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**ORIGINAL ARTICLE** 

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# **Do NH**<sub>4</sub>:NO<sub>3</sub> ratio and harvest time affect celery (*Apium graveolens*) productivity and product quality?

Said Saleh<sup>1,2</sup>, Guangmin Liu<sup>1</sup>, Mingchi Liu<sup>1</sup>, Wei Liu<sup>1</sup>, Hongju He<sup>1</sup>\*, Magdi T. Abdelhamid<sup>3</sup>\*

<sup>1</sup> Beijing Vegetable Research Center, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China
<sup>2</sup> Horticultural Crops Technology Department, National Research Centre, 33 EL Bohouth St, Dokki, Giza 12622, Egypt
<sup>3</sup> Botany Department, National Research Centre, 33 EL Bohouth St, Dokki, Giza 12622, Egypt

# ABSTRACT

Due to the remarkable health benefits of celery (*Apium graveolens*), its consumption has increased over time. A partial substitution of  $NO_3^-$  with  $NH_4^+$  is recommended to limit the accumulation of  $NO_3^-$  in leafy vegetables. Hence, a factorial experiment with two factors, consisting of six treatments as combinations of three  $NH_4$ : $NO_3^-$  ratios (0:100, 20:80 and 40:60) in nutrient solutions and two harvesting times (in the morning and in the evening), was conducted on celery plants in a soilless culture system. The results showed that 100%  $NO_3^-$  as a sole N source significantly increased plant height, leaf number, chlorophyll, fresh weight, N, K, Ca, Mg, Fe, Mn, Zn, protein, dietary fibre, soluble sugars, nitrate, vitamin C,  $\alpha$ -carotene,  $\beta$ -carotene and lutein of celery plants compared to either 80 or 60%  $NO_3^-$ . However, this increase was not significant compared to 20%  $NH_4^+$ :80%  $NO_3^-$  in terms of leaf number, fresh yield, N, Mg, Mn, protein, soluble sugars, vitamin C and  $\alpha$ -carotene. Harvesting in the evening significantly increased K, Mg, Fe, soluble sugars,  $\alpha$ -carotene and  $\beta$ -carotene, and lowered the nitrate level in celery plants. In conclusion, partial replacement of 20%  $NO_3^-N$  with 20%  $NH_4^-N$  and evening harvesting are recommended for a greater fresh yield, higher quality, and lower nitrate level.

Key words: growth, nitrate, nutrient solution, quality, time of day, yield

# **INTRODUCTION**

Nitrogen (N) is required by plants in the greatest quantity because it plays an essential role in plant productivity, since it is a major part of chlorophylls, proteins, nucleic acids and enzymes, and plays an important role in cell division, photosynthesis and accumulation of organic matter in plant tissues (Marschner, 1995; Abdelhamid et al., 2003; Stewart et al., 2005; Krężel and Kołota, 2011). Plants take up N in both anionic ( $NO_3^-$ ) and cationic ( $NH_4^+$ ) forms. However,  $NO_3^-$  is mostly preferred for maximum growth and development of leafy vegetable crops

(Santamaria et al., 1997; Wang et al., 2009; Urlic et al., 2017).

Up to a certain point, N fertilizer has a positive effect on plant productivity (Borowski and Michałek, 2008). Increased N use is almost always associated with high nitrate accumulation, especially in leafy vegetables (Premuzic et al., 2004; Santamaria, 2006; Prasad and Chetty, 2008; Fallovo et al., 2009; Saleh et al., 2010). The extra availability of nitrogen which is not tailored to plant requirements may reduce product quality through nitrate accumulation in the edible parts (Parente et al., 2006; Saleh, 2009; Liu et al., 2014). Nitrate residues in leafy vegetables



<sup>\*</sup>Corresponding author.

e-mail: hehongju@nercv.org (H. He); magdi.abdelhamid@yahoo.com (M. Abdelhamid).

may have indirect negative effects on human health. Nitrate is relatively non-toxic to humans, but its metabolites may cause carcinogenesis (IPCS Inchem, 2003; Santamaria, 2006). Because nitrate reduction results in nitrite in the human body, which can cause methaemoglobinaemia or act as a precursor in the endogenous formation of carcinogenic nitrosamines, an acceptable daily nitrate intake of 3.65 mg kg<sup>-1</sup> body weight has been set by the World Health Organization (WHO, 2002). Therefore, a 60 kg individual should not take in more than 219 mg  $NO_3^{-}$ , that is, he or she should not eat more than 100 g of a plant with an NO<sub>3</sub><sup>-</sup> content greater than 2190 mg/kg. Several factors affect nitrate accumulation in plants, such as the practice of fertilization (fertilizer application method and sources), light intensity and harvest timing, etc. (Reinink, 1991; Dejonckheere et al., 1994; Nicola et al., 2007; Zaki et al., 2012). In general, the veruse of mineral fertilizers causes pollution of the environment due to excessive accumulation and leaching of harmful elements into the groundwater (Ju et al., 2007; Parks et al., 2008; Abdelhamid et al., 2011; Liu et al., 2014). Consequently, nitrogen fertilization of vegetables must take into account not only agricultural economics, but also consumer preference and human health and environmental issues (FAO, 2002; Schenk, 2006; Ezzo et al., 2012; Saleh et al., 2016). Consumers are now more concerned about their health and high quality of food products, which should be rich in vitamins and minerals as well as free of chemical residues and heavy metals. The problem is that an excess of consumed nitrate may cause harmful effects, especially in high-risk groups, such as children and people with specific metabolic diseases (Anjana et al., 2007).

Celery (Apium graveolens), an Apiaceae family member, is an important herbal plant that has been grown since ancient times as a vegetable crop. Due to the scientific evidence of its remarkable health benefits, celery consumption has increased over time (El-Sayed et al., 2009; Shehata et al., 2010; El-Sayed et al., 2011; Kosterna et al., 2012). Because of its high levels of antioxidant compounds, vitamins and essential minerals, the health benefits of celery are substantial. Celery is classified as a leafy vegetable with respect to its use, thus accumulating excessive amounts of nitrate compared to other vegetables (Blom-Zandstra, 1989; Santamaria et al., 1999), which is the main quality index for leafy vegetables. The most obvious approach to nitrate reduction in leafy vegetables is to reduce nitrate

availability to plants (Gent, 2003). This parameter is therefore affected by the nitrogen fertilizer source with different  $NO_3$ -N:NH<sub>4</sub>-N ratios (Nicola et al., 2007; Wang et al., 2009; Saadatian et al., 2014; Abbasi et al., 2017; Urlic et al., 2017). Nitrate replacement with an ammonium fertilizer may lower plant tissue nitrate. Both  $NO_3$ -N and  $NH_4$ -N are used in nutrient solutions for hydroponics. The ratio of  $NO_3$ -N to  $NH_4$ -N is of great importance and can affect the growth and quality of plants (Anjana et al., 2007). To achieve optimum growth and maximum quality, adequate nutrient supply is required.

The importance of celery in terms of its health benefits had led us to reduce nitrate concentration by partially replacing  $NO_3$ -N with  $NH_4$ -N and selecting appropriate harvesting time. The aim of this study was therefore to evaluate the productivity of celery plants and their quality under three  $NH_4$ -N:NO<sub>3</sub>-N ratios in nutrient solution and two harvesting times in order to determine which ratio and harvesting time can lead to a high fresh yield accompanied by high product quality.

#### MATERIALS AND METHODS

#### **Experimental procedures**

The experiment was carried out in a greenhouse at the research station of Beijing Vegetable Research Center in Beijing, China, during the 2016/17 season. The maximum air temperature inside the greenhouse was 24°C, with a minimum night temperature of 14°C during the cultivation period. Relative humidity ranged from 60 to 65%.

Seeds of celery (*Apium graveolens* var. *dulce*; cv. Queen of France), produced by TÉZIER Seeds Company, France, were sown on July 4, 2016, into trays with  $5 \times 10$  cells, using black peat and perlite at 2:1 as a substrate. Uniform seedlings were transplanted into cultivation troughs filled with 8 L of a perlite substrate (2 seedlings per each trough) on September 1, 2016. The distance between the two plants in a trough was 25 cm, and the spacing between the troughs was 40 cm.

Celery plants were fertigated with three full nutrient solutions containing the same essential macro-nutrients (in mg L<sup>-1</sup>) at 140 N, 40 P, 210 K, 200 Ca, 48 Mg and 64 S, as well as the same essential micro-nutrients (in mg L<sup>-1</sup>) at 5 Fe, 0.5 Mn, 0.5 Zn, 0.5 B, 0.02 Cu and 0.01 Mo. The ratio between NH<sub>4</sub> and NO<sub>3</sub> as a source of nitrogen varied in the nutrient solutions and was 0:100, 20:80 and 40:60, which correspond to 0, 2,

and 4 mmol L<sup>-1</sup> NH<sub>4</sub>-N, and 10, 8, and 6 mmol L<sup>-1</sup> NO<sub>3</sub>-N, respectively, with 10 mmol L<sup>-1</sup> total N for each treatment. NO<sub>3</sub>-N was applied as Ca(NO<sub>3</sub>)<sub>2</sub> and KNO<sub>3</sub>, while NH<sub>4</sub>-N was applied as NH<sub>4</sub>Cl. At the end of the experiment, the celery plants were harvested at two different times of day, i.e., in the morning (7:00 a.m.), after complete darkness at night, and in the evening (6:00 p.m.), after having been fully exposed to daylight, on January 4, 2017.

The layout of the trial was that of a factorial experiment with two factors, replicated three times. The experiment consisted of six treatments as combinations of three NH<sub>4</sub> to NO<sub>3</sub> ratios and two harvesting times. The six treatments were: 1) NH<sub>4</sub>:NO<sub>3</sub> ratio of 0:100 with harvest in the morning (T1), 2) NH<sub>4</sub>:NO<sub>3</sub> ratio of 0:100 with harvest in the evening (T2), 3) NH<sub>4</sub>:NO<sub>3</sub> ratio of 20:80 with harvest in the morning (T3), 4) NH<sub>4</sub>:NO<sub>3</sub> ratio of 20:80 with harvest in the evening (T4), 5) NH<sub>4</sub>:NO<sub>3</sub> ratio of 40:60 with harvest in the evening (T6). Each sub-plot consisted of 15 troughs, containing 30 celery plants.

#### Measurements

The EC of nutrient solutions was 2.0-2.1 dS m<sup>-1</sup>, and the pH was adjusted to 5.9-6.2 using  $H_3PO_4$ . The total nutrient solution of 300 ml per plant was added into each trough three times a day, at 8:00 a.m., 12:00 noon and 4:00 p.m. Celery plants were harvested manually when they had reached their marketing stage on January 4, 2017, which was 126 days after transplanting to be grown in nutrient solution. Representative samples of ten plants from each treatment were randomly selected in the morning (7:00 a.m.) and in the evening (6:00 p.m.). Plant height (cm), number of leaves per plant, fresh weight per plant (g) and total chlorophyll content (SPAD values measured with a digital Minolta Chlorophyll Meter SPAD-502) were recorded.

Chemical composition: Other representative samples of ten celery plants from each treatment were randomly selected for chemical analysis to determine the macronutrients: N, P, K, Ca and Mg, and the micronutrients: Fe, Mn, Zn and Cu, according to the ICP-AES method, NY/T 1653-2008. Other chemical constituents related to quality parameters, including protein (Kjeldahl method), fibre (total dietary fibre), titratable acid (alkali titration), total soluble sugars (anthrone colorimetry), nitrate (colorimetry), vitamin C (2,6-dichloroindophenol titration),  $\alpha$ -carotene,  $\beta$ -carotene and lutein were determined according to Nielsen (2010).

#### Statistical analysis

All data were subjected to an analysis of variance for a factorial experiment with two factors in a completely randomized design (Gomez and Gomez, 1984), after testing for the homogeneity of error variances using Levene's test (1960). Statistically significant differences among means were compared at  $p \le 0.05$  using Duncan's multiple range test. The statistical analysis was carried out using GenStat 17th Edition (VSN International Ltd, Hemel Hempstead, UK). Correlation coefficient r was calculated to determine the relation between fresh weight yield and each of the physiological and chemical traits. Hierarchical cluster analysis was performed on the standardized data using a measure of Euclidean distance and Ward's minimum variance method as outlined by Ward (1963). Experimental data were also processed with a principal component analysis (PCA) using GenStat 17th Edition (VSN International Ltd, Hemel Hempstead, UK) in order to evaluate the existing relationships with original variables.

#### RESULTS

#### Changes in celery growth

The effects of the three different NH<sub>4</sub> to NO<sub>2</sub> ratios (0:100, 20:80 and 40:60) and two harvesting times (in the morning and in the evening), and their interactions on plant height, leaf number, SPAD value, and fresh weight of celery plants are shown in Table 1. The results showed that application of 100% NO<sub>3</sub> as a sole nitrogen source via nutrient solution significantly increased plant height, leaf number, SPAD value and fresh weight of celery plants. Significant gradual reductions in these traits were observed with decreasing NO<sub>2</sub> and increasing NH<sub>4</sub>. However, compared to 80% NO<sub>3</sub>, this reduction was not significant in leaf number and fresh weight (Tab. 1). There was no significant difference between harvesting celery plants in the morning and in the evening in terms of plant height, leaf number, SPAD value and fresh weight (Tab. 1). The interaction effects of the NH<sub>4</sub>:NO<sub>2</sub> ratios and harvesting times on plant height, leaf number, SPAD value and fresh weight of celery plants varied as shown in Table 1. Application of 100% NO<sub>3</sub> as a sole nitrogen source produced the highest biomass fresh weight of celery plants harvested in the morning, while the lowest fresh weight of the biomass was produced with the nutrient

Treatment		Plant height (cm)	Leaf No /plant	SPAD value	Fresh weight /plant (g)
NH <sub>4</sub> :NO <sub>3</sub> rat	tios (A):				
0:100		62.7**a	19.7 a	38.7 a	301 a
20:80		58.3 b	18.3 a	37.7 b	298 a
40:60		53.0 c	16.2 b	34.8 c	266 b
Harvest time	e (B):				
Mornin	g	57.9 a	18.2 a	37.0 a	289 a
Evening	g	58.1 a	17.9 a	37.1 a	288 a
Interactions	$(\mathbf{A} \times \mathbf{B})$ :				
0:100	Morning	63.0 a	20.0 a	38.3 b	302 a
	Evening	62.3 a	19.3 ab	39.1 a	300 b
20:80	Morning	58.0 c	18.7 bc	37.5 b	298 с
	Evening	58.7 b	18.0 cd	37.9 b	297 с
40:60	Morning	52.7 d	16.0 d	35.2 c	265 e
	Evening	53.3 d	16.3 d	34.4 d	266 d

**Table 1.** Effect of different  $NH_4:NO_3$  ratios and harvest time on plant height, leaf number per plant, SPAD value and fresh weight per plant of celery plants\*

\*Mean values within the same column for each trait with the same lower-case letter are not significantly different according to Duncan's multiple range test at  $p \le 0.05$ .

\*\*Measurements were made 126 d after transplanting

solution containing 40%  $NH_4$ -N plus 60%  $NO_3$ -N and the plants harvested in the morning (Tab. 1). At the  $NH_4$ :NO<sub>3</sub> ratio of 20:80, there was no significant difference between harvesting in the morning and harvesting in the evening for SPAD value and fresh weight yield.

#### Changes in celery chemical composition

The influence of the three NH<sub>4</sub>:NO<sub>3</sub> ratios (0:100,20:80 and 40:60) and harvesting time (in the morning and in the evening) on the concentration of macroelements: N, P, K, Ca and Mg, and microelements: Fe, Mn, Zn and Cu in celery plants is presented in Table 2. The results showed that application of 100% NO<sub>2</sub> as a sole nitrogen source via nutrient solution significantly increased the concentrations of N, K, Ca, Mg, Fe, Mn, Zn and Cu in celery plants compared to either 80% or 60% NO<sub>3</sub>. However, this increase was not significant compared to 80% NO<sub>3</sub> in terms of N, Mg and Mn (Tab. 2). Significant gradual reductions in these elements were observed with decreasing NO<sub>2</sub> and increasing NH<sub>4</sub>. Harvesting celery plants in the evening resulted in a significant increase in K, Mg and Fe compared to harvesting in the morning (Tab. 2). Regarding the interaction effects of the three NH<sub>4</sub> to NO<sub>3</sub> ratios and two harvesting times on the concentrations of elements, the differences among all of the six interaction combinations were statistically significant except for Cu (Tab. 2).

The application of 100% NO<sub>3</sub> as a sole nitrogen source produced the highest concentrations of N, Fe and Zn in celery plants harvested in the morning, and the highest K, Ca, Mg and Mn concentrations in celery plants harvested in the evening. At the NH<sub>4</sub>:NO<sub>3</sub> ratio of 20:80, the celery plants harvested in the morning contained more P compared to the other interaction combinations. By comparison, application of 40% NH<sub>4</sub>-N plus 60% NO<sub>3</sub>-N produced the lowest concentrations of N, Ca, Fe and Mn in the plants harvested in the morning, and the lowest concentrations of P, K, Mg and Zn in the plants harvested in the evening (Tab. 2).

#### Changes in celery nutritional composition

The effects of the three different  $NH_4$  to  $NO_3$  ratios (0:100, 20:80 and 40:60) and two harvesting times (in the morning and in the evening) and their interactions on protein, dietary fibre, titratable acidity, total soluble sugars, nitrate, vitamin C,  $\alpha$ -carotene,  $\beta$ -carotene and lutein of celery plants are shown in Table 3. The results showed that application of 100%  $NO_3$  as a sole nitrogen source via nutrient solution increased significantly the levels of protein, dietary fibre, total soluble sugars, nitrate, vitamin C,  $\alpha$ -carotene  $\beta$ -carotene and lutein in celery plants compared to either 80 or 60%  $NO_3$ . However, this increase was not significant compared to 80%  $NO_3$  in terms of protein, total soluble sugars, vitamin C and  $\alpha$ -carotene (Tab. 3).

**Table 2.** Effect of different  $NH_4:NO_3$  ratios and harvest time on the concentration of macroelements: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg), and microelements: iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) of celery plants\*

Treatments		Ν	Р	Κ	Ca	Mg	Fe	Mn	Zn	Cu
			(m	g 100 g <sup>-1</sup> F	W)			(mg kg	g <sup>-1</sup> FW)	
NH <sub>4</sub> :NO <sub>3</sub> ra	tios (A):									
0:100		317**a	107 a	516 a	72.0 a	67.6 a	11.9 a	1.10 a	1.92 a	0.97 a
20:80		305 a	111 a	472 b	69.3 b	66.5 a	11.7 b	1.03 a	1.86 b	0.95 a
40:60		281 b	105 a	469 b	63.0 c	58.4 b	11.0 c	0.94 b	1.73 c	1.01 a
Harvest time	e (B):									
Mornin	g	299 a	114 a	474 b	67.9 a	62.6 b	11.4 b	1.02 a	1.84 a	0.98 a
Evenin	g	302 a	102 b	497 a	68.3 a	65.8 a	11.6 a	1.03 a	1.83 a	0.97 a
Interactions	$(A \times B)$ :									
0:100	Morning	318 a	112 ab	483 b	71.7 a	64.3 b	11.9 a	1.08 a	1.95 a	0.95 a
	Evening	315 a	102 bc	548 a	72.3 a	70.9 a	11.9 a	1.12 a	1.88 ab	0.98 a
20:80	Morning	304 ab	120 a	468 b	69.3 a	64.1 b	11.6 b	1.05 ab	1.85 b	0.96 a
	Evening	305 ab	103 bc	477 b	69.3 a	68.9 a	11.8 ab	1.01 ab	1.87 ab	0.94 a
40:60	Morning	276 с	110 b	472 b	62.7 b	59.4 c	10.9 c	0.93 b	1.74 c	1.02 a
	Evening	285 bc	100 c	466 b	63.3 b	57.5 c	11.1 c	0.95 b	1.73 c	1.00 a

\*Mean values within the same column for each trait with the same lower-case letter are not significantly different according to Duncan's multiple range test at  $p \le 0.05$ .

\*\*Measurements were made 126 d after transplanting

**Table 3.** Effect of different  $NH_4$ : NO<sub>3</sub> ratios and harvest time on protein (PRT), dietary fibre (DF), titratable acidity (TA), total soluble sugars (TSS), nitrate (NIT), vitamin C (VC),  $\alpha$ -carotene ( $\alpha$ -C),  $\beta$ -carotene ( $\beta$ -C) and lutein (L) of celery plants\*

Treatment		PRT	DF	TA	TSS	NIT	VC	α-C	β-C	L
		(g 100	g-1 FW)	(%	6)	(mg kg <sup>-1</sup> FW)		(mg 100	g-1 FW)	
NH <sub>4</sub> :NO <sub>3</sub> ra	tios (A):									
0:100		1.98**a	1.52 a	0.50 a	0.36 a	2408 a	27.4 a	0.056 a	2.78 a	3.03 a
20:80		1.90 a	1.37 b	0.51 a	0.35 a	2172 b	25.1 ab	0.054 ab	2.69 b	2.87 b
40:60		1.75 b	1.18 c	0.49 a	0.32 b	2069 с	22.7 b	0.051 b	2.44 c	2.75 c
Harvest tim	e (B):									
Mornin	ıg	1.87 a	1.32 a	0.50 a	0.32 b	2282 a	27.3 a	0.050 b	2.58 b	2.87 a
Evenin	g	1.89 a	1.40 a	0.49a	0.36 a	2150 b	22.9 b	0.058 a	2.69 a	2.90 a
Interactions	$(\mathbf{A} \times \mathbf{B})$ :									
0:100	Morning	1.99 a	1.46 ab	0.52 a	0.34 b	2486 a	30.3 a	0.052 bc	2.83 a	3.04 a
	Evening	1.97 a	1.57 a	0.48 a	0.38 a	2329 b	24.5 bc	0.061 a	2.73 a	3.03 a
20:80	Morning	1.90 ab	1.32 bc	0.53 a	0.32 b	2230 c	27.3 ab	0.049 bc	2.62 b	2.85 bc
	Evening	1.91 ab	1.42 ab	0.49 a	0.37 a	2113 d	22.9 bc	0.060 a	2.77 a	2.89 b
40:60	Morning	1.72 c	1.16 d	0.46 a	0.30 c	2130 d	24.3 bc	0.048 c	2.30 c	2.74 d
	Evening	1.78 bc	1.20 cd	0.51 a	0.33 b	2007 e	21.2 c	0.055 ab	2.58 b	2.77 cd

\*Mean values within the same column for each trait with the same lower-case letter are not significantly different according to Duncan's multiple range test at  $p \le 0.05$ .

\*\*Measurements were made 126 d after transplanting

By comparison, titratable acidity was not affected by the three different  $NH_4$  to  $NO_3$  ratios. Harvesting celery plants in the morning resulted in a significant increase in nitrate and vitamin C, while celery plants harvested in the evening showed a significant increase in total soluble sugars,  $\alpha$ -carotene and  $\beta$ -carotene (Tab. 3). However, there were no significant differences between the two harvesting times on protein, dietary fibre, titratable acidity and lutein (Tab. 3). Regarding the interaction effects of

the three  $NH_4$  to NO<sub>3</sub> ratios and two harvesting times on the nutritional parameters of celery plants, the differences among all of the six interaction combinations were statistically significant except for titratable acidity (Tab. 3). The application of 100% NO<sub>3</sub> as a sole nitrogen source combined with harvesting in the morning produced significantly the highest concentrations of protein, nitrate, vitamin C,  $\beta$ -carotene and lutein, while the highest amounts of dietary fibre, total soluble sugars and  $\alpha$ -carotene were obtained with 100% NO<sub>2</sub> combined with harvesting in the evening. In contrast, the NH<sub>4</sub>:NO<sub>3</sub> ratio of 40:60 resulted in a significant reduction in protein, dietary fibre, total soluble sugars,  $\alpha$ -carotene,  $\beta$ -carotene and lutein in celery plants harvested in the morning, and in a significant reduction in nitrate and vitamin C in celery plants harvested in the evening.

#### **Correlation matrix**

Pearson's correlation coefficients (below the diagonal) among all the studied attributes of celery plants at the three different NH<sub>4</sub>:NO<sub>2</sub> ratios and two harvesting times (in the morning and in the evening) are shown in Table 4. There was strong correlation between fresh weight yield per plant and most of the other studied traits (plant height, leaf number, N, Ca, Mg, Fe, Mn, Zn, protein, dietary fibre, total soluble sugars, nitrate, vitamin C,  $\alpha$ -carotene,  $\beta$ -carotene and lutein) ( $p \le 0.05$  or  $p \leq 0.01$ ), which were highly positively correlated with one another in a linear way. There was also strong correlation between fresh weight yield and Cu ( $p \le 0.05$ ), which were highly negatively correlated with each other in a linear way (Tab. 4). However, there was correlation, but not significant, between fresh weight yield and P, K and titratable acidity, which were positively correlated with one another in a linear way. There was also correlation between Cu and all the other studied traits (plant height, leaf number, N, P, K, Ca, Mg, Fe, Mn, Zn, protein, dietary fibre, titratable acidity, total soluble sugars, nitrate, vitamin C, α-carotene, β-carotene and lutein) ( $p \le 0.05$  or non-significant), which were negatively correlated with one another in a linear way (Tab. 4).

#### Biplot graph

The biplot graph (Fig. 1) presents the relationships among the celery treatments using fresh weight yield and the other attributes studied. The biplot of the mean performance of the celery data explained 88.30% of the total variation of the standardized data. The first and second principal components (PC1 and PC2)



Figure 1. Polygon view of the ordination of treatments by trait biplots of principal component analysis outputs. Labels in the graph indicate the investigated treatments (green colour) and measured traits (blue colour).  $T1 = NH_4$ :NO<sub>3</sub> ratio of 0:100 with harvest in the morning;  $T2 = NH_4$ :NO<sub>3</sub> ratio of 0:100 with harvest in the evening;  $T3 = NH_4$ :NO<sub>2</sub> ratio of 20:80 with harvest in the morning;  $T4 = NH_4$ :NO<sub>3</sub> ratio of 20:80 with harvest in the evening;  $T5 = NH_4$ :NO<sub>3</sub> ratio of 40:60 with harvest in the morning;  $T6 = NH_4$ :NO<sub>3</sub> ratio of 40:60 with harvest in the evening. PH = plant height; LNo = leaf number per plant; FW = fresh weight per plant; N = nitrogen; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; Fe = iron; Mn = manganese; Zn = zinc; Cu = copper; PRT = protein;DF = dietary fibre; TA = titratable acidity; TSS = total soluble sugars; NIT = nitrate; VC = vitamin C;  $\alpha$ -C =  $\alpha$ -carotene;  $\beta$ -C =  $\beta$ -carotene; L = lutein

explained 71.45% and 16.85%, respectively. This relatively high proportion reflects the complexity of the relationships among the treatments and the measured traits. The lines perpendicular to the polygon sides facilitate comparison between the neighbouring vertex treatments. It is obvious that T1 recorded high values of plant height, leaf number, fresh weight yield, N, P, K, Fe, Zn, protein, nitrate, vitamin C,  $\beta$ -carotene and lutein. Also, T2 scored the highest values of SPAD, K, Ca, Mg, Mn, dietary fibre, total soluble sugars and  $\alpha$ -carotene. T3 recorded the highest values of P and titratable acidity. T5 scored the highest value of Cu, while T6 the lowest in terms of all measurements.

#### DISCUSSION

The current study revealed that celery plants, like many other leafy vegetables, prefer the  $NO_3^{-1}$  form of N (Tabs 1, 2 and 3). The use of 100%  $NO_3^{-N}$ 

	Hd	LNo	SPAD	z	Р	K	Ca	Mg	Fe	Mn	Zn	Cu	PRT	DF	TA	TSS	NIT	VC	α-C	β-C	Г
Hd	-																				
LNo	0.98	1																			
SPAD	0.94	$0.91^{**}$	1																		
z	0.98**	0.98**	0.93**	1																	
Р	$0.10^{ns}$	$0.28^{ns}$	$0.17^{\mathrm{ns}}$	$0.11^{\mathrm{ns}}$	1																
К	$0.61^{\mathrm{ns}}$	$0.50^{ns}$	$0.64^{ns}$	$0.54^{\mathrm{ns}}$	$-0.35^{\rm ns}$	1															
Ca	0.98**	0.97**	•••76.0	0.99**	$0.16^{\mathrm{ns}}$	$0.60^{ns}$	1														
Mg	0.79 <sup>ns</sup>	$0.72^{\rm ns}$	$0.92^{**}$	0.79 <sup>ns</sup>	$-0.10^{ns}$	$0.71^{\mathrm{ns}}$	$0.86^{*}$	1													
Fe	0.97**	$0.93^{**}$	0.95**	0.98**	$0.02^{ns}$	$0.59^{ns}$	$0.98^{**}$	$0.88^{*}$	1												
Mn	0.96**	$0.96^{**}$	0.93**	0.96**	$0.15^{\mathrm{ns}}$	$0.70^{\mathrm{ns}}$	0.97**	$0.80^{*}$	$0.94^{**}$	1											
Zn	0.97**	$0.96^{**}$	$0.93^{**}$	0.96**	$0.24^{\rm ns}$	$0.42^{\mathrm{ns}}$	0.95**	$0.75^{\mathrm{ns}}$	0.94**	$0.88^{*}$	1										
Cu	$-0.71^{ns}$	-0.72 <sup>ns</sup>	-0.73 <sup>ns</sup>	$-0.78^{ns}$	-0.1 $7^{ns}$	$-0.04^{ns}$	-0.76 <sup>ns</sup>	-0.68 <sup>ns</sup>	-0.81*	-0.61 <sup>ns</sup>	$-0.81^{*}$	1									
PRT	0.98**	0.98**	$0.93^{**}$	$1.00^{**}$	$0.11^{\mathrm{ns}}$	$0.54^{\mathrm{ns}}$	0.99**	0.79 <sup>ns</sup>	0.98**	0.96**	0.96**	-0.78 <sup>ns</sup>	1								
DF	0.95**	$0.88^{*}$	$0.94^{**}$	$0.94^{**}$	$-0.13^{ns}$	$0.76^{ns}$	0.95**	$0.91^{**}$	0.97**	0.94**	$0.88^{*}$	-0.66 <sup>ns</sup>	$0.94^{**}$	1							
TA	$0.35^{\mathrm{ns}}$	$0.50^{ns}$	$0.17^{ m ns}$	$0.46^{ns}$	$0.47^{\rm ns}$	$-0.34^{ns}$	$0.36^{\mathrm{ns}}$	-0.07 <sup>ns</sup>	$0.33^{\mathrm{ns}}$	$0.35^{ns}$	$0.41^{\mathrm{ns}}$	-0.50 <sup>ns</sup>	$0.46^{ns}$	$0.14^{\rm ns}$	-						
TSS	$0.67^{ns}$	$0.55^{\mathrm{ns}}$	$0.68^{ns}$	$0.71^{\mathrm{ns}}$	-0.54 <sup>ns</sup>	$0.67^{\mathrm{ns}}$	$0.70^{ns}$	$0.82^{*}$	0.79 <sup>ns</sup>	$0.67^{\mathrm{ns}}$	$0.56^{ns}$	-0.58 <sup>ns</sup>	$0.71^{\mathrm{ns}}$	$0.84^{*}$	$0.00^{\text{ns}}$	1					
NIT	$0.86^{*}$	$0.88^{*}$	$0.76^{\mathrm{ns}}$	$0.78^{ns}$	$0.39^{ns}$	$0.46^{*}$	$0.78^{ns}$	$0.48^{\rm ns}$	$0.71^{\mathrm{ns}}$	0.81*	$0.85^{*}$	-0.42 <sup>ns</sup>	$0.78^{ns}$	$0.70^{ns}$	$0.34^{\rm ns}$	$0.21^{\rm ns}$	1				
VC	$0.63^{ns}$	$0.73^{ns}$	$0.55^{\mathrm{ns}}$	$0.59^{ns}$	$0.73^{\mathrm{ns}}$	$0.04^{\mathrm{ns}}$	$0.58^{ns}$	$0.19^{ns}$	$0.47^{ns}$	$0.57^{ns}$	$0.72^{ns}$	$-0.40^{ns}$	0.59 <sup>ns</sup>	$0.38^{ns}$	$0.53^{ns}$	$-0.15^{ns}$	$0.89^{*}$	1			
α-C	$0.44^{\mathrm{ns}}$	$0.29^{ns}$	$0.46^{ns}$	$0.47^{\rm ns}$	-0.76 <sup>ns</sup>	$0.62^{\mathrm{ns}}$	$0.46^{ns}$	0.69 <sup>ns</sup>	$0.58^{\mathrm{ns}}$	$0.43^{ns}$	$0.32^{ns}$	$-0.40^{ns}$	$0.47^{ns}$	$0.67^{ns}$	-0.21 <sup>ns</sup>	0.95**	-0.02 <sup>ns</sup>	-0.41 <sup>ns</sup>	1		
β-C	$0.85^{*}$	0.83**	$0.74^{\rm ns}$	0.91**	$-0.12^{ns}$	$0.34^{\mathrm{ns}}$	$0.85^{*}$	$0.67^{\rm ns}$	0.91**	$0.77^{ns}$	$0.85^{*}$	-0.85*	0.91**	$0.83^{*}$	$0.54^{\rm ns}$	$0.78^{ns}$	$0.55^{ns}$	$0.37^{\mathrm{ns}}$	$0.61^{\mathrm{ns}}$	-	
Г	0.99**	$0.94^{**}$	06.0	0.95**	-0.02 <sup>ns</sup>	$0.68^{ns}$	$0.94^{**}$	$0.77^{ns}$	0.94**	0.94**	0.93**	-0.62 <sup>ns</sup>	0.95**	0.95**	$0.27^{\rm ns}$	0.69 <sup>ns</sup>	$0.85^{*}$	$0.58^{\mathrm{ns}}$	$0.50^{ns}$	$0.84^{*}$	1
FW	0.93**	0.95**	0.95**	0.96**	$0.29^{ns}$	$0.44^{\mathrm{ns}}$	0.98**	$0.84^{*}$	0.96**	0.91**	0.95**	-0.86*	0.96**	$0.88^{**}$	$0.44^{\rm ns}$	$0.63^{*}$	$0.73^{*}$	$0.61^{*}$	$0.57^{*}$	$0.83^{*}$	$0.86^{*}$
** and ' PH = pl zinc; Cu	* = signi ant heigh	ficant at ( ht; LNo = er: PRT =	.01 and 0 leaf num	0.05 level ther per proper	ls respecti blant; FW tarv fibre;	vely; ns = = fresh w TA = titrs	non-sign reight per	ufficant. plant; N ditv: TSS	= nitroge = total s	an; P = pl duble su	ars: NIT	s; K = pot = nitrate	tassium; • VC = v	Ca = cal itamin C	cium; Mg	ص المع ane (م-ر)	esium; F	e = iron	Mn = n	nangane	se; Zn =

resulted in the highest growth characteristics, fresh weight and concentrations of elements, and more nitrate content, while the ratio of 40% NH<sub>4</sub> : 60%NO<sub>3</sub> produced the lowest growth characteristics, concentrations of essential nutrients, antioxidant compounds and fresh weight of celery plants (Tabs 1, 2 and 3). Several earlier studies had reported progress in plant growth similar to that observed when NO<sup>2</sup> was the dominant source of N in the current study. In lettuce, for example, Urlic et al. (2017) reported that the yield of lettuce biomass increased as the concentration of  $NO_3^{-1}$  increased. In addition, Wang et al. (2009) had reported that spinach supplied with sole NO<sub>3</sub>-N had the highest biomass without noticeable weight differences between sole NO<sub>3</sub>-N and NH<sub>4</sub>:NO<sub>3</sub> ratio of 25:75. Furthermore, with 100% NO<sub>3</sub>-N, the growth of Swiss chard reached the highest value, and was inhibited by NH<sub>4</sub><sup>+</sup> nutrition. However, the growth of both celery and fennel was significantly less affected by the N chemical form than the growth of Swiss chard (Santamaria et al., 1999).

 $NO_3$  supply combined with low  $NH_4$  favours growth for most plant species (Mangel and Kirkby, 1987). However, using  $NH_{4}^{+}$  as part of total N stimulates plant growth due to a higher CO, assimilation rate per leaf and photo energy saving (Guo et al., 2007). It appears that assimilation of  $NH_{4}^{+}$  into plant metabolites requires less energy compared to that required for the assimilation of  $NO_3^{-}$  because  $NH_4^{+}$  does not have to be reduced. However, in terms of their uptake, the two forms are not independent of each other, where  $NH_4^+$  can suppress  $NO_3^-$  uptake, but not vice versa. The  $NH_4^+$ supply of 10-15% of total N is optimal for most crops grown hydroponically on inert substrates and should not exceed 25% of total N supply (Sonneveld, 2002). However, Savvas et al. (2006) had recommended that the nutrient solution for optimum growth of lettuce should contain 30% NH<sub>4</sub>-N of total N as lettuce preferably absorbs NH<sub>4</sub>-N over NO<sub>3</sub>-N and this may reduce the nitrate content of the leaves. The partial replacement of NO<sub>3</sub>-N with 20% NH<sub>4</sub>-N  $(20\% \text{ NH}_{A}: 80\% \text{ NO}_{3})$  in our current study resulted in a non-significant difference in the number of leaves per plant and fresh weight yield compared to 100% NO<sub>3</sub>-N. In addition, celery plants with 20% NH<sub>4</sub>: 80% NO<sub>3</sub> not only contained similar N, P, Mg, Mn, Cu, protein, titratable acidity and TSS, but also a reduced nitrate concentration compared to 100% NO<sub>3</sub>-N. Several researchers had reported a stimulating effect of NH<sub>4</sub>-N on plant growth and development if ammonium was not the main

source of N (Sonneveld, 2002; Savvas et al., 2003). Using ammonium as the sole or primary source of N generally results in impaired plant growth and reduced yields (Guo et al., 2002), and can have and intercellular toxic effect (Santamaria, 2006).

The concentrations of nitrate in plant leaves match the NO<sub>2</sub>-N proportion in the nutrient solution. For example, He et al. (2011) had previously reported the same pattern of increasing nitrate concentration in plant leaf following an increase in NO<sub>3</sub><sup>-</sup> in nutrient solution. As reported by several authors, leafy vegetables tend to accumulate nitrate in their leaves (Gent, 2003; Premuzic et al., 2004; Prasad and Chetty, 2008; Fallovo et al., 2009). A partial substitution of NO<sub>3</sub> with NH<sub>4</sub> is recommended in order to limit the accumulation of NO3<sup>-</sup> in leafy vegetables (Savvas et al., 2006). Nitrate absorption is inhibited by ammonium if both forms of N are present in the source (Savvas et al., 2006; Britto and Kronzuker, 2013). For example, supplying N in mixed form (NH<sub>4</sub> plus NO<sub>2</sub>) reduced levels of nitrate in leafy vegetables such as spinach (Zhang et al., 1990), endive (Santamaria et al., 1997), rocket (Santamaria et al., 2001), cabbage (Fallovo et al., 2009) and lettuce (He et al., 2011). In addition, Urlic et al. (2017) reported a significant decrease in nitrate concentration as NH<sub>4</sub> concentrations increased in a red lettuce cultivar. However, Wang and Li (2003) had reported that there was no significant effect of ammonium fertilizer on nitrate concentration in spinach and cabbage.

It is well known that N is absorbed in both forms:  $NH_{4}^{+}$  and  $NO_{3}^{-}$ . The N form supplied directly affects the pH of the rhizosphere and the absorption of nutrients (Forde and Clarkson, 1999). The addition of  $NH_{4}^{+}$  reduces the pH value in the root zone due to the uptake of  $NH_4^+$  cation, leading plants to release H<sup>+</sup> to maintain the lectrical neutrality of the plant, which results in a lower pH in the root environment (Sonneveld and Voogt, 2009). Further replacement of  $NO_3^-$  with  $NH_4^+$  may reduce the uptake of important cations, such as K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup>, which can be explained by the competition between the  $NH_{4}^{+}$ cation and these essential plant development nutrients (Sonneveld and Voogt, 2009). The extent of these effects depends on several factors, such as plant type, growing conditions and nutrient solution adjustments made in the ionic balance. Therefore, it is recommended that  $NH_{4}^{+}$  be used cautiously, particularly for sensitive Ca deficiency crops. In our current study, the concentrations of K<sup>+</sup> and Ca<sup>2+</sup> in celery plants were reduced due to the replacement of NO<sub>3</sub> with NH<sub>4</sub>.

Another potentially important factor in product quality is the time of day for harvesting leafy vegetables. Although most of the growth traits and fresh yield of celery plants were not significantly affected by either harvesting time in our current study (Tab. 1), harvesting in the evening is preferred due to a higher product quality in terms of more K, Mg, Fe, total soluble sugars,  $\alpha$ -carotene and β-carotene, and a much lower nitrate content (Tabs 2 and 3). Nitrate is reduced to ammonium and then the ammonium is incorporated into amino acids (mainly in the GS-GOGAT cycle), therefore, little nitrate accumulates in plants after exposure to full daylight, as leaves quickly convert nitrate into amino acids and protein (He et al., 2011). Environmental conditions such as light intensity and fertilizer mode (sources and methods) affect the nitrate content of various vegetables. For example, under low growth irradiance (cloudy and hazy days) or exposure to complete darkness at night (morning harvest), this balance may be disrupted so that the roots take up nitrate faster than the plant can convert nitrate into protein (He et al., 2011). Reduced nitrate reductase activity and nitrate accumulation are associated with low levels of light in leafy vegetables (Chadjaa et al., 1999). Therefore, in order to prevent high accumulation of nitrate in leaves, it may be a good practice to remove nitrate from nutrient solution during cloudy days or to extend the growing period of the plant a few more days before harvest under full sunlight. Orsini and Pascale (2007) reported that 50% shading of basil plants resulted in higher leaf nitrate than under normal light. Important improvements in the shelf-life after harvest could be achieved by rescheduling the time of day for harvesting leafy vegetables (Clarkson, 2005). Clarkson (2005) found that salad roquette and arugula had increased their shelf-life from 2 to 6 days after harvest, while lollo rosso lettuce and red chard had increased their shelf-life from 1 to 2 days after harvesting at the end of the day compared with early morning harvest. It has been shown that the post-harvest quality of lettuce cultivars is affected by harvest time (Moccia et al., 1998), but the best time of day to harvest appears to be variable.

The biplot of the mean performance of the celery data explained 88.30% of the total variation of the standardized data. The first and two principal components (PC1 and PC2) explained 71.45% and 16.85%, respectively (Fig. 1). This relatively high proportion reflects the complexity of the relationships among the treatments and the measured traits. Yan and Kang (2003) mentioned that the first two PC's

should reflect more than 60% of the total variation in order to achieve the goodness of fit for biplot model.

#### CONCLUSIONS

To sum up the significant results obtained, there was strong correlation between fresh weight yield and most of the other studied traits, i.e. plant height, leaf number, N, Ca, Mg, Fe, Mn, Zn, protein, dietary fibre, total soluble sugars, nitrate, vitamin C,  $\alpha$ -carotene,  $\beta$ -carotene and lutein. The use of 100% NO<sub>3</sub> as a sole nitrogen source significantly increased plant height, leaf number, chlorophyll, fresh weight, N, K, Ca, Mg, Fe, Mn, Zn, protein, dietary fibre, total soluble sugars, nitrate, vitamin C,  $\alpha$ -carotene,  $\beta$ -carotene and lutein in celery plants compared to the use of either 80 or 60% NO<sub>2</sub>. However, this increase was not significant compared with the use of 20% NH<sub>4</sub>: 80% NO<sub>3</sub> in terms of leaf number, fresh yield, N, Mg, Mn, protein, soluble sugars, vitamin C and  $\alpha$ -carotene. Harvesting in the evening significantly increased K, Mg, Fe, total soluble sugars, α-carotene and β-carotene, and lowered the nitrate content in celery plants. In conclusion, partial replacement of 20% NO<sub>3</sub>-N with 20% NH<sub>4</sub>-N and evening harvesting are recommended for a high fresh yield, high quality, and lower nitrate levels.

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## AUTHOR CONTRIBUTIONS

Said Saleh and Guangmin Liu contributed equally to this work. All authors discussed the results, commented on the paper, and approved the final manuscript.

## **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

#### REFERENCES

ANJANA S., UMAR M., IQBAL M., 2007. Nitrate accumulation in plants, factors affecting the process, and human health implications. A review. Agron. Sustain. Dev. 27, 45-57.

- ABBASI H.N., VASILEVA V., LU X., 2017. The influence of the ratio of nitrate to ammonium nitrogen on nitrogen removal in the economical growth of vegetation in hybrid constructed wetlands. Environments 4(24), 1-9.
- ABDELHAMID M., HORIUCHI T., OBA S., 2003. Evaluation of the SPAD values in faba bean (*Vicia faba* L.) leaves in relation to different fertilizer applications. Plant Prod. Sci. 6(3), 185-189.
- ABDELHAMID M.T., SELIM E.M., EL-GHAMRY A.M., 2011. Integrated effects of bio and mineral fertilizers and humic substances on growth, yield and nutrient contents of fertigated cowpea (*Vigna unguiculata* L.) grown on sandy soils. J. Agronomy 10(1), 34-39.
- BLOM-ZANDSTRA M., 1989. Nitrate accumulation in vegetables and its relationship to quality. Ann. Appl. Biol. 115, 553-561.
- BOROWSKI, E., MICHAŁEK S., 2008. The effect of nitrogen form and air temperature during foliar fertilization on gas exchange, the yield and nutritive value of spinach (*Spinacia oleracea* L.). Folia Hort. 20(2), 17-27.
- BRITTO D.T., KRONZUKER H.J., 2013. Ecological significance and complexity of N-source preference in plant. Ann. Bot. 112: 957-963.
- CHADJAA H., VEZINA L.P., GOSSELIN A., 1999. Effect of supplementary lighting on growth and primary nitrogen metabolism of greenhouse lamb's lettuce and spinach. Can. J. Plant Sci. 79, 421-426.
- CLARKSON J.J., 2005. End of day harvest extends shelf life. HortScience 40(5), 1431-1435.
- DEJONCKHEERE W., STEURBAUT W., DRIEGHE S., VERSTRAETEN R., BRAECKMAN H., 1994. Nitrate in food commodities of vegetable origin and the total diet in Belgium. Microbiol. Alim. Nutr. 12, 359-370.
- EL-SAYED A.A., ABDALLA A.M., OMER E.A., DARWESH M.A., EL-SAYED S.I., 2009. Response of growth, yield and essential oil of celery to partial or complete organic fertilization. Egypt J. Appl. Sci. 24(8B), 673-682.
- EL-SAYED S.M., GLALA A.A., ADAM S.M., 2011. Response of two celery cultivars to partial or complete organic nitrogen alternation strategies. Austral. J. Basic Applied Sci. 5(10), 22-29.
- EZZO M.I., GLALA A.A., SALEH S.A., OMAR N.M., 2012. Improving squash plant growth and yielding ability under organic fertilization condition. Austral. J. Basic Applied Sci. 6(8), 572-578.
- FALLOVO C., ROUPHAEL Y., CARDARELLI M., REA E., BATTISTELLI A., COLLA G., 2009. Yield and quality of leafy lettuce in response to nutrients solution composition and growing season. Intern. J. Food. Agric. Environm. 7, 456-462.
- FAO, 2002. Handling and processing of organic fruits and vegetables in developing countries (prepared by Heyes J. and Bycroft B.).
- FORDE B.G., CLARKSON D.T., 1999. Nitrate and ammonium nutrition of plants: Physiological and molecular perspectives. Adv. Bot. Res. 30, 1-90.

- GENT M.P., 2003., Solution electrical conductivity and ratio of nitrate to other nutrients affect accumulation of nitrate in hydroponic lettuce. HortScience 38(2), 222-227.
- GOMEZ K.A., GOMEZ A.A., 1984. Statistical Procedures for Agricultural Research, 2<sup>nd</sup> ed. John Wiley & Sons, Inc., New York, USA.
- GUO S., BRUCK H., SATTELMACHER B., 2002. Effects of supplied nitrogen form on growth and water uptake of French bean (*Phaseolus vulgaris* L.) plants. Plant Soil 239, 267-275.
- GUO S., ZHOU Y., SHEN Q., ZHANG F., 2007. Effect of ammonium and nitrate nutrition on some physiological processes in higher plants – growth, photosynthesis, photorespiration and water relations. Plant Biol. 9, 21-26.
- HE J., CHEOL L., QIN L., 2011. Nitrate accumulation, productivity and photosynthesis of temperate butter head lettuce under different nitrate availabilities and growth irradiances. Open Hort. J. 4, 17-24.
- CHINESE STANDARD NY/T1653-2008. Determination for mineral elements in vegetables, fruits and derived products by ICP-AES method. Standard of Ministry of Agriculture of the People's Republic of China, Beijing, China.
- IPCS INCHEM, 2003. Nitrate and potential endogenous formation of N-nitroso compounds. WHO Food additives series: 50. http://www.inchem.org/ documents/jecfa/jecmono/v50je06.htm. Accessed 1 June 2018.
- JU X.T., KOU C.L., CHRISTIE P., DOU Z.X., ZHANG F.S., 2007. Changes in the soil environment from excessive application of fertilizers and manures to two contrasting intensive cropping systems on the north China plain. Environ. Pollut. 145, 497-506.
- KOSTERNA E., ZANIEWICZ-BAJKOWSKA A., ROSA R. FRANCZUK J., 2012. The effect of AgroHydroGel and irrigation on celeriac yield and quality. Folia Hort. 24(2), 123-129.
- KRĘŻEL J., KOŁOTA E., 2011. Response of Chinese cabbage grown in the spring season to differentiated forms of nitrogen fertilisation. Folia Hort. 23(1), 55-59.
- LANGE D.D., CAMERON A.C., 1994. Postharvest shelf life of sweet basil (*Ocimum basilicum*). HortScience 29, 102-103.
- LEVENE H., 1960. Robust tests for equality of variances. In: Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling, I. Olkin et al. (Eds), Stanford University Press, USA, 278-292.
- LIU C., SUNG Y., CHEN B., LAI H., 2014. Effect of nitrogen fertilizers on the growth and nitrate content of lettuce (*Lactuca sativa* L.). Int. J. Environ. Res. Public Health 11, 4427-4440.
- MARSCHNER H., 1995. Mineral nutrition of higher plants. Academic Press, London, UK.
- MASSON J., TREMBLAY N., GOSSELIN A., 1991. Nitrogen fertilization and HPS supplementary lighting influence

vegetable transplant production. I. Transplant growth. J. Amer. Soc. Hort. Sci. 116(4), 594-598.

- MOCCIA S., FREZZA D., CHIESA A., 1998. Time of day at harvest effect on postharvest lettuce quality. Agr. Trop. Subtrop. 31, 83-86.
- NICOLA S., HOEBERECHTS J., FONTANA E., 2007. Ebb-and-Flow and floating systems to grow leafy vegetables: a review for rocket, corn salad, garden and purslane. Acta Hortic. 747, 585-592.
- NIELSEN S.S., 2010. Food Analysis Laboratory Manual. Springer, New York, USA.
- ORSINI F., PASCALE S.D., 2007. Daily variation in leaf nitrate content of two cultivars of hydroponically grown basil. Acta Hortic. 747, 203-210.
- PARENTE A., GONNELLA M., SANTAMARIA P., L'ABBATE P., CONVERSA G., ELIA A., 2006. Nitrogen fertilization of new cultivars of lettuce. Acta Hortic. 700, 137-140.
- PARKS SE., HUETT D.O., CAMPBELL L.C., SPOHR L.J., 2008. Nitrate and nitrite in Australian leafy vegetables. Austr. J. Agric. Res. 59, 632-638.
- PRASAD S., CHETTY A.A., 2008. Nitrate-N determination in leafy vegetables: Study of the effects of cooking and freezing. Food Chem. 106, 772-780.
- PREMUZIC Z., VILELLA F., GARATE A., BONILLA I., 2004. Light supply and nitrogen fertilization for the production and quality of butter head lettuce. Acta Hort. 659, 671-678.
- REININK K., 1991. Genotype × environment interaction for nitrate concentration in lettuce. Plant Breeding 107, 39-49.
- SALEH S.A., 2009. Precision stressing by supplemental Ca and *Bacillus subtilis* FZB24 to improve quality of lettuce under protected cultivation. Acta Hortic. 824, 297-302.
- SALEH S.A., GALALA A.A., EZZO M.I., GHONAME A.A., 2010. An attempt for reducing mineral fertilization in lettuce production by using bio-organic farming system. Acta Hortic. 852, 311-318.
- SALEH S.A., ZAKI M.F., TANTAWY A.S., SALAMA Y.A.M., 2016. Response of Artichoke productivity to different proportions of nitrogen and potassium fertilizers. Int. J. ChemTech Res. 9(3), 25-33.
- SANTAMARIA P., 2006. Nitrate in vegetables: Toxicity, content, intake and EC regulation. J. Sci. Food Agr. 86, 10-17.
- SANTAMARIA P., ELIA A., GONNELLA M., 1997. Changes in nitrate accumulation and growth of endive plants during light period as affected by nitrogen level and form. J. Plant Nut. 20(10), 1255-1266.
- SANTAMARIA P., ELIA A., SERIO F., GONNELLA M., PARENTE A., 1999. Comparison between nitrate and ammonium nutrition in fennel, celery and swiss chard. J. Plant Nut. 22(7), 1091-1106.
- SANTAMARIA P., GONNELLA M., ELIA A., PARENTE A., SERIO F., 2001. Ways of reducing rocket salad nitrate content. Acta Hortic. 549, 529-536.
- SAVVAS D., KARAGIANNI V., KOTSIRAS A., DEMOPOULOS V., KARKAMISI I., PAKOU P., 2003. Interactions between

ammonium and pH of the nutrient solution supplied to gerbera (*Gerbera jamesonii*) grown in soilless culture. Plant Soil 245, 393-402.

- SAVVAS D., PASSAM H.C., OLYMPIOS C., NASI E., MOUSTAKA E., MANTZOS L., ET AL., 2006. Effects of ammonium nitrogen on lettuce grown on pumice in a closed hydroponic system. HortScience 41, 1667-1673.
- SCHENK M.K., 2006. Nutrient efficiency of vegetable crops. Acta Hortic. 700, 21-33.
- SHEHATA S.M., ABDEL-AZEM H.S., ABOU EL-YAZIED A., EL-GIZAWY A.M., 2010. Interactive effect of mineral nitrogen and biofertilization on the growth, chemical composition and yield of celeriac plant. Eur. J. Sci. Res. 47(2), 248-255.
- SONNEVELD C., 2002. Composition of nutrient solutions. In: Hydroponic Production of Vegetables and Ornamentals. D. Savvas and H.C. Passam (Eds), Embryo Publications, Athens, Greece, 179-210.
- SONNEVELD C., VOOGT W., 2009. Plant nutrition of greenhouse crops. Springer, Dordrecht, Netherlands.
- STEWART M.W., DIBB W.D., JOHNSTON E.A., SMYTH J.T., 2005. The contribution of commercial fertilizer nutrients to food production. Agron. J. 97, 1-6.
- URLIC B., SPIKA M.J., BECKER C., KLARING H., KRUMBEIN A., BEN S.G., ET AL., 2017. Effect of NO<sub>3</sub> and NH<sub>4</sub> concentrations in nutrient solution on yield and nitrate concentration in seasonally grown leaf lettuce. Acta Agr. Scand. B-S. P. 67, 748-757.
- WANG Z.H., LI S.X., 2003. Effects of N forms and rates on vegetable grown and nitrate accumulation. Pedosphere 13, 309-316.
- WANG J., ZHOU Y., DONG C., SHEN Q., PUTHETI R., 2009. Effect of NH<sub>4</sub><sup>+</sup>-N/NO<sub>3</sub><sup>-</sup>-N ratios on growth, nitrate uptake and organic acid levels of spinach (Spinacia oleracea L.). Afr. J. Biotech. 8(15), 3597-3602.
- WARD J.J.H., 1963. Hierarchical grouping to optimize an objective function. J. Am. Stat. Assoc. 58, 236-244.
- WHO, 2002. The world health report 2002 Reducing Risks, Promoting Healthy Life. http://www.who.int/ whr/2002/en/. Accessed 1 June 2018.
- YAN W., KANG M.S. 2003., GGE Biplot Analysis: A Graphical Tool for Breeders, Geneticists, and Agronomists, CRD Press LLC, Boca Raton, USA.
- ZAKI M.F., TANTAWY A.S., SALEH S.A., HELMY Y.I., 2012. Effect of bio-fertilization and different levels of nitrogen sources on growth, yield components and head quality of two broccoli cultivars. J. Appl. Sci. Res. 8(8), 3943-3960.
- ZHANG C.L., GAO Z.M., ZHAO Y.D., TANG W.M., 1990. The effects of different nitrogen forms and their concentration combinations on the growth and quality of spinach. J. Nanjing Agric. Univ. 13, 70-74.

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