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Original article

Modifying of calcareous soil with some acidifying materials and its effect on *Helianthus annuus* (L.) growth

Sameera A. Alghamdi [a,*], Fatimah A. Al-Ghamdi [a], Manal El-Zohri [a,b], Amal A.M. Al-Ghamdi [a]

[a] Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[b] Department of Botany and Microbiology, Faculty of Science, Assiut University, Assiut 71516, Egypt

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ABSTRACT

Calcareous soils contain a high concentration of calcium carbonate ($CaCO_3$), which influences soil properties related to plant growth. Humic acid (HA) and ammonium molybdate (AM) were added as treatments for calcareous soils at concentrations of 0.1, 0.5 and 1 g/l respectively. The pots were divided into three groups. The first set of groups were irrigated with AM, while the second set of groups were irrigated with HA. As a control, the third group was irrigated using only tap water. Many soil properties and plant characteristics were measured during the experiment. The results showed that most of the studied treatments aided to increase organic carbon of calcareous soil and improved sunflower height, leaf area and shoot and root biomass. All investigated treatments significantly enhanced carbohydrates content in the sunflower shoots, except the treatment with 0.1 g/l AM, while only the with AM (under all studied concentrations) significantly enhanced carbohydrates content in roots higher than untreated. Proteins content in the shoots and roots of sunflower significantly increased when treated only with 1 g/l HA higher than control. The amino acid content of sunflower roots enhanced when treated with 0.1 and 1 m/l HA and 0.5 g/l AM Evidently, acidifying materials enhanced the calcareous soil and increased productivity.

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1. Introduction

There is a significant amount of calcium carbonate ($CaCO_3$) in calcareous soils, which influence plant growth characteristics, whether physical (soil water correlation and soil crusting) or chemical (availability of plant nutrients) (Hassan, 2012). This type of soil is common in arid areas where water scarcity, drought, hot weather, and wind erosion all have an impact on the extent of soil formation. In terms of the soil's chemical weather conditions, it runs slowly due to a lack of precipitation, and soluble products such as calcium carbonate remain because they are not washed away (Al-Saeedi, 2022). Moreover, plants in calcareous soils, where pH is high and $CaCO_3$ is dominant, suffer from low availability of P and K, resulting in far more serious problems than the deficiencies of not availing these nutrients is one of the critical objectives in plant nutrition (Al-Dubai et al., 2017). Additionally, Calcareous soils' cultivation is arduous, with challenges such as a high rate of infiltration, a poor structure, a low level of water organic matter, holding capacity, Content of clay, cation exchange capacity (CEC), and nutrients' availability especially nitrogen (N), phosphorous (P), and micronutrients), nutrients' loss via deep percolation and leaching, high pH, surface crusting and cracking, and nutritional imbalance between elements, e.g., magnesium (Mg), potassium (K), and calcium (Ca) (Pal et al., 2015).

In calcareous soils, low productivity is associated with poor physiochemical characteristics, low organic matter content, and insufficient nitrogen availability. Additionally, high pH values result in low availability of phosphate (P), zinc (Zn), and iron (Fe). Usually, chlorotic symptoms emerge in plants that grow in calcareous soils (Badawy, 2011). The growth of plants under punishing conditions (such as in Rhododendrons with high pH values and calcium contents) produces morphological and metabolic changes, which are most evident in leaf chlorosis and restricted root growth (Giel and Bojarczuk, 2011). Generally, calcareous soils

* Corresponding author.
E-mail addresses: saalghamdy1@kau.edu.sa (S.A. Alghamdi), falghamdi0517@stu.kau.edu.sa (F.A. Al-Ghamdi), melzohri@kau.edu.sa, mnzohri@aun.edu.eg (M. El-Zohri), aamghamdi@kau.edu.sa (A.A.M. Al-Ghamdi).

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are reclaimed by chemical modification (Abdel-Fattah, 2012; Cucci et al., 2012). In soils amended with calcareous materials, concentrations of Fe, K, Si, Ca, Mg, and Mn are higher due to their previous presence in carbonate rocks that make up the raw material of the amendments (Monfort-Salvador et al., 2015; Attia, 2019; Abd-El-Aziz, 2020). The amount of P in the soil solution increased with the addition of P from different P sources, such as rock phosphate and humic acid (HA), while the high pH and lime content in calcareous soils decreased it by causing its insoluble complexes (Izhar Shafi et al., 2020).

The second most important oil crop after soybeans is *Helianthus annuus* (Paniego et al., 2002). The name 'sunflower' derives from the image and size of the flower resembling that of the sun, as well as the fact that the flower rotates around it (Adeleke and Babalola, 2020). Usually blooming in midsummer, they grow rapidly and reach flowering maturity in about 80 to 120 days after germination. The only mandatory requirements for growing sunflowers are a sunny site and well-drained soil (Bantan and Abu-Zied, 2014). It contains dietary fiber and high-quality oil, which improves overall human health. Furthermore, the plant is used as a source of dietary fiber and medicinal purposes, as well as for ornamentation and livestock feed (Khan et al., 2015). Therefore, this study aims to assess whether using different concentrations of acids reduces the effect of insufficient availability of elements for sunflowers in limestone soils, considering the actual needs of the plant and locations so that high outputs can be obtained with minimal processors.

2. Materials and methods

2.1. Soil collection

The soil was gathered from the 10–20 cm depth at the east of Jeddah at 21°31'05.9"N 39°16'35.3"E. Soil alkalinity was tested according to Channarayappa and Biradar (2018), where the soil test gave effervescence, visibly releasing CO_2 gas when treated with acetic acid.

2.2. The experimental design

The pot experiment was conducted at King Abdulaziz University, Saudi Arabia. Sunflower seeds were sown in plastic pots with 3 kg of previously collected soil (chemical and physical aspects of the soil are shown in Table 1) under natural light condition in a greenhouse. The plants were watered with tap water using the soil's Field Capacity (FC)% level until the third true leaf appeared. Following that, the pots were divided into two groups. The first groups were irrigated with (AM) at three subgroup concentration levels (0.01, 0.5 and 1 g/l). Similarly, the second groups were irrigated with (HA) in three subgroups with varying concentrations (0.1, 0.5 and 1 g/l). The plants in the control group were only watered with tap water. Each treatment was irrigated twice a week at FC%. The experiment was conducted in a completely randomized design with three replicates. After two weeks of treatment, samples of plant tissues were collected for analysis.

Table 1
The physical and chemical characteristics of the calcareous soil used in the experiment before treatment.

Soil properties	Values	Soil Properties	Values
Soil Size Distribution		Soluble Cations	
Sand (%)	84.03	Ca^{++}	698.39
Silt (%)	15.89	Fe^{++}	587.42
Clay (%)	0.08	K^{+}	31.69
Soil Texture	Sandy	Mg^{++}	6759.58
Organic Matter	202.30	Zn^{++}	1.39
Field Capacity (%)	4.96	EC mm hos/cm (1:4)	1.64
Total Soluble Salts	4630.00	pH (1:4)	7.85

2.3. Soil analysis

The soil analysis was carried out for all soil samples taken during the period of experiment.

2.3.1. Soil texture

The soil samples were weighed at 100 g and sieved using various sieve diameters (mesh holes within 0.5 and 0.005 mm). The size of soil particles was assessed depending on the USDA classification was weighed separately and the relative percentage of clay, silt, and fine and coarse sand was gauged according to Al Yamani and El Desouki's method (2006).

2.3.2. Soil pH and EC

In a shaker, 10 g of soil and 40 ml of distilled water were shaken for 24 h. Afterwards, the solution was filtered with a filter paper according to Richards (1954). The pH meters and electrical conductivity meters were used to measure the pH and EC of soil solutions (McKeague, 1976).

2.3.3. Total soluble salts

The method used was American Public Health Association (APHA) 254 °C. (RICI MAAZ Chemical and Environmental Testing Laboratory, Dammam, Analytical Chemistry Unit ACAL)).

2.3.4. Organic carbon

The method (APHA) 5310B. (Analytical Chemistry Unit (ACAL), RICI MAAZ Chemical and Environmental Testing Laboratory, Dammam).

2.4. Plant analysis

2.4.1. Fresh and dry biomass of plants' shoots and roots

Following the experimental period, the samples were harvested, rinsed with distilled water, and dried with tissue paper. The harvested shoots and roots samples were then wrapped in tin foil and oven dried at 70 °C until constant weight of each sample was reached (approximately after 48 h), to assess the dry weight (g) (Shanker et al., 2005).

2.4.2. Shoot and root length

The heights (cm) of three randomly selected plants from each treatment were measured using a metric ruler (Shanker et al., 2005).

2.4.3. Leaf area

The leaf area (cm^2) was gauged in three plants from each treatment according to Larcher (1995).

2.4.4. Chlorophylls and carotenoids

The contents of chlorophyll *a*, chlorophyll *b* and total carotenes (mg/g FW) were assessed in a spectrophotometric manner in acetone extracts (Metzner et al., 1965).

2.4.5. Soluble carbohydrates

The colorimetric anthrone method was used to estimate the soluble carbohydrate concentration (mg/g DW) (Fales, 1951; Schlegel, 1956). A spectrophotometer with a wavelength of 620 nm was used to determine the amount of carbohydrates.

2.4.6. Soluble protein

Utilizing the Lowry protein quantification technique (Lowery et al., 1951), the soluble protein concentration (mg/g DW) of the shoot extract was determined. A spectrophotometer with a wavelength of 750 nm was used to measure the protein.

2.4.7. Free amino acids

The quantity of free amino acids concentration (mg/g DW) in the shoot extraction was estimated consistent with Moore and Stein (1948) using a spectrophotometer at wavelength 570 nm.

2.5. Statistical analysis

The obtained data underwent a One-Way Analysis of Variance (ANOVA) using the SPSS statistical package. To compare between means, Duncan's multiple range tests ($p < 0.05$) were employed.

3. Results

3.1. Soil analysis

The data in Fig. 1 (A) demonstrate the effect of the different treatments on soil pH value. All treatments used had insignificant differences. On the other hand, Fig. 1 (B) the treatments HA 0.1, HA 1, and AM 1 g/l did not affect EC of soil. EC decreased significantly by about 22.6 % and 42.5 % lesser than the control group when treated with HA 0.5 g/l and AM 0.1 g/l, respectively. However, a significant increase was found only in soils treated with AM 0.5 by about 33.7 % higher than control. Organic carbon in the soil samples treated with HA 1 g/l decreased by 5.06 %, same for soil treated with AM 1 g/l, which decreased by 3.23 % compared with control. However, the treatment with AM 0.1 g/l induced an immense surge in the percentage of organic carbon by 3.49 % higher than control Fig. 2 (A).

The data represented in Fig. 2 (B) shows the effect of various concentrations of treatments on the percentage of total soluble salt

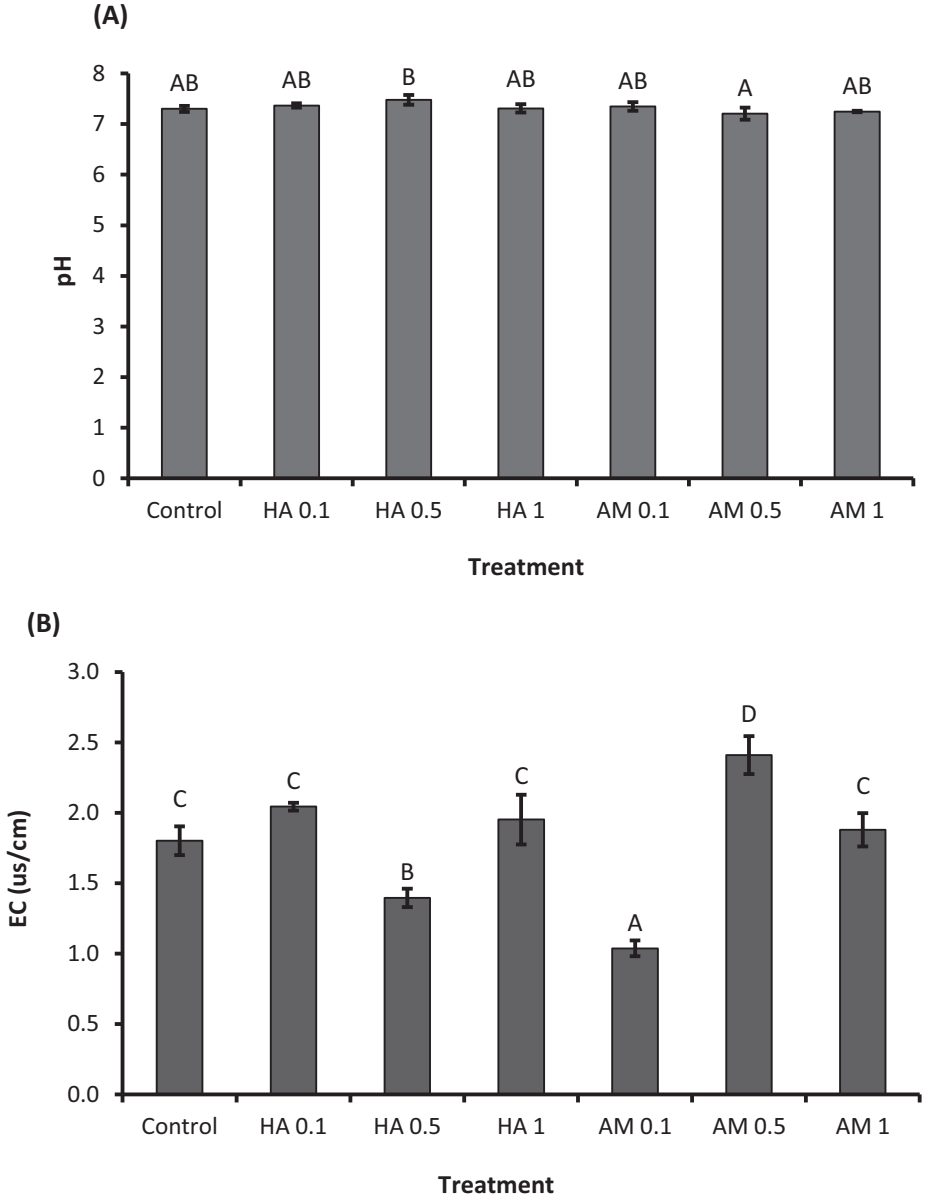

Fig. 1. Ph value (a) and ec value (b) of soil samples as affected with different concentrations of (ha) and (am), each columns is an average rate of three replicates and the vertical bars show a standard error. the statistical difference between the treatment effects is represented by different letters at $P < 0.05$.

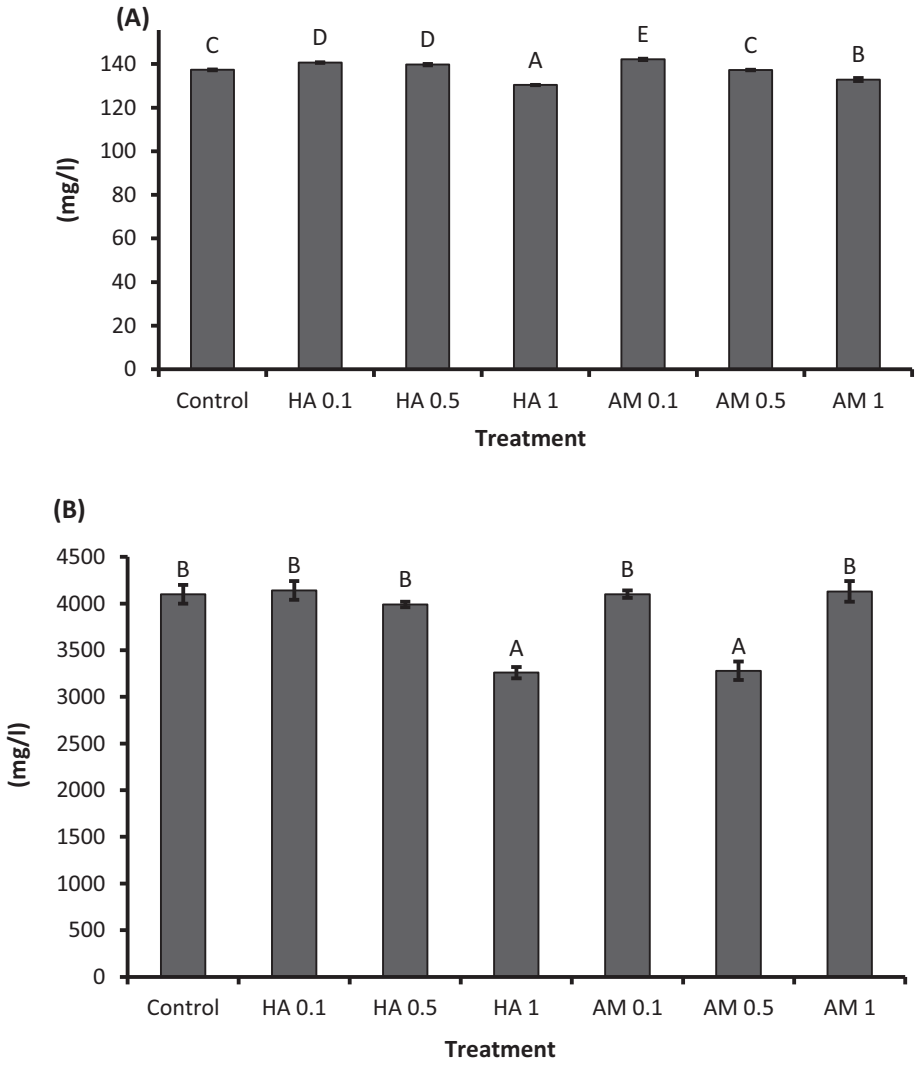

Fig. 2. Organic carbon (A) and total soluble salt (B) concentrations of soil samples as affected with different concentrations of (HA) and (AM), each column represents the average value of three replicates, and the vertical bars represent the standard error. The statistical difference between the treatment effects is represented by different letters at $P < 0.05$.

in the soil and control soil. The increase in the amount of total soluble salt resulting from calcareous soils was not affected by most treatments, except HA 1 g/l and AM 0.5 g/l where there was a significant decrease of 20.5 % and 20 %, respectively, lesser than control.

3.2. Plant analysis

3.2.1. Biomass

As shown in Fig. 3 (A), shoots' fresh weight increases significantly with the use of HA 1 g/l at all concentrations studied. The highest significant difference in the treatment AM 0.5 g/l was about 34.8 % higher than control. The fresh weight of the roots showed a positive significant difference with the treatments HA 1 g/l. Approximately 58 % higher increase than control was observed in treatment AM 0.5 g/l. Treatment HA 0.5 g/l showed significant reductions by 28 % less than control.

The dry weight of the sunflower roots and shoots was highly affected by most of the studied treatments when compared to the untreated control Fig. 3 (B). The results indicate that the treatment with concentration HA 1 g/l and all studied concentrations of AM increased the dry weight of the shoot by about 45.7 %, while the decrease was significant at concentration HA 0.5 g/l by an average of 17.5 % compared with control. However, there was a substantial surge in the dry weight of the roots at about 129.4 % higher than control in response to treatment with AM 0.1 g/l.

3.2.2. Shoot and root lengths

The length of the shoot, as shown in Fig. 4 (A), was not affected clearly in the treatments HA 1, AM 0.5, and AM 1 g/l. While the effect of treatment AM 0.1 g/l outperformed the average shoot height where a significant difference appeared in the length shoot by 7.5 %. There was a significant decrease in shoots length by 6.8 % and 9 % following treatment with HA 0.1 g/l and HA 0.5 g/l, respectively, which did not show a great effect on the shoot. On the other hand, Fig. 4 (A) illustrates the significant differences in root length between the different concentrations of treatments. The higher difference appeared in treatment HA 1 g/l, where it was about 29 % higher than control.

3.2.3. Leaf area

According to the analysis of variance in Fig. 4 (B), there was a significant difference between HA 0.5 g/l and AM 0.1 g/l on leaf area that was 34 % higher than control. While the decrease was sig-

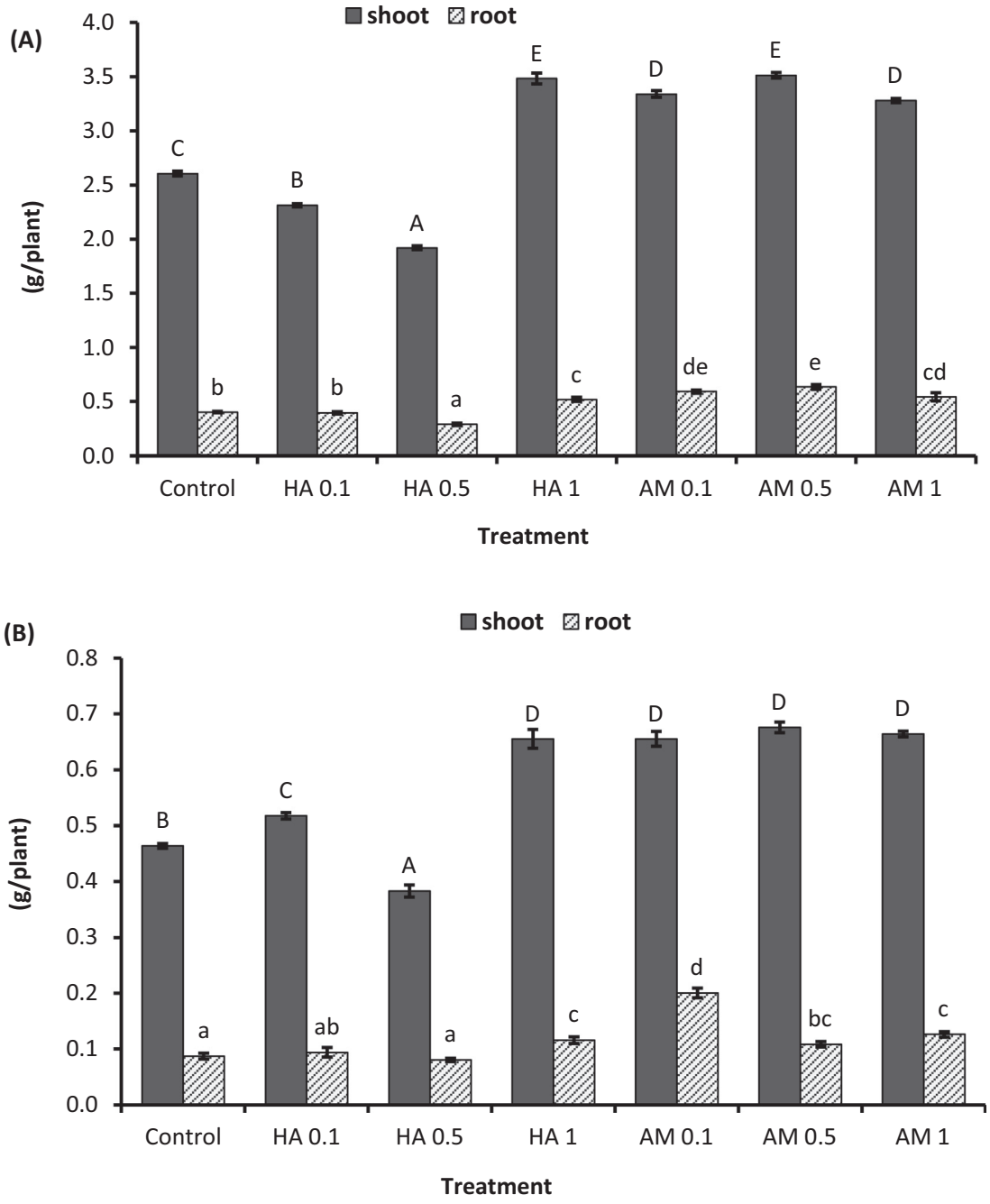

Fig. 3. Fresh weight (A) and dry weight (B) of shoots and roots of sunflower plant as affected with different concentrations of (HA) and (AM), each columns is an average rate of three replicates and the vertical bars show a standard error. The statistical difference between the treatment effects is represented by different letters at $P < 0.05$.

nificant for the two treatments AM 0.5 g/l and AM 1 g/l by about 15 % and 29 %, respectively, compared with control.

3.2.4. Pigments content

The effects of the treatments on the pigment content in sunflower plants can be seen in Fig. 5. The results indicate that the treatments with HA 0.1, HA 1, AM 0.1, and AM 1 g/l did not increase chlorophyll *A* levels. The concentration HA 1 g/l was the lowest in terms of significant difference; it was about 66.8 %. Meanwhile, the concentration AM 0.5 g/l significantly increased chlorophyll *A* concentration by about 30 %. A significant increase in chlorophyll *B* content was seen with treatment AM 0.5 g/l by approximately 12.4 % higher than with control. All other treatments had a negative effect on the amount of chlorophyll *B* in the plant compared with the control, and the addition of treatment HA 0.1 g/l was the lowest in terms of significant difference by about 37 %. In terms of carotenes, it appears the significant difference in all of them was less than the control, with the exception of treatment HA 1 g/l, which was equal to the control and showed no significant difference.

3.2.5. Primary metabolites content

The effect of various treatments on the amount of carbohydrates in the shoot sunflower plant is shown in Fig. 6 (A). All investigated treatment significantly enhanced carbohydrates content in plant shoot higher than un treated control, except the treatment

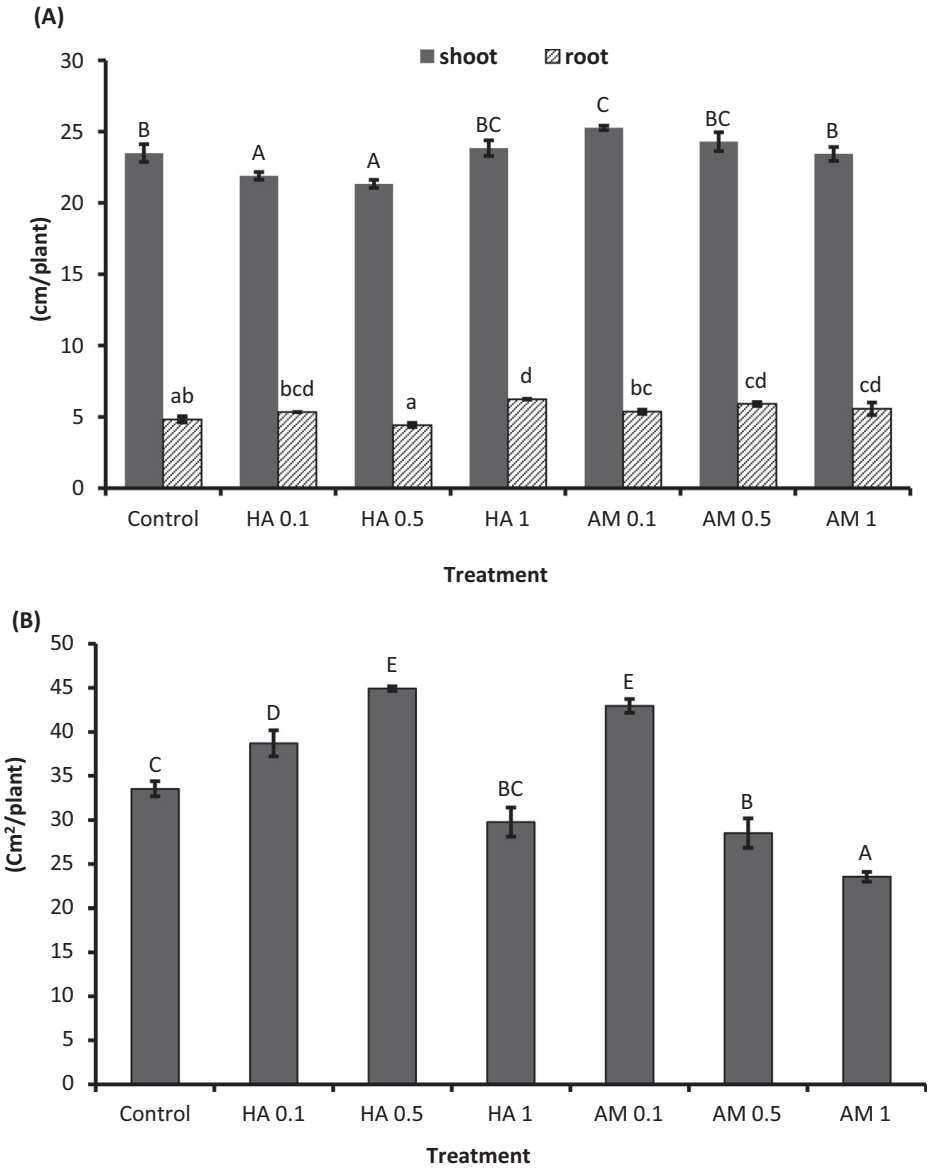

Fig. 4. Shoot and root length (A) and leaf area (B) of sunflower plant as affected with different concentrations of (HA) and (AM), each columns is an average rate of three replicates and the vertical bars show a standard error. The statistical difference between the treatment effects is represented by different letters at $P < 0.05$.

AM 0.1 g/l. The most significant induction was seen in treatments HA 1 g/l and HA 0.1 g/l, which increased carbohydrates content by approximately 67.9 % and 65.2 %, respectively. In terms of carbohydrates in roots, the results showed that there was no significant difference between treatments HA 0.5 g/l and AM 0.1 g/l when compared to the control. While a decreasing significant difference appeared in treatments HA 0.1 g/l and HA 1 g/l, which were about 26.3 % and 27.9 % less than the untreated control, respectively. The highest significant increase was found in the AM 1 g/l treatment, which was 48.6 % higher than the control.

The analysis of variance in Fig. 6 (B) shows that the protein content in the shoots of sunflower plants did not increase significantly under the influence of different treatments except when treated with concentration HA 1 g/l, and this significant increase is approximately 14.4 % higher than the control. The same treatment resulted in a 54 % increase in the percentage of protein in the roots. The remaining treatments had no positive effect on shoots, with the highest reduction was 61.3 % less than control when treated with AM 0.1 g/l.

The amino acid content of sunflower shoots was unaffected by any treatment. Fig. 6 (C) shows that the smallest significant decrease occurred on treatment HA 0.1 g/l, which was approximately 94 % less than the control. On the contrary, in the roots, this concentration increased the amount of amino acids by approximately 385.7 %, when treated with AM 0.1 g/l.

4. Discussion

The agricultural areas with calcareous soils are considered barren land and have limited yield in crops, and under these conditions plants suffer from a kind of stress due to the abundance of calcium carbonate (Mubarak et al., 2021; Salem, 2021). A goal of

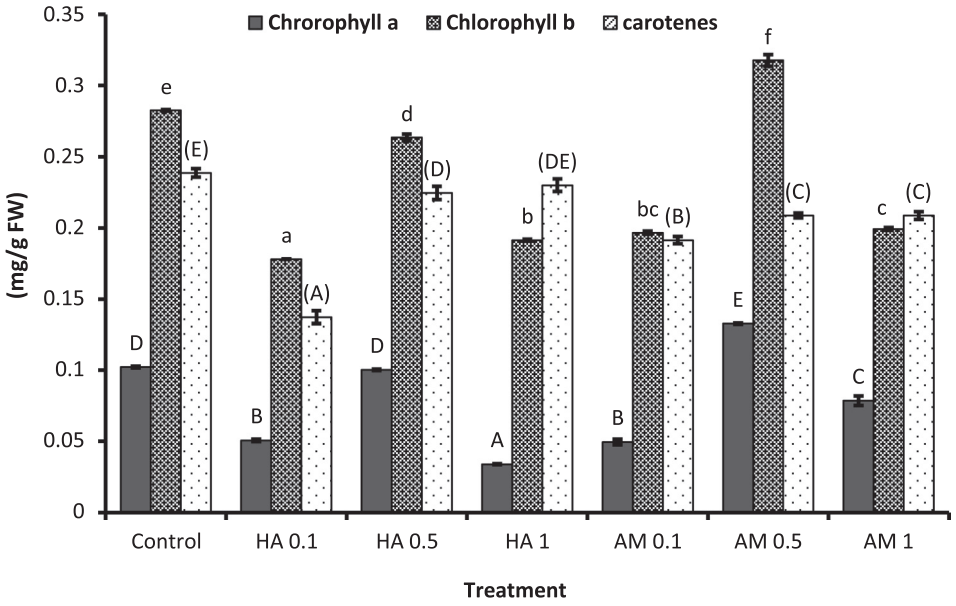

Fig. 5. Pigments concentration of sunflower plant as affected with different concentrations of (HA) and (AM), each columns is an average rate of three replicates and the vertical bars show a standard error. The statistical difference between the treatment effects is represented by different letters at $P < 0.05$.

this research is to investigate whether using different acid concentrations in limestone soil reduces the insufficient availability of elements for sunflowers, considering the actual needs of the plant and locations so that high outputs can be obtained with minimal processors. According to the results, humic acid treatment improved the properties of calcareous soil, leading to higher levels of soil organic carbon. The findings of Belal et al. (2019) confirm that humic acid can be added to calcareous soils to repair them. Humic acid application led to improving the reactions that form complexes of organic clays in limestone soils. These reactions take part in forming stable humus, which enhances the body and biological and chemical aspects of the soil (Syed-Bagheri, 2010). Also, the addition of ammonium molybdates at two concentrations of 0.5 and 1 1 g/l had a productive effect on soil properties (reducing pH of calcareous soil). As confirmed by Qu et al. (2011), ammonium molybdate treatment alters the pH of soil. When HA was added at a concentration of 1 g/l, a significant improvement was observed in sunflowers' biomass, consistent with Mahmoud et al. (2011). Humic substances have been proven to affect plant growth processes both in vitro and the field, increasing root growth and aerial growth in plants (Bezuglova and Klimenko, 2022). Introducing HA to calcareous soils enhanced the biological aspects of the soil and incited the remittance of nutrients from the indigenous available soil sources besides facilitating motion toward plants' roots, thus facilitating plant's absorption. The roots of sunflowers at 1 g/l HA were longer, presumably increasing biomass and increasing nutrient absorption, which is consistent with what was described previously Seiam and Sallam (2021).

In a study by Khattak and Dost (2010), plant height increased with the use of HA, suggesting that applying HA with fertilizers could enhance nutrient uptake and plant growth. This might be related to HA ability to enhance soil biochemical settings, including microbial and enzymatic activities, soil water retention, and cation exchange capacity. The outcomes of this study also showed that the ammonium molybdate increased biomass, as mentioned before by Qu et al. (2011) in their experiment on alfalfa plants. They observed that ammonium molybdate enhanced alfalfa plants through more biomass. The control was low in biomass yield compared with the high concentration treatments HA and AM.

When plant height was measured, the treatment with two concentrations of AM 0.5 g/l and 1 g/l, increased root length. This is consistent with the findings of Qu et al. (2011) who reported that, root lengths in alfalfa plants treated with ammonium molybdate are longer than in control plants. Humic acid increases the rate of plant growth and creates the best conditions for cell division (Pettit, 2004). The treatment with 0.1 and 0.5 g/l HA resulted in an increase in leaf area, according to the results. This is consistent with the findings of Saif El-Deen et al. (2011), who discovered that spraying humic acid on potato plants increased the strength and activity of vegetative growth as measured by plant height and leaf area. The increase in chlorophyll content in plants treated with ammonium molybdates at a concentration of 5.0 % may be attributed to the nitrogen component that is included in the composition of the porphyrin rings, which then enters into the formation of the chlorophyll molecule. Manganese, iron, and copper elements also Contribute to the synthesis and construction of chloroplasts, either directly or indirectly (Taiz and Zeiger, 2006).

It is also clear that HA can form natural chelating compounds with the elements, thus protecting the elements from washing or fixing. This increases the chance of absorption by the plant (Abu-Nekta and AL-Shatter, 2011), and this appeared to us more clearly when treated with a concentration of 1 g/l reflecting positively in the increase in the amount of manufactured carbohydrates and proteins needed to build plant tissues (Sanchez-Sanchez et al., 2002). It is clear that humic acids have a direct effect on the various vital processes of plants, including respiration, photosynthesis, protein synthesis, and enzymatic reactions, as their effect resembles that of plant hormones; humic acids can be considered stimuli for plant growth (Phuong and Tichy, 1976; Zandonadi et al., 2007).

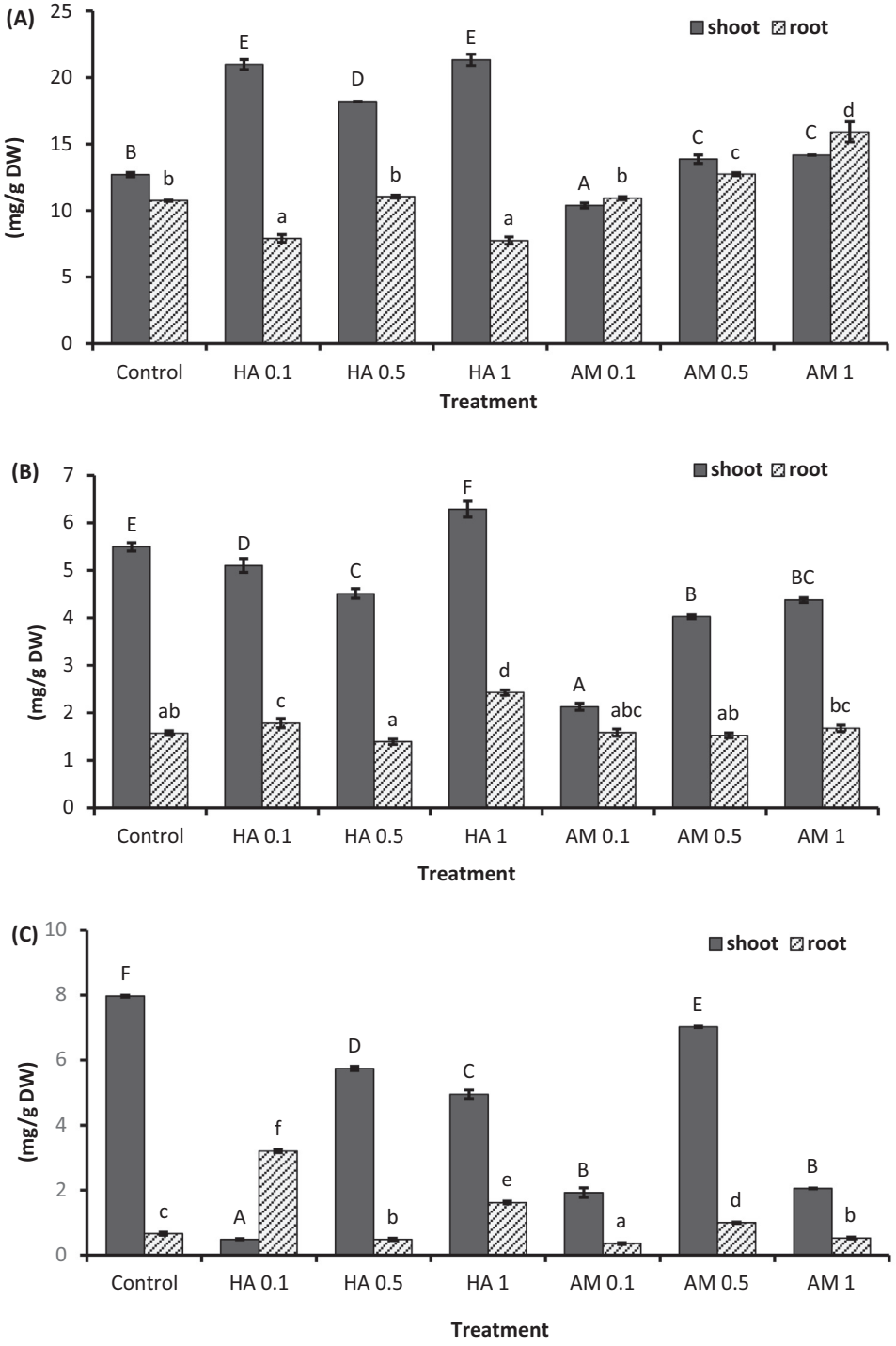

Fig. 6. Soluble carbohydrates (A), proteins (B) and amino acids (C) concentrations as affected with different concentrations of (HA) and (AM), each columns is an average rate of three replicates and the vertical bars show a standard error. The statistical difference between the treatment effects is represented by different letters at $P < 0.05$.

5. Conclusion

A goal of this research is to investigate whether using different acidifying agent's in limestone soil reduces the insufficient availability of elements for sunflowers, considering the actual needs of the plant and locations so that high outputs can be obtained with minimal processors. According to the results, humic acid treatment improved the properties of calcareous soil, leading to higher levels of soil organic carbon. Also, the addition of ammonium molybdates at two concentrations of 0.5 and 1 1 g/l had a productive effect on soil properties (reducing pH of calcareous soil). The treatment with 0.5 g/l and 1 g/l AM, increased root length. The treatment with 0.1 and 0.5 g/l HA resulted in an increase in leaf area. The treatment with 1 g/l HA and all studied concentrations of AM increased sunflower shoot and root biomass. All investigated treatments significantly enhanced carbohydrates content in the sunflower shoots, except the treatment with 0.1 g/l AM, while only the with AM (under all studied concentrations) significantly enhanced carbohydrates content in roots higher than untreated. Proteins content in the shoots and roots of sunflower

significantly increased when treated only with 1 g/l HA higher than control. The amino acid content of sunflower roots enhanced when treated with 0.1 and 1 m/l HA and 0.5 g/l AM.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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