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Impact of nano-chelated NPK and chemical fertilizers on the growth and productivity features of maize (*Zea mays* L.) under water-deficit stress

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Abstract

Objective: Drought stress is one of the most important factors limiting the development and production of maize worldwide. In this regard, using nano-fertilizers to control the release of nutrients can be a practical step towards achieving sustainable agriculture and environmental adaptation, and it is vital to induce drought stress tolerance in maize.

Methods: We aimed to evaluate the effect of nano-chelating-based nitrogen and NPK fertilizers on both agronomic and physiological characteristics of maize under water-deficit stress conditions. A split-plot experiment was conducted based on a randomized complete block design to test the effect of the different fertilizers. The main plots included two levels of irrigation: optimum irrigation and water-deficit stress (irrigation after 140 mm evaporation from a Class A pan). The subplots involved various combinations of nano-chelated fertilizers at five doses, alongside a control using conventional chemical fertilizers.

Results: Our results indicated that water-deficit stress adversely impacted various growth and productivity characteristics in maize. However, substituting conventional chemical fertilizers with nano-chelated fertilizers, even at a minimal level (10%), notably enhanced most studied traits compared to the control under water-deficit stress conditions. Specifically, treating plants with 222 kg/ha (Treatment 3 of the nano-chelated fertilizers (nano-chelated $N_{20}P_{20}K_{20}$ 96 kg/ha + nano-chelated nitrogen 126 L/ha) of nano-chelated fertilizers instead of 840 kg/ha of chemical fertilizers (300 kg/ha of triple superphosphate, 150 kg/ha of potassium sulfate, and 390 kg/ha of urea) resulted in a 33% increase in grain yield, overall improvements in yield components, and elevated nitrogen use efficiency under drought stress. Furthermore, nano-chelated fertilizers mitigated the impact of water-deficit stress through the chlorophyll a and b content while reducing leaf temperature.

Conclusion: our results indicated that nano-chelating-based macronutrient fertilizers could present a promising avenue within sustainable production systems, particularly under water-deficit stress conditions. Therefore, considering production costs and environmental problems, the application of nano-chelated $N_{20}P_{20}K_{20}$ 96 kg/ha + nano-chelated nitrogen 126 L/ha for the sustainable production of grain yield in the Fajr cultivar of maize will be sufficient, and higher levels of nano-fertilizers will be luxurious.

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Introduction

Drought stress is a critical obstacle to food security (Younas *et al.* 2022; Bakhoum *et al.* 2023). Also, it is the most limiting factor in the production of crops worldwide, especially maize (Henry *et al.* 2016; Bodnár *et al.* 2018; Hunter *et al.* 2021). Enhancing crop tolerance to drought stress is thus imperative for ensuring food security. However, the level of stress tolerance depends on various factors such as stress type and duration, plant species, varieties, and plant nutrition (Wang *et al.* 2017; Bakhoum *et al.* 2022; Hanafy and Sadak 2023). In a research, it was found that interruption in irrigation during the reproductive phase in maize led to elevated leaf temperature and protein level, simultaneously resulting in reduced leaf water content, moisture stress index, chlorophyll concentration, leaf area index, and yield of grain, oil, and protein (Ghassemi Golezani and Mousavi 2022). According to Ghassemi *et al.* (2020), drought stress considerably reduced the ear length, the number of kernels per ear, the number of kernel rows per ear, the number of kernels per row, the dry weight of husks, the 300-kernel weight, and the grain yield in maize.

Nutrition plays a fundamental role in plant growth and development. By enhancing the nutrient content, plants are encouraged to express their full potential regarding yield and secondary metabolites (El Omari *et al.* 2016). However, under drought-stress conditions, factors such as reduced soil moisture restrict nutrient accessibility and root absorption (Jose 2023). Therefore, efficient regulation of plant nutrients through accurate and balanced fertilization management helps increase crop productivity, achieve sustainable agriculture, improve plant tolerance to water deficits, and enrich soil fertility by increasing soil organic matter availability (Alzreejawi and Al-Juthery 2020; Younas *et al.* 2022).

Recently, the application of nanotechnology in agriculture has garnered attention due to its potential to augment agricultural production, increase input efficiency, and minimize associated risks (Shang *et al.* 2019; Sadak *et al.* 2022). For example, soil fertility is continuously reduced by the application of chemicals. Only a small fraction of these agrochemical inputs are used by plants, and the rest are unused chemicals that are detrimental to the ecosystem. The unused chemical fertilizers and pesticides are washed into the soil or carried by water into water bodies, causing chemical pollution to non-target organisms. The application of nanotechnology in agriculture reduces the cost of fertilizers and pesticides with the advancement of these tools. The application of nanotechnology-based techniques improves the properties of agricultural inputs such as targeted delivery, controlled release, solubility, and shelf life. These features not only make them more productive but also decrease the risk of environmental pollution (Chhipa 2019).

Nano-fertilizers characterized by high surface area, sorption capacity, and controlled-release kinetics to targeted sites, act as intelligent delivery systems, potentially boosting crop growth, conserving energy, and facilitating more efficient food production (Rameshaiah *et al.* 2015). These nano-fertilizers efficiently provide the bioavailable elements for plant use, reducing the leaching of mobile nutrients such as nitrate. This advantage and the controlled release of nutrients make them more cost-effective (Kah *et al.* 2018; Tarafder *et al.* 2020). Also, the substantial loss of applied fertilizers in the soil, ranging from 50 to 80% (with an efficiency of 20 to 50%), resulting in ecological issues such as diminished soil fertility and economic losses, is addressed through the use of nano-fertilizers (Ditta and Arshad 2016).

This study aimed to provide original evidence on the impact of nano-chelated fertilizers on maize growth and productivity under water-deficit stress.

Materials and Methods

Experimental site

The present study was conducted at the Agricultural Research Center, West-Azerbaijan (Saatloo Station), Iran, located at 45° 10′ 53″ E and 37° 44′ 180″ N, and 1338 m above sea level. Supplementary Table 1 presents the climatic features of the study area. The soil used for the experiment had a clay loam texture, a pH of about 8.3, and an electrical conductivity of about 0.8 S m^{-1} (Supplementary Table 2).

Experimental design

The research was conducted as a split-plot design based on the randomized complete block design with three replications. The main factor included optimum (irrigation after 70 mm evaporation from the class A evaporation pan) and deficit irrigation (irrigation after 140 mm evaporation from the class A evaporation pan) and the sub-factor consisted of five combinations of nano-chelated fertilizers with varying amounts and a chemical fertilizer treatment as the control (Table 1). Fertilizers were applied

Table 1. Fertilizer treatments used in the experiment in 2019.

Fertilizer	Before sowing	4 to 5-leaf stage	6 to 8-leaf stage	Tasseling stage
Chemical fertilizers	Triple superphosphate (300 kg/ha) + potassium sulfate (150 kg/ha)	Urea (130 kg/ha)	Urea (130 kg/ha)	Urea (130 kg/ha)
$N_{20}P_{20}K_{20}$ (160 kg/ha) + N (210 L/ha, containing 17% N) (nano-fertilizer 1)	$N_{20}P_{20}K_{20}$ (40 kg/ha)	$N_{20}P_{20}K_{20}$ (40 kg/ha) + N (70 L/ha)	$N_{20}P_{20}K_{20}$ (40 kg/ha) + N (70 L/ha)	$N_{20}P_{20}K_{20}$ (40 kg/ha) + N (70 L/ha)
$N_{20}P_{20}K_{20}$ (128 kg/ha) + N (168 L/ha, containing 17% N) (nano-fertilizer 2)	$N_{20}P_{20}K_{20}$ (32 kg/ha)	$N_{20}P_{20}K_{20}$ (32 kg/ha) + N (56 L/ha)	$N_{20}P_{20}K_{20}$ (32 kg/ha) + N (56 L/ha)	$N_{20}P_{20}K_{20}$ (32 kg/ha) + N (56 L/ha)
$N_{20}P_{20}K_{20}$ (96 kg/ha) + N (126 L/ha, containing 17% N) (nano-fertilizer 3)	$N_{20}P_{20}K_{20}$ (24 kg/ha)	$N_{20}P_{20}K_{20}$ (24 kg/ha) + N (42 L/ha)	$N_{20}P_{20}K_{20}$ (24 kg/ha) + N (42 L/ha)	$N_{20}P_{20}K_{20}$ (24 kg/ha) + N (42 L/ha)
$N_{20}P_{20}K_{20}$ (64 kg/ha) + N (84 L/ha, containing 17% N) (nano-fertilizer 4)	$N_{20}P_{20}K_{20}$ (16 kg/ha)	$N_{20}P_{20}K_{20}$ (16 kg/ha) + N (28 L/ha)	$N_{20}P_{20}K_{20}$ (16 kg/ha) + N (28 L/ha)	$N_{20}P_{20}K_{20}$ (16 kg/ha) + N (28 L/ha)
$N_{20}P_{20}K_{20}$ (32 kg/ha) + N (42 L/ha, containing 17% N) (nano-fertilizer 5)	$N_{20}P_{20}K_{20}$ (8 kg/ha)	$N_{20}P_{20}K_{20}$ (8 kg/ha) + N (14 L/ha)	$N_{20}P_{20}K_{20}$ (8 kg/ha) + N (14 L/ha)	$N_{20}P_{20}K_{20}$ (8 kg/ha) + N (14 L/ha)

through fertigation. Water-deficit stress was imposed from the two to four-leaf stages after the plants were fully established. Figure 1 displays the FTIR spectrum graph of nano-chelated nitrogen and nano-chelated NPK fertilizers. As the graph shows, there are no peaks in the 13.84 m^{-1} and 8.25 m^{-1} regions. These two regions belong to nitrate-nitrogen groups. One of the structural advantages of nano-chelated nitrogen and NPK 20-20-20 fertilizers is the absence of nitrate-nitrogen in their structure.

Irrigation was performed using 0.076 m polyethylene pipes. The amount of irrigation water for each plot was calculated according to the following formula:

$$I = W \times D \times f \times 10000$$

Where I = the amount of water that should be given in each irrigation ($m^3\ ha^{-1}$).

W = water storage capacity per cubic meter of soil (in the clay loam soil),

D = the depth of root development or the desired depth for wetting the soil, which should be 20 cm more than the depth of root development (0.7 m in this experiment).

f = easily accessible water coefficient that the plant can absorb water from the soil by its roots (0.5 in this study). Eight irrigations were carried out for the optimal irrigation treatment, while five rounds of irrigation were applied in the water-deficit stress treatments. The sowing of seeds was performed on 24 and 25 Jun 2019. Harvesting occurred in the first half of October (3 to 7 October) 2019.

Measured traits

Yield and yield components**:** To determine the biomass and its components, randomly taken samples were weighed after incubated at 72 °C for 48 hours. Afterward, the dry weight of the stems, leaves, ears, and cobs were measured separately.

The grain yield, which comprised of several components (i.e., the numbers of rows per ear, number of kernel rows, number of kernels per ear, and 1000-grain weight) were measured on 10 central selected ears (from a two m^2 area and extrapolating the results to tons per ha) (Karmollachaab *et al.* 2017).

Harvest index**:** The harvest index (HI) was calculated as follows:

$$\mathrm{HI}=\frac{\mathrm{G_Y}}{\mathrm{B_Y}}\times 100$$

Where G_Y and B_Y are grain yield and biomass, respectively.

Nitrogen use efficiency based on grain yield and biomass**:** The following equations were used to calculate the nitrogen use efficiency (NUE) based on grain yield and biomass (Bingham *et al.* 2012):

$$\mathrm{NUE_G}=\frac{\mathrm{GY}}{\mathrm{N}}$$

$$\mathrm{NUE_B}=\frac{BY}{\mathrm{N}}$$

Where NUE_G and NUE_B are nitrogen use efficiency based on grain yield (kg/kg), and biomass (kg/kg), respectively, and *N* is the amount of applied nitrogen (kg/ha).

Oil percentage (%)**:** The oil percentage of grains was measured using a seed analyzer (model Zx880 Near Infrared Food Analyzer, Berlin, Germany).

Plant height**:** The height of 10 randomly selected plants in each plot was measured by considering the distance from the ground surface to the end of the stem.

Stem diameter**:** The stem diameter of 10 randomly selected plants in each plot was measured by a caliper.

Ear height**:** Ear height was determined by measuring the distance from the soil surface, to the node of the upper ear in 10 randomly selected plants from the central rows of the experimental plots.

Ear length and diameter: Ten randomly selected ears in each plot were measured for their length and diameter using a ruler and a Vernier Caliper, respectively.

Grain depth: The following equation was used to calculate the depth of grains by using 10 randomly chosen ears (Khodarahmpour *et al.* 2012):

$$\text{Grain depth} = (\text{ear diameter} - \text{cob diameter})/2$$

Relative water content: First, the fresh leaf samples were submerged in distilled water. Then, their fresh weight was determined. Next, they were re-weighed after 24 hours. After that, the leaves were dried at 70 ºC for 48 hours and weighed again. The relative water content (RWC) was calculated according to the following equation (Dhopte and Livera-M 2002):

$$RWC = (FW-DW)/(TW-DW) \times 100$$

Where FW is the fresh weight, DW is the dry weight, and TW is the turgor weight of the leaf samples.

Proline content: Proline content was determined from 0.2 g samples of fresh leaves based on proline's reaction with ninhydrin (Bates *et al.* 1973; Anjum *et al.* 2017).

Chlorophylls a and b: As specified by Arnon (1949), one g fresh leaf was used to extract the pigments. Extraction was performed by adding 10 ml of 80% acetone. The extract was centrifuged at 5000–10000 rev min^{-1} for 5 min to separate the supernatants. This step was repeated until a clear upper liquid phase was acquired. The following equations were used to calculate the contents of chlorophyll a (Chl a) and chlorophyll b (Chl b) (mg/g FW).

$$\text{Chl a} = [12.7\ (D_{663}) - 2.59(D_{645})] \times \frac{V}{1000 \times W}$$

$$\text{Chl b} = [22.9\ (D_{645}) - 4.69(D_{663})] \times \frac{V}{1000 \times W}$$

Where V is the final volume of solution (ml) and W represents the sample weight (g); *D* indicates the absorbance level at the considered wavelengths (nm).

SPAD: The SPAD index was determined for 10 randomly selected leaves from each plot using a chlorophyll meter (Konica Minolta Model 502, Tokyo, Japan).

Carotenoid content: The amount of carotenoids was measured according to Voronin's method (Lichtenthaler 1987; Voronin *et al.* 2019) as follows:

$$C = \frac{(100 \times A_{470} - 1.82 \times Chla - 85.02 \times Chlb)}{198}$$

Where *C* is the content of carotenoids (mg g^{-1} FW) and A_{470} is the absorbance level at 470 nm wavelength.

Electrolyte leakage: For calculating the electrolyte leakage, fresh leaves (100 mg) were washed three times using distilled water and soaked in distilled water for one hour (25 ºC). Then, the electrical conductivity was measured by a conductivity meter (L_1). The container was then placed in a hot water bath (Bain-marie) for 10 min at 100 °C and its electrical conductivity was again determined (L_2) (Bai *et al.* 1996). Leaf electrolyte leakage was calculated as follows:

$$EL = \frac{L_1}{L_2} \times 100$$

Where EL, L_1, and L_2 represent the electrolyte leakage, the electrical conductivity of the fresh leaves at 25 ºC, and the electrical conductivity of the fresh leaves at 100 ºC, respectively.

Leaf temperature: Leaf temperature was measured in the field (at 12 to 2 pm) using an infrared thermometer (model 8889, *AZ* company, Taichung, Taiwan) (Costa-Filho *et al*. 2020).

Statistical analysis

The homogeneity of error variances was tested using the Bartlett test. To normalize the error residuals of all traits, those with nonnormal distribution were transformed using an appropriate transformation procedure in SPSS software (Ver. 22, New York, USA). The analysis of variance was performed by SAS software (Ver. 9.1). Means were compared by Tukey's test at a 5% significance level using MSTATC software.

Results

The analysis of variance (Supplementary Table 3) showed that irrigation affected ear height, plant height, stem diameter, ear length, ear diameter, number of kernels per row, number of kernels per ear, 1000-kernel weight, ear dry weight, and NUE_G. Also, nano-chelated fertilizers affected stem diameter, ear length, ear diameter, number of rows per ear, number of kernels per row, number of kernels per ear, 1000-kernel weight, dry weight of stems and leaves, ear dry weight, biomass, grain yield, harvest index, NUE_G, and NUE_B. The interaction of irrigation with nano-chelated fertilizers for ear length, dry weight of stems and leaves, harvest index, NUE_G, and NUE_B was also significant.

The water-deficit stress significantly reduced stem diameter, ear diameter, ear height, plant height, ear dry weight, number of kernels per row, number of kernels per ear, 1000-kernel weight, and grain yield compared to the plants under optimal irrigation (Table 2). The greatest grain yield (8.00 t/ha) resulted from the optimal irrigation conditions (Table 2). Treatment 3 of the nano-chelated fertilizers (nano-chelated $N_{20}P_{20}K_{20}$ 96 kg/ha + nano-chelated nitrogen 126 L/ha) significantly increased stem diameter, ear diameter, number of rows per ear, number of kernels per row, number of kernels per ear, 1000-kernel weight, ear dry weight, biomass, and grain yield compared to the application of chemical fertilizers on the average of irrigation conditions (Table 3). This fertilizer also showed higher ear length and the dry weight of stems and leaves than the chemical fertilizer under both optimum irrigation and water-deficit stress conditions, but the difference in ear length was not significant under water-deficit stress conditions (Table 4). The highest grain yield (8.47 t/ha) was associated with this fertilizer (Table 3).

Irrigation, nano-chelated fertilizer, and their interaction affected RWC, proline, chlorophyll a, chlorophyll b, SPAD, carotenoids, and leaf temperature. However, the electrolyte leakage was only affected by the main effects of irrigation and nano-chelated fertilizer (Supplementary Table 4).

Water-deficit stress decreased RWC, chlorophyll a, chlorophyll b, and SPAD, but increased proline, leaf temperature, carotenoids (Table 5), and electrolyte leakage (Table 2), but the increase in the carotenoid content was only significant concerning nano-fertilizer 1 and nano-fertilizer 3. Under optimum irrigation and water-deficit stress conditions, the application of nano-chelated fertilizer Treatment 3 increased RWC, chlorophyll a, chlorophyll b, and SPAD, but reduced proline content compared to chemical fertilizer application (Table 5), however, the difference was not significant concerning chlorophyll b in the water-deficit stress conditions, and SPAD in the optimal irrigation conditions. Also, this fertilizer had significantly lower leaf temperature than the control under water-deficit stress conditions (Table 5), and significantly lower electrolyte leakage on the average of both irrigation conditions (Table 3).

Mean comparisons concerning the interaction of irrigation with nano-chelated fertilizers showed that under water-deficit stress and normal conditions, the highest NUE based on grain yield and biomass was obtained from applying nano-chelated fertilizer No. 5 (Table 4). The highest (515.15 $kg.kg^{-1}$) and the lowest (29.84 $kg.kg^{-1}$) NUE based on grain yield was obtained from applying nano-chelated fertilizer 5 under optimum irrigation, and applying chemical fertilizer under water-deficit stress conditions, respectively (Table 4). Under optimum irrigation and water-deficit stress conditions, applying nano-chelated fertilizer Treatments 5, 4, 3, 2, and 1 increased NUE based on grain yield by 1200, 590, 500, 290, and 210% compared to chemical fertilizer, respectively (Table 4). These results highlight the superiority of the nano-chelated fertilizers for NUE.

Table 2. Mean comparison of different levels of irrigation for agronomic traits and electrolyte leakage in maize.

Irrigation	Stem diameter (mm)	Ear diameter (mm)	Ear height (cm)	Plant height (cm)	Ears dry weight (t/ha)	Biomass (t/ha)
Optimum irrigation	27.4^{a}	48.9^{a}	87.4^{a}	194.4^{a}	9.45^{a}	15.52^{a}
Water-deficit stress	25.8^{b}	45.5^{b}	75.5^{b}	167.5^{b}	7.43^{b}	12.48^{b}

Table 2 continued

Irrigation	Grain yield (t/ha)	No. of kernels per row	No. of kernels per ear	1000-kerenel weight (g)	Electrolyte leakage (%)
Optimum irrigation	8.00^{a}	36.48^{a}	570.88^{a}	199.09^{a}	56.58^{b}
Water-deficit stress	6.11^{b}	32.11^{b}	471.74^{b}	182.67^{b}	67.93^{a}

Means in each column with different letters are significantly different at 5% probability level based on the analysis of variance.

Discussion

The constant increase in the world population and the escalating rate of land degradation call for increasing yield per unit area of arable land (Senapati *et al.* 2019). However, stable crop production is adversely affected by various biotic and abiotic stresses, especially drought (Langridge 2013; Henry *et al.* 2016; El-Bassiouny *et al.* 2023). Therefore, improving crop tolerance to drought stress through adaptive strategies is critical to ensure food security. To achieve optimum yield without increasing the area of cultivated land, which is economically and ecologically restricted, attention must be paid to developing novel and efficient technologies to improve important traits related to plant productivity and its adaptation to environmental challenges.

In our study, water-deficit stress significantly reduced yield and yield components. Similar to our findings, other researchers reported that yield and yield components of maize decreased under drought stress conditions (Afzali *et al.* 2023). The number of kernels per ear is a crucial yield component sensitive to water scarcity (Schussler and Westgate 1991). Also, under water-deficit stress, 1000-grain weight, and dry weight decline following the decrease in photosynthesis rate and transporters activity (Kang *et al.* 2000; Sajedi and Sajedi 2009; Hasanzade *et al.* 2014). Eskandari *et al.* (2019) indicated that the impact of water deficit on the 1000-seed weight of maize was more

Table 3. Mean comparison of different nano-chelated fertilizers for agronomic traits and electrolyte leakage in maize.

Nano-fertilizer	Stem diameter (mm)	Ear diameter (mm)	No. of rows per ear	Ear DW (t/ha)	Biomass (t/ha)
Control	27.6^{b}	46.5^{de}	14.63^{b}	7.50^{de}	12.71^{cd}
Nano-fertilizer 1	27.0^{bc}	47.6^{bc}	15.09^{b}	8.90^{bc}	14.55^{b}
Nano-fertilizer 2	26.2^{cd}	47.8^{b}	15.33^{ab}	9.09^{b}	14.79^{b}
Nano-fertilizer 3	29.1^{a}	48.7^{a}	16.21^{a}	10.00^{a}	16.46^{a}
Nano-fertilizer 4	25.2^{de}	46.8^{cd}	14.78^{b}	8.05^{cd}	13.42^{c}
Nano-fertilizer 5	24.6^{e}	45.9^{e}	14.66^{b}	7.09^{e}	12.07^{d}

Table 3 continued

Nano-fertilizer	Grain yield (t/ha)	No. of kernels per row	No. of kernels per ear	1000-kerenel weight (g)	Electrolyte leakage (%)
Control	6.38^{cd}	31.83^{cd}	467.00^{c}	199.01^{b}	66.71^{b}
Nano-fertilizer 1	7.39^{b}	34.19^{bc}	517.62^{bc}	181.10^{cd}	60.92^{d}
Nano-fertilizer 2	7.49^{b}	35.60^{b}	546.79^{b}	176.77^{d}	57.72^{e}
Nano-fertilizer 3	8.47^{a}	39.32^{a}	640.15^{a}	213.38^{a}	53.96^{f}
Nano-fertilizer 4	6.63^{c}	33.30^{bcd}	494.35^{bc}	191.32^{bc}	64.25^{c}
Nano-fertilizer 5	5.97^{d}	31.5^{d}	461.95^{c}	183.71^{cd}	69.98^{a}

Means in each column followed by different letter(s) are significantly different at 5% probability level according to Tukey's Test; Nano-fertilizer 1: N20P20K20 (160 kg/ha) + N (210 L/ha), Nano-fertilizer 2: N20P20K20 (128 kg/ha) + N (168 L/ha), Nano-fertilizer 3: N20P20K20 (96 kg/ha) + N (126 L/ha), Nano-fertilizer 4: N20P20K20 (64 kg/ha) + N (84 L/ha) and Nano-fertilizer 5: N20P20K20 (32 kg/ha) + N (42 L/ha). The details of nano-fertilizer treatments are shown in Table 1.

than on other yield components of the crop. Consistent with other researchers (Sajedi *et al.* 2009), the results of the present study showed that water-deficit stress significantly decreased stem diameter, dry weight of stems and leaves, and plant height of maize It has been indicated that under water deficit, the secretion of cytokinin from the roots is reduced, resulting in reduced cell division and limited plant height (Lalinia *et al.* 2012). According to Danilevskaya *et al.* (2019), water-deficit stress effectively decreases ear growth and yield. The supply of photosynthetic material to plant tissues decreases under water-deficit stress. Therefore, insufficient translocation of photosynthates to sinks during water-deficit stress periods, hinders their growth.

In the present study, water-deficit stress reduced chlorophyll a, chlorophyll b, chlorophyll index, and RWC compared with normal irrigation. Similarly, another study reported that delayed irrigation reduced chlorophyll b content in maize compared to normal irrigation (Sarrafi *et al.* 2017). A possible explanation for the decrease in the chlorophyll index under water-deficit stress conditions is the production of free radicals during water-deficit stress that deteriorate chlorophylls (Tarighaleslami

Table 4. Interaction of different levels of irrigation and nano-chelated fertilizers on the dry weight of stem and leaves, harvest index, ear length, and nitrogen use efficiency in maize.

Irrigation	Nano-chelated fertilizers	DW of stem and leaves (t/ha)	Harvest index (%)	Ear length (mm)	NUE_G	NUE_B
Optimum irrigation	Control	5.59^{c-f}	52.18^{a}	198^{cde}	39.57^{h}	76.20^{h}
	Nano-fertilizer 1	6.13^{bc}	52.58^{a}	205^{bcd}	124.14^{f}	236.91^{fg}
	Nano-fertilizer 2	6.39^{b}	50.79^{ab}	216^{ab}	155.96^{e}	307.80^{ef}
	Nano-fertilizer 3	7.26 ^{a}	52.55^{a}	229^{a}	236.62^{d}	448.59^{d}
	Nano-fertilizer 4	5.77^{bcd}	50.82^{ab}	199^{cde}	270.85^{c}	536.36^{c}
	Nano-fertilizer 5	5.27^{d-g}	51.87^{ab}	193de	515.15^{a}	996.62^{a}
Water-deficit stress	Control	4.83^{fg}	48.09^{bc}	195^{cde}	29.84^{h}	62.02^{h}
	Nano-fertilizer 1	5.16^{d-g}	48.90^{abc}	200^{cde}	94.37^{g}	192.94^{g}
	Nano-fertilizer 2	5.01^{d-g}	50.70^{ab}	201^{b-e}	120.81^{fg}	238.43^{fg}
	Nano-fertilizer 3	5.65^{b-e}	50.46^{abc}	208^{bc}	180.82^{e}	358.32^{e}
	Nano-fertilizer 4	4.96^{fg}	48.14^{bc}	197^{cde}	219.18^{d}	455.16^{d}
	Nano-fertilizer 5	4.68^{g}	46.66^{c}	189^{e}	367.24^{b}	787.30^{b}

Means in each column followed by different letter(s) are significantly different at 5% probability level according to Tukey's Test; DW: Dry weight, NUE_G: Nutrient use efficiency based on grain yield, NUE_B: Nutrient use efficiency based on biomass; Nano-fertilizer 1: $N_{20}P_{20}K_{20}$ (160 kg/ha) + N (210 L/ha), Nano-fertilizer 2: $N_{20}P_{20}K_{20}$ (128 kg/ha) + N (168 L/ha), Nano-fertilizer 3: $N_{20}P_{20}K_{20}$ (96 kg/ha) + N (126 L/ha), Nano-fertilizer 4: $N_{20}P_{20}K_{20}$ (64 kg/ha) + N (84 L/ha) and Nano-fertilizer 5: $N_{20}P_{20}K_{20}$ (32 kg/ha) + N (42 L/ha). The details of nano-fertilizer treatments are shown in Table 1.

et al. 2017). Neisani *et al.* (2012) also stated that water-deficit stress significantly reduces RWC due to the accumulation of soluble carbohydrates under more negative soil water potential. Additionally, Nasrollahzade *et al.* (2018) reported that the RWC of maize was reduced by 20% under water-deficit stress, compared to regular irrigation.

Our findings in this research showed that water-deficit stress caused a decrease in grain yield and yield components but some nano-chelated fertilizers, especially treatment 3, significantly increased them as compared to the chemical fertilizer. Other studies have shown that nano-chelated fertilizers efficiently improve crop quality and quantity under unfavorable conditions such as drought. For example, a study by Astaneh *et al.* (2018) using nano-chelated nitrogen fertilizers under optimum irrigation and water-deficit stress conditions, effectively improved wheat grain yield and yield-related characteristics compared to conventional urea. Zaky *et al.* (2022) reported that applying mineral NPK along with nano-NPK produced maximum grain yield. They stated that reducing the consumption of chemical fertilizers reduces environmental pollution. Zahedifar and Zohrabi (2016) showed that using nano-chelated potassium fertilizer improves seed germination under drought-stress conditions.

Table 5. Interaction of different levels of irrigation and nano-chelated fertilizers on physiological characteristics in maize.

Irrigation	Nano-chelated fertilizer	RWC (%)	Proline (µmol/kg DW)	Chl a (mg/g FW)	Chl b (mg/g FW)	Chl index (SPAD)	Car (mg/g FW)	Leaf Temp (°C)
Optimum irrigation	Control	63.74^{c}	93.42^{def}	2.24^{e}	0.99^{cd}	52.96^{abc}	1.31^{c}	16.70^{ef}
	Nano-fertilizer 1	71.86^{b}	63.61^{g}	2.63^{c}	1.17^{bc}	58.33^{a}	1.18^{c}	16.10^{f}
	Nano-fertilizer 2	78.36^{a}	62.52^{g}	2.77^{b}	1.32^{ab}	56.64^{a}	0.80^{c}	16.03^{f}
	Nano-fertilizer 3	80.86^{a}	54.87 ^{g}	2.91^{a}	1.44^{a}	59.43^{a}	1.71^{abc}	15.63^{f}
	Nano-fertilizer 4	64.94^{c}	74.27^{efg}	2.42^{d}	1.18^{bc}	54.40^{ab}	1.29^{c}	16.26^{f}
	Nano-fertilizer 5	62.30^{c}	105.23^{bcd}	2.20^{e}	0.91^{d}	49.16^{bcd}	1.40^{bc}	16.76^{ef}
Water-deficit stress	Control	47.70^{f}	131.60^{b}	1.18^{i}	0.43^{e}	29.63^{f}	1.66^{abc}	18.86^{ab}
	Nano-fertilizer 1	50.83^{e}	116.83^{bcd}	1.31^{h}	0.59^{e}	41.93^{de}	2.34^{ab}	18.00^{bcd}
	Nano-fertilizer 2	55.80^{d}	100.96^{cde}	1.42^{g}	0.62^{e}	41.20^{e}	1.38^{bc}	17.63^{cde}
	Nano-fertilizer 3	57.30^{d}	66.21^{fg}	1.60^{f}	0.63^{e}	46.86^{cde}	2.42^{a}	17.33^{de}
	Nano-fertilizer 4	45.63^{fg}	122.03^{bc}	1.27^{h}	0.54^{e}	32.46^{f}	1.52^{abc}	18.53^{bc}
	Nano-fertilizer 5	43.66^{g}	168.54^{a}	1.14^{i}	0.48^{e}	27.83^{f}	1.24^{c}	19.59^{a}

Means in each column followed by the same letter(s) are not significantly different at 5% probability level according to Tukey's Test; RWC: Relative water content, Chla: Chlorophyll a, Chl b: Chlorophyll b; Chl index: Chlorophyll index; Car: Carotenoids; Leaf Temp: Leaf temperature; Nano-fertilizer 1: N20P20K20 (160 kg/ha) + N (210 L/ha), Nano-fertilizer 2: N20P20K20 (128 kg/ha) + N (168 L/ha), Nano-fertilizer 3: N20P20K20 (96 kg/ha) + N (126 L/ha), Nano-fertilizer 4: N20P20K20 (64 kg/ha) + N (84 L/ha) and Nano-fertilizer 5: N20P20K20 (32 kg/ha) + N (42 L/ha). The details of nano-fertilizer treatments are shown in Table 1.

Potassium has a role in anti-stress activity, enhancing plant resistance to water deficit and alleviating the negative impacts of this stress (Bahrami-Rad and Hajiboland 2017; Zahoor *et al.* 2017). The positive effects of potassium on maize yield and yield components are due to better absorption of nano-chelated fertilizers that enable the nutrient to perform its vital roles in the plant. Potassium plays a critical role in changing xylem sap hydraulic conductance and water dynamics in plants (Nardini *et al.* 2010; Oddo *et al.* 2011). Potassium increases leaf dry weight, shoot growth, and grain yield by regulating water use efficiency, increasing root growth, and increasing cell division (Amanullah *et al.* 2016). Supplying potassium to plants regulates the proper functioning of the stomata resulting in an enhanced carbon dioxide stabilization rate. Thus, an increase in the number of grains and grain yield with potassium intake can be explained by the role of potassium in increasing carbohydrate production and its rapid transfer to grains (Pettigrew 2008; Hasanuzzaman *et al.* 2018). Furthermore, Fakharzadeh *et al.* (2020) reported that utilizing nano-chelated iron fertilizer in paddy rice increased yield-related traits and bio-fortified white rice with iron. In addition, El-Bassiouny *et al.* (2020) stated that using nano-zinc in wheat enhanced growth and productivity.

Applying nano-chelated fertilizers (especially as the formulation in treatment 3) under normal irrigation and water-deficit stress, improved chlorophyll a, chlorophyll b, SPAD, and RWC in comparison to chemical fertilizer application. Vegetative growth and chlorophyll content are influenced by macro-nutrient absorption, especially nitrogen as an essential part of the chlorophyll molecule and phosphorus, essential for supplying ATP for chlorophyll production (Ye *et al.* 2019).

Based on our findings, nano-chelated fertilizers appear to be more efficient in supplying essential macronutrients to plants than chemical fertilizers. The greater efficiency of nano-chelated fertilizers in nutrient transfer enables smaller quantities of these inputs to enhance agronomic traits, chlorophyll content, and RWC compared to chemical fertilizers. Similar to our results, in the studies by Mir *et al.* (2015) and Ghahremani *et al.* (2014), applying nano-chelated potassium significantly improved the chlorophyll content. In addition, Astaneh *et al.* (2018) showed improvement in chlorophyll content of wheat under nano-chelated nitrogen treatments compared to urea.

Water-deficit stress increased proline content but the significant increase in carotenoid content was limited to nano-fertilizers 1 and 3. Carotenoids are a group of large isoprenoid molecules that play an essential role in photosynthesis and light protection. These compounds are divided into carotene hydrocarbons such as lycopene, beta-carotene, or xanthophyll. Two essential functions of carotenoids are the protection of chlorophyll from light oxidation and the absorption and transmission of short-wavelength photons onto chlorophyll a (Diedrick 2010). Studies have reported increasing the carotenoid content during drought stress (Dias *et al.* 2014). Moreover, research has shown that the amount of proline amino acid production increases with drought-stress intensity. Proline accumulation helps the plants resist the stress and maintain a suitable turgor pressure during drought stress (Soltani *et al.* 2012).

In the current study, water-deficit stress increased leaf temperature and electrolyte leakage. It seems that water-deficit stress affects stomata conductance and decreases the intra-tissue water content of leaves. The decrease in water content raises the canopy temperature. Canopy temperature has been shown to increase with longer irrigation intervals (Jahan *et al.* 2013). In addition, it has been reported that drought stress causes an increase in ion leakage to the extracellular space, with the increase being greater at 50% field capacity (Naghizadeh and Kabiri 2017).

Farmers mainly use mineral fertilizers such as di-ammonium phosphate, urea, and chemical NPK to increase and sustain crop yield. However, the nutrients in these fertilizers are poorly utilized due to various environmental and soil-related factors such as P-fixation, leaching, and volatilization of NO_3 and N_2O. The low nutrient use efficiency and high nutrient loss concerning conventional chemical fertilizers are important obstacles to increasing crop production, plant resistance against abiotic stresses, and agroecosystem and groundwater safety (Wang *et al.* 2013; Puntel *et al.* 2016).

In the present study, NUE was significantly increased in all nano-chelated fertilizer treatments compared to the chemical fertilizer. Notably, applying only 222 kg of nano-chelated fertilizer (Treatment 5) compared to 840 kg of chemical fertilizer (control), increased grain yield by 33%. In addition, applying 1.3 t/ha of nitrogen in nano-chelated form (nano-chelated nitrogen and $N_{20}P_{20}K_{20}$ fertilizers) in treatment 5, resulted in an NUE of 515 and 367 under normal irrigation and drought stress, respectively. These were 13 and 12.6 times greater than the NUEs observed in control chemical fertilizers, respectively. Indeed, despite the smaller quantity of fertilizers used in all nano-chelated fertilizer treatments compared to chemical fertilizer, the superior nutrient absorption and efficiency in this form resulted in greater yields and improved NUE. Zareabyaneh and Bayatvarkeshi (2015) indicated that nano-chelating based on nitrogen fertilizer is more resistant to leaching, has greater nitrogen use efficiency, and induces more yield when compared to urea in potatoes.

Increasing the amount of nano-chelated fertilizers significantly decreased NUE. This suggests an optimal level or limit for the performance of nano-chelated fertilizers. When the fertilizers are used below this threshold, they are effective in their intended purpose. However, if the threshold is exceeded, their effectiveness is hindered or reduced. It is important to note that even when used in quantities greater than the optimal level, nano-chelated fertilizers continue to outperform conventional chemical fertilizers.

Conclusion

This study found that water-deficit stress significantly reduced agronomic traits, especially grain yield and its components, photosynthetic pigments, RWC, and NUE. Contrarily, it increased proline content, carotenoids, leaf temperature, and electrolyte leakage. Application of nano-chelated fertilizers even in reduced amounts compared to chemical fertilizer, considerably enhanced maize agronomic traits, photosynthetic pigments, RWC, NUE, grain yield, and yield components while decreasing maize proline content, leaf temperature, and electrolyte leakage. The increased efficiency of such inputs enables the use of reduced doses of fertilizers that can reduce production costs and reduce the burden of chemical fertilizers on the environment. Lastly, replacing conventional chemical fertilizers with reduced doses of nano-chelated fertilizers improves the drought tolerance of the plants and facilitates the transition to sustainable agriculture in production systems.

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Conflict of Interest

Nano-chelated nitrogen and NPK 20-20-20 fertilizers were synthesized based on "Chelate Compounds" technology which is patented in USPTO (US8288587B2). Mohammad Hassan Nazaran is the owner of "Chelate Compounds" technology. There was no conflict of interest.

Artificial Intelligence Tools

In this study, artificial intelligence tools were not used.

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