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Original Research Article

A Novel Synergistic Strategy Using Nano-Ascorbic Acid and Green-Synthesized Iron Nanoparticles to Enhance Drought Tolerance in Cowpea (*Vigna unguiculata* L.)

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Abstract: One of the most serious limiting factors to cowpea (Vigna unguiculata L.) production in arid and semi-arid areas is drought stress. This paper has examined the elastic co-action of nano-ascorbic acid (nano-AsA) and green-synthesized iron nanoparticles (FeNPs) applied as a folaric acid on cowpea growth, physiology, and yield during varying irrigation regimes. The experiment was a field experiment that was performed during summer in the year 2024 in Najaf, Iraq and was split-plot in nature with three replications. Irrigation treatments included 50% (I_1), 60% (I_2), and 70% (I_3) depletion of available soil water, while foliar treatments consisted of distilled water (T_0), nano-AsA (50 mg L^{-1}; T_1), green-synthesized FeNPs (50 mg L^{-1}; T_2), and their combination (T_3). The FeNPs were synthesized through the green synthesis, which is environmentally friendly using the Moringa oleifera leaf extract, whereas the nano-AsA was synthesized by the ultrasonication. Findings revealed that the drought stress seriously diminished the height of plants, leaf area, number of pods and seeds per plant, dry biomass, and grain yield and increased proline content and activity of peroxidase. Application of nano-AsA and FeNPs (particularly their combination) alleviated the negative impact of drought through the promotion of antioxidant defenses, vegetative growth, and reproduction. Combined treatment (T 3) was always better than the individual application which led to delayed flowering, high biomass, and yield stability at stress levels. The results of the study indicate the possibility of combining nano-AsA and green-synthesized FeNPs as a new and greener approach to enhancing drought resistance and sustainable cowpea growth.

Keywords: Cowpea, Drought Stress, Nano-Ascorbic Acid, Green-Synthesized Iron Nanoparticles, Antioxidant Defense, Yield Stability.

INTRODUCTION

Cowpea (Vigna unguiculata L.) is a leguminous crop that is considered essential to semi-arid and arid lands due to the high level of dietary proteins, fiber, and vital micronutrients. Although it is rather resistant to drought than in other legumes, its productivity is significantly diminished during drought. Water scarcity -Climate change, sporadic rainfall, and increased temperatures are worsening water shortage, which has adverse impacts on photosynthesis, growth, and the development of reproduction and hence reducing yield potential [1.]

Stress/drought promotes the formation of reactive oxygen species (ROS), which damages cell membrane, proteins and nucleic acids. In spite of the existence of antioxidant defense system in plant like superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) systems, extreme or prolonged drought usually overwhelms the plants with these systems [2]. Thus, antioxidant compounds when used exogenously are deemed to be powerful measures that will enhance stress tolerance.

Ascorbic acid (AsA, vitamin C) is an important antioxidant molecule, which acts as an antioxidant, modulates photosynthesis, and osmotic balance through the accumulation of compatible solutes like proline and soluble sugars. It was demonstrated that AsA applied as a foliar has the effect of increasing drought tolerance in crops by increasing enzymatic and non-enzymatic antioxidant capacity [3]. Nevertheless, the stability and quick degradation of the conventional AsA limit its efficiency in most cases.

Advancement of nano-ascorbic acid (nano-AsA) preparations provides a new potential of stability improvement, penetration, and longevity of activity on plant tissues. In the recent years, nanotechnology in agriculture has been given growing interest, owing to the inherent physicochemical characteristics of nanoparticles like increased surface area and reactivity, which enable nanoparticles to interact more efficiently with the plant systems (6). After all, iron nanoparticles (FeNPs) in particular have been reported to stimulate chlorophyll biosynthesis, antioxidant activity, and drought tolerance on a variety of crops [4.]

In this regard, green synthesis of FeNPs with plant extracts has become a sustainable method, which is eco-friendly and does not require the use of unsafe reducing agents and which produces the nanoparticles at low costs [9]. The green-synthesized FeNPs have been effectively tested on spinach and common bean where they enhanced physiological performance and alleviated oxidative injury when stressed by drought [5]. As an illustration, Yilmaz (2024) showed that a joint foliar application of AsA and green-synthesized FeNPs has significant effects on improving the chlorophyll content, antioxidant activities of enzymes, and reduced lipid peroxidation in common bean placed under drought [6.]

In spite of these promising results, no study has been conducted on synergistic application of nano-AsA and green-synthesized FeNPs in cowpea. The combination of these two methods is likely to yield twofold benefits direct antioxidant protection by using nano-AsA and better physiological control by using FeNPs. Such a mixture may increase scavenging of ROS, accumulation of osmolites, pigment stability and water-use efficiency. Furthermore, it can also be used to trigger expression of drought responsive genes hence the sustainable enhancement of cowpea production in water-limited conditions.

Hence, the proposed research will focus on exploring the synergistic interaction between foliar-applied nano-ascorbic acid and green-synthesized iron nanoparticles and cowpea drought resistance. The study will target the physiological, biochemical, and molecular parameters such as leaf water content, chlorophyll retention, antioxidant enzyme activities, osmolyte concentration, and the expression of genes of drought tolerance. The proposed results can offer a new environmentally friendly approach to address the drought stresses in cowpea and help in the worldwide sustainable agricultural practices and food security.

MATERIALS AND METHODS

Experimental Site and Design

The field experiment was carried out in 2024 in a summer season in the experimental farm of the Najaf Governorate, Iraq (32 o0 00 N, 44 o19 E, 32 m a.s.l.). The soil was graded as silty loam with a field capacity of 0.32 cm^3 cm -3 and permanent wilting point of 0.15 cm 3 cm -3 which gave it an available water content of 0.17cm 3 cm -3. The study was randomly assigned to a trial that would be conducted in a split-plot design with three replications of both types: randomized complete block design (RCBD). The main plots were given irrigation regimes and the sub-plots foliar treatments.

Treatments

Main plots included three levels of irrigation based on available soil water depletion:

- I_1: 50% depletion (mild stress)
- I_2: 60% depletion (moderate stress)
- I_3: 70% depletion (severe stress)

Sub-plots received the following foliar treatments:

- T_0: Control (distilled water spray)
- T_1: Nano-ascorbic acid at 50 mg L^{-1}
- T_2: Green-synthesized iron nanoparticles (FeNPs) at 50 mg L^{-1}
- T_3: Combination of nano-ascorbic acid (50 mg L^{-1}) + FeNPs (50 mg L^{-1})

Foliar sprays were done at the six leaf stage and again when the leaves started flowering to ensure that the treatment did not wear off.

The experimental layout, irrigation regimes, foliar treatments, soil sampling procedure, and preparation methods for nano-ascorbic acid and green-synthesized FeNPs are illustrated in Figure 1.

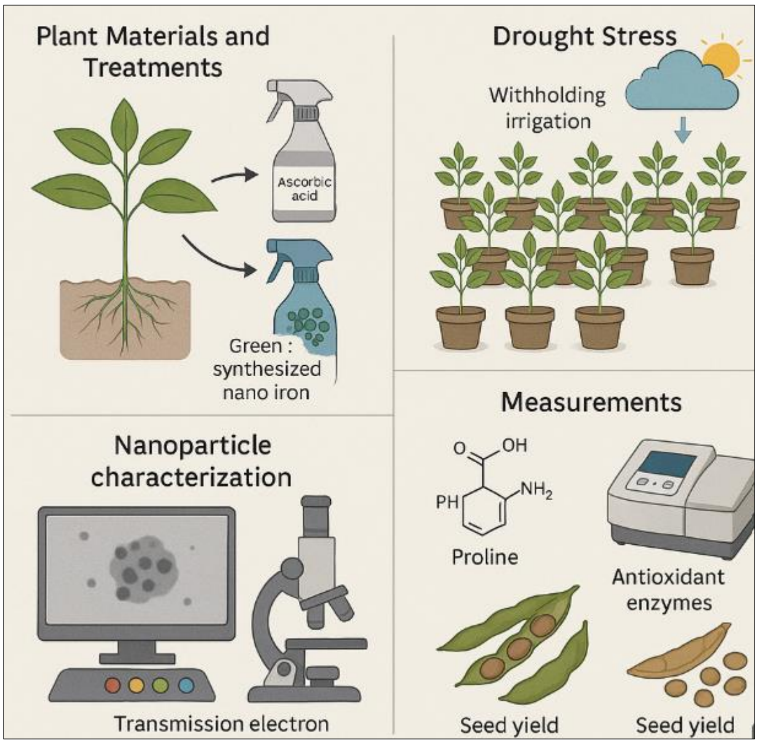

Figure 1: Graphical representation of the experimental design and methodology. (A) Field layout showing split-plot RCBD design with irrigation regimes (I_1 = 50%, I_2 = 60%, I_3 = 70% available water depletion) as main plots and foliar treatments (T_0 = control, T_1 = nano-ascorbic acid, T_2 = green-synthesized FeNPs, T_3 = combination) as sub-plots. (B) Foliar spraying at the six-leaf stage and flowering. (C) Soil sampling with auger at 0–20 and 0–30 cm depths. (D) Green synthesis of FeNPs using Moringa oleifera leaf extract. (E) Nano-ascorbic acid preparation by ultrasonication. (F) Flowchart of measured traits including morphological, yield, and biochemical parameters.

Crop Management

Seeds of cowpea (*Vigna unguiculata* L. cv. Bayader) were sown on March 15, 2024, at a spacing of 0.25 m between hills. Each experimental unit measured 3 × 2 m² and consisted of four ridges, each 3 m long. Thinning was performed one week after emergence to maintain one seedling per hill, while missing hills were re-seeded. Standard agronomic practices such as land preparation, weeding, and fertilization were applied equally across all treatments. Nitrogen fertilizer was supplied as urea (46% N) at a rate of 100 kg N ha^{-1}, split into three applications at sowing, elongation, and flowering.

Irrigation Scheduling and Soil Moisture Determination

Irrigation was delivered through a pump-driven plastic pipe system. Before stress induction, all plots were irrigated at 50% depletion of available soil water. Once seedlings reached the six-leaf stage, irrigation was scheduled according to the three depletion levels (50, 60, and 70%). Soil moisture was measured gravimetrically at depths of 0–20 cm during vegetative growth and 0–30 cm during flowering and pod filling stages. Fresh soil samples were collected with an auger, oven-dried at 105 °C until constant weight, and gravimetric water content was calculated and converted to volumetric content using soil bulk density. The irrigation depth was determined according to the FAO-56 method [7].

Preparation of Green-Synthesized FeNPs

Fresh leaves of *Moringa oleifera* were collected, washed with distilled water, air-dried at 40 °C, and ground into fine powder. Ten grams of the powder were boiled in 100 mL distilled water for 15 min, then filtered through Whatman No. 1 paper to obtain the aqueous extract. The extract was added dropwise to a 0.01 M $FeCl_3 \cdot 6H_2O$ solution (9:1 ratio, v/v) under magnetic stirring at 65 °C until the solution color changed from pale yellow to dark brown, confirming the formation of FeNPs. The suspension was allowed to cool, then stored at 4 °C until use. Particle size and morphology were confirmed by UV-Vis spectroscopy and dynamic light scattering analysis, showing FeNPs <100 nm in diameter.

Preparation of Nano-Ascorbic Acid

The sonochemical method was applied to the preparation of nano-ascorbic acid as a derivative of L-ascorbic acid powder (99 percent purity). Ultrasonication of aqueous solutions was conducted to 30 min resulting in stable nano-scale particles. The solution made was diluted afresh to 50 mg L -1 and applied to the foliage.

Characterization of Nanoparticles

A mixture of various physicochemical characterization methods was used to ensure the success of the synthesized green FeNPs and nano-ascorbic acid suspensions, as well as their stability. The surface plasmon resonance (SPR) peaks, which are characteristic of the nanoparticle synthesis, were recorded in UV–Visible spectroscopy (200800nm range) using a double-beam spectrophotometer (Shimadzu UV-1800, Japan). The hydrodynamic size distribution of the particle and the zeta potential that indicate colloidal stability were determined using dynamic light scattering (DLS) (Malvern Zetasizer Nano ZS90, UK). The morphology of particles and its surface structure were studied with the help of scanning electron microscopy (SEM) (JEOL JSM-6510LV, Japan), and the elemental composition of the particles, as well as the presence of iron in FeNPs, was verified with the help of energy-dispersive X-ray spectroscopy (EDX) combined with SEM. Furthermore, Fourier transform infrared spectroscopy (FTIR) (PerkinElmer Spectrum 100, USA) was also done to determine the functional groups of the Moringa oleifera extract attached to the FeNPs, which give evidence of bio-reduction and capping.

For nano-ascorbic acid, particle size and stability were confirmed using DLS and zeta potential analysis, while UV–Vis spectra were used to ensure retention of the characteristic absorption peak of ascorbic acid after nanosizing. Together, these analyses confirmed that both FeNPs (<100 nm) and nano-ascorbic acid were successfully synthesized and suitable for foliar application.

Measurements

Ten randomly selected plants per plot were used to measure:

- Days to 50% flowering
- Plant height (cm) at physiological maturity
- Number of branches per plant
- Leaf area (cm^2 $plant^{-1}$) at 50% flowering (Watson, 1953)
- Number of pods per plant and seeds per pod
- Dry biomass (g $plant^{-1}$) at flowering
- Seed yield (t ha^{-1}) adjusted to 14% standard moisture

Biochemical analyses included:

- Leaf proline content [8]
- Peroxidase (POD) activity [9]
- Malondialdehyde (MDA) as a marker of lipid peroxidation [10]

Statistical Analysis

The split-plot RCBD design was analyzed with the analysis of variance (ANOVA) on GenStat v20. The least significant difference test (LSD) was used to compare the means at the probability level of 5 percent [11].

RESULTS AND DISCUSSION

Days to 50% Flowering

Table 1 shows that regimes of irrigation, foliar treatments and their combination produced a significant effect on the number of days to 50 percent flowering in cowpea. Plants in both low and high irrigation levels took the longest time to flower (I 1 = 61.6 days), compared to those that were under severe stress (I 3 = 70% depletion) which flowered (56.9 days). The accelerated flowering rate during drought stress is an adaptive trait in numerous legumes, which allows plants to reduce the length of life cycle and avoid terminal drought [12].

There was a significant influence of foliar sprays on flowering time. Plants treated with nano-ascorbic acid (T 0) and FeNPs (T1 and T 2) delayed flowering (57.6 days and 59.0 and 60.1 days, respectively) as compared to the untreated control (T 0). The highest delay was recorded when nano-ascorbic acid and FeNPs were used together (T3: 61.6 days). This latency implies that exogenous supplementation prevented the acceleration in stress-induced phenology through protracted vegetative development before the onset of reproductive development. This is possible due to the properties of ascorbic acid to neutralize reactive oxygen species (ROS), protect photosynthetic pigments and regulate phytohormonal signals [13]. In a like manner, FeNPs have been reported to enhance chlorophyll biosynthesis and antioxidant enzyme activity that leads to the stability of phenology during droughts [14].

The relationship between foliar sprays and irrigation indicated the protective effect of treatments in stress conditions. In extreme drought (I3), the untreated plants (T0) flowered 50 percent in 55. 1 days, and the plants treated with the combined spray (T3) flowered in 59. 3 days. This four-day delay is physiologically important, and more assimilate can build up to reproduction. Similar findings were observed in faba bean and common bean in which flowering was postponed and the phenology stabilized in case of water deficit by foliar administration of ascorbic acid and green-synthesized FeNPs [15].

The LSD test at 5% probability level showed that the differences were significant: 1.46 days irrigation, 1.69 days foliar treatments and 0.93 days foliar treatments x irrigation. These outcomes emphasize the strength of the treatment effects that are observed.

The results, on the whole, indicate that the use of nano-ascorbic acid with green-synthesized FeNPs is an effective approach to reducing the water-induced increase in flowering in cowpea, which may lead to a greater yield stability in an environment with water limitations.

Table 1: Effect of foliar-applied nano-ascorbic acid, green-synthesized FeNPs, and their combination under different irrigation regimes on days to 50% flowering in cowpea

Foliar treatment	I_1 (50% AW depletion)	I_2 (60% AW depletion)	I_3 (70% AW depletion)	Mean
T_0: Control	59.3	58.4	55.1	57.6
T_1: Nano-AsA	61.6	59.9	55.5	59.0
T_2: FeNPs	62.1	60.4	57.9	60.1
T_3: Nano-AsA + FeNPs	63.3	62.3	59.3	61.6
Mean	61.6	60.3	56.9	—
LSD 0.05	Irrigation = 1.46	Foliar = 1.69	Interaction = 0.93	—

Plant Height (CM)

Irrigation regimes and foliar treatments also had a major influence on plant height and so did the interaction of the two (Table 2). The mean value indicated that the tallest growth was observed in well-watered plants (I 1 50% depletion), then moderate stress (I 2 45.57 cm), and the shortest growth was observed in severe stress (I 3 38.53 cm). The reduction of plant height in the drought stress is in line with the decrease in cell expansion and the internode elongation by lower turgor pressure and reduced supply of assimilates [16].

The drought stress had an adverse impact that was alleviated to diverse extents by foliar treatments. The control (T -) had a mean of 41.69 cm under irrigation conditions and nano-ascorbic acid (T -1) and FeNPs (T -2) raised the height of the plants to 44.1 and 45.66 cm respectively. The tallest plants were the combined treatment (T 3: nano-ascorbic acid + FeNPs), whose average measurement was 47.3cm. This enhancement in height of the plants can be explained by the antioxidative effect of nano-ascorbic acid, which preserves the structure of cells, and by the stimulation effect of FeNPs on chlorophyll biosynthesis and nutrient uptake in plants to maintain vegetative growth even in stressful conditions [17].

The effectiveness of the combined treatment was intense given the interaction between the irrigation and foliar sprays when the plants were stressed. Control plants (T 0) at extreme water shortage (I 3) were only 36.93 cm, compared to T 3 plants that grew to 40.0 cm. This height increase indicates effective use of water and cell turgor maintenance during drought, which has been reported previously in legumes and cereals subjected to nano-iron and antioxidant [18].

The LSD test in the 5% level established that there was a significant difference among the treatments: 2.42 cm in irrigation, 3.85 cm in foliar sprays, and 2.65 cm in their interaction. These findings underline the strength of the treatment effects and indicate that the combination of nano-ascorbic acid with the green-synthesized FeNPs is especially efficient in maintaining vegetative growth under the condition of water deficit.

Table 2: Effect of foliar-applied nano-ascorbic acid, green-synthesized FeNPs, and their combination under different irrigation regimes on plant height (cm) in cowpea

Foliar treatment	I_1 (50% AW depletion)	I_2 (60% AW depletion)	I_3 (70% AW depletion)	Mean
T_0: Control	46.3	41.9	36.9	41.7
T_1: Nano-AsA	49.3	45.0	38.0	44.1
T_2: FeNPs	51.2	46.6	39.2	45.7
T_3: Nano-AsA + FeNPs	53.1	48.8	40.0	47.3
Mean	50.0	45.6	38.5	—
LSD 0.05	Irrigation = 2.42	Foliar = 3.85	Interaction = 2.65	—

Number of Branches per Plant

Irrigation regimes, foliar applications and the interaction had a significant influence on the number of branches per plant (Table 3). Plants with less than 50 percent water depletion (I 1) had the highest number of plant branches (8.2), moderate stress (I 2, 7.6) and lowest numbers were recorded under severe stress (I 3, 6.0). Lessening in the number of branches in drought conditions could be explained by less photosynthetic activity and availability of assimilates restricting the development of axillary buds and elongation of stems [19].

The foliar application significantly increased the branching as compared to the untreated control (T 0: 6.0 branches). Nano-ascorbic acid (T 1) increased the average number of branches to 7.3, and FeNPs (T 2) increased the average number of branches to 7.7. The highest number of branches (8.1) was produced with the combined treatment (T 3: nano-ascorbic acid + FeNPs). The above-mentioned improvements may be attributed to the synergistic action of nano-ascorbic acid in the process of ROS scavenging and prevention of hormonal imbalance as well as the role of FeNPs in augmented chlorophyll synthesis and nutrient use, which consequently enhanced better vegetative growth and branching initiating [20].

The interaction resulted in the fact that with extreme stress (I 3) alone, control plants grew only 5.1 branches, and T 3 plants grew 6.8 branches, which is significantly recovered growth under water limitation. Like, positive reactions have been observed in legumes wherein antioxidant and nano-iron foliar sprays have improved branching and shoot vigor under stress [21].

The 5% level of LSD values of 0.62 of irrigation, 1.01 of foliar treatments, and 0.83 of their interaction indicate that the effect of the treatment in question was significant. The results of the above studies indicate that nano-ascorbic acid combined with green-synthesized FeNPs can effectively promote the branching capacity of cowpea, which is beneficial in enhancing a strong canopy formation during drought periods.

Table 3: Effect of foliar-applied nano-ascorbic acid, green-synthesized FeNPs, and their combination under different irrigation regimes on number of branches per plant in cowpea

Foliar treatment	**I_1 (50% AW depletion)**	**I_2 (60% AW depletion)**	**I_3 (70% AW depletion)**	**Mean**
T_0: Control	6.7	6.2	5.1	6.0
T_1: Nano-AsA	8.3	7.7	6.0	7.3
T_2: FeNPs	8.8	8.1	6.4	7.7
T_3: Nano-AsA + FeNPs	9.2	8.5	6.8	8.1
Mean	8.2	7.6	6.0	—
LSD 0.05	Irrigation = 0.62	Foliar = 1.01	Interaction = 0.83	—

Leaf Area (cm^2 $plant^{-1}$)

Irrigation regimes, foliar applications, and their interaction had a major effect on the leaf area (Table 4). The average data showed that under I one (50 percent available water loss, 201.07 cm 2), I two (60 percent water loss, 190.67 cm 2), and severe drought (I three, 160.34 cm 2), maximum leaf area was measured respectively. Such drought stress-induced decrease in leaf growth is not surprising, as the lack of water inhibits cell growth, decreases stomatal permeability, and inhibits photosynthesis [22].

The foliar sprays significantly increased the leaf area relative to the control (T 0: 152.93 cm 2). Nano-ascorbic acid (T 1) and FeNPs (T 2) alike raised the mean leaf area to 170.46 and 207.47 cm 2, respectively. Nano-ascorbic acid + FeNPs (T 3) gave the highest value of 205.26 cm 2 which is also significantly higher than the control. The impact of FeNPs on its own or synergistic action could be explained by the fact that it evoked chlorophyll biosynthesis and enzymatic antioxidant activity, which contributed to the maintenance of photosynthesis and the provision of more assimilates to the leaf expansion [23]. It is associated with the positive impact of nano-ascorbic acid on antioxidative processes and the maintenance of the osmotic balance regime and prevention of oxidative stress and maintenance of the turgor pressure of the cell under stress [24].

Combined foliar sprays were additionally demonstrated to have a better effect during drought with interaction effects. Untreated plants (T 0), at 70 percent depletion (I 3), had a leaf area of 132.6 cm 2 and T 2 and T 3 had leaf areas of 197.35 and 167.92 cm 2, respectively. This indicates that some of the stress-induced losses were overcome by the presence of supplementation, in line with the results in common bean and faba bean where exogenous antioxidants and nano-iron enhanced leaf growth and canopy photosynthesis during water deficit [25].

The LSD test (5%), indicated that there were critical differences of 28.15 cm 2 of irrigation, 62.66 cm 2 of foliar treatments, and 33.26 cm 2 of their interplay, which proved the significance of the observed responses.

Table 4: Effect of foliar-applied nano-ascorbic acid, green-synthesized FeNPs, and their combination under different irrigation regimes on leaf area (cm² plant⁻¹) in cowpea

Foliar treatment	I_1 (50% AW depletion)	I_2 (60% AW depletion)	I_3 (70% AW depletion)	Mean
T_0: Control	152.7	173.5	132.6	152.93
T_1: Nano-AsA	173.6	194.3	143.5	170.46
T_2: FeNPs	225.4	199.7	197.4	207.47
T_3: Nano-AsA + FeNPs	252.6	195.3	167.9	205.26
Mean	201.07	190.67	160.34	—
LSD 0.05	Irrigation = 28.15	Foliar = 62.66	Interaction = 33.26	—

Number of Pods per Plant

The irrigation regimes, foliar treatments, as well as their interaction, had a considerable effect on the number of pods per plant (Table 5). The greatest number of pods was obtained under I 1(50% water depletion, 17.26 pods plant -1), then I 2 (14.64 pods plant -1), and I 3 (13.25 pods plant -1). Such results have been in line with the overall phenomenon that drought decreases the supply of assimilates, the health of pollen, and reproductive success, resulting in a small number of pods per plant [26].

The Foliar sprays enhanced more pods than the control (T 0: 12.92 pods plant -1). Nano-ascorbic acid (T 1) raised the mean number of pods to 14.51, FeNPs (T 2) raised the number of pods to 15.99 and finally, the combined treatment (T 3) gave a value that was 16.78 pods per plant. These findings demonstrate the capacity of nano-ascorbic acid in alleviating oxidative stress and the enhancement of assimilates translocation to reproductive sinks, at the same time that FeNPs increase chlorophyll content and enzymatic antioxidant functions, which stimulates improved reproductive development under drought conditions [27].

The interaction analysis revealed that untreated plants (T 0) yielded 11.2 pods when stressed with severe stress (I 3) and 14.3 pods when treated with nano-ascorbic acid + FeNPs (T 3), indicating that a considerable recovery was observed in unfavorable stress. The same had been reported in faba bean and common bean, in which foliar application of ascorbic acid and nano-iron enhanced the reproductive activity and the number of pods under water stress [18].

The LSD test (5% level of significance) proved the significant differences of treatments: 0.68 between irrigation, 1.02 between foliar sprays, and 0.81 between these treatments and their interaction, which also supported the effectiveness of the treatments.

Table 5: Effect of foliar-applied nano-ascorbic acid, green-synthesized FeNPs, and their combination under different irrigation regimes on number of pods per plant in cowpea

Foliar treatment	I_1 (50% AW depletion)	I_2 (60% AW depletion)	I_3 (70% AW depletion)	Mean
T_0: Control	14.00	13.58	11.20	12.92
T_1: Nano-AsA	16.00	14.23	13.30	14.51
T_2: FeNPs	18.22	15.55	14.21	15.99
T_3: Nano-AsA + FeNPs	20.83	15.22	14.30	16.78
Mean	17.26	14.64	13.25	—
LSD 0.05	Irrigation = 0.68	Foliar = 1.02	Interaction = 0.81	—

Number of Seeds per Pod

Table 6 results show that the irrigation regimes, foliar sprays and their combination had a significant impact on the number of seeds per pod. Plants with I 1 (50 per cent depletion) had the highest number of seeds per pod (6.7), then I 2 (6.03) and the lowest number of seeds per pod was recorded in test under severe stress (I 3, 5.38). The drought decrease is an indication that water deficit has adverse effects on reproduction, fructification, and assimilate concentration to growing seeds [11].

The foliar sprays increased the seed set relative to the control (T 0: 5.36 seeds pod -1). Nano-ascorbic acid (T 1) alone increased the average seed count (5.9) and the addition of FeNPs (T 2) enhanced it to 6.2. The highest value attained was 6.66 seeds pod -1 which was obtained with the combined treatment (T3). This can be attributed to the fact that nano-ascorbic acid reduces oxidative stress, promotes carbohydrate partitioning but FeNPs increases photosynthesis and enzyme activities that subsequently increases reproductive success in stressing conditions [8].

It was found out that control plants yielded just 4.7 seeds per pod under severe water deficit (I III) as compared to 5.9 seeds per pod of T III plants. Such recovery under stress concentrates on the synergistic impact of nano-ascorbic acid and green-synthesized FeNPs. Equivalent results were also observed in legumes where the application of antioxidant and nanoparticle together led to improved fertilization and seed filling during the drought [28].

The 5% probability level LSD test proved that there were significant differences of 0.62 in case of irritation, 1.08 in case of foliar sprays and 0.89 in case of interaction. These findings indicate that foliar treatment using nano-ascorbic acid and FeNPs serves positively to terminate the reproductive characteristics of cowpea in water-stressed situations.

Table 6: Effect of foliar-applied nano-ascorbic acid, green-synthesized FeNPs, and their combination under different irrigation regimes on number of seeds per pod in cowpea

Foliar treatment	I_1 (50% AW depletion)	I_2 (60% AW depletion)	I_3 (70% AW depletion)	Mean
T_0: Control	5.9	5.5	4.7	5.36
T_1: Nano-AsA	6.5	5.9	5.3	5.90
T_2: FeNPs	6.8	6.2	5.6	6.20
T_3: Nano-AsA + FeNPs	7.6	6.5	5.9	6.66
Mean	6.70	6.03	5.38	—
LSD 0.05	Irrigation = 0.62	Foliar = 1.08	Interaction = 0.89	—

Dry Biomass (g plant⁻¹)

Regimes of irrigation, foliar applications, and interaction had a significant effect on dry biomass (Table 7). I was highest at the water content of I (50% depletion) at 85.0 g plant -1, I 2 (moderate stress) at 77.9 g plant -1 and I 3 (severe stress) at 59.8 g plant -1. The dry matter reduction caused by water deficit is direct result of the decrease of leaf area, photosynthetic efficiency and assimilate production [12].

The foliar sprays reduced the biomass loss in contrast to the untreated control (T 0: 73.0 g). The best result appeared with the combined treatment (T 3:78.4 g), then FeNPs (T 2:73.2 g), and nano-ascorbic acid (T 1:72.4 g). Such benefits can be explained by the factor of improved antioxidant protection, as well as improved nutrient use that nano-AsA and FeNPs provide and ensure that metabolic activity and carbon assimilation can be maintained during the stress [21].

The effects of interactions indicated that the untreated plants had a biomass production of only 62.2 g under extreme conditions of drought (I 3), but under the combined spray (T 3), the biomass production was 62.7 g. This is a small but important enhancement of the role played by exogenous treatments in maintaining biomass accumulation in adverse conditions. The same outcome was also indicated in the case of faba bean and common bean in which foliar antioxidant and nanoparticle application raised the partition of dry matter under water stress [15].

The LSD test at level of 5 percent showed that there were significant differences of 2.72 g in cases of irrigation, 5.13 g in case of foliar sprays and 3.83 g in interaction of the same.

Table 7: Effect of foliar-applied nano-ascorbic acid, green-synthesized FeNPs, and their combination under different irrigation regimes on dry biomass (g plant⁻¹) in cowpea

Foliar treatment	I_1 (50% AW depletion)	I_2 (60% AW depletion)	I_3 (70% AW depletion)	Mean
T_0: Control	80.5	76.3	62.2	73.0
T_1: Nano-AsA	83.5	74.4	59.4	72.4
T_2: FeNPs	85.1	79.4	55.1	73.2
T_3: Nano-AsA + FeNPs	91.0	81.6	62.7	78.4
Mean	85.0	77.9	59.8	—
LSD 0.05	Irrigation = 2.72	Foliar = 5.13	Interaction = 3.83	—

Grain Yield (kg plant⁻¹)

Grain yield per plant was also significantly affected by irrigation regimes, foliar applications, and their interaction (Table 8). The highest yield was obtained under I_1 (50% depletion, 1.19 kg plant^{-1}), while I_2 produced 1.15 kg plant^{-1}, and severe drought (I_3) reduced yield to 0.79 kg plant^{-1}. The yield decline under water deficit is consistent with the reduction in pod number, seed number, and biomass accumulation discussed earlier [29].

Foliar treatments enhanced yield compared with the untreated control (T_0: 0.96 kg plant^{-1}). The combined treatment (T_3) produced the maximum yield (1.14 kg plant^{-1}), followed by FeNPs (T_2: 1.05 kg) and nano-AsA (T_1: 1.03 kg). These findings suggest that foliar sprays improved reproductive efficiency and assimilate partitioning to seeds, consistent with previous reports in legumes treated with antioxidants and nanoparticles [7].

The interaction effect showed that under severe stress (I_3), yield in control plants was only 0.73 kg, while in T_3 it increased to 0.88 kg, indicating that combined application mitigated part of the yield loss under drought. The LSD test (5%) confirmed differences of 0.06 for irrigation, 0.13 for foliar sprays, and 0.07 for their interaction.

Table 8: Effect of foliar-applied nano-ascorbic acid, green-synthesized FeNPs, and their combination under different irrigation regimes on grain yield (kg plant^{-1}) in cowpea

Foliar treatment	I_1 (50% AW depletion)	I_2 (60% AW depletion)	I_3 (70% AW depletion)	Mean
T_0: Control	1.05	1.10	0.73	0.96
T_1: Nano-AsA	1.20	1.12	0.77	1.03
T_2: FeNPs	1.23	1.15	0.79	1.05
T_3: Nano-AsA + FeNPs	1.29	1.25	0.88	1.14
Mean	1.19	1.15	0.79	—
LSD 0.05	Irrigation = 0.06	Foliar = 0.13	Interaction = 0.07	—

Leaf Proline Content (µmol g^{-1} FW)

Proline content was significantly affected by irrigation regimes, foliar sprays, and their interaction (Table 9). The average results showed that proline concentration rose as water deficit went up, 0.416 µmol g -1 FW at I 1 (50 per cent depletion) at I 2 and 0.472 at extreme stress (I 3). This phenomenon is in line with the already established role of proline as an osmoprotectant that builds up during drought to sustain osmotic adjustment, stabilize proteins and membranes in addition to scavenging reactive oxygen species [20].

The foliar sprays resulted in minor yet significant differences relative to the control (T 0: 0.443 u mol g -1FW). Nano-ascorbic acid (T 1) produced 0.456 proline, and FeNPs (T 2) minimally decreased proline to 0.437. The combination treatment (T3) had 0.444 which is equivalent to the control. These results suggest that antioxidant sprays may partially mitigate stress, reducing the need for high proline accumulation. This is in line with previous studies where exogenous ascorbic acid or nanoparticles reduced proline levels by alleviating oxidative stress and improving water relations [22].

Interaction effects confirmed that the highest proline content (0.488 µmol g^{-1} FW) was observed in T_1 under I_3, while the lowest (0.410) occurred in T_2 under I_1. The LSD test at the 5% probability level showed critical differences of 0.012 for irrigation, 0.045 for foliar sprays, and 0.024 for the interaction.

Table 9: Effect of foliar-applied nano-ascorbic acid, green-synthesized FeNPs, and their combination under different irrigation regimes on proline content (µmol g^{-1} FW) in cowpea

Foliar treatment	I_1 (50% AW depletion)	I_2 (60% AW depletion)	I_3 (70% AW depletion)	Mean
T_0: Control	0.418	0.438	0.473	0.443
T_1: Nano-AsA	0.425	0.455	0.488	0.456
T_2: FeNPs	0.410	0.439	0.462	0.437
T_3: Nano-AsA + FeNPs	0.412	0.456	0.466	0.444
Mean	0.416	0.447	0.472	—
LSD 0.05	Irrigation = 0.012	Foliar = 0.045	Interaction = 0.024	—

Peroxidase (POD) Activity (U g^{-1} FW)

POD activity was significantly affected by irrigation regimes, foliar sprays, and their interaction (Table 10). On average, drought stress strongly increased POD activity, from 109.5 U g^{-1} FW under I_1 to 137.6 under I_2 and 179.8 under I_3. This reflects the upregulation of antioxidant enzymes as a natural defense against oxidative stress induced by water deficit [27].

Foliar sprays further enhanced POD activity beyond the control level (119.4 U g^{-1} FW). Nano-ascorbic acid (T_1) increased POD to 141.8, FeNPs (T_2) raised it to 148.2, while the combined treatment (T_3) produced the highest activity (159.7). These findings indicate a synergistic effect between nano-ascorbic acid and FeNPs in stimulating enzymatic antioxidant defenses. This is consistent with recent reports showing that nano-based treatments enhance peroxidase, catalase, and superoxide dismutase activities, thereby reducing oxidative damage under drought [19].

Interaction effects revealed that under severe stress (I_3), POD activity in untreated plants was 135.3 U g^{-1} FW, whereas T_3 reached 215.7, almost a 60% increase. The LSD test confirmed significant differences of 14.71 for irrigation, 28.49 for foliar sprays, and 16.54 for their interaction.

Table 10: Effect of foliar-applied nano-ascorbic acid, green-synthesized FeNPs, and their combination under different irrigation regimes on peroxidase activity (U g^{-1} FW) in cowpea

Foliar treatment	I_1 (50% AW depletion)	I_2 (60% AW depletion)	I_3 (70% AW depletion)	Mean
T_0: Control	102.6	120.4	135.3	119.4
T_1: Nano-AsA	115.0	135.3	175.2	141.8
T_2: FeNPs	112.1	139.4	193.2	148.2
T_3: Nano-AsA + FeNPs	108.3	155.3	215.7	159.7
Mean	109.5	137.6	179.8	—
LSD 0.05	Irrigation = 14.71	Foliar = 28.49	Interaction = 16.54	—

CONCLUSIONS

These findings showed that drought stress had a great impact on reducing growth, physiological performance, and yield characteristics of cowpea. The use of nano-ascorbic acid and the green-synthesized FeNPs, especially jointly, as foliar to reduce the harmful impacts of deficit of water was a successful approach. These treatments increased antioxidant defenses, vegetative and reproductive characteristics and increased yield stability among the stress. Thus, the incorporation of nano-based biostimulants can be a promising and green method of enhancing drought tolerance in cowpea crop production.

REFERENCES

1. Hall AE. Phenotyping cowpeas for adaptation to drought. *Front Physiol.* 2012;3:155. FrontiersPMC
2. Muchero W, Roberts PA, Diop NN, Drabo I, Cissé N, Close TJ, Maughan PJ, Huynh B-L, Wanamaker S, Pottorff M, Hearne S, Ehlers JD. Genetic architecture of delayed senescence, biomass, and grain yield under drought stress in cowpea. *PLoS One.* 2013;8(7):e70041. PLOSPubMed
3. Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem.* 2010;48(12):909–930.
4. Hasanuzzaman M, Bhuyan MHMB, Zulfiqar F, Raza A, Mohsin SM, Al Mahmud J, Fujita M. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of ROS signaling. *Plant Mol Biol.* 2020;103:493–523. PubMed
5. Akram NA, Shafiq F, Ashraf M. Ascorbic acid—a potential oxidant scavenger and its role in plant development and abiotic stress tolerance. *Front Plant Sci.* 2017;8:613.
6. Farooq A, Bukhari SA, Akram NA, Ashraf M, Wijaya L, Alyemeni MN. Exogenously applied ascorbic acid-mediated changes in plants under water deficit stress: pre- and post-stress applications. *Plants (Basel).* 2020;9(1):104.
7. Xiao Y, Miao J, Li Y, Basnet TB, Khanal S, Wei X. Roles of ascorbate and ascorbate peroxidase in plant development and abiotic stress responses. *Front Plant Sci.* 2021;12:667447.
8. El-Saadony MT, Desoky E-SM, Saad AM, El-Tahan AM, Rady MM, Soliman MH. Role of nanoparticles in enhancing crop tolerance to abiotic stresses: an updated overview. *Front Plant Sci.* 2022;13:967030.
9. Haider FU, Nasar J, Rehman A, Farooq M, Iqbal A, Naveed M, Khan N, Zohaib A. Harnessing plant extracts for eco-friendly synthesis of iron nanoparticles: recent advances and agricultural prospects. *Int J Biol Macromol.* 2024;263:130680.
10. Dola DB, Soliman MH, Elnahal AS, Tantawy DS, El-Mahdy MT, Hassan M, Abd El-Hamid EM. Nano-iron oxide accelerates growth, yield, and quality of soybean seeds under water deficit. *Front Plant Sci.* 2022;13:992535.
11. Yılmaz H. Foliar application of ascorbic acid and green-synthesized nano iron for enhancing drought tolerance in common beans. *Black Sea J Agric.* 2024;7(6):766–776.
12. Mahmoud AWM, Sadak MS, Abdalla AM, El-Sayed SAA. Foliar application of different iron sources improves growth and productivity of faba bean (*Vicia faba* L.). *Plants (Basel).* 2022;11(19):2605.
13. Etesami H, Jeong BR. Nanoparticle–plant interactions: uptake, translocation, and physiological responses under abiotic stress. *Ecotoxicol Environ Saf.* 2021;215:112112. (Article on nanoparticle–stress interactions).
14. Szabados L, Savouré A. Proline: a multifunctional amino acid. *Trends Plant Sci.* 2010;15(2):89–97.
15. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. *Plant Soil.* 1973;39(1):205–207. SpringerLink
16. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys.* 1968;125(1):189–198.
17. Chance B, Maehly AC. Assay of catalases and peroxidases. *Methods Enzymol.* 1955;2:764–775. (Methodological classic).
18. Uarrota VG, Moresco R, Coelho B, Nunes EC, Peruch LAM, Neubert EO, Rocha M, Maraschin M. The role of peroxidases in plant stress physiology: methodological notes for guaiacol peroxidase activity. *Food Chem.* 2015;197:737–746.
19. Allen RG, Pereira LS, Raes D, Smith M. Crop evapotranspiration—Guidelines for computing crop water requirements. *FAO Irrigation and Drainage Paper 56.* Rome: FAO; 1998. FAOHomeclimasouth.eu
20. Hillel D. *Fundamentals of Soil Physics.* New York: Academic Press; 1980. PagePlace
21. Watson DJ. The estimation of leaf area in field crops. *J Agric Sci.* 1937;27(3):474–483.

22. Bréda NJJ. Ground-based measurements of leaf area index: a review. *J Exp Bot.* 2003;54(392):2403–2417.
23. Kiwumulo HF, Kiggundu N, Babu SC, Nabirye D, Byarugaba J, Kizito S, Kizito E. Green synthesis and characterization of iron-oxide nanoparticles using *Moringa oleifera*. *BMC Res Notes.* 2022;15:149.
24. Katata-Seru L, Moremedi T, Aremu OS, Bahadur I. Green synthesis of iron nanoparticles using leaf and seed extracts of *Moringa oleifera*. *Carbohydr Polym.* 2018;181:144–150.
25. Gohari G, Akbari A, Panahirad S, Dadpour MR, Fotopoulos V, Kimura S. Foliar application of nanoparticles and antioxidants modulates drought stress responses in common bean. *Plant Physiol Biochem.* 2021;161:162–171.
26. Singh A, Prasad SM. Nanotechnology and its role in enhancing drought tolerance in plants: a review. *Plant Physiol Biochem.* 2020;155:285–301. (Review on nano-enabled drought tolerance).
27. Anjum SA, Tanveer M, Ashraf U, Hussain S, Shahzad B, Zohaib A, Abbas F, Saleem MF. Drought-induced changes in growth, leaf gas exchange, and chlorophyll fluorescence in cowpea (*Vigna unguiculata* L.). *Photosynthetica.* 2017;55(4):819–826. PubMed
28. Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA. Plant drought stress: effects, mechanisms and management. *Agron Sustain Dev.* 2009;29:185–212. PubMed
29. Elkhateeb O, Abdelhady H, El-Sayed W, Abdel-Rahim K, Abdallah Y. Biosynthesis of iron-oxide nanoparticles using plant extracts and their antimicrobial properties. *AMB Express.* 2024;14:65. (Green synthesis reference).