Journal of Medicinal Plants Research Vol. 6(10), pp. 1834-1841, 16 March, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.499
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Enhancing yield and aloin concentration of *Aloe vera* plants by simultaneous application of N and benzyladenine

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Accepted 10 May, 2011

***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 nitrogen (N) and Benzyladenine (BA) rates on the growth, yield and aloin concentration of *A. vera* plants. Treatments include four levels of N (0, 500, 1000 and 1500 mg pot^{-1}) and four levels of Benzyladenine (0, 500, 1000 and 1500 ppm). After 12 months of growth, morphological and physiological traits such as plant height, leaf number, leaf length, leaf diameter, leaf wide, leaf weight, gel fresh weight, fresh weight of leaf peel and gel to peel ratio were measured. The results showed that the concurrent application of N and BA increased the growth factors such as leaf number (23.16%), leaf thickness (23.81%), offset numbers (100%) and leaf weight (59.11%). The highest levels of aloin concentration and chlorophyll content were obtained in N1500+BA1000 ppm and N1500 mg per pot absolutely, respectively. Increasing N supply interacted positively with added BA by increasing *A. vera* yield, growth and aloin concentration.**

Key words: *Aloe barbadensis*, aloin, benzyladenine, growth, N, yield.

INTRODUCTION

Aloe barbadensis Miller is an important medicinal plant from Liliaceae family with African origin. Among 300 species, *Aloe vera* is considered as an important medicinal plant in many countries (Hasanuzzaman et al., 2008; Reynolds, 2004). The two major liquid sources of *A. vera* are yellow latex and clear gel, which is obtained from the large parenchymatic cells of the leaf (Ni et al., 2004). The main constituents of the latex are anthraquinones including the hydroxyathracene derivatives, aloin A and B, barbaloin, isobarbaloin and aloe amedin (Bradley, 1992). *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). Therefore, *A. vera* is a succulent plant and 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 *A. vera* under greenhouse conditions may be one of the strategies for increase in the yield of *A. vera* (Hasanuzzaman et al., 2008).

It has been reported that the application of N fertilizer enhanced the growth and yields of *A. vera* (Khandelwal et al., 2009; Van Schaik et al., 1997). Babatunde and Yongabi (2008) observed that the growth parameters of Aloe plants increased significantly, by increase in N levels. Cytokinins are important plant hormones that regulate various processes of plant growth and development with cell division and differentiation, enhancement of leaf extension and nutrient mobilization (Shudo, 1994). Plant species have different responses to

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Cytokinins. Application of Benzyladenine in *Codiaeum variegatum* plants significantly increased plant height, number of leaves and fresh weight of leaves in comparison to the control plants (Nahed and Aziz, 2007).

Interaction between N Fertilizers and BA resulted in significant increase in plant height, number of leaves, number of branches and leaf area in *C. variegatum* (Nahed and Aziz, 2007). Nevertheless, there has been no report on N and BA on the growth of Aloeaceae plant species. The objective of this study was to determine the effects of different levels of N and BA on the growth, yield and aloin concentration of *A. vera* plants under greenhouse conditions.

MATERIALS AND METHODS

Plant material and growth conditions

Pot experiment was carried out during the successive years 2009 to 2010 under greenhouse conditions. When the offsets of *A. vera* were 18 to 20 cm long and their weight 100 to 125 gr, these seedling were left to grow in each pot (12 inches in thickness and of 12 inches in height) containing 20 kg soil. The experiment was arranged in factorial based on complete randomized block design (RCBD) with four replications. Treatments included four levels of N from Urea source (N1: control, N2:500, N3:1000 and N4:1500 mg pot^{-1}) and four concentrations of BA (BA1: control, BA2: 500, BA3: 1000, BA4: 1500 ppm) and the combination of the above. 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 to field capacity during period of the experiments. After one year of growth, five plants per treatment randomly selected and four leaves per plant were harvested.

BA application

BA was dissolved with a small amount of 1 μ KOH and mixed with distilled water, that BA stock solution was diluted into 10 L aliquots of 0, 500, 1000 and 1500 ml L^{-1} BA, each containing 10 ml (0.1%) Tween-20 surfactant. All mixtures were formulated and sprayed in 17^{th} week after planting. Control plants were sprayed with Distilled water plus 10 ml L^{-1} Tween 20.

Data collection

Data was 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), 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 are bellowed:

$$V = (L/12)\ 3.14\ WT$$

The aloin concentration assay

The aloe juice was collected from leaf samples and freeze dried for 48 h. Then aloin content was determined using a high-performance liquid chromatography (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 µl. The chromatography was obtained by using HPLC equipped with a C18 column (4.6×250 mm, dp 10 µm). A diode array detector with two channels was used (channel A set at 275 nm, channel 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). 0.2 g of fresh leaf samples was ground with 10 ml of 80% acetone at 4°C and centrifuged at 2500 rpm for 10 min 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

Growth characteristics

Leaf number and length

Analysis of variance showed that N and BA levels had a significant effect on length and number of *A. vera* leaves ($P<0.05$) (Figure 1), but interaction between N and BA had no significant effect on leaf length. The highest number of leaves was observed in combined application of 1000 mg N with 1000 ppm BA treatment that was 23.16% over the control plants (Table 2).

Width and thickness of leaves

Application of N and BA significantly affected on leaves thickness ($P<0.05$). The highest leaf thickness was obtained in 1000 mg N and 1000 ppm BA and the lowest was obtained in control treatment (8.7%).

But it had no significant effect on leaf width. However, concurrent application caused increased thickness of leaves that the highest was observed in 1000 mg N with 1500 ppm BA that was 23.81% over the control plants (Figure 2).

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

Texture	pH	EC (mmhos/cm)	P(mg/kg)	O.C[1] (%)	T.N[2](%)	M. N[3] (mg/kg)	K(mg/kg)	Sulfur (mg/kg)
Sandy loam	7.5	1.68	17	1.09	0.09	17	433	48

1, 2 and 3 denotes the organic matter, total N and mineral N, respectively.

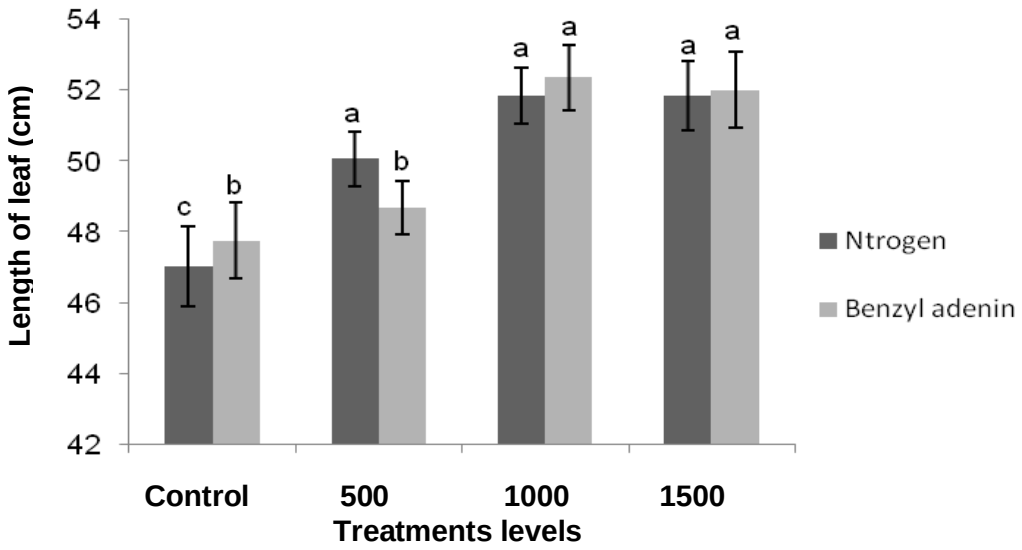

Figure 1. Effects of different rates of N and BA on leaf length of *Aloe vera* plants. Values are mean ± SE (n = 4) and differences between means were compared by Fisher's least significance test. Different letters indicate significant differences with control at P<0.05.

Volume of leaves

Analysis of variance results showed that the application of N and BA and their interaction had significant effect on the leaf volume of *A. vera* plants (P<0.05). The highest volume was obtained in concurrent application of 1500 mg N with 1500 ppm BA in comparison to the control treatment (47.66%) (Table 2).

Offset numbers

The application of N and BA and their interaction had significant effect on number of offsets. Means comparison showed that an increase in N and BA levels significantly increased the number of plant offsets. The highest number of offset was observed in the plants treated with 1500 mg BA alone, while the control treatment had no offsets (Figure 3).

Yield parameters

Leaf weight

The application of N and BA had a significant effect on leaf fresh weight of *A. vera* plants (P<0.01). Maximum leave weight was obtained in 1500 mg N with 1000 ppm BA (407.01 g) (Figure 4).

Gel and peel weight

Results of analysis of variance showed that application of N 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. Also, gel and peel weight were significantly affected by BA application (P<0.01). The maximum gel and peel weight was observed in 1000 ppm BA treatment. The concurrent application of N and BA had significant effects on gel and peel weight. The application of 1500 mg N with 1000 BA ppm resulted in the highest gel and peel weight compared to control treatment (64.49 and 47.51%), respectively (Table 2).

Gel/peel ratio

Analysis of variance showed that the application of N and BA had no significant effect on gel/peel ratio. The

Table 2. Results of means comparison the influence of application N whit foliar BA and their interaction on some characterize growth and yield Aloe vera plants.

Treatment	Leaf numbers	Leaf width (mm)	Gel weight (g)	Peel weight (g)	Leaf volume (cm^3)	Gel/peel ratio	Chlorophyll content (mg g^{-1})	
							A	B
N1*BA1	18.25±.25^{g}	68.05±4.95^{b-d}	99.54±11.92^{f}	66.88±7.77^{g}	113.2±13.68^{f}	1.49±.081^{d}	0.16±.0021^{g}	0.05±.0017^{i}
N1*BA2	20±.408^{f}	68.37±4.22^{b-d}	169.65±20.71^{de}	84.13±7.74^{fg}	139.82±13.68^{eg}	2.017±.17^{a-d}	0.20±.0083^{e-g}	0.06±.0055^{ih}
N1*BA3	22.25±.75^{bc}	74.44±2.18^{a-d}	257.36±15.54^{ab}	120.64±5.58 ^{ab}	184.62±16.25^{a-d}	2.13±.093^{a-c}	0.21±.0041^{d-f}	0.07±.0019^{gh}
N1*BA4	22±.40cd	67.04±2.98^{cd}	160.03±39.43^{de}	91.22±16.82^{d-g}	148.63±13.88^{e}	1.71±.21^{b-d}	0.17±.0053^{f-g}	0.09±.0082^{d-f}
N2*BA1	20.25±.47^{f}	68.47±2.17^{b-d}	205.71±22.85 ^{cd}	95.56±10.39 $^{c-f}$	152.3±10.21^{d-f}	2.33±.57^{ab}	0.20±.0055^{e-g}	0.08±.0084^{fg}
N2*BA2	20.25±.25 ^{f}	70.86±3.42 $^{a-d}$	153.75±7.33 e	95.23±2.29 $^{d-f}$	142.7±7.32 ^{eg}	1.61±.078^{cd}	0.21±.0085de	0.09±.0027^{fg}
N2*BA3	22.25±.47 ^{bc}	68.12±.82 $^{b-d}$	176.24±19.13 ^{de}	99.83±5.3 $^{b-f}$	157.1±10.36 $^{d-e}$	1.75±.13^{b-d}	0.28±.0035^{a}	0.12±.0035^{b}
N2*BA4	22.44± .019 ^{bc}	71.13± .80 $^{a-d}$	158.73±1.27 ^{de}	105±67.5 $^{a-f}$	168.59±6.75 $^{d-f}$	1.51±.038^{cd}	0.22±.0058de	0.09±.0041^{fg}
N3*BA1	21.25±.25 ^{de}	71.92±4.11 $^{a-d}$	207.48±18.99 $^{b-d}$	112.57±5.78 $^{a-e}$	171.17±14.58 $^{d-e}$	1.83±.07^{b-d}	0.23± .0022^{c-e}	0.08±.006^{f-h}
N3*BA2	20.67±.23^{e-g}	71.46±7.9^{a-c}	157.7±13.62d^{e}	102.43±10.47^{b-f}	158.49±19.46^{d-f}	1.58±.19^{cd}	0.21±.015^{d-e}	0.09±.0031^{e-g}
N3*BA3	23.75±.25^{a}	77.32±1.49^{a-c}	238.04±22.49^{a-c}	95.79±12.74^{c-f}	206.26±12.17^{ab}	2.58± .30^{a}	0.27±.011^{ab}	0.10±.0063^{c-e}
N3*BA4	23±0ab	69.76±2.54^{a-d}	244.88±4.11^{a-c}	115.44±3.93^{a-d}	189.7±8.3^{a-c}	2.13±.10^{a-c}	0.28±.0092^{a}	0.11±.013^{b-d}
N4*BA1	20.5±.41^{ef}	66.00±4.025^{a-d}	134.03±6.78^{ef}	89.06±4.38^{e-g}	136.6.7±8.26^{fg}	1.51±±.10^{cd}	0.27±.033^{a-b}	0.15±.0100^{a}
N4*BA2	20.25±.25^{f}	77.95±4.31^{ab}	168.7±25.83^{de}	84.13±5.14^{fg}	184.7±13.83 $^{a-d}$	2.07±.45^{ad}	0.24±.0068^{b-c}	0.11±.0060^{bc}
N4*BA3	23±.408^{ab}	70.093±2.5^{a-d}	280.35±18.28^{a}	127.41±10.63^{a}	183.5.4±7.63^{b-d}	2.25±.26^{ab}	0.28±.029^{ab}	0.11±.029^{bc}
N4*BA4	23±0^{ab}	79.53±3.83^{a}	258.6±25.76a^{b}	116.48±11.86^{a-c}	216.3±9.25^{a}	2.26±.22^{ab}	0.26±.012^{a-c}	0.09±.0041^{ef}
LSD at (%)	0.98	10.79	52.008	24.77	32.61	0.63	0.039	0.02

Values are mean ± SE (n = 4) and differences between means were compared by Fisher's least significance test. Means within the same column followed by the same letter were not significantly different. N1, N2, N3, N4 show 0, 500, 1000, 1500 mg N pot^{-1}, respectively. Also, BA1, BA2, BA3, BA4 show 0, 500, 1000, 1500 ppm *Benzyladenine,* respectively.

interaction of N with BA had a positive effect on gel to peel ratio. Also, the highest value was obtained in 1000 mg N with 1000 ppm BA (Table 2).

Effect on chemical constituents

Concentration of aloin

Application of N and BA significantly affected the aloin concentration. In this experiment, combined application of treatments significantly increased aloin concentration in *Al vera* plants. The highest concentration of aloin was obtained in combination of 1500 mg N with 1000 ppm BA (67.17%) Figure 5.

Chlorophyll contents

Analysis of variance showed that application of N and interaction of N with BA treatments had a significant effect on chlorophyll 'a', 'b' and total chlorophyll content. In this experiment, BA had significant effects on chlorophyll 'a' and total chlorophyll content, The highest chlorophyll a, b and total chlorophyll content were observed in the plant treated with 1500 mg N without BA (40, 66.6 and 50%, respectively) that considerably were more than control plants (Table 2 and Figure 6).

DISCUSSION

This experiment showed that application of N and BA had positive effect on the growth and aloin concentration of *A. vera* plants. Previous study inadequate level of N shortens the plant s life

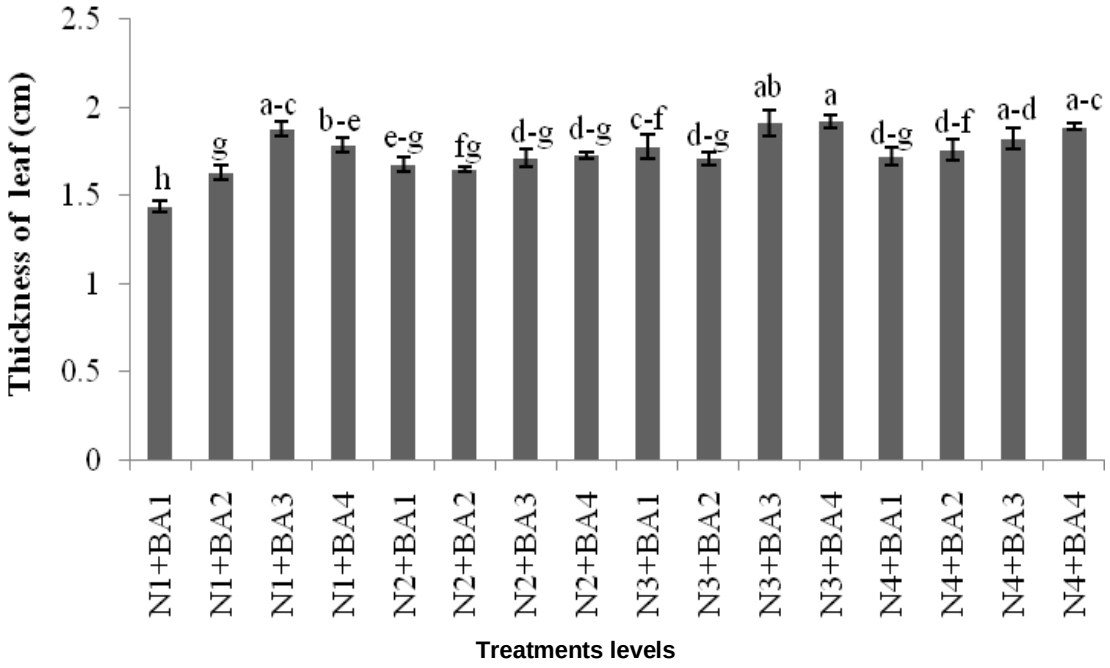

Figure 2. Effects of different rates of N and BA on leaf thickness of *Aloe vera* plants. Values are mean ± SE (n = 4) and differences between means were compared by Fisher's least significance test. Different letters indicate significant differences with control at P<0.05. N1, N2, N3, N4 show 0, 500, 1000 and 1500 mg N pot^{-1}, respectively. Also, BA1, BA2, BA3, BA4 show 0, 500, 1000 and1500 ppm b*enzyladenine,* respectively.

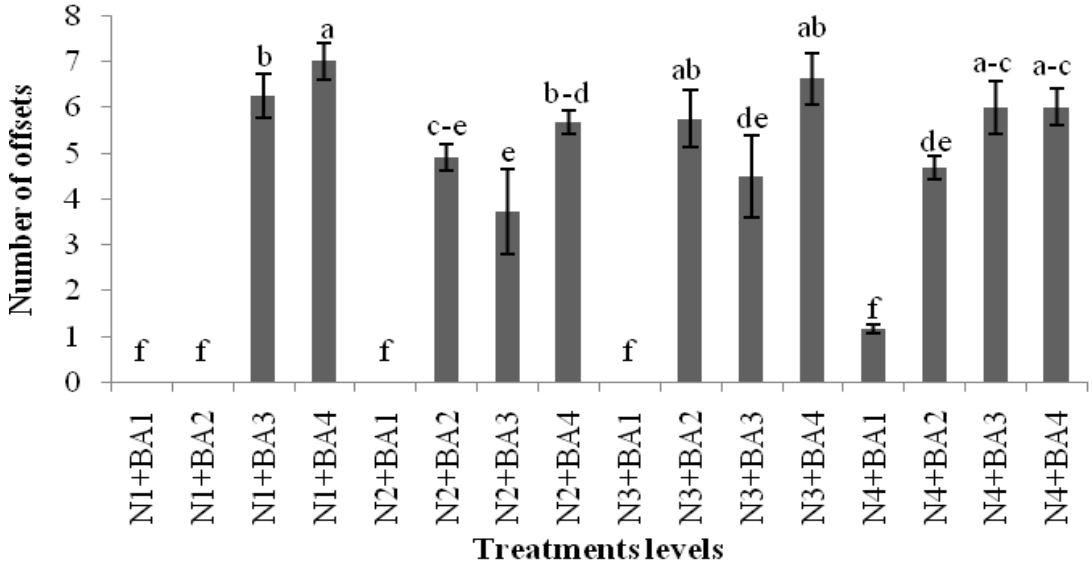

Figure 3. Effects of different rates of N and BA on number of offsets of *Aloe vera* plants. Values are mean ± SE (n = 4) and differences between means were compared by Fisher's least significance test. Different letters indicate significant differences with control at P<0.05. N1, N2, N3, N4 show 0, 500, 1000 and 1500 mg N pot^{-1}, respectively. Also, BA1, BA2, BA3, BA4 show 0, 500, 1000 and 1500 ppm b*enzyladenine,* respectively.

cycle, plant matures early and economic yield is generally poor. It is proven that N is often regarded as limiting for biomass production in natural ecosystems (Babatunde and Yongabi, 2008). In the present study, application of N

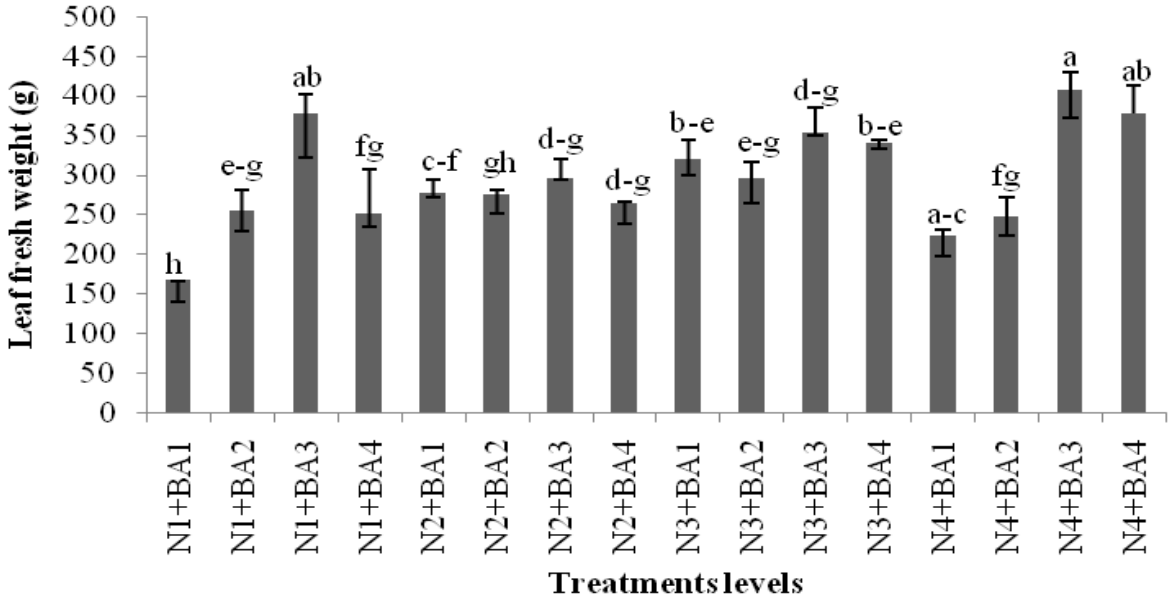

Figure 4. Effects of different rates of N and BA on the leaf fresh weight of *Aloe vera* plants. Values are mean ± SE (n = 4) and differences between means were compared by Fisher's least significance test. Different letters indicate significant differences with control at P<0.05. N1, N2, N3, N4 show 0, 500, 1000 and 1500 mg N pot^{-1}, respectively. Also, BA1, BA2, BA3, BA4 show 0, 500, 1000 and1500 ppm b*enzyladenine,* respectively.

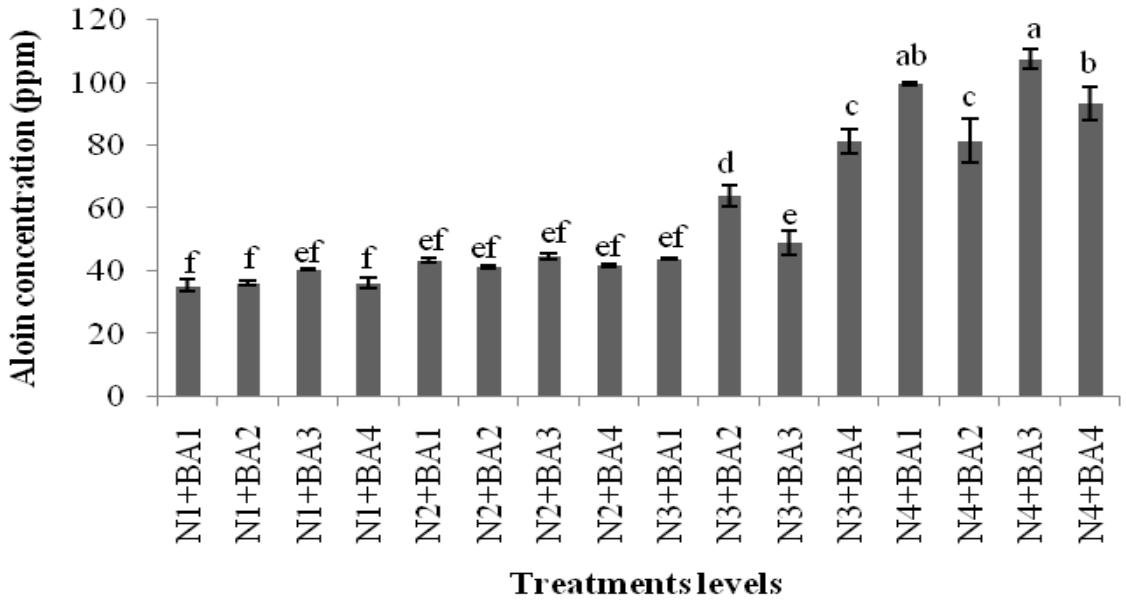

Figure 5. Effects of different rates of N and BA on the aloin concentration of *Aloe vera* plants. Values are mean ± SE (n = 4) and differences between means were compared by Fisher's least significance test. Different letters indicate significant differences with control at P<0.05. N1, N2, N3, N4 show 0, 500, 1000 and 1500 mg N pot^{-1}, respectively. Also, BA1, BA2, BA3, BA4 show 0, 500, 1000 and 1500 ppm b*enzyladenine,* respectively.

and BA sprayed on *A. vera* plant has increased the growth parameters. The best growth parameters were obtained in the plants treated with 1000 mg N and 1000 ppm BA foliar spray. In this regard, on the previous documents, growth parameter values were increased by enhancement of N levels. There is scarcity of information about the application of hormones sprayed on plants to this family. However, a number of studies showed that an

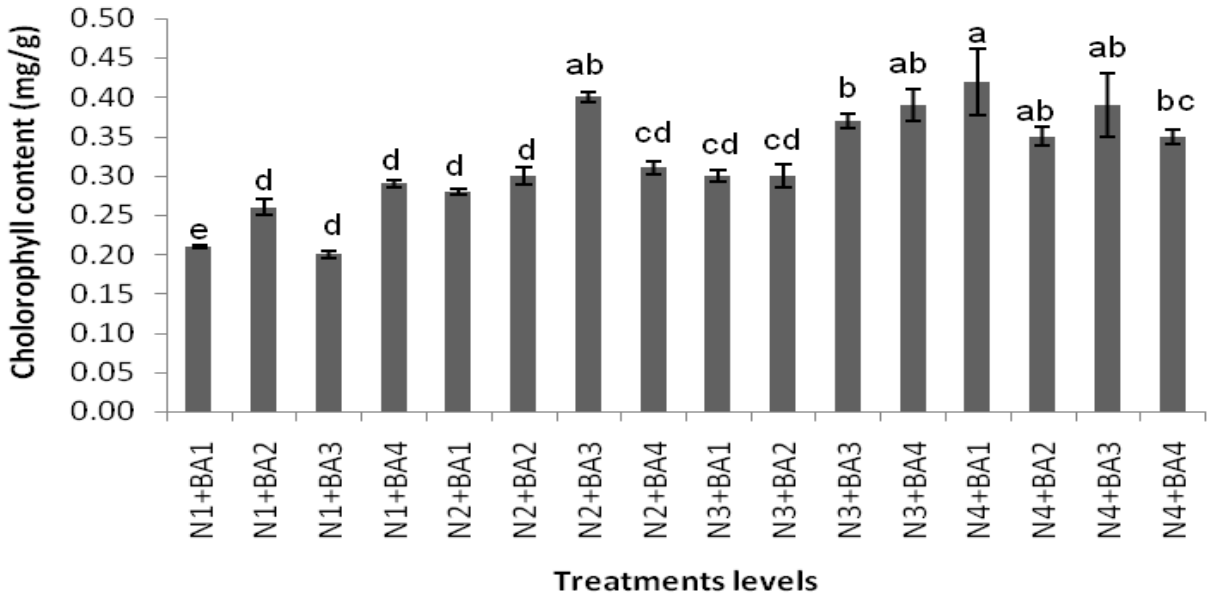

Figure 6. Effects of different rates of N and BA on the chlorophyll content of *Aloe vera* plants. Values are mean ± SE (n = 4) and differences between means were compared by Fisher's least significance test. Different letters indicate significant differences with control at P < 0.05. N1, N2, N3, N4 show 0, 500, 1000 and 1500 mg N pot^{-1}, respectively. Also, BA1, BA2, BA3, BA4 show 0, 500, 1000 and 1500 ppm b*enzyladenine,* respectively.

increase in N and BA led to increase in photosynthesis, chlorophyll content and cell division in apex meristem and cambium that caused an increase in the leaf yield and growth parameters in *A. vera* plants (Sakakibara et al., 2006; Halmann, 1990).

Also, by increase in the levels of N and BA growth parameters were increased, while in the plants treated with N or BA increase in the growth parameters was less observable and these results were similar with other studies. 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 *A. vera* is an important factor in yield determining in *A. vera* plant (Eshun and He, 2005). The application of 1000 mg N per pot had significantly increased the yield; similar results were obtained by Khandelwal et al. (2009). Increased N uptake from the soil by the root system of *A. vera* plant could be the reason for its higher gel content (Ray, 1999). Ji-Dong et al. (2006) reported that the N application increased leaf fresh weight and total biomass. On the other hand, Cytokinin can increase division, cell enlargement and distribution of assimilates in the succulent plants and thus cause to better development of the leaves and increase in gel weight (Carey, 2008). 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. It was observed that the BA increased the number of offsets in Aloe vera plants that might be due to the apical dominance and stimulation of branches in this experiment (Duck et al., 2004).

Similar results were obtained by Carey et al. (2008) on Echeveria and Sempervivum plants that belong to the Liliaceae family. Thus, BA may be used for increasing the number of offsets and the number of propagules or for reducing the apical dominance of the *A. vera* plants (Sakakibara et al., 2006). Phenolic compounds are considered to be secondary metabolites synthesized in plants and make a defense mechanism that reacts to different biotic and abiotic stress conditions (Dixon and Paiva, 1995). Aloin is an important phenolic compound in *A. vera* plants. Results showed that application of N and BA 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 *A. vera* with N increases. In another study, application of N increased the phenolic compounds (aloin and Barbaloin) in latex leaves (Saradhi et al., 2007). The treatment that had the highest level of yields had also the highest aloin concentration.

N is one of the most important elements of the chlorophyll structure; low rate of photosynthesis under conditions of N limitation can too often be attributed to the reduction of chlorophyll content (Toth et al., 2002). Exogenous cytokinin increased the chlorophyll content in the chloroplast (Davies, 2004). It was observed that the application of N increased the chlorophyll content in leaves of the *A. vera* plants. Generally, the highest levels of chlorophyll 'a' and 'b' were obtained in the highest

level of N. Therefore, the results of this study showed that the N and BA increased growth, yield and aloin concentration in *A. vera* plants. From the results of this investigation, it can be concluded that the interaction between of N with BA increased yield at *A. vera* plant so that the highest yield and aloin concentration were observed in the simultaneous application of 1500 mg N with 1000 ppm BA.

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