STABLE ISOTOPE ^{15}N AS PRECISION TECHNIQUE TO INVESTIGATE ELEMENTAL SULFUR EFFECTS ON FERTILIZER NITROGEN USE EFFICIENCY OF CORN GROWN IN CALCAREOUS SANDY SOILS

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ABSTRACT

Widespread use of urea was delayed in part due to its greater potential for nitrogen (N) loss via ammonia volatilization in calcareous soils. Elemental sulfur (S^0) can be applied to acidify calcareous soils which reduce N loss via ammonia volatilization and increases fertilizer nitrogen use efficiency. The present study was sought to examine the effect of S^0 combined with or without N on total dry matter (TDM), N uptake, percentages of N derived from fertilizer (%Ndff), Total fertilizer N recovery (%FNR), fertilizer N use efficiency (%FNUE) and fertilizer N loss (%FN loss) by corn plants grown in calcareous soils. Elemental S at rates of 0, 1, 5 and 10 t ha^{-1} were tested combined with or without labeled N-15 (10% atom enrichment) urea fertilizer at rates of 0 and 0.34 t ha^{-1} in greenhouse pot experiment using two soils differed in $CaCO_3$ content (Al Zaid soil, 38 % and Al Semaih soil, 69%). Addition of S^0 at the rate of 5 t.ha^1 in Al Zaid and Al Semaih soil combined with N fertilizer recorded improve in TDM, total N uptake, %Ndff, %FNR and %FNUE, and reduction in %NF loss. Collectively, the results indicate that S^0 fertilization is required to improve FNUE and thereby maintaining a sufficient availability of N, reduction of N loss and better growth of corn in sandy calcareous soils.

Keywords: **C**alcareous soil, Urea fertilizer, Elemental Sulfur, N-15, Fertilizer N-use efficiency, Corn.

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 $CaCO_3$ and alkaline pH that greatly reduce the solubility of Fe, Zn, Mn, and Cu thus characterizing as deficient in these micronutrients. The presence of $CaCO_3$ 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 (Neilsen, et al., 1993). The biochemical oxidation of S^0 produces $H_2SO_{4,\ \text{which}}$ decreases soil pH and solubilizes $CaCO_3$ 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 FNUE and that N deficiency can 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^0 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-cosystems 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^0 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 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^0 fertilization on N and S utilization, growth and fertilizer N use efficiency of corn grown in sandy calcareous soils using isotope N-15 as precision technique.

MATERIALS AND METHODS

Greenhouse experiments were conducted at Al-Foah Agricultural Experiment Station (27^0N and 22^0S latitude and 51^0W and 57^0E longitude), UAE University. Elemental S at rates of 0, 1, 5 and 10 t ha^{-1} were tested combined with or without N fertilizer at rates of 0 and 0.34 t ha^{-1} in pots under evaporative cooled greenhouse conditions. The treatment arrangements were as follows: S0N0=S 0 + N 0 (control), S0N1=S 0 + ^{15}N 0.34 t ha^{-1}, S1N0=S 1 t ha^{-1} + N 0, S1N1=S 1 t ha^{-1} + ^{15}N 0.34 t ha^{-1}, S2N0=S 5 t ha^{-1} + N 0, S2N1=S 5 t ha^{-1} + ^{15}N 0.34 t ha^{-1}, S3N0=S 10 t ha^{-1} + N 0 and S3N1=S 10 t ha^{-1} + ^{15}N 0.34 t ha^{-1}. The experiment was laid out in a factorial completely randomized design with three replications. With same set of treatments, two experiments were carried out simultaneously using each with normal irrigated water in Al Zaid and Al Semaih soils. Sandy calcareous soil samples were 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_3, CO_3 and SO_4) were determined as per the methods recommended in Page et al., (1982). Physicochemical properties of the soil are presented in Table 1.

Soils were air-dried before being used in the experimental pots. Free-draining polyethylene pots (height 25 cm x diameter 23 cm) were filled with 5 kg of sandy calcareous soil. Each pot was initially filled with 3.8 kg of soil. Prior to sowing each pot received extra 1.2 kg of soil mixed with P and K at the rates of 3.3 and 1.1 g pot^{-1} in the forms of single super-phosphate and potassium sulfate, respectively. Elemental S powder (particle size<150 µM) was collected from TAKREER Company, Abu Dhabi, UAE and applied as per treatment arrangement. According to treatment arrangement, urea- ^{15}N (10% atom enrichment) labeled fertilizer was applied at rates of 1.49 g per pot (~0.34 t ha^{-1}) at 10 and 17 days after germination (DAG) on the soil surface and irrigated by normal 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 irrigation water up to field capacity for proper germination and growth of maize plants. After emergence, all seedlings were kept until final harvest. Maize plants were selected at random and harvested after 35 DAG for N and S analysis and total dry matter accumulation. Roots and shoots were washed in deionized water and oven dried at 72°C for 48 h and grounded to

Table 1. Physicochemical properties of Al Zaid and Al Semaih soil

Soil properties	Al Zaid soil	AL Semaih soil
Texture		
Sand %	95.00	99.73
Silt + clay %	5.00	0.27
EC (dSm^{-1})	3.36	18.27
pH	8.48	8.60
Total $CaCO_3$ %	38.98	68.17
Active $CaCO_3$ %	3.50	12.50
O.C. %	0.17	0.14
Soluble cations (cmol L^{-1})		
Ca	1.60	28.60
Mg	1.40	12.60
Na	28.70	171.10
K	0.34	2.86
Soluble anions (cmol L^{-1})		
Cl	33.00	169.00
SO_4	3.40	25.18
HCO_3	2.90	1.40
CO_3	1.00	0.00

powder in a ball mill. Sulfur content was measured using ICP-AES, Varain model Vista MPX. The N concentration was measured by automatic distillation (FOSS, 2200 Kjeltic Auto Distillation) followed by acid titration (Munsinger and McKinney, 1982).

The ^{15}N-labeled corn plant and soil samples were analyzed for the atom percent of ^{15}N using a combustion continuous flow isotope ratio mass spectrometer in Iso-Analytical Limited laboratory, UK for ^{15}N analysis. The proportion of N derived from fertilizer (Ndff), total fertilizer N recovery (FNR), fertilizer N use efficiency (FNUE) and fertilizer N loss (FN Loss) were calculated as described in FAO/IAEA, 2001.

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.

RESULTS AND DISCUSSION

Total dry matter accumulation of corn

Total dry matter accumulation was affected significantly by application of S^0, N and their interaction (Fig. 1). The highest and lowest TDM accumulation was recorded by addition of S^0 at the rate of 5 t ha^{-1} with N and control treatment, respectively. Total dry matter accumulation was appreciably higher in Al Zaid soil than Al Semaih soil. Significantly higher Total dry matter accumulation was obtained by interaction effect of S^0 and N compared to control and other treatments, which did not receive N fertilizer. High dry matter production is one of the prerequisite for greater productivity in crop plants (Muchow, et al., 1993; Motior and Ahad, 1995). Use of adequate quantity of nutrients is one of the important strategies for increasing crop growth and yield (Fageria and Baligar, 1996). Dry matter yield of the present study indicated that application of S^0 at 5.0 t ha^{-1} with N is found suitable for corn grown in both tested soil, which may reflect on nutrient uptake ability and fate of nitrogen for corn growth.

Nitrogen uptake

Nitrogen uptake by corn plants was influenced significantly with application of S^0 and N fertilizer. The highest N uptake was recorded with application of S^0 at the rate of 5 t ha^{-1} combined with N fertilizer in both soils, while the lowest N uptake was obtained from control treatment (Fig. 2). Nitrogen uptake is closely correlated with dry matter yield which may reflect on nutrient uptake ability of N for maize growth. Significantly higher N uptake was obtained by interaction effect of S^0 and N compared to control and other treatment, which did not receive N fertilizer. Thus, the combined effect of S^0 and N fertilizer showed significant effect of N uptake by corn plants. These results coincide with the findings of Haneklaus et al. (1999) who reported that higher N concentration of

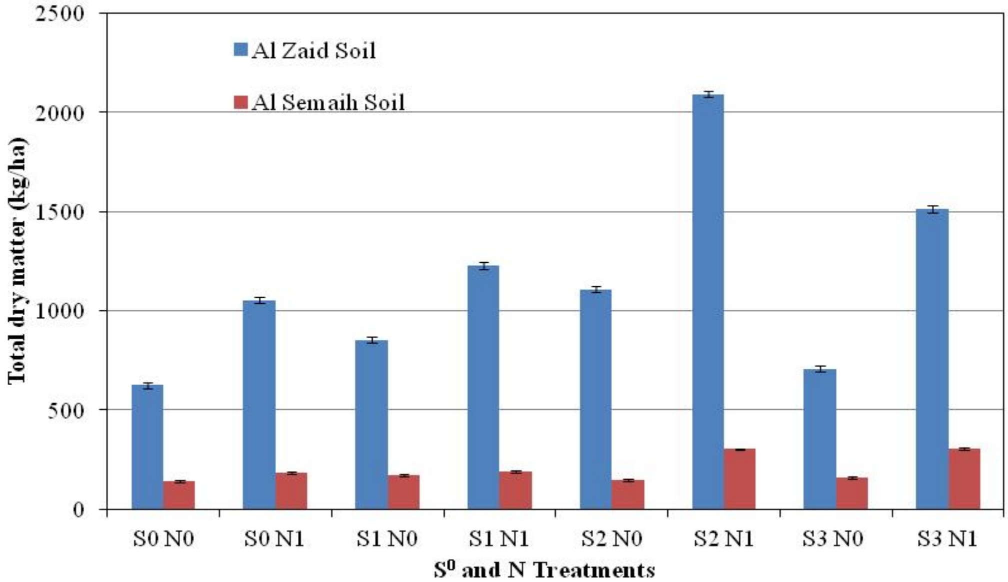

Fig. 1. Total dry matter (shoots and roots) accumulation of corn plant as affected by S^0 and N fertilization (Error bars denoted LSD value at 0.05 level).

groundnut was observed in calcareous soil using S application compared with zero S. For an environmentally sustainable production of maize, a sufficient supply of S is essential in order to minimize nitrogen losses to the environment. Therefore, our results revealed that application of S^0 at the rate of 5 t ha^{-1} with N fertilizer is seemingly better for corn plants in calcareous soils.

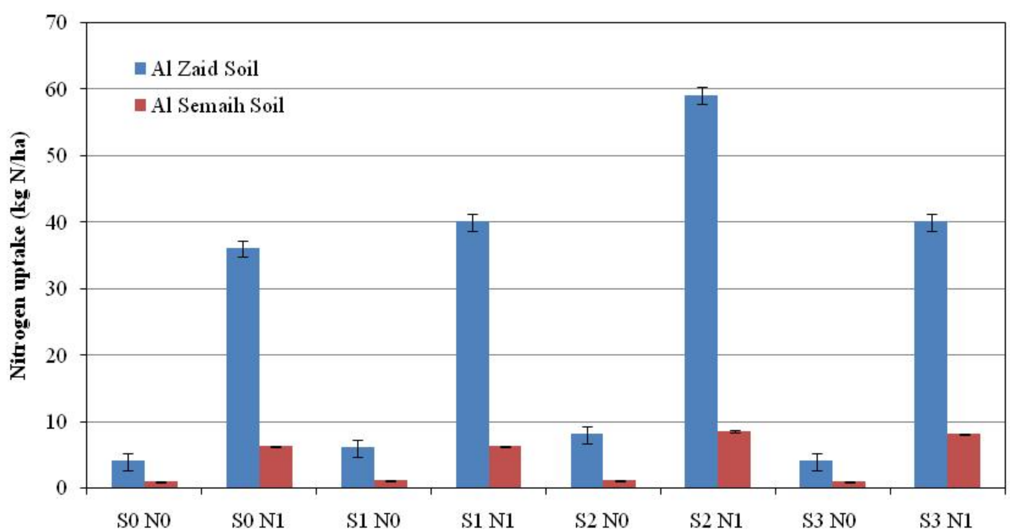

Fig. 2. Nitrogen uptake by corn plant as affected by S^0 and N fertilization (Error bars denoted LSD value at 0.05 level)

Sulfur uptake

Sulfur uptake was enhanced with application of S^0 and its interaction with N (Fig. 3). The highest and lowest uptake of S was recorded from S^0 at the rate of 5 t ha^{-1} plus N and control treatment, respectively under both types of soils. Intermediate S uptake was obtained from S^0 at the rate of 1 and 10 t ha^{-1} plus N when grown under both type of soils. In Al Zaid soil, S^0 at the rate of 5 t ha^{-1} plus N performed better in favor of S uptake while in Al Semaih soil no significance difference was observed under addition of S^0 at rates of 5 and 10 t ha^{-1} combined with N fertilizer. Our results coincide with the findings of Salvagiotti, et al., 2009, who reported that the S addition showed no effect at the lowest N rate, but N uptake was increased when S was applied, revealing a synergism between both nutrients.

Isotopic N-15 technique to study N fertilizer Use Efficiency

Percentage of nitrogen derived from fertilizer (%Ndff)

In Al Zaid soil, %Ndff was 55-87%. The highest and lowest %Ndff was achieved by addition of S^0 at the rate of 5 t ha^{-1} with N and zero S^0 with N fertilizer, respectively (Fig. 4). The %Ndff of Al Semaih soil was lower (42-70%). The highest and lowest NUE was achieved by addition of S^0 at the rate of 5 t ha^{-1} with N and zero S^0 with N fertilizer, respectively.

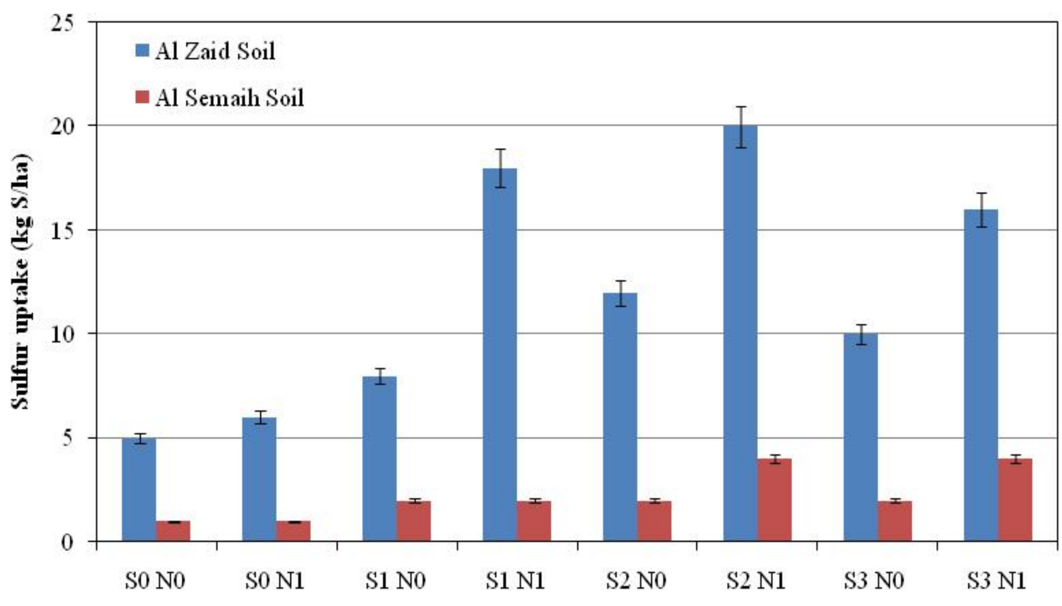

Fig. 3. Sulfur uptake by corn plant as affected by S^0 and N fertilization (Error bars denoted LSD value at 0.05 level)

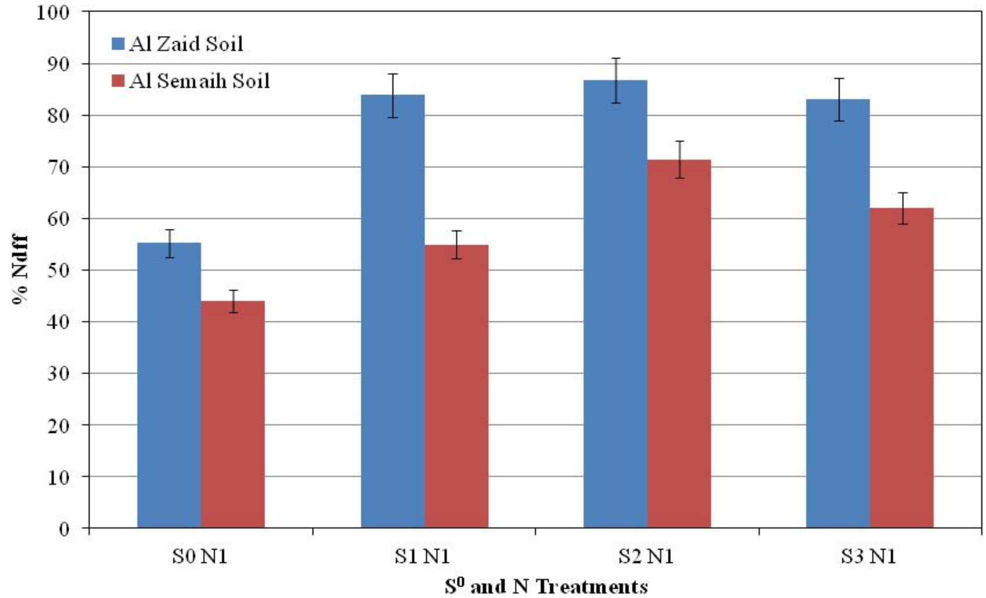

Fig. 4. % Ndff by corn plant as affected by S^0 and N fertilization (Error bars denoted LSD value at 0.05 level)

Fertilizer N use efficiency (FNUE)

The portion of corn N uptake derived from fertilizer as percentage of fertilizer N rate applied or fertilizer N use efficiency (FNUE) is presented in Fig. 5. The FNUE significantly affected by S^0 addition. The FNUE of Al Zaid soil was 18-30%. The FNUE of Al Semaih soil was estimated very low (2 - 4%). The highest and lowest FNUE was achieved by addition of S^0 at the rate of 5 t ha^{-1} with N and zero S^0 with N fertilizer, respectively in both soils. It has reported that, the world FNUE would be estimated at 33% (Raun and Johnson, 1999) and ranged from 42 and 29% in developed and developing nation, respectively (Keeney, 1982; van der Ploeg et al., 1997). Based on these observations, the application of S^0 at 5 t ha^{-1} increased the FNUE in Al Zaid soil to comparable value in developing country. In Al Semaih soil, the FNUE was considered very low even with applied S^0 at rate of 10 t ha^{-1}. This may be attributed to high $CaCO_3$ in this soil (Table. 1), which resulting in high N loss via NH_3 volatilization.

Total fertilizer N recovery in soil and plant (TFNR)

The sum of the portion of fertilizer N recovered in soil and plant as percentage of applied of N fertilizer is shown in Fig. 6. It is obvious that, the application of S^0 had significant effect on total fertilizer N recovery. Increasing S^0 rate from zero to 10 t ha^{-1} increased %TFNR from about 30% to 65% in Al Zaid soil and to 48% in Al Semaih soil. The %NFNR differences between S^0 rate 1 and 5 t ha^{-1} was highly significant in both

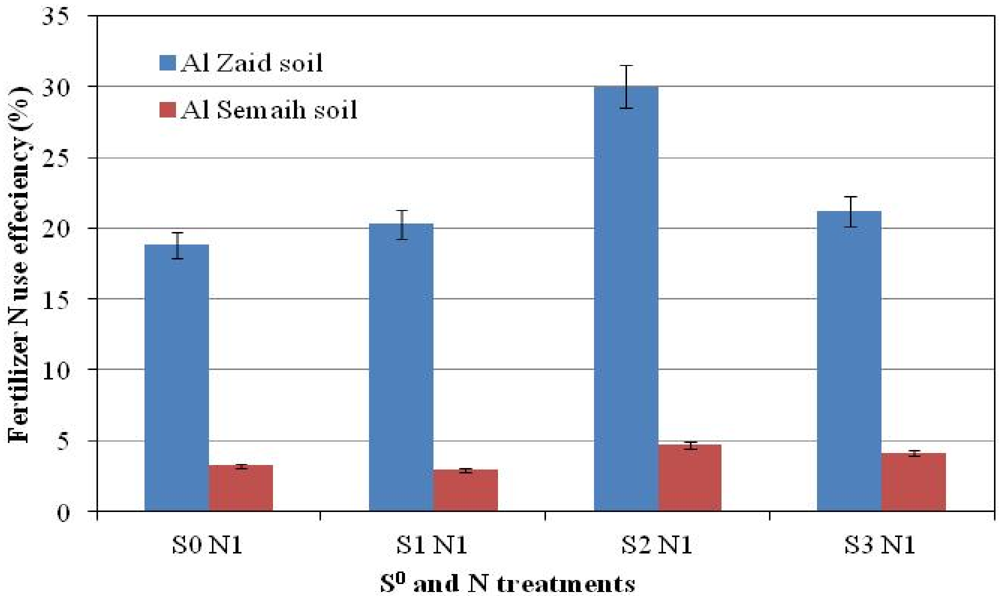

Fig. 5. Fertilizer N use efficiency by corn plant as affected by S^0 and N fertilization (Error bars denoted LSD value at 0.05 level)

soils. While % TFNR differences between S^0 rate 5 and 10 t, ha^{-1} was not significant in both soils. Therefore, S^0 application to Al Zaid soil at 5 t ha^{-1} was more economically than 10 t ha^{-1}. In Al Semaih soil, the application of S^0 had low effect on % TFNR, suggesting that beside S^0 application other agricultural management practices should be considered, such as timing of fertilizer application, fertilizer N form, in-season and foliar-applied N, fertigation, precision agriculture and application resolution, … etc.

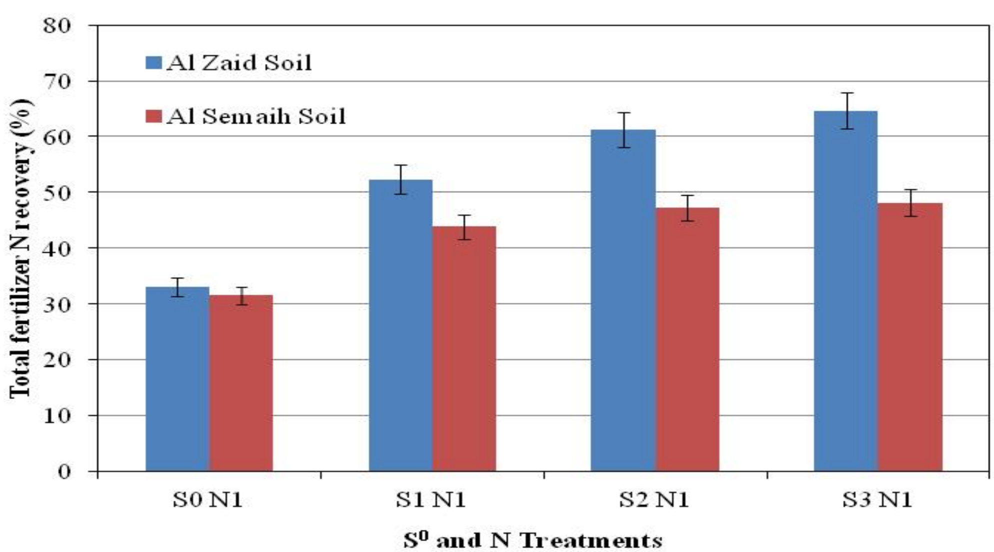

Fig. 6. Total fertilizer N recovery (%) in soil and corn plant as affected by S^0 and N fertilization (Error bars denoted LSD value at 0.05 level)

Fertilizer nitrogen loss (%FN loss)

The portion of fertilizer N unrecovered in soil and plant or % FN loss is presented in Fig. 7. The S^0 application combined with N fertilizer had significant effect on reducing of fertilizer N loss in both soils. The % FN loss decreased from about 68% to about 37% and 50% in Al Zaid and Al Semaih soil, respectively. The highest reduction in % FN loss was observed at S^0 rate of 10 t ha^{-1} in both soils. The differences in % FN loss between S^0 rate 5 and 10 t ha^{-1} was not significant in both soils. Therefore, S^0 application to Al Zaid soil at 5 t ha^{-1} was more economically than 10 t ha^{-1}. In Al Semaih soil, the application of S^0 had low effect on % FN loss, suggesting that beside S^0 application other agricultural management practices should be considered as previously mentioned.

Many ^{15}N recovery experiments have reported losses of fertilizer N in cereal production from 20 to 50%. These losses have been attributed to the combined effects of denitrification, volatilization, and/or leaching (Francis et al., 1993; Olson and Swallow, 1984; Karlen et al., 1996; Wienhold et al., 1995; Sanchez and Blackmer, 1988) when these factors were not measured separately. Scientists, also, documented that cereal plants release N from plant tissue, as NH_3 following anthesis (Harper et al., 1987; Francis et al., 1993). Plant N losses have accounted for 52 to 73% of the unaccounted N using ^{15}N in corn research (Francis et al., 1993).

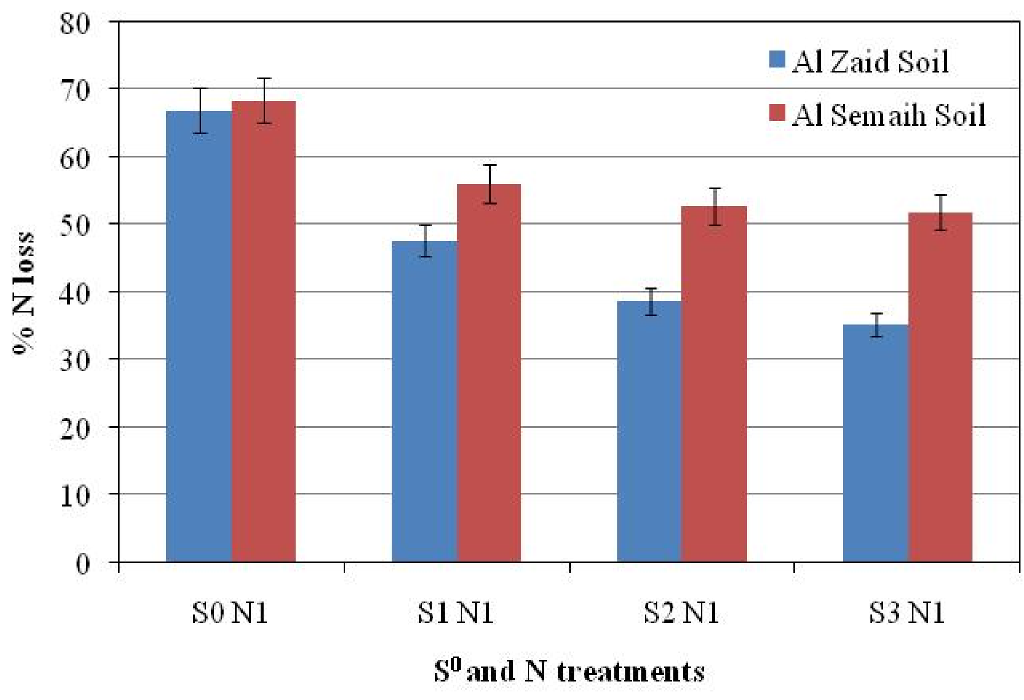

Fig. 7. Fertilizer N loss (%) from soil and corn plant as affected by S^0 and N fertilization (Error bars denoted LSD value at 0.05 level)

CONCLUSION

Addition of S^0 at the rate of 5 t ha^1 in Al Zaid and Al Semaih soil combined with N fertilizer recorded improve in TDM, total N uptake, %Ndff, %FNR and %FNUE, and reduction in %NF loss. Collectively, the results indicate that S^0 fertilization is required to improve FNUE and thereby maintaining a sufficient availability of N, reduction of N loss and better growth of corn in sandy calcareous soils.

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