effect of nano-micronutrient supplementation and glutathione on some vegetative traits of maize (*Zea mays* L.)

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Abstract

The study was conducted in one of the experimental fields at Field Crops Department, College of Agriculture, University of Kerbala, during the 2024 growing season. The aim was to investigate the effect of applying different concentrations of nano-micronutrient fertilizers and glutathione on vegetative growth yellow corn. A factorial experiment was carried out using a randomized complete block design (RCBD) with three replications. The experiment included two factors: the first factor involved foliar application of nano-micronutrient fertilizers at five concentrations (0, 0.5, 1, 1.5, and 2 g L^{-1}), denoted as (N0, N1, N2, N3, and N4) , respectively. The second factor consisted of glutathione foliar application at five concentrations (0, 40, 80, 120, and 180 mg L^{-1}), designated as (G0, G1, G2, G3, and G4) , respectively. The results revealed a significant superiority of treatment N4 (2 g L^{-1}) in most traits, as it achieved the highest mean leaf area (6148.6 cm^2)plant and chlorophyll content (48.581 SPAD). Meanwhile, treatment N3 (1.5 g L^{-1}) showed the highest mean for plant height and stem diameter (203.71 cm and 24.021 mm, respectively), compared to the control treatment. Furthermore, spraying with glutathione had a significant effect on all traits under study, with treatment G4 (180 mg L^{-1}) achieving the highest mean for plant height, number of leaves, stem diameter, leaf area, and chlorophyll index, with averages of (188.88 cm, 12.584 leaf $plant^{-1}$, 23.436 mm, 5980.3 cm^2, and 48.413 SPAD, respectively). The interaction treatment between adding nano-nutrients (N3) and spraying with glutathione (G4) was significantly superior in most of the vegetative growth traits under study.

Keywords: Maize, Nano-Micronutrient, Glutathione, Vegetative traits

Introduction

Maize (*Zea mays* L.) belonging to the Poaceae family is one of the important cereal crops, as it is widely cultivated in the world after wheat and rice. The crop is used as human nutrition and the production of edible oils, in addition to its use as green fodder. Iraq suffers from a low yield of maize, which may be due to factors of soil and crop service or a lack of superior genetic compositions that are not suitable for the different regions of Iraq [1]. The importance of maize comes from its entry into many industrial fields [2].

Some mineral elements in most of the lands of Iraq are exposed to many factors that determine their movement and availability for absorption. Therefore, researchers have turned to finding modern technical methods, including nano-fertilizers, for the purpose of adopting them in supplying plants with the necessary nutrients by spraying them on the plant to achieve a qualitative and quantitative improvement in production by reducing the obstacles facing nutrients in the soil that reduce their availability to the plant [3]. Researchers have recently started using modern technologies in the field of fertilization, especially nanofertilizers, and the fertilization efficiency of traditional fertilizers hardly exceeds 30-40% [4]. Nano-fertilizers are highly efficient in supplying plants with nutrients, as they play an important role in increasing carbon metabolism processes and intensifying crops' ability to resist diseases and reduce stress [5]. Nano-materials also act as catalysts for some vital aspects of the plant, as both the leaf and root surfaces of the plant are the center of vital processes for absorbing the main nutrients of the plant, which are easily penetrated by nano-materials [6]. Nano-fertilizers, including microelements (boron, copper, iron, manganese, etc.), play an important role in plant nutrition by adding them to the leaves and soil. Their slow release contributes to providing the plant with nutrients that include maintaining metabolic processes and improving the crop [7]. In recent years, nanoparticles have found wide applications in biological sciences because nanoparticles have a diameter smaller than the diameter of the cell membrane cavity, so they can easily pass through the membrane. Moreover, at the leaf level, they enter the plant through the pores of the leaves or through the cracks that are transferred to the various tissues [8].

Antioxidants play a vital role in protecting plants from stress resulting from harsh environmental conditions such as salinity and drought. These compounds reduce oxidative stress [9]. Antioxidants also help regulate osmotic pressure, which helps plants absorb water and nutrients more efficiently. In addition, antioxidants may help reduce the absorption of toxic elements in plants [10]. Glutathione is a short peptide consisting of three amino acids (glycine, glumatine and cysteine). It plays an effective role in reducing damage such as stress and strain to which plants are exposed, especially during the fertilization and flowering period, which is usually at high temperatures in July and August. Thus, it increases the plant's ability to withstand high temperatures. Given the real problems in the production of maize, this study aims to determine the best concentration of nano-nutrient with the aim of introducing them into plant growth and production improvement programs. In addition to determining the best concentration of glutathione, which plays a fundamental role in resisting oxidative stress and removing toxins, in addition to its role in regulating growth and enhancing the efficiency of photosynthesis, which is positively reflected in the growth and yield of maize.

Materials and Methods

A field experiment was conducted during the spring growing season of 2024 at Ibn Al-Bitar Vocational School in Al-Husayniyah District, Karbala (latitude 32°N, longitude 44°S) on a silty clay soil. The study aimed to investigate the effects of nano-micronutrient fertilization and glutathione on the growth and yield of maize. The experiment was arranged as a factorial design using a randomized complete block experiment (RCBD) with three replications, comprising a total of 25 experimental units per replication.

After performing soil preparation operations—including plowing, leveling, and smoothing—the field was divided into 3 × 3 m experimental units, each with an area of 9 m². Maize seeds of the Azwan variety were sown on July 14, 2024, in hills at the upper third of the ridge, with a spacing of 50 cm between hills. Seeds were sown alternately on both sides of the ridge, with three seeds per hill at a depth of 3–5 cm, and later thinned to one plant per hill once plants reached a height of 10–15 cm.

Nano-micronutrient fertilizers were applied as foliar sprays three months after planting, using five different concentrations: 0, 0.5, 1, 1.5, and 2 g L^{-1}, designated as N_0, N_1, N_2, N_3, and N_4, respectively. Similarly, glutathione was applied at five concentrations: 0, 40, 80, 120, and 180 mg L^{-1}, designated as G_0, G_1, G_2, G_3, and G_4, respectively. In addition to these treatments, standard agronomic practices such as weeding, irrigation, and fertilization were carried out as needed to support crop growth [11].

Data recorded

Five plants were randomly selected from each experimental unit to study the following traits:

Plant Height (cm): Plant height was measured at 100% flowering stage, from the soil surface to the base of the flag leaf.

Number of Leaves (leaf plant^{-1}): The total number of leaves was counted for the five sampled plants, and the average number of leaves per plant was determined.

Stem Diameter (mm): Stem diameter was measured using a vernier meter at the 100% flowering stage. The measurement was taken 1 mm below the second node on the stem, ensuring the removal of the leaf sheath before recording the diameter. The average stem diameter was then calculated [12].

Leaf area (cm²): Leaf area was determined using a fixed sample by measuring the length of the leaf located directly below the main ear. The El-Sahookie equation was applied to estimate the leaf area [13]:

Leaf area per plant = $(\text{Leaf length below the main ear})^2 \times 0.75$

Chlorophyll Index (SPAD): The chlorophyll index was measured using a SPAD Chlorophyll Meter. Readings were taken from four leaves per plant, and the average SPAD value was calculated from five sampled plants [14].

Statistical analysis

The results were statistically analyzed using the randomized complete block design (RBCD) and the means were compared using the least significant difference (LSD) at the probability level of 0.05 between the treatments and using the statistical program Genstat [15].

Results and Discussion

Plant Height (cm)

The results (Table 1) indicate a significant effect of nano-micronutrient fertilization, glutathione application, and their interaction on plant height. Table 1 shows a notable effect of micronutrients, where treatment N_3 recorded the highest average plant height of 203.71 cm, while the control treatment (N_0) resulted in the lowest average height of 168.57 cm. This increase in plant height may be attributed to the direct role of micronutrients, particularly zinc, in promoting the synthesis of tryptophan, an essential amino acid considered a key precursor for the biosynthesis of indole-3-acetic acid (IAA). IAA is crucial for cell elongation, which subsequently enhances plant height [16].

The results from the same table indicate that foliar application of glutathione had a significant effect on plant height. The treatment G_4 recorded the highest average of 188.88 cm, while the control treatment (G_0) resulted in the lowest average of 183.56cm. This increase in plant height may be attributed to the role of glutathione, which contains three essential amino acids: glutamine, cysteine, and glycine. These amino acids play a fundamental role in enhancing plant growth by stimulating various physiological processes that promote cell division and elongation, leading to a noticeable increase in plant height. These findings are consistent with the study by [17], which reported that glutathione foliar application in maize significantly increases plant height.

The results (Table 1) also indicate a significant interaction between glutathione foliar application and nano-micronutrient fertilization on plant height. The highest plant height 205.23 cm was observed in the treatment combining 180 mg L^{-1} glutathione (G_4) with 1.5 g L^{-1} nano-micronutrient fertilizer (N_3). In contrast, the control treatment (G_0N_0) resulted in the lowest plant height of 162.58 cm.

Table (1): Effect of nutrition with nano- micronutrient, glutathione and their interaction on the height of the maize plant (cm).

Glutathione Concentration (mg L^{-1})	Nano Micronutrient Concentration (g L^{-1})					Means
	N0	N1	N2	N3	N4	
G0	162.58	166.32	189.90	201.99	196.99	183.56
G1	169.25	170.46	193.74	203.76	198.67	187.17
G2	170.05	171.49	47 .194	204.79	199.95	188.15
G3	169.52	169.41	192.36	202.78	197.03	186.22
G4	171.43	171.93	195.14	205.23	200.69	188.88
Means	168.57	169.92	193.12	203.71	198.67	
L.S.D $_{0.05}$	Glutathione		Nano-micronutrient			Interaction
	0.710		0.710			1.587

Number of Leaves (leaf plant^{-1})

The results presented in Table 2 show significant differences in the number of leaves among treatments with nano-micronutrient foliar application. The treatment N2 recorded the highest average of 13.583 leaf plant^{-1}, The lowest average is treatment N1 recorded the of 10.713leaf plant-1. The superiority of the higher concentration in the number of leaves is attributed to the fact that treating maize plants with nano-fertilizer led to a significant increase in the rate of plant height and the number of leaves [18]. As for the effect of spraying with glutathione compound, based on the results of the table, It is noted the significant superiority of plants that treated with G4 with an average of 12.584 leaf plant-1 with a slight significant different compared with the rest treatments, where the lowest average recorded with the control treatment (G0). It is clear that adding glutathione as a spray on the maize plant caused a significant increase in leaves, and the reason is due to the great and effective role of the glutathione compound. The reason for the superiority of the spraying concentration with glutathione (180 mg L^{-1}) in giving the highest average for this trait may be attributed to its superiority in the trait of plant height (Table 1).

The interaction between the concentrations of nano-micronutrients and glutathione concentrations had a significant effect on this trait, as the treatment G4N2 gave the highest average of 14.230 leaf plant^{-1}, while the lowest interaction was in the treatment G0N1, which gave an average of 10.143 leaf plant^{-1}.

Table (2): Effect of nutrition with nano-micronutrient, glutathione and the interaction between them on the number of leaves in maize (leaf of plant^{-1}).

Glutathione Concentration (mg L^{-1})	Nano Micronutrient Concentration (g L^{-1})					Means
	N0	N1	N2	N3	N4	
G0	10.367	10.143	13.063	12.093	11.703	11.474
G1	10.447	10.457	13.293	12.323	12.187	11.741
G2	11.233	11.103	14.023	13.053	12.683	12.419
G3	10.473	10.387	13.307	12.337	11.607	11.622
G4	11.393	11.477	14.230	13.260	12.560	12.584
Means	10.783	10.713	13.583	12.613	12.148	
L.S.D $_{0.05}$	Glutathione		Nano-micronutrient			Interaction
	0.2341		0.2341			0.5236

Stem diameter (mm)

The results of Table (3) show a significant effect of the study factors on the average stem diameter, as the treatment N3 of the nano-nutrients significantly outperformed with the highest average of 24.021 mm, while the lowest average was in the control treatment (N0), which reached 20.115 mm. The superiority of this treatment may be attributed to the fact that foliar nutrition led to the activation of a number of physiological processes in which zinc and iron play a major role, whether in the carbon metabolism process or the activation of enzymes, which contributes to enhancing the plant's ability to grow and increase its biomass and thus increase the stem diameter. This result is consistent with [19].

The results of the same table indicated that there are significant differences between the concentrations of glutathione in the stem diameter, as the treatment G4achieved the highest average of 23.436 mm, while the control treatment (G0) gave the lowest average of 20.408 mm. The reason for this result may be that glutathione consists of three amino acids that lead to a decrease in the osmotic potential, which in turn causes to a decrease in the water potential of the cell, thus increasing the cell's ability to uptake water and dissolved nutrients, and then leading to an increase in the vegetative growth of the plant [20].

The results also showed that the interaction between the concentrations of the two factors had a significant effect on the stem diameter trait, as the highest result was achieved in the treatment N3G4 with an average of 25.740 mm, while the control treatment (N0G0) gave the lowest average of 19.040 mm.

Table (3): Effect of nutrition with nano-micronutrient, glutathione and the interaction between them on the stem diameter trait in maize (mm).

Glutathione Concentration (mg L^{-1})	Nano Micronutrient Concentration (g L^{-1})					Means
	N0	N1	N2	N3	N4	
G0	19.040	19.330	20.130	22.440	21.100	20.408
G1	19.170	20.057	20.857	23.167	21.827	21.015
G2	20.217	20.890	21.690	24.000	22.660	21.891
G3	21.167	21.650	22.450	24.760	23.420	22.689
G4	20.980	22.630	23.430	25.740	24.400	23.436
Means	20.115	20.911	21.711	24.021	22.681	
L.S.D $_{0.05}$	Glutathione		Nano-micronutrient			Interaction
	0.3232		0.3232			0.7227

Leaf area (Cm^2 $plant^{-1}$)

The results (Table 4) indicated a significant effect of spraying with nano micronutrients on the leaf area of plants, as treatment N4 achieved the highest average of 6148.6 $Cm^2 plant^{-1}$, while treatment N2 gave the lowest average of 5676.6 cm^2 $plant^{-1}$. Nano-elements have the ability to stimulate vegetative cells to elongate and divide as a direct effect on leaf formation areas, which is consistent with [21] who indicated the role of boron in cell division and the formation of pectin and lignin.

Effect of spraying with glutathione was significant in this trait, as the leaf area of the maize plant increased until the concentration G4 (180 mg L^{-1}) , which gave the highest average of 5980.3 cm^2 compared to the control treatment (G0) which gave the lowest average of 5837.0 $cm^2 plant^{-1}$. The superiority that occurred may be due to the role of glutathione in increasing the leaf area of the plant through the physiological role of the amino acids In its component and their effects, which is reflected in increasing the leaf area in plants.

As for the interaction between the concentration of the two factors, it was significant in this trait, as shown in Table (4), as the highest interaction was in the treatment N4G4, with an average of 6206.7 $cm^2 plant^{-1}$, compared to the treatment N2G0 plants, which gave the lowest average of 5591.0 cm^2 $plant^{-1}$.

Table (4): Effect of nutrition with nano-micronutrient, glutathione and the interaction between them on the leaf area trait in maize (cm^2).

Glutathione Concentration ($mg\ L^{-1}$)	Nano Micronutrient Concentration ($g\ L^{-1}$)					Means
	N0	N1	N2	N3	N4	
G0	5790.0	5804.0	5591.0	5937.0	6063.0	5837.0
G1	5882.3	5896.0	5683.0	6029.0	6155.0	5929.1
G2	5903.0	5907.7	5694.7	6040.7	6166.7	5942.5
G3	5863.0	5899.3	5679.7	6025.7	6151.7	5923.9
G4	5932.0	5947.7	5734.7	6080.7	6206.7	5980.3
Means	5874.1	5890.9	5676.6	6022.6	6148.6	
L.S.D $_{0.05}$	Glutathione		Nano-micronutrient			Interaction
	53.82		24.07			53.82

Chlorophyll index (SPAD)

The results of Table (5) show a significant effect of micronutrients on the chlorophyll trait, as treatment N4 recorded the highest average of 48.581 SPAD, while the control treatment (N0) recorded the lowest average of 43.736 SPAD. The reason for the superiority could be that micronutrients contain iron, which contributes to the formation of two compounds, Laevulinic and Protochlorophytic, which are essential for the chlorophyll synthesis pathway [22].

The same table also showed significant differences between the glutathione spray treatments on the chlorophyll index, as treatment G4 gave the highest average of 48.413 SPAD , while the control treatment (G0) recorded the lowest average of 45.000 SPAD. Glutathione plays an important role in improving the level of chlorophyll in the leaves, as it contributes to reducing oxidative stress and enhancing the efficiency of the photosynthesis process, which is positively reflected in increasing its content in the leaf [23].

The results also showed a significant interaction between the factors under study. From the results of Table 5, it was shown that the treatment G4N4 recorded the highest interaction of 50.500 SPAD, while the lowest interaction was in the G0N3 treatment with an average of 41.280 SPAD.

Table (5): Effect of nutrition with nano-micronutrient, glutathione and the interaction between them on the chlorophyll index trait in maize (SPAD).

Glutathione Concentration (mg L^{-1})	Nano Micronutrient Concentration (g L^{-1})					Means
	N0	N1	N2	N3	N4	
G0	45.870	46.290	47.200	41.280	44.360	45.000
G1	43.080	45.087	46.597	47.017	47.927	45.941
G2	44.093	45.920	47.430	47.850	47.760	46.611
G3	45.083	46.680	48.190	48.610	49.520	47.617
G4	45.143	47.660	49.170	49.590	50.500	48.413
Means	43.736	45.941	47.451	47.871	48.581	
L.S.D $_{0.05}$	Glutathione		Nano-micronutrient			Interaction
	0.5497		0.5497			1.2291

From the above, it can be concluded that the use of nano-micronutrient along with glutathione led to a significant improvement in morphological and even physiological traits. Therefore, these results can be used to improve field crop productivity by enhancing the use of nano-fine fertilizers as an effective source of nutrients, along with glutathione as a plant growth stimulant. it could be also recommend applying the optimal concentrations that showed the highest efficiency, with the need to conduct additional studies to evaluate the long-term effects of these treatments on crop productivity and quality in different environmental conditions.

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