

**EFFECT OF AMMONIUM NITRATE AND POTASSIUM HUMATE ON
VEGETATIVE GROWTH AND CHEMICAL CONSTITUENTS OF
CALOTROPIS PROCERA PLANTS.**

Selim, S. M.*; Hend, E. Wahba; Hassanen, M. A***. and Wafaa, M. Abd
El- Salam**

* Prof. of Floriculture, Medicinal & Aromatic plants, Hort. Dept., Fac. Agric., Fayoum Univ.

** Prof. of Medicinal & Aromatic plants, National Research Center.

*** Assistant Prof. of Floriculture, Medicinal & Aromatic plants, Hort. Dept., Fac. Agric., Fayoum Univ.

ABSTRACT:

The present work aimed to study the influence of three gradual rates of nitrogenous fertilizer (ammonium nitrate; NH_4NO_3) and potassium humate at four doses and their interactions on vegetative growth and chemical constituents of *Calotropis procera* plants. The obtained results showed that all morphological characteristics *i.e.* plant height, stem diameter, number of branches and leaves per plant, leaf area per plant, plant fresh and dry weights, root fresh and dry weights were increased by application of different rates of NH_4NO_3 and potassium humate fertilizer or their combinations. Also, plants treated with NH_4NO_3 and potassium humate fertilizers showed a significant increase in the contents of chlorophyll A and B, carotenoids, total carbohydrates(%), N%- P%- K% and total alkaloids of *Calotropis procera* leaves as compared untreated (control) plant. While total phenolic and total flavonoids contents were decreased with fertilization by different rates of NH_4NO_3 and potassium humate. The highest values of total phenolic and total flavonoids contents were obtained with applied the lowest fertilizer rates of NH_4NO_3 (1g/ plant) and potassium humate at zero level.

INTRODUCTION

Calotropis procera is a flowering shrub of the family *Asclepiadiaceae*. It is famous with different names based on its habitat, such as Dead Sea plant, Kisher and Usher in Arabic; Dead Sea fruit, Desert wick, Giant milkweed, Mudar fibre, Rubber tree, Rubber bush, Swallow-wort and Sodom apple in English; Akdo, Akada and Madar in Indian (Farooq *et al.*, 2014 ; Kumar *et al.*, 2013).

Calotropis procera is one of the important numbers of traditional herbal medicine in many countries. It has been reported to possess numerous medicinal properties. In the traditional Asian medical system, it has been used for bronchitis, pain, asthma and tumors. The plant is also known for its toxic

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properties that include dermatitis, iridocyclites, and acts like a poison and produces lethal effects (Muzammal, 2014). The root skin, latex, flowers, leaves and the ksara of arka are used for medicinal purposes. Milk weed is useful both, internally and externally (Khairnar et al., 2012). The latex of *Calotropis procera* extract is easily available and is used in medicine for treatment of many diseases (Kumar et al., 2013). It is also used against malaria and skin infection. The milky latex and flowers were considered to improve digestion, Catarrh, and increases appetite (Muzammal, 2014 and Seifu, 2004). Leaves and roots of this plant have been used to relieve pain under different conditions (Dewan et al., 2000).

Material and methods:

This study was designed to assess the main effects of three nitrogen fertilizer rates (1, 2 and 4 g of ammonium nitrate / plant) and application of potassium humate as a soil drench at four concentrations (0, 4,8 and 12 g/ plant) on vegetative growth and chemical composition of *Calotropis procera* wild plant . Tow pot experiments were conducted at a privet farm at Tezment village – Bani Sweif Governorate during the two successive seasons of 2015 and 2016. Seeds of *Calotropis procera* plant were collected from wild plants grown in Beni Suef desert. Black plastic bags 30×40 cm were prepared and field with 15 kg sandy clay loam soil. Before sowing the seed in the two seasons, soil samples were analyzed at Soil Testing Laboratory, Faculty of Agriculture, Fayoum University according to the standard published procedures (Wilde et al., 1985). Results of soil samples analysis are presented in Table 1.

EFFECT OF AMMONIUM NITRATE AND POTASSIUM HUMATE..... 105

Table (1): Some physical and chemical characteristics of the experimental site in two seasons.

Properties	2015	2016
Physical properties		
Clay (%)	27.3	25.7
Silt (%)	20.2	23.2
Fine sand (%)	52.5	51.1
Soil texture	Sandy Clay Loam	Sandy Clay Loam
Chemical properties		
PH	7.6	7.8
E _{Ce} (d _{sm} ⁻¹)	3.52	3.74
Organic matter (%)	2.56	2.86
Ca CO ₃ (%)	6.1	6.6
Cations (m. eq /L)		
Ca ⁺⁺	17.2	17.5
Mg ⁺⁺	6.8	6.5
N ⁺	13.2	13.6
K ⁺	0.8	0.7
Anions (m.eq/L)		
Cl ⁻	20.5	21.3
HCO ₃ ⁻	1.2	1.4
SO ₄ ⁻	16.3	17.1
Major elements (mg/L)		
N	14	13
P	22	20
K	72	69
Trace elements (mg/L)		
Fe	3.3	3.7
Zn	0.4	0.3
Mn	0.7	0.9

Four seeds were sown in each black plastic bag on 9th March, 2015 and 2016 seasons. After formation of 4 leaves, (about 45days) seedlings were thinned to one seedling per bag. All plants were fertilized with 3 g of super phosphate (15.5% P₂O₅) and 1 g of potassium sulphate (48% K₂O) . On the first of June, the plants were treated with Ammonium nitrate (33% N) at the rates of 1g (control), 2 and 4 g /plant. Potassium humate at rates of (0, 4, 8 and 12 g /plant) were applied as a soil drench for three times, monthly intervals and the first addition was done on 2th June, in the two seasons of study.

The experimental layout was a split-plot in randomized complete blocks design with three replicates each replicate was represented five pags. Nitrogen fertilizer rates were applied as the main plots whilst, potassium humate levels

were occupied the sub-plots and every treatment contained 5 plants. At the end of each growing season, (30th September) the following data were collected. Morphological characters, *i.e.* plant height (cm), number of branches plant⁻¹, stem diameter, number of leaves plant⁻¹, leaf area plant⁻¹ (cm²), fresh weight of the above – ground vegetative growth (g), dry weight of the above – ground vegetative growth (g), root fresh weight (g), root dry weight (g). Chemical analysis, *i.e.* chlorophyll A and B and carotenoids were calculated according to Moran (1982), leaf N content according to Hafez and Mikkelsen (1981), leaf P content according to A. O. A. C (1995), leaf K content according to Wilde *et al.* (1985), total carbohydrates content according to Herbert *et al.* (1971), total phenolics content according to (Ainsworth and Gillespie, 2007), total flavonoids content according to (Zhishen *et al.*, 1999) and total alkaloids content according to (Woo *et al.*, 1977). Appropriate analysis of variance was performed on results of each experiment. Comparisons among means of different treatments were performed using Least Significant Difference (L.S.D.) procedure at probability of 0.05 level as illustrated by Snedecor and Cochran (1980).

Results and Discussion

1. Morphological Characters:

1.1. Plant height (cm):

The data in Table (2) show that plant height increased as ammonium nitrate fertilizer rate increased and the most effective rate was 4 g / plant. This was true in the two seasons. This results were in accordance with Mohammadali *et al.* (2012) on tobacco plant (*Nicotiana tabacum* L.) and Ibrahim (2013) on Datura (*Datura innoxia* Mill.)

Table (2): Effect of nitrogen and potassium humate (KH) on plant height (cm) of *Calotropis procera* plant in two seasons.

Humic/g \ NH ₄ NO ₃ /g	first season (2015)				second season (2016)			
	1	2	4	Mean	1	2	4	Mean
0	42.7	46.9	50.7	46.8	39.7	42.6	47.4	43.2
4	57.5	61.8	71.2	63.5	46.0	50.8	59.7	52.1
8	65.0	74.9	77.8	72.6	57.9	64.4	71.0	64.4
12	71.3	77.4	78.9	75.9	63.8	67.1	72.5	67.8
Mean	59.1	65.2	69.6		51.8	56.2	62.6	
L.S.D 5%								
N	2.4				4.6			
KH	2.5				2.7			
N× KH	n.s				n.s			

EFFECT OF AMMONIUM NITRATE AND POTASSIUM HUMATE..... 107

The highest values were 75.9 and 67.8 cm obtained from plants treated with potassium humate at the highest rate (12g) per plant. This was true in the first and second seasons, respectively. Similar conclusions were reported by **Mohammadipour et al. (2012)** on (*Calendula officinalis* L.) plants, **Aisha et al. (2014)** on (*Brassica rapa*) plant and **Said-Al Ahl et al. (2016)** on dill plants.

The interaction between NH₄NO₃ and potassium humate was insignificant. The highest values were 75.9 and 72.5 obtained from plants treated with NH₄NO₃ at 4g and potassium humate 12g in the two seasons, respectively.

2. Number of branches per plant:

The tabulated data in Table (3) showed that when the rate of ammonium nitrate increased from 1g /plant to 2 g/ plant the number of branches per plant significantly increased but when the rate increased from 2 to 4 g/ plant the number of branches increased, but the increment not enough to be significant compared to 2g/ plant, while was significant compared with the lowest level (1g/ plant). This trend was obtained during the two seasons of the study. Similar conclusions were reported by **Rasmia et al., (2009)** on (*Catharanthus roseus* G. Don), **Azza et al. (2010)** on caraway (*Carum carvi* L.) plants, **Ahmadi (2010)** on (*Brassica napus* L.), **Rüveyde et al. (2011)** on fenugreek plants and **Ibrahim (2013)** on (*Datura innoxia* Mill.) plants.

Table (3): Effect of nitrogen and potassium humate (KH) on number of main branches /plant of *Calotropis procera* plant in two seasons.

Humic/g \ NH ₄ NO ₃ /g	first season (2015)				second season (2016)			
	1	2	4	Mean	1	2	4	Mean
0	2.4	3.1	3.4	3.0	2.5	2.7	2.9	2.7
4	3.4	4.0	4.7	4.0	3.1	3.6	4.0	3.6
8	3.9	4.8	5.7	4.8	3.4	4.7	5.0	4.4
12	4.9	5.5	5.6	5.3	3.8	5.0	5.1	4.6
Mean	3.7	4.3	4.8		3.2	4.0	4.3	
L.S.D 5%								
N	0.6				0.5			
KH	0.5				0.3			
N× KH	0.6				0.6			

Regarding the effect of potassium humate, there was a significant positive relationship in the first season between the levels of potassium humate and the number of branches per plant. As the levels of potassium humate increased the number of branches / plant increased significantly. In the second season, adding 4 or 8 g/plant of potassium humate caused a gradual significant increase in number of branches on the plant compared with the control, while adding 12 g /plant caused not significant increase in the number of branches compared with 8

or 4 g in the same time in which was significant compared to the untreated plants. The same trend was concluded by **Gomaa and Youssef (2008)** on caraway plants, **Bhatti et al.(2011)** on mustard plant, and **Mohammadipour et al. (2012)** on (*Calendula officinalis* L.) plants.

The interaction between NH_4NO_3 and potassium humate was significant. The most effect treatment in this respect was NH_4NO_3 at the rate of 4g and potassium humate at the rate of 8g in the first season, while in the second seasons. The most effect treatment in this respect was NH_4NO_3 at the rate of 4g/ plant and potassium humate at the rate of 812g/ plant.

3. Number of leaves per plant:

The results in Table (4) showed that the highest number of leaves per plant were (48 and 47 leaves) recorded in the seasons of 2015 and 2016, respectively with the treatment of the highest rate of ammonium nitrate (4g / plant). Similar results were reported by **Hosseini et al. (2011)** on (*Nicotiana tabacum* L.) plants, **Mohammad Ali et al. (2012)** on tobacco plants, **Gholizadeh et al. (2012)** on tobacco plants, **Ibrahim (2013)** on datura (*Datura innoxia* Mill.) plants and **Shafeek et al. (2016)** on (*Cucurbita pepo* cv. *Eskandarani*) plants.

Concerning to the effect of potassium humate treatment, the results clearly showed that all treatments with potassium humate significantly increased the number of leaves per plant and the highest level of potassium humate (12g / plant) recorded the highest number of leaves on the plant (51.4 and 49.0 leaves /plant) compared with the untreated plants (control) in the 2015 and 2016 seasons, orderly. These results agreed with those obtained by **Mohammadipour et al., (2012)** on (*Calendula officinalis* L.) plants, **Aisha et al. (2014)** on (*Brassica rapa*) plant, **Zeinali et al., (2015)** on garlic (*Allium sativum* L.) plants, and **Nadia et al. (2015)** on okra (*Hibiscus Esculentus*) plants.

The interaction between NH_4NO_3 and potassium humate was significant. The highest values were 58.7 and 57.9 obtained from plants treated with NH_4NO_3 at 4g/ plant and potassium humate 12g/plant in the two seasons, respectively.

EFFECT OF AMMONIUM NITRATE AND POTASSIUM HUMATE..... 109

Table (4): Effect of nitrogen and potassium humate (KH) on number of leaves / plant of *Calotropis procera* plant during the two seasons.

Nitrogen /g Humic/g	first season (2015)				second season (2016)			
	1	2	4	Mean	1	2	4	Mean
0	20.6	23.0	27.3	23.6	23.4	28.3	31.1	27.6
4	28.1	38.4	47.8	38.1	26.8	34.2	43.0	34.7
8	35.7	51.0	58.2	48.3	28.9	50.3	55.8	45.0
12	41.0	54.5	58.7	51.4	36.3	52.7	57.9	49.0
Mean	31.3	41.7	48.0		28.9	41.4	47.0	
L.S.D 5%								
N	3.2				1.7			
H	2.4				2.0			
N×KH	4.2				3.5			

4. Plant fresh weight (g)

Data in Table (5) showed that the highest the rate of fertilizer, the highest the fresh weight of the plant, so, addition of the highest rate of ammonium nitrate (4 g/ plant) produced 167.5 and 162.4 g / plant in 2015 and 2016 seasons, respectively.

In addition, a gradual increase of potassium humate was met with a significant gradual increase of the plant fresh weight. The highest level of potassium humate treatment (12 g / plant) produced the highest fresh weight of the vegetative foliage (188.3 g and 184.7g) in 2015 and 2016 seasons, respectively. Similar results were reported by **Renata et al. (2012)** on (*Ocimum basilicum L.*) Plants, **Ibrahim (2013)** on datura (*Datura innoxia Mill.*) plants, **Soha and Rabia (2014)** on (*Hibiscus sabdariffa L.*) and **Shafeek et al. (2016)** on summer squash plants.

Table (5) : Effect of nitrogen and potassium humate (KH) on plant fresh weight (g) of *Calotropis procera* plant in two seasons.

NH ₄ NO ₃ /g Humic/g	first season (2015)				second season (2016)			
	1	2	4	Mean	1	2	4	Mean
0	58.5	90.8	85.3	78.2	44.9	78.0	81.4	68.1
4	93.4	146.6	184.2	141.4	98.2	152.3	177.5	142.7
8	138.5	191.8	198.2	176.1	129.7	179.7	190.9	166.8
12	164.2	198.4	202.2	188.3	169.1	185.2	199.8	184.7
Mean	113.6	156.9	167.5		110.5	148.8	162.4	
L.S.D 5%								
N	7.98				3.53			
KH	4.58				5.77			
N× KH	7.94				10.00			

The interaction between NH₄NO₃ and potassium humate was significant. The most effect treatment in this respect was NH₄NO₃ at the rate of 4g / plant and potassium humate at the rate of 12 g/ plant. The most values were 202.2 and 199.8 in the two seasons, respectively.

5. Plant dry weight (g):

The results in Table (6) showed a significant increase in the dry weight of plant foliage with an increase in ammonium nitrate rate addition. The treatment with the highest rate of ammonium nitrate (4 g / plant) recorded the highest dry weight of plant foliage. The highest values were 37.5 and 37.1 g in the two seasons, respectively.

As for the effect of potassium humate, the results showed that the highest dry weight of the plant foliage were 40.8 and 39.9 g recorded from plants treated with potassium humate at 12g / plant during 2015 and 2016, respectively. These results agreed with those obtained by **Gholizadeh et al. (2012)** on tobacco, **Renata et al. (2012)** on (*Ocimum basilicum* L.) Plants, **Ibrahim (2013)** on datura (*Datura innoxia* Mill.) plants, **Hendawy et al . (2015)** on (*Mintha piperita*) var. Citrata and **Ebrahimi and Miri (2016)** on (*Borago officinalis*) and (*Cichorium intybus*) plants.

EFFECT OF AMMONIUM NITRATE AND POTASSIUM HUMATE..... 111

Table (6): Effect of nitrogen and potassium humate (KH) on dry weight of the above – ground vegetative growth (g)/ plant of *Calotropis procera* plant in two seasons.

NH₄NO₃/g Humic/g	first season (2015)				second season (2016)			
	1	2	4	Mean	1	2	4	Mean
0	14.0	19.0	23.2	18.7	15.2	18.2	21.4	18.3
4	19.7	25.5	35.5	26.9	20.3	25.0	34.9	26.7
8	29.1	36.3	45.3	36.9	28.3	35.2	46.5	36.7
12	32.4	43.8	46.2	40.8	31.6	42.7	45.4	39.9
Mean	23.8	31.1	37.5		23.9	30.3	37.1	
L.S.D 5%								
N	1.1				1.5			
KH	1.0				1.2			
N× KH	1.7				2.1			

The interaction between NH₄NO₃ and potassium humate was significant. The highest values was 46.2 obtained from plants treated with NH₄NO₃ at 4g/ plant and potassium humate 12g/plant in the first seasons, while in the second season was 46.5 obtained from plants treated with NH₄NO₃ at 4g/ plant and potassium humate 8g/plant.

This results may due to that nitrogenous and potassium humate fertilizer increase the nutrients uptake by plants and consequently increased all vegetative growth characteristic such as plant height (cm), number of branches per plant, number of leaves per plant , plant fresh weight (g), plant dry weight (g).

2. Chemical constituents

2.1. Total phenolic content:

Data in Table (7) show that the highest value of total phenolic content was 7.60 mg/g dry weight that obtained from plants treated by ammonium nitrat at 1g / plant, while the lowest record was obtained from plants treated with the highest rate of ammonium nitrate (4g/plant) during second season of study.

Table (7): Effect of nitrogen and potassium humate (KH) on total phenolic content in leaves of *Calotropis procera* plant(mg / g dry weight) during the second season (2016).

Humic/g \ NH ₄ NO ₃ /g	second season (2016)			
	1	2	4	Mean
0	8.45	6.58	6.16	7.07
4	8.32	6.68	6.20	7.07
8	7.01	7.10	5.79	6.63
12	6.60	7.82	5.77	6.73
Mean	7.60	7.05	5.98	
L.S.D 5%				
N	0.16			
KH	0.16			
N× KH	0.27			

Regarding to effect of potassium humate, the results showed that the treatment with potassium humate at the lowest level (0 g / plant) showed the highest content of total phenolic content (7.07 mg /g dry weight) as compared to fertilization with the highest rate (12g / plant). Our results were in harmony with many investigators such as **Phuong and Emily (2008)** on basil (*Ocimum basilicum* L.) plants, **Ibrahim et al. (2011)** on *Labisia pumila* Benth plants and **Munene et al. (2017)** on two amaranth varieties.

The interaction between NH₄NO₃ and potassium humate was significant. The most effect treatment in this respect was NH₄NO₃ at the rate of 0g / plant (control) and potassium humate at the rate of 0 g/ plant (control). The most values was 8.45 mg / g dry weight in the second seasons.

2.2. Total flavonoids content:

The results presented in Table (8) show that a significant decrease was obtained in total flavonoids content of plants with each level increase in ammonium nitrate addition. The treatment with the lowest rate of ammonium nitrate (1g / plant) recorded the highest total flavonoids content (8.41 mg / g dry weight) in plants. This trend was noted in 2016.

Table (8): Effect of nitrogen and potassium humate (KH) on total flavonoids content in leaves of *Calotropis procera* plant (mg / g dry weight) during the second season (2016).

Humic/g \ NH ₄ NO ₃ /g	second season (2016)			
	1	2	4	Mean
0	9.86	6.21	5.65	7.24
4	8.46	7.21	6.07	7.25
8	7.88	7.77	7.70	7.78
12	7.42	6.42	6.06	6.64
Mean	8.41	6.90	6.37	
L.S.D 5%				
N	0.34			
KH	0.40			
N× KH	0.69			

As regard to the effect of potassium humate , the results showed that the highest value of total flavonoids content was 7.78 mg /g dry weight that obtained from plants treated by potassium humate at 8g / plant. While, the lowest record was 6.64 mg /g dry weight, which obtained from plants treated with the highest rate of potassium humate at 12g / plant. Our results were in harmony with many investigators such as **Phuong and Emily (2008)** on *basil (Ocimum basilicum L.)* plants, **Ibrahim et al. (2011)** on *Labisia pumila* Benth plants and **Munene et al. (2017)** on two amaranth varieties.

The interaction between NH₄NO₃ and potassium humate was significant. The most effect treatment in this respect was NH₄NO₃ at the rate of 0g / plant (control) and potassium humate at the rate of 0 g/ plant (control). The most values was 9,86 mg / g dry weight in the second seasons. This trend was the same trend in total phenolic content.

2.3. Total alkaloids content:

The results in Table (9) showed that the highest rate (4 g/plant) of produced the highest values of total alkaloids content (0.781 g /100 g dry weight) in the season 2016 as compared to fertilization with the other rates (1 and 2 g / plant).

Table (9): Effect of nitrogen and potassium humate (KH) on total alkaloids content in leaves of *Calotropis procera* plant (g /100 g dry weight) during second season (2016).

Humic/g \ NH ₄ NO ₃ /g	second season (2016)			
	1	2	4	Mean
0	0.500	0.650	0.685	0.611
4	0.629	0.755	0.765	0.716
8	0.688	0.793	0.825	0.751
12	0.757	0.805	0.849	0.804
Mean	0.644	0.751	0.781	

Data also show that the gradual increase of potassium humate was met with a gradual increase of leaf total alkaloids content. The highest level of potassium humate treatment (12 g / plant) produced the highest values of total alkaloids content (0.804 g /100 g dry weight). These results agreed with those obtained by **Hosseini et al. (2011)** on (*Nicotiana tabacum* L.) plants, **Ibrahim (2013)** on datura plants, **Zinab and Rabee (2016)** on leaves of *Catharanthus roseus* and **Kizil et al. (2017)** on two henbane species (*Hyoscyamus reticulatus* L. and *Hyoscyamus niger* L.).

The interaction between NH₄NO₃ and potassium humate was significant. The highest value was 0.849 obtained from plants treated with NH₄NO₃ at 4g/ plant and potassium humate 12g/plant in the second seasons.

From the above mentioned results, it could be recommended to cultivate this wild plant in a systematic culture for producing the active principles including alkaloids and antioxidant materials including total phenolics and total flavonoids . Fertilizing this plant with nitrogenous and potassium humate should taken into our account to promote the vegetative growth and consequently chemical constituents.

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EFFECT OF AMMONIUM NITRATE AND POTASSIUM HUMATE..... 115

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EFFECT OF AMMONIUM NITRATE AND POTASSIUM HUMATE..... 117

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تأثير نترات الامونيوم وحامض الهيوميك علي النمو الخضري والمكونات الكيميائية في نبات العشار
أ.د / شكري محمود سليم*، د/ محمود علي حسنين*، أ.د/ هند السيد وهبة**، وفاء محمد عبد السلام
عبد اللطيف

استاذ الزينة و النباتات الطبية والعطرية المتفرغ – قسم البساتين – كلية الزراعة – جامعة الفيوم*،
استاذ الزينة و النباتات الطبية والعطرية المساعد – قسم البساتين – كلية الزراعة – جامعة الفيوم*،
أستاذ النباتات الطبية والعطرية بالمركز القومي للبحوث**

الخلاصة

أجريت هذه الدراسة علي نبات العشار في مزرعة خاصة بقرية تزممت الشرقية – بمحافظة بني سويف- مصر، خلال موسمين متتاليين ٢٠١٥-٢٠١٦ ويهدف البحث لإختبار تأثير التسميد بنترات الامونيوم (٣٣% ازوت) كمصدر للنيتروجين وهيومات بوتاسيوم علي النمو الخضري والمكونات الفعالة لنبات العشار (*Calotropis procera*).

حيث أستخدم سمد نترات الامونيوم بثلاث معدلات (١، ٢، ٤ جم/ نبات)، كما أستخدمت هيومات بوتاسيوم بمعدلات (صفر، ٤، ٨، ١٢ جم/ نبات).

يمكن تلخيص النتائج المتحصل عليها في الآتي :

أدي إستخدام التسميد بنترات الامونيوم و هيومات البوتاسيوم بمفردهما، أو متداخلان معا في تغذية نبات العشار إلي صفات النمو الخضري وهي (إرتفاع النبات ، سمك الساق، عدد الفروع/ نبات، عدد الأوراق / نبات، مساحة الورقة، الوزن الطازج والجاف لأجزاء النبات المختلفة من جذور وسيقان وأوراق)، مقارنة بالنباتات غير المعاملة خلال موسمي الدراسة .

أدت معاملة نبات العشار بالتسميد بنترات الامونيوم أو حمض الهيوميك بمفردهما أو متداخلان معا إلي زيادة في مكونات النبات الكيماوية مثل صبغات النبات المختلفة (كلوروفيل أ، ب و الكاروتينيدات الكلية)، الكربوهيدرات الذائبة الكلية ، وكذا محتوى الأوراق من المغذيات الكبرى (نيتروجين، فوسفور، بوتاسيوم كنسبة مئوية)، مقارنة بالنباتات غير المسمدة خلال موسمين الدراسة. أدي تطبيق التسميد بنترات الامونيوم و هيومات البوتاسيوم بمفردهما، أو متداخلان معا في تغذية نبات العشار إلي نقص في محتوى الفينولات الكلية و نقص في محتوى الفلافونيدات الكلية في أوراق نبات العشار خلال موسم ٢٠١٦، كما أدي تطبيق الأسمدة إلي زيادة محتوى الأوراق من القلويدات الكلية خلال موسم ٢٠١٦، مقارنة بالنباتات غير المسمدة خلال موسم ٢٠١٦.