J. Plant Production, Mansoura Univ., Vol. 9 (1): 109 - 115, 2018

Response of Spinach Plants to Foliar Application by Ascorbic Acid Under Different Sources of Nitrogen Fertilization

Metwaly, E. E.
Vegetable and Floriculture Dept., Faculty of Agric., Mansoura University. Mansoura, Egypt.

ABSTRACT

Two field experiments were carried out in the Experimental and Research unit, Faculty of Agriculture, Mansoura University in the two winter seasons of 2015 and 2016 to study the impact of foliar application by ascorbic acid (i.e. without, 150 and 300 ppm) under four nitrogen fertilization sources (ammonium nitrate, ammonium sulphate, calcium nitrate and urea) on growth and yield of spinach plants (cv. Dash). The results indicated that vegetative growth attributes (Plant height, plant fresh weight, leaves fresh weight, leaves number and leaves area) per plant, leaves chemical composition (N, P, K, chlorophyll a, chlorophyll b and carotenoids), yield and its chemical quality (blades and petioles fresh weight, dry matter % and NO_3 content), Vit. C, TSS, fertilizer use efficiency, hill weight and yield per fed.) were affected significantly by nitrogen form. Urea recorded the highest values of the previous parameters except NO_3 content to blades and petioles. As for the effect ascorbic acid, the data show that the mentioned attributes were increased compared to the control. The interaction between nitrogen form and ascorbic acid showed that urea form combined with 300 ppm ascorbic acid recorded the highest values for most effective previous characters and the lowest values for NO_3 content to blades and petioles.

Keywords: Spinach, ascorbic acid, sources of nitrogen and yield

INTRODUCTION

Spinach (*Spinacia oleracea* L.) which belongs to family Chenopodiacea is a popular leafy vegetable crops which are an important element of human diet and considered as excellent nutritive source of mineral and vitamins and they have not much calories (Rabie *et al*., 2014).

Spinach yield and quality are affected with many factors such as water deficit, environmental conditions, diseases, salinity, flooding, chemical toxicity, nutrients, ultraviolet radiation, and antioxidants (Wang *et al*., 2009).

Nitrogen (N) is one of the major nutrients that have many important roles as a basic element of nucleic acids, protein, growth hormones, chlorophyll and physiological process. Improved N management can be achieved by matching N supply with crop needs, using the optimum dose, selecting appropriate N forms, and improved timing of fertilizer application. Usually plants are able to take up N as nitrogen from soils in inorganic ions ammonium (NH_4^+), nitrate (NO_3^-) and organic forms mainly amino acids, but some may prefer one source or another depending on plant species (Kaymak,H.C., 2013). The most predominant N forms in commercial fertilizers in Egypt are ammonium nitrate, ammonium sulphate, calcium nitrate and urea. Several researchers studied the impact of N levels on various vegetable crops but no or little investigations had studied the effect of different N sources on growth and yield characters of spinach plants. The response of spinach plants to N sources varies where the highest increment of plant height, leaves numbers, leaves fresh and dry weights) and total yield was achieved in case of ammonium nitrate (Rabie, *et al*. 2014). Also, Alderfasi *et al*., (2015) reported that, both forms of N application in mixtures by the ratio of 50 % / 50 % nitrate/ ammonium recorded the maximum values of fresh weight and leaf area of spinach. In addition, Wang *et al*. (2009) mentioned that, the spinach biomass was decreased when the NH_4^+/ NO_3^- ratio was more than 50:50 in nutrient solution. The highest biomass registered by using NO_3^- - N as its sole nitrogen source, on contrast, there was no significantly difference in dry matter weight between 0:100 and 25: 75 of NH_4^+/ NO_3^- ratio. Moreover, Ghoname *et al*. (2009) mentioned that the maximum fruit yield of hot pepper was achieved in case of ammonium nitrate. On contrast, difference results were obtained by (Mirdad, 2009; Mirdad, 2011 and Kaymak, 2013) on spinach, Parsley and Purslane, respectively, they found that the highest yield was achieved by using urea fertilization. On the other hand, the highest yield for tomato fruits was recorded with application of the ammonium sulfate (Fouda, 2017).

Foliar spray applications with bio-stimulant products such as essential macro- and micronutrients, citrates, humates, amino acids and vitamins such as ascorbic acid are becoming more common for improving quantity and quality of production. Vitamins are compounds that are needs in relatively small quantities but that cannot be synthesized in amounts large enough to meet the normal required of the organism. Vitamins natural and safety bio-regulator compounds which at low concentrations influences upon many physiological processes. Ascorbic acid is one of the more important water soluble antioxidants in plants.

Ascorbic acid has beneficial effects on scavenging generation of reactive oxygen species (ROS) which produced during respiration and photosynthesis processes. Ascorbic acid (AsA) improves protection against the harmful worst impacts of light during photosynthesis by carbon dioxide conversion into plant matter (Pastori, *et al*., 2003). In addition, it enhances cell division, cell wall expansion, many other essential enzymatic and non-enzymatic reactions and regulating plant growth and development. Moreover, endogenous AsA has recently been suggested to be basic in the regulation of developmental senescence and plant defense against pathogens. Also, it is very essential for the regulation of flowering, photosynthesis and senescence (Dolatabadian *et al*., 2010).

Many investigates studied the effect of ascorbic acid on growth and yield of crops. El-Hifny and El-Sayed (2011) reported that AsA at (400 mg/L) recorded the highest values of growth attributes and total yield of sweet pepper plant. In the same respect, Taha *et al*. (2011) mentioned that foliar application of AsA led to increasing lettuce plant fresh and dry weight. Also, Esfahlan *et al*. (2013) studied the foliar application of ascorbic acid (0, 10, and 20 mM) on purslane plant, the results indicate that increase in ascorbic acid concentrations improved vegetative growth and yield characters. Shabana *et al*. (2015) found that, improving plant growth parameters, fruit setting, fruit quality and total yield of sweet pepper was done by using of individual seaweeds extract, salicylic acid, vitamin E or AsA and their mixed.

Therefore the aim of the present study was to evaluate the response of spinach plants to foliar application by ascorbic acid under different sources of nitrogen fertilization

MATERIALS AND METHODS

Two field experiments were carried out in the Experimental and Research unit, Faculty of Agriculture, Mansoura University in the two winter seasons of 2015 and 2016 to study the impact of foliar application by ascorbic acid (without, 150 and 300 ppm) under four nitrogen fertilization sources (ammonium nitrate, ammonium sulphate, calcium nitrate and urea) on growth and yield of spinach plants (cv. Dash) using surface irrigation system. Soil samples from the top layer (0-30 cm depth) were collected randomly for physical and chemical analysis before planting (Table 1).

Table 1. Physical and chemical characters during the both seasons of 2015 and 2016.

Seasons	Silt %	Clay %	Sand %	Texture soil	PH	E.C (dSm-1)	Organic matter %	$CaCO_3$ %	N ppm	P ppm	K ppm
2015	40.8	36.9	22.3	Clay loamy	8.22	1.63	1.99	3.27	52.9	6.2	286
2016	40.9	37.3	21.8	Clay loamy	8.17	1.68	2.04	3.31	53.3	6.3	279

Seeds of spinach were sown in dry soil on 20th and 24th of October in both seasons, respectively in hills spaced 30 cm apart on two sides of ridges which were (70 cm in width and 3 m length), Each sub plot included five rows, seeds at 4 kg per fed used as equal quantity for all experimental units and the plot area was 10. 5 m^2.

Four different sources of nitrogen fertilization (i.e. ammonium nitrate 33.5%N, ammonium sulphate 20.5%N, calcium nitrate 15.5%N and urea 46%N) were applied at the same rate (60 units of nitrogen per fed.) in equal dose, the first after 21 days after sowing and second addition was carried out 21 days later.

Foliar application with ascorbic acid include three levels (without, 150 and 300 ppm) were add two times at 21 and 35 day after sowing by using 200 l/fed. at the 1st and and 300 l/fed at the 2nd as spray solution volume. Ascorbic acid was obtained from (Al-Gomhoreya Co. for Chemical Industries, Mansoura, Egypt).

All treatments received 30 kg P_2O_5 and 25 kg K_2O kg/ fed. as mono calcium super phosphate (15.5 % P_2O_5), and potassium sulfate (50 % K_2O), respectively. Mono calcium super phosphate was added during preparation of the soil and potassium sulphate was added at one dose on the first irrigation time.

Experimental design:

A complete randomized blocks design with three replicates was used in a split plots experiment. Treatments of sources of nitrogen fertilization were designation in the main plots, while foliar applications with ascorbic acid were assigned in the sub plots.

Measurements:

Six plants were chosen at random from each sub plot at 60 days after sowing to determine the following parameters for the two seasons.

1- Vegetative growth attributes:

Plant height, plant fresh weight, leaves fresh weight, leaves number and leaves area) per plant.

2 – Leaves chemical composition:

N, P and K percent, chlorophyll a, b, carotenoids content were analysed according to AOAC (1990).

3 – Yield and its chemical quality:

Blades and petioles (fresh weight, dry matter % and NO_3 content), Vit. C, TSS were calculated according to (AOAC, 1990), fertilizer use efficiency (yield ton per fed. /nitrogen units), hill weight and yield per fed (plants weight of hill x number of hills per fed.) were also recorded.

Statistical analysis:

All data were statistically analyzed using the analysis of variance according to Snedecor and Cochran (1980). Least significant difference (LSD) at the probability of 5 % was used due to the procedure reported by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

1- Vegetative growth attributes:

Data presented in Table 2 indicated that vegetative growth criteria (plant height, plant fresh weight, leaves fresh weight, leaves number and leaves area per plant) were significantly affected by of nitrogen forms treatments. It could be noticed that the highest values of previous parameters were achieved in case of urea treatment followed by calcium nitrate and the reverse was true in case of ammonium nitrate treatments in the two seasons.

These results may be urea form resulted in that enhanced macro and micro nutrient uptake (zinc, manganese and copper in shoots and similar trend was observed in roots), increasing of stomatal conductance and transpiration rate (Sabir *et al*., 2013). Ammonium nutrition stimulates build-up of high levels of polyamines which act as precursors of secondary metabolites biosynthesis (Chen *et al*. 2011). Urea nitrogen enters the plant either directly, or in the form of ammonium or nitrate after urea degradation by urease enzyme resulted in producing ammonium. Ammonium carries a positive charge which decrease the loss by leaching and vice versa to nitrate. NH4+-N assimilation requires less energy than that of NO_3^--N, it is usually expected to be preferred by plants (Mirdad, 2009).

These results are in agreement with the findings of Mirdad (2009) on spinach; Mirdad (2011) on parsley and Sabir *et al*., (2013) on maize . On contrast, are not in the same trend with results obtained by Kaymak, (2013) who reported that ammonium sulphate form recorded the highest plant height and yield of purslane.

Table 2. Impact of Foliar application with ascorbic acid after 60 days from planting on vegetative growth characters of spinach under different sources of nitrogen fertilization during the two seasons of 2015 and 2016.

Treatments		Plant height (cm).		plant fresh weight g / plant		Leaves FW g / plant		Leaves No/ plant		Leaves area (cm^2) / plant	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Sources of nitrogen fertilization											
Ammonium nitrate		37.4	37.9	30.0	30.8	26.4	27.1	10.7	10.8	281.7	288.6
Ammonium sulphate		40.8	41.6	32.7	33.5	28.8	29.5	11.9	12.2	302.7	309.8
Calcium nitrate		43.7	44.7	35.5	36.5	30.9	31.6	13.0	13.6	318.5	325.8
Urea		45.7	46.9	39.0	40.4	32.3	33.3	14.7	15.3	341.5	351.7
LSD 5%		2.7	2.8	3.9	3.8	1.9	2.0	1.7	1.5	15.8	16.6
Foliar application with ascorbic acid (ppm)											
without		40.8	41.6	32.9	33.8	28.8	29.5	11.8	12.4	301.7	308.6
150		41.8	42.9	34.4	35.3	29.6	30.3	12.7	13.0	311.4	319.3
300		43.0	43.9	35.6	36.7	30.4	31.2	13.2	13.6	320.2	329.0
LSD 5%		1.9	1.6	3.0	3.3	1.3	1.5	N.S	N.S	11.4	11.7
Interaction											
Ammonium nitrate	without	35.2	35.8	28.2	28.7	24.8	25.3	9.8	10.0	268.5	273.4
	150	37.9	38.9	30.4	31.2	26.8	27.5	10.8	11.1	284.7	291.8
	300	39.1	39.1	31.4	32.4	27.7	28.5	11.5	11.5	291.7	300.5
Ammonium sulphate	without	40.1	40.5	32.2	32.8	28.3	28.9	11.6	12.0	297.4	303.4
	150	40.2	41.3	32.3	33.1	28.5	29.1	11.8	12.0	302.0	309.6
	300	42.0	43.1	33.7	34.6	29.7	30.4	12.3	12.6	308.5	316.5
Calcium nitrate	without	42.9	43.9	34.4	35.2	30.3	31.0	12.3	13.3	313.9	321.1
	150	43.4	44.5	35.8	36.7	30.7	31.4	13.1	13.6	316.7	324.7
	300	44.8	45.6	36.4	37.5	31.6	32.3	13.6	14.0	325.0	331.5
Urea	without	45.1	46.0	36.6	38.3	31.9	32.8	13.7	14.5	326.8	336.6
	150	45.8	47.0	39.2	40.2	32.4	33.2	15.0	15.1	342.1	351.0
	300	46.2	47.8	41.1	42.6	32.7	33.8	15.6	16.3	355.6	367.4
LSD 5%		3.8	3.3	6.1	6.6	2.7	3.0	2.8	2.3	22.8	23.3

As for the effect of spraying foliar application with ascorbic acid (AsA), results in Table 2 show that the attributes mentioned previously were significantly affected by application of AsA. The maximum values of these characters were achieved by application of AsA at 300 ppm in the two seasons. On contrast, the minimum values were observed by using without AsA treatment. This improvement in vegetative growth parameters could be due to exogenously application of AsA reducing or prevent the high activity of ROS. In addition, it enhances cell division, cell wall expansion, many other essential enzymatic and non-enzymatic reactions, regulating plant growth and development, Moreover, endogenous AsA has recently been suggested to be basic in the regulation of developmental senescence and plant defense against pathogens. Also, it is very essential for the regulation of flowering, photosynthesis and senescence and results in increasing in gibberellins and cytokinins.

These results are confirmed with the results obtained by Taha *et al*. (2011) on lettuce and Esfahlan *et al*. (2013) on purslane.

The interaction between Sources of nitrogen fertilization and spraying ascorbic acid showed that spinach plants which fertilized with urea and foliar sprayed by 300 ppm AsA were gave rise to the maximum values of all previous parameters. The minimum values were noticed with ammonium nitrate and without AsA in both seasons. These results were in harmony with those obtained by Mirdad (2009) on spinach; Shabana *et al*. (2015) on sweet pepper.

2- Leaves chemical composition parameters:

Results in Table 3 demonstrate that N, P, K, chlorophyll a, chlorophyll b and carotenoids content significantly affected by nitrogen forms. The urea form recorded the highest values of the N, P and K parameters followed by Ammonium sulphate. On contrast, the lowest values were recorded by calcium nitrate in the both seasons. On the other hand, chlorophyll a, chlorophyll b and carotenoids gave the maximum values by urea form followed by calcium nitrate, on contrary, the minimum values were recorded with ammonium nitrate.

These results may be urea form resulted in that enhanced macro and micro nutrient uptake (zinc, manganese and copper in shoots and similar trend was observed in roots), increasing of stomatal conductance and transpiration rate (Sabir *et al*., 2013). Ammonium nutrition stimulates build-up of high levels of polyamines which act as precursors of secondary metabolites biosynthesis (Chen *et al*. 2011). Urea nitrogen enters the plant either directly, or in the form of ammonium or nitrate after urea degradation by urease enzyme resulted in producing ammonium. Ammonium carries a positive charge which decreases the loss by leaching and vice versa to nitrate. $NH4^{+}$-N assimilation requires less energy than that of NO_3^{-}-N, it is usually expected to be preferred by plants (Mirdad, 2009) which lead to uptake higher amounts of nutrient such as N which are essential for chlorophyll pigments synthesis. Similar results were obtained by Sabir *et al*. (2013) on maize and Alderfasi *et al*. (2015) on spinach. On contrary, Fouda (2017) on tomato found that the maximum N.P.K and chlorophyll pigments content were recorded with ammonium sulphate form.

Data in Table 3 indicate that mentioned attributes were significantly increased in the two seasons by increasing AsA level. The high level of ascorbic acid 300 ppm registered the biggest values followed by 200 ppm. On contrary, minimum values of previous attributes were recorded by using the control of AsA in both seasons. These results may be due to exogenously application of AsA reducing or prevent the high activity of ROS. In addition, it is enhances cell division, cell wall expansion, many other important enzymatic, non-enzymatic reactions, increases gibberellins and cytokinins formation, ion uptake and membrane permeability. Also, AsA probably prevents chlorophyll oxidase enzymes therefore it will be inhibiting chlorophyll breakdown which cause increasing in photosynthesis. These results were conformity with those reported by El-Hifny and El-Sayed (2011) on sweet pepper Ismail *et al*. (2014) on Bitter lupine.

As for the interaction between sources of nitrogen fertilization and spraying ascorbic acid, data in Table 3 clear that the interaction combinations treatments significantly affected the previous criteria, the greater values of N, P and K parameters were obtained by using urea form combine with 300 ppm of AsA, but, the smallest values were observed with calcium nitrate and without AsA in both seasons. On the other hand, chlorophyll a, chlorophyll b and carotenoids gave the maximum values by urea form with 300 ppm of AsA. On contrary, the minimum values were recorded with ammonium nitrate combined with without AsA. Mirdad (2011) on parsley, Alderfasi *et al*. (2015) on spinach were found similar results.

Table 3. Impact of Foliar application with ascorbic acid after 60 days from planting on N, P, K percentage and pigments in leaves of spinach under different sources of nitrogen fertilization during the two seasons of 2015 and 2016.

Treatments		N %		P %		K %		Chl. a mg/100 FW		Chl.b mg/100 FW		Carotenoids mg/100g FW	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Sources of nitrogen fertilization													
Ammonium nitrate		2.56	2.62	0.324	0.331	2.79	2.88	111.0	114.3	53.8	55.1	33.4	34.2
Ammonium sulphate		2.67	2.81	0.337	0.346	2.90	3.02	121.0	123.8	57.7	59.0	37.8	38.7
Calcium nitrate		2.40	2.45	0.303	0.310	2.62	2.67	129.6	132.5	62.1	63.6	41.5	42.5
Urea		2.88	2.96	0.359	0.369	3.10	3.21	135.6	139.7	65.2	67.1	44.2	46.0
LSD 5%		0.01	0.02	0.002	0.005	0.02	0.08	8.1	6.8	3.9	3.6	3.5	3.3
Foliar application with ascorbic acid (ppm)													
without		2.56	2.64	0.322	0.330	2.77	2.86	121.1	123.9	58.1	59.5	37.8	38.7
150		2.62	2.70	0.331	0.339	2.86	2.95	124.1	127.7	59.7	61.2	39.2	40.1
300		2.70	2.78	0.340	0.349	2.93	3.02	127.7	131.1	61.3	62.9	40.7	42.2
LSD 5%		0.01	0.03	0.001	0.004	0.03	0.06	5.6	4.8	2.7	2.6	2.4	2.6
Interaction													
Ammonium nitrate	without	2.51	2.55	0.317	0.323	2.74	2.79	104.4	106.3	51.1	52.0	30.5	31.1
	150	2.58	2.64	0.326	0.334	2.81	2.91	112.5	117.0	54.0	55.4	34.1	34.9
	300	2.59	2.67	0.328	0.337	2.82	2.93	116.1	119.6	56.3	58.0	35.6	36.7
Ammonium sulphate	without	2.61	2.79	0.330	0.341	2.85	2.94	118.9	121.3	56.8	58.0	36.9	37.6
	150	2.68	2.81	0.339	0.348	2.92	3.06	119.4	122.4	57.0	58.5	37.1	38.0
	300	2.73	2.84	0.342	0.350	2.94	3.07	124.5	127.8	59.1	60.7	39.3	40.3
Calcium nitrate	without	2.33	2.38	0.295	0.301	2.54	2.60	127.3	130.2	59.9	61.2	40.5	41.4
	150	2.39	2.45	0.302	0.310	2.61	2.67	128.7	131.9	62.6	64.2	41.1	42.2
	300	2.48	2.53	0.314	0.320	2.71	2.76	132.9	135.5	64.0	65.3	43.0	43.8
Urea	without	2.79	2.87	0.345	0.356	2.98	3.12	133.8	137.8	64.8	66.7	43.4	44.7
	150	2.84	2.91	0.355	0.364	3.10	3.18	135.9	139.5	65.1	66.8	44.3	45.5
	300	3.01	3.11	0.376	0.388	3.24	3.35	137.2	141.7	65.7	67.9	44.9	48.0
LSD 5%		0.02	0.06	0.003	0.008	0.05	0.12	11.3	9.7	5.4	5.2	4.9	5.1

3- Yield and its chemical quality:

Obtained data of Table 4 revealed that fresh weight, dry matter % and NO3 content of blades and petioles of spinach leaves were affected significantly by nitrogen form. It is clear from the data that application of urea form gave the best results for the greater of fresh weight and dry matter % of blades and petioles of spinach leaves. And vice versa by using ammonium nitrate form. On other hand, It could be noticed that the lowest nitrate values were achieved in case of urea treatment and the reverse was true in case of ammonium nitrate form treatments in both blades and petioles of spinach leaves.

Such increases in case of urea form resulted in that enhanced macro and micro nutrient uptake (zinc, manganese and copper in shoots and similar trend was observed in roots), increasing of stomatal conductance and transpiration rate (Sabir *et al*., 2013). Ammonium nutrition stimulates build-up of high levels of polyamines which act as precursors of secondary metabolites biosynthesis (Chen *et al*. 2011). Urea nitrogen enters the plant either directly, or in the form of ammonium or nitrate after urea degradation by urease enzyme resulted in producing ammonium. Ammonium carries a positive charge which decrease the loss by leaching and vice versa to nitrate. NH4+-N assimilation requires less energy than that of NO_3^--N, it is usually expected to be preferred by plants (Mirdad, 2009). In addition, imbalance between nitrate absorption and reduction by nitrate reductase enzyme in plants caused nitrate accumulation in both blades and petioles of spinach leaves and when nitrate answer the supply in soil, plants usually absorb much more nitrate than they can reduce (Wang *et al*., 2008). Nitrogen as 60 units in this study may be the optimum dose which leads to scarcity of nitrate accumulation.

These results are in agreement with the findings of Mirdad (2009) and Alderfasi *et al*. (2015) on spinach. On the other hand, Rabie *et al*. (2014) reported that the maximum leaves fresh weight and the minimum of nitrate content were registered with ammonium nitrate form on spinach.

As for effect of ascorbic acid, data shown in Table 4 indicated that the influence of the higher level of ascorbic acid gave the maximum values of the previous parameters except NO_3 content in both blades and petioles of spinach leaves in both seasons. On contrary, the minimum values of these parameters were observed with without ascorbic acid in the two seasons except NO_3 content in both blades and petioles of spinach leaves in the two seasons. This may be attributed to exogenously application of AsA reducing oxygen free radicals or prevent the high activity of ROS. In addition, it is enhances cell division, cell wall expansion, many other important enzymatic, non-enzymatic reactions, increases gibberellins and cytokinins formation, ion uptake and membrane permeability. Also, AsA probably prevents chlorophyll oxidase enzymes therefore it will be inhibiting chlorophyll breakdown which cause increasing in photosynthesis. Taha *et al*. (2011) on lettuce and Shabana *et al*. (2015) on sweet pepper confirmed these results.

Respect to the interaction between nitrogen form and spraying ascorbic acid, the results showed that the combination consists of urea form and 300 ppm of AsA gave the highest values of all mentioned characteristics except NO_3 content. On contrary, the lowest values were recorded in case of ammonium nitrate form treatments and without AsA except NO_3 content in both blades and petioles of spinach leaves in the two seasons. These results were in accordance with those reported by Mirdad (2011) on parsley; El-Hifny and El-Sayed (2001) on sweet pepper; Sabir *et al*. (2013) on maize.

Table 4. Impact of Foliar application with ascorbic acid after 60 days from planting on Fresh weight dry matter % and NO_3 content of blades and petioles of spinach leaves under different sources of nitrogen fertilization during the two seasons of 2015 and 2016.

Treatments		Blades						Petioles					
		Fresh weight g / plant		Dry matter %		NO_3 mg/ kg D.W.		Fresh weight g / plant		Dry matter %		NO_3 mg/ kg D.W.	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Sources of nitrogen fertilization													
Ammonium nitrate		11.6	11.9	10.10	10.36	116.2	119.1	14.8	15.2	5.20	5.34	164.1	169.4
Ammonium sulphate		12.6	12.9	11.06	11.34	85.7	87.8	16.1	16.5	6.04	6.18	124.5	127.6
Calcium nitrate		13.5	13.8	11.90	12.18	103.0	99.8	17.3	17.7	6.78	6.94	144.1	146.4
Urea		14.1	14.6	12.48	12.36	38.4	39.4	18.1	18.6	7.22	7.44	51.7	53.0
LSD 5%		0.8	0.8	0.78	0.80	2.1	2.5	1.0	1.1	0.58	0.6	1.0	1.5
Foliar application with ascorbic acid (ppm)													
without		12.6	12.9	10.08	11.34	96.3	94.8	16.1	16.5	6.04	6.18	133.1	136.1
150		12.9	13.3	11.38	11.66	82.6	84.7	16.6	17.0	6.30	6.46	118.0	120.9
300		13.3	13.7	11.72	12.04	78.6	80.0	17.0	17.5	6.58	6.76	112.2	115.2
LSD 5%		0.5	0.7	0.54	0.56	1.4	1.5	0.7	0.8	0.46	0.48	1.2	1.4
Interaction													
Ammonium nitrate	without	10.9	11.1	9.46	9.64	127.1	130.9	13.9	14.2	4.60	4.68	180.9	186.4
	150	11.7	12.0	12.26	10.52	112.1	114.9	15.0	15.4	5.42	5.54	159.1	163.1
	300	12.1	12.5	10.60	10.92	109.5	111.4	15.5	15.9	6.60	5.78	152.2	158.8
Ammonium sulphate	without	12.4	12.7	10.88	11.10	91.1	93.9	15.9	16.2	5.86	5.98	135.2	139.2
	150	12.5	12.8	10.92	11.20	85.1	87.2	15.9	16.3	5.90	6.04	121.1	124.2
	300	13.0	13.3	11.42	11.70	80.8	82.2	16.6	17.0	6.34	6.52	117.2	119.3
Calcium nitrate	without	13.3	13.6	11.68	11.94	117.1	103.3	17.0	17.4	6.58	6.74	154.2	154.9
	150	13.4	13.8	11.82	12.12	98.2	100.7	17.2	17.6	6.72	6.88	141.1	144.6
	300	13.9	14.1	12.22	12.46	93.7	95.4	17.7	18.1	7.04	7.18	137.1	139.6
Urea	without	14.0	14.4	12.30	12.68	49.8	51.2	17.8	18.4	7.16	7.38	62.2	64.1
	150	14.2	14.6	12.52	12.84	35.1	35.9	18.1	18.6	7.22	7.40	50.6	51.8
	300	14.3	14.8	12.64	13.06	30.4	30.9	18.3	18.9	7.30	7.54	42.4	43.1
LSD 5%		1.1	1.4	1.08	1.12	2.8	2.9	1.5	1.6	0.94	0.98	2.4	2.8

4- Yield and its chemical quality

Data presented in Tables 5 illustrate that Vit. C, TSS, fertilizer use efficiency, hill weight and yield per fed. were affected significantly by nitrogen form. The urea form gave the highest values of the mentioned attributes followed by calcium nitrate. On the contrast, the lowest values were observed by using ammonium nitrate in both seasons.

These results may be attributed to urea form resulted in that enhanced macro and micro nutrient uptake (zinc, manganese and copper in shoots and similar trend was observed in roots), increasing of stomatal conductance and transpiration rate (Sabir *et al*., 2013). Ammonium nutrition stimulates build-up of high levels of polyamines which act as precursors of secondary metabolites biosynthesis (Chen *et al*. 2011). Urea nitrogen enters the plant either directly, or in the form of ammonium or nitrate after urea degradation by urease enzyme resulted in producing ammonium. Ammonium carries a positive charge which decreases the loss by leaching and vice versa to nitrate. NH_4^+-N assimilation requires less energy than that of NO_3^--N, it is usually expected to be preferred by plants (Mirdad, 2009) which lead to uptake higher amounts of nutrient such as N which are essential for chlorophyll pigments synthesis. which reflected positively to increases of leaves area and leaves fresh weight (Table 2 and 3), It could be a great impact vegetative growth and yield. Similar results were reported by Mirdad (2009) and Alderfasi *et al*. (2015) on spinach. On contrary, Ghoname *et al*. (2009) on hot pepper mentioned that ammonium nitrate form recorded the highest values of fruit yield and quality.

Spraying of ascorbic acid on spinach plants are indicated in Tables 5. Data revealed that, the mentioned criteria were increased compared to the control. The biggest values of these attributes were noticed with ascorbic acid at 300 ppm, in both seasons. On contrast, the smallest amounts of previous parameters values were registered by application of without acorbic acid treatment in the two seasons. This improvement in the yield and its component of spinach may be attributed to exogenously application of AsA reducing or prevent the high activity of ROS. In addition, its enhancement cells division, cell wall expansion, many other important enzymatic, non-enzymatic reactions, increases gibberellins and cytokinins formation, ion uptake and membrane permeability. Also, AsA probably prevents chlorophyll oxidase enzymes therefore it will be inhibiting chlorophyll breakdown which cause increasing in photosynthesis, leaves area and leaves number (Table 2 and 3) which leads to an increasing in yield and quality. These finding are similar to those obtained by Esfahlan *et al*. (2013) on purslane and Ismail *et al*. (2014) on bitter lupine.

Results in Table 5 shows that, the interaction was significant in the two seasons, the maximum values of previous parameters were registered with combination consist urea form and 300 ppm of ascorbic acid. On contrary, the minimum values were noticed with ammonium nitrate form and without ascorbic acid in both seasons. Similar findings were recorded by Mirdad (2011) on parsley; Alderfasi *et al*. (2015) on spinach and Shabana *et al*. (2015) on sweet pepper

Table 5. Impact of Foliar application with ascorbic acid after 60 days from planting on yield and its chemical quality of spinach under different sources of nitrogen fertilization during the two seasons of 2015 and 2016.

Treatments		Vit. C mg/100g F.W		TSS %		fertilizer use efficiency (Kg per unit)		Plants hill weight g / hill		Yield (ton) per fed.	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Sources of nitrogen fertilization											
Ammonium nitrate		60.6	62.1	4.56	4.67	210	215	315	322	12.60	12.91
Ammonium sulphate		66.1	67.6	5.11	5.23	229	235	344	352	13.77	14.10
Calcium nitrate		70.8	72.4	5.8	5.71	241	248	362	372	14.51	14.89
Urea		74.1	76.3	6.02	6.19	271	281	407	421	16.30	16.88
LSD 5%		4.4	4.1	0.41	0.26	16	19	25	28	1.01	1.15
Foliar application with ascorbic acid (ppm)											
without		66.1	67.7	5.11	5.23	228	235	343	352	13.73	14.11
150		67.8	69.5	5.28	5.41	238	244	358	367	14.32	14.68
300		69.7	71.6	5.55	5.71	247	254	371	382	14.85	15.29
LSD 5%		3.1	2.8	0.32	0.25	14	16	22	23	0.88	0.93
Interaction											
Ammonium nitrate	without	57.0	58.0	4.20	4.28	202	205	303	308	12.1	12.35
	150	61.4	63.0	4.65	4.76	208	213	313	320	12.5	12.84
	300	63.4	65.3	4.84	4.99	219	225	329	338	13.1	13.55
Ammonium sulphate	without	65.0	66.3	5.02	5.10	224	228	336	342	13.4	13.71
	150	65.2	66.9	5.02	5.15	231	237	347	356	13.9	14.24
	300	68.0	69.8	5.30	5.44	233	239	349	358	13.98	14.35
Calcium nitrate	without	69.5	71.1	5.45	5.58	234	239	350	358	14.03	14.35
	150	70.3	72.0	5.53	5.67	242	248	363	372	14.54	14.91
	300	72.6	74.0	5.76	5.87	249	256	373	385	14.95	15.40
Urea	without	73.0	75.2	5.81	5.98	255	267	382	400	15.31	16.04
	150	74.2	76.2	5.92	6.08	271	278	407	418	16.31	16.73
	300	74.9	77.4	6.32	6.52	288	297	432	446	17.29	17.8
LSD 5%		6.2	5.6	0.64	0.51	29	31	44	47	1.76	1.87

CONCLUSION

Under similar conditions of this study it can be concluded that; the foliar application of ascorbic acid at 300 ppm and soil addition of urea as form of N-fertilization is considered to be the most effective treatment for improve vegetative growth and recorded the highest safe yield quality of spinach plant.

REFERENCES

Alderfasi, A. A.; A. M. Aljuaid; A. E. Moftah and M. M. Selim (2015) Role of nitrification inhibitor combined with different nitrogen sources in decreasing injurious components in spinach. Adv Plants Agric Res 2(6): 00072. DOI: 10.15406/ apar.2015.02.00072.

AOAC (1990) Official Methods of Analysis.15th Ed. Association of Official Analytical Chemists, Inc., Virginia, USA.

Chen, L.; L. QQ; G. JY; Z. YL; Y. LF and C. Wang (2011) Effects of nitrogen forms on the growth and polyamine contents in developing seeds of vegetable soybean. J. of Plant Nutrition, 2 (34): 504–521.

Dolatabadian, A., S. A. M. Modarres Sanavy, and K. S. Asilan (2010) Effect of ascorbic acid foliar application on yield, yield component and several morphological traits of grain corn under water deficit stress conditions. Not. Sci. Biol., 2 (3): 45-50.

El-Hifny, I . M. and M.A. El-Sayed (2011) Response of sweet pepper plant growth and productivity to application of ascorbic acid and biofertilizers under saline conditions. Australian J. of Basic and Applied Sciences, 5(6): 1273-1283.

Esfahlan, E. N.; A. Pazoki; H. Rezaei; D. E. Asli and M. Usefirad (2013) Effects of ascorbate foliar application on morphological traits, relative water content and extract yield of Purslane (*Portulaca oleracea* L.) under salinity stress. Iranian J. of Plant Physiology, 4 (1): 889-898.

Fouda, K.F. (2017) Effect of interaction among N forms and calcium sources on quality and chemical composition of tomato (*Lycopersicon esculentum*). Egypt. J. Soil Sci., 57 (2): 61 -71.

Ghoname, A. A.; M. G. Dawood; G. S. Riad and W. A . El-Tohamy (2009) Effect of nitrogen forms and biostimulants foliar application on the growth, yield and chemical composition of hot pepper grown under sandy soil conditions. Res. J. Agric. & Biol. Sci., 5(5): 840-852.

Gomez, K.A. and A.A., Gomez (1984) Statistical Procedures for Agricultural Research. John Wiley & Sons Inc., Singapore 680.

Ismail, M. S.; G. M. Ghazal and G. S. Mohamed (2014) Effect of ascorbic acid and niacin on protein, oil fatty acids and antibacterial activity of *Lupinus termis* seeds. Inter. J. of Pharmacognosy and Phytochemical Res. 6(4); 866-873.

Kaymak, H. C. (2013) Effect of nitrogen forms on growth, yield and nitrate accumulation of cultivated purslane (*Portulaca oleracea* l.). Bulgarian J. of Agricultural Science, 19 (3): 444-449.

Maisarah, A.M.; Amira NB, Asmah R & Fauziah O (2013) Antioxidant analysis of different parts of Carica papaya. International Food Research Journal 20(3): 1043–1048.

Mirdad, Z. M. (2011) Effect of irrigation intervals, nitrogen sources and nitrogen levels on some characters of parsley (*Petroselinum crispum* Mill). Jkau: Met., Env. & Arid Land Agric. Sci., 22 (1): 3-17.

Mirdad, Z. M. (2009) spinach (*Spinacia oleracea*, l.) growth and yield responses to irrigation dates, mineral nitrogen - sources and levels – application. J. Agric. & Env. Sci. Alex. Univ., 8 (1):43-69.

Pastori, G. M.; G. Kiddle; J. Antoniw; S. Bernard; S. V. 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. The Plant Cell, 15:939-951.

Pietro, S. (1997) Producing nitrate- free endive head: effect of nitrogen form on growth, yield, and ion composition of endive. J. Amer. Soc. Hort. Sci, 122(1): 140-145.

Rabie, K; A. E.; H. M. Ashour and H. H. Manaf (2014) Influence of nitrogen forms on nitrate and nitrite accumulation in the edible parts of spinach (*Spinacia oleracea*, L.) plant with maintenance for yield production. J. of Horti. Sci & Ornamental Plants, 6 (3): 126-132.

Sabir, M.; M. M. Hanafi; M. T. Malik; T. Aziz; M. Z. Rehman; H. R. Ahmad; K. R. Hakeem and M. Shahid (2013) Differential effect of nitrogen forms on physiological parameters and micronutrient concentration in maize (*Zea mays* L.). AJCS, 7(12):1836-1842.

Shabana, A. I.; M. R. Shafeek; H. I. Ahmed and F. S. Abdel-Al (2015) Improving growth, fruit setting, total yield and fruit quality of sweet pepper plants (*Capsicum annum* L.) by using antioxidant and seaweed extracts. Middle East J. of Agric. Res. 4 (2): 154-161.

Snedecor, W.G. and G.W. Cochran (1980) Statistical Methods. 7th Ed., the Iowa State Univ. Press, Ames, Iowa, USA.

Taha, A. A.; A. A. Mosa and S. A. Ahmed (2011) role of micronutrients and antioxidants application in stimulating growth and yield of fresh edible vegetables. J. Soil Sci. and Agric. Eng., Mansoura Univ., 2 (2): 251 – 263.

Wang Z. H.; S. X. Li and S. Malhi (2008) Effects of fertilization and other agronomic measures on nutritional quality of crops. J. of the Science of Food and Agriculture, 88: 7-23.

Wang, J.; Y. Zhou; C. Dong; Q. Shen and R. Putheti (2009) Effects of NH_4^+-N/ NO_3^--N ratios on growth, nitrate uptake and organic acid levels of spinach (*Spinacia oleracea* L.). African J. of Biotechnology, 8 (15): 3597-3602.

استجابة نبات السبانخ للرش الورقى بحمض الاسكوربيك تحت مصادر ازوت مختلفة

السعيد السيد متولى
قسم الخضر والزينة- كلية الزراعة جامعة المنصورة

اجريت تجربتان حقليتان متتاليتان بوحدة التجارب والبحوث – كلية الزراعة جامعة المنصورة فى الموسم الشتوى من عامى 2015 و2016. لدراسة تاثير الرش الورقى بحمض الاسكوربيك (بدون - 200 – 300 جزء فى المليون) تحت اربع مصادر ازوت (نترات الامونيوم- سلفات الامونيوم- نترات الكالسيوم - اليوريا) على النمو والمحصول لنبات السبانخ صنف داش. اوضحت النتائج ان صفات النمو الخضرى (ارتفاع النبات – وزن النبات الطازج - الوزن الطازج للاوراق - عدد الاوراق – المساحة الورقية للنبات). التركيب الكيماوى للاوراق (نسبة النتروجين والفسفور والبوتاسيوم – كلورفيل أ – كلورفيل ب – الكاروتينيدات). المحصول وجودته الكيمائية (الوزن الطازج – المادة الجافة - محتوى النترات للانصال والاعناق– فيتامين ج - المواد الصلبة الذائبة الكلية- كفاءة استخدام السماد - وزن الجورة – المحصول الكلى) تأثر معنويا بمصادر الازوت. ولقد حققت اليوريا اعلى القيم للصفات السابقة ما عدا محتوى الانصال والاعناق من النترات. وبالنسبة للاسكوبيك اسد اوضحت النتائج زيادة الصفات السابقة بزيادة مستوى الاسكوربيك اسد. ولقد بين التفاعل بين مصادر الازوت والاسكوربيك اسد ان اليوريا + 300 جزء فى المليون حقق اعلى القيم ما عدا محتوى الانصال والاعناق من النترات.