Zagazig J. Agric. Res., Vol. 42 No. (4) 2015

http:/www.journals.zu.edu.eg/journalDisplay.aspx?JournalId=1&queryType=Master

YIELD AND QUALITY OF SPINACH AND PARSLEY AFFECTED BY NITROGEN FERTILIZER SOURCES AND DI-POTASSIUM HYDROGEN ORTHO-PHOSPHATE

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ABSTRACT

Two experiments were carried out during 2011/2012 and 2012/2013 seasons at the Experimental Farm, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt, to understand the effect of nitrogen forms (ammonium sulfate and urea) and di-potassium hydrogen ortho-phosphate (K_2HPO_4) on yield, nitrate and vitamin C content as well as nitrate reductase activity of spinach and parsley. Pot experiment results revealed that ammonium sulfate-fertilized plants produced significantly higher yield and nitrate content than urea-fertilized plants. K_2HPO_4 improved the yield with less accumulation of nitrate. Lower sodium and calcium were found in K_2HPO_4 fertilized-spinach plants. Whereas, the results of open field showed that the yield was increased by increasing K_2HPO_4 up to 15 and 20 mM in spinach and parsley, respectively. Previous effect was associated with a significantly lower nitrate and high nitrate reductase activity. K_2HPO_4 at 15 mM gave the highest vitamin C, 112.11 and 253.08 mg100 g^{-1} FW for spinach and parsley, respectively. The higher nitrate reductase activity was associated with lower nitrate ($r = -0.87$) in plants-treated with K_2HPO_4. It could be concluded that, supplementation of K_2HPO_4 could reduce the leaf NO_3^- concentration to safety levels for consumption in both spinach and parsley.

Key words: Spinach, parsley, di-potassium hydrogen orthophosphate, nitrate reductase activity, vitamin C, sodium content.

INTRODUCTION

Spinach (*Spinacia oleracea* L.) and parsley (*Petroselinum crispum* Mill.) are leafy vegetables that accumulate the highest free NO_3^- amounts because of a very efficient uptake system and an inefficient reductive system, or an unfavorable combination of both (Maynard *et al.*, 1976; Petropoulos *et al.*, 2008). Spinach was reported to contain more than 2500 mg kg^{-1} fresh weight of nitrate (Santamaria, 2006).

High dietary intake due to the high nitrate content generates concern about the possible health effects (Shao-Ting *et al.*, 2007; Ralt, 2009). The toxicity of nitrate per se is low, but in humans, 5–10% of the ingested nitrate is converted into the more toxic nitrite by salivary or gastrointestinal reduction (Boink and Speijers, 2001). Although earlier reports linking nitrates with the occurrence of cancer are largely un-substantiated. Other nitrate-induced syndromes, such as methaemoglobinaemia in infants (blue baby syndrome) have been confirmed (Addiscott and Benjamin, 2004; Fewtrell, 2004; Chan, 2011). The acceptable daily intakes (ADI) of nitrites and nitrates recommended By WHO is 0–0.06 mg NO_2 kg^{-1} and 0–3.7 mg NO_3 kg^{-1} body weight (Anjana and Iqbal, 2007; Anonymous, 2008). Therefore, assuming a 60 kg body weight, ingestion of only 100 g of fresh vegetables with a nitrate concentration of 2500 mg kg^{-1} fresh weights exceeds the acceptable daily intake for nitrate by approximately 13% (Anjana *et al.*, 2007).

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Nitrate accumulation in plants depends on three major groups: application of mineral fertilizer, treatment with physiologically active substances and sorbents, and the natural and anthropogenic changes in the soil environment. With respect to their impact on nitrate accumulation, these factors may be arranged in the following descending order: fertilizer > physiologically active substances > soil (Nazaryük *et al.*, 2002). It is well known that uptake and assimilation of nitrate are genetically determined (Ourry *et al.*, 1997).

It is well known that, type, amount, and form of nitrogen fertilizer influenced nitrate content in leafy vegetables (Lips *et al.*, 1990; Elia *et al.*, 1998; Vieira *et al.*, 1998; Hanafy *et al.*, 2000; Lasa *et al.*, 2001; Chen *et al.*, 2004; Hammad *et al.*, 2007; Stagnari *et al.*, 2007; Matraszek, 2008; Liu *et al.*, 2014). Plants can take up nitrogen in the form of inorganic ammonium or nitrate (Kandlbinder *et al.*, 1997). Intensive application of fertilizer caused an excess of nitrogen for crops. Plants absorb most of their nitrogen in the forms of NO_3^- and NH_4^+. Ammonium assimilation into plant metabolites requires less energy than nitrate assimilation, as it does not need to be reduced. 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.*, 2008).

Reduction of nitrate accumulation in fresh vegetable crops became an important task, which might be affected through specific treatments. Such treatments include the application of organic acids such as citric and salicylic acids (Hanafy, 1996; Fariduddin *et al.*, 2003); NPK fertilization (Hanafy *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 (NR) activity is considered a marker of nitrogen assimilation potential (Singh *et al.*, 2002). Literature showed a contradiction regarding NR activity, whereas, the lowest NR 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 NR activity in the absence of nitrate in *Clematis vitalba* L. but this stimulating effect does not occur in barley and tobacco plants grown under similar conditions (Bungard *et al.*, 1999; Munzarova *et al.*, 2006). That is why it was decided to investigate the nitrate reductase activity in plants supplied with NH_4^+ as a sole source of nitrogen.

Potassium (K) is the most prominent inorganic plant solute that plays important roles related to stomatal behavior, osmoregulation, enzyme activity, protein synthesis, cell expansion, neutralization of nondiffusible negatively charged ions and membrane polarization (Elumalai *et al.*, 2002). Increasing evidence suggests that improvement of K-nutritional status of plants can greatly lower the reactive oxygen species (ROS) production by reducing activity of nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidase and maintaining photosynthetic electron transport (Cakmak, 2005). Phosphorus (P) is used in many metabolic reactions and activities as a component of many organic compounds (Awad *et al.*, 1990).

Di-potassium hydrogen orthophosphate (K_2HPO_4) is a highly water-soluble salt, which is often used as a fertilizer and buffering solution and it is a common source of potassium (44.9%) and phosphorus (17.8%). In previous report by Elwan (2010) di-potassium hydrogen orthophosphate was used successfully to overcome salinity stress in eggplant.

Leafy vegetables are an important part in the human diet (Anjana *et al.*, 2007). Spinach and parsley are from the most important leafy vegetables as an important source of minerals worldwide (Kansal *et al.*, 1981; Petropoulos *et al.*, 2008; Proietti *et al.*, 2009).

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 (Toledo *et al.*, 2003; Proietti *et al.*, 2009). Anonymous (2001) stated that the higher levels of nitrate in crops have often been linked with lower ascorbic acid levels.

Elwan. (2010) reported that spraying of K_2HPO_4 lowered the sodium accumulation in eggplant fruits under salinity stress. It's well-known that, normal human blood pressure connected with foods containing low sodium and high potassium content (He and Mac Gregor, 2008; Ando *et al.*, 2010).

To our knowledge, a limited number of investigations have been carried out on the effect of potassium and phosphorus on leaf nitrate reduction (Hanafy *et al.*, 2000). However, there is currently no information available about the possible beneficial effects of di-potassium hydrogen ortho-phosphate (K_2HPO_4) 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 di-potassium hydrogen ortho-phosphate (K_2HPO_4) on the yield, nitrate content, vitamin C and some minerals of spinach and parsley plants grown in pots as well as open field.

MATERIALS AND METHODS

Two experiments (pot as well as open field) were conducted during 2011/2012 and 2012/2013 at the Experimental Farm of the Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. The experimental crops were two leafy vegetable genera: spinach (*Spinacia oleracea* L. cv. Balady) and parsley (*Petroselinum crispum* Mill. cv. Amaria). The environmental conditions were as follows: a 12 hr. photoperiod, temperature of 18-22/8-10°C day/night and a relative humidity of 60% to 65%.

Pot Experiment

Pot experiment was conducted during fall-winter of 2011 from 1 November to the end of December to examine the effect of source of nitrogen and application of K_2HPO_4 on yield and quality of spinach and parsley. Tested crop seeds were sown in 30 cm x 30 cm (diameter x depth) plastic pots filled with 7000 cm^3 sand (soaked overnight in tap water for washing, then dried up to approximately 60% moisture content before using) of pH 8.27, electrical conductivity (EC) 0.465 dSm^{-1}, calcium (Ca) 0.8 meq^{-1}, magnesium (Mg) 0.6 meq^{-1}, K 0.3 meq^{-1}, Na 3.0 meq^{-1}, bicarbonate (HCO_3) 1.6meq^{-1}, chloride (Cl) 3.0meq^{-1}, and sulfate (SO_4) 0.1meq^{-1} (Jackson, 1967).

Treatments, with three replicates (three pots for each replicate), consisted of (1) ammonium sulfate ≈ 20.5% N, (2) ammonium sulfate + 5 mM K_2HPO_4, (3) Urea ≈ 46% N, and (4) Urea + 5 mM K_2HPO_4. These treatments presented all possible combinations of two ammonium nitrogen sources (ammonium sulfate and urea) with two concentrations of K_2HPO_4 (0.0 and 5 mM).

Seeds of both leafy vegetables were sown in pots and the seedlings were thinned to ten and twenty per pot for spinach and parsley, respectively, after emergence for regular spacing and uniform plant size. The pots were irrigated two times per week with solution containing 4 g/l ammonium sulfate or 1.8 g/l urea with or without 0.87 (5 mM) g/l K_2HPO_4. Each pot received a total of 7.8 g ammonium sulfate or 3.5 g urea at the end of experiment. Application of K_2HPO_4 started from the second irrigation, whereas each pot received a total of 1.48 g K_2HPO_4 at the end of experiment. The pH was measured for water and K_2HPO_4 solutions and it was 6.5 and 7.7, respectively and adjusted to 7.0. Treatments were arranged as factorial experiment in completely randomized design.

Two months after sowing, the aerial parts of all plants per pot were harvested and washed in distilled water, dried with blotted paper and weighed to determine plants fresh weight; then dried for 72 hr., at 70 °C. Powdered material (0.5 g) was digested separately for each replicate using a mixture of sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) and then brought to a final volume of 50 ml with distilled water. The percentage (%) of potassium and sodium aerial part of plants were determined by flame photometer according to Brown and Lilleland (1946). Also, calcium aerial part of plants was determined by flame photometer according to Brown *et al.* (1948).

Open-Field Experiment

Field experiment (first of November 2012 to the mid of February 2013) with five treatments, each corresponding to a control receiving 0.0 K_2HPO_4, or K_2HPO_4 levels of 5, 10, 15 and 20 mM was conducted.

Randomized soil samples were collected from 0.0 to 50.0 cm depth, before plantation and homogenized together to determine the physicochemical characteristics of air-dried, crushed and sieved (<2mm) soil in accordance to the methods of Gee and Bauder (1986) and Sparks *et al.* (1996). Soluble cations Na^+, K^+, Ca^{2+} and Mg^{2+} and anions HCO_3^- and Cl^- were determined in the soil paste (1:2.5) (Richards, 1954). Sulfate (SO_4^{2-}) was precipitated by barium chloride as barium sulfate and gravimetrically determined (Jackson, 1967). 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 Richards (1954). 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, electrical conductivity (EC) 0.47 dSm^{-1}, calcium (Ca) 0.4 mM, magnesium (Mg) 0.3 mM, potassium (K) 0.3 mM, Na 3.0 mM, bicarbonate (HCO_3) 1.6 mM, chloride (Cl) 3.0 mM, and sulfate (SO_4) 0.05 mM. Before planting, the experimental location was cleared, ploughed, harrowed and divided into plots.

Randomized complete block design (one way) was used with four replicates, experimental unit area (plot) was 5 m x 1 m in size, including 10 or 15 rows with an inter-row distance of 10 and 7.0 cm for spinach and parsley, respectively. Urea as nitrogen source at 100 (46% N) 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 and 120 plants corresponding to a density of 120 and 360 plants m^{-2} for spinach and parsley, respectively.

Solutions containing 0, 5, 10, 15 and 20 mM K_2HPO_4 was added twice weekly after irrigation starting from the third week with the first addition of urea. The volume of solution ranged from 500 to 1000 ml and 350 to 700 ml per row each time, depending on plant size or development for spinach and parsley, respectively. Spinach plants received a total of 0, 5.2, 10.4, 15.6 and 20.8 g K_2HPO_4 per row, however parsley plants received a total of 0, 3.7, 7.4, 11.1 and 14.8 g K_2HPO_4 per row at the end of experiment. The pH was measured for water and K_2HPO_4 solutions and it was 6.5 and 7.7, respectively, and adjusted to 7.0.

Spinach plants were hand-harvested at the stage of marketable foliage size (two months) , however, parsley was cut by scythe after 60 days from sowing and the second cut was harvested 45 days from the first cut. The yield per plot was recorded and then the yield per faddan was calculated.

Chemical Analyses

Vitamin C

Extraction and determination of ascorbate (vitamin C) was performed using the protocol of Pearson (1970) by titration method using 2,6-dichlorophenolindophenol and calculated as mg 100 g^{-1} FW.

Nitrate content

NO_3^- (mg kg^{-1} FW) was determined spectrophotometrically at 540 nm as described by Singh (1988).

Nitrate reductase (NR) activity assay

The *in vivo* nitrate reductase activity in leaves of spinach and parsley cultivated under open field conditions were assayed using the method of Jaworski (1971), modified by Buczek (1985). Fifty milligram of crumbled plant material was put into test tubes containing 5 ml incubation buffer of pH 7.5 (0.1 M K_2HPO_4/ KH_2PO_4 with the addition of 0.1 M KNO_3), 0.1 ml *n*-propanol and 0.05% chloramphenicol. The final concentration of *n*-propanol and chloramphenicol in buffer was 1.86 and 0.00098%, respectively. Samples were vacuum-infiltrated for 30 min, and then incubated at 32°C for 1 hr., in a water bath. After that time, the tubes with the samples were placed into hot water (85°C) for 2-3 min. Then 1 ml of samples were taken from each test tube and 1ml of 1%

sulfanilamide solution at a HCl concentration of 3 M, 1 ml of 0.01% N-(1-naphtyl)-ethylendiamine hydrochloride and 2 ml of redistilled water were added. Sulfanillic acid is converted into corresponding diazo compound which couples with α-naphtylamine to form α-naphthyl-amine-P-azobenzene-P-sulphonic acid, a red azo-dye. After 30 min the absorbance of pink coloured solutions was read at 540 nm. NO_2^- concentration was calculated from the standard curve, which was prepared by dilution of 100 µM solution of $NaNO_2$. Nitrate reductase activity was expressed as µmol NO_2^- g^{-1} FW h^{-1}.

Statistical Analysis

The results were evaluated using descriptive statistics and analysis of variance (ANOVA). Using two-way ANOVA (pot experiment), the effect of nitrogen sources and K_2HPO_4 levels as well as their interactions were evaluated by Fisher's *F-test*, followed by Duncan's multiple range test at significance level α of 0.05 for comparing N x KP combinations. However, the results of open field experiments were analyzed using one-way (concentrations of di-potassium orthophosphate) ANOVA (Statsoft, 2001, Tulsa, OK, USA). Correlations between parameters (*e.g* nitrate and yield; nitrate and nitrate reductase activity) were evaluated using correlation matrices at significance level α of 0.001 (Statsoft, 2001).

RESULTS

Effect of Forms of Nitrogenous Fertilizers and K_2HPO_4 Levels and Their Interaction on Yield and Quality Characters of Spinach and Parsley (Pot Experiment)

The marketable yield, contents of vitamin C, nitrate, potassium, sodium and calcium in spinach and parsley fresh leaves are given in Tables 1, 2, 3 and 4. Main effect of nitrogen source and K_2HPO_4 affects marketable yield and nitrate content in both leafy vegetables, however the content of vitamin C was not affected by N-source, K_2HPO_4 and their interactions (Tables 1 and 2). The yield of spinach rose from 195.1 to 203.2 g pot^{-1}, while yield of parsley from 71.3 to 83.8 g pot^{-1}, when ammonium sulfate as nitrogen source was used instead of urea. However, urea as nitrogen source significantly decreased the nitrate content by 8.54% in spinach (Table 1), but significantly increased it in parsley by 7.95% comparing with ammonium sulfate (Table 2). Regarding the effect of K_2HPO_4, data presented in Tables 1 and 2 revealed that the application of 5 mM K_2HPO_4 markedly improved the yield by 4.58% and 23.38%, however, it decreased the content of nitrate by 38.9% and 18.47% in spinach and parsley, respectively. However, vitamin C content was not affected by the application of K_2HPO_4. Results showed that the highest yield was obtained with plants received ammonium sulfate as N-source and treated with 5 mM K_2HPO_4 in both tested leafy vegetable crops (Tables 1 and 2). However, mostly the lowest nitrate content was observed in spinach and parsley plants treated with K_2HPO_4 and received urea as N-source (Tables 1 and 2).

The amount of the other principal nutrients K, Na and Ca in spinach and parsley are given in Tables 3 and 4. N-source did not affect the content of all the detected ions in spinach and parsley, except Ca in spinach, which was higher in plants, received urea as N-source. Regarding the effect of K_2HPO_4, the results also showed that the treated spinach plants with K_2HPO_4 had higher potassium content and lower sodium and calcium contents (Table 3). The interaction effect showed that the lower sodium and calcium contents in addition to the highest potassium content were detected in spinach plants that received ammonium sulfate as N-source and supplied with K_2HPO_4. Also, results showed that no significant difference was found in spinach plants that received ammonium sulfate or urea as N-source at 5 mM K_2HPO_4; in case of potassium content (Table 3). The changes in contents of K, Na and Ca of parsley were not affected by N-source, K_2HPO_4 and their interactions (Table 4).

Regarding correlations between sodium and calcium or nitrate content, the results reveal that sodium content positively correlated at significant level with nitrate (r =0.7463***) and calcium (r =0.870***) contents.

Table 1. Marketable yield, contents of nitrate and vitamin C of spinach as affected by ammonium N sources, K_2HPO_4 levels and their interactions in pot experiment during 2011/2012 season

N-source	K_2HPO_4 conc. (mM)	Marketable Yield (g pot^{-1})	Vitamin C (mg 100 g^{-1} FW)	Nitrate (mg kg^{-1} FW)
Main effect of N-source				
Ammonium sulfate at 7.8 g pot^{-1}		203.22 a	74.44 a	765.5 a
Urea at 3.5 g pot^{-1}		195.05 b	75.55 a	700.1 b
Main effect of K_2HPO_4 (mM)				
	0.0	194.68 b	77.77 a	909.7 a
	5.0	203.6 a	72.22 a	555.8 b
Interaction effect of N-source and K_2HPO_4				
Amonium sulfate at 7.8 g pot^{-1}	0.0	198.54 b	75.55 a	942.6 a
	5.0	207.91 a	73.33 a	588.3 c
Urea at 3.5 g pot^{-1}	0.0	190.82 c	79.99 a	876.8 b
	5.0	199.29 b	71.10 a	523.3 d

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.

Table 2. Marketable yield, contents of nitrate and vitamin C of parsley as affected by ammonium N sources, K_2HPO_4 levels and their interactions in pot experiment during 2011/2012 season

N-source	K_2HPO_4 conc. (mM)	Marketable Yield (g pot^{-1})	Vitamin C (mg 100 g^{-1} FW)	Nitrate (mg kg^{-1} FW)
Main effect of N-source				
Ammonium sulfate at 7.8 g pot^{-1}		83.80 a	96.09 a	489.1 b
Urea at 3.5 g pot^{-1}		71.33 b	95.27 a	528.0 a
Main effect of K_2HPO_4 (mM)				
	0.0	69.45 b	95.26 a	560.3 a
	5.0	85.68 a	96.10 a	456.8 b
Interaction effect of N-source and K_2HPO_4				
Ammonium sulfate at 7.8 g pot^{-1}	0.0	75.00 b	97.20 a	543.1 a
	5.0	92.60 a	96.09 a	435.1 b
Urea at 3.5 g pot^{-1}	0.0	63.90 c	97.21 a	577.5 a
	5.0	78.75 b	95.27 a	478.5 b

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.

Table 3. **Potassium, sodium and calcium content in leaves of spinach as affected by ammonium N sources and K_2HPO_4 and their interactions in pot experiment during 2011/2012 season**

N-source	K_2HPO_4 conc. (mM)	K (%)	Na (%)	Ca (%)
Main effect of N-source				
Ammonium sulfate at 7.8 g pot^{-1}		5.76 a	2.60 a	0.40 b
Urea at 3.5 g pot^{-1}		5.13 a	2.80 a	0.52 a
Main effect of K_2HPO_4 (mM)				
	0.0	4.87 b	2.94 a	0.52 a
	5.0	6.02 a	2.46 b	0.40 b
Interaction effect of N-source and K_2HPO_4				
Ammonium sulfate at 7.8 g pot^{-1}	0.0	5.58 a	2.92 ab	0.48 b
	5.0	5.94 a	2.28 c	0.32 c
Urea at 3.5 g pot^{-1}	0.0	4.16 b	2.96 a	0.56 a
	5.0	6.10 a	2.64 b	0.48 b

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.

Table 4. **Potassium, sodium and calcium content in leaves of parsley as affected by ammonium N sources and K_2HPO_4 and their interactions in pot experiment during 2011/2012 season**

N-source	K_2HPO_4 conc. (mM)	K (%)	Na (%)	Ca (%)
Main effect of N-source				
Ammonium sulfate at 7.8 g pot^{-1}		3.24 a	1.52 a	0.67 a
Urea at 3.5 g pot^{-1}		3.89 a	1.43 a	0.76 a
Main effect of K_2HPO_4 (mM)				
	0.0	3.53 a	1.51 a	0.68 a
	5.0	3.50 a	1.44 a	0.75 a
Interaction effect of N-source and K_2HPO_4				
Ammonium sulfate at 7.8 g pot^{-1}	0.0	3.35 a	1.58 a	0.60 a
	5.0	3.12 a	1.46 a	0.73 a
Urea at 3.5 g pot^{-1}	0.0	3.71 a	1.44 a	0.76 a
	5.0	4.06 a	1.42 a	0.76 a

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.

Open-Field Experiment

Application of K_2HPO_4 at different concentrations (0, 5, 10, 15 and 20 mM) resulted in an increase in the foliage weight of spinach and parsley in comparison with the control treatment (Tables 5 and 6). In spinach, there was a steep increase in the yield until 15 mM K_2HPO_4, while a significant decrease in the yield was observed when the level of applied K_2HPO_4 increased from 15 to 20 mM and was similar as at 10 mM K_2HPO_4 (Table 5). Regarding the first cut of parsley, the results indicated that a significant steep increase in yield with increasing K_2HPO_4 concentration from 0.0 to 20 mM, however, in the second cut, the increasing was at non-significant level when the concentration of K_2HPO_4 was increased from 0.0 to 5 mM, from 5 to 10 mM and from 10 to 15 mM. At 20 mM K_2HPO_4, the yield of parsley in the second cut was significantly higher comparing with control and other K_2HPO_4 concentrations (Table 6).

Vitamin C content was at the highest in spinach plants treated with 15 mM of K_2HPO_4 in addition to 15 and 20 mM K_2HPO_4 in parsley (Tables 5 and 6). Plant nitrate content decreased up to 15 and 20 mM K_2HPO_4 in spinach and parsley, respectively (Tables 5 and 6). An opposite tendency was noted in the activity of nitrate reductase, whereas it was increased by increasing the concentration of K_2HPO_4 until 15 mM, decreased again in spinach plants (Fig. 1). While, linear increase in the activity of nitrate reductase with increasing the concentration of K_2HPO_4 up to 20 mM was observed, in case of parsley plants (Fig. 1).

Regarding correlations between yield and nitrate content, results reveal that the yield was negatively correlated at significant level with nitrate content in spinach (r = -0.871***) and parsley (r = -0.968***). Also, the results revealed a significant positive correlation between total yield of spinach (r = 0.860***) and parsley (r = 0.926***) with the activity of nitrate reductase. On the contrary, a highly significant negative correlation has been found between activity of nitrate reductase and nitrate content of spinach (r = - 0.874***) and parsley (r = - 0.959***).

DISCUSSION

The Interaction Effect Between Forms of Nitrogenous Fertilizers and K_2HPO_4 Levels on Yield and Quality Characters of Spinach and Parsley (Pot Experiment)

In general, the spinach and parsley plants fertilized with ammonium sulfate produced greater leaf fresh matter than those received urea as the N source in the absence of K_2HPO_4. This finding may be explained by the fact that ammonium 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 lower fresh matter produced using urea as N-source, this is partly because of urea toxicity and lower uptake rates of N (Marschner, 1995). In urea-fertilized plants, the concentration 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 lettuce (Khoshgoftarmanesh *et al.*, 2011) and broccoli (Elwan and Abd El-Hamed, 2011), however, the results of Guelser (2005) showed a similar yield in spinach fertilized with urea or ammonium sulfate.

Indicated results showed that the significantly highest yield in both tested leafy crops was obtained due to application of ammonium sulfate (Tables 1and 2). This finding may be explained by the fact that sulfur (anion sulfate in ammonium sulfate) increases nitrogen use efficiency as reported before by Salvagiotti *et al.* (2009). Also, nitrate content was significantly increased in spinach and non-significantly affected in parsley when ammonium sulfate as N-source in comparison to urea was used. These 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 lower application dose of ammonium sulfate increased

Table 5. Yield, nitrate and vitamin C contents in leaves of spinach as affected by K_2HPO_4 levels in open field experiment during 2012/2013 season

K_2HPO_4 concentration (mM)	Marketable yield (t fad^{-1})	Vitamin C (mg100 g^{-1} FW)	Nitrate content (mg Kg^{-1} FW)
0.0	4.30 d	93.24 c	953.9 a
5.0	7.78 e	95.46 bc	740.6 b
10	9.32 b	97.68 bc	725.8 b
15	10.28 a	112.11 a	545.1 c
20	8.71 b	102.12 b	714.0 b

Values are the means of four replicates. Values followed by the same letters within a column for each genus are not significantly different at the 5% level of probability according to Duncan's multiple range test.

Table 6. Yield, nitrate and vitamin C contents in leaves of parsley as affected by K_2HPO_4 levels in open field experiment during 2012/2013 season

K_2HPO_4 concentration (mM)	Marketable yield (t fad^{-1})		Vitamin C (mg 100 g^{-1} FW)	Nitrate content (mg Kg^{-1} FW)
	1st harvest	2nd harvest		
0.0	4.84 d	3.55 d	226.44 b	598.4 a
5.0	7.64 c	3.79 cd	226.44 b	727.3 b
10	8.94 b	4.21 bc	227.92 b	438.5 c
15	9.40 b	4.47 b	253.08 a	420.7 c
20	10.68 a	5.56 a	248.64 a	343.7 d

Values are the means of four replicates. Values followed by the same letters within a column for each genus are not significantly different at the 5% level of probability according to Duncan's multiple range test.

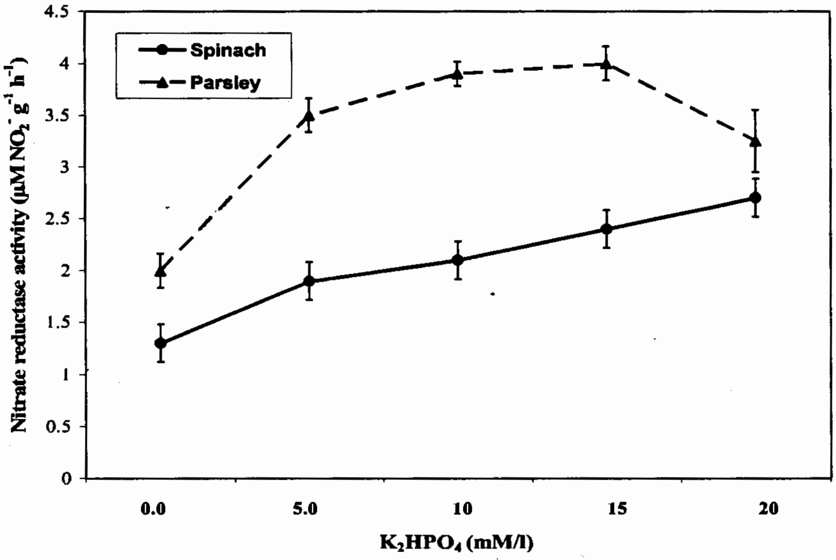

Fig. 1. Effect of K_2HPO_4 on nitrate reductase (NR) activity of the two leafy vegetables

nitrate content in spinach than urea compared to higher application dose of both fertilizers. However, the results were in contradiction with previous report on broccoli plants, which accumulated higher nitrate in plants received urea than plants received ammonium sulfate (Elwan and Abd El-Hamed, 2011).

In the present investigation, it was used only fertilizers containing N under forms not readily available to crop; *i.e.*, urea and ammonium sulfate, for lower accumulation of nitrate in leafy vegetables, as reported before by Stagnari *et al.* (2007).

The positive effect of di-potassium hydrogen ortho-phosphate on the yield of both tested leafy vegetables may be due to that ammonium in both N-sources is affected by K_2HPO_4 supplementation (Tables 1 and 2). The presence of potassium in the nutrient solution resulted in activation of nitrate reductase and reduction of nitrate accumulation as reported by Sharma and Agarwal (2002) in *Cicer arietinum* and Gairola *et al.* (2009) in beet spinach. Also, presence of phosphorus in the nutrient solution decrease soil pH which led to increase the availability and uptake of most essential elements (Guelser, 2005).

In line with this result, K_2HPO_4 supplement significantly decreased the nitrate concentration in leaves of fertilized plants with urea or ammonium sulfate. The highest nitrate level has been found in the leafy vegetables with about 500–3500 mg NO_3^- per kg fresh leaves (Karlowski, 1990; Santamaria, 2006). Reducing such level to less than 500 mg kg^{-1} fresh leaves is highly recommended for human health. In the present experiment, K_2HPO_4 supplementation could reduce the leaf NO_3^- concentration to safety levels for consumption. Little attention has been paid to the significance of potassium and phosphorus for improving quality, and safety of leafy vegetables for human consumption (Hanafy *et al.*, 2000; Gairola *et al.*, 2009), however no reports used K_2HPO_4 to improve yield and quality of leafy vegetables was found. The significant reduction of nitrate in the leaves of fertilized both leafy vegetable plants by K_2HPO_4 addition is an important crucial issue. The increased activation of nitrate reductase enzyme by application of nutrient solution containing potassium converting nitrate to amino acids is probably the reason for nitrate reduction in the leaves as found in the present results (Tables 1 and 2) as well as reported by Marschner (1995) and Epstein and Bloom (2005). Also, nutrient solution containing phosphorus decreased nitrate accumulation in both tested leafy vegetables (Tables 1and 2), due to assimilate nitrate in amino acids and protein form (Guelser, 2005).

Obtained results showed a similar trend for nitrate accumulation, sodium and calcium content in spinach. Whereas, better results with a reduction of nitrate (Table 3) accumulation were attributed with a lower content of both sodium and calcium (Table 2). The lower sodium content could be explained by decreasing absorption of sodium from the soil in plants treated with K_2HPO_4 as reported before by Barker and Pilbeam (2007) who found that the sodium concentration decreased in the grass with potassium supplying. This finding could be explained as K^+/Na^+ replacement relation or dilution effect (Malvi, 2010). However, obtained results regarding Ca^{2+} content was supported by the findings of Grimme *et al.* (1975) who found that, with an increase of K^+ availability in the soil , the uptake of Mg^{2+} and Ca^{2+} by Oat (*Avena sativa* L.) was reduced.

In spinach, the addition of K_2HPO_4 resulted in an increase in leaf potassium (Table 3) content (especially the plants fertilized with urea) which was accompanied by increased rates of photosynthesis, photorespiration and RuBP carboxylase activity as reported by Cakmak (2005). However, the vitamin C was not affected by the N-source with or without K_2HPO_4 in both tested leafy crops; this may be connected with the not optimal concentration of K_2HPO_4.

Open-Field Experiment

In this experiment the results showed that there was an increase in the yield as well as nitrate reductase activity by increasing K_2HPO_4 level up to 15 mM in spinach and 20 mM in parsley connected with lower nitrate content (Tables 5 and 6 and Fig. 1). Also, the significantly higher vitamin C was found in both tested crops with the levels of K_2HPO_4 which produced the highest yield.

The growth promotion of both tested leafy crops by K_2HPO_4 supplementation is related to the improvement of nitrogen assimilation as reported by Brown *et al.* (1993) and Ruiz and Romero (2002). Enhancing growth of plants with increasing K_2HPO_4 concentration up to 15 mM in spinach and 20 mM in parsley is due to increasing the activity of nitrate reductase in the leaves (Tables 5 and 6). Also, this effect connected with the fact that the K^+ maintain high cell turgor pressure which affects cell elongation for growth and most importantly regulates the opening and closing of the stomates which affect transpirational cooling and CO_2 uptake for photosynthesis (Malvi, 2010). However, the reduction of the spinach yield at 20 mM K_2HPO_4 may be due to the potassium antagonistic effect on other cations or increased photorespiration of C3 plants (Cakmak, 2005).

The favorable K_2HPO_4 concentration reduced the content of nitrate to 545.1 mg in spinach and 343.7 mg kg^{-1} FW in parsley instead of 953.9 mg and 598.4 mg in both crops respectively. There is a reverse relationship between nitrate content and nitrate reductase activity or yield as found in this study (Fig. 1; Tables 5 and 6) and by previous reports (Olday *et al.*, 1976). So, accumulation or assimilation of nitrate in cell depends upon activity of nitrate reductase (NR). By comparing nitrate and NR activity of different treatments, we found that increased NR activity decreased nitrate accumulation in leaves of both leafy vegetables. Maximum nitrate was found in plants non-received K_2HPO_4. It shows that application of N fertilizer without K_2HPO_4 can increase nitrate accumulation in leaves. Results of this study suggest that potassium phosphate application reduces nitrate accumulation, which is in accordance with the findings of Hanafy *et al.* (2000) and Ruiz and Romero (2002) who reported that increase in the rate of potassium application facilitates uptake and transport of nitrate towards the aerial parts of the plant, promotes the metabolism and utilization of nitrate and ultimately, reduce nitrate accumulation in vegetable crops. Phosphorus, potassium and sulfur have major roles in production of proteins, thereby decrease nitrate within the plant (Brown *et al.*, 1993).

Concerning the content of vitamin C, the indicated results supported the results in the first experiment (pot experiment), whereas the application of 5 mM K_2HPO_4 did not affect the content of vitamin C in both experiments and crops even the yield was significantly increased. Only K_2HPO_4 at level of 15 mM gave the significant highest vitamin C in both crops, this may be due to this concentration (15 mM) is a critical level to improve leaf vitamin C content. However, vitamin C content was significantly reduced again at 20 mM K_2HPO_4 in comparison to 15 mM. This reduction may be due to K^+ regulates the stomatal opening which lead to entrance of O_2 between leaves cells, responsible for oxidizing ascorbic acid (Lee and Kader, 2000). These results were supported by the results of Anonymous (2001) and Pokluda (2008) who found that the higher levels of nitrate in crops have often been linked with lower ascorbic acid levels.

Conclusion

The significant highest yield was associated with ammonium sulfate fertilizer. The accumulation of nitrate in both tested vegetables under different nitrogen forms was crop depended. Application of K_2HPO_4 increased the yield of tested leafy vegetable crops in pot as well as field experiment. K_2HPO_4 supplementation greatly reduced the nitrate accumulation and increased nitrate reductase in the leaves in both tested leafy vegetables. Supplementation of K_2HPO_4 at 5 mM reduced the content of sodium and calcium in leaf of spinach, however the significant highest vitamin C required high K_2HPO_4 (15 mM) concentration as shown in field experiment. Production of low nitrate and sodium as well as high vitamin C and potassium containing leafy vegetables are more safety for human health against cancer, methaemoglobinaemia and high pressure blood.

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تأثير مصادر السماد النتروجينى وفوسفات البوتاسيوم على محصول وجودة السبانخ والبقدونس

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أجريت تجربتان خلال موسمى ٢٠١١-٢٠١٢ و ٢٠١٢-٢٠١٣ فى المزرعة البحثية بكلية الزراعة جامعة قناة السويس بالإسماعيلية لدراسة تأثير مصدر النتروجين (كبريتات الأمونيوم – اليوريا) وإضافة فوسفات البوتاسيوم على المحصول وتركيز النترات وفيتامين ج وكذلك نشاط إنزيم اختزال النترات فى كلا من السبانخ والبقدونس، فى تجربة الأصص أعطت النباتات المسمدة بكبريتات الأمونيوم أعلى محصول وأعلى محتوى نتراتى معنوى مقارنة بمثيلتها المسمدة باليوريا، كما حسن إضافة فوسفات البوتاسيوم كمية المحصول مع تراكم كميات اقل من النترات فى كلا المحصولين تحت الدراسة، أيضاً أدت إضافة فوسفات البوتاسيوم إلى انخفاض تركيز الصوديوم والكالسيوم فى نباتات السبانخ فقط، وفى تجربة الحقل ارتبط زيادة المحصول مع زيادة تركيز فوسفات البوتاسيوم حتى ١٥ مليمول و ٢٠ مليمول فى كل من السبانخ والبقدونس على التوالى، كما ارتبط زيادة المحصول بنقص تركيز النترات وزيادة نشاط إنزيم اختزال النترات، كما أوضحت النتائج أن إضافة ١٥ مليمول من فوسفات البوتاسيوم أعطى أعلى تركيز معنوى لفيتامين ج (١١٢,١١ و٢٥٣,٠٨ ملجرام / ١٠٠ جرام وزن طازج) لكل من السبانخ والبقدونس على التوالى، كما أظهرت النتائج ان اعلى نشاط لإنزيم اختزال النترات ارتبط بأقل تركيز لتراكم النترات فى النباتات المضاف إليها فوسفات البوتاسيوم ($r = 0.87$)، ويمكن استنتاج أن إضافة فوسفات البوتاسيوم قد أدى إلى خفض تركيز النترات فى حدود المعدلات الآمنة للاستهلاك فى كل من السبانخ والبقدونس.

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