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Responses of sulfur, nitrogen and irrigation water on Zea mays growth and nutrients uptake

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### Abstract

Availability of both native and applied nutrients is one of the major constrains for plants growth in sandy calcareous soils. Elemental sulfur ( $S^0$ ) is often applied to acidify calcareous soil which increases the availability of nutrients in soils. The present study was sought to examine the effect of  $S^0$  combined with or without N under acidified (pH 6.5) and normal (pH >7.5) irrigation water on growth, apparent N-use efficiency (NUE) and uptake availability of nitrogen (N), phosphorus (P), sulfur (S), iron (Fe), zinc (Zn) and manganese (Mn) by corn plants grown in calcareous soils. Elemental S at rates of 0, 1, 5 and 10 t ha<sup>-1</sup> were tested combined with or without N at rates of 0 and 0.34 t ha<sup>-1</sup> in pots using normal and acidified irrigation water under Al Zaid and Al Semaih soils in evaporative cooled greenhouse conditions. Total dry matter (TDM) accumulation and nutrients uptake had positive relation while soil pH showed negative correlation with TDM and uptake availability of all nutrients. Addition of S<sup>0</sup> at the rate of 5 t ha<sup>-1</sup> combined with N fertilizer recorded improved NUE, superior TDM and maximum uptake of all nutrients under both types of irrigation water and soils. Collectively, the results indicate that S fertilization is required to improve NUE and thereby maintaining a sufficient availability of nutrients and growth of corn in sandy calcareous soil.

**Keywords:** Calcareous soil, Corn, Sulfur, Nitrogen, Irrigated water, Nutrients uptake **Abbreviations:** N-nitrogen, NUE-nitrogen use efficiency, P-phosphorus, S-sulfur, S<sup>0</sup>-elemental sulfur, Fe-iron, Zn-zinc, Mnmanganese, TDM-total dry matter, UAE-United Arab Emirates

## Introduction

Major agricultural soils in United Arab Emirates (UAE) are dominated by sandy calcareous type which is relatively low in organic matter content with high pH value that showed marked influence on the nutrients availability for plant growth (Abdou, 2006). Soil pH has an important role in the loss of N and or fixation of most nutrients and therefore different nutrient management practices are required for crop production in calcareous and non-calcareous soils. Calcareous soil has high CaCO<sub>3</sub> and alkaline pH that greatly reduce the solubility of Fe, Zn, Mn, and Cu thus characterizing as deficient in these micro nutrients. The presence of CaCO3 in soils also directly or indirectly affects the availability of N, P, Mg, and K (Brady and Weil, 2002). Additions of  $S^0$  are used as a nutrient and acidifier which can alter physicochemical properties of soil (Neilson et al., 1993). The biochemical oxidation of  $S^0$  produces  $H_2SO_4$ which decreases soil pH and solubilizes CaCO3 in alkaline calcareous soils to make soil conditions more favorable for plants growth including the availability of plant nutrients (Lindemann et al., 1991; Abdou, 2006; El-Tarabily et al., 2006). Nitrogen, P and K are frequently the most limiting

nutrients for plant growth in numerous ecosystems (Olivera et al., 2004). Intensive cropping systems requires important amounts of N, P, K and S fertilizers and among these N fertilizer plays significant role. Crop deficiencies of S have been reported with increasing frequency in the last decade, caused by decreasing anthropogenic S input and by the lack of input through S fertilization to compensate for exportation (Scherer, 2001). Sulfur is accumulated in plants in low concentrations compared to N, but is an essential element as a constituent of proteins, Cysteine-containing peptides such as glutathione, or numerous secondary metabolites (Scherer et al., 2008; Abdallah et al., 2010). Sulfur deficiency can reduce NUE and that N deficiency can also reduce S-use efficiency (Fismes et al., 2000). Nitrogen and S both involved in protein synthesis and play an important role in the protection of plants against nutrient stress and pests (Luit et al., 1999) and synthesis of vitamins and chlorophyll in the cell (Kacar and Katkat, 2007). The severity of S deficiency is aggravated by higher rates of N application. Plants grown without N fertilizer showed no apparent S stress, whereas plant receiving N fertilizer,

particularly at higher rate without S, showed symptoms suggesting severe physiological disorder in N nutrition (Janzen and Bettany, 1987; Kopriva and Rennenberg, 2004). Increased application of N fertilizer increasing S response resulting its N/S ratio leading to a reduction of protein-N and an increase in nitrate-N and other non-protein N fractions and crop quality may adversely affected (Jackson, 2000). Seed yield decreased due to insufficient supply of S nutrition while an excessive supply of S can affect quality of meal by increasing glucosinolates content in seed (Rosa and Rodrigues, 1998). The poor efficiency of N caused by insufficient S needed to convert N into biomass may increase N losses from cultivated soils (Schnug et al., 1993; MacGrath and Zhao, 1996). Conversely, N addition increased seed yield in S-rich conditions, and maximum yield responses to both N and S applications are obtained when the amounts of available N and S are balanced (Joshi et al., 1998). Corn as an oilseed crop is highly responsive to S; making corn an ideal crop for S application in the forms of S<sup>0</sup> and ammonium sulfate or urea, especially in alkaline and calcareous soils (Ghosh et al., 2000). Nutrients availability and uptake ability in calcareous soil can be enhanced by acidification which has large cumulative effects on the overall N balance and amount of soil N reserves (Cassman et al., 2002). Sulfur uptake efficiency is increased and deficiency symptom is disappeared by the application of N fertilizer in the form of urea in S deficient soil (Murphy, 1999). The interaction of nutrients is of great importance because decline in S supply from the atmosphere has already caused substantial losses of N from agro-ecosystems to the environment (Luit et al., 1999). Therefore, a strong focus on reducing N leads to arid environments and the interaction between N and S metabolism needs more clarification with a view to improve environment friendly fertilizing techniques. Based on these observations, sufficient supply of S is required to maintain the optimum growth and nutrient uptake ability of plants. For this purpose, the use of S<sup>0</sup> fertilizer is gaining importance, because besides the inhibitory actions on N, it contains high S concentration. Substantial information on N and S nutrition of plant is available (Fismes et al., 2000) but the data related to both N and S interaction with irrigation water either acidified or normal are still insufficient, especially for corn cultivation in sandy calcareous soils of UAE. Accounting for the above observations, this research was undertaken to investigate the impact of increasing levels of S<sup>0</sup> fertilization on N and S utilization, growth and nutrient uptake ability of corn grown in sandy calcareous soil with acidified and normal irrigation water.

#### Materials and methods

#### **Experimental** design

Greenhouse experiments were conducted at Al-Foah Agricultural Experiment Station  $(27^0N \text{ and } 22^0S \text{ latitude and } 51^0W \text{ and } 57^0E \text{ longitude})$ , UAE University in 2005. Elemental S at rates of 0, 1, 5 and 10 t ha<sup>-1</sup> were tested combined with or without N at rates of 0 and 0.34 t ha<sup>-1</sup> in pots under evaporative cooled greenhouse conditions. The treatment arrangements are as follows: S 0+N 0 (control), S 0+N 0.34 t ha<sup>-1</sup>, S 1 t ha<sup>-1</sup>+N 0, S 1 t ha<sup>-1</sup>+N 0.34 t ha<sup>-1</sup>, S 5 t ha<sup>-1</sup>+N 0, S 5 t ha<sup>-1</sup>+N 0.34 t ha<sup>-1</sup>,

 Table 1. Physicochemical properties of Al Zaid and Al Semaih

 soils

SOIIS		
Soil properties	Al Zaid soils	AL Semaih soils
EC (d Sm <sup>-1</sup> )	3.36	18.27
pН	9.08	9.01
Total CaCO <sub>3</sub> %	38.98	68.17
Active CaCO <sub>3</sub> %	3.50	12.50
O.C. %	0.17	0.14
Texture:		
Sand %	95.00	99.73
Silt + clay %	5.00	0.27
Soluble cations (meq L <sup>-</sup>		
<sup>1</sup> ):		
Ca	1.60	28.60
Mg	1.40	12.60
Na	28.70	171.10
К	0.34	2.86
Soluble anions (meq $L^{-1}$ ):		
Cl	33.00	169.00
$SO_4$	3.40	25.18
HCO <sub>3</sub>	2.90	1.40
CO <sub>3</sub>	1.00	0.00

S 10 t ha<sup>-1</sup>+N 0, S 10 t ha<sup>-1</sup>+N 0.34 t ha<sup>-1</sup>. The experiment was laid out in a factorial completely randomized design with three replications. With same set of treatments, four experiments were carried out simultaneously using each with normal (pH >7.5) and acidified irrigated water (pH 6.5), respectively in Al Zaid and Al Semaih soils. Sandy calcareous soil was collected from the areas of Al Zaid and Al Semaih in Abu Dhabi, UAE. Based on the name of soil collection sites, tested soils were designated as Al Zaid and Al Semaih soils. A proportion of soil was separated and sieved through 1-mm stainless steel sieve and stored in plastic bags for physicochemical analysis. Soil pH was determined from the prepared soil suspension (1:2.5 soil water ratios) by using combined pH meter model 900A (Thermo Orion, Ontario, Canada) (Thomas, 1996). Electrical conductivity (EC) was measured by the saturation extracts of soil samples using Orion model 120 microprocessor conductivity meters (Thermo Scientific, USA). Water soluble cations (Ca, Mg, Na, and K) and anions (Cl, HCO<sub>3</sub>, CO<sub>3</sub> and SO<sub>4</sub>) were determined as per the methods recommended in Page et al., (1982). Physicochemical properties of the soil are presented in Table 1. After harvesting of corn plants, soil samples were also collected from each pot to determine Na, K, Cl, EC and pH. The analytical results are presented in Tables 2 and 3.

### Management practices

Soils were air-dried before being used in the experimental pots. Free-draining polyethylene pots (height 25cm x diameter 23 cm) were filled with 5.0 kg of sandy calcareous soil. Each pot (25cm x 23 cm) was initially filled with 3.8 kg of soil. Prior to sowing each plot received extra 1.2 kg of soil mixed with P and K at the rates of 3.3 and 1.1 g pot<sup>-1</sup> in the forms of single superphosphate and potassium sulfate, respectively. Elemental S powder (particle size < 150  $\mu$ m) was collected from TAKREER Company, Abu Dhabi, UAE and applied as per treatment schedule. According to treatment schedule N was applied at rates of 1.49 g per pot (~ 0.34 t ha-1) at 10 and 17 days after

Treatment	Na (cn	nol L <sup>-1</sup> )	K (cn	nol L <sup>-1</sup> )	Cl (cm	ol L <sup>-1</sup> )
	NIW*	AIW*	NIW	AIW	NIW	AIW
Al Zaid soil <u>:</u>						
S0N0	30.85	35.99	0.69	1.99	126.0	43.33
S0N1	29.31	26.35	1.05	2.02	86.00	37.00
S1N0	24.02	27.14	1.62	1.95	76.00	35.00
S1N1	28.57	31.72	1.74	2.62	72.00	31.00
S2N0	22.54	26.37	1.88	2.26	66.00	29.33
S2N1	23.97	31.13	2.05	2.48	56.00	29.00
S3N0	18.99	25.07	1.91	2.34	70.00	27.00
S3N1	26.51	29.72	2.40	3.03	69.00	22.00
LSD (0.05)	3.65	3.65	0.40	0.40	12.5	8.25
Al Semaih soil:						
S0N0	132.93	95.87	6.00	5.29	368.00	122.00
S0N1	134.97	102.53	6.39	5.80	363.00	119.00
S1N0	146.23	90.10	5.42	4.44	456.00	116.33
S1N1	113.77	112.20	5.60	5.24	333.00	107.00
S2N0	84.67	101.53	4.98	5.20	291.00	116.33
S2N1	106.83	78.27	6.35	5.19	341.00	101.6
S3N0	92.30	104.30	5.18	4.88	425.00	100.6
S3N1	140.07	86.40	6.54	5.56	328.00	80.33
LSD (0.05)	8.50	8.85	0.35	1.61	25.70	21.50

Table 2. Na, K and Cl content in soil as affected by S<sup>0</sup>, N and irrigation water after harvesting of corn plants

\*NIW- normal irrigation water, \*AIW-acidified irrigation water

**Table 3.** EC and pH of soil as affected by S<sup>0</sup>, N fertilizer and irrigation water after harvesting of corn plants

Treatment	EC (	dSm <sup>-1</sup> )	1	ьH
	NIW*	AIW*	NIW	AIW
Al Zaid soil:				
S0N0	3.87	5.53	8.35	8.24
S0N1	4.72	5.65	7.93	8.11
S1N0	4.74	5.66	7.95	8.10
S1N1	4.81	5.92	7.76	8.01
S2N0	4.90	5.80	7.84	7.97
S2N1	5.86	6.95	7.64	7.92
S3N0	5.76	6.11	7.66	7.90
S3N1	6.31	7.20	7.56	7.88
LSD (0.05)	0.42	0.25	0.14	0.12
Al Semaih soil:				
S0N0	17.59	15.75	8.15	8.08
S0N1	17.31	15.17	8.12	8.05
S1N0	16.17	14.61	7.96	7.91
S1N1	14.60	13.62	7.96	7.84
S2N0	14.36	13.07	7.75	7.80
S2N1	13.30	13.06	7.74	7.72
S3N0	13.33	12.95	7.71	7.70
S3N1	12.21	10.66	7.60	7.68
LSD (0.05)	1.08	0.99	0.15	0.12

\*NIW- normal irrigation water, \*AIW-acidified irrigation water

germination on the soil surface and irrigated by normal and acidified water, respectively. Ten corn seeds [cv. Merit (Asgrow vegetable seeds, CA, USA)] were sown per pot at a depth of 5 mm into the soil. The pots were saturated with normal (pH >7.5) and acidified irrigation water (pH 6.5) up to field capacity for proper germination and growth of corn plants. After emergence all seedlings were kept until final harvest.

## Plant analysis

Corn plants were selected at random and harvested after 35 days of germination for nutrient analysis and TDM

accumulation. Roots and shoots were washed in deionized water and oven dried at 72°C for 48 hours and grounded to powder in a ball mill. The plant samples were then digested by the dry ashing method (Jones and Case, 1990) for the determination of total content of micronutrients (Fe, Mn and Zn) using atomic absorption spectrophotometer (Varian, model SpectrAA 220 FS). Sulfur content was measured using ICP-AES, Varain model Vista MPX. Phosphorus was determined colorimetrically according to the method described by Kuo (1996). The N concentration was measured by automatic distillation (FOSS, 2200 Kjeltic Auto Distillation) followed by acid titration (Munsinger and McKinney, 1982). The apparent

Treatment	N (mg g <sup>-1</sup> )		P (m	g g <sup>-1</sup> )	S (1	ng g <sup>-1</sup> )
	NIW*	AIW*	NIW	AIW	NIW	AIW
Al Zaid soil:						
S0N0	5.65	6.68	5.70	8.42	4.75	6.71
S0N1	28.22	29.94	7.99	8.68	6.39	7.79
S1N0	7.03	6.98	9.38	8.37	8.21	8.98
S1N1	32.6	29.95	10.83	9.99	10.83	9.84
S2N0	7.22	7.45	9.56	9.51	9.09	9.65
S2N1	34.19	31.12	14.67	11.68	12.71	12.20
S3N0	6.39	7.01	10.58	9.20	9.91	10.20
S3N1	26.44	30.10	14.12	11.71	13.85	13.71
LSD (0.05)	0.75	0.68	0.51	0.55	0.52	0.40
Al Semaih soil:						
S0N0	5.40	7.05	6.29	6.71	5.38	6.5
S0N1	28.15	29.41	6.51	8.29	7.14	7.43
S1N0	6.71	7.11	11.73	8.39	10.53	7.71
S1N1	28.15	28.19	12.21	9.64	11.56	9.64
S2N0	7.14	7.32	12.72	9.01	12.42	10.00
S2N1	32.47	30.66	13.3	11.2	13.20	11.05
S3N0	6.42	7.20	12.93	8.97	13.07	10.56
S3N1	26.47	28.88	13.50	11.55	13.61	13.38
LSD (0.05)	0.50	0.50	0.30	0.40	0.35	0.60

Table 4. N, P and S uptake by corn plant as affected by S<sup>0</sup> and N fertilizer and irrigation water

\*NIW- normal irrigation water, \*AIW-acidified irrigation water

N-use efficiency (ANU) of corn plant was estimated to examine the effect of S supply on N uptake by using the following equation (Fismes et al., 2000). ANU = [(N uptake from fertilized plots-N uptake from controls)/N fertilizer applied].

### Statistical analysis

Statistical analysis was carried out by one-way ANOVA using general linear model to evaluate significant differences between means at 95% level of confidence (SAS, 2003). Further statistical validity of the differences among treatment means was estimated using the least significant differences (LSD) comparison method. MS Excel was used for regression analysis and graphical presentations.

### Results

## Soil properties

Soil properties changed significantly by addition of  $S^0$  and N under both types of irrigation water at both soils. In Al Zaid soil, Na contents reduced significantly by additions of  $S^0$  and N fertilizer under both types of irrigated water (Table 2). In Al Zaid soil, the average reduction of Na content was more pronounced in those soils irrigated with normal water. Na content was also reduced significantly in Al Semaih soil as a result of  $S^0$  addition. The higher reduction rate of Na content was obtained by addition of  $S^0$  at rates of 5 and 10 t ha<sup>-1</sup> in absence of N fertilizer under normal irrigation water while Na content reduced more by addition of  $S^0$  at the rate of 5 t ha<sup>-1</sup> combined with N fertilizer under acidified irrigation water. In Al Semaih soil, the average reduction rate of Na content was higher in the soils irrigated by acidified irrigation water than

normal water. Potassium enrichment was observed in both Al Zaid and Al Semaih soils due to the addition of  $S^0$  and N irrespective of the pH of irrigation water (Table 2). The enrichment of K in soil was directly related with the addition of higher levels of S<sup>0</sup> (5 or 10 t ha<sup>-1</sup>) and N fertilizer in both soils. Chloride (Cl) content increased significantly by the addition of S<sup>0</sup> and N in both the soils irrigated by normal water and decreased when irrigated by acidified water (Table 2). Soil pH and EC changed significantly by addition of  $S^0$ , N and their interaction. In Al Zaid soil, initial EC was 3.36 d Sm<sup>-1</sup> (Table 1) and rose significantly to 4.72-6.31 d Sm<sup>-1</sup> by addition of S<sup>0</sup> and N fertilizer under both types of irrigation water (Table 3). By application of normal irrigation water, maximum EC was obtained by addition of S<sup>0</sup> at the rate of 5 and 10 t ha<sup>-1</sup> combined with N and S<sup>0</sup> at the rate of 10 t ha<sup>-1</sup> without N. On the contrary, maximum EC was recorded by application of S<sup>0</sup> at rates of 5 and 10 t ha<sup>-1</sup> plus N under acidified irrigation water. The lowest EC was recorded from control treatment under normal irrigation water but when corn plant was irrigated by acidified water minimum EC was recorded by addition of S<sup>0</sup> at the rate of 1 t ha<sup>-1</sup> and zero S<sup>0</sup> with or without N. In Al Semaih soil, EC changed significantly by addition of S<sup>0</sup> at rates of 5 and 10 t ha<sup>-1</sup> combined with or without N (Table 3). In Al Zaid soil, pH decreased significantly by addition of  $S^0$  at the rate of 10 t ha combined with or without N followed by  $S^0$  at the rate of 5 t ha<sup>-1</sup> plus N under normal irrigation water while soil pH reduced substantially by addition of by addition of S<sup>0</sup> at rates of 5 and 10 t ha<sup>-1</sup> combined with or without N under acidified irrigation water. In Al Semaih soil, pH was reduced significantly by addition of S<sup>0</sup> and N regardless of levels under acidified and normal irrigation water. In case of acidified irrigation water, soil pH decreased in the absence of S<sup>0</sup> while under normal irrigation water soil pH decreased with the presence of  $S^0$  and N fertilizer in both soils (Table 3).

Treatment	Fe (n	ng g <sup>-1</sup> )	Zn (n	ng g <sup>-1</sup> )	Mn	$(mg g^{-1})$
	NIW*	AIW*	NIW	AIW	NIW	AIW
Al Zaid soil:						
S0N0	0.01	0.02	0.06	0.05	0.06	0.06
S0N1	0.86	1.12	0.08	0.09	0.66	0.90
S1N0	0.01	0.02	0.08	0.07	0.10	0.13
S1N1	1.14	1.12	0.10	0.07	1.31	1.08
S2N0	0.02	0.02	0.08	0.05	0.11	0.10
S2N1	1.63	1.86	0.12	0.10	1.43	1.41
S3N0	0.03	0.01	0.07	0.05	0.14	0.08
S3N1	1.56	1.43	0.10	0.08	1.01	1.09
LSD (0.05)	0.05	0.04	0.005	0.01	0.03	0.05
I Semaih soil:						
S0N0	0.04	0.03	0.04	0.03	0.07	0.07
S0N1	0.97	0.43	0.05	0.06	0.75	0.82
S1N0	0.04	0.03	0.03	0.04	0.06	0.20
S1N1	0.84	0.77	0.05	0.06	1.56	1.22
S2N0	0.04	0.03	0.03	0.05	0.20	0.14
S2N1	1.31	1.20	0.06	0.08	1.79	1.60
S3N0	0.03	0.03	0.03	0.06	0.12	0.15
S3N1	1.06	1.16	0.12	0.07	0.96	1.00
LSD (0.05)	0.02	0.04	0.004	0.01	0.04	0.04

Table 5. Fe, Zn and Mn uptake by corn plants as affected by  $S^0$  and N fertilizer and irrigation water

\*NIW- normal irrigation water, \*AIW-acidified irrigation water

Table 6. Interrelationships among TDM accumulation, EC, soil pH and nutrients uptake under irrigation water in Al Zaid soils. Normal irrigation water:

	EC	pН	Ν	Р	S	Fe	Zn	Mn
TDM	0.62*	-0.63*	0.84**	0.84**	0.82**	0.87**	0.96**	0.84**
EC		-0.91**	0.91**	0.87**	0.59*	0.38	0.26	0.15
pН			-0.56*	-0.77*	-0.76*	-0.42	-0.02	-0.31
N				0.59*	0.57*	0.94**	0.83**	0.96**
Р					0.99*	0.75*	0.87**	0.72*
S						0.72*	0.87**	0.72*
Fe							0.88**	0.95**
Zn								0.91**

\* Significant at 0.05 level of probability; \*\* Significant at 0.01 level of probability

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Acidified	irrigatioi	i water:

	EC	pН	Ν	Р	S	Fe	Zn	Mn
TDM	0.65*	-0.50*	0.82**	0.65*	0.57*	0.88**	0.67*	0.91**
EC		-0.47	0.57*	0.61*	0.63*	0.46	-0.18	0.59*
pН			-0.72*	-0.79*	-0.69*	-0.57*	-0.13	-0.62*
N				0.60*	0.50*	0.94**	0.80**	0.96**
Р					0.92**	0.76*	0.48	0.60**
S						0.62*	0.48	0.60**
Fe							0.89**	0.96**
Zn								0.87**

\* Significant at 0.05 level of probability; \*\* Significant at 0.01 level of probability

# Apparent N-use efficiency, macronutrients uptake and TDM accumulation

In Al Zaid soil, ANU was 9-16% and 7-12% when corn plant was irrigated by normal and acidified water, respectively. The highest and lowest ANU was achieved by addition of  $S^0$  at the rate of 5 t ha<sup>-1</sup> with N and zero  $S^0$  with N fertilizer, respectively under normal irrigation water. In case of acidified irrigation water, maximum ANU was obtained from  $S^0$  at rates of 1 and 5 t ha<sup>-1</sup> combined with N while the lowest ANU was recorded from zero  $S^0$  with N fertilizer. The ANU of Al Semaih soils was estimated very low (1.3 – 3.5%) irrespective of the pH of irrigation water. However, plants received acidified irrigation water showed comparatively higher ANU values than those received normal irrigation water regardless of fertilizer levels in Al Semaih soil (Fig. 1). Total dry matter accumulation was affected significantly by application of S<sup>0</sup>, N and their interaction (Fig. 2). The highest and lowest TDM accumulation was recorded by addition of S<sup>0</sup> at the rate of 5 t ha<sup>-1</sup> with N and control treatment, respectively under both types of irrigation and soils. In Al Zaid soil, appreciably higher TDM accumulation was obtained under normal irrigation water while plant irrigated by acidified water accumulated greater TDM in Al Semaih

**Table 7.** Interrelationships among TDM, EC, soil pH and nutrients uptake under irrigation water in Al Semaih soils. Normal irrigation water:

	EC	pН	Ν	Р	S	Fe	Zn	Mn
TDM	0.49	-0.56*	0.87**	0.92**	0.91**	0.95**	0.51*	0.62*
EC		-0.96*	0.60*	0.35	0.39	0.39	0.66*	0.54*
pН			-0.72*	-0.87**	-0.79*	-0.58*	-0.04	-0.73*
N				0.69*	0.63*	0.96**	0.15	0.92**
Р					0.99**	0.80**	0.14	0.36
S						0.77*	0.63*	0.36
Fe							0.43	0.78*
Zn								0.17

\* Significant at 0.05 level of probability; \*\* Significant at 0.01 level of probability

Acidified irrigation water:

	EC	pН	Ν	Р	S	Fe	Zn	Mn
TDM	0.54*	-0.77*	0.90**	0.96**	0.93**	0.80**	0.68*	0.78*
EC		-0.58*	0.67*	0.56*	0.65*	0.54*	-0.30	0.63*
pН			-0.84**	-0.85**	-0.89**	-0.70**	-0.23	-0.81**
N				0.87**	0.86**	0.92**	0.67*	0.95**
Р					0.97**	0.70*	0.59*	0.76*
S						0.69*	0.59*	0.77*
Fe							0.81**	0.96**
Zn								0.77*

\* Significant at 0.05 level of probability; \*\* Significant at 0.01 level of probability

**Table 8.** Regression equation and coefficients of determination ( $R^2$ ) for relationship between TDM accumulation,  $S^0$  and N uptake under normal and acidified irrigation water in Al Zaid and Al Semaih soils

	Regression equation	$R^2$
Al Zaid soil:		
Normal water	$y = -21.605 + 0.0403x_1$	0.7935
Normal water	$y = -0.3279 + 0.0099x_2$	0.6992
Acidified water	$y = -31.399 + 0.0513x_1$	0.8887
Acidified water	$y = -0.8403 + 0.0093x_2$	0.8623
Al Semaih soil:	-	
Normal water	$y = -4.9048 + 0.045x_1$	0.7485
Normal water	$y = -0.9475 + 0.0159x_2$	0.8224
Acidified water	$y = -5.101 + 0.0411x_1$	0.8018
Acidified water	$y = -0.2247 + 0.0109x_2$	0.8671

 $y = TDM, x_1 = N, x_2 = S^0$ 

soil. Total dry matter accumulation was appreciably higher in Al Zaid soil than Al Semaih soil. Nutrients uptake by corn plant was influenced significantly by addition of S<sup>0</sup>, N and their interaction under both types of irrigation water and soils. The highest N uptake was recorded in the plants receiving S<sup>0</sup> at the rate of 5 t  $ha^{-1}$  plus N under both types of irrigation water and soils (Table 4). Intermediate N uptake was recorded from S<sup>0</sup> at the rate of 10 and 1 t ha<sup>-1</sup> plus N by use of normal and acidified irrigation water, respectively for both soils. The lowest N uptake was obtained from control treatment where neither S<sup>0</sup> nor N was applied regardless of irrigation water types. In Al Zaid soil, the highest and lowest uptake of P was obtained from S<sup>0</sup> at the rate of 5 t ha<sup>-1</sup> plus N fertilizer and control treatment, respectively under both types of irrigation water. In Al Semaih soil, maximum P uptake was obtained from S<sup>0</sup> at the rate of 10 t ha<sup>-1</sup> plus N followed by 5 t ha<sup>-1</sup> combined with N under acidified irrigation water while the highest uptake of P was obtained from  $\overline{S}^0$  at the rate of 10 t ha plus N fertilizer under normal irrigation water. The lowest P uptake was obtained from control treatment (Table 4). In Al Zaid soil, both N and P uptake was higher only under normal

irrigation water while in Al Semaih soil uptake was more in plants irrigated with acidified water. The highest and lowest uptake of S was recorded from  $S^0$  at the rate of 10 t ha<sup>-1</sup> plus N and control treatment, respectively under both types of irrigation water and soils. Intermediate S uptake was obtained from  $S^0$  at the rate of 5 t ha<sup>-1</sup> plus N when grown under both type of water and soils (Table 4). In Al Zaid soil, normal irrigation water performed better in favor of S uptake while in Al Semaih soil no appreciable difference was observed under normal irrigation water by addition of  $S^0$  at rates of 5 and 10 t ha<sup>-1</sup> combined with N fertilizer.

#### Micronutrients uptake

In this study, we focused only on three micronutrients such as Fe, Mn and Zn. The uptakes of these micronutrients were affected significantly by addition of  $S^0$ , N fertilizer under both types of irrigation water and soils. In Al Zaid soil, significantly higher Fe, Zn and Mn uptake was recorded from  $S^0$  at the rate of 5 t ha<sup>-1</sup> combined with N under both types of irrigation water. In Al Semaih soil, maximum Fe uptake was obtained

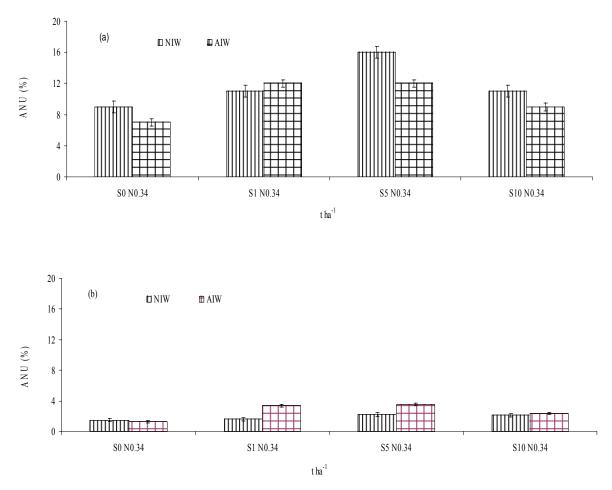


Fig. 1. Apparent nitrogen use efficiency of corn plant as affected by elemental S, N and irrigation water (a) Al Zaid soil (b) Al Semaih soil Error bars denoted SE±

from S<sup>0</sup> at the rate of 5 and 10 t ha<sup>-1</sup> plus N fertilizer when plant was irrigated by normal and acidified water, respectively (Table 5). The lowest uptake of Fe, Zn and Mn was recorded in the plants grown with zero S and N in both soils regardless of irrigation water types. The Fe uptake was significantly higher in Al Zaid soil, than that in Al Semaih soil. Acidified irrigation water contributed more towards Fe uptake than normal irrigation water regardless of  $S^0$  and N fertilizer levels. Addition of S<sup>0</sup> at rates of 1 and 10 t ha<sup>-1</sup> plus N recorded intermediate and identical Zn uptake under both types of irrigation water (Table 5). In Al Semaih soil, the highest Zn uptake was obtained from  $S^0$  at the rate of 10 t ha<sup>-1</sup> plus N under both types of irrigation water. In Al Zaid soil, Zn uptake was favored by normal irrigation water while no significant variation was observed between normal and acidified irrigation water in Al Semaih soil. Intermediate uptake of Mn was recorded by S<sup>0</sup> at the rate of 1 t ha<sup>-1</sup> under both types of irrigation water and soils (Table 5). In Al Zaid soil, Mn uptake was significantly higher than Al Semaih soil. Both types of irrigated water and soils acted equally in favor of Mn uptake.

## Regression analysis of nutrient uptake, soil pH, EC and biomass accumulation

In Al Zaid soil, TDM accumulation showed strong positive correlations with N ( $r^2 = 0.82$  to 0.84, P<0.01), Fe ( $r^2 = 0.87$  to 0.88, P<0.01) and Mn ( $r^2$ = 0.84 to 0.91, P<0.01) under both types of irrigation water (Table 6). A strong positive correlation was also found between TDM accumulation with P ( $r^2 = 0.84$ , P < 0.01) and S ( $r^2 = 0.82$ , P < 0.01) under normal irrigation water while a week positive relationship was observed under acidified irrigation water. In Al Semaih soil, TDM accumulation showed strong positive correlations with N ( $r^2 = 0.87$  to 0.90, P<0.01), P  $(r^2 = 0.92 \text{ to } 0.96, P < 0.01)$ , S  $(r^2 = 0.91 \text{ to } 0.93, P < 0.01)$ , and Fe  $(r^2 = 0.80 \text{ to } 95, P < 0.01)$  under normal irrigation water (Table 7). A positive correlation was observed between TDM accumulation with Zn ( $r^2 = 0.51$  to 0.68, P<0.05) and Mn  $(r^2=0.62 \text{ to } 0.78, P<0.05)$  under both types of irrigation water and soils. In both soils, EC showed a strong negative correlation with pH ( $r^2$ = -0.91 to -0.96, P<0.01) under normal irrigation water while negative and week correlation was observed ( $r^2$ = -0.47 to -0.58, P<0.05) under acidified irrigation

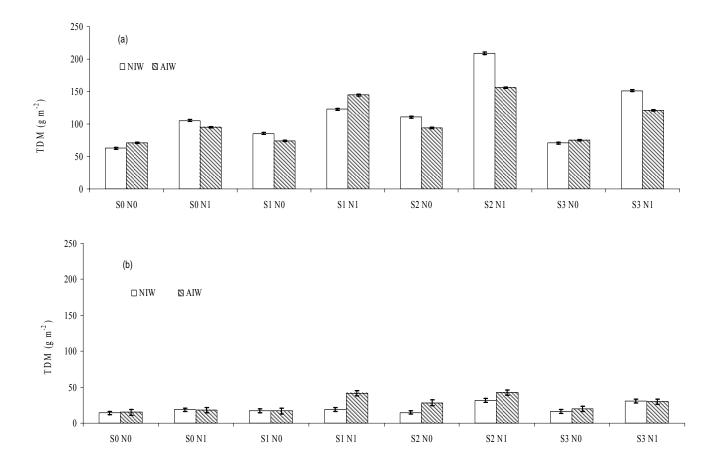


Fig. 2. Total dry matter accumulation of corn plant as affected by elemental S, N fertilizer and irrigation water (a) Al Zaid soil, (b) Al Semaih soil Error bars denoted LSD value at 0.05 level

water in both soils. Soil pH showed negative correlations among nutrients and EC under both types of irrigation water and soils (Tables 6 and 7). The relationship of EC with Zn was negative ( $r^2$ = -0.18 to -0.66, P<0.05) under both irrigation water and soils. The interrelationship among the nutrients was positive in both the soils. In Al Zaid soil, a highly significant positive linear relationship was observed among TDM accumulation and nutrients uptake of N, P, S, Fe, Zn and Mn under normal irrigation water (Table 6). On the contrary a highly significant positive linear relationship was observed among TDM accumulation and nutrients uptake of N, P, S and Fe under acidified irrigation water in Al Semaih soil (Table 7). In both soils, polynomial equation of 3rd order showed best fitted curve ( $R^2$ = 0.81 to 0.98, P<0.01) under normal irrigation water. In case of acidified irrigation water, polynomial equation of 3rd order also showed best fitted curve ( $R^2 = 0.65$  to 0.77, P<0.05) but relationship was week (Fig. 3). The slopes of the equation indicated a TDM yield of 40-51 g for every 1.0 g of N uptake and 9-10 g biomass yield for every 1.0 g of S uptake for both types of irrigation water and soils (Table 8). Regression

analysis of TDM accumulation clearly indicated higher rate of  $S^0$  application is required for corn growth in sandy calcareous soils of UAE.

#### Discussion

#### Soil properties

The reduction rate of EC was distinct compared with initial soil EC. This was caused by a combined effect of acidified irrigation water, addition of higher levels of by addition of  $S^0$  and N fertilizer. Even only application of  $S^0$  or N under acidified water caused appreciable decline in EC but the rate of change was slightly poor in those soils irrigated with normal water. Soil pH affected the availability of nutrients. Trace metals such as Fe, Zn and Mn are more available at lower pH than most nutrients. In this study, both pH and EC decreased significantly by addition of  $S^0$  and N fertilizer. The soil pH dropped from an initial value of 9.08 to 7.56 by addition of  $S^0$  and N fertilizer. In the present study, soil pH

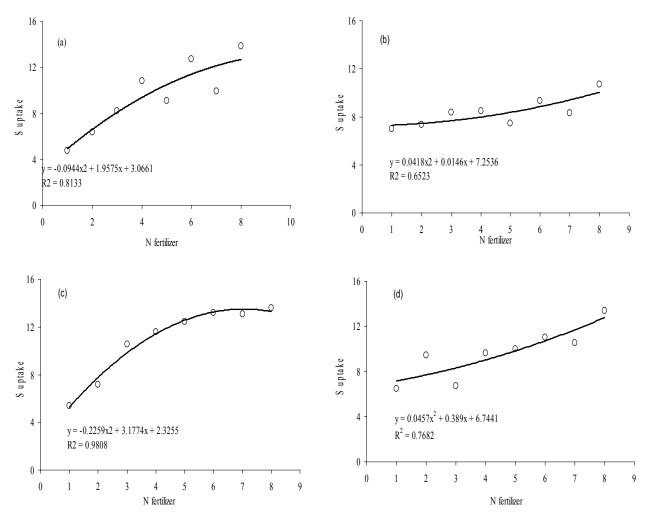


Fig. 3. Relationship between elemental S and N under irrigation water (a) Al Zaid soil with normal water, (b) Al Zaid soil with acidified (c) Al Semaih soil with normal water (d) Al Semaih soil with acidified water

decreased more than 1.0 unit and our findings are at par with the findings of Soliman et al., (1992) where a decrease of soil pH by 0.2, 0.5, and 0.9 units was reported as a result of increasing S applications. In addition to supplying  $S^0$  as a nutrient. S compounds are also used as soil amendments. These compounds act as soil acidifiers neutralizing CaCO<sub>3</sub> with acid; thus, in turn, may lead to a lowering of soil pH and improved nutrient availability. The rates of soil acidifiers required to cause a plant response depend on the amount of CaCO<sub>3</sub> in the soil (El-Tarabily et al., 2006). Calcareous soils are alkaline because of the presence of CaCO3 which dominates the physicochemical properties of the soil. Soil pH is the most important factor which can regulate Zn and Mn supply in calcareous soils. Low levels of soluble Zn are found in alkaline soils and a negligible amount can be exchangeable form  $(Zn^{2+})$  for plants. Both Zn and Mn deficiencies are pHdependent and their concentration in solution decreases 100fold for each unit increase in pH (Brady and Weil, 2002).

## Apparent N-use efficiency, nutrients uptake and TDM accumulation

Elemental S had a significant impact on ANU especially at higher rate of S addition in soil. The ANU value obtained by addition of S<sup>0</sup> at the rate of 5 t ha<sup>-1</sup> with N was 16% versus 9% of ANU with N fertilizer alone. Similar results were recorded with chemical fertilizers for which the averaged values shifted from 25% to 40% when S was added with N fertilizer (Fismes et al., 2000). The plants receiving S<sup>0</sup> at higher dose of 5 t ha<sup>-1</sup> showed significantly higher N uptake than those receiving lower or no S during the growth period. In cereal crops, the ANU values are generally higher and can reach the range of 75 to 90% (Delogu et al., 1998). The same tendency was obtained for the apparent S-use efficiency with low values not exceeding 8% (Fismes et al., 2000). In this study, S-use efficiency in sandy calcareous soil was too low. Therefore, our results compare favorably with values varying between 9 and 16%

obtained from the S-sufficient soils. Nitrogen uptake was appreciably higher at Al Zaid soil compared to Al Semaih soil resulted of higher accumulations of TDM. In Al Semaih soil, TDM accumulation was lower consequently N uptake was quite poor by corn plant. Nitrogen uptake is closely correlated with dry matter yield which may reflect on nutrient uptake ability of N for corn growth. Significantly higher N uptake was obtained by interaction effect of S<sup>0</sup> and N compared to control and other treatment which did not receive N fertilizer. Thus the combined effect of S<sup>0</sup> and N showed significant effect on N uptake by corn plants. These results are coincides with the findings of (Haneklaus et al., 1999) and they reported that higher N concentration of groundnut was observed in calcareous soil using S application compared with zero S. For an environmentally sustainable production of corn, a sufficient supply of S is essential in order to minimize nitrogen losses to the environment. Therefore, our results revealed that application of S<sup>0</sup> at the rate of 5 t ha<sup>-1</sup> with N fertilizer is seemingly better for corn plants in calcareous soils. In Al Semaih soil, P uptake and TDM accumulation in corn was poor. These results indicated that TDM yield and P uptake are closely correlated. Phosphorus uptake improved due to amendment of soil by addition of higher levels of  $S^0$  and N fertilizer. Phosphorus uptake showed a strong positive correlation with S uptake under both types of irrigation water and soils. The higher rate of S<sup>0</sup> concentrated in a small volume of calcareous soil creates an acidic zone and increases the availability of P and micronutrients to roots growing zone (Obreza et al., 1993). Sulfur uptake was enhanced by the application of S<sup>0</sup> and its interaction with N and had a strong positive effect on TDM accumulation. The results clearly showed that Zn and Mn uptake increased significantly by corn plants when grown with moderate levels of S<sup>0</sup> with N, whereas zero S<sup>0</sup> and N recorded poor uptake of Mn. These results coincided with the findings of Kayser et al., (2001) who reported that higher application of  $S^0$ markedly increased Zn uptake by Helianthus annuus grown in calcareous soil. Application of S<sup>6</sup> increased Zn solubility due to soil amendment and Zn concentration in sunflower and peanut plants significantly improved in calcareous soil (Kayser et al., 2001). Kaplan and Orman (1998) also reported that application of S<sup>0</sup> at the rate of 2 t ha<sup>-1</sup> increased Mn and Zn content in the shoots of sorghum plants under calcareous soils in Turkey. Total dry matter showed profound influence on uptake availability of all nutrients by application of S<sup>0</sup>, N and irrigation water. Similar results were obtained by Varin et al., (2010) who reported that application of sulfate increased whole plant dry mass, root length and nodule biomass in white clover while Besharati and Rastin (1999) reported that S application had significant effects on root and shoot dry matter of corn grown in calcareous soils under greenhouse conditions. Total dry matter was not affected by low concentration of sulfate but affected when by the complete absence of S (Koralewska et al., 2007). Total dry matter accumulation and nutrient uptake was increased by application of S<sup>0</sup> at the rate of 5 t ha<sup>-1</sup> plus N fertilizer. The oxidation of S<sup>0</sup> resulted in both direct chemical changes through lowering soil pH, EC and increasing sulfate concentration. The results suggest that the application of S<sup>0</sup> in calcareous soil is a good alternative for the improvement of soil properties, especially considering its beneficial effects on nutrients uptake pattern. Based on the above findings it is recommended that application of S<sup>0</sup> at the rate of 5 t ha<sup>-1</sup> with N is economically and technically suitable to grow corn in

calcareous soil of UAE. Considering chemical properties of soil, TDM accumulation and nutrients uptake, both normal and acidified irrigation water is suitable for Al Zaid and Al Semaih soils, respectively.

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#### References

- Abdallah M, Dubousset L, Meuriot F, Etienne P, Avice JC, Ourry A (2010) Effect of mineral sulphur availability on nitrogen and sulphur uptake and remobilization during the vegetative growth of *Brassica napus* L. J. Expt. Bot. 61(10): 2335-2346
- Abdou AS (2006) Effect of applied elemental sulfur and sulfur-oxidizing bacteria (*Parococcus versutus*) into calcareous sandy soils on the availability of native and applied phosphorus and some micronutrients. In:18th World Congress of Soil Science, Philadelphia, Pennsylvania, USA. July 9-15, 2006
- Brady NC, Weil RR (2002) The Nature and Properties of Soils. 14th ed. Prentice Hall, Upper Saddle River, New Jersey
- Besharati H, Rastin NS (1999) Effect of application *Thiobacillus* spp. Inoculants and elemental sulfur on phosphorus availability. Iran. J. Soil Water Sci. 13:23-39
- Cassman KG, Dobermann A, Walters DT (2002) Agro ecosystems, nitrogen use efficiency, and nitrogen management. Royal Swedish Academy of Sciences. Ambio 31(2):132-140.
- Delogu G, Cattiveli L, Pecchioni N, De Falcis D, Maggiore T, Stanca AM (1998) Uptake and agronomic efficiency of nitrogen in winter barley and winter wheat. Eur. J. Agron. 9:11-20
- El-Tarabily KA, Abdou AS, Maher ES and Satoshi M (2006) Isolation and characterization of sulfur-oxidizing bacteria, including strains of *Rhizobium* from calcareous sandy soils and their effects on nutrient uptake and growth of maize. Aus. J. Agril. Res. 57(1):101-111
- Fismes J, Vong PC, Guckert A, Frossard E (2000) Influence of sulfur on apparent N-use efficiency, yield and quality of oilseed rape (*Brassica napus* L.) grown on a calcareous soil. Eur. J. Agron. 12(2):127-141
- Ghosh PPK, Hati KM, Mandal KG, Misra AK, Chaudhary RS, Bandyopadhyay KK (2000) Sulphur nutrition in oilseed based cropping systems. Fert. News. 45:27-40
- Haneklaus S, Paulsen HM, Gupta AK, Bloem E, Schung E (1999) Influence of sulfur fertilization on yield and quality of oilseed rape and mustard. New Horizons for an old crop. Proceedings of the 10<sup>th</sup> International Rapeseed Congress, Canberra, Australia

- Jackson GD (2000) Effects of nitrogen and sulfur on canola yield and nutrient uptake. Agron. J. 92(4):644-649
- Janzen HH, Bettany JR (1987) Oxidation of elemental sulfur under field conditions in central Saskatchewan. Can. J. Soil Sci. 67:609-618
- Jones JB, Case VW (1990) Sampling, Handling, and Analyzing Plant Tissue Samples. In: Westerman (ed.) "Soil Testing and Plant Analysis" Book Series no. 3. Soil Sci. Soc. Am., Madison WI, pp. 389-427
- Joshi NI, Mali PC, Saxena A (1998) Effect of nitrogen and sulphur application on yield and fatty acid composition of mustard (*Brassica juncea* L) oil. Agron. Crop Sci. 180:59-63
- Kacar B, Katkat AV (2007) Plant Nutrition. 3th ed. Nobel Press; Ankara, Turkey
- Kaplan M, Orman S (1998) Effect of elemental sulphur and sulphur containing west in a calcareous soil in Turkey. J. Plant Nut. 21:1655-1665
- Kayser A, Schroder TJ, Grunwald A, Schulin R (2001) Solubilization and plant uptake of zinc and cadmium from soils treated with elemental sulfur. Inter. J. Phytorem. 3:381-400
- Kopriva S, Rennenberg H (2004) Control of sulphate assimilation and glutathione synthesis: interaction with N and C metabolism. J. Exp. Bot. 55:1831-1842
- Koralewska A, Posthumus FS, Stuiver CEE, Buchner P, Hawkesford MJ, De Kok LJ (2007) The characteristic high sulphate content in *Brassica oleracea* is controlled by the expression and the activity of sulphate transporter. Plant Biol. 9:654-661
- Kuo S (1996) Phosphorus. In: Sparks DL (ed) "Methods of Soil Analysis, Part 3 Chemical Methods" Book Series no. 5. Soil Sci. Soc. Am. 869-919. Mad. WI
- Lefroy DB, Sholeh, Blair G (1997) Influence of sulfur and phosphorus placement and sulfur particle size on elemental sulfur oxidation and the growth response of corn (*Zea mays*). Aust. J. Agril. Res. 48:485-496
- Lindemann WC, Aburto JJ, Haffner WM, Bono AA (1991) Effect of sulfur source on sulfur oxidation. Soil Sci. Soc. Am. J. 55:85-90
- Luit J, De K, Grill D, Hawkesford MJ, Schnug E, Stulen I (1999) Plant Sulfur Research. Fundamental, agronomical and environmental aspects of sulfur nutrition and assimilation in plants. Prog. Rep. 1997/1998
- MacGrath SP, Zhao FJ (1996) Sulphur uptake, yield responses and the interactions between nitrogen and sulphur in winter oilseed rape (*Brassica napus*). J. Agr. Sci. 126:53-62
- Munsinger RA, McKinney R (1982) Modern Kjeldahl systems. Am. Lab. 14:76-79
- Murphy MD (1999) The interaction of applied S with N, P, Se and Mo in soils and on the uptake of these elements by plants. Plant Sulfur Research. Luit J, De K, Grill D, Hawkesford MJ, Schnug E, Stulen I (eds) Prog. Rep. 1997/1998
- Neilsen D, Hogoe LK, Hoyt PB (1993) Oxidation of elemental sulphur and acidulation of calcareous orchard soils in southern British Colombia. Can. J. Soil Sci. 73:103-114
- Obreza TA, Alva AK, Calvert DV (1993) Citrus fertilizer management on calcareous soils. Circular 1127, Soil and Water Science Department, Florida Cooperative Extension Service, IFAS, University of Florida. http://edis.ifas.ufl. edu/BODY\_CH086

- Olivera M, Tejera N, Iribarne, Ocana A, Luch C (2004) Growth, nitrogen fixation and ammonium assimilation in common bean (*Phaseolus vulgaris*): effect of phosphorus. Physiol. Planta. 121:498-505
- Page AL, Miller RH, Keenly DR (1982) Methods of Soil Analysis (Part 2): Chemical and Microbiological Properties. 2<sup>nd</sup> ed. American Society of Agronomy, Inc. and Soil science Society of America, Inc. Publisher, Agronomy Series no. 9. Madison, Wiscosin, USA
- Rosa EAS, Rodrigues PMF (1998) The effect of light and temperature on glucosinolate concentration in the leaves and roots of cabbage seedlings. J. Sci. Food Agric. 78:208-212
- SAS (2003) SAS Institute, SAS Version 9.1.2(c) 2002-03. SAS Institute, Inc., Cary, NC pp. 449-453
- Scherer HW (2001) Sulphur in crop production: invited paper. Eur. J. Agron. 14:81-111
- Scherer HW, Pacyna S, Spoth KR, Schulz M (2008) Low levels of ferredoxin, ATP, and leghemoglobin contribute to limited N<sub>2</sub> fixation of peas (*Pisum sativum* L.) and alfalfa (*Medicago sativa* L.) under S deficiency conditions. Biol. Fert. Soils 44:909-916
- Schnug E, Haneklaus S, Murphy DPL (1993) Impact of sulphur fertilization on fertilizer nitrogen efficiency. Sulphur Agr. 17:8-12
- Soliman MF, Kostandi SF, van Beusichem ML (1992) Influence of sulfur and nitrogen fertilzer on the uptake of iron, manganese, and zinc by corn plants grown in calcareous soil. Comm. Soil Sci. Plant Anal. 23(11):1289-1300
- Thomas GW (1996) Soil pH and soil acidity. Sparks DL (ed) In: Methods of soil analysis, Part 3-Chemical methods, Soil Sci. Soc. Am. Book Series # 5. Madison, Wisconsin, USA, pp: 475-490
- Varin S, Cliquet JB, Personeni E, Avice JC, Servane LL (2010) How does sulphur availability modify N acquisition of white clover (*Trifolium repens* L.) J. Exp. Bot. 61(1):225-234