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IMPACT OF ACIDIC AGENTS ON NUTRIENTS AVAILABILITY, GROWTH AND CROP PRODUCTION OF *VICIA FABA* (L.) PLANT IN CALCAREOUS SOIL

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Abstract

The purpose of the present study was to overcome unfavorable alkaline characteristics of calcareous soil, via acidification, for cultivating faba bean (*Vicia faba* L.). *V. faba* seeds were grown for 90 days in calcareous soil that collected from Jeddah region, Saudi Arabia. Soil was treated with acidifying compounds; namely, ammonium sulfate (AS), calcium sulfate (CS), elemental sulfur (ES) and acetic acid (AA) at two concentration levels of 200 and 400 mg/Kg for each (100- and 200-ml acetic acid), to neutralize the hyper-alkaline nature of the calcareous soil. Germination, growth, crop yield, metabolites (chlorophyll, total proteins, carbohydrates, and free amino acids) and soil minerals (K, Ca, Mg, N, P, Cl and P_2O_5) contents exhibited significantly enhancing impact of the applied treatments as well as soil validation for agriculture. Improvement in soil characteristics, as field capacity and pH, were also recorded. Ammonium sulfate at a concentration of 400 mg/Kg soil induced generally the highest significant enhancement in most of the studied parameters and hence it is considered the most recommended treatment and dose. The other treatments induced enhancement in some or several of the parameters. The least efficient treatment was acetic acid as it induced minor significance in most of the studied parameters. In general, the applied acidification treatments relieved the drawbacks of calcareous soil as inferred from *V. faba* performance, according to the following order: AS4 > AS2 > CS4 > CS2 > ES4 > ES2 > AA2 > AA1 (AS ammonium sulfate, CS calcium sulfate, ES elemental sulfur; 2 and 4 refers to 200 and 400 mg/g soil, respectively).

Key words: Faba bean; Alkaline soil; Acidification; Crop yield; Growth; Metabolites; Mineral elements

Introduction

Calcareous soils are widely spread in arid and semi-arid regions and cover almost one-third of the world's land surface area (Bolan *et al.*, 2023). These soils are characterized by high content of $CaCO_3$ ranging from few percentages to 95%; more than 30% of the earth surface is calcareous (Singare *et al.*, 2022). Due to their alkaline nature, availability of mineral elements in calcareous soils is limited compared with non-calcareous ones. Solubility or fixation of mineral elements are dependent on soil pH and calcium, which affects base saturation (100% in calcareous soils) and exchange complex. For instance, phosphorus availability for plants in calcareous soils is usually limited and, in this context, calcium carbonate was reported an excellent phosphate binder (Jing *et al.*, 2024). Bioavailability of phosphorus is pH and calcium sensitive; it is most available for living cells at pH values of 6–7. Inorganic phosphorus readily reacts and fixed as calcium phosphates hydroxylapatite, $[Ca_5(OH)(PO_4)_3]$ at pH values > 7.3 whereas at pHs < 6 it occurs as aluminum phosphates and at pH<5 as iron phosphates (Bhattacharya, 2019); meaning it is only sparingly available for plant uptake, causing a mineral deficiency to plants. Elevation of the pH of waters containing typical concentrations of calcium leads to the formation of apatite type of phosphate precipitates (Ren *et al.*, 2021).

The high calcium concentrations in calcareous soils not only suppress phosphate absorption but also magnesium uptake; translocation of magnesium from the roots to the upper plant parts is also suppressed (Yuncong, 2001). In addition, crops grown on calcareous soil usually exhibit symptoms of potassium, magnesium or iron deficiency, despite their levels are abundant or adequate but not bioavailable. Furthermore, calcareous soils are low in organic matter, nitrogen, iron, Zn, Mn and Cu owing to limited solubility at alkaline pH values (Zekri *et al.*, 2010; Alghamdi *et al.*, 2023). Supplemental mineral application is inefficient because they are quickly refixed into soil particles in unavailable forms.

Chlorosis is a common symptom in plants grown in calcareous soils. In calcareous soils, mineral N-fertilizers are most commonly used for supplying nitrogen requirements of plants, and ammonium fertilizers are superior compared with nitrate fertilizers due to their effect as soil acidifiers (Maqsood *et al.*, 2016). However, when ammonium fertilizers such as ammonium sulfate are added to calcareous soils, nitrogen may release to the atmosphere via gasification of ammonium to ammonia gas (Bonten *et al.*, 2016). Nevertheless, the alkaline pH of the calcareous soils is alternatively important in precipitating heavy metals; thus, protecting plants and humans from their toxicity. In this respect, very low pH, significantly elevated aluminum, manganese, cadmium and lead releases can lead to dramatically high concentrations of these elements in soil solution, accumulating in crops which in turn cause root damage and yield decline (De Vries & McLaughlin, 2013; Mok *et al.*, 2015; Zhou, 2015).

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Trials to improve characteristics of soils having calcareous nature are based exclusively on two ideas, that is acidifying treatments and/or cultivating tolerant plants. In this respect, special plants such as asparagus, beets, cabbage, cauliflower, celery, carrot, lettuce, parsley, and spinach were suggested for soils with pH values of neutral–alkaline (7.0–8.0) (Cooperative Extension, solutions to soil problems ii. high pH [alkaline soil] reviewed 2010 Utah State University). Soil treatment is urging in many countries having calcareous soil, as their population increases and actually suffering food shortage. In this respect, DeLuca *et al*., (1989) reported that application of elemental sulfur (S°) and inoculation with *Thiobacillus thioparus* (sulfur oxidizing bacteria) increased soil acidity and increased phosphorus availability and uptake by wheat and corn plants. Elemental sulfur is oxidized to sulfuric acid, thus lowering soil pH while gypsum (calcium sulfate) may lower the content of sodium in the soil; calcium replaces and leaches sodium. Wiedenfeld (2011) recorded gradual long-lasting effects of sulfur application to sugarcane (*Saccharum* spp.) on soil pH, resulting after four years in substantial declines in soil pH. In agreement, Soaud *et al*., (2011) recorded significantly lowered pH of calcareous soils following sulfur application. As a result, soil available phosphate and sulfate (SO_4)-S levels increased, and sugarcane plant growth and yields increased. Sulfur application enhanced the uptake of mineral elements (Kayser *et al*., 2000; El-Bakry *et al*., 2024). However, Sulfur applications changed the concentrations of heavy metals in calcareous soils, but variably dependent on sulfur and soil sources (Abdou *et al*., 2011; Skwierawska *et al*., 2012).

Application of sulfur-containing fertilizers results in reducing the pH but temporarily and locally in calcareous soils. This prevents the formation of PO_4^{-3} from HPO_4^{-2} or $H_2PO_4^{-}$, thus increasing phosphorus availability for plant uptake (Taalab *et al*., 2008). Islam *et al*., (2011) applied three levels (0, 15 & 30 kg sulfur/ ha) of sulfur as gypsum and ammonium sulfate on chickpea productivity. In this concert, an extension publication concerning soil acidification for crop production has been issued by Horneck *et al*., (2007).

Acidic fertilizers such as ammonium sulfate or compost and manures are useful in acidifying alkaline soils. In a long-term study, Rasmussen and Dick (1995) reported that soil pH (Oregon) decreased at the rate of 0.03 to 0.05 unit per year upon applying ammonium sulfate at the rate of 135 lb N/acre. Compost application is also repeatedly recorded to lower the pH value of the soil. Higher organic matter contents lower soil pH (Cooperative Extension, solutions to soil problems ii. high pH [alkaline soil] reviewed 2010 Utah State University). Irrigation using wastewater resulted in high concentrations of N, P and K in the topsoil, which then decreased within depth, and the increase of salts in topsoil was proportional to its content in the wastewater used (El-Zohri *et al*., 2014). Saleh *et al*., (2015) reported that organic and bio-fertilizers stimulated yield of *Nigella sativa* L. grown in calcareous soil; seed oils and fatty acid fractions were also enhanced.

The aim of the current work was to overcome unfavorable alkaline characteristics of calcareous soil, via acidification, for cultivating faba bean (*V. faba* L.) plants. Mineral availability and water holding capacity of such soils are anticipated to improve, and hence plant growth, subsequent to soil acidification. The case study plant, *V. faba*, is leguminous and thus important as food, feed and for land reclamation, as it contains nitrogen fixing nodules which is important to overcome one of the major problems of calcareous soils i.e., nitrogen availability. Remediation of the alkalinity problem of calcareous soils by acidification at such laboratory work may be modeled to expand the cultivable area in the desert of Kingdom of Saudi Arabia.

Material and Methods

Experimental set up and treatments: A pot experiment was conducted at King Abdulaziz University, Saudi Arabia. Seeds of *V. faba* (faba bean) plants were sown for 90 days at plastic pots of 5 Kg capacity; each pot contains 4 Kg of calcareous soil collected from Jeddah region. The used soil was analyzed before and after addition of treatments, for their pH, field capacity, organic matter, available potassium, available phosphorus, sulfur, sodium, nitrogen, calcium and magnesium. The plants were grown under natural light condition in a greenhouse, there was a moderate humidity, at the overnight and early in the morning, the lowest temperature recorded within the greenhouse was 20°C, while the highest recorded temperature per day was 28°C.

The plants were watered with tap water using the soil's field capacity level until the third true leaf appeared. Following that, the pots were divided into 9 groups. Two groups supplemented with ammonium sulfate at concentrations of 200 and 400 mg/Kg soil; 2 groups supplemented with calcium sulfate at concentrations of 200 and 400 mg/Kg soil; 2 groups supplemented elemental sulfur at concentrations of 200 and 400 mg/Kg soil and 2 groups supplemented with acetic acid at concentrations of 100 and 200 ml/Kg soil. While, the control group did not receive any of the above treatments.

Soil analysis: pH of the soil was monitored using pH meter, field capacity and organic matter was estimated using procedures previously described by other investigators (Yousef, 1999; Conklin, 2005), available potassium, available phosphorus, sulfur, sodium, nitrogen, calcium and magnesium were prepared as and estimated using the atomic absorption spectrometer at the Analytical Chemistry Unit (ACAL), RICI MAAZ Chemical & Environmental Testing Laboratory, Dammam, KSA.

Plant growth: Germinating seeds were counted. After four weeks of treatment (40-days-old plants), samples of plant tissues were collected for vegetative growth measurements. The number of leaves per plant, shoot and root lengths were measured and the average of 10 shoots or roots ± SE were recorded, fresh mass of 10 shoots or roots ± SE were recorded, dry mass of 10 shoots or roots ± SE were recorded, nodule number were recorded. At the end of the experimental period, 90 days, crop yield traits (plant and seeds fresh and dry weights) were recorded.

Plant metabolic pools: Chlorophyll (a and b) and carotenoids contents of 40-days-old plants were assessed in 95% ethyl alcohol extracts of 0.1 g of the 3rd leaf according to Su *et al*., (2010), calculated and expressed as mg. g^{-1} leaves fresh weight. A definite fresh mass of leaves (0.5 g) of each treatment was boiled for 2 hours in distilled water in a water bath; after cooling the extractants were centrifuged at (2.1xg) for 20 minutes and the supernatants were separated. Reducing sugars were determined as Schlegel (1956), amino acids according to the method of Moore and Stein (1948) and soluble protein contents were estimated using the method of Lowry *et al*., (1951) spectrometrically with a Lamda 25 UV-Vis spectrophotometer.

Statistical analysis

Each experiment was repeated three times and the mean values of three replicates ± standard errors (se) are presented. Statistical analysis of the data was conducted using ANOVA one-way test (analysis of variance) by SPSS program version 21, and Duncan values were determined at 0.05 levels.

Results

Validating improvement in characteristics of calcareous soil by supplemented acidifying agents was checked by following their anticipated positive impact on *Vicia faba*; germination, growth, crop yield and metabolite contents of the plant were followed as the measurables of assessment.

Soil Analysis

Field capacity was significantly elevated by all the applied treatments relative to the original field capacity of the soil, the highest at ammonium sulfate concentration of 400 mg/Kg soil (Table 1). Soil pH was not severely altered following the application of the acidifying treatments, only at decimals of digits (Table 1). The highest drop in the soil pH of 0.2 unit was brought about at the concentration of elemental sulfur (ES4) followed by ES2 (0.14 unit) and AS4 (0.15). Organic matter percentage was highly significantly increased according to the following order of treatments: AS4 > ES4 > AS2 > ES2 > CS4 > A1 > CS2; AS4 and ES4 almost tripled organic matter contents. At control and A2 treated soils, organic matter content was lowered relative to that of the original soil (Table 1).

Available potassium was mostly lowered by the applied treatments relative to that before sowing the plants attaining the highly significantly least levels at acetic acid; it is only elevated at A4 (Table 2). Such lowered levels of available K may be a result of consumption by the cultivated plants. Available P was increased at AS2 > C > ES4 but lowered at other treatments (Table 2). Sulfur displayed its highest content at soils treated with elemental sulfur, followed by ammonium sulfate; calcium sulfate induced moderate increment while acetic acid induced higher content (Table 2). Sodium content was highly significantly increased at elemental sulfur and ammonium sulfate while lowered at acetic acid (Table 2). Phosphorus pentoxide (P_2O_5) content was highly significantly elevated by ammonium sulfate and the control followed by the other treatments (Table 4). Chloride content was highly significantly higher at ammonium sulfate, elemental sulfur and calcium sulfate (Table 2). Calcium content exhibited significant increase at acidification treatments; the highest level was recorded at elemental sulfur followed by calcium sulfate and ammonium sulfate (Table 2). Magnesium content of the used soil was several times less than that of calcium and the impact of treatments was less influential on magnesium alterations. Magnesium was lowered by calcium sulfate and acetic acid, moderately increased by elemental sulfur while the highest content was recorded at ammonium sulfate, AS4 > AS2 (Table 2).

Germination and growth of *V. faba*: Germination of faba seeds at ammonium sulfate concentration of 200 and 400 mg/Kg soil was significantly the highest relative to any of the other applied treatments, followed by calcium sulfate of 200 and 400 mg/Kg soil or elemental sulfur of 200 and 400 mg/Kg soil; the lowest was at control cultures (Table 3). Acetic acid treated soils did not exert any significant change concerning the number of germinated seeds compared with control (untreated) soil. Also, leaf numbers per plant were significantly increased by ammonium sulfate, calcium sulfate and elemental sulfur regardless of the concentration applied (indicates that minimum level is enough in the case of leaf numbers) while acetic acid did not induce any alterations in leaf numbers (Table 3). Shoot length was highly significantly enhanced by ammonium sulfate (2.38 and 1.67 times that of the control at 400 and 200 mg/Kg soil, respectively). It was also significantly enhanced by calcium sulfate and elemental sulfur. Acetic acid application was not accompanied with significant alteration in leaf number compared with the control plants. Root length was also enhanced by all the applied acidifying treatments except the 200 ml acetic acid; the highest enhancement was recorded at ammonium sulfate concentration of 400 mg/Kg soil. Fresh and dry weight of shoots and roots exhibited their significantly highest values at ammonium sulfate followed by calcium sulfate and elemental sulfur; the least enhancement accompanied acetic acid application (Table 3). Plant fresh and dry weight exhibited their highest values in response to ammonium sulfate treatments while the lowest values were also recorded in acetic acid treated cultures (Table 3).

Nodule number was the highest at ammonium sulfate concentration of 400 mg/Kg soil; it was 2.3 times that of the control (Fig. 1). Generally, the number of nodules responded to the applied treatments i.e., were increased by the sulfur containing treatments regardless of the concentration not by acetic acid (Fig. 1). Collectively, the applied treatments and concentrations exerted relief to *V. faba* plants from the drastic characteristics of calcareous soils.

Crop yield: Crop yield of *V. faba*, estimated as vegetative growth (plant fresh and dry weight) or as seeds (fresh and dry weight) of 90 days old plants as influenced by various acidifying agents to calcareous soil is presented in Figs. 2&3. Sulfur containing compounds highly significantly enhanced vegetative growth in the following order: AS4 > AS2 > ES4 > ES2 > CS4 > ES2. Acetic acid treatment, however, was inhibitory to growth of *V. faba* (Fig. 2A&B). Similarly, fresh and dry weight of seeds was highly significantly enhanced by the applied treatments at a similar order (Fig. 3A&B).

Table 1. Characteristics of the used calcareous soil as influenced by various acidifying agents before and after cultivation of *Vicia faba* plants for 90 days.

Treatment*	Field capacity (%)	pH	Organic matter (%)
B	25.3 ± 1.4^{a}	7.8 ± 0.1^{d}	0.29 ± 0.0^{b}
C	25.7 ± 2.5^{abc}	7.6 ± 0.0^{c}	0.25 ± 0.0^{a}
AS2	26.9 ± 0.5^{de}	7.8 ± 0.0^{d}	0.68 ± 0.0^{g}
AS4	28.0 ± 1.3^{e}	7.6 ± 0.0^{c}	0.74 ± 0.0^{h}
CS2	26.5 ± 1.2^{bcd}	7.8 ± 0.1^{d}	0.34 ± 0.0^{c}
CS4	25.4 ± 1.6^{ab}	7.8 ± 0.0^{d}	0.53 ± 0.0^{e}
ES2	26.8 ± 0.9^{cd}	7.6 ± 0.0^{c}	0.65 ± 0.0^{f}
ES4	25.9 ± 2.5^{abcd}	7.6 ± 0.0^{c}	0.70 ± 0.0^{g}
AA1	26.9 ± 1.7^{de}	7.4 ± 0.0^{b}	0.44 ± 0.0^{d}
AA2	27.0 ± 1.2^{de}	7.3 ± 0.1^{a}	0.26 ± 0.0^{a}

*B is the soil contents before treatment and cultivation, C is the control (untreated calcareous soil), AS2 is 200 mg ammonium sulfate /Kg soil, AS4 is 400 mg ammonium sulfate /Kg soil, CS2 is 200 mg calcium sulfate/Kg soil, CS4 is 400 mg calcium sulfate/Kg soil, ES2 is 200 mg elemental sulfur /Kg soil, ES4 is 400 mg elemental sulfur /Kg soil, AA1 is 100 ml acetic acid/Kg soil, AA2 is 200 ml acetic acid/Kg soil. Different small letters following digits indicate significant differences between treatments.

Table 2. Minerals content (mg/Kg soil) of the used calcareous soil as influenced by various acidifying agents before and after cultivation of *Vicia faba* plants for 90 days.

Treatment*	Available K	Available P	S	Na	P_2O_5	Cl	Ca	Mg
B	64.2 ± 3.2^{h}	23.9 ± 1.3^{cd}	35.3 ± 2.1^{c}	45.7 ± 2.5^{b}	53.4 ± 4.3^{cd}	173.5 ± 6.2^{a}	96.7 ± 4.3^{c}	15.3 ± 0.8^{c}
C	6.7 ± 0.4^{b}	51.9 ± 3.2^{f}	5.1 ± 2.6^{a}	65.4 ± 3.1^{d}	121.6 ± 5.2^{f}	166.3 ± 8.1^{a}	66.6 ± 5.8^{a}	12.2 ± 0.3^{b}
AS2	33.0 ± 1.7^{g}	56.7 ± 2.8^{g}	57.2 ± 3.1^{e}	107.2 ± 5.2^{f}	130.6 ± 6.0^{g}	241.6 ± 7.3^{c}	80.1 ± 4.6^{b}	35.2 ± 2.1^{f}
AS4	66.8 ± 1.3^{i}	30.1 ± 1.8^{e}	76.3 ± 4.3^{g}	106.3 ± 2.7^{f}	131.8 ± 3.6^{g}	290.7 ± 6.8^{e}	96.8 ± 4.6^{c}	44.9 ± 2.4^{g}
CS2	17.7 ± 1.1^{e}	18.5 ± 0.8^{a}	43.7 ± 2.6^{d}	58.5 ± 1.9^{c}	51.1 ± 2.8^{bc}	170.6 ± 4.9^{a}	119.6 ± 6.2^{d}	12.6 ± 0.5^{b}
CS4	13.6 ± 0.5^{d}	21.3 ± 0.5^{b}	30.8 ± 3.2^{bc}	53.1 ± 4.8^{c}	43.8 ± 2.2^{a}	174.7 ± 8.3^{a}	96.6 ± 5.8^{c}	13.4 ± 0.2^{b}
ES2	17.6 ± 0.6^{e}	26.1 ± 2.1^{d}	86.8 ± 3.2^{h}	80.5 ± 3.2^{e}	57.8 ± 3.1^{d}	202.9 ± 5.7^{b}	375.7 ± 11.5^{f}	21.3 ± 0.5^{e}
ES4	24.5 ± 1.2^{f}	56.8 ± 3.5^{g}	131.0 ± 5.4^{i}	125.5 ± 5.7^{g}	68.8 ± 4.6^{e}	262.6 ± 8.4^{d}	345.5 ± 12.3^{e}	20.4 ± 0.3^{e}
AA1	9.2 ± 0.4^{c}	22.3 ± 1,5^{bc}	27.4 ± 3.1^{b}	67.3 ± 4.2^{d}	52.2 ± 2.9^{bc}	169.3 ± 7.3^{a}	100.5 ± 5.6^{c}	18.9 ± 0.7^{d}
AA2	1.3 ± 0.0^{a}	21.2 ± 0.8^{b}	68.5 ± 4.2^{f}	37.5 ± 2.1^{a}	47.1 ± 2.4^{ab}	161.8 ± 8.1^{a}	60.8 ± 3.8^{a}	7.7 ± 0.5^{a}

*Legends and abbreviation are identical to those in table 1

Table 3. Germination and vegetative growth of 40 days old *Vicia faba* plants as influenced by various acidifying agents to calcareous soil.

Treatment*	Germination	Leaves No. /plant	Shoot length (cm)	Root length (cm)	Shoot FW (g/plant)	Root FW (g/plant)	Shoot DW (g/plant)	Root DW (g/plant)
C	8.7 ± 0.2^{bc}	6.7 ± 0.1^{a}	16.5 ± 0.5^{c}	7.2 ± 0.3^{b}	6.9 ± 0.4^{a}	13.6 ± 1.2^{a}	0.4 ± 0.0^{a}	0.2 ± 0.0^{a}
AS2	10.0 ± 0.2^{d}	8.7 ± 0.3^{c}	39.3 ± 2.1^{g}	10.7 ± 0.2^{c}	13.7 ± 0.8^{d}	3.8 ± 0.1^{d}	1.6 ± 0.1^{g}	0.6 ± 0.0^{cd}
AS4	10.0 ± 0.1^{d}	8.7 ± 0.1^{c}	27.7 ± 1.9^{f}	13.3 ± 0.5^{d}	16.2 ± 1.1^{e}	4.9 ± 0.2^{e}	1.6 ± 0.1^{g}	0.6 ± 0.0^{cd}
CS2	9.0 ± 0.6^{cd}	8.0 ± 0.5^{bc}	26.6 ± 2.4^{e}	9.7 ± 0.2^{c}	9.5 ± 0.5^{b}	2.8 ± 0.0^{b}	1.0 ± 0.0^{c}	0.4 ± 0.0^{ab}
CS4	8.3 ± 0.3^{ab}	8.0 ± 0.3^{bc}	25.7 ± 1.7^{e}	11 ± 0.3^{c}	11.8 ± 1.2^{cd}	3.1 ± 0.0^{bc}	1.3 ± 0.1^{e}	0.5 ± 0.0^{bcd}
ES2	9.3 ± 0.5^{cd}	8.0 ± 0.2^{bc}	24.7 ± 1.2^{d}	9.7 ± 0.1^{c}	10.1 ± 0.7^{bc}	5.2 ± 0.1^{e}	1.1 ± 0.0^{d}	0.64 ± 0.0^{ab}
ES4	10.0 ± 0.3^{d}	8.7 ± 0.2^{bc}	26.5 ± 1.0^{e}	13.1 ± 0.2^{d}	13.6 ± 0.6^{d}	3.4 ± 0.1^{c}	1.4 ± 0.1^{f}	0.7 ± 0.0^{d}
AA1	8.6 ± 0.2^{b}	6.7 ± 0.1^{a}	15.5 ± 0.3^{b}	9.8 ± 0.3^{c}	6.4 ± 0.3^{a}	3.3 ± 0.0^{c}	1.3 ± 0.0^{e}	0.5 ± 0.0^{bcd}
AA2	7.3 ± 1.3^{a}	6.7 ± 0.1^{a}	10.7 ± 0.1^{a}	5.8 ± 0.1^{a}	6.2 ± 0.4^{a}	3.6 ± 0.1^{d}	0.9 ± 0.0^{b}	0.4 ± 0.0^{bc}

*Legends and abbreviation are identical to those in table 1

Table 4. Metabolic pools (mg/g FW) of *Vicia faba* plants grown for 40 days in calcareous soil as influenced by various acidifying agents.

Treatment*	Pigments			Total proteins		Total carbohydrates		Frees amino acids	
	Chl. a	Chl. b	Carotenoids	Shoot	Root	Shoot	Root	Shoot	Root
C	0.57 ± 0.0^{a}	0.37 ± 0.0^{d}	0.51 ± 0.0^{bc}	10.4 ± 0.2^{a}	29.4 ± 2.3^{a}	27.1 ± 2.5^{b}	65.0 ± 5.^{7c}	16.25 ± 0.9^{a}	299.4 ± 10^{a}
AS2	0.58 ± 0.0^{a}	0.38 ± 0.0^{de}	0.52 ± 0.0^{bc}	23.8 ± 1.3^{d}	35.5 ± 1.9^{b}	12.0 ± 0.8^{a}	111.0 ± 8.4^{g}	139.4 ± 10.2^{g}	424.6 ± 16de
AS4	0.56 ± 0.0^{a}	0.49 ± 0.0^{g}	0.57 ± 0.0^{d}	15.1 ± 0.4^{bc}	37.1 ± 2.5^{bc}	39.0 ± 2.8^{d}	89.5 ± 4.5^{e}	44.8 ± 5.2^{b}	394.5 ± 12^{c}
CS2	0.57 ± 0.0^{a}	0.32 ± 0.0^{6c}	0.48 ± 0.0^{ab}	11.2 ± 0.6^{a}	35.0 ± 3.4^{b}	29.0 ± 2.1^{bc}	32.2 ± 1.7^{a}	72.3 ± 6.1^{d}	419.6 ± 9.5^{d}
CS4	0.57 ± 0.0^{a}	0.30 ± 0.0^{bc}	0.47 ± 0.0^{ab}	12.3 ± 0.3^{a}	37.5 ± 1.5^{bcd}	14.4 ± 1.2^{a}	73.0 ± 4.5^{d}	23.5 ± 1.2^{a}	434.7 ± 14^{e}
ES2	0.57 ± 0.0^{a}	0.41 ± 0.0^{ef}	0.53 ± 0.0^{cd}	12.8 ± 0.6^{ab}	42.6 ± 2.5^{d}	47.1 ± 5.1^{e}	55.0 ± 3.8^{b}	64.7 ± 5.1^{c}	473.9 ± 13^{f}
ES4	0.56 ± 0.0^{a}	0.41 ± 0.0^{f}	0.53 ± 0.0^{cd}	12.4 ± 0.2^{a}	51.5 ± 4.0^{e}	60.0 ± 4.7^{f}	78.9 ± 5.6^{d}	96.1 ± 5.6^{e}	537.4 ± 11^{g}
AA1	0.58 ± 0.0^{a}	0.27 ± 0.0^{a}	0.45 ± 0.0^{a}	16.0 ± 1.1^{c}	41.7 ± 2.6^{cd}	30.5 ± 1.9^{c}	101.4 ± 5.9^{f}	113.2 ± 4.7^{f}	637.5 ± 23^{h}
AA2	0.57 ± 0.0^{a}	0.29 ± 0.0^{ab}	0.46 ± 0.0^{a}	12.0 ± 0.8^{a}	27.6 ± 1.5^{a}	13.9 ± 0.9^{a}	57.0 ± 3.1^{b}	49.6 ± 5.2^{b}	312.5 ± 15^{b}

*Legends and abbreviation are identical to those in table 1.

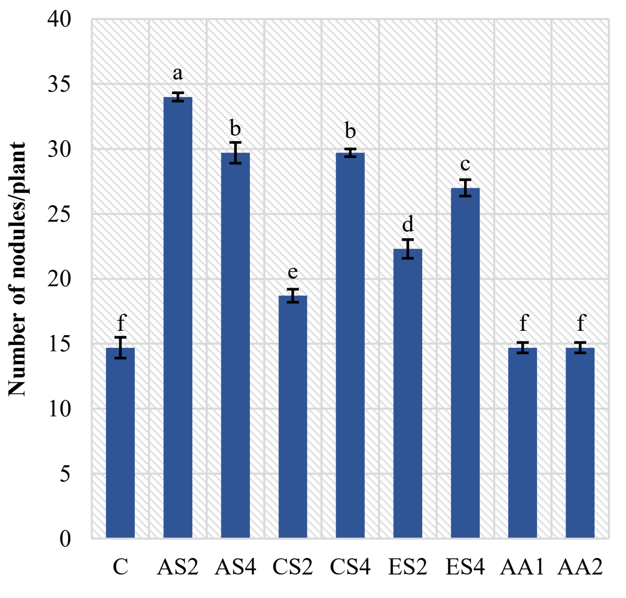

Fig. 1. Number of nodules per plant as influenced by various acidifying agents. C is the control (untreated calcareous soil), AS2 is 200 mg ammonium sulfate /Kg soil, AS4 is 400 mg ammonium sulfate /Kg soil, CS2 is 200 mg calcium sulfate/Kg soil, CS4 is 400 mg calcium sulfate/Kg soil, ES2 is 200 mg elemental sulfur /Kg soil, ES4 is 400 mg elemental sulfur /Kg soil, AA1 is 100 ml acetic acid/Kg soil, AA2 is 200 ml acetic acid/Kg soil. Each column is an average value of three replicates and the vertical bars show a standard error. the statistical difference between the treatment effects is represented by different letters at $p<0.05$.

Metabolic pools: Chlorophyll a per unit leaf fresh mass did not exhibit significant alteration in response to any of the imposed treatments and remained as much as those of plants grown on un-remediated soils (Table 4). Chlorophyll b and carotenoids, however, were significantly enhanced by ammonium sulfate and elemental sulfur treatments at concentrations of 400 mg/Kg soil while they have been inhibited at acetic acid application.

Shoot proteins were not significantly altered by the applied treatments except a significant enhancement at AS4 whereas root proteins were significantly enhanced by ES2, ES4 and AA1 (Table 4). Root proteins were maximally enhanced at ES4, ES2 and AA1, in a manner more or less similar to those of shoots. Total carbohydrates of shoots were significantly increased by AS4, ES2, ES4 and AA1 while those of roots were enhanced by AS2, AS4, ES4 and AA1 (Table 4). Total free amino acids exhibited their lowest contents at control plant shoots, CS4 and AA2; other treatments significantly enhanced amino acids, the highest contents were recorded at AS2 followed by ES4 (Table 4). Roots amino acids content were several folds higher than those of the shoots. Further and significant enhancement at root contents was induced by most of the applied treatments.

Discussion

To face drought and desertification for more food production worldwide, including the Kingdom of Saudi Arabia, it is mandatory to find out solutions to expand the agricultural area; one of which is improving soil characteristics of the desert including calcareous soil, which are vast and abundant in the Kingdom of Saudi Arabia. However, these soils have so many characteristic defects hindering them from being cultivated. They are alkaline and permeable, in addition to being at hot and dry regions. Alkalinity, in turn, causes mineral deficiency, primarily nitrogen, phosphorus and iron (Hartemink & Barrow, 2023). The present work aims to remediate the alkalinity problem, specifically, to render such soils more cultivable. Acidifying the desert soil with acidic fertilizers (ammonium sulfate, gypsum "calcium sulfate"), elemental sulfur, or household vinegar was applied. Faba beans (*V. faba* L.), was cultivated as the case study plant at a laboratory scale in the current work. This plant is proteinaceous and thus it is an important plant for food and feed. In addition, *V. faba* is also beneficial for land reclamation because it contains nitrogen fixing nodules. The main anticipated results from this work such as lowed soil pH, higher water holding capacity, higher soluble minerals and stimulated plant growth have been achieved. Generally, the investigated acidifying materials at their tested concentrations induced significant relief to *V. faba* plants promoting from relieved harsh characteristics of calcareous soils. The ameliorative effect of treatments and concentrations appeared in the following order: ammonium sulfate 400 mg/Kg soil>, ammonium sulfate 200 mg/Kg soil > calcium sulfate 400 mg/Kg soil > calcium sulfate 200 mg/Kg soil > elemental sulfur 400 mg/Kg soil > elemental sulfur 200 mg/Kg soil > acetic acid. Germination, vegetative growth, crop yield and metabolite contents generally exhibited enhancement according to such order. The remarkably higher efficiency of ammonium sulfate, inferred from plant performance i.e., enhanced germination, growth and crop yield. This may indicate that ammonium sulfate served, in addition to soil acidification, as a nitrogen supplement compensating for nitrogen deficiency, which is a prominent characteristic of calcareous soils. On the other hand, acetic acid application showed the least effective treatment to plant relief.

Chlorosis is a common symptom in plants grown in calcareous soils, due to nitrogen deficiency (Dong *et al*., 2018). In the current work, however, increased chlorophyll b and carotenoids but not of chlorophyll a were recorded at some acidification treatments and concentrations. In calcareous soils, mineral N-fertilizers are most commonly used for supplying nitrogen requirements of plants, and ammonium fertilizers are superior compared with nitrate fertilizers due to their effect as soil acidifiers (Maqsood *et al*., 2016). However, when ammonium fertilizers such as ammonium sulfate are added to calcareous soils, nitrogen may release to the atmosphere via gasification of ammonium to ammonia gas (Bonten *et al*., 2016). Loss of N through volatilization was estimated lowest from NH_4NO_3 and NH_4Cl as compared to $(NH_4)_2SO_4$ (Turner *et al*., 2012). Chlorosis can be caused by lime and fertilizers that increase bicarbonate (HCO_3^-) concentration in soil solution. Carbonate (lime) or bicarbonate raises pH, inhibiting plants' ability to obtain iron from the soil solution. Bicarbonate can also reduce a plant's capability to use iron already within the plant (Marschner, 1995). Lime-induced chlorosis has been observed in blueberries. Lower soil pH is desired in many production systems to increase the availability of nutrients; at farm level different sulfur containing compounds such as ammonium-sulfate or elemental sulfur are used to achieve this purpose. Ammonium sulfate reduces volatilization by lowering pH and delaying the urease activity (Chien *et al*., 2011).

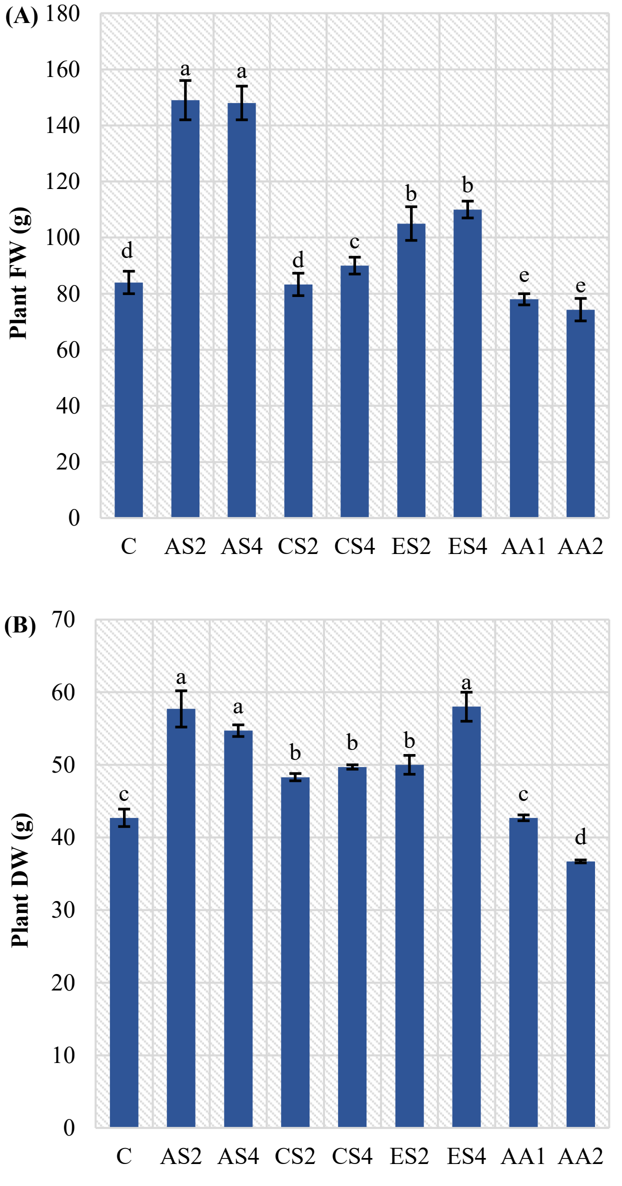

Fig. 2. Plant biomass (g) as influenced by various acidifying agents as: (**A**) fresh weight (FW); (**B**) dry weight (DW). C is the control (untreated calcareous soil), AS2 is 200 mg ammonium sulfate /Kg soil, AS4 is 400 mg ammonium sulfate /Kg soil, CS2 is 200 mg calcium sulfate/Kg soil, CS4 is 400 mg calcium sulfate/Kg soil, ES2 is 200 mg elemental sulfur /Kg soil, ES4 is 400 mg elemental sulfur /Kg soil, AA1 is 100 ml acetic acid/Kg soil, AA2 is 200 ml acetic acid/Kg soil. Each column is an average value of three replicates and the vertical bars show a standard error. the statistical difference between the treatment effects is represented by different letters at $p<0.05$.

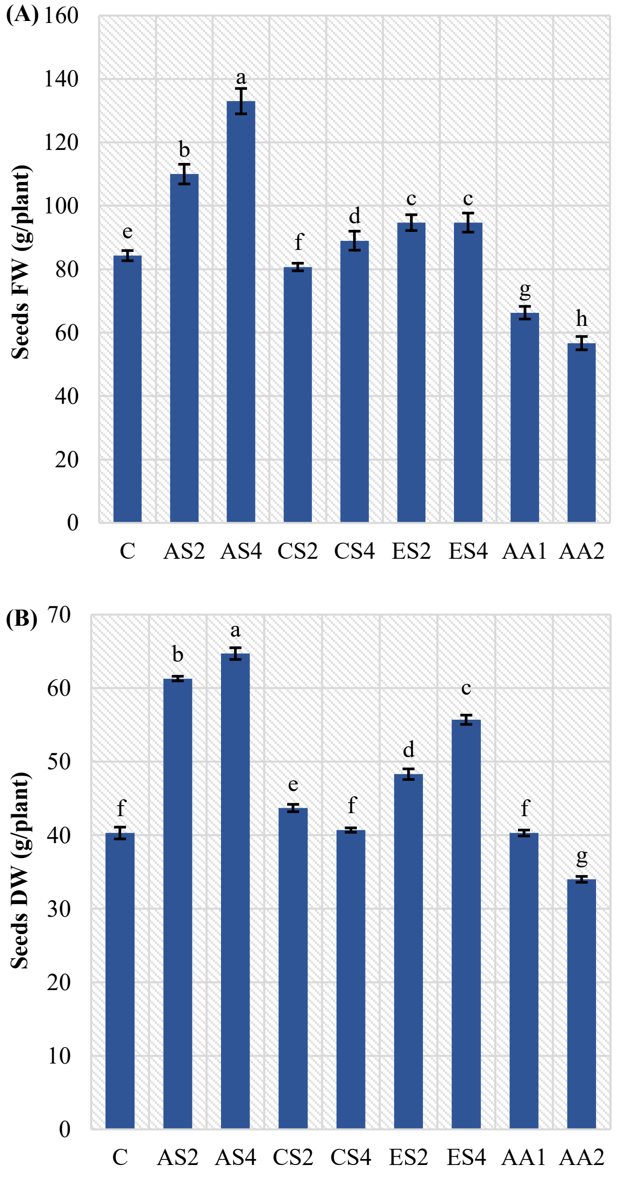

Fig. 3. Seeds weight (g) as influenced by various acidifying agents as: (**A**) fresh weight (FW); (**B**) dry weight (DW). C is the control (untreated calcareous soil), AS2 is 200 mg ammonium sulfate /Kg soil, AS4 is 400 mg ammonium sulfate /Kg soil, CS2 is 200 mg calcium sulfate/Kg soil, CS4 is 400 mg calcium sulfate/Kg soil, ES2 is 200 mg elemental sulfur /Kg soil, ES4 is 400 mg elemental sulfur /Kg soil, AA1 is 100 ml acetic acid/Kg soil, AA2 is 200 ml acetic acid/Kg soil. Each column is an average value of three replicates and the vertical bars show a standard error. the statistical difference between the treatment effects is represented by different letters at $p<0.05$.

In the current work, soil pH was noticeably altered, only at two decimals of digits, following the application of the acidifying treatments; the highest drop in the soil pH of 0.2 unit was brought about at the concentration of elemental sulfur (ES4) followed by ES2 (0.14 unit) and AS4 (0.15). In this respect, Zhu *et al*., (2018) reported the averaged pH decrease for all soil crop combinations was 0.44 units during the period 1980–2010, which is comparable with the reported results of 0.50 units by Guo *et al*., (2010) during 1980s to 2000s, which implies that the model results are plausible. Soil acidification is mainly driven by nitrogen (nitrate) induced base cation leaching, and the net removal of base cations by harvest (Duan *et al*., 2004; 2011). The acidification is expected to accelerate in the future because of increasing N fertilizer application and decreasing N use efficiency (Guo *et al*., 2010). The calcium carbonate buffering system can keep pH at a high level for >150 years (Zhu *et al*., 2018), while in non-calcareous soils a moderate to strong decline in both base saturation and pH is predicted for the coming decades in the “business as usual (BAU)” scenario.

Soil characteristics of water holding capacity, pH and availability of mineral elements, namely, K, P, N, Ca and Mg have been improved by the applied acidifying compounds, which may justify improved performance of *V. faba*. Furthermore, soil acidification can be applied for several years on the same soil samples to elucidate the cumulative effectiveness of the treatments in validating soil characteristics for agriculture. In this respect, Horneck *et al*., (2007) stated that soil acidification usually is a long-term, expensive process and difficult to assess.

Soil with pH greater than 8.4 requires the addition of elemental sulfur to lower pH and the addition of gypsum (calcium sulfate) to lower soil sodium content while soil with pH less than 8.4 generally requires only elemental sulfur to lower pH (Horneck *et al*., 2007). Elemental sulfur is oxidized by *Thiobacillus thioparus* to sulfuric acid, which reacts with calcium (carbonate) to form $CaSO_4$ (gypsum); gypsum reacts with sodium e.g., sodic soil, producing sodium sulfate, which is leachable. Therefore, 33 tons of elemental S would be needed to neutralize the calcium carbonate present in the top foot of soil. This assumes no additional carbonate is added by irrigation water. Applying enough elemental S or gypsum for soil acidification will also fulfil the needed plant nutrition. However, salinity problems may arise following sulfur application at rates higher than those necessary for maximum yields, which needs to be leached or otherwise would reduce yields. Salts in the topsoil will negatively affect soil microorganisms, soil and plant productivity (Papadopoulos, 1995).

Collectively, acidification of the whole soil profile is usually a long-term, expensive process and accumulates salts in topsoil. Instead, localized acidification, which may be accomplished by banding or creating acidifying small holes or zones directly in contact with the root system of crop plants or nearby roots of fruit trees is more applicable. For example, Wiedenfeld (2011) recommended a single addition of elemental sulfur at up to 1120 kg per hectare for sugarcane directly below the seed cane at planting in a calcareous soil.

Conclusion

In conclusion, the acidifying agents at their applied concentrations induced significant relief to *V. faba* plants benefiting from relieved drastic characteristics of calcareous soils. The ameliorative effect of treatments and concentrations can be arranged, on general basis, as follows: ammonium sulfate 400 mg/Kg soil > ammonium sulfate 200 mg/Kg soil > calcium sulfate 400 mg/Kg soil > calcium sulfate 200 mg/Kg soil > elemental sulfur 400 mg/Kg soil > elemental sulfur 200 mg/Kg soil. Acetic acid treatment, however, was the least effective treatment to plant relief and thus it may be excluded from an ameliorative program or, otherwise, higher concentrations would be tested for better effectiveness.

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References

Abdou A.S., F.H. Al Darwish, M.E. Saleh, K.A. El-Tarabily, M. Sofian-Azirun and R.M. Monitor. 2011. Effects of elemental sulfur, phosphorus, micronutrients and *Paracoccus versutus* on nutrient availability of calcareous soils. *Aust. J. Crop Sci.*, 5: 554-561.

Alghamdi, S.A., F.A. Al-Ghamdi, M. El-Zohri and A.A.M. Al-Ghamdi. 2023. Modifying of calcareous soil with some acidifying materials and its effect on *Helianthus annuus* (L.) growth. *Saudi J. Biol. Sci.*, 30(4): 103568.

Bhattacharya, A. 2019. Changing environmental condition and phosphorus-use efficiency in plants. In: (Eds.): Choudhary, K.K., A. Kumar and A.K. Singh. Changing Climate and Resource Use Efficiency in Plants. Academic Press, Cambridge, USA, pp. 241-305. https://doi.org/ 10.1016/ B978-0-12-816209-5.00005-2.

Bolan, N., P. Srivastava, C.S. Rao, P.V. Satyanaraya, G.C., Anderson, S. Bolan and M.B. Kirkham. 2023. Distribution, characteristics and management of calcareous soils. *Adv. Agron.*, 182: 81-130.

Bonten L.T., G.J. Reinds and M. Posc. 2016. A model to calculate effects of atmospheric deposition on soil acidification, eutrophication and carbon sequestration. *Environ. Model Softw*., 79: 75 84.

Chien, S.H., M.M. Gearhart and S. Villagarcía. 2011. Comparison of ammonium sulfate with other nitrogen and sulfur fertilizers in increasing crop production and minimizing environmental impact: a review. *Soil Sci.*, 176(7): 327-335.

Conklin, A.R. 2005. Introduction to Soil Chemistry. Analysis and Instrumentation, 3rd ed., Hoboken: John Wiley and Sons. P.218.

De Vries, W. and M.J. McLaughlin. 2013. Modeling the cadmium balance in Australian agricultural systems in view of potential impacts on food and water quality. *Sci. Total Environ.*, 461: 240-257.

Deluca, T.H., E.O. Skogley and R.E. Engel. 1989. Band-applied elemental sulfur to enhance the phytoavailability of phosphorus in alkaline calcareous soils. *Biol. Fertil. Soils*., 7(4): 346-350.

Dong, Y., W. Chen, Y. Zhuge, Y. Song, G. Hu, Y. Wan and X. Li. 2018. Effect of application of exogenous nitric oxide at different critical growth stages in alleviating Fe deficiency chlorosis of peanut growing in calcareous soil. *J. Plant Nutr*., 41(7): 867-887.

Duan, L., Y. Huang, J. Hao, S. Xie and M. Hou. 2004. Vegetation uptake of nitrogen and base cations in China and its role in soil acidification. *Sci. Total Environ.*, 330: 187-198.

Duan, Y., M. Xu, B. Wang, X. Yang, S. Huang and S. Gao. 2011. Long-term evaluation of manure application on maize yield and nitrogen use efficiency in China. *Soil Sci. Soc. Amer. J.*, 75: 1562-1573.

El-Bakry, F.A., A.E. El-Sonbaty, M.A. El-Sherpiny and N.R. Hashish. 2024. Synergistic Effects of Elemental Sulfur Soil Addition and Beneficial Elements Spraying on Onions. *J. Soil Sci. Agric. Eng.*, 15(6): 135-141.

El-Zohri M., A.F. Hifney, T. Ramadan and R. Abdel-Basset 2014. Use of sewage in agriculture and related activities. In: (Ed.): Pessarakli M. *Handbook of Plant and Crop Physiology*. 3rd ed. CRC Press, Taylor & Francis, pp. 931-966, Print ISBN: 978-1-4665-5328-6, eBook ISBN: 978-1-4665-5329-3.

Guo, J., X. Liu, Y. Zhang, J. Shen, W. Han and W. Zhang. 2010. Significant acidification in major Chinese croplands. *Science*, 327: 1008-1010.

Hartemink, A.E. and N.J. Barrow. 2023. Soil pH-nutrient relationships: the diagram. *Plant & Soil*, 486(1): 209-215.

Horneck, D., D. Wysocki, B. Hopkins, J. Hart and R. Stevens. 2007. Acidifying soil for crop production: Inland Pacific Northwest. PNW 599-E. A Pacific Northwest Extension publication Oregon State University, University of Idaho, Washington State University.

Islam, M., S. Mohsan, S. Afzal, S. Ali, M. Akmal and R. Khalid. 2011. Phosphorus and sulfur application improves the chickpea productivity under rainfed conditions. *Int. J. Agric. Biol.*, 13: 713-718.

Jing, T., J. Li, Y. He, A. Shankar, A. Saxena, A. Tiwariand and M.K. Awasthi. 2024. Role of calcium nutrition in plant Physiology: Advances in research and insights into acidic soil conditions-A comprehensive review. *Plant Physiol. Biochem.*, 108602.

Kayser, A., K. Wenger, A. Veller, W. Attinger, H.R. Felix and S.K. Gupta. 2000. Enhancement of phytoextraction of Zn, Cd and Cu from calcareous soil: the use of NTA and sulfur amendments. *Environ. Sci. Tech*., 34: 1778-1783.

Lowry, O.H., N.J. Rosebrough, A.L. Fare and R.J. Randall. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.

Maqsood M.A., U.K. Awan, T. Aziz, H. Arshad, N. Ashraf and M. Ali. 2016. Nitrogen management in calcareous soils: problems and solutions. *Pak. J. Agri. Sci.*, 53(1): 79-95.

Marschner, H. 1995. Mineral Nutrition of Higher Plants. 2nd Edition, Academic Press, London, England.

Mok, J.S., H.D. Yoo, P.H. Kim, H.D. Yoon, Y.C. Park and T.S. Lee. 2015. Bioaccumulation of heavy metals in oysters from the southern coast of Korea: Assessment of potential risk to human health. *Bull. Environ. Contam. Toxicol.*, 94: 749-755.

Moore, S. and W.W. Stein. 1948. Amino acid free photometric ninhydrin method for use in chromatography of amino acids. *J. Biol. Chem.*, 176: 367-388.

Papadopoulos, I. 1995. Wastewater management for agriculture protection in the Near East Region, Technical Bulletin, FAO, Regional Office for the Near East, Cairo, Egypt.

Rasmussen, P.E. and R.P. Dick. 1995. Long-term management effects on soil characteristics and productivity. In: Proceedings of the Western Nutrient Management Conference. Potash and Phosphate Institute Salt Lake City, UT, March 9-10.

Ren, C., Y.F. Li, Q. Zhou and W. Li. 2021. Phosphate uptake by calcite: Constraints of concentration and pH on the formation of calcium phosphate precipitates. *Chem. Geol.*, 579: 120365.

Saleh F.E., M. El-Zohri, N. Medhat and R. Abdel-Basset. 2015. Stimulatory Influence of organic and bio-fertilizers on yield, seed oils and fatty acid fractions of *Nigella sativa* L. grown in calcareous soil. *American-Eurasian J. Agric. & Environ. Sci.*, 15(10): 1984-1995.

Schlegel, H.G. 1956. Die verwertung organischer säuren durch *Chlorella* im licht. *Planta*, 47(5): 510-526.

Singare, P., N. Meshram and H. Kausadikar 2022. Calcareus soil and their management: A review. *Just Agric.*, 3: 1-6.

Skwierawska, M., L. Zawartka, A. Skwierawski and A. Nogalska. 2012. The effect of different sulfur doses and forms on changes of soil heavy metals. *Plant Soil Environ*., 58(3): 135-140.

Soaud, A.A., F.H. Al Darwish, M.E. Saleh, K.A. El-Tarabily, M. Sofian-Azirun and M.M. Rahman. 2011. Effects of elemental sulfur, phosphorus, micronutrients and *Paracoccus versutus* on nutrient availability of calcareous soils. *Aust. J. Crop Sci.*, 5: 554-561.

Su, X.; A.B., Howell and D.H. D'Souza. 2010. Antiviral effects of cranberry juice and cranberry proanthocyanidins on foodborne viral surrogates–a time dependence study *In vitro*. *Food Microbiol.*, 27: 985-991.

Taalab, A.S., F.A. Hellal and M.A. Abou-Seeda. 2008. Influence of phosphatic fertilizers enriched with sulfur on phosphorus availability and corn yield in calcareous soil in arid region. *Ozean J. App. Sci.*, 1: 105-115.

Turner, D.A., R.B. Edis, D. Chen, J.R. Freney and O.T. Denmead. 2012. Ammonia volatilization from nitrogen fertilizers applied to cereals in two cropping areas of southern Australia. *Nutr. Cyc. Agroecosys.*, 93: 113-126.

Wiedenfeld, B. 2011. Sulfur application effects on soil properties in a calcareous soil and on sugarcane growth and yield. *J. Plant Nutr.*, 34: 1003-1013.

Yousef, A.F. 1999. Analysis methods and devices for soil and water. King Saud University, Riyadh.

Yuncong, L. 2001. Calcareous soils in Miami-dade county. Fact. Sheet SL 183, a publication of the Soil and Water Science Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Zekri M., T. Obreza and K. Morgan. 2010. Nutrient management of citrus trees on alkaline soils. *Citrus Indus.,* 91(10): 10-13.

Zhou, H. 2015. Effects of cropland soil acidification and remediation in Jiaodong of Shangdong Province. Thesis. China Agricultural University, Beijing.

Zhu Q., X. Liu, T. Hao, M. Zeng, J. Shen, F. Zhang and W. De Vries. 2018. Modeling soil acidification in typical Chinese cropping systems. *Sci. Total Environ.*, 614: 1339-1348.