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ARTICLE

Contribution of Elemental Sulfur to Soil Acidification, Iron Release and Uptake by Corn (*Zea mays* L.)

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ABSTRACT

A glasshouse experiment was conducted to determine the effects of elemental sulfur (S) applications on soil acidity, the solubility of soil iron, and the uptake of iron (Fe) by corn (*Zea mays* L.). Soil samples were treated with four rates of sulfur and incubated for 0, 20, and 40 days before corn plantation. While one unit increase in S application rate corresponded to a soil pH decrease of approximately 1.52 units, the solubility of the Fe was significantly increased. Fe concentrations in leaves and stems increased as soil pH decreased from 7.03 to 5.42, but further soil acidification decreased Fe concentrations in plant tissues. Overall, applying S at a rate of 0.5 g S kg^{-1} soil may to enhance corn performance by 45 percent while posing minimal risk to groundwater or crops.

1. Introduction

Micronutrient availability in soils depends on soil chemical factors, such as soil acidity and mineralogy [1, 2]. Iron deficiencies in plants generally occur in calcareous and alkaline soils, in which high pH depresses the solubility of iron (Fe) and zinc (Zn) and decreases nutrient uptake by plant roots [3]. Applying elemental sulfur to these soils can increase iron solubility and uptake but may also acidify the soil rhizosphere. For every pH unit decrease, there is a corresponding increase in the solubility of Fe and Zn by a factor of 10 to 1000 [4]. Elemental sulfur is especially useful as a soil amendment because it is ready availability and acidulates at a gradual rate [5]. Elemental sulfur acidulates soil as microbial and chemical oxidation convert it to sulfuric acid over time [6]. It has been reported that applying sulfur above adequate concentrations may improve iron use efficiency in wheat [7].

Applications of elemental sulfur are likely to be improve yields in crops grown under severe iron limitations. It was found that high elemental sulfur supply increased the Fe concentrations in shoots, and that concentrations of iron and sulfur in leaves were significantly correlated, irrespective of iron availability in nutrient solution. Increased sulfur availability may stimulate iron accumulation via increases in methionine, which in turn facilitates the production of elevated levels of phytosiderophores and nicotianamine [7].

There are contrasting reports on the effects of elemental sulfur applications on soil pH and nutrient availability [8-10]. As previously noted, applications of elemental sulfur have been found to decrease soil pH and increase nutrient solubility [14, 15, 16], while some studies have found that nutrient solubitliy decreased in acidified soils [11-13]. The increased release

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of soil nutrients from unavailable to available pools could be due to soil pH changes as reported by Ye et al. (2011) and Ye et al. (2010) [15, 16]. They also showed that plant nutrient availability increased due to soil pH reductions caused of S applications, and that the mobile fraction of soil heavy metals increased in more acidic soils. The displacement of cations from the exchange complex on clay minerals and soil organic matter occurs via hydrogen ions, and the ion solubility and oxidation state are strongly affected by soil acidity [17].

Because soils vary in their responses to elemental sulfur applications [18], it is necessary to find an optimal application rate for each one. While the general effects of elemental sulfur applications on soil micronutrient release was elucidated by Karimizarchi et al. (2016) [19], the specific impacts of elemental sulfur applications on soil acidity, iron solubility, and iron uptake by plants in the Bintang Series soils have not been studied. Our objective is to quantify the effects of elemental sulfur on the uptake and distribution of iron in corn (*Zea mays* L.) growing in acidified Bintang Series soil.

2. Material and Methods

The Bintang Series soil was amended with 0.0, 0.5, 1.0 and 2.0 g S kg^{-1}soil and incubated for 0, 20 and 40 days before corn plantation. The corn plants were grown for 45 days under glasshouse conditions. Soil samples were retrieved at the planting and harvesting stages and were subjected to nutrient analysis. In addition, corn leaves, stems and roots were analyzed separately to determine the nutrient content of each part.

2.1 Soil Characterization and Site Description

Soil samples were collected from the surface horizon (0-20 cm) of Bintang Series soil. Collections were made in Bukit Bintang, Perlis, Malaysia (6° 31′ 01.61″ N, 100° 10′ 12.43″ E). The collection area was under natural vegetation and its soils were derived from limestone parent materials (Karimizarchi et al., 2014a). The soil samples were air-dried, ground to a particle size of < 2 mm, and shaken for 30 minutes on a reciprocal shaker. The samples were then rested for 24 hours before soil pH and electrical conductivity were measured in a soil-water suspensions (10 g soil to 25 ml deionized water).

2.2 Plant Growth and Management

Seeds of the sweet corn cultivar Masmadu were provided by the Malaysian Agricultural and Development Research Institute [19]. Seeds were germinated in laboratory conditions and after 24 hours were transplanted into plastic pots 30 cm in diameter and 50 cm in height. Each pot contained 10 kg of Bintang Series soil and received three plants. After one week the plants were thinned to one. Seedlings were grown for 45 days in a greenhouse located at the University Putra Malaysia (UPM). Each pot was weighed every day, and the soil moisture content was adjusted to 90 percent of field capacity. The plants were irrigated daily. Chemical fertilizers were applied at rates recommended by MARDI [20], including 40 kg K_2O per ha in the form of muriate of potash, 60 kg P_2O_5 per ha in the form of triple superphosphate, and 120 kg N per ha in the form of urea.

2.3 Soil and Plant nutrient Extraction and Determination

Since buffered extractants may interfere with the effect of sulfur on nutrient solubility, the available fraction of soil nutrients were extracted using calcium chloride [16, 21]. Soil solutions were centrifuged for approximately 15 minutes at 3000 rpm and were then filtered. Plant parts, including root, shoot and leaf tissues, were separately washed with dionized water, dried at 65 °C, and weighed. The tissues were then ground, ashed in a muffle furnace at 480 °C for 10 hours, and dissolved in diluted acid [21]. ICP-OES (Perkin Elmer, Optima 8300) was used to determine nutrient concentrations.

2.4 Statistical Analysis

The relationships between soil properties and plant traits were analyzed using various regression models (p = 0.05) in Sigmaplot software. Differences among treatments were analyzed using ANOVAs in SAS 9.1, and Duncan's test at $\alpha = 0.05$ was used to identify significance differences among treatment means.

3. Results and Discussion

Soil pH was affected by both the rates and timing sulfur applications (Table 1). For example, the pH values of soil samples incubated for 40 days after being amended with sulfur at rates of 0.5, 1.0, and 2.0 g sulfur per kg soil decreased a background value of 7.51 to 6.66, 5.45 and 4.80, respectively. Plant growth stages also had a significant effect on soil pH (Table 1).

Table 1. Effect of elemental Sulfur application rates (G S kg^{-1} soil) and timing on soil pH

Sulfur rate	Soil pH							
	At planting				At harvest			
	0	20	40	Mean	0	20	40	Mean
0	7.51Aa	7.44Aab	7.42Ab	7.45Aa	6.99Aa	6.92Aa	6.88Aa	6.93Ab
0.5	7.26Ba	6.75Bb	6.66Bb	6.89Ba	6.30Ba	6.23Ba	6.34Ba	6.29Bb
1	7.22Ca	6.27Cb	5.45Cc	6.31Ca	5.35Ca	5.27Ca	5.17Ca	5.26Cb
2	7.34Ca	5.44Db	4.80Db	5.86Da	3.90Db	3.86Db	4.06Da	3.94Db
Mean	7.33Aa	6.48Ab	6.08Ac		5.63Ba	5.57Ba	5.61Ba	

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Averaged across time, soil pH for sulfur application rates of 0.0, 0.5, 1.0 and 2.0 g kg^{-1} soil decreased from 7.45, 6.89, 6.31 and 5.86 at planting to 6.93, 6.29, 5.26 and 3.94 at harvest, respectively. The relationships of soil pH to incubation time and growth stage suggest oxidation of elemental sulfur occurs gradually and that an incubation time of 20 days was not enough for time the elemental sulfur to oxidize completely. There was no significant difference in soil pH among incubation times for all sulfur application rates at harvest (Table 1). This indicates that the elemental sulfur had been totally oxidized to sulphate at harvest under conditions of this experiment.

The relationship between sulfur rate and soil pH was modelled in order to predict the effects of elemental sulfur additions to Bintang Series soils (Figure 1). The relationship between soil pH and sulfur application rate was linear at the time of harvest, when soil pH values ranged from 6.94 to 3.94. For each unit increase in the rate of elemental sulfur application, the soil pH decreased by approximately 1.52 units. When averaged across three sample dates, the soil pH values were 7.03, 6.29, 5.26 and 3.94 for sulfur application rates of 0.0, 0.5, 1.0 and 2.0 g S kg^{-1} soil, respectively. The relationship between S application rates and soil pH change is of special interest and needs to be studied for each specific soil. A previous study found that the relationship between S application rate and soil pH was best described using an exponential model, in which applications of 4 tons of S ha^{-1} decreased soil pH from 7.0 to 4.8, while applications of 12 tons of S ha^{-1} further decreased soil pH to only 4.2 [22].

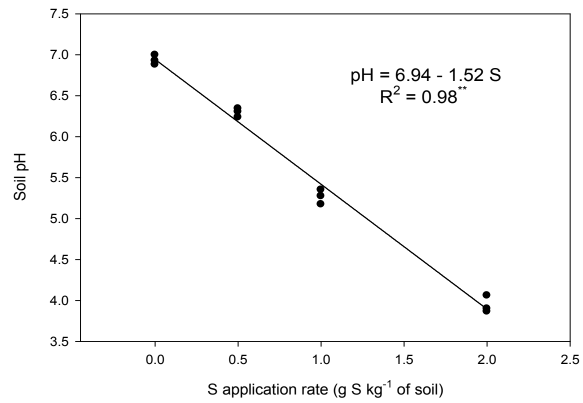

Figure 1. Soil pH changes in response to elemental sulfur application rate

Sulfur applications decreased soil pH and may affect the release of soil Fe, so the correlation between soil nutrient availability and soil pH was studied. A significant correlation of -0.60** was found between soil pH and soil iron. Decreasing soil pH increases the release of soil Fe. This agrees with the general consensus on the positive effect of soil acidification on soil Fe solubility [23, 24, 25].

3.1 Soil acidity and Fe Solubility

The bioleaching of soil nutrients as a function of elemental sulfur application rate and timing in Bintang Series soil was elucidated (Table 2). Additionally, since the acidity produced by oxidation of elemental sulfur in soil is known to increase the solubility of micronutrients [26], the relationship between soil pH and Fe release for Bintang Series soil was quantified (Figure 2). There is no significant change in extractable Fe due to incubation days at planting at each sulfur rate (Table 2). Application of elemental S at 1 and 2 g kg^{-1} significantly increased extractable Fe only at incubation days of 20 and 40. For instance the concentration of Fe at 40 days of incubation significantly increased from 0.11 mg kg^{-1} in unamended soil to 0.21 and 0.24 mg kg^{-1} in soils treated with 1 and 2 g S kg^{-1} soil, respectively. The extractability of Fe also was significantly affected by growth stage. For instance, averaged across timing, the concentration of Fe increased around 4 times from planting to harvest for highest sulfur application rate. The efficacy of elemental sulfur applications to reduce soil pH and increase soil Fe availability has been previously documented [26]. Our data are in agreement with these results and showed that applications of elemental sulfur decreased soil pH (Table 1). At the same time, decreasing soil pH from 7 to 5 only slightly affected the soil Fe concentration. However, under the conditions of our experiment, further pH reductions increased Fe solubility in Bintang Series soil (Figure 2). This is in line with the observations of other researchers [23], who reported low soil Fe solubility even under very acidic conditions [23].

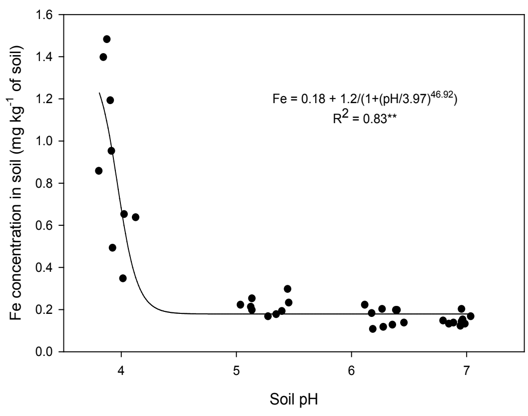

Figure 2. Soil Fe concentration as a function of soil pH

Our data was fitted with non-linear regression model, in which Fe Concentration = $0.18+1.2/(1+(pH/3.97))^{46.92}$, $R^2= 0.83^{**}$. The relationship between –Log (Fe Concentration) and soil acidity was fitted with a linear regression model, in which pFe = 0.25 pH - 0.78, $R^2= 0.75^{**}$. Although this function is similar to the stability diagrams for Fe as function of pH that were developed by Khan and Mazid (2011) [24], in which Log Fe^{2+} = 15.75 - (pe + pH) - 2pH. However, we found that each unit decrease in soil pH corresponded to a 0.25 unit increase in Log(Fe Concentration), while Lindsay [23] found the each unit decrease in soil pH corresponded to a 2 unit increase in Log(Fe Concentration). This difference in the observed rate of Fe concentration change due to soil pH reduction can be attributed to differences in soil properties, as well as the assumptions that were considered, between the two studies.

3.2 Fe as an Immobile Nutrient in Corn

Our data showed that the relationship between elemental sulfur application rates and leaf Fe concentrations followed a non-linear quadratic regression model (Y = 61.5 +12.58 X-5.85 X^2 R^2=0.67*). While increasing elemental sulfur application rates to 2 g S kg^{-1} soil increased soil Fe concentrations (Table 2), leaf Fe concentrations increased at an elemental sulfur application rate of 1 g S kg^{-1} soil and decreased at an elemental sulfur application rate of 2 g S kg^{-1}(Figure 3). The same trend was found for Fe concentration in the corn stem and root (Figure 3). This reflects the fact that corn actively but not passively controls Fe absorption from the soil solution. Our finding is in line with the Marschner (2012) [1], who stated that both passive and active mechanisms function in Fe transport across plant cell membranes. In addition, there were differences in Fe concentration in different parts of the corn plants. While the Fe concentrations in roots ranged from 500 to 1500 mg kg^{-1}, Fe concentrations in stem and leaves ranged from 30 to 42 and 59 to 69 mg kg^{-1}, respectively (Figure 3). This observation is in agreement with the results of Barker and Pilbeam (2007) [25], who reported that Fe is an immobile element in corn. The highest concentration of leaf Fe, 69 mg kg^{-1}, occurred in leaves at the two intermediate sulfur application rates, and a comparison of Fe concentrations in our corn plants to shoot values (50-300 mg kg^{-1}) [25, 1] accepted as adequate shows that Fe was not toxic under the conditions of our experiment.

Table 2. Soil Fe changes in response to elemental Sulfur timing (0, 20, and 40 days application before planting) and application rates (g S kg^{-1} soil) at planting and at harvest.

Sulfur rate	Soil Fe (mg kg^{-1} soil)							
	At planting				At harvest			
	0	20	40	Mean	0	20	40	Mean
0	0.21Aa	0.14BCa	0.11Ba	0.15BCa	0.14Ba	0.16Ba	0.13Ba	0.14Ba
0.5	0.14Aa	0.12Ca	0.09Ba	0.12Cb	0.17Ba	0.17Ba	0.14Ba	0.16Ba
1	0.12Aa	0.18Ba	0.21Aa	0.18ABa	0.25Ba	0.18Bb	0.20Bb	0.21Ba
2	0.15Aa	0.25Aa	0.24Aa	0.21Ab	0.94Aab	1.17Aa	0.54Ab	0.88Aa

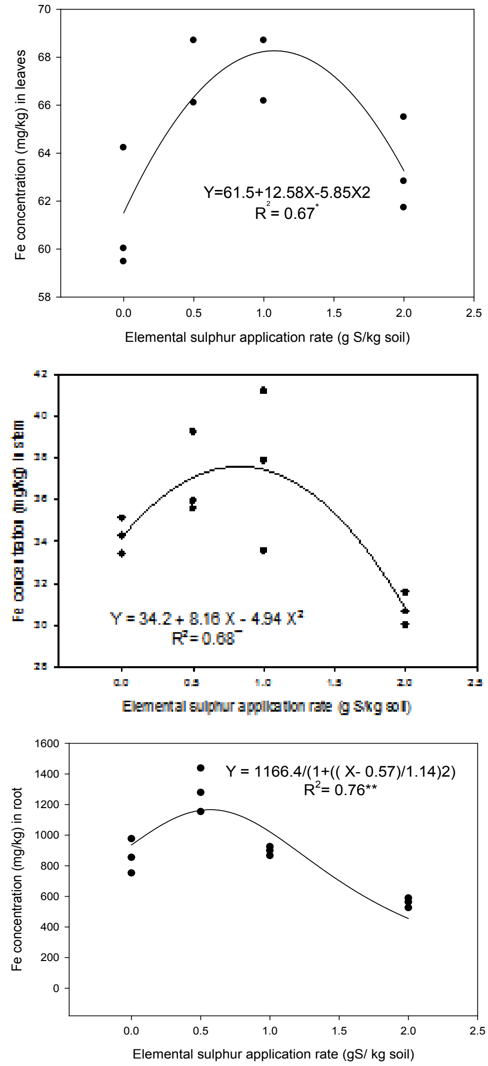

Figure 3. Relationship of elemental sulfur application rate and iron concentration in different parts of corn

3.3 Corn Performance as a Function of Fe Concentration

There was no significant relationship between leaf dry weight and leaf Fe concentration. Leaf Fe concentrations ranged from 60 to 75 mg kg^{-1} (Figure 3), while the recommended range of corn leaf Fe concentrations range from 50 to 300 mg kg^{-1} [1]. However, there is a significant relationship, Y = 19.3/((1+((X-37.6)/5.4)^2)), R^2 = 0.695**, between Fe concentrations in stem and stem dry weight. According to this model, the maximum performance of corn, 19.3 g pot^{-1}, was obtained at Fe concentrations of 37.6 mg kg^{-1} in the stem (Figure 4). Because maximum corn production, in terms of all leaves, stem and root weight, was not obtained at maximum Fe content, it can be concluded that the Fe was not the main limiting factor for corn growth under the conditions of our experiment.

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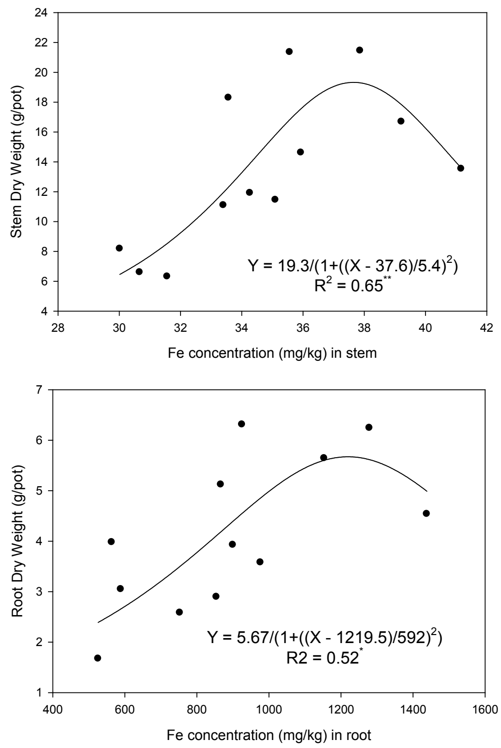

Figure 4. Relationship between Fe concentration in corn and corn performance

4. Conclusion

As soil pH decreased by 1.52 units with each unit addition of elemental sulfur, it can be concluded that elemental sulfur is an appropriate means for lowering pH in Bintang Series soils. In addition, our results demonstrated that extractable Fe was not significantly affected by soil acidification due to elemental sulfur application until soil pH decreased to approximately 4, at which point hydrous oxide precipitates, and applications of 2 g S kg^{-1} soil increased the $CaCl_2$ extractable Fe by 5.57 times. Moreover, our results showed that this increases in soil Fe solubility failed to increase Fe concentration in corn leaves, stem and root. In conclusion, applying of elemental sulfur at a rate of 0.5 g S kg^{-1} soil is recommended to improve corn performance by 45 percent while posing minimal risks to corn production and groundwater quality.

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