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Article

Potential of Fulvic Acid and Quercetin with and without Zinc Ferrite Nanoparticles to Alleviate Salinity Stress in Maize

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Abstract

Salinity stress disrupts water balance, leading to dehydration, reduced nutrient uptake, and hindering essential metabolic processes, thereby affecting plant growth and productivity. The use of quercetin (QC), fulvic acid (FA), and zinc ferrite nanoparticles (ZnFNP) approach can serve as an effective strategy to counteract the issue. Quercetin and fulvic acid enhance plant nutrient absorption, promote healthy soil, improve water retention, strengthen roots, and enhance stress resilience. Zinc ferrite nanoparticles enhance plant growth by efficiently delivering essential nutrients, improving nutrient absorption, boosting enzymatic activities and metabolic processes, and exhibiting antioxidant properties, thereby promoting sustainable crop productivity. Their combined application as an amendment against salinity still needs scientific justification. This study evaluated the individual and combined effects of QC, FA, and ZnFNP on maize under salinity stress. Combined application (15µM QC + 2 mgL^{-1} FA + ZnFNP) significantly enhanced biomass, chlorophyll content, nutrient uptake, and antioxidant regulation compared with the control. Treatments were applied in 4 replications following a completely randomized design. Results exhibited that 15 µM QC+2mgL^{-1} FA with ZnFNP showed significant improvement in maize shoot and root fresh and dry weight (17.25%, 39.34%, 15.37%, and 26.65%) over control under salinity stress. Significant enrichment in maize chlorophyll a, b, and total chlorophyll (38.22%, 63.79%, and 51.20%) over the control under salinity stress validates the effectiveness of 15 µM QC+2mgL^{-1} FA with ZnFNP. Furthermore, improvements in N, P, and K concentrations in roots and leaves verified the productive functioning of 15 µM QC + 2 mgL^{-1} FA relative to the control under salinity stress. In conclusion, 15 µM QC+2mgL^{-1} FA with ZnFNP is the recommended amendment for mitigating salinity stress in maize.

Keywords: Fulvic acid; Nanoparticles; Chlorophyll content; Quercetin; Growth attributes.

1. Introduction

Soil salinity represents one of the major drivers of land degradation worldwide, ranking second only to soil erosion in its impact on agricultural productivity (Ashraf and Chen, 2023). Approximately 1.0 billion hectares, accounting for nearly 7% of the global land area, are currently affected by salinization (Musie and Gonfa, 2023). This problem continues to intensify, with an estimated 2,000 hectares of fertile agricultural land lost each day due to salinity, posing a serious threat to global food security (Nicolas et al., 2023). This occurrence results in a significant 10–25% reduction in crop yields, with severe conditions potentially leading to desertification (Liu *et al.*, 2024). As a result, it is imperative to implement measures to mitigate salinity's impact, not only to preserve arable land but also to sustainably enhance crop production (Mishra *et al.*, 2023). Such measures are crucial for ensuring food security amid the continuous growth of the global population.

Quercetin (QC), a plant flavonoid, has positive effects on growth by acting as an antioxidant, protecting against environmental stress (Mansour *et al.*, 2023). It

supports seed germination, root elongation, and photosynthesis efficiency. Quercetin's role in nutrient uptake enhances overall plant vitality, making it a potential tool in agriculture for improved crop yield and resilience (Singh *et al.,* 2024). Fulvic acid (FA) enhances nutrient uptake, making essential minerals more available to plants (Shaltout *et al.,* 2023). It also helps prevent metal toxicity, promotes a healthy soil environment, and improves water retention. With fulvic acid, plants develop stronger roots, absorb nutrients more efficiently, and become more resilient to stress. Fulvic acid contributes to healthier crops, potentially increasing productivity (Jesmin *et al.,* 2023).

Zinc ferrite nanoparticles positively influence plant growth by serving as efficient carriers of essential nutrients, particularly zinc (Tombuloglu *et al.,* 2023). These nanoparticles enable controlled zinc ion release, enhancing nutrient absorption, enzymatic activities, and metabolic processes. Additionally, they exhibit antioxidant properties that mitigate oxidative stress in plants (Thakur and Thakur, 2023). The encouraging results suggest their potential as eco-friendly nanofertilizers, promoting sustainable crop productivity.

Maize, a fundamental cereal crop, holds enormous significance due to its dual role as a primary food source and a key contributor to various industries (Ali *et al.,* 2023). Widely consumed worldwide, maize provides vital nutrition for humans and livestock, contributing significantly to food security and economic stability (Shahid *et al.,* 2023). Despite its fundamental role, maize cultivation is increasingly threatened by salinity stress, an environmental factor that adversely affects plant growth (Li *et al.,* 2023). Salinity stress disrupts the plant's water and nutrient balance, hampering overall development and diminishing yields. Salinity stress poses a significant challenge to maize cultivation, impacting both farmers' economic returns and the supply chain of the sugar and bioenergy industries (Alotaibi, 2023). QC, FA, and ZnFNP can be suitable approaches to mitigate salinity stress in maize.

Although the detrimental effects of salinity are widely documented, most studies have evaluated individual mitigation strategies rather than combined biochemical amendments. However, little is known about the combined effects of QC, FA, and ZnFNP on maize tolerance to salinity stress. That's why the current study aims to explore the potential of QC, FA, and ZnFNP to mitigate the salinity stress on maize. This research evaluates the individual and combined effects of QC, FA, and ZnFNP on maize growth under salinity stress by addressing this research gap and proposing an environmentally sustainable approach to mitigate the detrimental effects of salinity on maize cultivation.

2. Materials and Methods

2.1. Experimental site

In 2022, an experiment was conducted in a research area to examine the effectiveness of fulvic acid and quercetin, with and without zinc ferrite nanoparticles, in alleviating salinity stress in maize. Soil samples were collected from the research site, air-dried, and passed through a 2-mm sieve for the assessment of their physicochemical properties. The detailed physicochemical characteristics of the soil and irrigation water are presented in Table 1.

Table 1. Pre-treatment physicochemical attributes of soil and irrigation water

Soil	**Values**
pH	8.12
SOM (%)	0.50
Available Phosphorus (µg/g)	6.20
Total Nitrogen (%)	0.002
EC*e* (dS/m)	5.31
Extractable Sodium (µg/g)	111
Texture	Clay Loam
Extractable Potassium (µg/g)	125
Irrigation	**Values**
pH	7.93
Bicarbonates (meq./L)	4.62
Carbonates (meq./L)	0.00
Chloride (meq./L)	0.02
EC (µS/cm)	885
Sodium (mg/L)	163
Ca+Mg(meq./L)	4.15

2.2. Synthesis of ZnFNP

Initially, solutions were prepared by dissolving 0.2 M $Zn(NO_3)_2 \cdot 6H_2O$ and $Fe(NO_3)_3$ separately in deionized water with continuous stirring. enugreek (*Trigonella foenum-graecum* L.) seed extract was produced by thoroughly washing the seeds, grinding them into a fine paste, and filtering the mixture to obtain a clear extract. For biosynthesis, equal volumes of the metal salt solution and plant extract were mixed in a 1:1 ratio with continuous stirring. The pH of the reaction mixture was gradually adjusted to 10 using NaOH solution. The appearance of a dark brown to black coloration confirmed the formation of $ZnFe_2O_4$ nanoparticles. Following synthesis, the nanoparticles were recovered by centrifugation at 5000 rpm for 10–15 minutes and subsequently washed several times with deionized water to eliminate residual impurities. The purified $ZnFe_2O_4$

nanoparticle precipitate was then dried in a hot air oven at 60–80 °C for 4–5 hours.

2.3. Treatments

The experimental treatments consisted of an untreated control, 15µM quercetin (QC), 2 mgL^{-1} fulvic acid (FA), and a combined application of 15µM QC + 2 mgL^{-1} FA. Each treatment was evaluated both in the absence (No ZnFNP) and presence of ZnFNP. Foliar applications were initiated 4 weeks after germination, with 2 sprays applied at 15-day intervals. The experiment was arranged in a completely randomized design (CRD) with four replications per treatment. Foliar treatments were uniformly applied to both adaxial and abaxial leaf surfaces until runoff to ensure thorough coverage.

2.4. Seed procurement, sterilization, and sowing

The maize seeds of the Gohar-19 variety were procured from a licensed seed trader authorized by the Government of Punjab, Pakistan, ensuring compliance with regulatory standards. After the initial preparation, five seeds were sown in each pot containing 15 kg of soil. Following germination, seedlings were thinned to retain two healthy plants pot^{-1}.

2.5. Fertilizer

The soil was amended with nitrogen, phosphorus, and potassium at the rates of 119 kg ac^{-1} for nitrogen (2.20 g/15 kg soil), 69 kg ac^{-1} for phosphorus (1.28 g/15 kg soil), and 50 kg ac^{-1} for potassium (0.93 g/15 kg soil). Urea served as the nitrogen source, while single superphosphate was used for phosphorus and potassium, as specified.

2.6. Irrigation and Soil Salinity

Throughout the experiment, soil moisture was maintained at 65% of field capacity using a Cubilan 4-in-1 soil moisture meter. Soil salinity was monitored weekly with a portable EC meter, and irrigation water salinity was adjusted to maintain a consistent EC using NaCl: $MgCl_2$, and $CaCl_2$ (1:1:1 ratio) throughout the experimental period.

2.7. Data collection

Sixty days after sowing, samples were collected to obtain the required data. The data-collection process involved assessing various factors, including fresh and dry shoot and root weights immediately after harvest. For the dry weight analysis, the drying procedure consisted of 48 hours of oven-drying at 65°C. Parameters such as chlorophyll content and antioxidant levels were evaluated in freshly collected leaves 27 days after germination. Additionally, the concentrations of nitrogen (N), phosphorus (P), and potassium (K) were determined in leaves collected 60 days after germination.

2.8. Estimation of Chlorophyll

Initially, 0.5 g of fresh leaves was grinded in a pestle-mortar with 20 ml of 80% acetone. After filtration, absorbance was measured at 663 and 645 nm using a spectrophotometer (Arnon, 1949).

$$\text{Chlorophyll a (mg/g)} = ((12.7\times A663) - (2.69\times A645)\times V)/(1000\times W) \quad (1)$$

$$\text{Chlorophyll b (mg/g)} = ((22.9\times A645) - (4.68\times A663)\times V)/(1000\times W) \quad (2)$$

$$\text{Total Chlorophyll (mg/g)} = (20.2\ (A645) + 8.02\ (A663)\times V)/(1000\times W) \quad (3)$$

2.9. Antioxidant

The assessment of superoxide dismutase (SOD) activity involved measuring the inhibition of nitro blue tetrazolium (NBT) reduction at 560 nm, as described in a previous study (Dhindsa *et al.,* 1982). For Peroxidase (POD) activity evaluation, the standard protocol at 420 nm was followed (Hori *et al.,* 1997). Catalase (CAT) activity was determined by measuring the breakdown of hydrogen peroxide (H2O2) and the resulting reduction in absorbance at 240 nm, indicative of H_2O_2 decomposition (Aebi, 1984). To quantify malondialdehyde (MDA), an indicator of lipid peroxidation, the sample extract was reacted with thiobarbituric acid (TBA) to form a colored complex. The absorbance of this complex was measured at 532 nm (Cakmak and Horst, 1991).

2.10. Ascorbic acid (AsA)

Inspired by the methodology of Mukherjee and Choudhuri (1983), we assessed AsA levels in maize leaves (0.25 g). The extraction process involved using 10 mL of 6% TCA. To this, we added one drop of thiourea (10%, dissolved in 70% ethanol) and 2% dinitrophenyl hydrazine (2 mL in 9 N H_2SO_4) to a four mL aliquot of the sample. After a 15-minute incubation, the solution was cooled, and the optical density (OD) was measured at 530 nm after adding 80% H_2SO_4 (5 mL).

2.11. Relative water contents (RWC)

The fresh weights of young leaf samples were determined, followed by immersion in water for an hour to record turgid weights. Subsequently, the leaf samples were dried to assess their dry weights. The Relative Water Contents (RWC) of the youthful leaf samples were computed using the methodology outlined by Barrs and Weatherley (1962).

$$\text{RWC (\%)} = [(FW\text{-}DW) / (TW\text{-}DW)] \times 100 \quad (4)$$

2.12. Proline content

The proline concentration (mgg^{-1}) was determined using the method described by Bates et al. (1973). This involved employing the acid ninhydrin reagent and measuring the absorbance of the toluene chromophore at 520 nm (Watanabe *et al.*, 2000).

2.13. Total phenolics

A leaf sample weighing 100 mgwas finely ground in 80% acetone (5mL), followed by centrifugation. Subsequently, a 0.1 mL aliquot was combined with 1 mL of Folin-Ciocalteau's reagent and 2 mL of deionized water. After thorough mixing, 5 mL of 20% sodium carbonate was added, and the final volume was adjusted to 10 mL with distilled water. Total phenolics were quantified at 750 nm (Julkunen-Tiitto, 1985).

2.14. N, P, and K leaf and roots

Samples were digested using sulfuric acid for N analysis (Mills and Jones, 1991), while a diacid mixture was used for P and K (Miller, 1997). The nitrogen content was assessed through a customized micro-Kjeldahl method (Steyermark and McGee, 1961). Potassium content was measured using a flame photometer. Simultaneously, phosphorus content was quantified at 420 nm using a spectrophotometer, employing the yellow color method (Mills and Jones, 1991).

2.15. Statistical analysis

We conducted standard statistical analyses to compare the data. A two-way analysis of variance (ANOVA) was used to assess the treatment effect. Paired comparisons for treatment were performed using the Tukey test at a significance level of $p \leq 0.05$. For the cluster plot, convex hull, hierarchical cluster plot, and Pearson correlation, we used OriginPro (OriginLab Corporation, 2021).

3. Results

3.1. Growth attributes

In the absence of ZnFNP, 15 µM QC increased shoot fresh weight by 9.63%, 2mgL^{-1} FA by 3.61%, and the combined treatment by 16.30% compared to the control. With ZnFNP, 15 µM QC resulted in an 11.07% increase, 2mgL^{-1} FA in a 5.32% rise, and the combination in a significant 17.25% increase related to the control (Figure 1A). Compared with the control group without ZnFNP, shoot dry weight increased by 33.37% with 15 µM QC. The addition of 2mgL^{-1} FA resulted in a 12.13% increase in shoot dry weight, while the combined treatment of 2mgL^{-1} FA and 15µM QC led to a substantial 54.94% increase over the control with no ZnFNP. For the ZnFNP-treated plants, shoot dry weight increased by 24.61% with 15 µM QC, 13.64% with 2mgL-1 FA, and 39.34% with the combined treatment of 2mgL^{-1} FA and 15 µM QC, values that were parallel to the control (Figure 1B). The root fresh weight increased by 14.25%, 6.60%, and 24.66% when treated with 15 µM QC, 2mgL^{-1} FA, and 2mgL^{-1} FA + 15 µM QC, respectively, parallel to the control without ZnFNP. Exposure to 15 µM QC+ZnFNP caused a 12.13% rise in the root fresh weight, while 2mgL^{-1} FA+ZnFNP treatment resulted in an 8.12% increase over the control. The combined application of 2mgL^{-1} FA and 15 µM QC with ZnFNP led to a notable 15.37% rise in root fresh weight related to the ZnFNP control (Figure 1C). In the case of root dry weight, the 15 µM QC, 2mgL^{-1} FA, and 2mgL^{-1} FA+15 µM QC treatment without ZnFNP showed 22.34%, 12.06%, and 35.12% and with ZnFNP resulted in 14.97%, 6.33%, and 26.65% increase parallel to the control (Figure 1D).

3.2. Chlorophyll and Leaf Relative Water Content

Compared to the control group, chlorophyll a content increased by 35.47% with 15 µM QC treatment without ZnFNP, by 58.65% with 2mgL^{-1} FA, and by 98.39% with the combined treatment of 2mgL^{-1} FA and 15 µM QC. In ZnFNP, the 15 µM QC resulted in a 21.33% increase, and the application of 2mgL^{-1} FA and 2mgL^{-1} FA+15 µM QC led to an 11.11% and 38.22% increase in chlorophyll a content more than the control (Figure 2A). The chlorophyll b content exhibited a 47.79% increase in the presence of 15 µM QC with no ZnFNP as opposed to the control. The addition of 2mgL^{-1} FA led to a 26.10% rise, while the combined treatment of 2mgL^{-1} FA and 15 µM QC resulted in a significant 67.47% elevation with no ZnFNP above the control. In the ZnFNP-treated samples, the chlorophyll b levels increased by 50.00% with 15 µM QC, 23.49% with 2mgL^{-1} FA, and 63.79% with the combined treatment of 2mgL^{-1} FA and 15 µM QC, as related to the ZnFNP control (Figure 2B). Regarding total chlorophyll content, the 15 µM QC treatment showed a 68.32% increase with no ZnFNP and a 35.89% increase with ZnFNP over the control. Adding 2mgL^{-1} FA resulted in a 37.43% increase in total chlorophyll content for no ZnFNP and a 17.40% increase for ZnFNP. In contrast to the control, when 2mgL^{-1} FA was combined with 15µM QC, the total chlorophyll content significantly increased by 98.43% in the absence of ZnFNP and 51.20% with ZnFNP (Figure 2C). The relative leaf water content increased by 10.42%, 1.54%, and 19.66% with 15 µM QC, 2mgL^{-1} FA, and 15 µM QC+2mgL^{-1} FA treatments in the absence of ZnFNP, respectively, more than the control. The 15 µM QC, 2mgL^{-1} FA, and 15 µM QC+2mgL^{-1} FA treatment with ZnFNP led to a notable 16.74%, 8.65%, and 23.50% increase in leaf relative water content over the ZnFNP control (Figure 2D).

3.3. MDA, H_2O_2, AsA, and total phenolics

The 15 μM QC with no ZnFNP resulted in an 18.56% decrease in MDA content; 2mgL^{-1} FA led to a 10.13% decrease; and 2mgL^{-1} FA + 15 μM QC resulted in a notable 27.60% decrease, more than the control. In the ZnFNP treatments, 15 μM QC resulted in a 27.58% decrease in MDA content, 2mgL^{-1} FA led to an 11.91% decrease, and 2mgL^{-1} FA+15 μM QC resulted in a substantial 35.93% decrease above the control (Figure 3A). Under no ZnFNP, 15 μM QC exhibited a 31.79% decrease, while 2mgL^{-1} FA resulted in a 10.544% decrease in H_2O_2 above the control, and 2mgL^{-1} FA+15 μM QC led to a significant 69.52% decrease. In the presence of ZnFNP, 15 μM QC showed a 59.25% decrease in H2O2 compared with the control; 2mgL^{-1} FA led to a 26.82% increase; and their combination resulted in a substantial 99.86% decrease (Figure 3B). The AsA content in samples treated with 15 μM QC decreased by 11.5% without ZnFNP and 16.0% with ZnFNP over the control. Adding 2mgL^{-1} FA decreased AsA content by 53.0% with no ZnFNP and 6.0% with ZnFNP, parallel to the control. Combining 2mgL^{-1} FA and 15 μM QC led to a substantial 32.7% decrease in AsA content with no ZnFNP and 27.6% with ZnFNP compared to the control (Figure 3C). In comparison to the control, 15 μM QC alone decreased total phenolics by 16.40%, while 2mgL^{-1} FA led to a 7.25% decrease with no ZnFNP. The combined treatment of 2mgL^{-1} FA and 15 μM QC resulted in a 26.13% decrease compared to the control under no ZnFNP. With ZnFNP, the 15 μM QC showed a 20.00% reduction in total phenolics, and 2mgL^{-1} FA led to a 6.74% decrease; a combination of 2mgL^{-1} FA+15 μM QC with ZnFNP resulted in a significant 34.04% decrease parallel to the control (Figure 3D).

3.4. POD, SOD, CAT, and leaf-free proline

Peroxidase activity (POD) showed varied responses across different experimental conditions; without ZnFNP, 15 μM QC resulted in a 29.41% decrease, whereas 2mgL^{-1} FA led to a 13.04% reduction compared with the control. The 2mgL^{-1} FA + 15 μM QC treatment showed a 55.92% decrease in POD activity compared to the control without ZnFNP. In the presence of ZnFNP, 15 μM QC showed a substantial 73.27% decrease, 2mgL^{-1} FA led to a 29.79% reduction, and the combined treatment resulted in a remarkable decrease in POD activity compared with the control (Figure 4A). In the case of SOD, 15 μM QC showed a 31.51% decrease, 2mgL^{-1} FA displayed a 14.88% decrease, and the combination of 2mgL^{-1} FA with 15 μM QC resulted in a significant 43.06% decrease with no ZnFNP. In contrast, the ZnFNP group exhibited a 26.15% decrease with 15 μM QC, a 16.32% decrease with 2mgL^{-1} FA, and a substantial 42.70% decrease with the combined treatment of 2mgL^{-1} FA and 15 μM QC, parallel to the control (Figure 4B). With no ZnFNP, CAT activity decreased by 19.35%, 11.39%, and 27.52% with 15 μM QC, 2mgL^{-1} FA, and a combination of both over the control. In the presence of ZnFNP, CAT activity decreased by 19.83%, 9.87%, and 31.00% with 15 μM QC, 2mgL^{-1} FA, and 15 μM QC+2mgL^{-1} FA treatment, respectively, compared to the control (Figure 4C). In the absence of ZnFNP, 15 μM QC led to a 14.31% decrease in leaf-free proline, 2mgL^{-1} FA resulted in an 8.18% decrease, and the combination of 2mgL^{-1} FA+15 μM QC demonstrated a substantial 27.43% decrease, rivaled to the control group. The 15 μM QC with ZnFNP resulted in a remarkable 43.41% decrease, while 2mgL^{-1} FA with ZnFNP led to a notable 21.52% decrease in leaf-free proline parallel to the control. The most substantial reduction was observed in the group treated with 2mgL^{-1} FA and 15 μM QC, along with ZnFNP, which showed a remarkable 72.13% decrease in leaf-free proline content compared to the control (Figure 4D).

3.5. Leaf N, P, and K

The 15 μM QC treatment showed a 12.22% increase in leaf N over the control without ZnFNP; 2mgL^{-1} FA resulted in a 4.58% rise, while the combination of 2mgL^{-1} FA and 15 μM QC led to a 21.90% increase. In the ZnFNP, 15 μM QC showed a 12.07% increase in leaf N, 2mgL^{-1} FA led to a 6.63% rise, and the combination of 2mgL^{-1} FA and 15 μM QC resulted in a 16.18% increase, contrasted to the ZnFNP control (Figure 5A). Leaf P showed varying responses compared to the control across treatments. In the absence of ZnFNP, 15 μM QC showed a notable increase of 33.72% in leaf P, while 2mgL^{-1} FA resulted in a rise of 17.05% in contrast to the control. The combined treatment with 2mgL^{-1} FA and 15 μM QC resulted in a 63.57% increase over the control without ZnFNP. In the presence of ZnFNP, 15 μM QC induced a significant rise of 27.87% in leaf P, 2mgL^{-1} FA resulted in a 12.09% increase, and the combination of 2mgL^{-1} FA and 15 μM QC showed a marked elevation of 44.67% parallel to the control (Figure 5B). Compared to the control group, leaf K exhibited a 32.39% increase with 15 μM QC treatment, a 2.83% increase with 2mgL^{-1} FA treatment, and a substantial 55.47% increase with the combined treatment of 2mgL^{-1} FA and 15 μM QC in the absence of ZnFNP. In the presence of ZnFNP, the leaf K showed an 18.18% increase with 15 μM QC treatment, a 6.94% increase with 2mgL^{-1} FA treatment, and a notable 29.90% increase with the combined treatment of 2mgL^{-1} FA and 15 μM QC over the control (Figure 5C).

3.6. Root N, P, and K

With no ZnFNP, the addition of 15 μM QC resulted in a 32.65% increase in root N content, while the inclusion of 2mgL^{-1} FA led to a 14.29% elevation in contrast to the

control. Combining 2mgL^{-1} FA with 15 µM QC resulted in a 47.96% increase in root N compared to the control with no ZnFNP. In the presence of ZnFNP, the application of 15 µM QC resulted in a 17.72% increase in root N, and the addition of 2mgL^{-1} FA resulted in a 9.49% increase relative to the control. The combination of 2 mgL^{-1} FA and 15 µM QC with ZnFNP exhibited the most significant effect, with a 36.08% increase in root N contrasted to the control (Figure 6A). The root P (%) showed a 57.14% increase in the 15 µM QC treatment compared with the control without ZnFNP. With 2mgL^{-1} FA treatment, there was a 28.57% increase in root P, and the combined treatment of 2mgL^{-1} FA and 15 µM QC resulted in a substantial 100% increase. For ZnFNP, the root P in the 15 µM QC treatment showed a 29.41% increase relative to the control; the 2mgL^{-1} FA treatment showed 17.65%; and the combined treatment of 2mgL^{-1} FA and 15 µM QC resulted in a 52.94% increase (Figure 6B). Adding 15 µM QC increased root K by 6.29% compared with the control with no ZnFNP, by 3.09% with 2mgL^{-1} FA, and by 12.56% with the combination. In ZnFNP samples, 15 µM QC increased K by 8.21%, 2mgL^{-1} FA by 3.28%, and the combination by 15.06% evaluated to the control (Figure 6C).

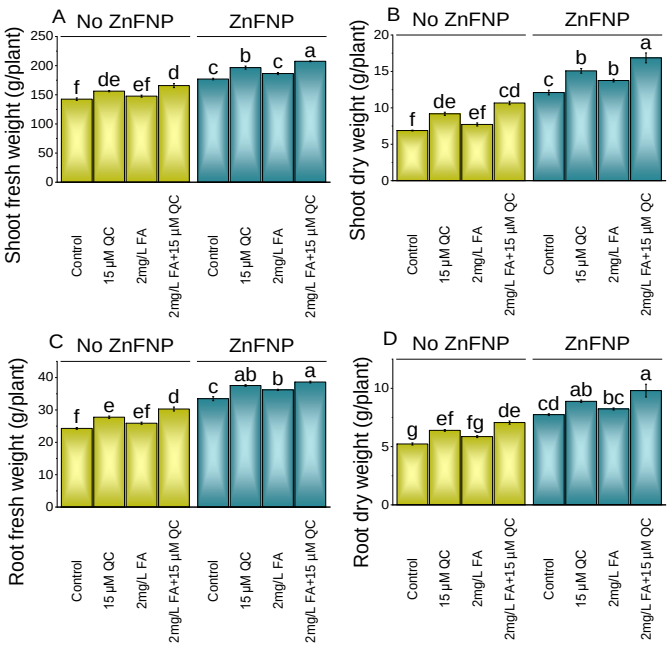

Figure 1. Impact of fulvic acid and quercetin on fresh and dry weight of maize shoot (A&B) and root (C&D) cultivated with and without ZnFNP. Bars represent mean values ± standard error [n=4]. Different letters (on bars) indicate significant differences compared at $p < 0.05$ applying Tukey's test.

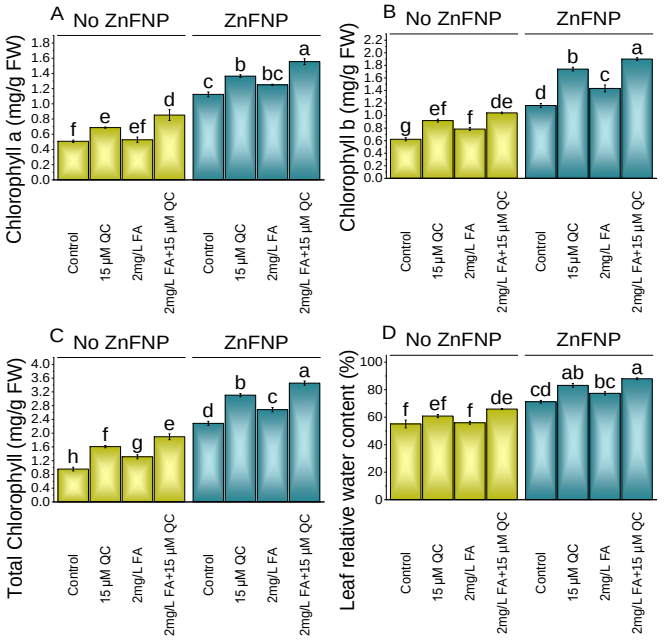

Figure 2. Impact of fulvic acid and quercetin on chlorophyll of maize leaves (A,B&C) and relative water content (D) cultivated with and without ZnFNP. Bars represent mean values ± standard error [n=4]. Different letters (on bars) indicate significant differences compared at $p < 0.05$ applying Tukey's test.

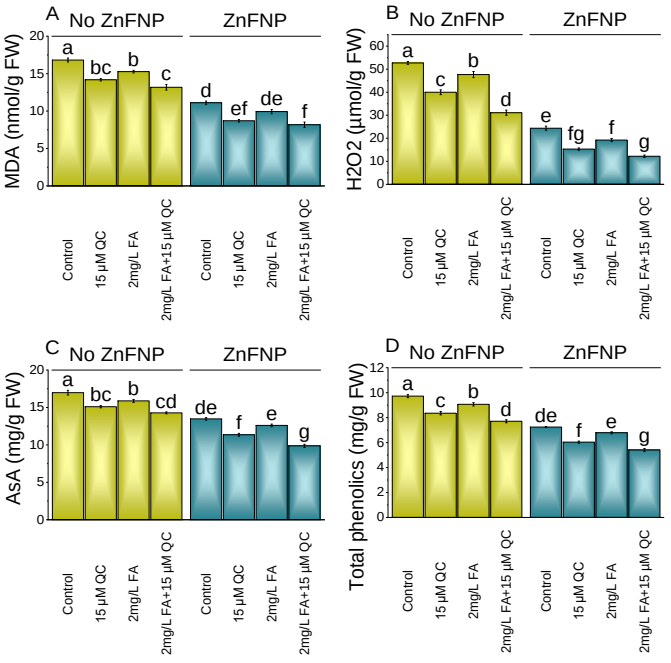

Figure 3. Impact of fulvic acid and quercetin on malondialdehyde; MDA (A), hydrogen peroxide; H_2O_2 (B), ascorbic acid; AsA (C), and total phenolics (D) cultivated with and without ZnFNP. Bars represent mean values ± standard error [n=4]. Different letters (on bars) indicate significant differences compared at $p < 0.05$ applying Tukey's test.

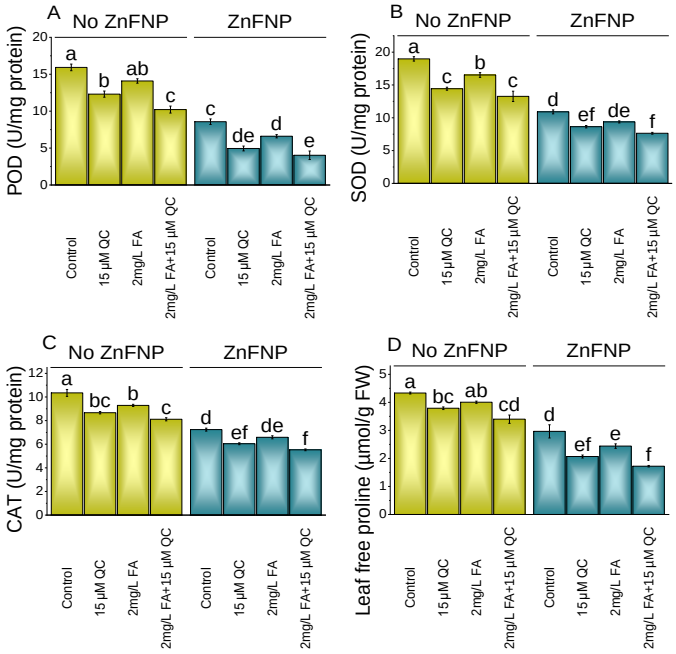

Figure 4. Impact of fulvic acid and quercetin on peroxidase; POD (A), superoxide dismutase; SOD (B), catalase; CAT (C), and leaf-free proline (D) cultivated with and without ZnFNP. Bars represent mean values ± standard error [n=4]. Different letters (on bars) indicate significant differences compared at $p < 0.05$ applying Tukey's test.

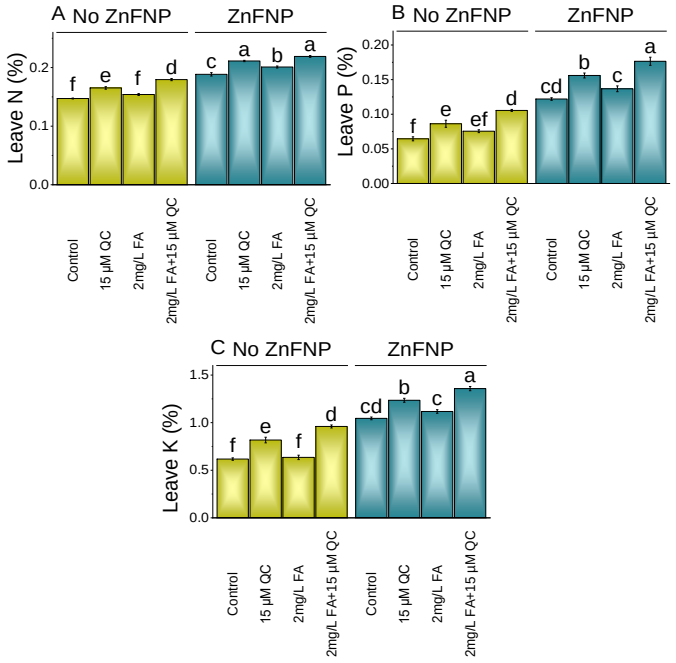

Figure 5. Impact of fulvic acid and quercetin on leaf nitrogen, phosphorus, and potassium (A,B&C) cultivated with and without ZnFNP. Bars represent mean values ± standard error [n=4]. Different letters (on bars) indicate significant differences compared at $p < 0.05$ applying Tukey's test.

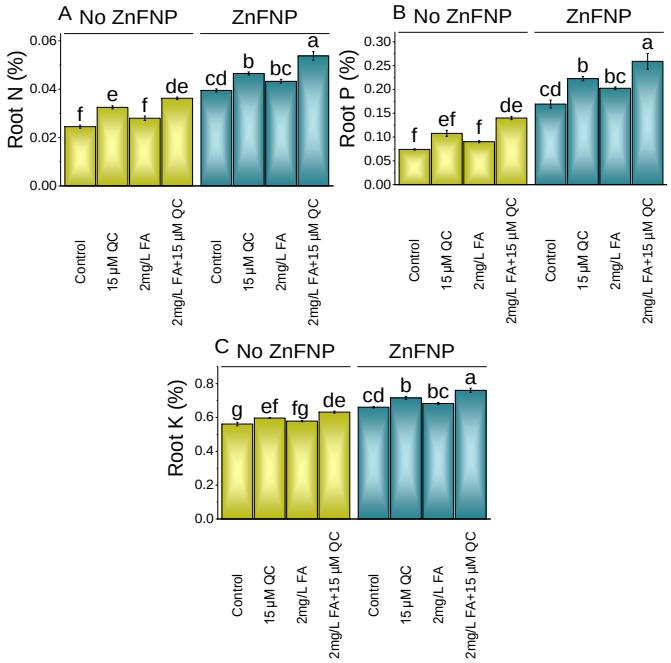

Figure 6. Impact of fulvic acid and quercetin on root nitrogen, phosphorus, and potassium (A,B&C) cultivated with and without ZnFNP. Bars represent mean values ± standard error [n=4]. Different letters (on bars) indicate significant differences compared at $p < 0.05$ applying Tukey's test.

3.7. Convex Hull and Hierarchical Cluster Analysis

The convex hull analysis reveals distinct clustering patterns among the treatments in the principal component space (PC 1 and PC 2). The control group is characterized by a large convex hull with 98.04% and 0.57% contributions in PC 1 and PC 2, respectively. Samples treated with 15 µM QC form a separate cluster, with negative PC1 scores dispersed across PC2. The 2mgL^{-1} FA treatment exhibits a distinct grouping with positive PC 1 scores. In contrast, the combination of 2mgL^{-1} FA and 15µM QC creates a cluster extending into both positive and negative PC1 values. Examining individual scores, the control samples consistently exhibit negative PC 1 values, suggesting a commonality in their response. In contrast, 15 µM QC-treated samples exhibit negative PC1 scores, highlighting their divergence from the control. Samples treated with 2mgL^{-1} FA exhibit positive PC 1 scores, indicating a unique response. The combined treatment of 2mgL^{-1} FA and 15µM QC shows a complex pattern with both positive and negative PC1 values, reflecting a nuanced impact on the samples (Figure 7A). The convex hull analysis provided valuable insights into the distribution of scores across principal components (PC1 and PC2). PC1 accounted for 98.04% of the observed variation, while PC2 accounted for 0.57%. The scores and associated labels were examined within the Convex Hull for no ZnFNP and ZnFNP treatments. For the no ZnFNP treatment, data points exhibited a clear clustering pattern

with scores from -7.87972 to -1.00236 along PC1 and from 0.01837 to 0.40286 along PC2. This clustering suggests a cohesive grouping of samples within the no ZnFNP. In contrast, the ZnFNP treatment displayed a broader distribution of scores along both PC1 and PC2. Scores ranged from -0.2018 to 8.74652 along PC1 and from -0.48716 to 1.50767 along PC2. Notably, the ZnFNP treatment showed a distinct separation from the no-ZnFNP cluster, particularly evident in the positive direction along PC1 (Figure 7B). The results of the hierarchical cluster analysis (HCA) unveiled meaningful associations among various plant parameters, shedding light on their interrelationships. The variables and their pairwise similarities, along with corresponding labels when available, are summarized below: The first cluster includes root dry weight and total, displaying a notable similarity of 0.08413. In the second cluster, leaf K and total Chlorophyll exhibit a similarity of 0.13792, suggesting a potential connection between these traits. Moving to the third cluster, shoot dry weight and leaf K to share a similarity of 0.22205, indicating a degree of association between shoot biomass and leaf potassium content. The fourth cluster involves leaf N and root P, demonstrating a strong similarity of 0.27541, suggesting a correlation between nitrogen and phosphorus levels in plant tissues. The fifth cluster comprises MDA and POD, showing a similarity of 0.29892, potentially reflecting a shared response to oxidative stress. In the sixth cluster, shoot fresh and root K exhibit a similarity of 0.32718, hinting at a relationship between leaf area and root potassium content. The seventh cluster involves CAT, demonstrating a similarity of 0.34357, suggesting a potential connection between catalase activity. The eighth cluster consists of total phenolics and AsA, with a high similarity of 0.44476, suggesting potential co-regulation of these biochemical components. Chlorophyll b forms the ninth cluster with a substantial similarity of 0.53401. In the tenth cluster, SOD and leaf N show a similarity of 0.56582, suggesting a connection between superoxide dismutase activity and leaf nitrogen content. Moving to the twelfth cluster, H2O2 and chlorophyll demonstrate a high similarity of 0.83598, suggesting a potential link between hydrogen peroxide levels and chlorophyll a content. In the thirteenth cluster, leaf P and leaf-free proline are like 0.99199, hinting at a potential correlation between phosphorus content and free proline levels in leaves. The last cluster involves leaf-free proline and the last two variables, forming a distinct subgroup with higher similarities ranging from 1.54442 to 98.76066 (Figure 7C).

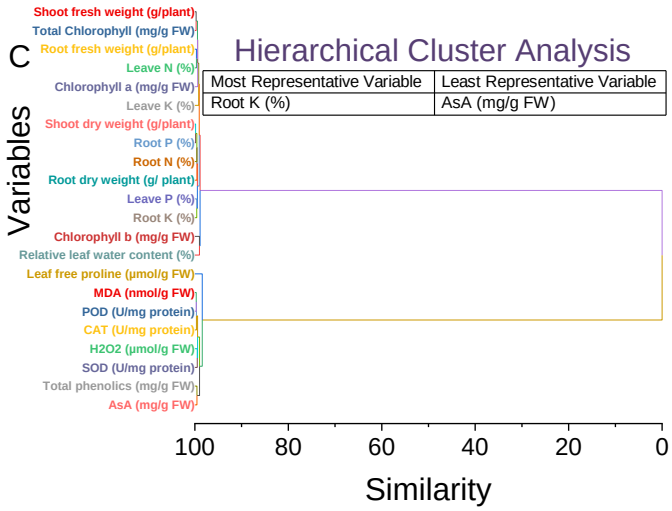

Figure 7. Cluster plot with convex hulls illustrating treatments (A), ZnFNP levels (B), and a hierarchical cluster plot (C) for the analyzed attributes.

3.8. Pearson Correlation Analysis

The pearson correlation analysis provided valuable insights into the intricate relationships among various plant parameters. A strong positive correlation (0.99446) was observed between shoot and root dry weight, indicating a closely linked growth pattern. Additionally, shoot fresh weight showed strong positive correlations with shoot dry weight (0.98986), root dry weight (0.99244), and other growth-related variables, underscoring a cohesive association between leaf area and overall plant biomass. Pigment-related parameters, such as chlorophyll a, b, and total chlorophyll, demonstrated positive correlations with each other, underscoring their role in the photosynthetic process (0.96561-0.99834). Conversely, leaf-free proline exhibited negative correlations with several parameters, particularly MDA and H_2O_2, suggesting a potential role in stress-related responses (-0.96834 to -0.99196). Antioxidant-related parameters, including total phenolics, AsA, POD, and CAT, displayed negative correlations with oxidative stress markers, indicating potential antioxidant roles (-0.97923 to -0.99482). Nutrient content correlations were evident among elements in leaves and roots. Leaf N, P, and K exhibited positive correlations, reflecting potential relationships between leaf nutrient contents (0.96479 to 0.99573). Similarly, roots N, P, and K showed positive correlations, suggesting potential coordination in nutrient levels within the root system (0.95633 to 0.99455) (Figure 8). HCA revealed clear grouping between treatments with ZnFNP and those without, with growth- and chlorophyll-related traits clustering together, while oxidative stress markers formed a distinct group

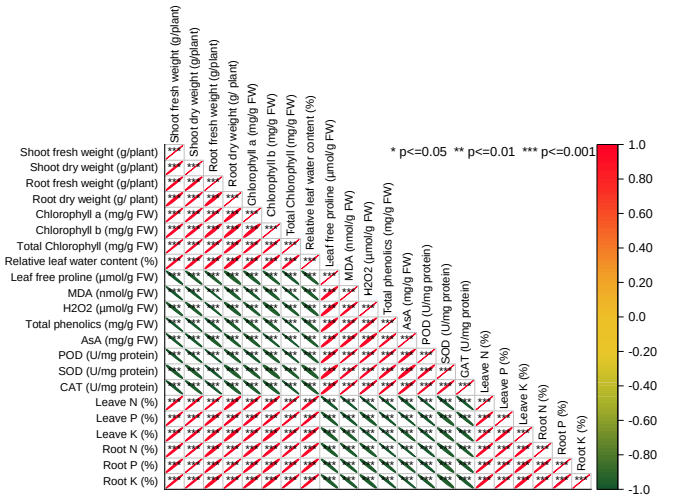

Figure 8. Analysis of Pearson correlations for the measured parameters.

4. Discussion

Salinity stress can adversely affect plant growth by disrupting water balance, leading to dehydration and reduced nutrient uptake (Soni *et al.*, 2023). Elevated soil salt levels can also hinder essential metabolic processes, thereby impeding overall plant development and productivity (Wang *et al.*, 2023). The substantial increase in shoot and root fresh and dry weight, especially with the application of 2mgL^{-1} FA and 15 µM QC, can be attributed to quercetin's synergistic effects on nutrient uptake and utilization. QC may facilitate nutrient absorption, increasing biomass (Qiu *et al.*, 2023). With its chelating properties, Fulvic acid could enhance nutrient availability by forming stable complexes with essential ions, promoting overall plant growth (Jiang *et al.*, 2023). In the presence of ZnFNP, the observed increase in shoot and root dry weight suggests a potential mitigative effect of 15 µM QC and 2mgL^{-1} FA, possibly alleviating oxidative stress induced by ZnFNP. The rise in chlorophyll content, linked to the antioxidant properties of QC and nutrient-chelating capabilities of FA, suggests a potential mitigation of nanoparticle-induced (Ren *et al.*, 2023). In the presence of ZnFNP, the moderate increase in chlorophyll content under the treatments indicates a potential counteraction of negative effects on chlorophyll synthesis and stability. The increase in leaf relative water content with 15 µM QC, 2mgL^{-1} FA, and their combination may be linked to QC's antioxidant properties, which preserve cell membrane integrity and promote water retention (Zeng *et al.*, 2024).

In the presence of ZnFNP, the observed increase in leaf-relative water content suggests that the applied treatments mitigated nanoparticle-induced water stress, highlighting the key roles of QC and FA in overcoming adverse effects on leaf water status (Hayat *et al.*, 2023). The substantial reduction in leaf-free proline in the presence of ZnFNP suggests that ZnFNP may enhance stress tolerance by modulating proline metabolism. ZnFNP may achieve this by activating or regulating enzymes involved in proline degradation, or by influencing stress signaling pathways, thereby decreasing proline accumulation. The decrease in MDA levels with ZnFNP indicates an effective antioxidant defense system (ur Rehman *et al.*, 2023). MDA serves as a marker of lipid peroxidation and oxidative stress, and the mechanism involves ZnFNP enhancing the activity of antioxidant enzymes or directly scavenging reactive oxygen species (ROS), thereby reducing lipid peroxidation and MDA formation (Jyothish and Jacob, 2023). The decrease in H_2O_2 levels with the combined treatment and ZnFNP suggests an efficient ROS scavenging system. H_2O_2 serves as a signaling molecule and a byproduct of oxidative stress, and ZnFNP may enhance the activity of enzymes involved in H_2O_2 breakdown or activate antioxidant pathways, leading to reduced H_2O_2 accumulation (Lourenço *et al.*, 2023). The decrease in AsA levels suggests a potential utilization of AsA in scavenging ROS or activating alternative antioxidant pathways (Kamran *et al.*, 2023). AsA is a key antioxidant, and its mechanism involves ZnFNP stimulating the activity of enzymes involved in AsA recycling or activating pathways that utilize AsA for ROS detoxification. The decrease in total phenolics with ZnFNP suggests a complex interplay between ZnFNP and phenolic metabolism. As secondary metabolites with antioxidant properties, Phenolics may be influenced by ZnFNP, potentially through the modulation of gene expression in phenolic biosynthesis or the activity of enzymes in the phenolic pathway, leading to changes in total phenolic content (Metwally and Abdelhameed, 2023). The enhanced performance of the combined treatment suggests a synergistic interaction where QC improves redox balance, FA enhances nutrient chelation and transport, and ZnFNP facilitates micronutrient availability, collectively strengthening physiological resilience under salinity stress.

Regarding POD, SOD, and CAT activities, the decrease in POD activity with ZnFNP suggests a modulation of the ROS detoxification system (Haydar *et al.*, 2023). POD, involved in peroxide detoxification, may be influenced by ZnFNP in terms of gene expression or activity, leading to decreased POD activity. Similarly, the decrease in SOD activity with ZnFNP suggests a modification of the antioxidant defense system, where ZnFNP may regulate the expression or activity of SOD. The decrease in CAT activity with ZnFNP suggests a controlled ROS-scavenging mechanism. ZnFNP may influence CAT-related pathways, ensuring an optimal level of CAT activity for efficient hydrogen peroxide detoxification without causing excessive depletion. For leaf nitrogen (N), phosphorus (P), and potassium (K)

content, the increases with applied treatments indicate a positive impact on nutrient uptake and assimilation (Li *et al.*, 2021). QC and FA may enhance nutrient uptake, influence root architecture, or modulate nutrient transporters, increasing leaf N, P, and K content. Similarly, the increase in root nitrogen (N), phosphorus (P), and potassium (K) content suggests a positive influence on root development and nutrient absorption. The combined treatment of $2mgL^{-1}$ FA and 15 μM QC, especially in the presence of ZnFNP, may enhance root growth, activate nutrient transporters, or modulate rhizospheric processes, increasing root N, P, and K content (Afzal *et al.*, 2022). Although ZnFNP showed positive effects, potential risks such as nanoparticle accumulation in soil, long-term impacts on microbial communities, and cost considerations should be acknowledged. Future field-scale trials are essential to assess environmental safety and economic feasibility.

5. Conclusions

In conclusion, using $2mgL^{-1}$ FA + 15 μM QC with ZnFNP shows potential to enhance maize growth under salinity stress. Applying $2mgL^{-1}$ FA + 15 μM QC with ZnFNP notably enhances the absorption of essential nutrients, such as N, P, and K, in both shoot and root systems, thereby improving maize growth under salinity stress. Moreover, the $2mgL^{-1}$ FA + 15 μM QC with ZnFNP treatment has the potential to regulate antioxidant levels under salinity, thereby mitigating the detrimental effects of salinity on maize. Further comprehensive field studies are encouraged to assess the efficacy of $2mgL^{-1}$ FA + 15 μM QC with ZnFNP as a prime solution for alleviating salinity stress in maize. The combined QC–FA–ZnFNP treatment demonstrates strong potential for integration into salinity-affected cropping systems. Its scalability through foliar application makes it a viable candidate for field testing to improve maize resilience in saline regions.

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Abbreviations

Abbreviations used in this manuscript include:

AsA	Ascorbic acid
CAT	Catalase
FA	Fulvic acid
K	Potassium
MDA	Malondialdehyde
N	Nitrogen
OD	Optical density
P	Phosphorus
POD	Peroxidase
QC	Quercetin
RWC	Relative water contents
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
ZnFNP	Zinc ferrite nanoparticles

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