatment means were compared by Fisher’s least-significant difference test (LSD) at P < 0.05. The number of replications (n=4) in the table/figures denotes individual plants from each treatment measured for each parameter.

**Results**

1. **Growth Characteristics**

   **Leaf number and length**

   Analysis of variance showed that N levels had a significant effect on length and number of Aloe vera leaves (P< 0.05) (table 2), in this experiment, N application was significant on the number of leaves (P< 0.05). However, the highest leaf length was obtained in the plants treated with 1000 mg N (9.28% over the control).
Width and Thickness of Leaves
Application of Nitrogen had significant effects on leaves’ thickness ($P < 0.05$). The highest leaf thickness was obtained in 1000 mg N and the lowest was obtained in control treatment (8.7%). But it had no significant effect on leaf width.

Volume of leaves
The results also showed that the application of N had significant effect on the leaf volume of Aloe vera plants ($P < 0.05$). As a result, the application of N had increased the volume of leaves. The highest volume was obtained in application of 1500 mg N (Table 2).

Offset numbers
The application of nitrogen had a significant effect on number of offsets. The results of means comparison showed that an increase in levels significantly increased the number of plant offsets. The highest number of offset was observed in the plants treated with 1000 mg (Table 3).

2. Yield parameters
Leaf weight
The application of nitrogen had a significant effect on leaf fresh weight of Aloe vera plants ($P < 0.01$). The highest leaf weight was observed in 1000 mg nitrogen (Table 2).

Gel and Peel weight
Results of ANOVA showed that application of nitrogen had significant effect on gel weight ($P < 0.05$) but had no significant effect on peel weight. The highest gel weight was obtained in 1000 mg N (Table 2).

Gel / Peel ratio
The results of the study also showed that the application of nitrogen and benzyl adenine had no significant effect on gel/peel ratio. Also, the highest value was obtained in 1000 mg N (Table 2).

3. Effect on Chemical constituents
Concentration of aloin
Application of nitrogen had significantly impacts on the aloin concentration, so that the highest concentration was obtained in plants treated with 1500 mg N (Table 3).

Chlorophyll contents
The results in table 3 showed that application of N had a significant effect on chlorophyll ‘a’, ‘b’ and Total chlorophyll content, by increase in the levels of N, chlorophyll ‘a’, ‘b’ and total chlorophyll content was increased. The highest chlorophyll a, b and total chlorophyll content were observed in the plant treated with 1500 mg N (table 3).

Discussion
This experiment showed that application of N had positive effect on the growth and aloin concentration of Aloe vera plants. Mengel and Kirkby (1987) reported that inadequate level of nitrogen, shortens the plants life cycle, plant matures early and economic yield is generally poor. The reason for the significant increase in yield parameters of Aloe vera is recognized to be the fact that nitrogen is often regarded as limiting for biomass production in natural ecosystems (Babatunde and Yongabi, 2008). In this study, application of nitrogen on Aloe vera plant has increased the growth parameters. The best growth parameters were obtained in the plants treated with 1000 mg nitrogen. In this regard, Tawaraya et al (2007) showed that shoot fresh weight was increased by AM colonization and can increase the nutrient. Babatunde and Yongabi (2008) reported that the growth parameter values were increased by enhancement of nitrogen levels. In our study, volume of leaf increased as a result of increase in length and thickness of leaves. Thus, leaf volume can be an important factor for the determination of leaf yield and leaf fresh weight (Hernández-Cruz et al., 2002).
A leaf of aloe vera is an important factor in yield determining in Aloe vera plant (Eshun and He, 2005). The application of 1000 mg nitrogen per pot had significantly increased the yield. This result was
confirmed by Khandelwal et al. (2009). Increased nitrogen uptake from the soil by the root system of Aloe vera plant could be the reason for its higher gel content (Ray, 1999). Ji-Dong et al. (2006) reported that the nitrogen application increased leaf fresh weight and total biomass. Hernández-Cruz et al. (2002) showed that the yield of aloe gel was better with a low frequency of watering and a high amount of fertilizer.

Phenolic compounds are considered to be secondary metabolites synthesized in plants through the phenylpropanoid pathway. These make a defense mechanism that reacts to different biotic and abiotic stress conditions (Dixon and Paiva, 1995). Aloin is an important phenolic compound in Aloe vera plants. Results showed that application of nitrogen caused aloin concentrations to be increased. Similar results were obtained by Ji-Dong et al. (2006). They showed that the amount of aloin was enhanced in Aloe vera with nitrogen increases. In another study, application of N increased the Phenolic compounds (aloin, Barbaloin) in latex leaves (Saradhi et al., 2007). Nitrogen is one of the most important elements of the chlorophyll structure; low rate of photosynthesis under conditions of nitrogen limitation can too often be attributed to the reduction of chlorophyll content (Toth et al., 2002). It was observed that the application of nitrogen increased the chlorophyll content in leaves of the Aloe vera plants. Generally, the highest levels of chlorophyll ‘a’ and ‘b’ were obtained in the highest level of nitrogen.

In fact, the unique role of N in photosynthesis, energy compound and important physiological processes increases the content of chlorophyll, and is indirect cause of growth and yield enhancing and chemical compound (aloin) in Aloe vera plant (Nahed and Aziz, 2007). Therefore, the results of this study showed that the nitrogen increased growth, yield and aloin concentration in Aloe vera plants.

Table 1. Physic and chemical properties of soil used in pot experiments

<table>
<thead>
<tr>
<th>Texture</th>
<th>PH</th>
<th>EC (mhmhos/cm)</th>
<th>P (mg/Kg)</th>
<th>O.C (^1) (%)</th>
<th>T.N (^2) (%)</th>
<th>M. N (^3) (mg/Kg)</th>
<th>K (mg/Kg)</th>
<th>Sulfur (mg/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandy loam</td>
<td>7.5</td>
<td>1.68</td>
<td>17</td>
<td>1.09</td>
<td>0.09</td>
<td>17</td>
<td>433</td>
<td>48</td>
</tr>
</tbody>
</table>

1, 2 and 3 denotes the organic matter, total nitrogen and mineral nitrogen, respectively.
### Table 2. The effect of nitrogen application on growth and yield aloe vera plants

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Length of Leaf (cm)</th>
<th>Number of Leaf</th>
<th>Thickness of Leaf (cm)</th>
<th>Wide of Leaf (cm)</th>
<th>Leaf Fresh Weight (g)</th>
<th>Gel Weight (g)</th>
<th>Peel Weight (g)</th>
<th>Volume Leaf (cm³)</th>
<th>Gel/Peel</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>47.043±1.12 c</td>
<td>20.62±0.47 c</td>
<td>1.68±0.047 b</td>
<td>69.48±1.83 a</td>
<td>262.36±24.85 b</td>
<td>171.65±18.35 b</td>
<td>90.71±6.85 b</td>
<td>146.5±9.22 b</td>
<td>1.84±0.92 a</td>
</tr>
<tr>
<td>N2</td>
<td>50.07±0.77 a</td>
<td>21.3±0.32 b</td>
<td>1.69±0.017 b</td>
<td>69.65±1.19 a</td>
<td>271.82±8.37 b</td>
<td>173.67±8.61 b</td>
<td>98.90±2.92 b</td>
<td>154.15±4.43 b</td>
<td>1.81±1.15 a</td>
</tr>
<tr>
<td>N3</td>
<td>51.86±0.79 a</td>
<td>22.17±0.33 a</td>
<td>1.84±0.034 a</td>
<td>72.62±2.20 a</td>
<td>317.52±14.24 a</td>
<td>212.02±11.65 a</td>
<td>106.55±4.50 a</td>
<td>182.04±7.95 a</td>
<td>2.03±3.13 a</td>
</tr>
<tr>
<td>N4</td>
<td>51.85±0.98 a</td>
<td>21.68±0.34 b</td>
<td>1.79±0.028 a</td>
<td>73.4±1.98 a</td>
<td>312.92±23.33 a</td>
<td>210.42±18.22 a</td>
<td>104.31±6.24 a</td>
<td>184.99±7.23 a</td>
<td>2.02±3.15 a</td>
</tr>
<tr>
<td>LSD 5%</td>
<td>2.18</td>
<td>0.49</td>
<td>0.062</td>
<td>5.39</td>
<td>34.17</td>
<td>26</td>
<td>12.38</td>
<td>16.3</td>
<td>0.31</td>
</tr>
</tbody>
</table>

N1, N2, N3, N4 show 0, 500, 1000, 1500 mg nitrogen pot⁻¹, respectively. Means within the same column followed by the same letter were not significantly different according to LSD (P<0.05), Values are mean ± SE (n = 4) and differences between means were compared by Fisher’s least significance test.

### Table 3. The effect of application nitrogen on Aloin and chlorophyll content

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Aloin</th>
<th>Chlorophyll</th>
<th>Total Chlorophyll</th>
<th>Offset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>37/012±d</td>
<td>0.19±0.0053 c</td>
<td>0.068±0.0048 c</td>
<td>25±0.0069 c</td>
</tr>
<tr>
<td>N2</td>
<td>42.85±c</td>
<td>0.23±0.0087 ab</td>
<td>0.093±0.0043 b</td>
<td>.32±0.012 b</td>
</tr>
<tr>
<td>N3</td>
<td>59.55±b</td>
<td>0.25±0.0086 a</td>
<td>0.093±0.0047 b</td>
<td>.34±0.0118 a</td>
</tr>
<tr>
<td>N4</td>
<td>95.56±a</td>
<td>0.26±0.109 a</td>
<td>0.115±0.00708 a</td>
<td>.38±0.015 a</td>
</tr>
</tbody>
</table>

N1, N2, N3, N4 show 0, 500, 1000, 1500 mg nitrogen pot⁻¹, respectively. Means within the same column followed by the same letter were not significantly different according to LSD (P<0.05), Values are mean ± SE (n = 4) and differences between means were compared by Fisher’s least significance test.
REFERENCES


