Rebalance the Nutritional Status and the Productivity of High CaCO$_3$-Stressed Sweet Potato Plants by Foliar Nourishment with Zinc Oxide Nanoparticles and Ascorbic Acid

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1. Introduction

Micronutrients and phosphorus (P) availability are revoked by calcium carbonate in calcareous soils [1,2]. Therefore, the strategy of balanced fertilization with macro and micronutrients in plant nutrition is essential for crop production in arid and semi-arid regions and the Mediterranean basin [3]. The calcareous soil is known to contain an amount of calcium carbonate (CaCO$_3$) that distinctly affects the physical, chemical, and biological soil-related properties of plant growth and soil–water relations. For example, soil crust caused by calcium carbonate prevents root expansion and the unavailability of nutrients [4]. Although micronutrients are needed in small quantities, they play vital roles in the development of plants. The great importance of micronutrients is due to their stimulatory and catalytic effects on metabolic processes and their positive effects on plant yield and quality [5,6].

Zinc (Zn), listed as a very essential micronutrient, is a component of various enzyme systems for energy production, and it maintains the structural integrity of biomembranes and regulates growth [5]. Zn is required by many enzymes for nitrogen metabolism, energy transfer, and protein synthesis [7]. Sandy soils with high carbonate content mainly...
suffer from zinc deficiency, which is also proven in alkaline soils with high pH, P, and iron (Fe) [8]. To improve the efficiency of fertilizer use, the nanotechnology strategy has been examined, but not on a large scale yet. The privilege of using nutrients, including Zn in the nano-scale, is to increase their ability towards diffusion through the pores of plant cells, and mutagens can occur in the plant via these nanoparticles (NPs), which improve plant growth and yield [9,10]. Nanotechnology in the field of agriculture focuses on targeted farming, which involves applying NPs with peerless properties to enlarge crop productivity [11]. The small size of NPs, whose diameters range from 1 to 100 nm in length, has an enormous surface area that modifies their physical and chemical properties compared with bulk materials [12,13]. NPs can be manufactured from a variety of bulk materials and their mode of action can be explained based on chemical properties and particle size and shape [14].

Zinc oxide nanoparticles (ZnONPs) are used as a strategy to fertilize plants through their foliage making Zn more available for rapid and efficient absorption through the leaves while minimizing the rate of Zn added [15]. Oxidative damage is attenuated along with increasing Zn content and other nutrients and plant productivity in various crops by applying ZnONPs as leafy nourishment [15–17].

Several stress-relieving studies use ascorbic acid (ASA) as an indicative compound [18]. ASA is one of the most effective growth regulators and plays multiple roles in many developmental processes including cell division and cell wall expansion in favor of plant growth under different stressors [19,20]. ASA is a pivotal member of the ASA–glutathione cycle, and it has a beneficial effect on the plant under stress conditions. In stressed plants, ASA plays an antioxidative role in scavenging the free radicals, increasing crop yields, and protecting plants from various environmental stresses [21].

Sweet potato (Ipomoea batatas L.) belonging to the family of Convolvulaceae has become the most widely distributed crop in most developing countries concerning its important role in ensuring food security and incomes for developing countries. It is grown globally on an area of about 8.1 million hectares, yielding 106.6 million tons; with an average yield of about 13.15 tons per hectare. Based on this information, sweet potato is the seventh most important food crop in the world in terms of production [22].

At the field level, the response of sweet potato grown under a high level of carbonate (calcareous soil conditions) to leafy nourishment with ZnONPs, ASA, or mixtures thereof has not yet been investigated. Because of the increased proportion of soils that suffer from a high content of calcium carbonate that has adverse effects on plants, especially in dry areas, solutions had to be found to overcome this problem. This study hypothesized that foliar nourishing with nutrients such as Zn in nanoparticles along with antioxidants such as ASA will help sweet potato plants to overcome the adverse effects of high calcium carbonate content. Therefore, this study aimed at exploring the potential positive influences of foliar-applied ZnONPs, ASA, or ZnONPs+ASA in attenuating the stress effects of calcareous conditions on the nutritional status and productivity of sweet potato plants.

2. Materials and Methods

2.1. Experimental Location, Timing, Planting Material, and Weather Conditions

The present study was fulfilled utilizing the experimental Farm at Fayoum University (29° 17’N; 30° 53’E), Egypt, during the summer seasons of 2019 and 2020. The cultivation of the tested sweet potato was achieved by utilizing vine cuttings, which were transplanted on 10 April 2019 and 19 April 2020. The climatic data as an average of the two growing seasons (April to August) of the region are provided in Table 1. The soil textures tested were in both seasons, respectively, sandy clay loam and sandy loam, and classified as typic torripsamment, siliceous, and hyperthermic [23]. A standard variety of sweet potato (Ipomoea batatas L.) cv. Beauregard was tested by collecting vine cuttings from AGROFOOD Farm at Nubaria District, Egypt.
Table 1. Average weather data for the Fayoum region, Egypt, over the sweet potato growing seasons.

| Month | Average Day Temperature (°C) | Average Night Temperature (°C) | Average Relative Humidity (%) | Average Speed of Wind (ms⁻¹) | Average Measured Pan Evaporation Class A (mm d⁻¹) | Average Precipitation (mm d⁻¹) |
|-------|------------------------------|------------------------------|-------------------------------|-----------------------------|-----------------------------------------------|-------------------------------|
| April | 32.9                         | 16.1                         | 32.3                          | 1.87                        | 5.58                                           | 0.02                          |
| May   | 35.8                         | 18.9                         | 34.1                          | 1.88                        | 6.87                                           | 0.00                          |
| June  | 40.0                         | 19.8                         | 38.4                          | 1.49                        | 7.56                                           | 0.00                          |
| July  | 41.1                         | 24.8                         | 37.9                          | 2.03                        | 6.88                                           | 0.02                          |
| August| 41.3                         | 25.6                         | 36.8                          | 1.78                        | 6.78                                           | 0.00                          |

2.2. Treatments and Experimental Design

Three levels of ascorbic acid (ASA; 0, 250, and 500 mg L⁻¹) and three other levels of zinc oxide nanoparticles (ZnONPs; 0, 1000, and 1500 mg L⁻¹) were prepared individually or in combinations as described in Table 2. All spray solutions were foliar-applied three times: 30, 45, and 60 days after transplanting (DAT) the cuttings. ZnONPs (≤100 nm; Figure 1) and ASA were purchased from Sigma-Aldrich, St. Louis, MO, USA.

Table 2. The studied experimental treatments.

| Symbol | Treatment | Ascorbic Acid | Zinc Oxide Nanoparticles |
|--------|-----------|---------------|----------------------------|
| T₀₀    | ASA₀, ZnONP₀ | No ascorbic acid was applied | No zinc oxide nanoparticles was applied |
| T₀₁    | ASA₀,ZnONP₁ | No ascorbic acid was applied | 1000 mg L⁻¹ of zinc oxide nanoparticles |
| T₀₂    | ASA₀,ZnONP₂ | No ascorbic acid was applied | 1500 mg L⁻¹ of zinc oxide nanoparticles |
| T₁₀    | ASA₁,ZnONP₀ | 250 mg L⁻¹ of ascorbic acid | No zinc oxide nanoparticles was applied |
| T₁₁    | ASA₁,ZnONP₁ | 250 mg L⁻¹ of ascorbic acid | 1000 mg L⁻¹ of zinc oxide nanoparticles |
| T₁₂    | ASA₁,ZnONP₂ | 250 mg L⁻¹ of ascorbic acid | 1500 mg L⁻¹ of zinc oxide nanoparticles |
| T₂₀    | ASA₂,ZnONP₀ | 500 mg L⁻¹ of ascorbic acid | No zinc oxide nanoparticles was applied |
| T₂₁    | ASA₂,ZnONP₁ | 500 mg L⁻¹ of ascorbic acid | 1000 mg L⁻¹ of zinc oxide nanoparticles |
| T₂₂    | ASA₂,ZnONP₂ | 500 mg L⁻¹ of ascorbic acid | 1500 mg L⁻¹ of zinc oxide nanoparticles |

Figure 1. TEM image of zinc oxide nanoparticles (ZnONPs).

The main experimental plots were colonized by the three ASA levels. Each main plot was subsequently subdivided into three subplots of the three levels of ZnONPs. Thus, there
were $3 \times 3 = 9$ treatment combinations. The size of each subplot was $3.0 \text{ m} \times 3.5 \text{ m}$ (10.5 m$^2$). There were four rows in each subplot and each row of ten cuttings (plants) 30 cm apart, for a total of 40 plants per sub-plot and a total of 1080 plants for this study. Through both seasons, the trials were put in order according to the split-plot structure for a randomized complete block design (RCBD), with three replicates. For commercial production of the sweet potato crop, all agricultural practices, including irrigation and control of weeds, pests, and diseases, were carried forward as recommended.

2.3. Soil Sampling and Determinations

Soil samples were collected at a depth of 0–25 cm before transplanting to determine some physical and chemical properties of the studied soil as described in [24]. The resultant data are shown in Table 3.

Table 3. Some soil physical and chemical characters.

| Soil Property | 2019   | 2020   |
|---------------|--------|--------|
| Particle size distribution |        |        |
| Sand %        | 66.8%  | 63.6%  |
| Silt %        | 16.5%  | 7.8%   |
| Clay %        | 16.7%  | 28.6%  |
| Soil texture  | Sandy clay loam | Sandy loam |
| pH in soil paste | 7.77   | 7.19   |
| ECe (dSm$^{-1}$) in soil paste extracted | 4.24   | 3.95   |
| Organic matter (OM %) | 1.03   | 0.90   |
| CaCO$_3$ (%)  | 11.3   | 10.8   |
| Soluble ions (mmol L$^{-1}$) |        |        |
| CO$_3$−       | 2.70   | 2.03   |
| HCO$_3$−      | 25.6   | 21.1   |
| Cl$^-$        | 18.3   | 20.3   |
| Na$^+$        | 31.3   | 31.6   |
| K$^+$         | 0.88   | 0.65   |
| Ca$^{++}$     | 7.47   | 7.11   |
| Mg$^{++}$     | 6.98   | 4.03   |
| Total N (mg kg$^{-1}$) | 515   | 450    |
| Available-P mgkg$^{-1}$ (Extractable with NaHCO$_3$, pH = 8.5) | 4013   | 3424   |
| Available-K mgkg$^{-1}$ (Extractable with NH$_4$OAC pH = 7.0) | 1237   | 1816   |
| Fe             | 4.15   | 6.03   |
| Mn             | 10.7   | 18.2   |
| Zn             | 0.04   | 0.07   |
| Cu             | 0.40   | 0.68   |

2.4. Leaf and Tuber Nutrient Contents

At 90 DAT, a random sample containing 5 plants from each experimental unit (subplot) was collected and washed with distilled water. Additionally, upon harvesting (120 DAT), ten uniform tubers from each treatment (subplot) were collected, cleaned, and sliced. The samples of leaves and tuber slices were oven-dried at 70 °C and ground to determine total P [25]. The total content of Fe, Mn, Zn, and Cu in both leaves and tubers was determined using an inductively coupled plasma–optical emission spectrometry (ICP-OES, Perkin-Elmer OPTIMA-2100 DV, Norwalk, CT, USA) according to the methods described in [26].

2.5. Tuber Yield Evaluation

Upon harvesting (120 DAT), the tubers were collected from all remaining plants in each subplot (treatment), weighed, and the resulting weights were calculated as the total yield of tuberous roots (ton ha$^{-1}$).
2.6. Statistical Analysis

The analysis of variance (ANOVA) and LSD were calculated by using the GEN STAT statistical package, version 12.1. For the ANOVA analysis, treatments were considered fixed, while replicates were considered random.

3. Results

3.1. Influence of Zinc Oxide Nanoparticles (ZnONPs) on the Nutrient Content of Sweet Potato Leaves and Tuberous Roots

The influence of ZnONPs on sweet potato leaf nutrient contents such as phosphorus (P), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) in the 2019 and 2020 seasons are shown in Table 4. The highest P content values (2.6 and 2.9%) were recorded in the untreated plants (NP₀) and treated plants with 1000 mg L⁻¹ of ZnONPs (ZnONP₁). The maximum values of nutrient content (586.9 vs. 583.4 for Fe; 105.8 vs. 105.7 for Mn; 28.1 vs. 25.0 for Zn; and 26.5 vs. 23.0 mg kg⁻¹ for Cu) were obtained with ZnONP₁ treatment for Fe and Mn and with ZnONP₀ for Zn in both growing seasons. The highest values of leaf contents of Cu were obtained using ZnONP₀ treatments and 1500 mg L⁻¹ of ZnONPs (ZnONP₂) in both seasons, respectively. Results depicted in Table 4 indicated that the increased contents of 7.4 vs. 10.8% for Fe and 10.6 vs. 2.2% for Mn were obtained from ZnONP₁ compared with the control treatment in both seasons, respectively. The results of the ANOVA indicate that all treatments had no significant effects on the leaf P and Cu contents in the first season only and had significant influences (at p ≤ 0.01) on the leaf Fe, Mn, and Zn contents in both seasons, while on the leaf P and Cu contents only in the second season.

Table 4. Influence of zinc oxide nanoparticles on leaves and tuberous root nutrients content of stressed sweet potato in 2019 and 2020 seasons.

| Treatment  | Parameters     | Leaves          | Tuberous Roots    |
|------------|----------------|-----------------|-------------------|
|            |                | P (%)           | Fe (mg kg⁻¹)      | Mn (mg kg⁻¹) | Zn (mg kg⁻¹) | Cu (mg kg⁻¹) | P (%)   | Fe (mg kg⁻¹) | Mn (mg kg⁻¹) | Zn (mg kg⁻¹) | Cu (mg kg⁻¹) |
| ZnONPs     |                |                 |                   |              |             |             |        |               |             |             |               |
| ZnONP₀     | 2.6a           | 543.4b          | 94.6c             | 28.1a        | 26.5a       | 3.5b         | 677.6b   | 11.80a       | 23.2a       | 17.2a       |
| ZnONP₁     | 2.5b           | 586.9a          | 105.8a            | 24.7b        | 26.5a       | 3.4c         | 684.9b   | 11.54a       | 20.8b       | 17.2a       |
| ZnONP₂     | 2.5b           | 496.4c          | 99.3b             | 21.9c        | 24.0a       | 3.7a         | 511.8a   | 10.79b       | 21.0b       | 17.5a       |
| LSD₀.₀₅   | 0.09           | 9.3             | 1.8               | 1.7          | ns          | 0.07         | 15.7     | 0.65         | 0.25        | ns          |

2019 growth season

2020 growth season

| ZnONPs     | P (%) | Fe (mg kg⁻¹) | Mn (mg kg⁻¹) | Zn (mg kg⁻¹) | Cu (mg kg⁻¹) |
|------------|-------|--------------|--------------|--------------|--------------|
| ZnONP₀     | 2.9a  | 520.2b       | 103.4a       | 25.0a        | 20.3b        |
| ZnONP₁     | 2.9a  | 583.4a       | 105.7a       | 24.6a        | 21.3b        |
| ZnONP₂     | 2.8a  | 494.5c       | 98.5b        | 25.0a        | 23.0a        |
| LSD₀.₀₅   | ns    | 8.8          | 2.7          | ns           | 1.21         |

Means in the same column denoted by a different letter indicate significant difference between treatments at p ≤ 0.05.

On the other hand, the data in Table 4 show the reversible effect of ZnONPs on tuberous root nutrient contents (i.e., P, Fe, Mn, Zn, and Cu) of sweet potato. The maximum values of P (3.7 vs. 4.3%) and Cu (17.5 vs. 39.9 mg kg⁻¹) contents were obtained using ZnONP₂ and ZnONP₀ treatments, and those of Fe content (684.9 vs. 999.8 mg kg⁻¹) were obtained using ZnONP₁ and ZnONP₀ treatments. Similar resulting data were obtained for both leaf Mn and Zn contents, however, ZnONP₀ treatment induced the highest values of Mn (11.8 vs. 32.8 mg kg⁻¹) and Zn (23.2 vs. 24.5 mg kg⁻¹) contents in both seasons. Analysis of variance showed highly significant increments in the tuberous root contents of P, Fe, Mn, and Zn with all treatments, which had no significant effects on the root Cu content in both seasons.
3.2. Influence of Ascorbic Acid (ASA) on the Nutrient Content of Sweet Potato Leaves and Tuberous Roots

The influence of ASA levels on nutrient contents in leaves and tuberous roots of sweet potato is presented in Table 5. Leafy nourishing with 250 mg ASA L\(^{-1}\) gave the highest values for P (3.0 vs. 3.3%) and Fe (567.6 vs. 568.6 mg kg\(^{-1}\)) in both seasons. The untreated plants with 500 mg ASA L\(^{-1}\), and with 250 mg ASA L\(^{-1}\), gave the maximum values for leaf Mn (117.8 vs. 108.9 mg kg\(^{-1}\)), Zn (27.6 vs. 27.2 mg kg\(^{-1}\)), and Cu (33.1 vs. 23.0 mg kg\(^{-1}\)). It is obvious from statistical analysis that the application of ASA treatments had highly significant effects for leaf Fe, Mn, and Zn contents in both seasons, and furthermore, for leaf P and Cu contents in the 2020 season, while there were no significant differences for P and Cu contents in the 2019 season. As depicted in Table 5, the increment rates were 37.9 vs. 51.6%; 11.2 vs. 16.0; 45.5 vs. 10.5; 18.3 vs. 19.3, and 61.4 vs. 17.7 mg kg\(^{-1}\) for P, Fe, Mn, Zn, and Cu contents in the 2019 and 2020 seasons, respectively.

**Table 5.** Influence of ascorbic acid on leaves and tuberous root nutrients content of stressed sweet potato in 2019 and 2020 seasons.

| Treatment | Parameters | Leaves | Tuberous Roots |
|-----------|------------|--------|----------------|
| ASA       | P (%)      | Fe mg kg\(^{-1}\) | Mn mg kg\(^{-1}\) | Zn mg kg\(^{-1}\) | Cu mg kg\(^{-1}\) | P (%) | Fe mg kg\(^{-1}\) | Mn mg kg\(^{-1}\) | Zn mg kg\(^{-1}\) | Cu mg kg\(^{-1}\) |
| ASA\(_0\) | 2.1a       | 510.5b | 117.8a | 23.7b | 33.1a | 2.8c | 631.7b | 11.0b | 22.1b | 20.3a |
| ASA\(_1\) | 3.0a       | 567.6a | 81.0c  | 23.4b | 23.4a | 4.2a | 730.9a | 15.3a | 23.6a | 16.6a |
| ASA\(_2\) | 2.5a       | 550.5a | 101.0b | 27.6a | 20.5a | 3.5b | 511.7c | 7.8c  | 19.3b | 15.0a |
| LSD\(_{0.05}\) | ns | 21.0 | 2.6 | 2.5 | ns | 0.23 | 35.2 | 0.74 | 0.26 | ns |

2019 season

| ASA\(_0\) | 2.2c       | 480.6b | 108.9a | 24.6b | 19.6b | 2.3c | 676.5c | 12.5b | 17.7c | 18.9c |
| ASA\(_1\) | 3.3a       | 568.6a | 98.6b  | 22.8c | 23.0a | 4.9a | 997.4a | 25.4a | 27.0a | 28.4b |
| ASA\(_2\) | 3.2b       | 548.9a | 100.1b | 27.2a | 22.0ab | 3.8b | 856.2b | 23.1a | 26.4b | 38.0a |
| LSD\(_{0.05}\) | 0.08 | 20.5 | 4.4 | 0.17 | 2.7 | 0.04 | 1.1 | 4.12 | 0.50 | 0.58 |

2020 season

Means in the same column denoted by a different letter indicate significant difference between treatments at \(p \leq 0.05\).

There were highly significant differences among the treatments on tuberous root contents of P, Fe, Mn, and Zn contents in 2019 and 2020 seasons, excluding Cu content recorded only in the 2020 season. It is evident from Table 5 that ASA\(_1\) was the superior treatment for P (4.2 vs. 4.9%), Fe (730.9 vs. 997.4 mg kg\(^{-1}\)), Mn (15.3 vs. 25.4 mg kg\(^{-1}\)), and Zn (23.6 vs. 27.0 mg kg\(^{-1}\)) contents in both seasons; however, the Cu content (20.3 vs. 38.0 mg kg\(^{-1}\)) was recorded by ASA\(_0\) and ASA\(_2\) treatments in 2019 and 2020 seasons, respectively.

Similarly, the general trend of data portrayed in Table 5 indicates that all studied nutrient contents were higher in the 2020 season than those in the 2019 season. The obtained data indicate that the rate of increases reached 52.4 vs. 111.6%, 42.8 vs. 47.4%, 96.2 vs. 102.4%, 22.9 vs. 52.7%, and 35.8 vs. 101.1% for P, Fe, Mn, Zn, and Cu contents in the 2019 and 2020 seasons, respectively.

3.3. Influence of ZnONPs and ASA Interaction on the Nutrient Content of Sweet Potato Plants

Results in Figures 2–11 showed the influence of interaction of ZnONPs and ASA on the leaf and tuberous root nutrient (P, Fe, Mn, Zn, and Cu) contents of sweet potato plants. Analysis of variance showed that all studied nutrient contents were affected by highly statistically significant differences with all treatments, except for P-leaf content in the first season. In this investigation, the results revealed that the best values for P and Fe contents (3.7 vs. 3.6% and 693.4 vs. 686.4 mg kg\(^{-1}\)) were obtained by spraying plants with 250 mg ASA L\(^{-1}\) individually (T\(_{10}\))
and spraying plants with 250 mg ASA L\(^{-1}\) + 1000 mg ZnONPs L\(^{-1}\) (T\(_{11}\)) in both seasons, respectively.

**Figure 2.** Influence of interaction between zinc oxide nanoparticles and ascorbic acid on leaf P content of stressed sweet potato plants in 2019 and 2020 seasons. Bars with a different letter indicate significant difference between treatments at \(p \leq 0.05\).

**Figure 3.** Influence of interaction between zinc oxide nanoparticles and ascorbic acid on root P content of stressed sweet potato plants in 2019 and 2020 seasons. Bars with a different letter indicate significant difference between treatments at \(p \leq 0.05\).

**Figure 4.** Influence of interaction between zinc oxide nanoparticles and ascorbic acid on leaf Fe content of stressed sweet potato plants in 2019 and 2020 seasons. Bars with a different letter indicate significant difference between treatments at \(p \leq 0.05\).
Figure 5. Influence of interaction between zinc oxide nanoparticles and ascorbic acid on root Fe content of stressed sweet potato plants in 2019 and 2020 seasons. Bars with a different letter indicate significant difference between treatments at $p \leq 0.05$.

Figure 6. Influence of interaction between zinc oxide nanoparticles and ascorbic acid on leaf Mn content of stressed sweet potato plants in 2019 and 2020 seasons. Bars with a different letter indicate significant difference between treatments at $p \leq 0.05$.

Figure 7. Influence of interaction between zinc oxide nanoparticles and ascorbic acid on root Mn content of stressed sweet potato plants in 2019 and 2020 seasons. Bars with a different letter indicate significant difference between treatments at $p \leq 0.05$. 
Figure 8. Influence of interaction between zinc oxide nanoparticles and ascorbic acid on leaf Zn content of stressed sweet potato plants in 2019 and 2020 seasons. Bars with a different letter indicate significant difference between treatments at $p \leq 0.05$.

Figure 9. Influence of interaction between zinc oxide nanoparticles and ascorbic acid on root Zn content of stressed sweet potato plants in 2019 and 2020 seasons. Bars with a different letter indicate significant difference between treatments at $p \leq 0.05$.

Figure 10. Influence of interaction between zinc oxide nanoparticles and ascorbic acid on leaf Cu content of stressed sweet potato plants in 2019 and 2020 seasons. Bars with a different letter indicate significant difference between treatments at $p \leq 0.05$. 
Dissimilar data for leaf Mn, Zn, and Cu contents, however, the maximum values (125.7 and 125.2) were obtained by spraying plants with 500 mg ASA L\(^{-1}\) + 1500 mg ZnONPs L\(^{-1}\) (T\(_{22}\)) and spraying plants with ASA individually (T\(_{10}\)) for Mn (26.2 and 31.9). The untreated plants (T\(_{00}\)) and plants sprayed with maximum level of ASA (T\(_{20}\)) gave the highest contents of Zn (44.8 vs. 27.5), and spraying plants with 1000 mg ZnONPs L\(^{-1}\) individually (T\(_{01}\)) and 500 mg ASA L\(^{-1}\) individually (T\(_{10}\)) recorded the highest Cu content in both seasons.

The impact of ASA and ZnONPs interaction on the aforementioned nutrients in tuberous roots of sweet potato is presented in Figures 2–11. The ASA and ZnONPs mixture was not useful, since T\(_{00}\) (the control) treatment gave the best values (909.5 vs. 29.0 mg kg\(^{-1}\)) for Fe and Cu contents in the 2019 season. The (T\(_{10}\)) treatment was also considered the superior treatment for Mn, Zn, and Cu in the 2020 season, even though the statistical analysis showed a highly significant influence for all treatments. The highest values (5.0 vs. 6.0%) were obtained for tuberous root P content with plants treated with 250 mg ASA L\(^{-1}\) + 1500 mg ZnONPs L\(^{-1}\) (T\(_{12}\)) treatment. Furthermore, (T\(_{11}\)) treatment was the superior treatment for Fe content in the 2019 season and Mn content in the 2020 season.

### 3.4. Influence of ZnONPs and ASA Applied Individually or in Interaction on Total Tuberous Root Yield of Sweet Potato

It is clear from Figure 12 that ZnONPs treatments significantly affect the tuberous root yield of sweet potato plants. The maximum values (16.7 and 17.6 ton ha\(^{-1}\)) were obtained with plants nourished with ZnONP\(_1\) treatment followed by ZnONP\(_2\) treatment compared with the control treatment (13.3 vs. 14.5 ton ha\(^{-1}\); 25.5 vs. 20.8%) in both seasons, respectively. Concerning the influence of ASA, shown in Figure 13, the data obtained indicated that the ASA\(_4\) treatment is the best in both seasons by giving 20.0 vs. 20.0 ton ha\(^{-1}\) with an increment percentage of 63.5 vs. 44.3% compared with the ASA\(_0\) treatment.

The influence of the interaction of ASA and ZnONPs on the total yield of sweet potato tubers is portrayed in Figure 14. The results indicated that the highest values (21703.3 vs. 23643.3 kg ha\(^{-1}\)) were obtained by nourishing plants with T\(_{11}\) treatment (250 mg ASA L\(^{-1}\) + 1000 mg ZnONPs L\(^{-1}\)).
3.5. Correlation and Regression Analyses

Table 6 shows Pearson’s correlation coefficients between TY with nutrients, namely P, Fe, Mn, Zn, and Cu in leaf and root tissues of stressed sweet potato exogenously sprayed with zinc oxide nanoparticles and ascorbic acid in both growing seasons. Significant ($p \leq 0.05$) negative correlations were obtained between TY and potato leaf’s Zn (−0.575 ** and −0.452 *) in both 2019 and 2020 seasons, respectively, as well as Mn (−0.407 *) and Cu (−0.525 **) only in 2019 growing season. However, significant ($p \leq 0.05$) positive correlations (0.462 * and 0.658 **) were obtained between TY and each of P and Fe content in potato leaves. Among the nutrients in potato’s root, P content had positively ($p \leq 0.01$) correlated (0.563 ** and 0.579 **) for both the 2019 and 2020 seasons, respectively, in addition to Mn content (0.402 *) for the 2019 season only with TY trait. *,** indicate, respectively, differences at $^* p \leq 0.05$ probability level, $^{**} p \leq 0.01$. 

Table 6. Pearson’s correlation coefficients between total yield (TY) and each of leaf and root nutrient content of stressed sweet potato plants exogenously sprayed with different levels of zinc oxide nanoparticles and ascorbic acid in both the 2019 and 2020 seasons.

| Nutrient      | TY (t ha⁻¹) | 2019 | Probability | Pearson r | Probability | Pearson r | Probability |
|---------------|-------------|------|-------------|-----------|-------------|-----------|-------------|
|               |             |      |             |           |             |           |             |
| Leaves nutrients content |             |      |             |           |             |           |             |
| Phosphorus (P) | 0.263 **    | 0.186 |             | 0.462 *   | 0.015       |
| Iron (Fe)      | 0.279 **    | 0.159 |             | 0.658 **  | <0.001      |
| Manganese (Mn) | −0.407 *    | 0.035 | −0.220 ns   | 0.271      |
| Zinc (Zn)      | −0.575 **   | 0.002 | −0.452 *    | 0.018      |
| Copper (Cu)    | −0.525 **   | 0.005 | 0.333 ns    | 0.090      |
| Root nutrients content |             |      |             |           |             |           |             |
| Phosphorus (P) | 0.563 **    | 0.002 |             | 0.579 **  | 0.002       |
| Iron (Fe)      | 0.113 ns    | 0.574 |             | 0.334 ns  | 0.089       |
| Manganese (Mn) | 0.402 *     | 0.038 |             | 0.164 ns  | 0.413       |
| Zinc (Zn)      | −0.016 ns   | 0.939 |             | 0.214 ns  | 0.283       |
| Copper (Cu)    | −0.262 ns   | 0.186 |             | 0.097 ns  | 0.631       |

*, **, and ns indicate, respectively, differences at \( p \leq 0.05 \) probability level, \( p \leq 0.01 \) probability level, and not significant.

Stepwise regression analysis in Table 7 and Figures 15–21 indicates that 57.1% of variations in TY of sweet potato are explained by variations in four leaf nutrients (i.e., Fe, Mn, and Zn) in the 2019 season, and 72.4% by variations in four leaf nutrients (i.e., Fe, Mn, Zn, and Cu) in the 2020 season.

Table 7. Proportional contribution in predicting total tuberous root yield (RY) using stepwise multiple linear regression for stressed sweet potato plants sprayed with different levels of ascorbic acid and zinc oxide nanoparticles in both the 2019 and 2020 seasons.

| Year  | r     | Adjusted \( R^2 \) | SEE  | Significance | Fitted Equation |
|-------|-------|---------------------|------|--------------|----------------|
| 2019  | 0.788 | 0.571               | 2.627| ***          | \( RY = 21.346 + 0.280Fe - 0.068Mn - 0.538Zn \) |
| 2020  | 0.876 | 0.724               | 1.862| ***          | \( RY = 12.498 + 0.032Fe - 0.056Mn - 0.403Zn - 0.133Cu \) |

Figure 15. Contribution of Fe content to the quadratic response of stressed sweet potato to different levels of ascorbic acid and zinc oxide nanoparticles in the 2019 season. *** indicates differences at \( p \leq 0.001 \) probability level.
Figure 16. Contribution of Mn content to the quadratic response of stressed sweet potato to different levels of ascorbic acid and zinc oxide nanoparticles in the 2019 season. *** indicates differences at $p \leq 0.001$ probability level.

Figure 17. Contribution of Zn content to the quadratic response of stressed sweet potato to different levels of ascorbic acid and zinc oxide nanoparticles in the 2019 season. *** indicates differences at $p \leq 0.001$ probability level.

Figure 18. Contribution of Fe content to the quadratic response of stressed sweet potato to different levels of ascorbic acid and zinc oxide nanoparticles in the 2020 season. *** indicates differences at $p \leq 0.001$ probability level.
Figure 19. Contribution of Mn content to the quadratic response of stressed sweet potato to different levels of ascorbic acid and zinc oxide nanoparticles in the 2020 season. *** indicates differences at $p \leq 0.001$ probability level.

Figure 20. Contribution of Zn content to the quadratic response of stressed sweet potato to different levels of ascorbic acid and zinc oxide nanoparticles in the 2020 season. *** indicates differences at $p \leq 0.001$ probability level.

Figure 21. Contribution of Cu content to the quadratic response of stressed sweet potato to different levels of ascorbic acid and zinc oxide nanoparticles in the 2020 season. *** indicates differences at $p \leq 0.001$ probability level.
4. Discussion

For a long time, plants of different crops have suffered from one or more environmental stresses, with numerous attempts by researchers and plant breeders to find solutions that make plants adapt to these stresses. However, with continuous climate change to a degree that we have not realized before has led to an increase in the suffering of different crop plants as a result of the adverse effects of environmental stresses, which has led to weakening or inhibiting the growth and performance of plants and thus productivity. In the arid and semi-arid regions of the world, this agricultural issue is being discussed more often by researchers [15,27–39]. One of the aspects of this agricultural issue that strongly affects the growth and performance of different crop plants and thus crop productivity is the high percentage of calcium carbonate (CaCO$_3$) in agricultural soil (calcareous soil) [2,40]. This study addressed the issue of calcareous content in sandy (clay) loam soil located in a semi-arid region (Egypt) and attempted to identify a successful solution for plants to adapt and coexist with the problem of high CaCO$_3$ content. High CaCO$_3$ is a harsh type of environmental stress that has severe effects on plant performance, especially on the ability of plants’ to uptake nutrients. In previous studies that addressed this agricultural issue, efforts have been made to find solutions that are wholly or partially successful, including phosphorus nanoparticles, organic fertilizers, and bio-fertilizers [2,40]; however, the use of zinc oxide nanoparticles (ZnONPs) and ascorbic acid (ASA) applied as leafy nourishment for sweet potato plants at field level has not yet been investigated under a high level of CaCO$_3$ (calcareous soil).

4.1. The Influence of ZnONPs on Sweet Potato Performance

Nano micronutrient fertilizers stand out as one of the most useful means for their high efficiency. One of them, ZnONPs, has been previously applied as leafy nourishment in numerous studies using different crop plants or trees grown in normal conditions or under stress conditions such as pomegranate [16], peanut [41,42], common dry bean [9], wheat [17,43], mango [44], and eggplant [15] and several positive influencing results have been reported.

The calcareous state of the tested soil restricts its water content and minimizes or prevents the plants from absorbing soil water and thus the nutrients are fixed in an insoluble form [2,40]. However, under stress that restricts water in the soil, plants can absorb water and nutrients due to the provision of plants with ZnONPs that enhance the activity and expression of genes related to plant hormones (for example, ABA and cytokinin), which regulate root growth. These findings lead to an increase in the absorbing surfaces of the roots while improving carbohydrate and protein metabolism to positively modify the osmoticum state in plant cells to increase water and nutrient absorption [15,42,43,45]. The findings of this study displayed that sweet potato plants grown with water restriction induced by the calcareous condition of the tested soil collected lower contents of P, Mn, Fe, Zn, and Cu (Tables 4 and 5, Figures 2–11). The insufficient amount of water available for plant roots in calcareous soils leads to fixation and less diffusion of nutrients around the roots of plants, resulting in reduced absorption, active transport, membrane permeability, and transpiration flow [15,46]. On the other hand, Grangah et al. [47] reported on sorghum plants that the leafy application of ZnONPs resulted in higher contents of various nutrients as disclosed in this report (Tables 4 and 5, Figures 2–11). In the same trend, previous reports have portrayed that ZnONPs can raise various macro and micronutrients in pinto beans and sorghum plants [48,49]. As a consequence, the findings of these reports are in agreement with our findings that the stress has injurious influences on the nutritional status of the plant causing an imbalance in the nutrient contents. However, the leafy nourishment containing ZnONPs positively altered this imbalance in the contents of the plant nutrients and attenuated the adverse influences of stress due to improved plant water status and stability of cellular membranes [15,46].

In the El-Metwally et al. [42] report, the increase in root weight due to peanut leaf nourishment with ZnONPs was obtained through improved root enlargement to enable
plants to take in more water and nutrients for growth and productivity. These findings were further explained in [50], in which they found relations between different ions of minerals and their translocation from leaves to the fruits, reflected in plant yield. The results of the present study are in good agreement with those in [51]; however, the best effect of Fe in leaves is shown with plant spraying with ZnONPs, resulting in a transfer from root to shoots [52]. Similar findings were reported in [53], noting that these findings might be due to the role of the elements in metabolic processes and the penetration ability of ZnO as a nanomaterial to the plant cell. In addition, the study of Prasad et al. [41] found that the use of fertilizers in nanoparticles is completely controlled, which increased their total and improved their quality characteristics.

Although improvements in nutrient contents were determined by nourishing sweet potato leaves with ZnONPs under the stress of calcareous conditions, there was a decline in P, Zn, and Cu contents that might be a result of the interaction of Zn with some other elements such as Mn and Cu [54]. In some cases, ZnONPs application tended to reduce the contents of Fe, Mn, and Cu compared with the control (Figures 4, 5, 7 and 11). This result may be because foliar nourishment with ZnONPs resulted in a significant increase in the content of Zn in plant tissues which resulted in the antagonism of zinc with other nutrients, including Fe, Mn, and Cu [55,56]. Additionally, in this study, the reduction in P uptake under calcareous soil conditions was due to several reasons: (a) absorption processes causing strong fixing of \( \text{PO}_4^{3-} \) in soil solution; (b) reduced \( \text{PO}_4^{3-} \) activity as a result of the action of ionic strength; and (c) Ca and P minerals have low solubility. Therefore, calcareous conditions (10.8–11.3%) and moderate salt stress (3.95–4.24 dS m\(^{-1}\)) of the current study (Table 3) caused nutrient fixation along with the osmotic stress induced by the soil solution which all caused the ions to be unbalanced [2,57]. However, nourishing sweet potato leaves with ZnONPs generally attenuated the adverse calcareous and saline effects and rebalanced plant nutrients (Tables 4 and 5, Figures 2–11), which was positively reflected in the increased plant yield (Figures 12–14).

### 4.2. The Influence of ASA on Sweet Potato Performance

Many desired processes related to plant biology are activated when the plant experiences stress such as calcareous conditions [2,40], which is the stress addressed in this study. As one of the pivotal approaches to all aspects of the plant, nutrient uptake by plant roots is one of these desired processes, which requires soil without any restrictions for it to work well. Under stress conditions, such as the high percentage of calcium carbonate (CaCO\(_3\)), the plant cannot absorb nutrients from the calcareous soil, so the plant needs adjuvants that help it strengthen its ability to extract the fixing nutrients in such conditions. Thus, several low-molecular-mass antioxidants are elevated in the stressed plant such as ascorbic acid (ASA) to help it shed over productive reactive oxygen species (ROS) as a pivotal role in the plant defense system [58]. ASA is a pivotal member of the ASA-glutathione (GSH) cycle as an important part of the plant defense system for eliminating ROS in the stressed plant through utilizing ASA as an electron donor [2,58]. These findings indicate a pivotal role of the ASA-GSH cycle, including ASA in the high CaCO\(_3\)-stressed sweet potato plant in the elimination of ROS to strengthen the plant’s ability to uptake nutrients from the tested calcareous soil (Tables 4 and 5, Figures 2–11), which was reflected in increased sweet potato yield (Figures 12–14).

As one of the pivotal strategies for sustaining and developing agricultural productivities under various stresses, plants should be foliar-provided with ASA to reinforce tolerance to the high soil CaCO\(_3\) content in sweet potato and minimize the deleterious influences of calcareous conditions to uptake sufficient quantities of nutrients and thus produce satisfactory returns (yields) in environments with excess CaCO\(_3\). Since ASA is a pivotal antioxidant that is involved in several activities related to plant biology, including plasma membranes [19,58], it has been suggested that it plays a central role in cellular membrane permeability and nutrient uptake [59]. As a finding, foliar nourishment with ASA for sweet potato plants is expected to raise the ASA content [58] and elevate plants'
various nutrient content (Tables 4 and 5, Figures 2–11), which is positively reflected in higher plant yield (Figures 12–14). Furthermore, the role of ASA in developing processes including cell division and cell wall expansion leads to enhanced plant growth characters [20], which is also positively reflected in higher plant yield. The rise in nutrients in our report is consistent with the findings in [60], who attributed this nutrient elevation (e.g., N, P, K, Ca, Zn, Mn, Fe, and Cu) in tomato leaves to implicating Zn in auxin (IAA) biosynthesis in favor of promoting plant rooting, thus raising the nutrient amounts absorbed and translocated to different plant organs, which is reflected in plant growth and productivity. Our findings regarding leaf Zn and Cu contents may be because ASA as an antioxidant had an influence on plant growth regulators and their fundamental roles in activating both cellular elongation and division in meristematic tissues, as well as the roles played in many aspects of radix control in antioxidant activity in the plant cells [61]. Therefore, ASA intercepts free radicals and protects the cell from oxidative damage that leads to aging and disease [62], and furthermore, improves the role of ASA in many metabolic and physiological processes and enhances the synthesis of carbohydrates [63]. Although improvements in nutrient contents were made by nourishing sweet potato leaves with ASA under the stress of calcareous conditions, there was a decline in Mn and Cu contents that might be due to the interaction of P with these two nutrients.

4.3. Possible Mechanisms of Synergistic Action of ZnONPs and ASA against Abiotic Stress

The best results were obtained concerning sweet potato leaf and tuberous root contents of nutrients and plant yield with leaf nourishing using a mixture of 1000 mg ZnONPs L$^{-1}$ and 250 mg ASA L$^{-1}$ under the stress conditions of calcareous soil. These best results may be attributed to the integrative roles of Zn and ASA which enable the plant to collect more nutrients and increase yield; Zn to expand the absorbent root surfaces through its role in phytohormones biosynthesis; and ASA to eliminate ROS and their adverse influences resulting from soil stressed with high CaCO$_3$ content. Possible mechanisms of action of ZnONPs against abiotic stress can be summarized as follows: ZnONPs enhance the activity and expression of plant hormone genes (e.g., ABA and cytokinin), which increase root growth and thus absorbing root surfaces while improving carbohydrate and protein metabolism to positively modify the osmoticum state in plant cells to increase water and nutrient uptake [15,42,43,45]. As a consequence, ZnONPs positively altered the imbalance in the contents of the plant nutrients and attenuated the adverse influences of stress due to improved plant water status and stability of cellular membranes [15,46]. The increase in root weight by leafy nourishment with ZnONPs may be due to the improved root growth and enlargement to enable plants to take in more water and nutrients for growth and productivity [42]. The improved plant contents of nutrients by ZnONPs is positively reflected in increased plant yield due to the nutrient roles in metabolic processes and the penetration ability of ZnO as a nanomaterial to the plant cell. In some cases, the application of ZnONPs reduces Fe, Mn, and Cu contents because foliar nourishing with ZnONPs increases in Zn content in plant tissues and antagonizes Fe, Mn, and Cu [55,56]. In general, nourishing plant leaves with ZnONPs generally attenuates the adverse effects of calcareous and saline soils and rebalances plant nutrients in favor of plant productivity.

Possible mechanisms of action of ASA against abiotic stress can be summarized as follows: ASA as a powerful antioxidant has a pivotal role in plant defense systems by helping plants eliminate overproduced reactive oxygen species (ROS) [58]. It is a pivotal member as an electron donor in the ASA–glutathione (GSH) cycle as an important part of the plant defense system for eliminating ROS in stressed plants [2,58]. Thus, the ASA–GSH cycle, including ASA, has a key role in eliminating ROS in CaCO$_3$-stressed sweet potato plants by strengthening plants’ ability to uptake nutrients from calcareous soils in favor of plant productivity. As one of the pivotal strategies for sustaining and developing agricultural productivities under various stresses, plants should be foliar-provided with ASA to reinforce plant tolerance to the high soil CaCO$_3$ content and minimize the deleterious
influences of calcareous conditions to uptake sufficient quantities of nutrients and thus produce satisfactory returns (yields) in environments with excess CaCO$_3$. Since ASA is a pivotal antioxidant that is involved in several activities related to plant biology, including plasma membranes \cite{19,58}, it has been suggested that it plays a central role in cellular membrane permeability and nutrient uptake \cite{59}. Additionally, the role of ASA in developing processes including cell division and cell wall expansion leading to enhanced plant growth and yield characters is also important \cite{20,60}. The increase in Zn content as a result of leafy nourishing with ASA contributes to auxin (IAA) biosynthesis to promote plant rooting, thus raising the nutrient amounts absorbed and translocated to different plant organs in favor of plant productivity. ASA as an antioxidant had an influence on plant growth regulators and their fundamental roles in activating both cellular elongation and division in meristematic tissues, as well as the roles played in many aspects of radix control in antioxidant activity in the plant cells \cite{61}. Therefore, ASA intercepts free radicals and protects the cell from oxidative damage that leads to aging and disease \cite{62}. Furthermore, the improved role of ASA in many metabolic and physiological processes enhances the synthesis of carbohydrates \cite{63}.

The incorporation of both enhanced ZnONPs and ASA mechanisms in stressed plants can occur in conjunction to increase water and nutrient uptake under the harsh conditions of calcareous soils in favor of stressed plants for improved performance and production.

5. Conclusions

It has been proven that nutrients are fixed in insoluble form in calcareous soils, making them in an unbalanced status. However, nourishing the plant leaves with ZnONPs at a level of 1000 mg L$^{-1}$ recorded the highest values for the leaf Fe and Mn contents in both seasons, while the untreated control plants gave the maximum values for the leaf P and Cu content in the first season, and the leaf Zn content in both seasons. On the other hand, nourishing the plant leaves with ASA at a level of 250 mg L$^{-1}$ recorded the highest values for the leaf P and Fe contents, while the control plants and applying the level of 500 mg ASA L$^{-1}$ collected the highest leaf Mn, Cu, and Zn contents. Corresponding results were obtained for tuberous root content of nutrients by spraying plants with 250 mg ASA L$^{-1}$ in both seasons, excluding the Cu content. In summary, it can be concluded that the best results for the leaf nutrient contents and yield were obtained with sweet potato plants grown under stressful conditions of high soil CaCO$_3$ content with a mixture of 1000 mg ZnONPs L$^{-1}$ and 250 mg ASA L$^{-1}$.

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