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Response of Yield and Quality of Garlic to Nitrogen Sources and Foliar Spray with Sulfur Treatments

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ABSTRACT

Two experiments were carried out during 2014/2015 and 2015/2016 seasons, at the Experimental Farm, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt, to study the effect of nitrogen forms (ammonium nitrate and urea) and sulfur treatments (0, 5, 10 and 15 mM) from two sources (potassium sulphate and calcium sulphate) on garlic yield, chlorophyll, carbohydrates, phenols, nitrate and elements content. Results revealed that ammonium nitrate-fertilized plants had the highest nitrate content in the absence of sulfur treatments. However, plant growth, yield, chlorophyll, phenols, NPK, Ca and S reached the maximum values and nitrate content decreased when plants were sprayed with potassium sulphate at 10 mM. Potassium sulphate was more favorable than calcium sulphate for improving the plant growth, yield and other bulb quality such as phenols and S content. Urea-fertilized plants showed less bulb yield and nitrate content than ammonium nitrate-fertilized plants, but higher than un-fertilized plants. Chlorophyll, nitrogen, phenols and S content were lower and the carbohydrates content was higher in plants grown with un-fertilized than in well fertilized with nitrogen. The results showed that N and S nutrition during the growth and bulb production are strongly coupled. Their interactions are synergistic at suitable form of nitrogen (ammonium nitrate) as well as at optimum rate of potassium sulphate (10 mM). However, excessive level of potassium sulphate (15 mM) seems to be antagonistic. Together, the results indicate that S spraying is required to improve N-use efficiency and thus increasing the bulb yield with high minerals nutrient such S and N content which are very important for S-containing bioactive compounds in garlic.

Keywords: *Allium sativum*, N-forms, potassium sulphate, yield, nitrate content

INTRODUCTION

Garlic (*Allium sativum* L.) belongs to the family of Alliaceae. It is one of the most important bulb vegetables throughout the world and it has been cultivated for thousands of years. According to FAO, the total harvested area was1,547,381ha and an annual production of 24,939,965 tonnes of bulbs in the world in 2014 and harvested area was 10,997 ha and production was 263,167 tonnes in Egypt (FAO, 2014). Garlic is an important vegetable, not only for local consumption and export, but also for its the medicinal value, such as anti-cancer, antidiabetic, antimicrobial, anti-inflammatory, immunomodulatory activities, cardioprotective and antioxidant (Kumar *et al.*, 2013; Chen *et al.*, 2013; Yun *et al.*, 2014; Martins *et al.*, 2016). Therefore, increasing yield and improving quality of garlic are necessary aims for growers and consumers.

Garlic requires intensive and complete fertilization system to obtain high yield and good quality. The application of 92kg N, 40kg P and 30kg S ha^{-1} gave optimum yield and quality of garlic (Diriba-Shiferaw *et al.*, 2014). Nitrogen is a major essential element to increase yield and quality of vegetables. Nitrogen has many functions in growth of plants. It is a component of protein, nuclic acids, chlorophyll and alkaloids. Nitrogen improves absorption of water and nutrients. It is absorbed as NO_3^- and NH_4^+ by roots of plant, the most annual crops grow best when nitrogen supply mixture of NO_3^- and NH_4^+in controlled conditions (Fageria and Baligar, 2005). Ammonium assimilation requires less energy (5 ATP mol^{-1} of NH_4^+) than nitrate assimilation (20 ATP mol^{-1} of NO_3^-), this mechanism of energy saving may be responsible for higher nitrogen use efficiency in NH_4^+-N (Salsac *et al.*, 1987). Regarding to nitrogen fertilizer on garlic, most literatures focused on the effect of nitrogen levels (Kakar *et al.*, 2002; Naruka and Dhaka, 2002; Naik and Hosamani 2003; Farooqui *et al.*, 2009; El-Zohiri and Abdou 2009; Hore *et al.*, 2014; Zaki *et al.*, 2014), however a few literatures studied the effects of nitrogen sources (Nori *et al.*, 2012). The results of Nori *et al.* (2012) indicated that no great differences between both examined sources (Ammonium sulphate and Urea) in the case of yield and nitrate content. While, the nitrogen sources significantly affected the yield and nitrate content in broccoli (Elwan and Abd El-Hamed, 2011) and spinach (Elwan and Elhamahmy, 2015a&b).

Sulfur is an important element in attributes of garlic quality. In Allium species, more than 80% of the total sulfur may be bound to sulfur-containing compounds such as amino acids (e.g., cysteine, methionine), proteins, alliin, coenzymes and other secondary plant products (Marschner, 1995). Sulfur fertilization on garlic was mostly studied as soil application (Farooqui *et al.*, 2009; Zaman *et al.*, 2011; Diriba-Shiferaw *et al.*, 2014; Imen *et al.*, 2013; Hore *et al.*, 2014), however, few literatures were found on the effect of sulfur sources fertilization as foliar spray on garlic. Soil fertilization of rosy garlic plants with sulfur resulted in improving the flavor, increasing of total polyphenol and decreasing of reduced carbohydrates (Imen *et al.*, 2013). The highest bulb yield of garlic was observed with 45 kg S/ha (Zaman *et al.*, 2011).

Foliar application of fertilizers is an important tool for the sustainable and production management of plant crops, and is a commercial importance worldwide (Fernández and Brown, 2013). Foliar spray with nutrients have 5 to 30 times more efficient than soil fertilization, depending on the nutrient, soil and crop. Additionally, it saves the money of grower because it applies with low amount than the soil application (Lovatt, 2013).

A few literatures are available on the effect of a combination between nitrogen and sulfur levels on garlic (Farooqui *et al.*, 2009). Therefore, the aims of this study were to investigate the effect of two different forms of nitrogen (ammonium nitrate and urea) as soil application, foliar application of sulfur sources and their interaction on growth, yield and chemical components of garlic plants.

MATERIALS AND METHODS

1. Plant materials and treatments

The experiment was conducted at Research Experimental Farm of the Faculty of Agriculture, Suez Canal University, Ismailia, Egypt, during the two consecutive winter seasons of 2014-2015 and 2015-2016 to study the effect of different sources of nitrogen and sulfur on vegetative growth, yield and chemical components of garlic *Allium sativum* L. "cv. Seds 40". Soil texture was sandy (92 % sand, 6 % clay and 2 % loam), with pH 7.67, EC 0.361 dSm^{-1}, available N 60 ppm, available P 28.1 ppm, available K 70 ppm, Ca^{+2}2 $meql^{-1}$, Mg^{+2}1 $meql^{-1}$, Na^{+}0.60 $meql^{-1}$, K^{+} 0.1 $meql^{-1}$, HCO_3^{-}1.60 $meql^{-1}$, Cl^{-}1.40 $meql^{-1}$ and SO_4^{-2}0.70 $meql^{-1}$ and CO_3^{-2} 0.00 $meql^{-1}$ organic matter 0.14%, organic carbon 0.081 %.

Experimental soil was cleared, ploughed and harrowed, and then drip irrigation system was placed. The organic manure at the rate of 20 m^3/fad. and ordinary superphosphate 15.5% P_2O_5 were added at soil preparation. Uniform and healthy cloves were soaked in running water for 24h. The planting was carried out by hand on 20th and 23rd of October in the first and second season, respectively. Experimental unit area (plot) was 3 m x 2 m in size (two rows), including approximately 60 plants. Cloves were sown at 10 cm apart and 100 cm between rows.

The experiment was consisting of two factors, the first factor was nitrogen sources and the second one was sulfur treatments. Two nitrogen forms (ammonium nitrate 33.5% and urea 46%) were applied as recommended dose at 285 kg N/ha (El Sayed and El Morsy, 2012) in addition to control treatment (0 kg N) as soil application, and seven sulfur treatments from two sulfur sources (potassium sulphate and calcium suphate) were applied as foliar spray. Three concentrations (5, 10 and 15 mM) from each source (K_2SO_4 or $CaSO_4$. $2H_2O$) plus 0 mM sulfur as control treatment were used.

Nitrogen sources were applied five times, the first dose was added after three weeks from cultivation of cloves and the rest doses were supplied with 2 weeks intervals.

The solutions of sulfur treatments were sprayed five times, began after 30 days from planting, with 2 weeks intervals on the whole foliage in the morning (10–11 a.m.). The volume of solution was ranged from156 to 468 L per feddan each time, depending on plant size or development. The sprays were carried out with a manual pump. The other normal agricultural treatments for growing garlic plants were practiced.

2. Measurements

Nine plants in appropriate maturity stage from each replicate were harvested (end of March) when older leaves turned yellowish green and started withering to measure all parameters.

Vegetative growth

Plant height was measured from base of swelling sheath to the tip of the largest linear blade in plant. Leaves fresh weight was estimated with gravimetric method after removing the bulbs at neck zone.

Bulb characteristics and yield

Bulb fresh weight was estimated after removing the foliage at neck zone. Bulb diameter was measured at equatorial of bulb and neck diameter was measured at neck zone using a precision caliper 0/0001 m. bulbing ratio was calculated according to the formula described by Mann (1952) as follow:. Bulbing ratio = (bulb neck diameter (cm)/bulb diameter (cm))

Total yield (t/ha): Plants of each experimental plot were harvested after 180 days from sowing, weighted and total yield of whole plants was calculated.

Chemical composition

Organic compounds

Total chlorophylls were determined spectro photometrically according to Lichenthaler and Wellburn (1983). Total Carbohydrates and total phenols were determined according to Mazumdar and Majumder (2003).

Minerals determination

Three bulbs from each replicate were dried at 70 ºC till constant weight, then ground, 0.5 g of fine ground materials was digested with a mixture of sulfuric acid and hydrogen peroxide and then brought to a final volume of 100 ml with distilled water to determine nitrogen, phosphorus, potassium and calcium.

A) Nitrogen was measured at wavelength of 650 nm using a spectrophotometer (UNICO UV/Visible 2100, USA) according to method of Baethgen and Ally (1989).
B) Phosphorus: P was analyzed by chlorostannus reduced molybdophosphoric blue color method, in sulfuric acid system at 660 nm using a spectrophotometer (UNICO UV/Visible 2100, USA) as described by Jackson (1973).
C) Potassium and Calcium were determined using a Perkin-elmer, Flame photometer (Page, 1982).
D) Sulphur: 0.5 g of fine ground was digested with a mixture of nitric acid and perchloric acid and then brought to a final volume of 100 ml with distilled water. Sulfur was estimated according to Novozamsky and Van Eck (1977).

Nitrate content: nitrate was measured according to Cataldo *et al.* (1975).

3 Statistical analysis

The experiments were organized in a completely randomized block design (CRBD) with a split plot arrangement, with three replications, in which each replication considered as a block. Nitrogen forms were randomly distributed in the main plots and sulfur treatments were randomly distributed in the sub-plots. Data were analyzed by analysis of variance (ANOVA) using CoStat version 6.303 1998–2004 CoHort software, 798 Lighthouse Ave, PMP 320, Monterey, CA 93940, USA. Duncan's test used to compare means at the 5% significance level. Standard error (SE) of means were calculated for presentation in figure using Sigma Plot version 10.0 (Systat Software Inc., Hounslow, UK)

RESULTS

1. Plant growth and yield

The main effect of nitrogen source significantly influenced plant growth and yield in both seasons (Table 1 & Fig. 1). Results indicated that the significant highest plant growth and yield were associated with ammonium nitrate compared with urea and no-fertilizer.

Garlic plant growth and yield significantly affected by the main effect of sulfur treatments (Table 1 & Fig. 1). Where, sulfur treatments gave higher plant growth and yield comparing with non-treated one. The application of potassium sulphate was more effective than calcium sulphate for enhancing plant growth and yield. The foliar spray of sulfur at 10 mM as potassium sulphate increased plant height and yield by approx. 9.5% and 41 %, respectively, comparing with non-treated plants. However, yield increased by only 21% when sulfur applied as calcium sulphate at 10 mM. The yield decreased when sulfur concentration increased from 10 to 15 mM and this decreasing was non-significant in case of potassium sulphate and significant for calcium sulphate.

The interaction effects between nitrogen sources and sulfur treatments are presented in Table (1) and figure (1c&d). Garlic plants that were fertilized with ammonium nitrate and were sprayed with 10 mM potassium sulphate had the highest plant growth and yield compared with other combinations. The increasing percentage was approx. 214% and 227.1% for yield, 214.9% and 222.9% for bulb weight and 36.5% and 37.5% for plant height in both seasons, respectively, when garlic plants received ammonium nitrate and treated with 10 mM potassium sulphate in comparison with non-treated plants.

The correlations data presented in table (5) illustrated that the yield positively correlated at significant level with most of other presented parameters (chlorophyll, phenols, nitrate, N, P, K, Ca and S), however, negatively correlated at significant level with carbohydrates content.

2. Chlorophyll, carbohydrates and total phenols

The main effects of nitrogen sources and sulfur treatments as well as their interaction on chlorophyll content, carbohydrates and total phenols are presented in Table (2). With regard to the main effect of nitrogen source, the significant highest chlorophyll content was observed in plants received ammonium nitrate. Total phenols did not differ significantly using both nitrogen sources, however, non-fertilized plants had the significant lowest values. In contrast to the previous results regarding to phenols content, un-fertilized plants had the significant highest carbohydrates content than the nitrogen-fertilized plants with any nitrogen forms in both seasons.

Concerning the main effect of sulfur treatments, the potassium sulphate at 10 mM gave the significant highest chlorophyll and total phenols in comparison with other sulfur treatments, however, no sulfur treatment had the highest carbohydrates, and in most cases, the effect was at significant level. At 15 mM sulfur from both sources, the chlorophyll and phenols decreased in comparison to favorable treatment of sulfur (10 mM) and mostly this decreasing was at significant level. However, the carbohydrates increased by increasing the level of sulfur from 10 to 15 mM in both sources.

With respect to the interaction effect between nitrogen sources and sulfur treatments, the results showed that the garlic plants fertilized with ammonium nitrate and sprayed with 10 mM potassium sulphate had the significant highest chlorophyll content and total phenols in comparison with other combinations. For instance, the above-mentioned treatment increased chlorophyll content by 128% and 94% as well as total phenols by 108.37% and 103.87 % in both seasons, respectively. However, the combination between no-nitrogen and no-sulfur treatment resulted in a highest carbohydrates content compared with other combinations. In the same line, fertilized plants with urea as well as non-fertilized with nitrogen, which sprayed with 15 mM sulfur, gave also high carbohydrates content (Table 2). Chlorophyll and phenols contents are positively correlated (Table 5) at significant level to each other and with minerals contents (N, P, K and S). Both negatively correlated at significant level with carbohydrates content.

Table 1. Main effects of nitrogen source, sulfur treatments and their interactions on plant height, weight of leaves and blubbing ratio of garlic "cv. Seds 40".

Parameters	Plant H. (cm)			Mean	Leaves W. g/plant			Mean	Blubbing Ratio			Mean
N. Source	0 N	A nitrate	Urea		0 N	A nitrate	Urea		0 N	A nitrate	Urea	
S Treat	2014/2015 season											
S0	68.38h	83.56de	80.63e	77.52d	30.38j	73.73c-f	54.54h	52.88e	0.249i	0.271e-g	0.260f-i	0.260d
KS5	72.67fg	87.32bc	88.00bc	82.66b	39.37ij	82.72bc	70.27ef	64.12bc	0.264e-i	0.280c-e	0.271e-g	0.272bc
KS10	75.38f	93.33a	86.14cd	84.95a	44.21i	99.26a	81.90bd	75.12a	0.268e-h	0.309a	0.295a-c	0.291a
KS15	73.88f	90.00ab	89.50bc	84.46ab	40.93i	81.15b-d	84.92b	69.00b	0.277d-f	0.277d-f	0.290b-d	0.281ab
CaS5	73.25f	88.88bc	88.00bc	83.38ab	37.57ij	79.11b-e	72.89d-f	63.19c	0.251hi	0.303ab	0.275d-f	0.277bc
CaS10	74.22f	86.11cd	87.63bc	82.65b	42.40i	69.63fg	77.05b-f	63.03cd	0.249i	0.303ab	0.289b-d	0.280b
CaS15	69.25gh	88.00bc	82.13e	79.79c	35.95ij	76.86b-f	60.44gh	57.75de	0.254g-i	0.278c-e	0.268e-h	0.267cd
Mean	72.43c	88.17a	86.00b		38.69c	80.35a	71.71b		267.21c	359.60a	325.11b	
	2015/2016 season											
S0	68.91h	86.00de	80.86f	78.59c	30.65i	80.89b-d	56.47f	56.00d	0.245k	0.278d-g	0.265g-j	0.263d
KS5	72.86g	88.43b-d	87.43cd	82.90b	39.45g-i	90.42b	73.56c-e	67.81b	0.271f-h	0.280d-g	0.274e-h	0.275c
KS10	75.91g	94.71a	87.61cd	86.08a	45.47g	101.79a	89.18b	78.82a	0.267f-i	0.309a	0.303ab	0.293a
KS15	75.13g	91.29ab	89.79bc	85.40a	44.77g	87.99b	90.40b	74.39a	0.276d-g	0.282c-f	0.298a-c	0.285ab
CaS5	73.04g	87.70cd	88.57b-d	83.10b	37.66g-i	76.97cd	71.77de	62.13bc	0.251i-k	0.300ab	0.270f-h	0.274c
CaS10	74.00g	87.43cd	87.38cd	82.93b	42.10gh	71.33de	83.06bc	65.49b	0.250jk	0.291b-d	0.288b-e	0.276bc
CaS15	67.04h	87.43cd	82.59ef	79.02c	33.65hi	74.05c-e	64.29ef	57.33cd	0.259h-k	0.270f-h	0.273e-h	0.267cd
Mean	72.41c	88.99a	86.32b		39.11c	83.35a	75.53b		0.259b	0.287a	0.282a	

Values are the means of three 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. S0= no sulfur spray; KS= potassium sulphate; CaS= calcium sulphate.

Table 2. Main effects of nitrogen source, sulfur treatments and their interactions on chlorophyll, carbohydrate and total phenols of garlic "cv. Seds 40".

Parameters	Chlorophyll mg/100FW			Mean	Carbohydrate mg/gDW			Mean	T.Phenols mg/gDW			Mean
N. Source	0 N	A nitrate	Urea		0 N	A nitrate	Urea		0 N	A nitrate	Urea	
S Treat					**2014/2015 season**							
S0	69.95m	103.39c-i	101.25d-j	91.53e	248.76a	218.96f-h	238.33a-d	235.35a	2.63i	3.09hi	3.24g-i	2.99d
KS5	96.22g-k	107.56b-e	93.56j-l	99.11d	204.67hi	217.95fg-i	243.04ab	221.89bc	2.82i	4.64bc	4.37b-e	3.94b
KS10	95.50h-k	159.55a	105.36c-f	120.14a	223.44d-f	224.59d-f	226.92c-f	224.99bc	3.00hi	5.48a	4.72b	4.40a
KS15	98.82f-k	107.62b-e	110.91bc	105.78b	247.94a	206.23g-i	236.01a-e	230.06ab	3.00hi	4.08b-f	4.48b-d	3.85b
CaS5	95.17i-k	103.82c-g	103.47c-h	100.82cd	242.72a-c	226.83d-f	206.32g-i	225.29bc	2.58i	3.94d-f	3.59f-h	3.37c
CaS10	91.74kl	114.41b	108.72b-d	104.96bc	230.04b-f	228.66b-f	202.33i	220.34c	2.77i	4.15b-f	3.84d-g	3.59bc
CaS15	86.17l	99.60e-k	108.93b-d	98.23d	244.51ab	222.89d-f	221.62e-g	229.67ab	2.99hi	4.02c-f	3.78e-g	3.60bc
Mean	90.51c	113.71a	104.60b		234.58a	220.87b	224.94b		2.83b	4.20a	4.00a	
					2015/2016 season							
S0	72.05k	104.45d-g	103.13e-h	93.21e	256.27a	225.44fgh	244.81a-d	242.17a	2.84j	3.40h-j	3.55g-i	3.26d
KS5	98.34g-i	110.74b-d	96.78hi	101.95cd	212.18h	224.43fgh	249.52a-c	228.71b	3.04ij	4.75b-d	4.68b-e	4.16b
KS10	97.69g-i	139.80a	108.58b-e	115.36a	230.95d-f	233.64d-f	233.40d-f	232.66b	3.22ij	5.79a	5.27ab	4.76a
KS15	100.95f-i	110.80b-d	114.23b	108.66b	255.45a	212.71gh	242.49a-e	236.88ab	3.06ij	4.39c-f	4.88bc	4.11b
CaS5	97.04hi	106.68c-f	106.14c-f	103.28cd	250.23a-c	233.31d-f	212.80gh	232.11b	2.80j	4.25d-f	3.90f-h	3.65c
CaS10	93.63ij	112.98bc	111.49b-d	106.03bc	237.55b-f	235.14c-f	212.84gh	228.51b	2.99ij	4.46c-f	4.15d-g	3.87bc
CaS15	87.74j	102.45e-h	110.86b-d	100.35d	252.02ab	229.37d-f	228.10e-g	236.50ab	3.21ij	4.33c-f	4.09e-g	3.88bc
Mean	92.49c	112.56a	107.32b		242.09a	227.72b	231.99b		3.02b	4.48a	4.36a	

Values are the means of three 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. S0= no sulfur spray; KS= potassium sulphate; CaS= calcium sulphate.

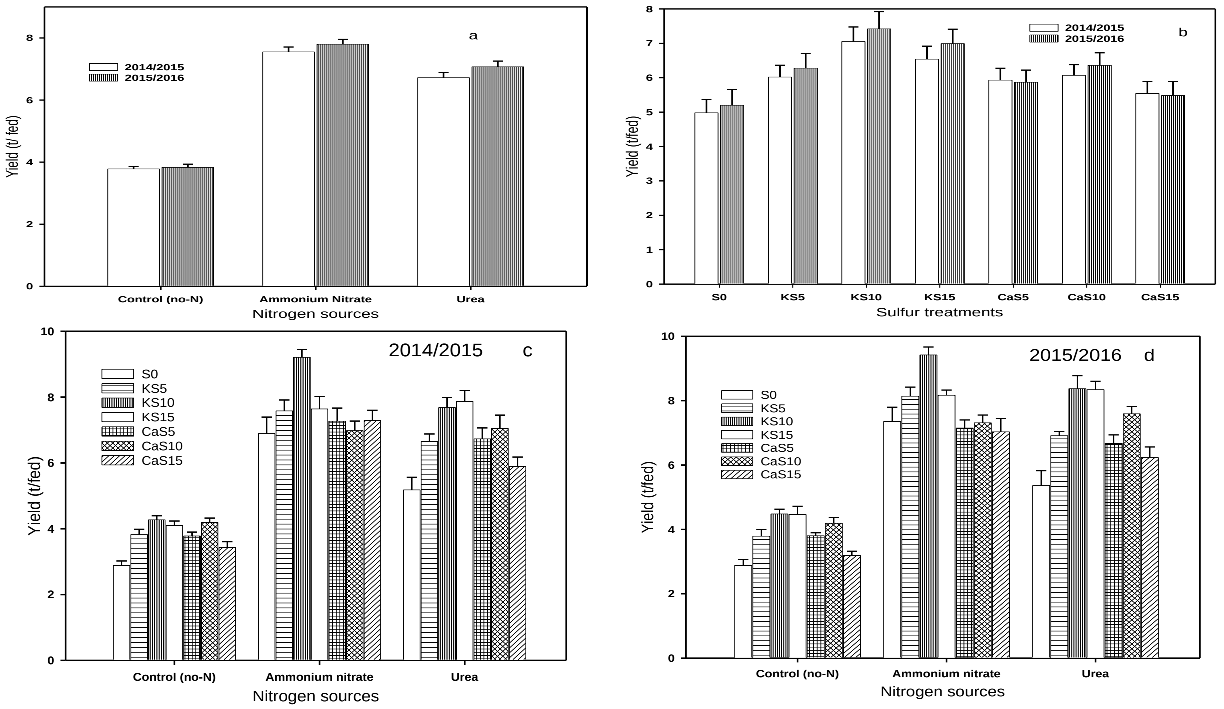

Figure 1. Main effects of nitrogen source (a), sulfur treatments (b) and their interactions (c&d) on yield of garlic "cv. Seds 40".

3. Nitrate content

The main effects of nitrogen sources and sulfur treatments as well as their interaction on nitrate content are presented in table (4). Ammonium nitrate provided the significant highest nitrate content followed by urea, however, non-fertilized plants with nitrogen had the lowest nitrate content over all sulfur treatments in both seasons. Un-sprayed plants with sulfur over all nitrogen sources had the significant highest nitrate content, while, the lowest nitrate content was obtained in sulfur sprayed plants with 10 mM as potassium sulphate (Table 4). With respect to the interaction between nitrogen sources and sulfur treatments, the highest values of nitrate content were found in ammonium nitrate-fertilized plants followed by plants received ammonium nitrate and sprayed with sulfur at concentration of 10 or 15 mM as calcium sulphate. However, non-fertilized plants that sprayed with sulfur at 10 mM as potassium sulphate gave the lowest nitrate content in both seasons (Table 4).

4. Mineral contents

Data regarding to the main effects of nitrogen source and sulfur treatments as well as their interaction effect on mineral contents (N, P, K, Ca and S) are presented in tables (3&4). The results indicated that the significant lowest nitrogen, phosphorus, potassium, calcium and sulfur were found in non-fertilized plants, however non-significant differences were detected between both nitrogen forms regarding all measured mineral contents overall sulfur treatments in both seasons. Regarding to the main effect of sulfur treatments overall nitrogen sources, the potassium sulphate at all concentrations calcium sulphate at 10 mM gave the significant highest nitrogen content in the bulb comparing to no-sulfur treatment. The foliar spray of potassium sulphate at 10 or 15 mM gave the significant highest potassium content in the bulbs. Concerning phosphorus content, potassium sulphate at 5 mM gave the significant highest value. Bulb sulfur content increased by increasing sulfur concentration from 0 to 10 mM as potassium sulphate, then decreased at 15 mM. Generally, foliar spray of calcium sulphate increased the bulb calcium content compared with other treatments.

Concerning the interaction effects, the highest nitrogen, phosphorus and sulfur contents were noticed in ammonium nitrate-fertilized plants that were sprayed with potassium sulphate at 5 or 10 mM. In addition, two different combinations (ammonium nitrate + 10 mM calcium sulphate and urea + 15 mM potassium sulphate) gave the same statistical values of nitrogen content as previous best combinations in both seasons. Sprayed plants with 15 mM as potassium sulphate and fertilized with both nitrogen forms gave the highest potassium content. While, only the combination of ammonium nitrate and 10 mM potassium sulphate gave the same statistical potassium values in both seasons. All combinations containing both nitrogen forms plus foliar spray with calcium sulphate at any concentration had the non-significant highest bulb calcium content in comparison with some cases using potassium sulphate in both seasons.

Table 3. Main effects of nitrogen source, sulfur treatments and their interactions on N, P and K content of garlic "cv. Seds 40".

Parameters	N mg/gDW			Mean	P mg/gDW			S treat. mean	K mg/gDW			Mean
N. Source	0 N	A nitrate	Urea		0 N	A nitrate	Urea		0 N	A nitrate	Urea	
S Treat						**2014/2015 season**						
S0	10.98j	22.13h	22.52gh	18.54c	3.54f	4.23bc	4.40ab	4.06bc	13.01h	14.26ef	14.28ef	13.85e
KS5	15.01i	28.68ab	27.23b-e	23.64a	4.19b-d	4.75a	4.44ab	4.46a	13.82f-h	15.38bc	15.80b	14.99b
KS10	14.21i	29.83a	27.53b-d	23.85a	3.80ef	4.42ab	4.26bc	4.16bc	14.07e-g	17.05a	15.63b	15.58a
KS15	14.45i	26.22d-f	28.15a-c	22.94a	4.27bc	4.23bc	4.22bc	4.24b	14.10ef	17.23a	16.68a	16.00a
CaS5	13.47i	24.42fg	24.74f	20.88a	3.50f	4.30bc	3.84d-f	3.88d	13.29gh	14.76c-e	15.10b-d	14.38cd
CaS10	14.78i	28.49a-c	26.69c-e	23.32a	4.08b-e	3.99c-e	4.27bc	4.11bc	13.23h	15.47bc	15.45bc	14.72bc
CaS15	13.12i	25.55ef	22.60gh	20.42a	3.80ef	4.17b-e	4.00c-e	3.99cd	13.12h	14.41d-f	15.10b-d	14.21de
Mean	13.72b	26.47a	25.64a		3.88b	4.30a	4.20a		13.52b	15.51a	15.43a	
						2015/2016 season						
S0	12.02k	23.87i	24.26hi	20.05c	3.82i	4.75b-e	4.79b-e	4.45c	12.75l	14.47g-i	14.62g-i	13.95d
KS5	16.00 j	30.55a-c	29.03b-d	25.19a	4.69c-f	5.43a	5.05bc	5.06a	13.99ij	16.09b-d	16.39bc	15.49b
KS10	15.30j	31.67a	29.37b-d	25.445a	4.31gh	5.07ab	4.90b-d	4.76b	14.25hi	17.73a	16.62b	16.20a
KS15	15.12j	29.07b-d	31.00ab	25.06a	4.69c-f	4.84b-e	4.89b-d	4.81b	14.28g-i	17.97a	17.34a	16.53a
CaS5	14.49j	26.22f-h	26.56e-g	22.42b	3.90i	4.85b-e	4.34f-h	4.37c	13.53jk	15.30ef	15.66de	14.83c
CaS10	15.59j	30.29a-c	28.51c-e	24.80a	4.56d-g	4.67d-g	4.81b-e	4.68b	13.11kl	14.89f-h	15.80c-e	14.60c
CaS15	14.09jk	27.32d-f	24.44g-i	21.95a	4.16hi	4.65d-g	4.50e-h	4.44c	12.94kl	14.26hi	14.92fg	14.04d
Mean	14.66b	28.43a	27.60a		4.30b	4.89a	4.76a		13.55b	15.81a	15.91a	

Values are the means of three 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. S0= no sulfur spray; KS= potassium sulphate; CaS= calcium sulphate.

Table 4. Main effects of nitrogen source, sulfur treatments and their interactions on Ca, S and nitrate content of garlic "cv. Seds 40".

Parameters	Ca mg/gDW			Mean	S mg/gDW			Mean	Nitrate mg/kg FW			Mean
N. Source	0 N	A nitrate	Urea		0 N	A nitrate	Urea		0 N	A nitrate	Urea	
S Treat						**2014/2015 season**						
S0	3.07d-f	3.40b-e	3.33c-e	3.27bc	4.81i	5.26gh	5.63fg	5.23d	291.88c-h	461.36a	403.58b	385.61a
KS5	2.90ef	3.93a-c	3.87a-c	3.57ab	5.18hi	6.61ab	6.25b-d	6.01b	263.62f-h	299.72c-g	292.19c-h	285.18cd
KS10	3.40b-e	3.40b-e	4.00ab	3.60ab	5.50f-h	7.00a	6.65ab	6.38a	246.62h	275.01e-h	276.93d-h	266.19d
KS15	3.60a-d	2.50f	2.80ef	2.97c	5.52f-h	6.35bc	6.51b	6.13b	253.71gh	319.99c-e	310.23c-f	294.64c
CaS5	3.60a-d	4.20a	4.0ab	3.93a	5.34gh	6.04c-e	6.44bc	5.94b	264.54f-h	328.72c	335.49c	309.58c
CaS10	3.60a-d	3.80a-c	3.87a-c	3.76a	5.36gh	6.43bc	6.48b	6.09b	273.34e-h	412.32ab	332.47c	339.38b
CaS15	3.11d-f	4.00ab	4.00ab	3.70a	5.14hi	5.79ef	5.89d-f	5.61c	276.73d-h	420.09ab	324.86cd	340.56b
Mean	3.32b	3.60a	3.70a		5.26b	6.21a	6.26a		267.21c	359.60a	325.11b	
						2015/2016 season						
S0	2.76i	3.40f-h	3.26f-i	3.14c	4.93l	5.94h-j	6.00h-j	5.63d	312.88f-h	500.36a	439.91b	417.72a
KS5	3.00g-i	4.06a-d	4.00b-e	3.69b	5.74jk	7.27b	6.92cd	6.64b	286.28hi	340.72c-f	331.52d-g	319.51cd
KS10	3.46e-h	3.53d-g	4.04a-e	3.67b	6.17f-h	7.68a	7.33b	7.06a	269.62i	312.68f-h	317.60e-h	299.96d
KS15	3.54d-g	2.92hi	3.34f-i	3.27c	6.07g-i	7.33b	7.36b	6.92a	278.04hi	357.65c-e	312.50f-i	316.06d
CaS5	3.73c-f	4.62a	4.54ab	4.30a	5.80ij	6.72de	7.10bc	6.54b	289.20g-i	367.72cd	371.49cd	342.80c
CaS10	3.72c-f	4.22a-c	4.41ab	4.12a	5.81ij	6.89cd	6.93cd	6.54b	299.67f-i	456.65b	360.80cd	372.38b
CaS15	3.24f-i	4.42ab	4.54ab	4.07a	5.49k	6.34fg	6.44ef	6.09c	302.07f-i	463.09ab	377.52c	380.89b
Mean	3.35b	3.88a	4.02a		5.72b	6.88a	6.87a		291.11c	399.84a	358.76b	

Values are the means of three 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. S0= no sulfur spray; KS= potassium sulphate; CaS= calcium sulphate.

Table 5. Correlation among characters of garlic "cv. Seds 40" treated with nitrogen sources and sulfur treatments as well as their interactions.

Characters	Chlorophyll	Carbohydrate	T. Phenols	Nitrate	N	P	K	Ca	S
Yield	0.69*	-0.28*	0.79*	0.34*	0.87*	0.49*	0.72*	0.30*	0.75*
Chlorophyll		-0.31*	0.63*	0.12	0.63*	0.41*	0.64*	0.17	0.66*
Carbohydrate			-0.16	-0.15	-0.30*	-0.03	0.29*	-0.05	-0.22*
T. Phenols				0.09	0.80*	0.56*	0.76*	0.25*	0.79*
Nitrate					0.41*	0.21*	0.08	0.25*	0.14
N						0.53*	0.78*	0.35*	0.88*
P							0.46*	0.19	0.62*
K								0.06	0.82*
Ca									0.35*

DISCUSSION

In many plant species such as onion, broccoli, spinach, parsley and cabbage, the plant growth, yield and quality were affected by the source of nitrogen (Inal and Tarakcioglu, 2001; Turan and Sevimli, 2005; Elwan and Abd El-Hamed, 2011; Elwan and Elhamahmy, 2015a&b). However, in garlic only a few investigations were examined the effects of nitrogen form on garlic yield and nitrate content (Nori *et al.*, 2012). In the present study, only non-sulfur containing nitrogen sources (ammonium nitrate and Urea) to avoid the disturbance sulfur nitrogen-containing source (ammonium sulphate) for our sulfur treatments were used. In general, the garlic plants fertilized with ammonium nitrate produced greater plant growth and yield than those received urea in the absence of sulfur treatments. The highest plant growth and yield for ammonium nitrate applied in both seasons may have been associated with better N use efficiency in intensive growth period of bulb. The NH_4^{+} and NO_3^{-} forms have equally effective for plants, but the relative advantage of both forms on plant growth also strongly depends on the external conditions (Marschner, 1995; Neeteson and Carton, 2001). Ammonium is less mobile in the soil than nitrate and offers the possibility to cover the nitrogen demand in one application. On the other hand, ammonia volatilization may contribute to poor N use efficiency. Nitrate is generally the favored source for plant growth, but the degree of favorite varies within plant species and other environmental factors (Barker and Mills, 1980). Regarding lower plant growth and yield for urea applied may have been attributed to urea toxicity and lower uptake rates of N (Marschner, 1995) and may be due to urea is not suitable for cultivation under the conditions of sandy soil. 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). 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). Similar results have been reported by Nori *et al.* (2012), Turan and Sevimli (2005), Khoshgoftarmanesh *et al.* (2011), Elwan and Abd El-Hamed, (2011) and Elwan and Elhamahmy (2015 a&b) in garlic, cabbage, lettuce, broccoli and leafy vegetables (spinach and parsley), respectively. Their results indicated that the higher plant growth and yield were associated with the application of ammonium nitrate or ammonium sulphate as N-sources comparing with urea. Also, Fertilization with ammonium nitrate resulted in an increase in chlorophyll content which was accompanied by increased rates of photosynthesis process (Toth *et al.*, 2002). Additionally, the nitrification of NH_4 releases the H that decrease the pH of soil and increase the elements uptake especially in alkaline soil (Liu *et al.*, 2014).

Sulfur is the most important nutrient in terms of garlic quality since it is highly involved in the bioactive compounds biosynthesis, firstly through its incorporation in the amino acid cysteine and finally with the formation of alliin (Martins *et al.*, 2016). The foliar spray of sulfur, either in the form of potassium sulphate, or calcium sulphate resulted in increasing the yield compared to no sulfur treatment, however, the potassium sulphate form was more efficient than calcium sulphate. A part from this efficiency of potassium sulphate due to 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 stomata which affect transpirational cooling and CO_2 uptake for photosynthesis (Malvi, 2010). Also, it's well reported that the higher valence of a cation, the lower its ability to move into the cells (Mengel, 2002). Therefore, the uptake of calcium (Ca^{2+}) by the epicuticular cells is lower than potassium (K^{+}).

No nitrogen treatment induced carbohydrates accumulation and decreased the chlorophyll, total phenols (Table 2). It is well reported that the expiration of photosynthetic genes is repressed by the accumulation of carbohydrates that accelerate the decrease in photosynthetic capacity under nitrogen deficiency (Araya *et al.*, 2010). Garlic plants that were fertilized with ammonium nitrate had the highest chlorophyll perhaps that nitrogen is a structural element of chlorophyll molecule, and thereby affects formation and accumulation of chlorophyll (Bojovic and Markovic, 2009).

As expected, the highest content of polyphenol compounds was found in plants sprayed with 10 mM potassium sulphate, while the lowest amount of phenol compounds was found in plants grown without sulfur treatment. Our indicated results are in agreements with the results of Imen *et al.* (2013) on rosy garlic who found a positive correlation between application of higher S concentration and higher containing of phenols. Enhanced phenol synthesis in bulbs by application of potassium sulphate at 10 mM might be due to higher nutrients content such as NPK and S (Table 3&4) that promote the growth. However, a negative relationship between S treatments and carbohydrates especially the favorable treatment (10 mM potassium sulphate) for yield and quality. This finding is supported by the results of Imen *et al.* (2013) who observed the same relationship between sulfur treatments and carbohydrates content in rosy garlic. Garlic plant growth such as plant height and leaves weight is restricted in non-sulfur treated plants. Under these conditions, only small amounts of carbohydrates are translocated from the leaves, and consequently carbohydrates accumulate in the leaves.

In spite of ammonium nitrate allowed the highest garlic yields, but it also increased particularly nitrate concentration. Our results were in accordance with the results of Elwan and Abd El Hamed (2011) who indicated that ammonium nitrate produced high yield with high nitrate content in broccoli. The higher nitrate content as well as yield may be due to the nitrate absorption by plant roots is available for translocation to the shoots, storage in vacuoles and assimilation into reduced N products (Huber *et al.*, 1996).

Our results proved that significant higher nitrate content connected with non-sulfur treated plants (Table 5). These findings supported by the results of Hu *et al.* (1991) and Dannehl *et al.* (1995). They reported that under S deficient conditions, the amount of amino acids and nitrate in leaves increases dramatically and protein degradation within chloroplasts was occurred. Also, sulfur deficiency results in limiting protein synthesis by reducing the amount of methionine and cysteine available to assembly new proteins (Sexton *et al.*, 1997; Blake-Kalff *et al.*, 1998). Sulfur treatment at 10 mM as Potassium sulphate lowered the accumulation of nitrate in bulbs (Table 5). Our results supported by the results of Lappartient and Touraine, (1996) who reported that the pathway of S nutrition is the reduction of SO_4^{-2} to cysteine in assimilatory sulphate reduction pathway, which tightly linked to assimilatory nitrate reduction (Kast *et al.*, 1995). Also, sulfur is an essential component of enzymes of nitrate reductase and nitrite reductase which reduce nitrate and nitrite in nitrogen assimilation. Its availability results in decreasing nitrate content (Campbell, 1999; Swamy *et al.*, 2005).

There is a positive relationship between S-fertilization and elements content such as nitrogen (Table 3, 4&5). S-fertilization increase the nitrogen content and enhance the protein synthesis where S is a constituent of the initiation amino acid, methionine and cysteine (Ahmad and Abdin, 2000). The combined effects of nitrogen fertilization and sulfur supply on garlic plant growth, yield, chlorophyll, phenols and mineral contents (especially N and S) are found to be synergistic. However, it was antagonistic in the case of carbohydrates content. The highest plant growth, yield, chlorophyll, phenols, mineral content such as N and S were achieved in ammonium nitrate-fertilized plants that were sprayed with 10 mM potassium sulphate. Further increase of potassium sulphate to 15 mM tended to decrease the previous mentioned parameters. Our results supported by Fismes *et al.* (2000) on oilseed who indicated that the interactions between N and S are synergistic at optimum rates and antagonistic at excessive levels of one of the both. Also, Both nitrogen and sulfur have a role in regulating ATP sulphurlase and nitrate reductase, the incorporation of the sulphide moiety into O-acetylserine during the cysteine synthesis appears to the meeting point between nitrogen and sulfur metabolism (Ahmad and Abdin, 2000). So, the increasing in nitrogen content with foliar spray of sulfur may be due to the metabolic coupling between sulfur and nitrogen assimilation.

CONCLUSION

The significant highest yield, chlorophyll and nitrate content were associated with ammonium nitrate than urea as nitrogen sources over all sulfur treatments. Fertilized-garlic plants with both sources gave the significant highest phenols content, NPK, Ca, S than un-fertilized plants, however the response of carbohydrates content was contradictory over all sulfur treatments. The foliar application of potassium sulphate at 10 mM, mostly gave the highest yield, chlorophyll, phenols, NPK and S and the lowest nitrate content than other sulfur treatments over all nitrogen sources, however the highest carbohydrates was found in un-sulfur treated plants. The superior combination treatment for yield, chlorophyll, phenols, NPK and S was ammonium nitrate and foliar spray with potassium sulphate at 10 mM. The highest nitrate content was measured in ammonium nitrate-fertilized plants without sulfur spraying, however, the highest carbohydrates was measured in control plants (without N and S nutrition). The overall findings indicate that S fertilization contributes to improved noticeable N-use efficiency for high yield, N and S content as well as low nitrate content.

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استجابة محصول و جودة أبصال الثوم لمصادر النيتروجين و الرش الورقي بمعاملات الكبريت

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أجريت تجربتين حقليتين خلال موسمى ٢٠١٥/٢٠١٤ و ٢٠١٦/٢٠١٥م بمزرعة التجارب كلية الزراعة جامعة قناة السويس بالإسماعيلية و ذلك لدراسة تأثير مصادر النيتروجين (نترات النشادرو اليوريا) و الرش بالكبريت بتركيزات (٠، ٥ ، ١٠ و ١٥ مليمول) من مصدرين للكبريت (سلفات البوتاسيوم و سلفات الكالسيوم) على محصول الثوم و محتواه من الكلوروفيل و الكربوهيدرات و الفينولات و النترات و العناصر المعدنية. أوضحت النتائج أن أعلى محتوى من النترات كان في النباتات المسمدة بنترات النشادر في غياب الرش بالكبريت بينما النباتات التي سمدت بنترات النشادر و رشت بالكبريت بمعدل ١٠ مليمول انخفض محتواها من النترات و أعطت أعلى قيم للنمو الخضري و المحصول و الكلوروفيل و الفينولات و العناصر المعدنية. استخدام سلفات البوتاسيوم كمصدر للكبريت أفضل من استخدام سلفات الكالسيوم لتحسين النمو الخضري و المحصول و صفات جودة أبصال الثوم مثل الفينولات الكلية و محتوى الكبريت. أظهرت النتائج أن النباتات المسمدة باليوريا أعطت أقل محصول أبصال و أقل محتوى من النترات مقارنة بالنباتات المسمدة بنترات النشادر و لكنها أعلى في محتواها من النترات مقارنة بالنباتات التي لم تسمد (معاملة المقارنة). وجد أعلى محتوى من الكربوهيدرات في أبصال النباتات التي لم تسمد بأى من مصدرى النيتروجين فى حين أنها احتوت على أقل محتوى من الكلورفيل و النيتروجين و الفينولات و الكبريت. أوضحت النتائج أن التسميد بالنيتروجين و الكبريت خلال النمو الخضري و إنتاج أبصال الثوم ترافقت بقوة. وجد أن هناك تفاعل تعاونى بين التسميد بالنيتروجين و الرش بالكبريت و ذلك باستخدام المصدر المناسب من النيتروجين (نترات النشادر) و المعدل المثالى من الكبريت (سلفات البوتاسيوم ١٠مليمول). في حين أن تأثير المستوى الأعلى من سلفات البوتاسيوم (١٥مليمول) يبدو أنه متضاد. أفادت النتائج المتحصل عليها أن الرش بالكبريت متطلب أساسي لتحسين كفاءة استخدام النيتروجين مما يؤدى إلى زيادة محصول الأبصال و محتواها من العناصر المعدنية خاصة الكبريت و النيتروجين حيث انهما من العناصر الهامة جداً في المركبات الكبريتية الحيوية في الثوم.