Reduction of Nitrate Content in Response to Salicylic Acid in Spinach and Parsley Fertilized with Two Different N-Sources

Elwan M. W. M.[1] and M. A. M. Elhamahmy[2]

[1] Suez Canal University, Faculty of Agriculture, Department of Horticulture, Ismailia, Egypt.

[2] Suez Canal University, Faculty of Agriculture, Department of Agricultural Botany, Ismailia, Egypt.

Received: *8/3/2015*

Abstract: Spinach and parsley are hyper-nitrate accumulator vegetables, thereby constituting a possible human health risk. For that, pot and two field experiments were conducted to investigate the effect of exogenous salicylic acid (SA) application on yield, nitrate content and another quality parameters in spinach and parsley using two different ammonium fertilizers as ammonium sulfate (A. sulfate) and urea. The results of pot experiment (factorial, 2 x 2) showed that, A. sulfate-fertilized plants produced maximum yield compared to urea-fertilized ones, which companied with high level of nitrate content (up to 942.6 and 604.5 mg Kg^{-1} FW in spinach and parsley, respectively). Application of 5 µM of SA reduced nitrate content by about 18 and 10 % in A. sulfate-fertilized plants and by 50 and 7 % in urea-fertilized plants, in both spinach and parsley, respectively. Under field conditions, using only urea fertilizer, nitrate was decreased to minimum levels, 679.0 and 395.6 mg kg^{-1} FW, in spinach and parsley, sprayed with 20 and 5 µM-SA, respectively. This reduction was associated with induction of nitrate reductase (NRase) activity. The maximum percentage of NRase activity over control (74%) was recorded in spinach treated with 20 µM of SA and reached to 60 % in parsley treated with 5 µM-SA. Also, spraying of SA increased marketable yield, vitamin C and total free amino acids contents in both tested leafy vegetables. It was concluded that, SA application preserved nitrate content in safe limit for human consumption.

Keywords: Nitrogen, leafy vegetables, vitamin C, yield, nitrate reduction

INTRODUCTION

Plants uptake nitrogen in the form of NO_3^- or NH_4^+ (Barker and Pilbeam, 2007). There is strong evidence that some of the excess nitrogen taken up by the plant is not converted to protein but remains as non-protein nitrogen (Wang *et al.,* 2002). High levels of free NO_3^- are accumulated in shoots due to both it's an efficient uptake and an inefficient reductive systems (Petropoulos *et al.,* 2008). Ammonium assimilation into plant metabolites requires less energy than nitrate assimilation, as it does not need to be reduced (Hopkins and Huner, 2004). It is well known that uptake and assimilation of nitrate are genetically determined (Ourry *et al.,* 1997). Santamaria (2006) reported that, spinach and parsley are classified as very higher NO_3^- accumulator vegetables (239-3872 mg Kg^{-1} FW for spinach and 1000-2500 mg Kg^{-1} FW for parsley). Whereas, harmful effects may occur when livestock and humans consume rich-nitrate plant materials; they may suffer from methemoglobinemia or carcinoma by converting nitrate to nitrite of nitrosamines. In the human diet, about 80% of nitrates are provided from vegetables, while nitrate levels in fruits, cereals and legumes are very low because of higher nitrogen assimilation efficiency (Cornée *et al.,* 1992).

Nutritional, environmental and physiological circumstances are mainly factors responsible for nitrate accumulation in plants (Anjana *et al.,* 2007). Nitrate accumulation in plants depends on three factors, arranged in the following descending order, application of mineral fertilizer; treatment with physiologically active substances and sorbents; natural and anthropogenic changes in the soil environment (Nazaryuk *et al.,* 2002). It is well known that, type, amount, and form of nitrogen fertilizer influenced nitrate content in leafy vegetables (Hanafy *et al.,* 2000; Chen *et al.,* 2004; Stagnari *et al.,* 2007; Matraszek, 2008).

In recent decades, extensive researches have been done to reduce the nitrate accumulation in vegetables as nutrient management, horticultural technology and breeding. Such treatments include the application of organic acids such as citric and salicylic acids (Fariduddin *et al.,* 2003); NPK fertilization (Hanafy Ahmed *et al.,* 2000); foliar application of mixed amino acids in radish (Xing-Quan *et al.,* 2008) as well as proline in rocket (Barbieri *et al.,* 2011).

Nitrate reductase (NRase), which catalyzes the first enzymatic step in nitrate assimilation in higher plants, involving reduction of nitrate to nitrite, is a highly regulated enzyme (Hopkins and Huner, 2004) and it is considered to be a limiting factor for the growth, development and protein production in plants (MacKintosh and Meek, 2001). Several metabolic and environmental signals regulate NRase activity (Kaiser *et al.,* 1999). At transcriptional level, NRase activity is regulated by the availability of substrate NO_3^- and by the end product of the nitrogen assimilation pathway, glutamine (Hopkins and Huner, 2004). The activity of NRase is considered a marker of nitrogen assimilation potential (Singh *et al.,* 2002). Literature showed contradictory results regarding NRase activity, whereas, the lowest NRase activity was attributed with nitrogen form of NH_4^+-N than NH_4NO_3 form as reported in New Zealand spinach and lettuce by Matraszek (2008). However, there are evidences that ammonium ions can dramatically stimulate the NRase activity in the absence of nitrate in *Clematis vitalba*, but this stimulating effect does not occur in barley and tobacco plants grown under similar conditions (Bungard *et al.,* 1999; Munzarova *et al.,* 2006; Bloom *et al.,* 1992). That is why it was

[1]Elwan_wasfy@yahoo.com; [2]mhamahmy@yahoo.com

decided to investigate the NRase activity in plants supplied with NH_4^+ as a sole source of nitrogen.

Salicylic acid (SA), an endogenous plant growth regulator has been found to generate a wide range of metabolic and physiological responses in plants, thereby affecting their growth and development (Hayat *et al.,* 2010) such as ion uptake and transport (Harper and Balke, 1981). Hanafy *et al.* (2000) found that, 50 and 100 ppm of SA increased both fresh and dry yield of rocket leaves in both 1st and 2nd cuts. In this context, Kumar *et al.* (2010) found that, 50 µM of SA increased nitrate assimilation through the induction of NRase activity in isolated cotyledons in *Cucumis sativus* L.

Over-fertilization should be avoided but correct fertilization can have a positive effect on the quality of agricultural produce (Isherwood, 2000). In the recent market economy, product quality has become increasingly important. More than 90 % of the vitamin C in human diet is supplied by fruits and vegetables. Vitamin C is one of the most important nutritional quality factors in many horticultural crops and has many biological activities in the human body (Lee and Kader, 2000). Ascorbic acid is a well-known antioxidant and enzyme cofactor with many roles in human health (Conklin, 2004). Spinach is low in calories and a good source of vitamins C and A, and minerals, especially iron (Proietti *et al.,* 2009). Ascorbic acid (vitamin C) can afford protection conversion of nitrate to nitrite and nitrosamines, so it is of some interest that the higher levels of nitrate in crops have often been linked with lower ascorbic acid levels (Anonymous, 2001).

There isn't enough information available about the possible beneficial effects of SA on the yield and quality such as nitrate content and vitamin C in leafy vegetables. Therefore, the objective of this work was to investigate the impact of SA on the yield, nitrate content, vitamin C and NRase activity and some minerals of spinach and parsley plants grown in pots as well as open field.

MATERIALS AND METHODS

Pot and two field experiments were conducted at the Experimental Farm of the Faculty of Agriculture, Suez Canal University, Ismailia, Egypt (30º 58′ N latitude, 32º 23′ E longitude and 13 m above sea level). Two leafy vegetables, spinach (*Spinacia oleracea* L. cv. Balady) and parsley (*Petroselinum crispum* Mill. cv. Amaria) were used. The environmental conditions were as follows: a 12 h photoperiod, temperature fluctuated between 18-22/8-10°C day/night and a relative humidity ranged from 60% to 65%.

Pot experiment was conducted during 2011/2012 to investigate the effect of ammonium nitrogen sources and application of salicylic acid (SA) on yield and quality of spinach and parsley. Plastic pots (30 cm diameter) were filled with 7 kg sandy soil. The chemical analysis of the soil used (Page *et al.,* 1982) are: pH 8.27, electrical conductivity (EC) 0.465 dSm^{-1}, calcium (Ca) 0.8 meq^{-1}, magnesium (Mg) 0.6 meq^{-1}, potassium (K) 0.3 meq^{-1}, sodium (Na) 3.0 meq^{-1}, bicarbonate (HCO_3) 1.6 meq^{-1}, chloride (Cl) 3.0 meq^{-1}, and sulfate (SO_4) 0.1 meq^{-1}.

Treatments, with three replications (5 pots for each replicate) in randomized complete block design, consisted of (1) ammonium sulfate (A. sulfate) (≈20.5%N), (2) A. sulfate + 5 µM SA applied foliarly once a week. (3) Urea (46%), and (4) Urea + 5 µM SA applied foliarly once a week. Application of SA was done five times after 2 weeks of sowing.

Seeds of both spinach and parsley were provided by the Egyptian Crops Research Center, Ministry of Agriculture, Egypt, and sown in fall-winter of 1st November to the end of December, 2011. After emergence, the seedlings were thinned to leave ten or twenty per pot for spinach or parsley, respectively distributed at regular spacing and uniform seedling size. The pots were irrigated twice a week with 4 g l^{-1} of A. sulfate or 1.8 g l^{-1} of urea solution. Each pot were received a total of 7.8 g A-sulfate or 3.5 g urea at the end of the experiment. The volume of sprayed solution of SA ranged from 80 to 160 ml per pot each time, depending on plant size. The same amount of water was pulverized to the control plants. The pH was measured for water and SA solutions and it was 6.5 and 7.7, respectively, and adjusted to 7.0. All these sprays were applied in the morning (8:00-9:00 a.m.).

Sixty days after sowing, the aerial parts of all plants per pot were harvested, washed in distilled water, dried with blotted paper and weighed to determine plants fresh weight; then dried for 72 h at 70ºC. Powdered materials (0.5g) were digested separately for each replicate using a mixture of sulfuric acid and hydrogen peroxide and then brought to a final volume of 50 ml with distilled water (Page *et al.,* 1982). The percentage (%) of K^+ and Na^+ in aerial part of plants were determined by flame photometer according to Brown and Lilleland (1946). Also, Ca^{2+} in aerial part of plants was determined by flame photometer according to Brown *et al.* (1948).

According to the results obtained, two field experiments were carried out during 2012/2013 and 2013/2014 (1st Nov. to end of Feb.) with five treatments of SA denoted 0.0 (control), 5, 10, 15 and 20 µM.

Randomized soil samples were collected at 0.0-50 cm depth, before each plantation and homogenized together to determine the physicochemical characteristics. Electrical conductivity of the saturated soil paste extract expressed as dSm^{-1} was measured using a conductivity meter model Jenway 3310 (Jenway Ltd., Essex, Cambridge, UK) according to Page *et al.* (1982). Soil pH was determined by bench type Beckman glass electrode pH meter, in 1: 2.5 soil–water suspensions according to Page *et al.* (1982). The soil of the experimental site was sandy soil (85.21% sand, 11.5% silt and 3.29% clay) with pH 8.27 and electrical conductivity (EC) 0.47 dSm^{-1}. Before planting, the experimental location was cleared, ploughed, harrowed and divided into plots.

The experiment was arranged on a randomized complete block design with four replicates. Each replicate (plot) consisted of a 5m x 1m area, including 10 or 15 rows with an inter-row distance of 10 or 7cm for spinach and parsley, respectively. Urea as nitrogen source at 100 kg $fad.^{-1}$ was applied in three equal parts, after two, four and six weeks from sowing the seeds.

When the second leaf appeared, the leafy vegetable in each row was thinned to 60 or 120 plants corresponding to a density of 120 or 360 plants m^{-2} for spinach and parsley, respectively.

SA or distilled water as control was applied foliarly once a week. Application of SA was done five times after 2 weeks of sowing. The volume of sprayed solution ranged from 1 to 2 liter per plot each time, depending on plant size or development. The pH was measured and adjusted to 7.0 and all sprays were applied in the morning (8:00-9:00 a.m.).

Spinach plants were hand-harvested at the stage of marketable foliage size (eight weeks) and parsley was cut by scythe after 60 days from sowing; the second cut was performed 45 days from the first cut. The yield per plot was measured and then the yield per fad. was calculated.

Chemicals analysis:

Vitamin C, Nitrate and Nitrate reductase activity were determined in the leaves of both leafy vegetables. The extraction and determination of ascorbate (vitamin C) were performed using the protocol of Pearson (1970) and expressed as mg $100g^{-1}$ FW. NO_3^- (mg kg^{-1} FW) was determined spectrophotometrically at 540 nm, as described by Singh (1988). Nitrate reductase (NRase) activity was assayed using the method of Jaworski (1971). NRase activity was expressed as µmol NO_2^- g^{-1} FW h^{-1}. The total amino acids concentration was also estimated using the method of Rosen (1957) spectrophotometricaly at 650 nm in ethanolic extract (96% ETOH) in leaves, extracted according to Abdel-Rahman *et al.* (1975). All spectrophotometric measurements were conducted using UV/VIS spectrometer, T80, PG instrument Ltd, USA.

Statistical analysis:

The results were evaluated using descriptive statistics and analysis of variance (ANOVA). In the pot experiment, the effect of nitrogen sources and SA levels as well as their interaction were evaluated using two-way ANOVA by Fisher's F-test, followed by Duncan's multiple range test. Results of field experiments were analyzed using one-way ANOVA (concentrations of SA). All tests were performed at significance levels of 0.001, 0.01 and 0.05. Calculations carried out using the software package Statistica. TM for Windows version 6.1 (Statsoft, 2001, Tulsa, OK, USA).

RESULTS

Pot experiment:

The main effect of N-source (Table 1) showed that spinach plants fertilized with Ammonium sulfate (A. sulfate) had a significant highest yield and nitrate content with significant lowest vitamin C content. While, the significant highest yield, nitrate and vitamin C content were recorded with A-sulfate fertilizer in parsley plants. Regarding to main effect of SA, results in the same table showed that supplementation of SA significantly improved yield and vitamin C content whereas lowered nitrate content in both tested vegetable crops. The interaction effect revealed that the significant highest yield was found in plants received A. sulfate and treated with 5 µM SA in both tested crops (Table 1). However, the lowest nitrate content was observed in plants received urea and treated with 5 µM SA in both tested crops. In contrary to foliage yield, urea-fertilized spinach and treated with 5 µM SA showed maximum amount of vitamin C (88.88 mg 100 g^{-1} FW) compared with A. sulfate-fertilized plants (59.9 mg 100 g^{-1} FW) and non-treated with SA, respectively (Table 1). However, A. sulfate-fertilized and treated with 5 µM SA parsley plants recorded maximum vitamin C (115.54 mg 100 g^{-1} FW) compared to urea-fertilized and non-treated ones (91.1 mg 100^{-1} g FW).

Regarding to measured elemental concentrations, data in Table (2) showed that, only spinach plants differed significantly in measured minerals content under both N-sources and SA treatments. Results of the main effect of N-source showed that the significant highest K^+ and significant lowest Na^+ and Ca^{2+} were found in A. sulfate-fertilized plants. Concerning the main effect of SA, the data revealed that sprayed plants had the significant highest K^+ and significant lowest Na^+ content. However, Ca^{2+} content did not affected significantly by SA application. The interaction effect proved that fertilized plants with A. sulfate and treated with 5 µM SA had the highest K^+ and lowest Na^+ content, and the highest Ca^{2+} content was found in urea-fertilized plants and sprayed with 5 µM SA.

Field experiment:

SA applications resulted in an increase in the foliage weight of spinach and parsley in comparison with the control in both seasons (Table 3). In spinach, there was a significant increase in the yield until 20 µM SA. The same treatment had the significant highest vitamin C, nitrate reductase (NRase) activity and total amino acids with lowest nitrate content. The results indicated also that, the highest yield of first and second cut of parsley were noticed in treated plants only with 5 µM SA. The same treatment gave the significant highest NRase activity and total amino acids as well as significant lowest nitrate content, while significant highest vitamin C was found in plants treated with 10 µM SA.

DISCUSSION

The present investigation aimed to evaluate two nitrogen, ammonium sulfate ≈ 20.5 % N and urea ≈ 46.5 % N fertilizer forms on foliage yield and nutritional quality (in terms of vitamin C and nitrate content) in spinach and parsley, which classified as higher nitrate accumulator vegetables (Santamaria, 2006). Only fertilizers containing N under forms not readily available to crop, i.e. urea and A. sulfate were used to low accumulation of nitrate in leafy vegetables, as reported before by Stagnari *et al.* (2007). Results of pot experiment showed that, there was a significant difference between fertilization with A. sulfate and urea on foliage yield of spinach and parsley. Fertilization with A. sulfate (1.1g kg^{-1} soil) accumulated about 38 and 6 g over the mass yield in spinach and parsley, respectively compared to urea-fertilizer (0.5 g kg^{-1} soil).

Table (1): Main effects of N-sources and SA levels as well as their interaction on marketable yield, contents of nitrate and vitamin C of spinach and parsley (pot experiment).

Crop	N-Source	Marketable Yield (g pot^{-1})			Vitamin C (mg 100 g^{-1} FW)			Nitrate (mg kg^{-1} FW)		
		SA^0	SA^5	Mean	SA^0	SA^5	Mean	SA^0	SA^5	Mean
Spinach	A-Sulf.	136.92 c	198.33 a	167.63 a	59.99 b	75.55 a	67.77 b	942.6 a	744.1 c	858.4 a
	Urea	98.54 d	186.79 b	142.66 b	79.99 a	88.88 a	84.44 a	876.8 b	437.2 d	657.0 b
	Mean	117.73 b	192.56 a		69.99 b	82.22 a		909.7 a	590.7 b	
	P value									
	N	0.00082***			0.010239*			0.00018***		
	SA	0.0000***			0.02846*			0.000036***		
	N*SA	0.008**			0.4122 ns			0.000856***		
Parsley	A-Sulf.	73.69 b	81.94 a	77.82 a	106.66 ab	115.54 a	111.10 a	604.5 a	544.2 bc	574.4 a
	Urea	67.54 c	75.21 b	71.37 b	91.10 c	99.99 bc	95.55 b	558.8 b	517.8 c	538.3 b
	Mean	70.62 b	78.58 a		98.88 b	107.765 a		581.7 a	531.0 b	
	P value									
	N	0.014547*			0.003429**			0.00908**		
	SA	0.007**			0.023897*			0.00266**		
	N*SA	0.861766 ns			1.000 ns			0.2764 ns		

SA^0= without salicylic acid, SA^5= with 5 µM salicylic acid, A-Sulf. = Ammonium sulfate, N= Nitrogen –Source, SA= salicylic acid. Values followed by the same letter within a column are not significantly different at the 5% level of probability according to Duncan's multiple range test. ***,** and * significant at 0.1%, 1% and 5%; ns: not significant.

Table (2): Main effects of N-sources and SA levels as well as their interaction on potassium, sodium and calcium content in leaves of spinach and parsley (pot experiment).

Crop	N-Source	K%			Na%			Ca%		
		SA^0	SA^5	Mean	SA^0	SA^5	Mean	SA^0	SA^5	Mean
Spinach	A-Sulf.	4.86 b	5.58 a	5.22 a	2.92 b	2.60 b	2.76 b	0.48 b	0.48 b	0.48 b
	Urea	4.16 c	5.40 a	4.78 b	3.40 a	2.96 b	3.18 a	0.56 ab	0.60 a	0.56 a
	Mean	4.51 b	5.49 a		3.16 a	2.78 b		0.52 a	0.54 a	
	P value									
	N	0.003396**			0.016804*			0.011056*		
	SA	0.000157***			0.02327*			0.921648 ns		
	N*SA	0.021261*			0.602607 ns			0.921648 ns		
Parsley	A-Sulf.	4.44 a	4.44 a	4.44 a	1.32 a	1.32 a	1.32 a	0.76 a	0.84 a	0.80 a
	Urea	4.78 a	4.34 a	4.56 a	1.44 a	1.32 a	1.38 a	0.84 a	0.80 a	0.82 a
	Mean	4.61 a	4.39 a		1.38 a	1.32 a		0.80 a	0.82 a	
	P value									
	N	0.68752 ns			0.648797 ns			0.724659 ns		
	SA	0.47198 ns			0.648797 ns			0.724659 ns		
	N*SA	0.47198 ns			0.648797 ns			0.320188 ns		

SA^0= without salicylic acid, SA^5= with 5 µM salicylic acid, A-Sulf. = Ammonium sulfate, N= Nitrogen –Source, SA= salicylic acid. Values followed by the same letter within a column are not significantly different at the 5% level of probability according to Duncan's multiple range test. ***,** and * significant at 0.1%, 1% and 5%; ns: not significant.

Table (3): Yield, nitrate, vitamin C, nitrate reductase activity and total amino acids in leaves of spinach and parsley, as affected by salicylic acid levels in field experiments.

Crop	SA concentration (µM)	Marketable yield (t fad.$^{-1}$) 1st harvest 2012/13	1st harvest 2013/14	2nd harvest 2012/13	2nd harvest 2013/14	Vitamin C (mg 100 g^{-1} FW)	Nitrate content (mg Kg^{-1} FW)	Nitrate reductase (µmol g^{-1} h^{-1} FW)	Total amino acids (mg 100 g^{-1} FW)
Spinach	0.0	2.60 c	3.15 d	-	-	103.6 b	1040.8 a	1.095 d	600 d
	5.0	3.64 b	5.20 c	-	-	104.3 b	935.6 b	1.483 c	3000 c
	10	3.54 b	6.15 c	-	-	100.6 b	868.2 b	1.494 c	3600 bc
	15	3.95 b	7.42 b	-	-	101.0 b	770.3 c	1.733 b	3900 ab
	20	7.82 a	9.36 a	-	-	115.4 a	679.0 d	1.908 a	4500 a
Parsley	0.0	4.08 c	4.01 d	2.09 d	2.35 d	170.9 c	704.2 a	1.350 c	2700 c
	5.0	8.93 a	9.60 a	4.55 a	5.60 a	199.8 b	395.6 c	2.170 a	3600 a
	10	8.29 a	6.99 b	3.51 b	4.33 b	222.0 a	407.4 c	1.870 b	3600 a
	15	5.51 b	6.03 bc	3.11 bc	3.69 bc	179.8 c	571.7 b	1.510 d	3000 b
	20	5.56 b	5.31 c	2.70 cd	3.31 c	164.3 c	579.7 b	1.550 d	2400 d

Values are the means of four replicate. Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple range test.

Positive effect of A. sulfate (containing 24% sulfur) on yield may be explained by the fact that A. sulfate as N-form decrease soil pH, which might favor elements availability and uptake by plants in slightly alkaline soils (Guelser, 2005; Fageria *et al.,* 2010). Regarding to low fresh matter produced using urea as N-source, this is partly because of urea toxicity and low uptake rates of N (Marschner, 1995). In urea-fertilized plants, the concentrations of the nitrogen storage and transport of amino acids increased resulting in higher total amino acid concentrations and growth depression (Witte, 2011). Urea toxicity in plants is probably resulted from the NH_4 released during urea assimilation (Luo *et al.,* 1993) or by urea itself (Krogmeier *et al.,* 1989). Similar results have been reported for parsley (Petropoulos *et al.,* 2008), rape, chinese cabbage and spinach (Chen *et al.,* 2004) and rocket (Hanafy *et al.,* 2000). However, the results of Guelser (2005) showed a similar yield in spinach fertilized with either urea or A. sulfate.

The obtained results showed also that, the significant highest yield in both tested leafy vegetables was attributed to the application of A. sulfate (Table 1). This finding may be explained by the fact that sulfur (anion sulfate in A. sulfate) increases nitrogen use efficiency, as reported before by Salvagiotti *et al.* (2009). Also, nitrate content was significantly increased in spinach and parsley when A. sulfate as N-source was used. This results may be explained by the fact that NH_4^+ is rapidly oxidized to NO_3^- by nitrification in sandy soil so that nitrate is the major available source for plants (Barker and Pilbeam, 2007; Canali *et al.,* 2014). This finding was agreed with the results of Guelser (2005) who found that low dose of A. sulfate increased nitrate content in spinach than urea compared to high dose of both fertilizers. However, the results were in contradiction with our previous report on broccoli, which accumulated high nitrate in plants (Elwan and Abd-El Hamed, 2011).

The effect of SA application was more pronounced on foliage yield in spinach plants compared to parsley under both forms of ammonical N (Table 1). Under field conditions, it was found also that, response of foliage yield in spinach and parsley depended on SA concentration. Spinach yield increased under high level of SA (20 µM) but low level of SA (5 µM) gave the highest yield in parsley. SA application may be increase plant growth by, promote plant cell division and enlargement (Hayat *et al.,* 2005), altered phytohormones balance (Shakirova *et al.,* 2003), induced source-sink mediated invertase (Elwan and Elhamahmy, 2009), increased photosynthesis rate (Shi *et al.,* 2006) or indirectly regulates both local disease resistance mechanisms, including host cell death, defense gene expression and systemic acquired resistance. However, effect of SA application on yield of urea-fertilized spinach was more obvious than on A. sulfate-fertilized ones. This may indicate the protective role of exogenous SA against the urea toxicity. SA increased the activity of antioxidant enzymes which may be prevent the toxicity effect of urea on plant (Shakirova *et al.,* 2003; Hayat, 2005). Similarly, Hanafy *et al.* (2000) found that, 50 and 100 ppm of SA resulted in increased both fresh and dry yield of rocket leaves in both 1st and 2nd cuts.

Although urea-fertilized spinach showed about 20 mg100 g^{-1} FW of vitamin C more than A. sulfate ones, but A. sulfate-fertilized parsley had 15.5 mg $100g^{-1}$ FW over than urea-fertilized plants. These results coordinated with Mozafar (1993) who reported that, the content of ascorbic acid in vegetables is affected by growth conditions and the application of nitrogen fertilizers; its concentration is high in mature leaves with fully developed chloroplast.

Treatment with 5 μM-SA increased vitamin C content in spinach and parsley under both nitrogen sources in pot experiment. Under field conditions, vitamin C responded to high levels (20 μM) of SA in urea-fertilized spinach plants but affected with moderate level (10 μM) in urea-fertilized parsley. SA such as many phenolics was reported to protect vitamin C against oxidative decomposition in fruit juices (Miller and Rice-Evans, 1997). Raskin 1992 cited that SA increased the antioxidant capacity in many plants. In addition, vitamin C accumulated in metabolically active tissues such as leaves acts as a signaling molecule that coordinates a protective mechanism of the oxidative system (Pastori *et al.,* 2003).

The status of leaf mineral (Na^+, Ca^{2+} and K^+) contents under nitrogen fertilizers and SA application was apparent only in spinach plants grown in pots. Urea fertilizer increased Na^+ and Ca^{2+} contents but decreased amount of K^+ in spinach leaves. Moreover, foliar application of SA increased level of K^+ and decreased Na^+ content. SA as a signal transductor, may be induced membrane transport protein mediating Na^+, K^+ and Ca^{2+} such as, Na^+/H^+ antiporter which exclude Na^+ ions into apoplast and the K^+ influx channels or inhibit Na^+ influx transporter or Na^+-non selective cation channels, which need more investigations (Hopkins and Huner, 2004; Barker and Pilbeam, 2007). Raskin (1992) mentioned that enhancing effect of SA on the availability and movement of nutrients could result in stimulating different nutrients in the leaves. Also, SA in low concentration retarded K^+ influx and increased Ca^{2+} and alters proton influxes (Hayat, 2005). Harper and Balke (1981) found that SA inhibited K^+ absorption in excised oat root tissue. The degree of inhibition was both concentration and pH dependent. With decreasing pH, the inhibitory effect of SA increased, suggesting that the protonated form of SA was more active than its charged form. Results were agreed with Hanafy *et al.* (2000) who found that, high nitrogen fertilization increased total nitrogen, potassium and iron content but decreased phosphorus, manganese and zinc levels in rocket leaves. Also, 50 and 100 ppm of SA increased nitrogen, manganese and zinc but decreased phosphorus and potassium.

Excessive use of nitrogen fertilizers may have a double negative effect on the quality of plant foods, increases the concentration of NO_3 and decreases of amino acids (Mozafar, 1993). Pot urea-fertilized-spinach and parsley plants, showed minimum amount of nitrate accumulation compared to A. sulfate-fertilized ones. Additionally, SA-treated plants showed associated reduction in nitrate levels when fertilized with A. sulfate and urea in both spinach and parsley. SA might be involved in mobilization of internal tissue NO_3^- and chlorophyll biosynthesis to increase the functional state of the photosynthetic machinery in plants (Shi *et al.,* 2006).

Urea-fertilized spinach and parsley plants showed gradual reduction in nitrate concentration with increasing of SA levels under field conditions. Over third of nitrate content was decreased in 20 and 5 μM-SA-treated spinach and parsley plants, respectively. The nitrate concentration in both the metabolic pool and the storage pool of the leaf blades in three leafy vegetables (rape, Chinese cabbage and spinach) grown in plastic pots increased with nitrate supply (Chen *et al.,* 2004). Nitrate concentration in the leaf petioles of rocket plant was significantly increased by 41.2 and 75.0% in both 1st and 2nd cut, respectively when fertilized with the high level of nitrogen (Hanafy *et al.,* 2000) while 50 ppm of SA treatments significantly decreased it in the 1st and 2nd cut. There are evidences that NH_4^+ ions can dramatically stimulate the NRase activity in the absence of nitrate in *Clematis vitalba*, but this stimulating effect does not occur in barley and tobacco plants grown under similar conditions (Munzarova *et al.,* 2006; Bloom *et al.,* 1992).

Data in present investigation showed that, reduction of nitrate was correlated with induction of NRase activity in both studied leafy vegetables, which also recorded high values with increasing of SA application. Furthermore, urea-fertilized spinach and parsley showed the highest values of NRase activity under 20 and 5 μM of SA, respectively. NRase has been shown to be regulated by many environmental factors as well as endogenous factors such as metabolites and plant growth regulators as SA (Guerrero *et al.,* 1981).

The metabolism of NH_4^+ into amino acids and amides is the main mechanism of assimilation and detoxification of NH_4^+ (Barker and Pilbeam, 2007). Application of SA increased amino acids content in both urea-fertilized spinach and parsley. Total free amino acids were increased many times in 20 and 5 μM-SA treated spinach and parsley, respectively. Similar results were reported by Kumar *et al.,* (1999) who indicated that, the total protein content was increased in soybean plants sprayed with SA and this increase might be due to enhanced activity of NRase. Also, a significant increase in the activity of NRase was observed in both roots and leaves of the plants raised from the wheat grains soaked in lower concentration (10^{-5} M) of SA (Hayat *et al.* 2005). Such a lower concentration of SA when sprayed to the foliage of mustard plants enhanced their NRase activity. However, at higher concentrations (10^{-3} or 10^{-4} M), SA proved to be inhibitory (Fariduddin *et al.,* 2003). The treatment of maize plants with low concentrations of SA also enhanced the uptake of nitrogen and activity of NRase, whereas, high concentrations were proved to be inhibitory (Jain and Srivastava, 1981). The activity NRase was enhanced in the leaves of wheat following the exogenous application of SA and protected the enzyme from the action of proteinases and trypsin (Rane *et al.,* 1995). In urea-fertilized plants, the concentrations of the nitrogen storage and transport of amino acids are increased resulting in higher total amino acid concentrations and growth depression (Witte, 2011).

CONCLUSION

It may be concluded from the results that, using of ammonium sulfate increased foliage yield of spinach and parsley under local circumstances compared to urea fertilizer. However, shoots of spinach and parsley fertilized by urea recorded minimum levels of nitrate. Also, application of salicylic acid at low level reduced the nitrate content in both vegetable crops, accompanied with activation of nitrate reductase. Furthermore, vitamin C and free amino acid contents were increased in shoots of both leafy studied vegetables.

REFERENCES

Abdel-Rahman, T., H. Thomas, G. J. Doss and L. Howell (1975). Changes in endogenous plant hormones in cherry tomato fruits during development and maturation. Physiologia Plantarum, 34(1): 39-43.

Anjana, S.U., M. Iqbal and Y. B. Abrol (2007). Are nitrate concentration in leafy vegetables within the safe limits? Current Science, 92(3): 355–360.

Anonymous (2001). Organic Farming, Food Quality and Human Health. A Review of the Evidence. Soil Association.

Barbieri, G. A., E. Bottino, D. Stasio, S. Vallone and A. Maggio (2011). Proline and light as quality enhancers of rocket (*Eruca sativa* Miller) grown under saline conditions. Scientia Horticulturae, 128: 393–400.

Barker, A.V. and D. J. Pilbeam (2007). Handbook of Plant Nutrition. Taylor and Francis Group Press, Boca Raton, FL.

Bloom, A. J., S. S. Sukrapanna and R. L. Warner (1992). Root respiration associated with ammonium and nitrate absorption and assimilation by barley. Plant Physiology, 99: 1294–1301.

Brown, J. D. and O. Lilleland (1946). Rapid determination of potassium and sodium in plant material and soil extract by flame Photometry. Proceedings of the American Society for Horticultural Science, 73: 813.

Brown, J. G., O. Lilleland and R. K. Jackson (1948). The determination of calcium and magnesium in leaves using flame methods and a quartz spectophotometer. Proceedings of the American Society for Horticultural Science, 52: 1–6.

Bungard, R. A., A. Wingler, J. D. Morton, M. Andrews, M. C. Press and J. D. Scholes (1999). Ammonium can stimulate nitrate and nitrite reductase in the absence of nitrate in *Clematis vitalba*. Plant Cell Environment, 22: 859–866.

Canali, S., M. Diacono, C. Ciaccia, O. Maseti, F. Tittarelli and F. Montemurro (2014). Alternative stratiegies for nitrogen fertilization of overwinter processing spinach (*Spinacia oleracea* L.) in southern Italy. European Journal of Agronomy, 54: 47-53.

Chen, B. M., Z. H. Wang, S. X. Li, G. X. Wang, H. X. Song and X. N. Wang (2004). Effects of nitrate supply on plant growth, nitrate accumulation, metabolic nitrate concentration and nitrate reductase activity in three leafy vegetables. Plant Science, 167: 635–643.

Conklin, P. L. (2004). Ascorbic acid: an essential micronutrient provided by plants. In: Encyclopedia of Plant and Crop Science. Mercel Dekker.

Cornée, J., D. Lairon, J. Velema, M. Guyader and P. Berthezene (1992). An estimate of nitrate, nitrite and N-nitrosodimethylamine concentrations in French food products or food groups. Journal of Science Aliments, 12: 155–197.

Elwan, M. W. M. and K. E. Abd El-Hamed (2011). Influence of nitrogen form, growing season and sulfur fertilization on yield and the content of nitrate and vitamin C of broccoli. Scientia Horticulturae, 127(3): 181-187.

Elwan, M. W. M. and M. A. M. Elhamahmy (2009). Improved productivity and quality associated with salicylic acid application in greenhouse pepper. Scientia Horticulturae, 122: 521-526.

Fageria, N. K., A. B. Dos Santos and M. F. Moraes (2010). Influence of urea and ammonium sulfate on soil acidity indices in lowland rice production. Communications in Soil Science and Plant Analysis, 41: 1565–1575.

Fariduddin, Q., S. Hayat and A. Ahmad (2003). Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity and seed yield in *Brassica juncea*. Photosynthetica, 41: 281–284.

Guelser, F. (2005). Effects of ammonium sulfate and urea on NO_3^- and NO_2^- accumulation, nutrient contents and yield criteria in spinach. Scientia Horticulturae, 106: 330–340.

Guerrero, M. G., J. M. Vega and M. Losada (1981). The assimilatory nitrate reducing system and its regulation. Annual Review of Plant Physiology, 32: 169–204.

Hanafy, A. H., M. K. Khalil and A. Farrag (2000). Nitrate accumulation, growth, yield and chemical composition of rocket (*Eruca vesicaria* subsp. Sativa) plant as affected by NPK fertilization, kinetin and salicylic Acid. ICEHM, Cairo University, Egypt, 495- 508.

Harper, J. R. and N. E. Balke (1981). Characterization of the inhibition of K^+ absorption in oats roots by salicylic acid. Plant Physiolgy, 68: 1349–1353.

Hayat, Q., S. Hayat, M. Irfan and A. Ahmad (2010). Effect of exogenous salicylic acid under changing environment: A review Environmental and Experimental Botany, 68: 14–25.

Hayat, S., Q. Fariduddin, B. Ali and A. Ahmad (2005). Effect of salicylic acid on growth and enzyme activities of wheat seedlings. Acta Agronomica Hungrica 53: 433–437.

Hopkins, W. G. and N. P. A. Huner (2004). Introduction to Plant Physiology. 3rd Edition. John Wiley and Sons, Inc. USA, pp: 576.

Isherwood, K. F. (2000). Mineral fertilizer use and the environment. In: International Industry

Association, United Nations Environment Program, Paris.

Jain, A. and H. S. Srivastava (1981). Effect of salicylic acid on nitrate reductase activity in maize seedlings. Physiolgia Plantarum, 51: 339–342.

Jaworski, E. G. (1971). Nitrate reductase assay in intact plant tissues. Biochemical Biophysical Research Communications, 43:377–379.

Kaiser, W. M., H. Weiner and S. C. Huber (1999). Nitrate reductase in higher plants: a case study for transduction of environmental stimuli into control of catalytic activity. Physiolgia Plantarum, 105: 385–389.

Krogmeier, M. J., G. W. McCarty and J. M. Bremner (1989). Phytotoxicity of foliar applied urea. Proceedings of the National Academy of Sciences of U.S.A. 89: 8189–8191.

Kumar, P., S. D. Dube and V. S. Chauhan (1999). Effect of salicylic acid on growth, development and some biochemical aspects of soybean (*Glycine max* L. Merrill). Indian Journal of Plant Physiology, 4: 327–330.

Kumar, S. P., C. V. Kumar and B. Bandana (2010). Effects of salicylic acid on seedling growth and nitrogen metabolism in cucumber (*Cucumis sativus* L.). J. of Stress Physiology and Biochemistry, 6(3): 102-113.

Lee, S. K. and A. A. Kader (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biology and Technology, 20: 207–220.

Luo, L., Z. H. Lian and X. L. Yan (1993). Urea transformation and the adaptability of three leafy vegetables to urea as a source of nitrogen in hydroponic culture. Journal of Plant Nutrition, 16: 797–812.

Matraszek, R. (2008). Nitrate reductase activity of two leafy vegetables as affected by nickel and different nitrogen forms. Acta Physiologia Plantarum, 30: 361–370.

MacKintosh, C and S. E. M. Meek (2001). Regulation of plant NRase activity by reversible phosphorylation, 14-3-3 proteins and proteolysis. Cellular and Molecular Life Sciences, 58: 205–214.

Marschner, H. (1995). Mineral Nutrition of Higher Plants, Academic Press, London, pp. 229–312.

Miller, N. and C. A. Rice-Evans (1997). The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. Food Chemistry, 60: 331–337.

Mozafar, A. (1993). Nitrogen fertilizers and the amount of vitamins in plants: a review. Journal of Plant Nutrition, 16(12): 2479–2506.

Munzarova, E., B. Lorenzen, H. Brix, L. Vojtiskova and O. Votrubova (2006). Effect of NH_4^+/NO_3^- availability on nitrate reductase activity and nitrogen accumulation in wetland halophytes *Phragmites australis* and *Glyceria maxima*. Environmental and Experimental Botany, 55: 49–60.

Nazaryuk, V. M., M. I. Klenova and F. R. Kalimullina (2002). Ecoagrochemical approaches to the problem of nitrate pollution in agroecosystems. Russian Journal of Ecology, 33: 392–397.

Ourry, A., A. J. Gordon and J. H. Macduff (1997). Nitrogen uptake and assimilation in roots and root nodules, in: Foyer C.H. and W. P. Quick (Eds.), A molecular approach to primary metabolism in higher plants, Taylor and Francis, London, pp. 237–253.

Page, A. L., R. H. Miller and D. R. Keeney (1982). Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties. ASA, Madison, WI.

Pastori, G. M., G. Kiddle, J. Antoniw, S. Bernard, S. Veljovic-Jovanovic, P. J. Verrier, G. Noctor and C. H. Foyer (2003). Leaf vitamin C contents modulate plant defense transcripts and regulate genes that control development through hormone signaling. Plant Cell, 15: 939–951.

Pearson, D. (1970). The Chemical Analysis of Foods, 6th ed. T.A Constable, London.

Petropoulos, S. A., C. M. Olympios and H. C. Passam (2008). The effect of nitrogen fertilization on plant growth and the nitrate content of leaves and roots of parsley in the Mediterranean region. Scientia Horticulturae, 118: 255–259.

Proietti, S., S. Moscatello, F. Famiani and A. Battistelli (2009). Increase of ascorbic acid content and nutritional quality in spinach leaves during physiological acclimation to low temperature. Plant Physiology and Biochemistry, 47: 717–723.

Rane, J., K. C. Lakkineni, P. A. Kumar and Y. P. Abrol (1995). Salicylic acid protects nitrate reductase activity of wheat leaves. Plant Physiology and Biochemistry 22 (2): 119–121.

Raskin, I. (1992). Role of salicylic acid in plants. Annual Review of Plant Physiology and Plant Molecular Biology, 2: 439-463.

Rosen, H. (1957). A modified ninhydrin colorimetric analysis for amino acids. Archives of Biochemistry and Biophysics, 67: 10-15.

Salvagiotti, F., J. M. Castellarin, D. J. Miralles and H. M. Pedrol (2009). Sulfur fertilization improves nitrogen use efficiency in wheat by increasing nitrogen uptake. Field Crop Research, 113: 170-177.

Santamaria, P. (2006). Nitrate in vegetables: Toxicity, content, intake and EC regulation. Journal of the Science of Food and Agriculture, 86: 10–17.

Shakirova, F. M., A. R. Sakhabutdinova, M. V. Bezrukova, R. A. Fatkhutdinova and D. R. Fatkhutdinova (2003). Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. Plant Science, 164: 317–322.

Shi, Q., Z. Bao, Z. Zhu, Q. Ying and Q. Qian (2006). Effects of different treatments of salicylic acid on heat tolerance, chlorophyll fluorescence and antioxidant enzyme activity in seedlings of *Cucumis sativa* L. Plant Growth Regulation, 48(2): 127-135.

Singh, J. P. (1988). A rapid method for determination of nitrate in soil and plant extracts. Plant Soil, 110: 137–139.

Singh, D. B., S. Varma and S. N. Mishra (2002). Putrescine effect on nitrate reductase activity, organic nitrogen, protein, and growth in heavy metal and salinity stressed mustard seedlings. Biologia Plantarum, 45(4): 605–608.

Stagnari, F., V. D. Bitetto and M. Pisante (2007). Effects of N fertilizers and rates on yield, safety and nutrients in processing spinach genotypes. Scientia Horticulturae, 114: 225–233.

Statsoft, Inc. (2001). STATISTICA for Windows. Version 6. http://www. Statsoft.com.

Wang, Z. H., Z. Q. Zong, S. X. Li and B. M. Chen (2002). Nitrate accumulation in vegetables and its residual in vegetable fields. Environmental Science 23: 79–83.

Witte, C. (2011). Urea metabolism in plants. Plant Science, 180: 431–438.

Xing-Quan, L., C. Hui-yun, N. Qin-xue and L. K. Seung (2008). Evaluation of the Role of Mixed Amino Acids in Nitrate Uptake and Assimilation in Leafy Radish by Using 15N-Labeled Nitrate. Agricultural Sciences in China, 7(10): 1196-1202.

خفض المحتوى النتراتى في السبانخ والبقدونس كإستجابة للرش بحمض السلسيلك تحت ظروف التسميد بنوعين من السماد النيتروجينى

محمد وصفى محمد علوان[١]- محمد على محمود الحماحمى[٢]

[١]قسم البساتين – كلية الزراعة – جامعة قناة السويس

[٢]قسم النبات الزراعي – كلية الزراعة – جامعة قناة السويس

تعتبر السبانخ والبقدونس من محاصيل الخضر عالية التخزين للنترات وبذلك تشكل خطورة على صحة الإنسان. صممت تجربة أصص وتجربتان حقليتان لدراسة تأثير الرش بحمض السلسيلك على المحصول والمحتوى النتراتى وصفات الجودة الأخرى على السبانخ والبقدونس تحت ظروف التسميد بكبريتات الامونيوم واليوريا. أوضحت نتائج تجربة الأصص تفوق محصول النباتات المسمدة بكبريتات الامونيوم مقارنة بالتسميد باليوريا ولكنه ارتبط بارتفاع المحتوى النتراتى ليصل إلى ٩٤٢.٦ و٦٠٤,٥ مليجرام / كجم وزن طازج للأوراق في كلا من السبانخ والبقدونس على التوالي. أدت معاملة النباتات بحامض السلسيلك بتركيز ٥ ميكرومول إلى خفض كمية النترات بحوالي ١٨ و ١٠ % في النباتات المسمدة بكبريتات الامونيوم وبنسبة ٥٠ و٧% في النباتات المسمدة باليوريا في كلا من السبانخ والبقدونس على التوالي. تحت ظروف الحقل والتسميد باليوريا فقط انخفض مستوى النترات ليصل الى٦٧٩.٠ و٣٩٥,٦ مليجرام / كجم وزن طازج في كلا من السبانخ والبقدونس تحت تأثير الرش بحمض السلسيلك بتركيز ٢٠ و ٥ ميكرومول على التوالي. كما ارتبط هذا الانخفاض بزيادة نشاط انزيم اختزال النترات. زاد نشاط الانزيم بنسبة٧٤% في السبانخ المعاملة بـ ٢٠ ميكرومول من حمض السلسيلك و٦٠% في البقدونس المعامل بـ ٥ ميكرومول . كما أن المعاملة بحمض السلسيلك أدت إلى زيادة المحصول وكمية فيتامين ج والأحماض الامينية الحرة في كلا محصولي الخضر الورقية تحت الدراسة. والخلاصة أن المعاملة بحمض السلسيك تحافظ على تركيز النترات في الحدود الآمنة للاستهلاك الآدمي في السبانخ والبقدونس.