Improvement of yield and quality of two *Spinacia oleracea* L. varieties by using different fertilizing approaches

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Abstract The effects of different fertilizers [the control with no fertilizer (C), inorganic fertilization (I), combined inorganic and organic fertilizer (IOHmax1) and (IOHmax2)] on yield and nutrients contents of two spinach varieties (“Balady and Virofly”) were investigated. Significant effects of variety were observed on vegetative growth and nutrients contents. While Virofly had significantly higher leaf area (236.96 cm²), stem diameter (7.43 mm) and fresh weight of vegetative and radical parts (15.05 and 0.96 g, respectively), Balady had significantly higher chlorophyll and carotene contents (0.0023 and 0.0018 g/g fw, respectively). No significant impacts of variety on vitamin C, nitrite, nitrate and oxalates contents were observed. IOHmax2 treatment (4 g/l of Humax + 100 mg/l of NH₄NO₃ per plant fertigation⁻¹) enhanced stem diameter and root growth and significantly improved the yield by produced plants with higher stem length, leaf number and surface area. This treatment improved the quality of plant by increasing vitamin C content and reducing nitrite and oxalates contents. No significant effects of different fertilizers were observed on NO₃⁻ content. A fairly balanced yield/NO₃⁻ and oxalates content can be achieved with combined inorganic and organic fertilizer (IOHmax1) and (IOHmax2).

Keywords Spinach · Organic · Inorganic · Yield · Nitrate · Nitrite · Oxalates

Introduction

Spinach is classed as an edible leafy vegetable belonging to *Chenopodiaceae* family. It has been naturalized in central parts of Asia as an annual plant (rarely biennial) (Kallo and Bergh 1993; Kawazu Okimura et al. 2003). This vegetable contains considerable amounts of vitamins, especially C, E, folic acid and mineral components; it is also extremely rich in antioxidants such as beta-carotene and lutein (Gupta and Wagle 1988; Watanabe et al. 1994). These antioxidants have anticancer properties; moreover, beta-carotene improves lung operation and decreases risk of diabetes, while lutein improves vision in old ages (Moghadam et al. 2012). Spinach leaf has 3.2% protein, 0.65% fiber, 0.6% fat and linolenic acid (omega-3) and linoleic acid (omega-6) (Salunkhe and Kadam 1998).

Unfortunately, as in other leafy vegetables, spinach shows a tendency to accumulate oxalate (Heaney et al. 1988; Bohn et al. 2004), nitrates (NO₃⁻) and nitrites (NO₂⁻) (Takebe et al. 1995; Jaworska 2005) which differ according to the variety (Cantliffe 1992; Stagnari et al. 2007). Such compounds are unwanted in the human diet. Nitrogen fertilizers have been identified as the major factors that influence nitrate content in vegetables (Cantliffe 1973). Nitrate can be reduced, in the human body to nitrite which combines with hemoglobin and oxidizes Fe²⁺ to Fe³⁺, preventing the oxygen absorption and inducing methemoglobinemia (Stagnari et al. 2007). Moreover, nitrate can form cancer-causing compounds (Walker 1990; Gangolli et al. 1994). In 1997, the European Commission defined the maximum acceptable level of nitrate accumulation in spinach (3000 and 2500 ppm on fresh weight basis for crops harvested from 1 November to 31 March and from 1 April to 31 October, respectively). Oxalic acid is an organic acid that occurs naturally in many plants. It binds

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to Ca^{2+} , Fe^{2+} , and Mg^{2+} and interferes with the bioavailability of these minerals (Rubatzky and Yamaguchi 1997). Spinach contains relatively high levels of oxalate (400–900 mg/100 g FW) as compared to other vegetables (Savage et al. 2000).

For maximum performance of this plant, as other agricultural plants, its needs optimized agricultural managements as well as appropriate weather and soil conditions.

In Syria, the use of chemically synthesized fertilizer is in large proportion. The farmers of spinach focus only on the N chemical fertilization without considering the vegetables response to N rate and forms. Some studies showed that the increments in nitrogen doses increased NO_3 and NO_2 contents and yield of spinach significantly (Elia et al. 1999; Canali et al. 2014), but usually decreased P, Zn and Mn contents (Gulser 2005). The chemical inputs are used to promote plant growth and increase crop production. However, the potential impacts of chemical fertilizers on plant production and quality, human health and the well-being of ecosystem raised the research concerns (Ubalua 2009; Vignesh et al. 2012). Moreover, the chemical fertilizers have only high consumption elements (nitrogen, phosphorus, potassium), while low consumption elements (Fe, Zn, Mg, B etc.) are rare. The low consumption elements are basic elements in plant metabolism processes and consequently in plant yield and quality (Moustafa and Omran 2006; Briat et al. 2007), however, soil micronutrients availability to crop plants changes under the influence of environmental conditions, in particular soil pH (Orlovius 2001; Dords 2009). Hence, there is an immediate need to reduce the chemical fertilizers usage.

In recent years, efforts have been made to enhance productivity of vegetables without compromising food quality standards (Chaudhry et al. 2006). Humic substances, due to their multiple growth and physiological functions, are widely recognized as a promised agricultural practice (Saruhan et al. 2011). Several studies showed that humic substances enhance root, leaf and shoot growth, and also stimulate the germination of various crop species (Jones et al. 1991). Since the humic substances are easy preparation and environmentally friendly nature (Sary et al. 2009), such compounds can be the most precious material to fulfill the nutritional needs of leafy plants. Earlier researches have registered a number of positive effects on the growth and productivity of some plants through foliar supplementation (Ezz El-Din and Khalil 2003).

Due to continuously changing environmental attributes, the risk factors related to sustainable agriculture and product safety are being increasingly investigated. In this context, the objective of this research is to investigate the growth, yield and quality of two spinach varieties as affected by chemical and organic fertilizers.

Table 1 Physical and chemical properties of the soil sampled at a depth of 0–25 cm, and used for planting two *Spinacia oleracea* L. varieties

Soil characteristics	Value
pH (soil–water 1:2)	8.6
EC (μSm^{-1})	0.26
Organic carbon content	1.6%
Macroelements	
N	5.8 ppm
P	19 ppm
K	142 ppm
Microelements	
Zn	1.1 ppm
B	0.22 ppm
Fe	1.2 ppm
Mg	2 ppm
Cu	0.6 ppm

Materials and methods

Plant material and culture conditions

The seeds of two varieties of spinach (*Spinacia oleracea* L.): the equity limb spinach “Balady” and the splayed limb spinach “Virofly”, were planted at the beginning of October in plastic pots (4L) containing a mixture of soil and perlite (1:1; v/v). The properties of soil sampled at a depth of 0–25 cm were analyzed (Table 1) by standard analytical techniques (Miller and Keeney 1982). This soil is very low quality which contains less quantity of macronutrients as well as micronutrients.

The pots were placed in a research field at the university of Damascus (altitude 743 meters above sea level, 33°29'N, 36°14'E). As classified by UNESCO-FAO (1960), the climate is accentuated thermo-Mediterranean. The winter temperatures can fall below 0 over night and the summer temperatures can rise above 40 °C.

The plants were irrigated once a week. When the 2nd leaf appeared, four fertilization treatments were applied: (1) the control treatment (C) in which no fertilizer was applied, (2) the inorganic-N fertilization (I) in which 150 mg/l of NH_4NO_3 per plant were added via irrigation, (3) the first combined treatment of inorganic (125 mg/l of NH_4NO_3 per plant) and organic (IOHumax1) in which 2 g/l of Humax (JH. Biotech. Inc. Ventura, CA, U.S.A) were sprayed for each plant fertigation⁻¹ and (4) the second combined treatment of inorganic (100 mg/l of NH_4NO_3 per plant) and organic (IOHumax2) in which 4 g/l of Humax were sprayed for each plant fertigation⁻¹. The treatments were applied twice per seven days until a week before harvest. It should be noted that Humax fertilizer consists of

extract potassium humate (250 g/l), organic matter (200–250 g/l), fulvic acids (≤ 20 g/l), amino acids (≤ 20 g/l), nitrogen (≤ 50 g/l), phosphorus (P_2O_5 , ≤ 50 g/l), potassium (K_2O , ≤ 50 g/l) and trace elements (Mg, Fe, Zn, B, 10–15 g/l).

Morphological and chemical measurements

The harvest was carried out after 45 days of germination (at least 6 true leaves). Plants were harvested by cutting the stems of each plant separately at 1 cm over ground. The measurements were achieved on fully-developed middle leaves of spinach plant. The morphological measurements were achieved on 5 plants/replicate. The length of root and stem as well as the stem diameter were recorded. Leaves number and area per plant were recorded using a Li-Cor 3100 area meter (Li-Cor, Lincoln, NE, USA). Radical and vegetative fresh weights were measured immediately using a sensitive balance (Sartorius, 0.1 ± 0.001 g, India). The dry weights of radical and vegetative parts were measured after drying in oven at a temperature of 70°C until the stability of weight.

For the biochemical analysis, leaves and petioles were placed in liquid nitrogen and stored at a temperature of -20°C . To determine the leaf chlorophyll content (a & b), 0.2 g of leaves was homogenized by adding 7 ml of acetone (80%). The mixture was centrifuged at 3400 rpm for 20 min at 4°C (Tabletop model, IEC 215, USA). The resulting supernatant was used to determine the absorbance at 664 and 647 nm using the spectrophotometer (Shimadzu Mini-1240 UV–Vis, USA). The leaves chlorophyll content was determined according to Najla et al. (2012):

$$[\text{Chlorophyll a}] = -13.19 \times \text{Abs}_{664} - 2.57 \times \text{Abs}_{647}$$

$$[\text{Chlorophyll b}] = 22.1 \times \text{Abs}_{647} - 5.26 \times \text{Abs}_{664}$$

where: A_{647} and A_{664} are the absorbance at 647 and 664 nm, respectively.

To determine β -carotene content, Teow et al. (2007) method was used. The carotene content was analyzed by high performance liquid chromatography (HPLC, Hamilton, UK). The samples were placed in the sample tray cooled to 6°C and covered by aluminum foil to minimize light. Samples (20 μl) were injected onto a reverse phase column (Chandler and Schwartz 1987). Separation was performed at 35°C with a mobile phase of methanol, acetonitrile and chloroform (42.5/42.5/15 v/v). The flow rate was maintained at 1.2 ml/min. Peaks were monitored at 450 nm. Standard solutions of β -carotene with concentration from 0.5 to 10 $\mu\text{g/ml}$ were used to obtain a standard curve.

Vitamin C content was measured according to Murshed et al. (2008). 0.5 g sample of frozen ground leaf

were homogenized in 1 mL of cold trichloroacetic acid (TCA) (6%) (w:v). The homogenate was centrifuged at 16 000 g at 4°C for 15 min. 10 μL of extract were added to 10 μL of 10 mM DL-dithiothreitol (DTT) and 20 μL of 0.2 mM phosphate buffer (pH 7.4). After incubation for 15 min at 42°C , 10 μL of N-ethylmaleimide (NEM) (0.5%) (w:v) was added with additional incubation for 1 min at room temperature to remove excess DTT. This was followed by adding 150 μL of a reagent prepared just before use by mixing 50 μL of 10% TCA, 40 μL of 42% (v:v) of orthophosphoric acid (H_3PO_4), 40 μL of 4% (w:v) 2,2-bipyridyl dissolved in ethanol (70%) and 20 μL of 3% (w:v) ferric chloride. After further incubation for 40 min at 42°C , the absorbance was measured in the spectrophotometer at 525 nm. Commercial L-ascorbic acid was used to generate a standard curve.

To estimate leaf and petioles nitrate content, 0.225 g of homogenized leaf were used for colorimetric determination of nitrate at 410 nm by the spectrophotometer following the nitration by salicylic acid method described by Cataldo et al. (1975). The complex formed by nitration of salicylic acid under highly acidic conditions absorbs maximally at 410 nm in basic solutions (pH > 12). Absorbance of the chromophore is directly proportional to the amount of nitrate present. The nitrite content was determined using the ISO method (ISO/6635, 1984).

Total oxalate content was measured using the enzyme method as described by Ruan et al. (2013). 2.0 g of leaf plant were homogenized with 1.6 mL of HCl (0.5 mol/L) and then diluted with 1 mL distilled water. The homogenate was transferred into 10 mL graduated tubes and heated in a boiling water bath for 20 min. After cooling, the homogenate was completed to 10 mL with distilled water. The next day, 1 mL of the homogenate was clarified by centrifugation (12,000 rpm, 10 min) at 4°C . To create an oxalate extract, 0.016 mL of NaOH (2 mol/L) was added accurately to 0.5 mL supernatant. To measure the oxalate content in the oxalate extracts, 20 mg oxalate oxidase in the form of dry powder was first placed in a 2 mL test tube. Then 0.54 mL distilled water, 0.8 mL color reagent (pH 4.0) and 0.04 mL (150 U/mL) horseradish peroxidase (Bio Life Science & Technology Co., Shanghai, China) were added, then 0.1 mL oxalate extract was added to initiate the reaction. After incubation at room temperature for 90 min, the absorbance at 555 nm of the reaction mixture was read in a spectrophotometer and oxalate content was determined with reference to the standard curve which was prepared by adding 0, 2, 4, 6, 8 and 10 mg oxalic acid into 1 mL reaction system, respectively. The data are presented as mg oxalate/100 g FW.

Experimental design and statistical analysis

The experiment was set up as a randomized complete block with 6 replicates (5 plants/replicate, $n = 30$). Using the R-Project statistical software, data were subjected to ANOVA and differences between control and treatments were considered statistically significant at $P \leq 0.05$ using Fisher test.

Results

Leaves number (leaf/plant) and area (cm²) per plant

The different fertilization treatments affected both leaf number and area (Table 2). No significant differences were recorded between the two varieties for leaf number. In the Balady variety, the leaf number increased significantly in the IOHmax1 and IOHmax2 by 27 and 41%, respectively as compared to the control (10.65 leaf/plant). This increase was not significant in Virofly variety in any of the treatments.

The leaf area of Virofly variety (236.96 cm²) was significantly higher as compared to Balady variety (157.33 cm²). The leaf area did not vary according to the fertilization treatment in Balady variety, while it increased significantly in Virofly variety by twofolds in IOHmax2 as compared to the control (166.73 cm²).

Stem length (cm) and diameter (mm)

No significant differences between the two varieties were observed for the stem length. Significant differences were observed according to the treatment in Balady variety only (Table 3), where the IOHmax2 treatment increased significantly the stem length as compared to the control

Table 2 The effect of different fertilization treatments on leaf number (leaf/plant) and area (cm²) for two *Spinacia oleracea* L. varieties

Variety	Treatment	Leaf number	Mean	Leaf area	Mean
Balady	C	10.65 ^b	17.88 ^a	132.91 ^a	157.33 ^b
	I	14.61 ^{ab}		146.03 ^a	
	IOHmax1	14.74 ^a		145.90 ^a	
	IOHmax2	18.00 ^a		172.25 ^a	
LSD _{5%}		4.07		39.35	
Virofly	C	12.38 ^a	14.60 ^a	166.73 ^b	236.96 ^a
	I	18.07 ^a		209.21 ^b	
	IOHmax1	18.57 ^a		211.02 ^b	
	IOHmax2	19.94 ^a		364.76 ^a	
LSD _{5%}		8.20	3.08	131.56	45.73

* The different characters indicate the significant differences between the treatments and the varieties at 95% confidence level

Table 3 The effect of different fertilization treatments on stem length (cm) and diameter (mm) for two *Spinacia oleracea* L. varieties

Variety	Treatment	Stem length	Mean	Stem diameter	Mean
Balady	C	27.76 ^b	34.34 ^a	4.51 ^c	6.26 ^b
	I	33.44 ^{ab}		5.74 ^{bc}	
	IOHmax1	32.69 ^{ab}		5.89 ^b	
	IOHmax2	45.74 ^a		8.74 ^a	
LSD _{5%}		14.99		1.26	
Virofly	C	27.66 ^a	28.50 ^a	5.29 ^b	7.43 ^a
	I	24.14 ^a		7.30 ^{ab}	
	IOHmax1	25.05 ^a		8.38 ^a	
	IOHmax2	25.88 ^a		8.55 ^a	
LSD _{5%}		11.53	7.44	2.40	1.01

* The different characters indicate the significant differences between the treatments and the varieties at 95% confidence level

(27.76 cm). However, no significant differences were recorded between the other treatments (Table 3).

Virofly variety had significantly a higher stem diameter (7.43 mm) as compared to the Balady variety (6.26 mm). The stem diameter was increased generally according to the fertilization treatment. In Balady variety, it increased significantly by 1.9 and 1.3 folds in IOHmax2 and IOHmax1 treatments, respectively as compared to the control (4.51 mm), while no significant differences were observed between inorganic treatment “I” (5.74 mm) and the control. Besides, in Virofly variety the stem diameter increased significantly by 1.6 folds in both IOHmax1 and IOHmax2 treatments, respectively as compared to the control (5.29 mm). No significant differences were observed between inorganic treatment “I” (7.30 mm) and the control.

Root length (mm)

Significant difference in the root length was recorded between the varieties (64.03 and 78.70 mm in Balady and Virofly, respectively). The root length increased according to the treatment only in Balady (Table 4). It increased significantly in IOHmax2 treatment (74.13 mm) as compared to inorganic treatment “I” and control (59.03 and 56.79 mm, respectively).

Vegetative and radical fresh weights (g)

The Table 5 shows the variation of vegetative and radical fresh weights (g) for two *Spinacia oleracea* L. varieties according to the fertilization treatments. Virofly had significantly higher vegetative and radical fresh weight (15.05 and 0.96 g, respectively) as compared to Balady (8.05 and 0.51 g, respectively).

Table 4 The effect of different fertilization treatments on root length (mm) for two *Spinacia oleracea* L. varieties

Variety	Treatment	Root length	Mean
Balady	C	56.79 ^b	64.03 ^b
	I	59.03 ^b	
	IOHmax1	59.84 ^{ab}	
	IOHmax2	74.13 ^a	
LSD _{5%}		15.08	
Virofly	C	68.60 ^a	78.70 ^a
	I	76.66 ^a	
	IOHmax1	87.50 ^a	
	IOHmax2	84.03 ^a	
LSD _{5%}		19.61	8.84

* The different characters indicate the significant differences between the treatments and the varieties at 95% confidence level

The vegetative fresh weight of Balady increased following fertilization treatment, however, this increase was significant only in IOHmax2 treatment (9.27 g) as compared to the control (7.03 g), but not for I and IOHmax1 treatments (8.13 and 7.75 g, respectively). The same pattern was observed for Virofly (Table 5), where a significant increase of vegetative fresh weight was only recorded in IOHmax2 treatment (20.05 g) as compared to the control (9.30 g).

The radical fresh weight did not vary according to the fertilizer treatment in Balady. However, significant increases by 121, 109 and 94% were recorded in the IOHmax2 treatment as compared to the control, inorganic “I” and IOHmax1 treatments (0.71, 0.75 and 0.81 g, respectively) in Virofly.

Dry weight of vegetative and radical parts (g)

The vegetative dry weight was significantly higher in Virofly (1.79 g) as compared to Balady (1.05 g) (Table 6). The different fertilizer treatments did not affect this

parameter in Balady where it reached to 0.81, 0.95, 0.82 and 1.03 g in the control (C), inorganic (I), IOHmax1 and IOHmax2, respectively. However, for Virofly, a significant increase was observed in IOHmax2 treatment (2.59 g) as compared to the control (C) (0.97 g) only, but not with inorganic (I) (1.71 g) and IOHmax1 treatment (1.78 g).

Concerning the radical dry weight (Table 6), it was significantly higher in Balady (0.59 g) than Virofly (0.34 g). In Balady, the radical dry weight increased significantly by 2, 4 and 1.9 folds in IOHmax2 treatment as compared to the control (C), inorganic (I) and IOHmax1 (0.57, 0.25 and 0.61 g, respectively). In Virofly, this parameter was significantly higher in IOHmax2 (0.55 g) as compared only to the control and inorganic (I) treatments (0.17 and 0.20 g, respectively), while no significant difference was recorded as compared to IOHmax1 (0.31 g).

Plant chlorophyll (a & b) and β -carotene content (mg/g fw)

The Table 7 shows the effects of fertilizer treatments in chlorophyll and β -carotene contents in the two varieties. Balady had significantly a higher content of chlorophyll a, chlorophyll b and β -carotene (2.2, 2.1 and 1.8 mg/g fw) as compared to Virofly (1.7, 0.7 and 1.1 mg/g fw, respectively). Concerning the fertilizer treatments, significant increases in chlorophyll and β -carotene contents were observed (Table 7). For example, a significant increases of chlorophyll a content by 7, 6.8 and 6.5 folds were observed in inorganic treatment (I), IOHmax1 and IOHmax2, respectively, in Balady as compared to the control (0.4 mg/g fw). While in Virofly, a significant difference in the chlorophyll a content was only observed between IOHmax2 treatment (1.3 mg/g fw) and the control (0.4 mg/g fw). However, neither Balady nor Virofly showed significant differences related to chlorophyll b content between

Table 5 The effect of different fertilization treatments on vegetative and radical fresh weight (g) for two *Spinacia oleracea* L. varieties

Variety	Treatment	Vegetative fresh weight	Mean	Radical fresh weight	Mean
Balady	C	7.03 ^b	8.40 ^b	0.41 ^a	0.51 ^b
	I	8.13 ^{ab}		0.45 ^a	
	IOHmax1	7.75 ^{ab}		0.44 ^a	
	IOHmax2	9.27 ^a		0.57 ^a	
LSD _{5%}		2.03		0.18	
Virofly	C	9.30 ^b	15.05 ^a	0.71 ^b	0.96 ^a
	I	15.47 ^{ab}		0.75 ^b	
	IOHmax1	16.59 ^{ab}		0.81 ^b	
	IOHmax2	20.05 ^a		1.57 ^a	
LSD _{5%}		6.79	2.35	0.64	0.22

* The different characters indicate the significant differences between the treatments and the varieties at 95% confidence level

Table 6 The effect of different fertilization treatments on vegetative and radical dry weight (g) for two *Spinacia oleracea* L. varieties

Variety	Treatment	Vegetative dry weight	Mean	Radical dry weight	Mean
Balady	C	0.81 ^a	1.05 ^b	0.57 ^{bc}	0.59 ^a
	I	0.95 ^a		0.25 ^c	
	IOHmax1	0.82 ^a		0.61 ^b	
	IOHmax2	1.03 ^a		1.16 ^a	
	LSD _{5%}	0.27		0.34	
Virofly	C	0.97 ^b	1.79 ^a	0.17 ^b	0.34 ^b
	I	1.71 ^{ab}		0.20 ^b	
	IOHmax1	1.78 ^{ab}		0.31 ^{ab}	
	IOHmax2	2.59 ^a		0.55 ^a	
	LSD _{5%}	0.88		0.25	

* The different characters indicate the significant differences between the treatments and the variety at 95% confidence level

Table 7 The effect of different fertilization treatments on plant chlorophyll (a & b) and β -carotene content (mg/g fw) for two *Spinacia oleracea* L. varieties

Variety	Treatment	Chlorophyll a content	Mean	Chlorophyll b content	Mean	β -carotene content	Mean
Balady	C	0.4 ^b	2.2 ^a	0.3 ^a	2.1 ^a	1.2 ^b	1.8 ^a
	I	2.9 ^a		2.7 ^a		1.5 ^b	
	IOHmax1	2.7 ^a		2.8 ^a		1.2 ^b	
	IOHmax2	2.6 ^a		2.4 ^a		3.1 ^a	
	LSD _{5%}	1.9		2.5		1.2	
Virofly	C	0.4 ^b	1.7 ^b	0.4 ^a	0.7 ^b	0.7 ^b	1.1 ^b
	I	1.0 ^{ab}		0.9 ^a		1.2 ^{ab}	
	IOHmax1	0.6 ^b		0.4 ^a		0.9 ^{ab}	
	IOHmax2	1.3 ^a		1.0 ^a		1.6 ^a	
	LSD _{5%}	0.6		1.4		0.8	

* The different characters indicate the significant differences between the treatments and the variety at 95% confidence level

treatments and control (Table 7). Concerning β -carotene, significant increases by 2.6 and 2.3 folds were observed between IOHmax2 treatment and the control (1.2 and 0.9 mg/g fw) in Balady and Virofly, respectively.

Plant contents of vitamin C (mg/100 g fw), nitrate (mg/kg fw) and nitrite (mg/kg fw)

Both Balady and Virofly varieties did not show any significant difference related to the contents of vitamin C (30.93 and 31.61 mg/100 g fw, respectively), nitrate (216.727 and 213.279 mg/kg fw, respectively) and nitrite (0.366 and 0.349 mg/100 g fw, respectively) (Table 8).

The IOHmax2 treatment has the higher significant content of vitamin C in Balady (30.33 mg/100 g fw) as compared to the other treatments and in Virofly (36.01 mg/100 g fw) as compared to the control (31.49 mg/100 g fw) and inorganic treatment (I) (32.31 mg/100 g fw) but not with the IOHmax1 treatment (33.90 mg/100 g fw).

While no significant effect on NO₃ content was observed between fertilizer treatments, regardless the

variety, the inorganic treatment “I” had a significant effect on NO₂ content in Balady (0.42 mg/kg fw) as compared to the control (0.37 mg/kg fw) and in Virofly (0.39 mg/kg fw) as compared to all other treatments.

Oxalates content of leaf and petiole (mg/100 g fw)

The Figs. 1 and 2 show no significant effect of variety on the oxalates content either for leaf or petiole. Significant effects on oxalates content of leaf and petiole of Balady plants were observed according to the fertilizer treatment (Fig. 1). While the inorganic treatment “I” induced an increase in oxalates content of both leaf and petiole (485 and 250 mg/100 g fw, respectively) as compared to the control (250 and 210 mg/100 g fw, respectively), the oxalates content was decreased in both IOHmax1 and IOHmax2 treatments. However, only the IOHmax2 decreased significantly the leaf and petiole oxalates contents (390 and 185 mg/100 g fw), as compared to the control and inorganic treatment “I”.

The Fig. 2, show the effects of fertilizer treatments on the oxalates content of leaf and petiole of Virofly variety. The

Table 8 The effect of different fertilization treatments on plant content of vitamin C (mg/100 g fw), nitrate-N ($\text{NO}_3\text{-N}$, mg/kg fw) and nitrite (NO_2 , mg/kgfw) for two *Spinacia oleracea* L. varieties

Variety	Treatment	Vitamin C content	Mean	NO_3 content	Mean	NO_2 content	Mean
Balady	C	29.57 ^b	30.93 ^a	220.18 ^a	216.72 ^a	0.37 ^b	0.37 ^a
	I	29.25 ^b		223.83 ^a		0.42 ^a	
	IOHumax1	29.35 ^b		224.82 ^a		0.41 ^{ab}	
	IOHumax2	30.33 ^a		227.18 ^a		0.41 ^{ab}	
	LSD _{5%}	0.85		11.58		0.04	
Virofly	C	31.49 ^b	31.61 ^a	205.79 ^a	213.27 ^a	0.31 ^b	0.35 ^a
	I	32.31 ^b		206.49 ^a		0.39 ^a	
	IOHumax1	33.90 ^{ab}		207.99 ^a		0.31 ^b	
	IOHumax2	36.01 ^a		208.65 ^a		0.30 ^b	
	LSD _{5%}	2.41	0.9	18.42	7.31	0.06	0.03

* The different characters indicate the significant differences between the treatments and the variety at 95% confidence level

Fig. 1 The effect of different fertilization treatments on leaf and petiole content of oxalates (mg/100 g fw) for *Spinacia oleracea* L. Balady variety

inorganic treatment “I” increased the leaf and petiole contents of oxalates by 10.4 and 19.8%, respectively as compared to the control (451 and 215 mg/100 g fw, respectively). However, this indicator is decreased for leaf and petiole respectively by 2.2 and 5%, for the IOHumax1 and by 10.8 and 26.3%, respectively for IOHumax2 as compared to the control. Significant differences were recorded between the IOHumax2 treatment and all the other treatments (Fig. 2).

Discussion

Most of Syrian soils suffer from microelements deficiencies (Table 1). Moreover, the shortage of some elements, despite of being abundant, is depending on pH of soil. For

example, iron deficiencies are common for plants on alkaline soils. Actually, the chemical fertilizers are used to improve vegetable nutritional quality in Syrian farmers. However, some of these fertilizers have potentially adverse impacts on human health and ecosystem. Other products appear to be better choice since they may be economically viable, easily biodegradable and less or non-phytotoxic (Ubalua 2009). The use of humates foliar spray is gaining a worldwide interest because they represent a safe method for improving stress resistance, yield and quality of the plants (De Lucia and Vecchiatti 2012).

The results from this study showed that the different varieties and forms of fertilizers had a significant effect on vegetative growth and nutrient concentration in spinach plants. Concerning the effect of variety, Virofly plants had

Fig. 2 The effect of different fertilization treatments on leaf and petiole content of oxalates (mg/100 g fw) for *Spinacia oleracea* L. Virofly variety

significantly higher leaf area (236.96 cm²), stem diameter (7.43 mm), root diameter (78.70 mm), fresh weight of vegetative and radical parts (15.05 and 0.96 g, respectively) and dry weight of vegetative part (1.97 g) as compared to Balady. Balady plants had significantly higher chlorophyll and carotene contents (1.8 and 1.1 mg/g fw, respectively). However, no significant impacts of variety on vitamin C, nitrite, nitrate and oxalates contents were observed. Many works demonstrated that the effects of fertilizer were dependent on plant species, cultivar, environment, dose and time of application (Kunicki et al. 2010).

Recently, some studies indicated that about 40–70% of nitrogen of the applied irrigation fertilizers is lost to environment and cannot be absorbed by plants, causing not only substantial economic losses but also environment pollution (Saigusa 2000). Moreover, N-supplying fertilizers tend to accumulate nitrate and nitrite in plant (Bovis and Touchton 1998; Abubaker et al. 2010). Therefore, fertilizers spray may be successfully used under suboptimal environmental conditions to avoid unnecessary costs and nitrate accumulation in spinach leaves.

High concentration of Humax (4 g/l) significantly improved the yield by produced spinach plants with higher leaf number, surface area stem length (Table 2 and 3). These effects on yield were already observed in vegetables (Vernieri et al. 2005). Improving leaf number and surface area might induce an increase of photosynthesis rate and thus chlorophyll content. The chlorophyll increment could be attributed to Mg content in Humax, which is the center of the chlorophyll particle (Cakmak and Kirkby

2008). Increased chlorophyll contents show greater production yield in crops as photosynthesis and chlorophyll contents are positively correlated (Wang et al. 2008). Moreover, Humax (4 g/l) treatment enhanced stem diameter (Table 3) and the root growth (Table 4), and subsequently the capacity of water and mineral absorption and translocation. It was reported that the application of humates increased the synthesis and activity of hormones which play an important role in promoting the plant growth (Abdel-Mawgoud et al. 2007; Saruhan et al. 2011). Moreover, recent studies showed that the photosynthesis process assimilation proceeds more rapidly and the nitrate formation is averted when humate was applied (Ajalli et al. 2013). Our results show that Humax application was particularly safe; they result in significant rise in yield but marginal nitrate, nitrite accumulation and a significant decrease of oxalates. Nitrate accumulation in spinach leaves was roughly in inorganic fertilization treatment (I) compared to the other treatments. This is apparently explained by the higher N availability in the inorganic fertilizer applications and rapid release of N from the chemical fertilizers beyond the ability of plant usage (Nosengo 2003; Abubaker et al. 2010). The plant nitrate concentration seems to be resulted from an imbalance between the uptake and translocation of nitrate, and the reduction of this compound to ammonia which is subsequently incorporated into amino acids (Maynard et al. 1976).

In some countries, the nitrates content in fresh vegetables such as spinach is regulated by the Minister of Health (Jaworska 2005). The regulation permits the content of

2500 mg NO₃[−]/kg fw. In the present experiment, this level was lower (205–227 mg NO₃[−]/kg fw) but it was similar to the NO₃[−] values reported in different studies (Leuzzi et al. 1996). However, some works concerning spinach show that this species of vegetables can accumulate over 5000 mg NO₃[−]/kg fw (Watanabe et al. 1994). Concerning the nitrite content, it was ranged from 0.30 and 0.41 mg NO₂[−]/kg fw. Similar levels were reported in the New Zealand spinach (Jaworska 2005) and parsley (Lisiewska and Kmiecik 1997).

Oxalates compounds are unwanted in vegetables because of their unfavorable effects on the humans (Kabaskalis et al. 1995). The content of oxalates depends on species and cultivar, forms of fertilizers (especially nitric ones), phases of plant growth (Kabaskalis et al. 1995; Takebe et al. 1995) and usable part of plant (Jaworska 2005). Similarly to the results of Stagnari et al. (2007), our investigations showed that spinach accumulated much more oxalate in blades than petioles in general by about 1–4 times (Figs. 1, 2). Tanaka et al. (2001) suggested that the main function of oxalate in spinach is neutralization of OH[−] released during nitrate reduction. Ammonium nitrate is a fast-release fertilizer, it is quick to dissolve in water and the released nitrate ion can be directly used by plants, therefore, ammonium nitrate is suitable for the short life cycle plants (such as spinach), but it stimulates the oxalate accumulation (Stagnari et al. 2007). From our study, it emerges that a fairly balanced yield/NO₃[−] and oxalates content can be achieved with either IOHumax 1 treatment (2 g/l of Humax + 125 mg/l of NH₄NO₃ per plant fertigation^{−1}) or IOHumax2 treatment (4 g/l of Humax + 100 mg/l of NH₄NO₃ per plant fertigation^{−1}). Other considerations for commercial cropping such as the availability and security of various organic fertilizer as well as their costs, will be worthy of further research.

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