

## Effect of Nitrogen Fertilization on Rocket Plants Tolerance to Cadmium and Their Chemical Composition

<sup>1</sup>Abou Hussein, E.A. and <sup>2</sup>Salwa AR. Hammad

<sup>1</sup>Soil Sci. Dept. and <sup>2</sup>Agric. Botany Dept., Faculty of Agric., Minufiya Univ., Shibin El-Kom, Egypt.

**Abstract:** This research was carried out to study the effect of both individual and combined treatments of N fertilization (rates and forms) and cadmium on rocket plants growth, tolerance to high levels of Cd and chemical composition of the plants. So, pots experiment was carried out on alluvial clay soil at the Experimental Farm, Fac. of Agric., Minufiya Univ., for two seasons (2007 and 2008). Nitrogen fertilizers were used as calcium nitrate ( $\text{NO}_3^-$ -N) and ammonium sulphate ( $\text{NH}_4^+$ -N) at application rates of 0, 100 and 200% of the recommended dose (RD) of N for rocket plant, where Cd was applied as cadmium acetate at rates of 0, 100, 150 and 300% of Cd toxic level. The obtained data show a significant increase of fresh and dry matter yield, plant height, leaves area and the content of N with the increase of added N. These increases were more clear with the treatments of  $\text{NH}_4^+$ -N compared to those resulted from  $\text{NO}_3^-$ -N treatments. Also, the values of dry matter relative increase of rocket plants fertilized by  $\text{NH}_4^+$  were higher than those of plants fertilized by  $\text{NO}_3^-$ -N. Also, agronomic efficiency of  $\text{NH}_4^+$ -N fertilizer was higher than that of  $\text{NO}_3^-$ -N fertilizer. On the other hand, increasing the added N resulted in a decrease of plant concentrate of Cd, where this decrease was more clear with the treatments of  $\text{NH}_4^+$ -N. Increasing the added Cd resulted in a significant decrease of fresh weight, plant height, leaves area, dry matter yield and its relative increase and N content, where such decreases were lower in the plants fertilized by  $\text{NH}_4^+$ -N as compared with those fertilized by  $\text{NO}_3^-$ -N. On the other hand, increasing the added Cd resulted in a clear increase of plant content of Cd and thus a decrease of Cd agronomic efficiency. In this respect, the obtained results were similar for both shoots and roots in the two growth seasons. All rates of Cd significantly decreased photosynthetic pigments, total soluble sugars and total free amino acids in leaves, however the high level showed a negative effect on these chemical contents. Adding N fertilizers to plants decreased the harmful effect of Cd and increased the mentioned parameters. These increases were more pronounced with the application of  $\text{NH}_4^+$ -N as compared with  $\text{NO}_3^-$ -N. Application of Cd increased the enzymatic activities (peroxidase and polyphenoloxidase) and proline concentration in leaves, compared to the control treatments, while the different N fertilization sources and rates treatments decreased the effect of Cd. Generally,  $\text{NH}_4^+$ -N at the highest rate gave the best results as compared with  $\text{NO}_3^-$ -N. From the obtained data, it could be recommended that, in the soils contaminated by Cd must be fertilized by  $\text{NH}_4^+$ -N at high rates to reduce the harmful and toxic effect of Cd on plants.

**Key words:** Rocket, Nitrogen, Cadmium, Vegetation parameters, Chemical composition.

## INTRODUCTION

Rocket (*Eruca vesicaria* sub sp. *sativa*) plant fresh leaves are consumed by the Egyptian public as a fresh green salad. Besides its importance as a green salad, it is used as a source of a medicinal oil.

Cadmium is one of several metals that has become in focus in recent years as environmental contaminants harmful to human health. The big concern in Cd pollution is due to application of sewage sludge to soil and thus enters the food chain through uptake by plants<sup>[1,2]</sup>. Moreover, phosphate fertilizers are a main source of metal contamination in modern agriculture, which contain discrete amounts of heavy metals especially cadmium impurities<sup>[3]</sup>. Cadmium causes a number of toxic symptoms in plants, e.g. growth reduction, inhibition of photosynthesis, induction of enzymes, altered stomatal action, efflux of cations and generation of free radicals<sup>[4]</sup>. Page *et al.*<sup>[5]</sup> reported that, at 0.1 mg/l Cd concentration in nutrient solution, the concentration of Cd in plant leaves of different crops varied between 9 and 90 mg/kg for bean and corn respectively.

Since the vertical expansion in the agricultural area is very important in Egypt, nitrogen (N) fertilization is used in large quantities. Source and rate of N fertilization are the most important nutritional factors affecting the growth, flowering and chemical constituents of plants. Many authors studied the effect of different rates of calcium nitrate and ammonium sulphate on various vegetative growth traits and herb weight of different herbaceous plants and obtained positive responses (Badran *et al.*)<sup>[6]</sup> on guar and (Melegy)<sup>[7]</sup> on corn plants.

The main aims of the present work were to study: (1) The effect of both different forms and application rates of N fertilizers; (2) The effect of different application rates of Cd and (3) The combined effect of both N and Cd on: a) Plant growth, b) The content of N and Cd on rocket plant growth and contents on some chemical constituents and physiological parameters of plants as well as the hazardous effect of high rates of Cd.

## MATERIALS AND METHODS

A pot experiments were conducted at the greenhouse of the Experimental Farm, Faculty of Agriculture, Minufiya University, Shibin El-Kom, during the winter seasons of 2007 and 2008 to study the individual and the combined effects of both different forms and application rates of nitrogen

fertilization under different levels of Cd, on rocket plant growth and composition.

An alluvial clay soil was used soil in this study. Soil samples were collected from the surface layer (0 – 20 cm), air dried, ground, good mixed, sieved through a 2- mm<sup>2</sup> sieve and analyzed for some physical and chemical properties and also for its contents of some nutrients and cadmium, according to the standard methods of soil analysis<sup>[8-10]</sup>. The obtained data were recorded in Table (1a, b and c).

Plastic pots (72 pot) of 25 cm inner diameter and 20 cm depth were used in this study. Each pot was filled with 4.5 kg of served alluvial soil. The pots were planted by rocket (*Eruea vesicaria* subsp. *sativa*) seeds of local Egyptian cv. "Balady" on 25<sup>th</sup> January, each year of 2007 and 2008, at the rate of 0.5 g seeds per pot.

**Experiment Design:** The used pots were divided into two main groups (36 pot / main group). The pots of each main group were divided into three sub groups (12 pot / sub group). The pots of the first three sub groups were fertilized by calcium nitrate [Ca (NO<sub>3</sub>)<sub>2</sub>], as a nitrate form of nitrogen (20% N) at application rates of 0, 100 and 200% of the recommended dose of nitrogen [0, 2.64 and 5.28 g Ca (NO<sub>3</sub>)<sub>2</sub> / pot], respectively. The other three sub groups were fertilized by ammonium sulphate [(NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub>], as on ammonium form of nitrogen (21.5% N) at the prementioned percentages of the recommended dose of nitrogen which equals 0, 2.12 and 4.24 g fertilizer / pot, respectively. The pots of each sub group were further divided into four sub sub group (3 pots / sub sub group). The pots of each sub sub groups were treated by one of the tested Cd levels. Cadmium was added as cadmium acetate Cd (CH<sub>3</sub> COO)<sub>2</sub>. The tested Cd levels were 0, 100, 150 and 300% of the toxic level of Cd which equals 0, 75.98, 151.18 and 303.37 mg Cd (CH<sub>3</sub> COO)<sub>2</sub> / pot, respectively. Before planting, the pots were fertilized by superphosphate (15.5% P<sub>2</sub>O<sub>5</sub>) as phosphorus fertilizer at the recommended dose of P. Also, the pots were fertilized by potassium sulphate (48% K<sub>2</sub>O) at the recommended dose of K after 15 days of planting. The treatments of Cd were carried out after 7 days of planting with irrigation water. The treatments of N were carried out after 20 days of planting. The pots were moistened by tap water every three days up to the level of 60% of WHC of the used soil. Before planting, all pots were fertilized by calcium superphosphate (15.5% P<sub>2</sub>O<sub>5</sub>) as P fertilizer at rate of 300 kg / fed. (1.35 g/pot). After 15 days of planting potassium sulphate (48% K<sub>2</sub>O) was added to

**Table 1:** Analytical data of the studied soil.

a) Physical properties:													
Particles size distribution (%)						Texture grade	WHC (%)						
C. sand		F. sand		Silt	Clay								
7.9		13.9		33.2	45.0	Clayey	62						
b) Chemical properties:													
pH (2.5 soil/water sus.)		EC (dSm <sup>-1</sup> )	Soluble cations (meq / 100 g)				Soluble anions (meq / 100 g)				OM (%)	CaCO <sub>3</sub> (%)	CEC (meq/100g)
7.2		0.83	Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Cl <sup>-</sup>	CO <sub>3</sub> <sup>2-</sup>	HCO <sub>3</sub> <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	2.25	2.15	41.5
			5.2	0.6	1.6	0.9	6.5	0.0	0.3	1.5			
c) Contents of available nutrients and cadmium:													
Macronutrients (mg/kg)						Micronutrients (mg/kg)				Cd			
N	P	K		Fe	Mn	Zn	Cu						
8.90	1.50	750		2.75	2.21	0.80	0.50		0.15				

each pot at rate of 200 kg/fed. (0.9 g/pot). The pots were arranged in a split block design with three replicates. Nitrogen forms and their application rates represented the main plot (A), where the Cd treatments represented the sup plot (B).

After 60 days from sowing, the plant materials were harvested as a whole plant from each pot. The roots of the harvested plants were separated from shoots. The fresh weight of both shoots and roots were measured. Fresh materials of each pot were divided at a ratio of 3 : 1. The first one was taken to determine the dry matter yield (DMY). The oven dried materials were finely ground and used for some chemical analysis. On the other hand, the following measurements were made on the other portion of the fresh materials.

**1. Vegetation Parameters:** Plant height (cm), fresh weight of shoots, roots and whole plants (g / pot) were recorded. Leaves area (cm<sup>2</sup> / pot) using the dry weight method, according to Aase<sup>[11]</sup>.

**2. Dry Matter Yield of Plants:** Plant samples were oven-dried at 70°C for about 48 h to determine the dry matter yield, then the relative increase (RI%) was calculated by the next equation:

$$RI (\%) = \frac{\text{DMY of treated plants} - \text{DMY of untreated plants}}{\text{DMY of untreated plants}} \times 100$$

Also, the agriculture efficiency (AE) was calculated according to the equation of Sisworo *et al.*<sup>[12]</sup>, as follows:

$$AE = \frac{\text{DMY of treated plants} - \text{DMY of untreated plants}}{\text{Added (N or Cd) as (g or mg / kg)}}$$

### 3. Physiological Aspects of Plants:

- Photosynthetic pigments: Chlorophyll a + b and carotenoids were determined in fresh leaves by the method described by Moran<sup>[13]</sup>.
- Total soluble sugars (TSS), total amino acids (TAA) and proline concentrations: TS and TAA in dry leaves were determined using the methods of Dubois *et al.*<sup>[14]</sup> and Rosen,<sup>[15]</sup> respectively. Proline concentration in fresh leaves was measured using the method described by Bates *et al.*<sup>[16]</sup>.
- Enzyme activities: Peroxidase and phenoloxidase activities were measured in fresh leaves using the methods described by Fehrman and Dimond<sup>[17]</sup> and Broesh<sup>[18]</sup>, respectively.

**4. Chemical Composition of Plants:** A 0.2 g of each oven-dried, plant sample was digested using 5 ml of acid mixture: solution (3 : 1, conc. H<sub>2</sub>SO<sub>4</sub> : conc. HClO<sub>4</sub>) until the digestion become clear<sup>[9]</sup>. The digestion was diluted using distilled water up to volume of 100 ml. The concentration of both N and Cd was determined according to the standard methods<sup>[9,10]</sup>. The obtained data were statistically analyzed according to Gomez and Gomez<sup>[19]</sup>.

## RESULTS AND DISCUSSION

**1. Vegetation Parameters of Rocket Plants:** The presented data in Tables (2 and 3) show plant height and leaves area of rocket plants as affected by the studied treatments of N and Cd. The measured vegetation parameters were increased significantly with the increase of added N. These increases were more significant with the treatments of NH<sub>4</sub><sup>+</sup> -N form. These results reveal that, NH<sub>4</sub><sup>+</sup> -N form enhanced the plant growth more than that of NO<sub>3</sub><sup>-</sup> -N<sup>[7]</sup>. The

**Table 2:** Plant height (cm) and leaf area (cm<sup>2</sup> / pot) of rocket plant as affected by the studied treatments of Cd and N fertilization.

Growth season	Added Cd (% of toxic level)	Plant height (cm)					Leaves area (cm <sup>2</sup> / pot)						
		Control	Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		Control	Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>			
			100	200	100	200		100	200	100	200		
First	0	19.00	22.32	24.00	25.00	26.00	106.30	13.70	17.70	19.60	20.33		
	100	16.33	21.00	22.00	23.33	24.00	105.90	146.31	149.01	178.90	186.50		
	150	15.00	20.00	21.00	22.00	23.00	98.90	137.40	146.10	104.80	172.70		
	300	11.00	15.00	16.00	17.00	18.00	74.60	109.10	117.70	121.70	124.40		
L.S.D 5%		A = 0.94		B = 0.77		AB = 1.28		A = 3.82		B = 3.94		AB = 3.75	
Second	0	18.33	23.11	26.70	30.05	31.61	119.50	162.10	180.50	193.70	207.40		
	100	19.10	22.65	24.01	28.50	29.11	95.40	103.30	115.10	166.10	182.30		
	150	15.25	21.33	22.00	23.00	24.50	82.30	130.40	147.20	162.10	170.50		
	300	14.11	14.65	15.67	16.33	17.00	60.10	97.30	108.80	113.80	115.30		
L.S.D 5%		A = 0.65		B = 0.96		AB = 0.95		A = 3.35		B = 2.85		AB = 5.529	

**Table 3:** Fresh matter yield (g / pot) of rocket plant (roots and shoots) affected by the studied treatments.

Growth season	Added Cd (% of toxic level)	Roots					Shoots						
		Control	Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		Control	Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>			
			100	200	100	200		100	200	100	200		
First	0	1.92	2.00	2.18	2.64	2.36	18.70	20.60	27.14	29.26	32.00		
	100	1.74	1.98	2.03	2.11	2.50	16.70	19.95	20.56	21.93	22.00		
	150	1.67	1.90	2.00	2.05	2.50	14.57	18.95	19.50	20.10	21.00		
	300	1.64	1.70	1.75	1.85	1.90	13.67	15.60	16.10	17.00	17.50		
L.S.D 5%		A = 0.05		B = 0.05		AB = 0.08		A = 0.54		B = 0.49		AB = 0.81	
Second	0	2.37	2.63	2.70	2.98	3.55	21.25	23.11	26.75	30.05	31.61		
	100	1.92	2.46	2.56	2.70	3.08	19.11	22.65	24.01	28.50	29.11		
	150	1.77	2.40	2.23	2.51	2.80	15.25	21.75	23.15	26.01	27.35		
	300	1.65	1.82	2.09	2.18	2.20	14.11	15.44	17.01	17.92	18.31		
L.S.D 5%		A = 0.04		B = 0.04		AB = 0.04		A = 0.56		B = 0.24		AB = 0.67	

high positive effect of NH<sub>4</sub><sup>+</sup>-N compared to NO<sub>3</sub><sup>-</sup>-N may be resulted from NH<sub>4</sub><sup>+</sup>-N adsorption by the negative charges on both soil colloids and plant roots, while NO<sub>3</sub><sup>-</sup>-N may be removed or leached from rhizosphere with irrigation water<sup>[7,20]</sup>. Effect of the tested N fertilization treatments on the measured vegetation parameters was similar in both growth seasons. These results are in agreement with those obtained by Martinetti *et al.*<sup>[21]</sup>, Abdel-Ati and Abbas<sup>[22]</sup> on lettuce, Abou Hussien and Barsoum<sup>[23]</sup> on some oil crops and Melegy<sup>[7]</sup> on corn.

Data of the effect of the studied, Cd treatments on plant fresh weight, plant height and leaves area, recorded in Tables (2 and 3) show that, these

parameters were decreased significantly with the increase of added Cd individually, where the highest negative effect was found with 300% of Cd toxic level in the two growth seasons. Also, these data show that the tested levels of Cd had an inhibitory effect on rocket plants growth. Similar results had been obtained by El-Gamal<sup>[24]</sup> on spinach and pea, Tantawy<sup>[25]</sup> on sesame, sorghum and pea and El-Chinbihi<sup>[26]</sup> on wheat. The interactions among N and Cd treatments on the studied vegetative parameters, as recorded in Tables (2 and 3), show that, the obtained values of these parameters lied in the intermediate level of each individual treatment of either of N or Cd. Hence, it could be concluded that, N fertilization reduced the harmful and toxic effect of Cd on the studied vegetation parameters of rocket plants. This effect was more pronounced with increasing the added N. Also, this beneficial effect of N fertilization may be resulted from the enhancement of rocket plant growth. The

obtained data also show that,  $\text{NH}_4^+$ -N form reduced the harmful effect of Cd more than that  $\text{NO}_3^-$ -N, due to  $\text{NH}_4^+$  ions adsorption by the negative charges of roots, which in turn reduces the absorbed amounts of Cd<sup>[27]</sup>.

**2. Dry Matter Yield of Rocket Plants:** The presented data in Tables (4 to 6) show the effect of the studied treatments of N and Cd on rocket dry matter yield (roots and shoots) and the relative change and agronomic efficiency of N and Cd. The obtained data show that, increasing the amounts of both forms of N resulted in a clear and significant increase of rocket plants, where these increases were more greater with

the treatments of  $\text{NH}_4^+$ -N. This superior effect of  $\text{NH}_4^+$ -N resulted from the absorbed amounts of  $\text{NH}_4^+$  compared to  $\text{NO}_3^-$ . This appeared clearly from the calculated values of dry matter RI (%) of both  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N. The values of RI increased with the increasing the added N ( $\text{NO}_3^-$  or  $\text{NH}_4^+$ ). These results are in agreement with the findings of Abou Hussein and Barsoum<sup>[22]</sup> and Melegy<sup>[7]</sup>.

Dry matter yield (roots and shoots) of rocket plants and its RI were negatively affected by the individual treatments of Cd and its combination with N fertilizers, where the lowest values were associated the individual treatments with 300% of Cd toxic level (Tables 4 to 6). This negative effect of Cd was reduced with increasing the amounts of applied of N fertilizers, particularly with  $\text{NH}_4^+$ -N. This trend was found in

the two growth seasons for both shoots and roots. Also, the negative effect of the Cd treatments on rocket plants may be supported by the calculated values of the relative and absolute changed of dry matter yields (Tables 4, 5) which were more negative with the individual treatments of Cd, followed by those added

with low levels of  $\text{NO}_3^-$ -N, then with  $\text{NH}_4^+$ -N.

This may be resulted from the competition between  $\text{Cd}^{2+}$  and  $\text{NH}_4^+$  ions in absorption on the negative

charges of roots. In general, such effect of Cd could be due to the disturbance of enzyme activities as, explained by Mengel and Kirkbly<sup>[28]</sup>. Similar effects of Cd on plant dry matter yield had been found by Kovacevic *et al.*<sup>[29]</sup>, El-Shikha<sup>[30]</sup>, El-Kassas *et al.*<sup>[31]</sup> and Tantawy<sup>[25]</sup>.

Data in Tables (4 to 6) show that, in both growth seasons, changes of the dry matter yield of roots were higher than those of shoots. These results may be attributed to the lower yield of roots of the untreated plants, as compared with those of shoots of the same plants. These results are in agreement with the findings of Abou El-Khir *et al.*<sup>[32]</sup>

Agronomic efficiency (AE) of both N fertilizers and Cd treatments, recorded in Tables (7 and 8), show that, the highest values of AE were found with the

individual treatments of  $\text{NH}_4^+$ -N followed by those of  $\text{NO}_3^-$ -N, where the lowest values were resulted

from the individual treatments of Cd. Application Cd at different levels reduced AE values of N fertilizer treatments, where this decrease was more clear with the

treatments of  $\text{NO}_3^-$ -N compared to those of  $\text{NH}_4^+$ -N. This trend was found for both roots and

shoots in the two growth seasons. Also, the obtained data show that, AE of both shots and roots decreased with increasing of added N or Cd. Also, with the different treatments under study, the values of AE of shoots were higher than those of roots. The values of AE also reveals that, the adverse effect of Cd on plant growth greatly differed from shoots to roots, where this effect being more obvious on roots than on shoots, presumably due to its effect on plasma membrane of root cells<sup>[33]</sup>. These results are in agreement with those obtained by Tantawy<sup>[25]</sup> and Abou El-Khir *et al.*<sup>[32]</sup>.

### 3. Physiological Aspects of Rocket Plants (Biochemical Constituents):

**A) Photosynthetic Pigment Contents:** Data given in Table (9) indicate that, total chlorophyll (Chl. a + b) and carotenoids contents significantly decreased with increasing cadmium levels, as compared with control in both seasons. Application of higher Cd rate (300% of toxic level) reduced "Chl. a + b" by 61.93 and 59.92%, as well as carotenoids by 46.71 and 54.54% in the first and second seasons, respectively. These results are confirmed by Gil *et al.*<sup>[34]</sup>, Shenker *et al.*<sup>[35]</sup> on wheat and barley and El-Ghinbihi<sup>[26]</sup> on wheat. The mechanism of Cd toxicity on photosynthetic pigments were attributed to inhibition of the biosynthesis of the aminolevulinic acid (ALA), a precursor of chlorophyll

**Table 4:** Dry matter yield (g / pot) of rocket plant (roots and shoots) affected by the studied treatments.

Growth season	Added Cd (% of toxic level)	Roots					Shoots						
		Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		Control	Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>				
		100	200	100	200		100	200	100	200			
First	0	0.22	0.51	0.52	0.60	0.76	2.64	4.40	4.80	4.85	5.43		
	100	0.19	0.41	0.44	0.52	0.61	2.26	3.72	4.02	4.10	4.60		
	150	0.17	0.36	0.39	0.40	0.46	2.10	3.33	3.60	3.75	4.10		
	300	0.11	0.22	0.24	0.25	0.27	1.52	2.23	2.53	2.35	2.59		
L.S.D 5%		A = 0.012		B = 0.009		AB = 0.011		A = 0.16		B = 0.21		AB = 0.16	
Second	0		0.25	0.60	0.63	0.71	0.80	2.72	4.45	5.05	5.00	5.30	
	100	0.21	0.48	0.51	0.60	0.65	2.30	3.80	4.10	4.00	4.35		
	150	0.18	0.35	0.43	0.47	0.50	2.11	3.40	3.60	3.70	3.90		
	300	0.11	0.20	0.25	0.27	0.30	1.54	2.26	2.58	2.40	2.65		
L.S.D 5%		A = 0.029		B = 0.025		AB = 0.04		A = 0.14		B = 0.13		AB = 0.23	

**Table 5:** Relative increase (RI%) of rocket dry matter yield as affected by different treatments of nitrogen fertilization.

Growth season	Added Cd (% of toxic level)	Roots				Shoots			
		Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	
		100	200	100	200	100	200	100	200
First	0	130.9	136.4	177.7	246.5	69.3	81.8	83.7	105.7
	100	115.7	131.2	163.2	221.1	61.6	77.9	81.4	103.5
	150	111.8	129.4	135.3	170.2	58.6	71.4	78.6	98.1
	300	100.0	119.2	127.3	145.5	46.7	66.4	54.7	70.4
Second	0	140.0	152.0	184.0	220.0	66.9	95.7	89.8	95.0
	100	129.6	142.9	185.7	209.5	65.2	78.3	77.9	89.2
	150	94.4	139.9	161.1	177.7	61.2	70.6	75.4	85.0
	300	81.9	127.3	145.2	172.7	43.0	67.6	55.8	72.8

**Table 6:** Relative increase (RI%) of rocket dry matter yield as affected by different application rates of cadmium.

Growth season	Added Cd (% of toxic level)	Roots					Shoots				
		Control	Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		Control	Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	
			100	200	100	200		100	200	100	200
First	100	-13.7	-19.7	-15.4	-13.3	-19.7	-15.0	-6.4	-16.3	-15.5	-15.3
	150	-22.3	-29.4	-25.0	-33.3	-31.5	-20.4	-24.3	-25.0	-22.7	-24.5
	300	-50.0	-56.0	-53.9	-59.3	-64.5	-42.4	-49.3	-47.3	-51.6	-52.3
Second	100	-16.0	-20.0	-19.0	-12.7	-18.8	-15.4	-14.6	-19.9	-20.0	-17.9
	150	-28.0	-41.7	-33.3	-33.8	-37.5	-22.4	-21.3	-29.7	-26.0	-35.9
	300	-56.0	-66.7	-57.1	-61.9	-62.5	-43.3	-49.2	-48.9	-52.0	-50.00

**Table 7:** Agronomic efficiency (AE g / g) of rocket dry matter yield as affected by different treatment of nitrogen fertilization.

Growth season	Added Cd (% of toxic level)	Roots				Shoots			
		Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	
		100	200	100	200	100	200	100	200
First	0	1.129	0.575	1.495	0.909	9.723	5.320	11.603	6.495
	100	0.907	0.487	1.244	0.730	8.230	4.447	9.809	5.502
	150	0.796	0.332	0.957	0.550	7.357	3.992	8.612	4.904
	300	0.486	0.265	0.598	0.323	4.934	2.799	6.053	3.098
	0	1.327	0.697	1.699	0.957	9.845	5.596	11.962	6.400
	100	1.062	0.554	1.459	0.778	8.407	4.595	9.594	5.203
	150	0.775	0.476	1.124	0.598	7.522	3.992	8.851	4.665
	300	0.442	0.277	0.646	0.359	6.106	2.500	5.742	3.170

**Table 8:** Agronomic efficiency (AE mg / gm) of rocket dry matter yield as affected by different application rates of cadmium.

Growth season	Added Cd (% of toxic level)	Roots				Shoots					
		Control	Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		Control	Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	
			100	200	100	200		100	200	100	200
First	100	8.444	18.222	19.556	23.111	27.111	100.494	165.333	178.667	182.222	204.444
	150	5.037	10.667	11.556	11.852	13.630	62.222	98.667	106.667	111.111	121.481
	300	1.630	3.359	3.556	3.704	4.000	22.519	33.037	37.481	34.815	43.704
Second	100	9.333	19.111	22.667	26.667	28.889	102.222	160.889	182.222	177.778	193.333
	150	5.333	16.660	12.701	13.926	14.915	62.520	100.741	106.667	169.630	115.556
	300	1.603	2.963	3.704	4.000	4.444	22.815	33.481	38.333	35.556	39.259

(Thomas and Singh, [36] and or enhancing the activity of chlorophyllase and thus chlorophyll degradation (Abdel-Basset *et al.*, [37] and Moya and Picazo, [38]

Results in the same table concerning the contents of “Chl. a + b” and carotenoids in rocket leaves also show a significant increase due to the use of N-forms at the different rates. The highest increase occurred on

using the high rate of NH<sub>4</sub><sup>+</sup> -N as compared with

the untreated plants in both seasons. Similar results obtained by Ashraf [39] on sunflower and Abdou *et al.* [40] on pot marigold. Nitrogen is a major component of all amino acids, and hence proteins acting in building up the chloroplasts Marschner, [41], thus its favourable effect on chlorophyll content is quite expected.

As for the interaction between Cd treatments and application of N-forms and rates, it can be noticed that, N treatments alleviated the harmful effects of Cd

pollution and significantly improved photosynthetic pigments especially at lower Cd concentration.

NH<sub>4</sub><sup>+</sup> -N showed superiority over NO<sub>3</sub><sup>-</sup> -N.

**b) Total Soluble Sugars (TSS), Total Free Amino Acids (TAA) and Proline Concentrations:** In both seasons, results in Table (10) indicate that, Cd levels significantly affected the total soluble sugars (TSS), total free amino acids (TAA) and proline concentrations. TSS and TAA concentrations in leaves of Cd polluted plants significantly decreased, while there was a remarkable increase in praline, as compared with the control plants. Similar results were obtained by Delauney and Verma [42], Nagoor and Vyas [43], El-Gamal [24] and El-Ghinbihi [26]. The inhibitory effect of Cd on TSS maybe attributed to its negative effects on photosynthesis process [44]. Moreover, Kavita and Dubey [45] found that, Cd at rates of (50 – 500 mM) decreased amino acid levels in rice seedlings.

**Table 9:** Photosynthetic pigments concentrations (mg / g dwt.) in rocket leaves as affected by the studied treatments.

The studied characters	Added Cd (% of toxic level)	First season					Second season				
		Control	Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		Control	Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	
			100	200	100	200		100	200	100	200
Chlorophyll (a + b)	0	5.63	6.00	6.72	7.11	8.10	6.05	6.75	7.15	7.62	8.51
	100	4.46	5.91	6.08	6.65	7.25	4.95	6.58	6.71	7.05	8.00
	150	3.82	5.84	5.95	6.03	7.01	3.61	6.45	6.57	6.82	7.15
	300	3.00	5.00	5.14	5.21	5.34	2.75	4.32	4.47	4.56	4.66
L.S.D 5%		A = 0.22	B = 0.16	AB = 0.22			A = 0.23	B = 0.20	AB = 0.39		
Carotenoids	0	2.48	3.20	3.52	3.65	3.95	2.62	3.35	3.68	3.81	4.57
	100	2.05	2.82	3.04	3.20	3.40	1.95	3.00	3.25	3.47	3.68
	150	1.62	2.53	2.59	2.65	2.67	1.56	2.75	3.00	3.09	3.28
	300	0.95	1.45	1.62	1.75	1.81	1.05	1.51	1.78	1.85	1.92
L.S.D 5%		A = 0.15	B = 0.12	AB = 0.15			A = 0.14	B = 0.12	AB = 0.13		

**Table 10:** Total soluble sugars, total free amino acids (mg / g dwt.) and proline concentrations (mg/g dwt.) in rocket leaves as affected by the studied treatments.

The studied characters	Added Cd (% of toxic level)	First season					Second season				
		Control	Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		Control	Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	
			100	200	100	200		100	200	100	200
Total soluble sugars	0	25.51	27.31	29.48	31.70	33.71	24.68	28.20	29.12	30.50	31.62
	100	20.80	27.00	28.81	30.00	31.05	21.10	26.34	27.20	29.30	30.05
	150	16.30	26.10	27.40	29.23	30.00	15.75	25.71	26.59	27.60	28.40
	300	10.35	17.61	18.30	19.77	21.40	11.33	15.82	17.65	18.45	21.66
L.S.D 5%		A = 0.45	B = 0.31	AB = 0.45			A = 0.36	B = 0.39	AB = 0.35		
Total free amino acids	0	85.13	88.11	95.18	105.13	122.13	71.40	80.20	91.31	99.61	115.31
	100	78.13	87.10	92.00	101.20	105.80	65.20	75.40	87.00	91.13	104.12
	150	63.00	86.10	90.05	98.00	108.11	50.30	74.33	80.20	85.15	91.40
	300	65.11	60.12	65.15	72.30	75.10	40.15	44.20	56.04	60.09	64.00
L.S.D 5%		A = 1.90	B = 1.49	AB = 1.86			A = 1.52	B = 1.57	AB = 1.50		
Proline	0	308.10	290.30	283.60	272.30	261.10	282.30	271.40	265.10	265.30	211.80
	100	307.30	307.00	306.10	305.20	300.00	350.10	280.30	275.80	265.40	271.30
	150	567.40	408.20	401.10	384.30	365.00	475.40	311.30	305.40	288.30	291.80
	300	615.20	608.10	590.20	575.10	502.60	558.30	428.30	405.10	382.30	375.40
L.S.D 5%		A = 1.90	B = 2.50	AB = 1.86			A = 2.03	B = 2.02	AB = 1.99		

It is obvious from the same table that, nitrogen forms had significant effects in TSS, TAA and proline concentrations of rocket plants, compared to the control in both seasons. Generally, ammonium sulphate (NH<sub>4</sub><sup>+</sup> N) was more effective in increasing TSS and

TAA concentrations, as compared with calcium nitrate (NO<sub>3</sub><sup>-</sup> - N). Moreover, addition of N fertilizers caused a reduction in the concentration of proline. Nitrogen improved the content of total sugars via a



favourable effect on chlorophyll synthesis that positively reflected the intensity of photosynthesis. The relation between chlorophyll content and photosynthesis intensity was previously reported by Midan<sup>[46]</sup>.

Significant differences in the concentrations of TSS, TAA and proline concentrations were noticed due to the interaction among Cd levels and N fertilizers (forms and rates) in both seasons. The highest values of TSS and TAA were recorded with the use of high level of N (200% of RD) under the lowest level of Cd (100 mg/L), meanwhile, a marked reduction in the accumulation of proline in rocket leaves was recorded.

**C) Enzymes Activities:** It is obvious from Table (11) that, there are a remarkable increase in peroxidase and phenoloxidase activities under Cd pollution conditions, when compared with the unpolluted plants in both seasons. These findings were supported by those obtained by Kativa and Dubey<sup>[45]</sup> and El-Gamal<sup>[24]</sup>. It is in this regard that Chen and Kao<sup>[47]</sup> reported that, cadmium increased the capacity of several enzymes, e.g. peroxidase, glutamate dehydrogenase, glutamine synthetase and proteolytic enzymes. There is an evidence that, an increase in peroxidase could appear as a metabolic response to various stress conditions resulting in the pollution of soil by heavy metals (Devos *et al.*,<sup>[48]</sup> Phenoloxidase enzyme acts as oxidoreductase enzyme, which helps in oxidation of phenolic compounds during the metabolic processes.

Data in both seasons show that, nitrogen fertilizers resulted significant decreases in enzymes activities. The lowest values were recorded for the application rate of 200% of the recommended N dose, compared to the control and the polluted plants by Cd. Nitrogen fertilization increases nitrogen amounts taken up by the plants and thus contributes directly to the assimilation of enzymes and their activities<sup>[49]</sup>.

Significant differences in enzymes activity were noticed due to the interaction among sources and rates of nitrogen treatments and Cd pollution. Ammonium sulphate showed superiority over calcium nitrate under all levels of Cd. Plants treated with Cd (300% of toxic

level) and  $\text{NH}_4^+$ -N at 200% of RD resulted in the reduction of peroxidase activity to 32.98 and 35.26% and the reduction in phenoloxidase was 36.06 and 36.84% in the first and second season, respectively.

#### 4. Element Contents of Rocket Plants:

**a) Nitrogen:** Nitrogen concentration and its uptake by

rocket plants (shoots and roots) were clearly affected by the studied treatments. The presented data in Table (12) show that, both N concentration and uptake were increased with the increase of added N. These increases were found for both individual and combined treatments of N fertilization, but those with the individual treatments were higher than those associated with the combined treatments of N and Cd. The highest increases of N content were found with the high rates (200% of RD) of the individual application

of  $\text{NH}_4^+$ -N. The superiority of  $\text{NH}_4^+$ -N than of

$\text{NO}_3^-$ -N may be resulted from the greater adsorption

of  $\text{NH}_4^+$  on the negative charges of both plant roots

and soil colloids and also to  $\text{NO}_3^-$  leaching from the rhizosphere<sup>[28]</sup>. N concentration and its uptake by shoots were higher than those detected for the roots. The content of N as affected by the individual treatments of N was similar in the two growth seasons. These results are in agreement with those obtained by El-Fiki<sup>[50]</sup>, Abou Hussein and Barsoum<sup>[23]</sup> and Melegy<sup>[7]</sup>.

The recorded data in Table (12) show that, clear decreases of N content of rocket shoots and roots were associated with the increase of added Cd. Such decreases resulted from the individual treatments of Cd were greater than those found with for the combined treatments of N and Cd. This trend was found in both growth seasons for the shoots and roots. These results reveal that, the treatments of Cd at the highest toxic levels had an inhibitory effect on uptake of N by rocket plants. This effect was reduced by N fertilization

and with  $\text{NH}_4^+$ -N better than with  $\text{NO}_3^-$ -N.

Similar negative effect of Cd on plant content of N was found by El-Habet<sup>[51]</sup> and Zein<sup>[52]</sup>.

**b) Cadmium:** The presented data in Table (13) show Cd concentration and its uptake by rocket plants (shoots and roots) as affected by the studied treatments. These data indicate that, Cd content of rocket plants increased with the increase of added Cd. This increase was more clear with individual treatments of Cd, especially at the higher application rates. This trend was found for both shoots and roots in the two growth seasons. These results are in agreement with the findings of Eissa and El-Kassas<sup>[53]</sup> and Abou El-Khair *et al.*<sup>[32]</sup>.

**Table 11:** Peroxidase and phenoxidase activity (O.D / g fwt.) in rocket leaves as affected by the studied treatments.

The studied characters	Added Cd (% of toxic level)	First season						Second season					
		Control		Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		Control		Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	
		100	200	100	200	100	200	100	200	100	200	100	200
Peroxidase	0	0.52	0.47	0.39	0.34	0.27	0.45	0.37	0.34	0.30	0.23		
	100	0.71	0.49	0.45	0.40	0.36	0.62	0.41	0.39	0.35	0.29		
	150	0.25	0.51	0.50	0.48	0.45	0.79	0.31	0.29	0.27	0.25		
	300	0.97	0.81	0.75	0.71	0.05	0.85	0.49	0.46	0.41	0.39		
L.S.D 5%		A = 0.003	B = 0.002	AB = 0.005			A = 0.008	B = 0.006	AB = 0.014				
Phenoxidase	0	0.29	0.25	0.23	0.19	0.16	0.33	0.28	0.25	0.23	0.21		
	100	0.33	0.26	0.24	0.20	0.19	0.40	0.30	0.27	0.25	0.23		
	150	0.44	0.27	0.25	0.22	0.21	0.46	0.31	0.29	0.27	0.25		
	300	0.57	0.39	0.38	0.37	0.36	0.61	0.49	0.46	0.41	0.39		
L.S.D 5%		A = 0.007	B = 0.009	AB = 0.007			A = 0.008	B = 0.008	AB = 0.008				

**Table 12:** Nitrogen (N) concentration (%) and its uptake (mg / pot) of rocket plants (roots and shoots) as affect by the studied treatments of N and Cd.

Growth season	Added Cd (% of toxic level)	Roots												Shoots									
		Control		Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		Control		Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		Control		Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>					
		100	200	100	200	100	200	100	200	100	200	100	200	100	200	100	200	100	200				
		Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)
First	0	1.50	3.30	1.70	8.67	1.85	9.62	1.76	10.56	1.93	14.67	1.65	43.56	1.82	80.08	1.95	93.60	1.90	92.15	2.25	122.18		
	100	1.45	2.76	1.67	6.85	1.81	7.96	1.72	8.94	1.88	11.47	1.60	36.16	1.75	65.10	1.90	76.38	1.85	75.85	2.11	97.06		
	150	1.38	2.35	1.50	5.40	1.70	6.63	1.65	6.60	1.82	8.37	1.50	31.50	1.68	55.94	1.80	64.90	1.73	64.88	1.85	75.85		
	300	1.29	1.42	1.40	3.08	1.65	3.96	1.52	3.80	1.65	4.96	1.43	21.74	1.52	33.90	1.70	43.01	1.62	38.07	1.75	45.33		
Second	0	1.55	3.88	1.68	10.08	1.87	11.78	1.78	12.64	1.95	15.60	1.65	44.88	1.82	80.99	1.97	99.49	1.92	96.00	2.25	119.25		
	100	1.42	2.98	1.65	7.10	1.80	9.18	1.72	10.32	1.85	12.03	1.60	36.80	1.77	67.25	1.93	79.13	1.85	74.00	2.14	93.09		
	150	1.40	2.52	1.50	5.25	1.73	7.44	1.63	7.61	1.80	9.00	1.51	31.86	1.68	57.12	1.78	64.97	1.75	64.75	1.88	73.32		
	300	1.30	1.45	1.42	2.84	1.63	4.08	1.50	4.05	1.68	5.04	1.40	21.56	1.55	35.03	1.70	43.86	1.64	39.36	1.78	47.17		

**Table 13:** Cadmium concentration (mg / kg) and its uptake (mg / pot) of rocket plant (roots and shoots) as affect by the studied treatments of N and Cd.

Growth season	Added Cd (% of toxic level)	Roots												Shoots									
		Control		Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		Control		Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		Control		Ca (NO <sub>3</sub> ) <sub>2</sub>		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>					
		100	200	100	200	100	200	100	200	100	200	100	200	100	200	100	200	100	200				
		Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)	Conc. (mg/kg)	Uptake (mg/pot)
First	0	11.51	0.0025	10.75	0.0056	10.05	0.0052	9.61	0.0058	9.40	0.0071	10.15	0.0258	10.05	0.0442	9.61	0.0461	9.50	0.0468	9.25	0.0502		
	100	16.17	0.0031	15.50	0.0064	14.72	0.0065	14.25	0.0074	12.11	0.0074	15.71	0.0355	15.42	0.0574	14.50	0.0504	13.58	0.0557	12.65	0.0582		
	150	25.80	0.0044	24.50	0.0088	23.15	0.0099	21.85	0.0087	19.50	0.0092	24.11	0.0506	23.88	0.0752	21.00	0.0755	21.11	0.0792	18.20	0.0746		
	300	36.92	0.0041	35.11	0.0084	32.90	0.0079	31.23	0.0078	27.90	0.0075	35.80	0.0544	34.33	0.0766	32.15	0.0595	30.65	0.0721	27.10	0.0702		
Second	0	11.75	0.0029	10.80	0.0065	10.10	0.0064	9.60	0.0068	9.50	0.0042	10.05	0.0273	9.81	0.0437	9.45	0.0477	9.31	0.0466	9.15	0.0485		
	100	16.20	0.0034	16.05	0.0069	14.18	0.0072	14.50	0.0087	12.35	0.0080	15.80	0.0363	15.51	0.0576	14.50	0.0595	13.80	0.0552	12.40	0.0539		
	150	25.65	0.0046	21.75	0.0076	23.30	0.0100	21.60	0.0101	19.72	0.0099	24.32	0.0513	24.05	0.0818	23.22	0.0836	21.25	0.0786	18.35	0.0716		
	300	37.25	0.0041	35.40	0.0071	32.50	0.0081	32.05	0.0065	28.11	0.0069	36.05	0.0555	34.50	0.0780	31.90	0.0818	31.32	0.0752	27.00	0.0721		

Rocket plants (shoots and roots) content of (application rates and forms), when applied as cadmium was greatly affected by N fertilization individual or combined with Cd. In both growth

seasons, Cd concentration in shoots and roots decreased with the increase of added N. Such decrease was more obvious with  $\text{NH}_4^+$ -N forms. In most treatments under study, Cd concentration of roots was higher than that of shoots, except for the plants untreated with N and Cd. Cd uptake by rocket plants increased with the increase of added N. This trend was correlated with the dry matter yield. Nitrogen application in combination with Cd, resulted in decreases of Cd concentration, especially with the treatments of  $\text{NH}_4^+$ -N. From the previous discussion, it may be concluded that, rocket plants are characterized by high tolerance to Cd pollution<sup>[54]</sup>. Consequently, in case of cultivation of rocket plants in a Cd polluted soil, it is recommended to apply the N fertilizers as  $\text{NH}_4^+$ -N form.

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