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Effect of Ammonium Nitrate on Yield of Leaves, Gel and Crude Aloin of *Aloe vera* L.

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Abstract
Aloe vera L. (*A. vera*) belongs to family Liliaceae, used for different medical (anticancer, anti-diabetes, antioxidant, antimicrobial and anti-cardiovascular diseases) and cosmetic purposes under cosmetic purposes. Ammonium nitrate (NH_4NO_3) is a common nitrogen (N) source. The effect of NH_4NO_3 (0, 150, 250 and 450 kg ha^{-1}) on the number of leaves and leaves weight, gel content and crude aloin composition of *A. vera* were investigated during the years of 2013:2014 and 2014:2015. The vegetative growth characters (i.e., number of leaves and its fresh weight) in general increased significantly with different doses of NH_4NO_3 doses compared with one check (control). The highest vegetative growth characters were recorded in the 250 kg ha^{-1} of NH_4NO_3. Doses of NH_4NO_3 affected significantly for the multifarious changes in changes in gel and crude aloin contents of offsets, mother plant and the whole plant during the first and second years.

Keywords**:** *Aloe vera* L., ammonium nitrate (NH_4NO_3), number of leaves, weight of leaves, gel, crude aloin.

1. Introduction
A. vera belongs to family *Liliaceae*, used medically and for cosmetic applications since ancient times [1]. The *A. vera* gel possesses various biological and physiological activities: healing ability of skin burns and cutaneous injuries; prophylactic effect against radiation leucopenia; anti-ulcer; inhibitory action against some bacteria and fungi; inflammation-inhibiting effect; inhibition of the prostaglandin synthesis by anthraquinone type compounds; and inhibition of the AIDS virus by acemannan [2]. *A. vera* aloin used for some pharmacological properties such as anticancer, anti-diabetes, antioxidant, antimicrobial and anti-cardiovascular diseases [3].
Fertilization with inorganic nutrients especially nitrogen (N) enhances the vegetative characters, gel and aloin contents of *A. vera* [4-6]. Ammonium nitrate (NH_4NO_3) has been a common N source. It is an odorless salt with 33 to 34% N. It can be surface-applied or incorporated into the soil. It contains both ammonium and nitrate resulting in reduce volatilization risk as compared to urea, and the nitrate provides a directly available N source. Since it contains ammonium, this fertilizer lowers the pH of the soil resulting in increasing the availability of other soil nutrients [7]. Many reports have shown that vegetative measurements of medicinal plants were significantly increased when the respective N source was NH_4NO_3 [8-9]. Alipoor [10] indicted that NH_4NO_3 (200 kg ha^{-1}) has highly significant effect for increase leaf yield of *A. vera*. NH_4NO_3 caused a significant increase in the oil extracted from sweet basil (*Ocimum basilicum* L.) plants [11]. Omer *et al.* [12] revealed that application of NH_4NO_3 (60 kg N ha^{-1}) resulted in the highest amounts of monoterpenic compositions of American basil (*Ocimum americanum* L.).
The main constituent (thymol) of thyme (*Thymus vulgaris* L.) had the maximum value (63.6%) when the plants received 50 mg N (as NH_4NO_3 formula) [13]. Shirdel *et al.* [14] reported that NH_4NO_3 had a significant effect

on the axillary shoot of dog rose (*Rosa canina*). Gendy [15] indicated that NH_4NO_3 increased the vegetative growth characters and chemical constituents of guar plants. NH_4NO_3 treatments produced the highest yield of basil plant (cultivar of 'Fino Verde') [16].
In this study, we investigate the possible effect of NH_4NO_3 on the numbers of leaves and its weight, gel content and aloin composition of *A. vera*, as an important medicinal plant.

2. Materials and methods

2.1. *Experimental site*
The experiment was carried out at the Experimental Farm in a new reclaimed area, Salhiya region, Egypt during the years of 2013:2014 and 2014:2015. The soil analyses used in this study were presented in Table 1. Offsets of *A. vera* were kindly provided by the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Giza, Egypt. The offsets were transplanted into the open field (one offset per hill) in the first week of April 2013. The experimental design was a complete randomized block with three replicates. The experimental area (plot) was 6 m² [2 m x 3 m] (each plot includes 3 ridges); the distance between hills was 40 cm and 60 cm apart between hills. All agronomic cultural practices operations other than experimental treatments were performed according to the recommendations of the Ministry of Agriculture, Egypt.

Table 1. Mechanical and chemical properties of soil

Items	Values
Texture	Sand
pH	8.6
EC (dSm^{-1})	1.2
$CaCO_3$ (%)	8.4
P_2O_5 (mg g^{-1})	2.1
N (mg g^{-1})	255
Available Mn (mg g^{-1})	5.1
Available Cu (mg g^{-1})	0.5
Available Zn (mg g^{-1})	0.9
Available Fe (mg g^{-1})	1.7

2.2. *Treatments*
Plots were divided into four main groups. The first group was not subjected to any treatments (control). The second, third and fourth groups were subjected to different levels of (NH_4NO_3 (33.5% N) by 150, 250 and 450 kg ha^{-1}.

2.3. *Vegetative growth measurements*
Number of leaves ($plant^{-1}$) and weight of leaves (kg $plant^{-1}$ & ton ha^{-1}) were recorded in offsets and mother plant than were calculated for the whole plant during the 2013:2014 and subsequent 2014:2015 years.
2.4. *Chemical constituent measurements*
Aloe gel was prepared from Aloe leaf according to McAnalley [17]. Phenolic compounds (as crude aloin) were determined according to Mahran [18]. Gel and crude aloin (percentage, g $plant^{-1}$ & ton ha^{-1}) were recorded in offsets and mother plant and were calculated for whole plant during the first and second year.

2.5. *Statistical analysis*
For the purpose of analyses, one factor was considered: four NH_4NO_3 doses. For each treatment there were 3 replicates. The experimental design followed a complete random block design according to Snedecor [19]. The averages of data were statistically analyzed using 1-way analysis of variance (ANOVA-1). Significant values were determined according to LSD at 0.05 ($P > 0.05$). The applications of that technique were according to the STAT-ITCF program [20].

3. Results

3.1. *Effect of NH_4NO_3 on vegetative growth characters*

Vegetative growth characters such as number of leaves (plant^{-1}) and leaves weight (kg plant $^{-1}$ & ton ha^{-1}) were affected by changes in NH_4NO_3 doses (Table 2). Thus the various vegetative growth characters in general increased significantly under the various NH_4NO_3 doses as compared with control. The highest vegetative growth characters were recorded in the 250 kg ha^{-1} of NH_4NO_3. The greatest values of the number of leaves (plant^{-1}) were 8, 11; 25, 24.1; 33, 35.1 of offsets, mother plant & whole plant during the first and second years, respectively. The highest values of weight of leaves (kg plant $^{-1}$ & ton ha^{-1}) were 1.1, 5 & 46.7, 208.4; 3.3, 7.1 & 139, 281.8; 2.2, 12.1 & 185.7, 490.2 of offsets, mother plant & whole plant during the first and second years, respectively.

Table 2. Effect of NH_4NO_3 on vegetative growth characters

Treatments (kg ha^{-1})	No of leaves (plant^{-1})		Weight of leaves			
			kg plant $^{-1}$		ton ha^{-1}	
	Age (year)		Age (year)		Age (year)	
	One	Two	One	Two	One	Two
OFFSETS						
Control	5.0	8.0	0.8	2.8	42.3	115.8
150	6.0	10.0	1.0	5.2	43.5	214.1
250	8.0	11.0	1.1	5.0	46.7	208.4
450	7.0	10.0	0.9	3.6	37.4	163.8
LSD (0.05)	1.9	1.7	0.2	1.2	9.1	6.2
MOTHER PLANT						
Control	16.0	22.0	2.5	4.5	103.7	185.0
150	19.0	24.0	2.7	5.7	107.9	223.6
250	25.0	24.1	3.3	7.1	139.0	281.8
450	19.0	24.0	2.7	6.8	114.3	310.1
LSD (0.05)	3.0	3.1	0.2	0.3	7.7	10.3
WHOLE PLANT						
Control	21.0	30.0	3.3	7.3	146.0	300.8
150	25.0	34.0	3.7	10.9	151.4	437.7
250	33.0	35.1	4.4	12.1	185.7	490.2
450	26.0	34.0	3.6	10.5	151.7	473.9
LSD (0.05)	2.7	3.7	0.3	0.3	3.6	177.2

3.2. *Effect of NH_4NO_3 on gel content*

NH_4NO_3 doses caused some significant changes in gel contents (percentage, g plant^{-1} & ton ha^{-1}) of offsets, mother plant & whole plant during the first and second years (Table 3). The highest values of gel percentage were recorded under 250 kg ha^{-1} of NH_4NO_3 during the first year of offsets and whole plant with the values of 0.4 and 0.4; during the second year the highest percentages of gel were recorded under 450 kg ha^{-1} of NH_4NO_3 with the values of 1.8, 1, and 1.4 of offsets, mother plans and whole plant, respectively.

The highest gel contents (g plant^{-1}) were recorded from the treatment of 450 kg ha^{-1} of NH_4NO_3 with the values of 2.7, 69.4; 9.1, 71.8; 11.8, 141.2 of offsets, mother plant & whole during the first and second years, respectively. The highest gel contents (ton ha^{-1}) obtained from the plants treated with 250 kg ha^{-1} of NH_4NO_3 with the values of 0.2, 0.4 and 0.6 of offsets, mother plant & whole plant during the first year; during the second year the highest gel contents (ton ha^{-1}) recorded at 450 kg ha^{-1} of NH_4NO_3 with the values of 2.7, 2.8 and 5.5.

3.3. *Effect of NH_4NO_3 on crude aloin content*

Some significant changes were found in crude aloin contents which calculated as g plant^{-1} or ton ha^{-1} under NH_4NO_3 treatments but no changes in crude aloin percentages were found of offsets, mother plant & whole

plant (Table 3). The highest values of crude aloin (g plant^{-1}) were recorded under 250 kg ha^{-1} of NH_4NO_3 during the first year of offsets, mother and whole plant with the values of 2.7 and 3.7; during the second year the highest percentages of crude aloin were recorded under 450 kg ha^{-1} of NH_4NO_3 with the values of 10.3, 16.6 and 26.9 of offsets, mother plants and whole plant, respectively. No changes in crude aloin (ton ha^{-1}) during the first year; during the second year the highest crude aloin as ton ha^{-1} were recorded under 450 kg ha^{-1} of NH_4NO_3 with the values of 0.5, 0.6 and 1.1 of offsets, mother plants and whole plants,.

Table 3. Effect of NH_4NO_3 on gel and crude aloin contents,

OFFSETS												
Treatments (kg ha^{-1})	Gel contents						Crude aloin contents					
	%		g plant^{-1}		t ha^{-1}		%		g plant^{-1}		t ha^{-1}	
	Age (year)		Age (year)		Age (year)		Age (year)		Age (year)		Age (year)	
	One	Two	One	Two	One	Two	One	Two	One	Two	One	Two
Control	0.3	1.4	2.4	39.3	0.1	1.5	0.2	0.2	1.3	5.1	0.1	0.2
150	0.3	0.9	2.6	47.2	0.1	1.8	0.2	0.2	1.6	9.9	0.1	0.4
250	0.4	1.1	4.0	57.1	0.2	2.2	0.2	0.2	2.7	8.2	0.1	0.4
450	0.3	1.8	2.7	69.4	0.1	2.7	0.2	0.2	1.7	10.3	0.1	0.5
LSD (0.05)	0.1	0.1	1.2	20.9	NS	0.8	NS	NS	0.6	3.8	NS	0.1
MOTHER PLANT												
Control	0.3	0.9	6.3	41.2	0.2	1.6	> 0.1	0.2	0.4	8.6	> 0.1	0.3
150	0.3	0.9	6.8	49.3	0.3	1.9	> 0.1	0.2	0.6	10.4	> 0.1	0.4
250	0.3	0.8	7.5	59.8	0.4	2.3	> 0.1	0.2	1.0	13.7	> 0.1	0.5
450	0.3	1.0	9.1	71.8	0.3	2.8	> 0.1	0.2	0.6	16.6	> 0.1	0.6
LSD (0.05)	NS	0.1	2.0	1.1	NS	NS	NS	NS	0.1	0.4	NS	NS
WHOLE PLANT												
Control	0.3	1.2	8.7	80.5	0.3	3.1	0.2	0.2	1.7	13.7	0.1	0.5
150	0.3	0.9	9.4	96.5	0.4	3.7	0.2	0.2	2.2	20.3	0.1	0.8
250	0.4	1.0	11.5	116.9	0.6	4.5	0.2	0.2	3.7	21.9	0.1	0.9
450	0.3	1.4	11.8	141.2	0.4	5.5	0.2	0.2	2.3	26.9	0.1	1.1
LSD (0.05)	NS	0.1	0.4	2.9	NS	0.4	NS	NS	0.2	1.5	NS	0.1

4. Discussion

The significant effect of NH_4NO_3 on number and weight of leaves, gel and crude aloin content may be due to NH_4NO_3 a salt with high N (33 to 34% N) [7]. Adding N plays necessary roles in the plant metabolisms through the action of different enzymes [21-23]. Application of N increased the vegetative growth measurements as well as gel and crude aloin contents of *A. vera* [24]. The highest growth characters and chemical constituents of some medicinal plants (anise, coriander and sweet fennel) obtained under 82 kg ha^{-1} of N [25].

The positive effect of NH_4NO_3 on growth character and chemical constituents may be due to NH_4NO_3 its ammonium contents, so, this fertilizer lowers the pH of the soil resulting in increased the availability of other soil elements for plant [7]. On the other hand the increase in growth characters and chemical constituent's values of *A. vera* under NH_4NO_3 doses may be due to the increase in the dry matter contents [26]. Ammonium nitrate (NH_4NO_3) has been a common N source. It is an odorless salt with 33 to 34% N. It can be surface-applied or incorporated into the soil. It contains both ammonium and nitrate resulting in reduce volatilization risk as compared to urea, and the nitrate provides a directly available N source. Since it contains ammonium, this fertilizer lowers the pH of the soil resulting in increasing the availability of other soil nutrients [7]. Many reports have shown that vegetative measurements of medicinal plants were significantly increased when the respective N source was NH_4NO_3 [8-9]. Alipoor [10] indicted that NH_4NO_3 (200 kg ha^{-1}) has highly significant effect for increase leaf yield of *A. vera*. NH_4NO_3 caused a significant increase in the oil extracted from sweet basil (*Ocimum basilicum* L.) plants [11]. Omer *et al.* [12] revealed that application of NH_4NO_3 (60 kg N ha^{-1}) resulted in the highest amounts of monoterpenic compositions of American basil (*Ocimum americanum* L.).

NH_4NO_3 has a high of N (33.5%), The positive effects of N fertilization quantity may be due to the important physiological role of N on molecule structure as porphyrin. The porphyrin structure is found in such metabolically important compounds as the chlorophyll pigments and the cytochromes, which are essential in photosynthesis and respiration. Coenzymes are essential to the function of many enzymes. Accordingly, Nitrogen plays an important role in synthesis of the plant constituents through the action of different enzymes activities and protein synthesis [27] that reflected in the increase in growth parameters of plants such as anise, coriander and sweet fennel plants.

These results are in accordance with those obtained by previous studies Khalid [22] who reported that N fertilization increased the growth and chemical constituents of some aromatic plants. Nitrogen fertilization increased the amount of leaf yield and oil content of *Ocimum basilicum* L. [28]. Hellal *et al.* [29] indicated that N fertilization increased the growth and oil yield of dill. Sarab *et al* [30] obtained oil concentration from herb in case of the high dose of N application. Kandil *et al.* [31] recorded highest basil yields when the highest N rates were applied. The enhanced accumulation of oil under the conditions when plants are well supplied with nitrogen results from the increased production of biomass as well as from the direct impact on the biosynthesis of oil substance [32]. The aforementioned experimental results and present attempt and the present study proved significantly with high doses of nitrogen on the significant effect of an increased amount of nitrogen on the concentration of chemical composition of the essential oil obtained from the basil herb [33]. The increase for N resulted in accumulation of oil, as well as a rise in chemical constituents [34]. Also, these results are in accordance with those obtained by Khalid and Shedeed [35] on *Nigella sativa* L. plants, they reported that N fertilizer treatments were superior to the control and significantly improved the vegetative growth characters of *Nigella sativa* L. plants. The results of chemical constituents were similar to those of Mohammed [36]

Conclusion

On the basis of results obtained and concluded that different agronomic characters were increased significantly by the application of NH_4NO_3 doses as compared to control and changes have also been occurred for gel and crude aloin contents of *Aloe vera* L. plants.

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