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Effect of Spraying with Humic and Ascorbic Acids and their Interaction on Some Vegetative Characteristics of Maize

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Abstract

Maize (*Zea mays* L.) is one of the world's most important cereal crops, and improving its growth and productivity depends on using modern agricultural techniques and biostimulants that enhance the efficiency of the plant's physiological processes. This study aimed to evaluate the effect of foliar spraying with humic acid and ascorbic acid and the interaction between them on some vegetative characteristics of yellow corn. The experiment was conducted to study the effect of three concentrations of humic acid (0, 75, and 150 mg. L^{-1}) and three concentrations of ascorbic acid (0, 150, and 300 mg .L^{-1}) on some vegetative characteristics of maize. The studied characteristics included plant height (cm), stem diameter (cm), number of leaves, vitamin C content in leaves (mg. g^{-1} fresh weight), and dry weight (g. m^{-2}). The data were statistically analyzed to determine the effect of the studied factors and their interactions. The results showed a significant increase in all studied traits with increasing concentrations of humic acid and ascorbic acid. The highest values for plant height (174.05 cm), stem diameter (24.01 cm), number of leaves (16.56 leaves), leaf vitamin C content (6.42 mg.g^{-1}), and dry weight (414.81 **g/m²**) were recorded with the interaction treatment of 150 **mg.L^{-1}** of humic acid and 300 mg.L^{-1} of ascorbic acid, while the lowest values were recorded in the control treatment. results of the study indicate that foliar spraying with humic acid and ascorbic acid has a positive effect on improving the vegetative and physiological characteristics of maize, and that the interaction between them can enhance vegetative growth and dry matter accumulation, which may contribute to improved crop yield.

Keywords: Humic acid, Ascorbic acid, Foliar spraying, maize

I. Introduction

Maize (*Zea mays* L.) is a vital agricultural crop, cultivated for grain and silage production, in addition to its essential role in human and animal nutrition(Revilla et al., 2022). Iraq has witnessed a significant expansion in corn cultivation areas in recent years. However, corn farming in this region faces numerous environmental challenges that negatively impact productivity. The selection of unsuitable varieties is a major contributing factor to these problems, as it leads to increased moisture retention within the grains, potentially jeopardizing safe ripening at harvest time. Therefore, selecting corn varieties appropriate to Iraq's specific environmental conditions is crucial for achieving high yields and ensuring crop quality at harvest(Sharma & Rayamajhi, 2022). Foliar applied ascorbic acid has been reported to alleviate stress-induced effects in maize through improvement of photosynthetic capacity, osmolytes production and antioxidant defence system(Pinna & Husted, 2025). Ascorbate is crucial for transporting photosynthates from the source tissues to sink organs, enhancing net photosynthesis rates, inhibiting auxin oxidation and enhancing plant antioxidant potential. These activities aid in promoting plant growth and increasing drought resistance(Wadas & Kondraciuk, 2025). In addition, ascorbic acid promotes growth of maize plants through enhancing water use efficiency and bio-synthesis of photosynthetic pigments, which are factors in eventual higher yield. It plays an important protective role as reducing agent in plant tissues against oxidative damage produced by water deficits(Zhu et al., 2024). In addition, ascorbic acid can increase drought resistance by the regulation of photosynthetic pigments, accumulation of osmoprotective substances and induction of antioxidant systems in maize plant to prevent damage from drought stress especially at early growth stages. Furthermore, ascorbic acid acts as a cofactor of phytohormones, enzymic activities and cell development-related processes suggesting its effective role in mitigation of water deficit stress(Asl et al., 2024). Humic acid (HA) is widely acknowledged as a major group of naturally occurring antioxidant substances present in soil and aquatic environments. These compounds are actively involved in numerous redox processes that regulate ecosystem dynamics, and they may constitute a substantial proportion of dissolved organic matter in natural water systems(Dauletbay et al., 2024).

The application of humic acid, either as a foliar spray or as a seed treatment prior to sowing, has been reported to markedly enhance plant biomass, chlorophyll concentration, and proline accumulation. Foliar application is generally more effective in increasing biomass production, chlorophyll content, and nutrient uptake, whereas seed treatment has a stronger influence on modifying proline levels in leaves(Vašková et al., 2023). Humic acid has also been shown to improve plant tolerance to salinity stress by stimulating biomass production, enhancing chlorophyll and mineral nutrient content, and activating antioxidant enzyme systems(Abu-Ria et al., 2023). Furthermore, the combined application of humic acid with ascorbic acid represents a cost-effective strategy for promoting seedling establishment and early plant growth under drought-prone soil conditions. Incorporation of humic acid into the soil has been associated with sustained maize growth during periods of limited water availability. The growth-promoting effects of humic acid are largely attributed to its hormone-like activity, particularly through the stimulation of root development and increased root proliferation.

II. Materials and Methods

A field experiment was conducted during the winter season, starting from 2 March 2025 to 7 April 2025, in a plastic greenhouse affiliated with the College of Science, Department of Biology. The experiment employed a completely randomized design with two replicates. 18 plants were used, divided equally among treatments. The experiment was arranged as a factorial experiment using a randomized complete block design (RCBD) with three replicates. The impact of two factors (i.e., humic acid at three levels (0, 150 and 300 mg L^{-1}) and ascorbic acid at three levels (0, 75 and 150 mg·L-1)). Both factors in combination generated a total of nine treatment combinations (9 treatments × 3 replicates = 27 experimental units). Spraying treatments were applied according to the assigned concentrations during vegetative growth and all other agronomic practices were uniformly carried out for all experimental units throughout the growing season.

Table 1: Selected chemical composition of the study soil before planting

No.	Property	Value	Unit	Acceptable Limits
1	Sand	**44.5**	%	—
2	Silt	**45.42**	%	—
3	Clay	**10.07**	%	—
4	Soil Texture (Loam)	---	---	---
5	Field Capacity	28.4	%	—
6	Organic Matter	0.75	%	> 1.2
7	Electrical Conductivity (EC)	2.19	dS/m	> 5
8	Soil pH	7.4	—	—
9	Bulk Density	1.23	g/cm³	—
10	Permanent Wilting Point	9.5	%	—
11	Gypsum	17.5	%	—

12	Nitrogen (N)	12.2	mg/kg	> 20
13	Phosphorus (P)	4.91	mg/kg	8–15
14	Potassium (K)	102	mg/kg	> 50
15	Calcium Carbonate ($CaCO_3$)	16	—	—
16	Sodium (Na)	—	—	—

Studied characteristics

1- **Plant height (cm):** The plant height was measured from the point of contact with the ground to the end of the spike, excluding the awns, using a graduated ruler. It was measured at the end of the season for ten randomly selected plants, and then their average was taken.
2- **Stem diameter:** Stem diameter was measured in three replicates using a digital micrometer. The measurement was taken at a height of 50 cm from the soil surface, using two perpendicular planes to minimize the error of non-circular shape. The average stem diameter for each replicate was then calculated by averaging the values of the selected plants.
3- **Number of leaves(leaf)** : The number of leaves was measured by counting the complete leaves of a number of randomly selected plants from each treatment, and then calculating the average number of leaves per plant.
4- **Vitamin C content in leaves (mg. g^{-1} fresh weight):** Estimate the vitamin C content in vegetable weight using the standard curve for vitamin C according to the method of Bajaj and Kaur (1981).
5- **Dry weight (g. m^{-2}):** A plant sample was taken from a known area, then dried in an oven at 70°C for 48 hours until the weight stabilized. After that, the sample was weighed, and the dry weight per square meter was calculated.

Statistical analysis

The Statistical Packages of Social Sciences-SPSS (2019) program was used to detect the effect of difference factors (Factorial experiment-ANOVA one way) in study parameters. Least significant difference-LSD test was used to significant compare between means in this study.

Results and Discussion

Plant height(m): Table 2 presents the effect of humic acid and ascorbic acid spraying on plant height (cm) of maize plants. The results indicate an increase in plant height with increasing concentrations of both factors. The mean plant height increased from 158.22 cm at 0 mg/L humic acid to 171.06 cm at 150 mg/L. Similarly, increasing ascorbic acid concentration resulted in higher plant height, where the mean values were 159.73, 164.81, and 168.22 cm at 0, 150, and 300 mg/L, respectively.

The interaction between the two factors showed that the highest plant height (174.05 cm) was obtained with the combination of 150 mg/L humic acid and 300 mg/L ascorbic acid, whereas the lowest value (153.71 cm) was recorded in the control treatment (0 × 0).

Table 2. Effect of spraying with humic acid and ascorbic acid on high plant (cm)

Humaic acid / Ascorbic acid	0	150	300	Mean
0	153.71	159.88	161.08	**158.22**
75	157.69	163.23	169.53	**163.48**
150	167.80	171.33	174.05	**171.06**
Mean	**159.73**	**164.81**	**168.22**	

Stem diameter(cm)

Table 3 shows the interaction effect of humic acid and ascorbic acid (vitamin C) spraying on the stem diameter of maize plants. The results showed statistically significant differences, with a gradual increase in stem diameter values observed with increasing levels of humic acid and ascorbic acid. The average stem diameter at 0 mg/L humic acid was 14.28 cm, rising to 19.83 cm at 150 mg/L. Regarding the effect of ascorbic acid concentration, the average stem diameter at 0 mg/L was 13.42 cm, increasing to 16.60 cm at 150 mg/L, and reaching a maximum average of 20.48 cm at 300 mg/L. The interaction results also indicate that the highest stem diameter value, 24.01 cm, was observed when 150 mg/L humic acid was applied with 300 mg/L ascorbic acid. The results show that the stem diameter values increased gradually with increasing levels of both factors.

Table 3. Effect of spraying with humic acid and ascorbic acid on stem diameter (cm)

Humaic acid / Ascorbic acid	0	150	300	Mean
0	10.87	14.65	17.31	**14.28**
75	13.24	15.81	20.13	**16.39**
150	16.15	19.33	24.01	**19.83**
Mean	**13.42**	**16.60**	**20.48**	

Number of leaves(leaf)

Table 4 presents the effect of humic acid and ascorbic acid spraying on number of leaves (leaf. mg.L)of maize plants. The results indicate an increase in number of leaves with increasing concentrations of both factors. The mean number of leaves increased from 11.20 cm at 0 mg/L humic acid to 13.91 cm at 150 mg/L. Similarly, increasing ascorbic acid concentration resulted in higher number of leaves, where the mean values were 10.34, 12.51, and 14.82 leaf. mg.L at 0, 150, and 300 mg/L, respectively.

The interaction between the two factors showed that the highest number of leaves (16.56) was obtained with the combination of 150 mg/L humic acid and 300 mg/L ascorbic acid, whereas the lowest value (9.31) was recorded in the control treatment (0 × 0).

Table 4. Effect of spraying with humic acid and ascorbic acid on Number of leaves (leaf. mg.L)

Humaic acid / Ascorbic acid	0	150	300	Mean
0	9.31	11.27	13.01	**11.20**
75	10.01	12.81	14.89	**12.57**
150	11.71	13.45	16.56	**13.91**
Mean	**10.34**	**12.51**	**14.82**	

Vitamin C Content (mg. g^{-1} fresh weight)

Table 5 shows the interaction effect of humic acid and ascorbic acid (vitamin C) spraying on the vitamin C content in maize leaves (mg g^{-1} fresh weight). The results indicated a gradual increase in vitamin C content with increasing levels of both humic acid and ascorbic acid. The average vitamin C content in leaves at 0 mg/L humic acid was 4.29 mg g^{-1}, which increased to 5.20 mg g^{-1} at 75 mg/L and reached 6.04 mg g^{-1} at 150 mg/L. Regarding the effect of ascorbic acid concentration, the average vitamin C content was 4.74 mg g^{-1} at 0 mg/L, increasing to 5.10 mg g^{-1} at 150 mg/L and reaching 5.70 mg g^{-1} at 300 mg/L. The highest interaction between humic acid and ascorbic acid (6.42 mg/g) was recorded when 150 mg/L of humic acid was combined with 300 mg/L of ascorbic acid. In general, the vitamin C content of corn leaves increased gradually with increasing concentrations of both humic and ascorbic acids.

Table 5 . Effect of spraying with humic acid and ascorbic acid on content of Vitamin C in leaf (mg. g^{-1}. fresh weight)

Humaic acid / Ascorbic acid	0	150	300	Mean
0	3.81	4.29	4.78	**4.29**
75	4.69	5.01	5.89	**5.20**
150	5.71	5.99	6.42	**6.04**
Mean	**4.74**	**5.10**	**5.70**	

Dry weight (g.m-2)

Table 6 shows the interaction effect of humic acid and ascorbic acid (vitamin C) spraying on the dry weight of maize plants (g m^{-2}). The results indicate a gradual increase in dry weight values with increasing levels of both humic acid and ascorbic acid. The average dry weight at 0 mg/L humic acid was 335.92 g m^{-2}, and increased to 393.15 g m^{-2} at 150 mg/L. Regarding the effect of ascorbic acid concentration, the average dry weight was 344.23 g m^{-2} at 0 mg/L, increasing to 401.14 g m^{-2} at 300 mg/L.

The interaction between humic acid and ascorbic acid, the highest value (414.81 g m^{-2}) was recorded when 150 mg/L humic acid was combined with 300 mg/L ascorbic acid. Overall, the dry weight values increased progressively with increasing concentrations of both humic acid and ascorbic acid.

Table 6. Effect of spraying with humic acid and ascorbic acid on dry weight (g. m^{-2})

Humaic acid / Ascorbic acid	0	150	300	Mean
0	289.11	331.09	387.55	**335.92**
75	374.81	389.92	401.07	**388.60**
150	368.77	395.86	414.81	**393.15**
Mean	**344.23**	**372.29**	**401.14**	

Plant height is one of the most important physiological indicators of vegetative growth in cereal crops such as corn, as it reflects the plant's efficiency in photosynthesis, nutrient absorption, and biomass formation, which directly affects crop productivity at the end of the season.

The results of the current study indicated a significant increase in plant height when sprayed with humic acid and ascorbic acid. This increased height can be explained by the complementary effect of humic and ascorbic acids on physiological functions. Humic acid improves nutrient absorption and facilitates nutrient translocation within the plant. It also increases cell wall permeability and stimulates the activity of plant hormones (such as auxins) that promote cell division and stem elongation(Ahmed et al., 2023). These mechanisms lead to an overall increase in plant height compared to untreated crops, results consistent with other studies demonstrating humic acid's ability to enhance vegetative growth and increase plant height in maize and other crops(Al-Akabi et al., 2025). Ascorbic acid (vitamin C) plays a crucial role as an antioxidant within plants, helping to reduce free radicals and protect cells from oxidation during periods of rapid growth. It also enhances the efficiency of metabolic processes, particularly photosynthesis(Zahid et al., 2023). These functions support increased energy availability for growth and promote cell elongation in developing tissues, resulting in an overall increase in plant height(Sarwar et al., 2021). The findings of the current study are consistent with several other studies indicating that foliar spraying with ascorbic acid promotes vegetative growth even under environmental stress conditions(Ahmed et al., 2022; Al-Hamdani, 2025). The interaction between humic acid and ascorbic acid in the current study indicates a synergistic effect; both compounds enhance each other in supporting essential plant physiological functions, A study conducted by (Yaquby et al., 2024) in Afghanistan confirmed that the effect of humic acid (HA) and ascorbic acid (AsA) was significant on cucumber growth under different levels of soil salinity.

The results of the current study showed statistically significant differences in stem diameter. The findings indicated a gradual increase in stem diameter with increasing concentrations of humic and ascorbic acids. This can be explained by the fact that increased nutrient uptake, particularly of nitrogen and potassium, leads to increased formation of vascular and supporting tissues in the stem, resulting in increased stem thickness(Abd-Elhakim, 2023). Ascorbic acid also enhances the activity of enzymes involved in cell wall synthesis and reduces oxidative stress within plant cells, which contributes to increased lateral stem growth(Narra et al., 2024). These results are consistent with a study that demonstrated that adding humic acid improved vegetative growth characteristics of maize, including stem diameter, due to increased photosynthetic efficiency and nutrient transport within the plant.

The study results showed a significant increase in **leaf number** with increasing concentrations of humic and ascorbic acids at p (0.05). This increase can be explained by the fact that humic acids stimulate cell division activity in apical buds and increase nutrient uptake, leading to increased leaf formation and expanded leaf area(Amiri Forotaghe et al., 2022). Ascorbic acid also contributes to regulating physiological processes within the plant and acts as an antioxidant, maintaining the integrity of plant cells, which improves leaf growth and increases leaf count. These results are consistent with a study that showed humic acid treatment increased leaf number and leaf area in maize plants due to improved plant physiological processes(Ahmed, 2023; Rostami et al., 2022).

The study results showed a gradual increase **in vitamin C content in corn leaves** with increasing concentrations of humic acid and ascorbic acid. Given that ascorbic acid is one of the most important parts of the antioxidant defense system in plants, it has a role in regulating growth and protecting cells from oxidative stress accompanying intensive metabolic processes, this increase can be regarded as desirable(Kadhum et al., 2025). In addition, exogenous application of ascorbic acid induces its concentration in the leaves and stimulates the activity of some enzymes active in the ascorbate-glutathione cycle responsible for scavenging free radicals resulting in increased concentrations of ascorbic acid in plant tissues(Darvishan et al., 2013). Moreover, as the application of humic acid can enhance plant mineral nutrition, it may also induce important processes in plants that resulted in an increased synthesis of antioxidant substances such as vitamin C. A study by (Ivanov, 2014) indicated that ascorbic acid plays a pivotal role in protecting chloroplasts and regulating photosynthesis in plants, contributing to improved plant physiological performance and increased antioxidant content.

Dry weight of maize plants significantly increased with the increasing concentrations of humic acid and ascorbic acid. The higher values for dry weight of the plants after spraying with humic and ascorbic acids can be explained because these compounds function as biostimulants, affecting several physiological processes regarding the plant(Chen et al., 2022). Responsible for better absorption of nutrients, increasing the permeability of cell membranes and stimulating root growth, humic acid promotes the availability of essential elements such as nitrogen, phosphorus and potassium needed to synthesize organic compounds in plants(Kadhim & Hamza, 2021).

Ascorbic acid (vitamin C) is the main antioxidant in chloroplasts, where it plays a role against oxidative stress and increases photosynthetic efficiency and carbohydrate synthesis (Foyer et al., 2020). Higher photosynthetic efficiency to process more carbon dioxide, along with enhanced nutrient absorption, induces more sugars and organic compounds (e.g. cellulose & proteins) produced in plant tissues as dry matter. Using vegetative growth traits (plant height, plant leaf number) in combination with the leaf area increases. Increased biomass accumulation process also leads to higher dry weight and rates of photosynthesis due to better light energy absorption aided by the ability of leaves to grow more(Bilska et al., 2019).

Numerous studies have indicated that humic substances act as biostimulants for plants by enhancing nutrient uptake and activating metabolic processes, leading to increased plant growth and biomass. Ascorbic acid, in particular, plays a crucial role in regulating photosynthesis and boosting the antioxidant defense system in plants, both of which contribute to improved growth and dry matter accumulation(Chen et al., 2003; Dolatabadian et al., 2008).

III. Conclusions

Foliar application of maize with humic acid and ascorbic acid resulted in a marked improvement in its vegetative and physiological characteristics, with the combined application proving more effective than either acid alone. The interaction between 150 mg/L humic acid and 300 mg/L ascorbic acid achieved the best overall performance, suggesting its potential as an effective strategy for enhancing maize growth and yield.

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