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Effect of application of humic acid and zinc at different concentrations and their interaction on some vegetative growth characteristics of chilli pepper *Capsicium annuum L*

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ABSTRACT: The field experiment was conducted in an agricultural area in Mansouriya Al-Shatt, Diyala Governorate, to investigate the impact of foliar application of humic acid and zinc sulfate on the vegetative growth of pepper plants. A factorial experiment was structured using a completely randomized block design with three replications. The first factor included humic spray concentrations (0, 6, 12) g. L^{-1}, The second factor was spraying zinc sulphate concentrations (0, 1, 2) g. L^{-1}. The results showed that foliar fertilization with humic acid at the level of 12 g. L^{-1} was significantly superior in giving the highest averages for all vegetative traits, as there was a significant superiority in the trait of plant height, chlorophyll, number of branches, and leaf area, which reached 44.70 cm, 65.17 SPAD, 5.00 branches. $plant^{-1}$, and 74.25 cm^2, respectively. Zinc spraying at 2 $gm.L^{-1}$ was superior in plant height and chlorophyll with an average of 44.31 cm and 62.70 SPAD, respectively, while the concentration of 1 $gm.L^{-1}$ for zinc spraying gave the highest average in the number of branches with an average of 4.55 $branches.plant^{-1,}$ while the comparison treatment for zinc spraying was superior in leaf area with an average of 72.99 cm^2. The interaction between the two experimental factors had a significant effect on all studied traits.

Keywords: Pepper, foliar spray, humic acid, zinc

1. INTRODUCTION

Sweet pepper (*Capsicum annuum* L.) is one of the main vegetable crops belonging to the Solanaceae family. It is one of the important crops for daily use and export (1). It is a source of many vitamins, such as vitamins A and C, and many nutrients, such as calcium, iron, phosphorus, zinc, and manganese. It also contains both proteins and carbohydrates (2, 3). According to the statistics of the Iraqi Ministry of Planning, the total cultivated area in 2022 amounted to 20,273 dunums, with a total production of 43,904 tons. Foliar nutrition depends on providing the plant with nutrients by spraying on the green group of the plant to get rid of signs of micronutrient deficiency immediately, such as yellowing of leaves, spotting, and weak nodes, and it resorts to ensure a rapid and effective response by the plant (4). Many studies have proven the success of this method when applied to different types of crops (5). Humic acid is a one of humic organic acid derived from the decomposition of organic matter, including plant and animal waste. It is rich in nutrients and enhances the physical and chemical qualities of soil, leading researchers to incorporate it into soil and crops (6). Zinc is one of the micronutrients for plants that play an effective role in many physiological processes (7), as it regulates the work of plant cells and regulates the growth process by controlling some growth regulators such as NAA, which causes the stems to elongate, leading to faster growth (2). It plays an important role in the formation and fixation of pollen grains on flower stigmas (8), and zinc deficiency causes the plant to stop growing and a decrease in the number of flowers and fruits (9). Awalin *et al*. reported that spraying zinc at a concentration of 100 mg.L^{-1} on the shoots of pepper plants led to a significant increase in the height of the pepper plant, as the average plant height reached 58.32 cm compared to the no-add treatment, which gave an average plant height of 50.80 cm. Al- Al-Musawi found that using nano-zinc at a concentration of 6 g. L^{-1} gave the highest average of chlorophyll index, amounting to 45.2 SPAD, compared to the no-addiction treatment, which gave an average of 32.4 SPAD. The same concentration also gave the highest average leaf area, reaching 1846.1 cm^2, while the comparison treatment recorded 1688.1 cm^2 (2). Fathima *et al.* showed in a field study that using humic spray on pepper plants at a concentration of 4 ml. L^{-1} gave the highest average number of branches, reaching 23 branches per plant, compared to the control treatment, which recorded 19 branches per plant (10). El-Sayed *et al.* indicated in a field experiment on pepper plants that using humic for two agricultural seasons at a concentration of 2 gm. L^{-1} gave the highest average of the total chlorophyll index for the two seasons, reaching 1.465 and 1.449 SPAD, while the comparison treatment for the two seasons gave 1.247 and 1.339 SPAD, respectively. It also gave the highest average number of branches, reaching 5.76 branches. Plant-1, while the non-addition treatment amounted to 5.27 branches. $Plant^{-1}$ (1).

2. MATERIALS AND METHODS

The field experiment was conducted in the Mansouriya Al-Shatt region of Diyala Governorate, located 35 km from Baghdad Governorate, during the summer season of 2024 in clay-textured soil. The objective was to examine the impact of foliar application of humic acid and zinc on various quantitative and qualitative traits of the pepper plant Capsicum annuum L. Random samples were collected from diverse areas of the field soil at a depth of 0-30 cm for laboratory analysis prior to planting, as detailed in Table (1). An experiment utilizing a randomized complete block design (RCBD) was executed with three replications. The experimental unit encompassed an area of 4 m^2, with dimensions of 2 m by 2 m. It contained four holes, with a spacing of 60 cm between each hole and 30 cm between the holes and the boundaries of the experimental unit. The distance between each copy was 1 meter, resulting in a total of 28 plants within the experimental unit.

The experiment consisted of two components: the first component involved spraying three different concentrations of humic acid (0, 6, and 12) g.L^{-1}. The second component involved spraying zinc sulfate at three different concentrations (0, 1, and 2) g.L^{-1}on the vegetative group of the plant in two batches. The first batch was applied one month after the seedlings were planted, and the second batch was applied one month after the first batch. California pepper seedlings obtained from one of the nurseries affiliated with Baghdad Governorate/Al-Basatin neighborhood were planted on 20/3/2024 and then given germination irrigation. All soil and crop service operations were carried out according to the recommended plant irrigation and fertilization needs. Until the plant reached the harvest stage, which was on 29/5/2024. The data were statistically analyzed using the analysis of variance method, employing the SPSS statistical software. The means of the coefficients were compared utilizing Duncan's multiple range test at a significance level of 0.05 (11).

2.2 Studied attributes

2.2.1 Plant height (cm)

Measure the height of the plant using a measuring tape from ground level to the top of the plant and take the average of the height of ten plants (4).

2.2.2 Number of branches (branch. plant^{-1})

Count the number of branches in the ten plants taken from the two median lines and extract the average number of branches for each plant (5).

2.2.3 Leaf area of plant (cm^2)

The leaf area was calculated using an area meter Am 300 as an average of ten plants from each experimental unit by taking ten leaves from each plant representing the different sizes of one plant and extracting the average area of one leaf from it, then multiplying it by the average number of leaves for one plant to obtain the total leaf area of the plant (12).

2.2.4 Leaf Chlorophyll Index (SPAD) Estimation

It was estimated using the Chlorophyll.meter502 device by taking ten plants from the central lines and recording the readings for the samples (12).

Table 1. Some physical and chemical properties of field soil.

Attributes	pH	Electrical conductivity	Organic matter	N Ready	P Ready	K Ready	Zn Ready	sand	silt	clay	textile
Value	7.4	3.49	11	77.6	9.6	130		111.2	299.2	589.7	Clay
Unit	-	Decismins-1	gm.kg^{-1}	mg.kg^{-1}				gm.kg^{-1}			

Soil properties were analyzed by referring to (13, 14)

3. RESULTS AND DISCUSSION

3.1 Plant height (cm)

The results of Table (2) show that foliar fertilization with humic acid led to a significant increase in the average plant height, as the highest average reached 44.70 cm at a concentration of 12 g.L-1, an increase of 9.69% compared to the no-spray treatment, which recorded 40.75 cm. Perhaps the reason is that humic acid is one of the decomposed organic fertilizers rich in major and minor elements necessary for building plant cells and carrying out vital processes, in addition to the fact that the effect of humic acids is similar to the effect of plant hormones that achieve the best conditions for cell division, which leads to an increase in the plant growth rate, which leads to an increase in the plant height (15).

The results of the table also indicated a significant difference in the increase in the average trait when foliar feeding with zinc, as the spraying treatment at the level of 2 g. L^{-1} gave the highest average of 44.31 cm, an increase of 6.41%

compared to the non-spraying treatment, which gave the lowest level of 41.64 cm. The reason is the role played by zinc in the formation of the amino acid tryptophane, which is responsible for the formation of indole acetic acid AAA (9), which plays a role in increasing the length of the stem through division and expansion in the cells of the apical meristem(16).

The interaction between humic acid and zinc led to a significant increase in the average of the trait, as the interaction treatment at the level of 12 $g.L^{-1}$ of humic acid and zinc at a concentration of 2 $g.L^{-1}$ gave the highest average of 46.53 cm, with an increase of 21.42% compared to the control treatment, which gave the lowest average of 38.32 cm.

Table (2) The impact of foliar application of humic acid and zinc, on the mean plant height (cm)

Zinc concentrations $gm.l^{-1}$	Humic acid concentrations $gm.l^{-1}$			Mea.of zinc
	0	**6**	**12**	
0	38.32 c	42.60 b	44.00 ab	41.64 b
1	41.32 bc	42.52 b	43.59 ab	42.48 ab
2	42.61 b	43.79 ab	46.53 a	44.31 a
Mean of Humic acid	40.75b	42.97 a	44.70 a	

*Means that share similar letters do not have statistically significant differences at a probability level of 0.05, according to Duncan's multiple range test.

3.1 Leaf Chlorophyll Index (SPAD)

The results of Table (3) show that the average chlorophyll index increased significantly when spraying with humic acid at the level of 12 $g.L^{-1}$, giving the highest average of 65.17 SPAD, with an increase rate of 24.84% compared to the non-spray treatment, which recorded 52.20 SPAD. The reason for the increase is due to the role of the acid in increasing the magnesium content of the leaves and to the role of this element in increasing chlorophyll by entering into its composition or by activating and stimulating the processes related to its production (17).

The results also showed a significant difference in the trait when spraying with zinc at the level of 2 $g.L^{-1}$, giving the highest average of SPAD 62.70 compared to the non-spraying treatment, which recorded 50.42 SPAD, with an increase rate of 24.35%. The reason is that zinc contributes directly to the process of manufacturing chlorophyll through the formation of carbohydrates and energy compounds(7). Also in activating the enzyme carbonic anhydrase which plays a regulating role for the pH inside the chloroplasts which leads to protecting proteins from losing their nature and vitality (18). The interaction between humic acid and zinc resulted in a significant increase in the trait, as the interaction treatment at the level of 12 g. L^{-1} of humic acid and zinc at a concentration of 2 g. L^{-1} gave the highest average for the chlorophyll index reaching 68.90 SPAD compared to the control treatment which recorded the lowest value reaching 43.66 SPAD and the percentage of increase reached 57.81%.

Table (3) The impact of foliar application of humic acid and zinc, on the average total chlorophyll index (SPAD).

Zinc concentrations gm.l^{-1}	Humic acid concentrations gm.l^{-1}			Mean of zinc
	0	6	12	
0	43.66 d	44.56 d	63.03 b	50.42 b
1	56.70 c	62.46 b	63.60 ab	60.92 a
2	56.23 c	62.96 b	68.90 a	62.70 a
Mean of Humic acid	52.20 c	56.66 b	65.17 a	

*Means that share similar letters do not have statistically significant differences at a probability level of 0.05, according to Duncan's multiple range test.

3.3 Number of main branches (branch.plant^{-1})

The results of Table (4) showed that foliar feeding with humic acid at a concentration of 12 g.L^{-1} gave a significant increase in the average number of branches, reaching 5.00 branches.plant-1 compared to the non-spraying treatment, which recorded the lowest average of 3.77 branches.plant^{-1}, with an increase rate of 32.62%. The reason may be attributed to the availability of nutrients absorbed by the plant, which causes an increase in the height of the plant and then an increase in the number of branches (19).

The table indicated a significant increase in the average of this trait when spraying with zinc, as the concentration of 1 g.l^{-1} gave the highest average number of branches, reaching 4.55 branches.plant-1, compared to the treatment without spraying, which gave the lowest average, reaching 3.93 branches.plant^{-1}, and the percentage of increase was 15.78%. Perhaps the reason is the role of zinc in the formation of auxin, which participates in interaction with gibberellin in the division of cambium cells and the differentiation of xylem and bark tissue cells. Thus, vascular differentiation of the lateral buds occurs, which become connected to those in the main stem, which is positively reflected in the increase of branches(16) . The interaction between humic acid and zinc led to a significant increase in this trait, as the interaction treatment at the level of 12 g.L^{-1} of humic acid and zinc at a concentration of 1 g.L^{-1} gave the highest average number of branches, the highest average of 5.26 branches.plant^{-1}, with an increase rate of 49.00% compared to the control treatment, which gave the lowest average number of branches, which amounted to 3.53 branches.plant^{-1}.

Table (4) The effect of foliar feeding with humic acid and zinc and the interaction between them on the average number of branches per plant (branch.plant^{-1})

Zinc concentrations gm.l^{-1}	Humic acid concentrations gm.l^{-1}			Mea.of zinc
	0	**6**	**12**	
0	3.53 c	3.73 bc	4.53 ab	3.93 b
1	4.00 b c	4.40 abc	5.26 a	4.55 a
2	3.80 b c	4.06 bc	5.20 a	4.35 ab
Mean of Humic acid	3.77 b	4.06 b	5.00 a	

*Means that share similar letters do not have statistically significant differences at a probability level of 0.05, according to Duncan's multiple range test.

3.4 Leaf area of plant (cm^2)

The results of Table (5) showed that foliar feeding with humic acid led to an increase in the average leaf area, as the highest average reached 74.25 cm^2 at a concentration of 12 gm.L^{-1}, with an increase rate of 4.35% over the non-spraying treatment, which recorded 71.15 dm^2. The reason is the role of acid in increasing vegetative growth by increasing the manufactured materials in the leaves and their transfer to the rest of the plant parts, which leads to an increase in the leaf area due to what it contains of major and minor elements and plant hormones, which lead to an increase in enzymatic activity and then photosynthesis in the leaf (20).

The table showed no significant difference in this trait when foliar feeding with zinc, as the comparison treatment gave the highest average of 72.99 dm^2/1 compared to the spraying treatment at concentrations of 1 and 2 gm.L^{-1}, which gave an average of 72.85 and 70.97 dm^2/2. Perhaps the reason is that the nutrients were transferred to other growth parts such as height, number of branches, and chlorophyll in abundance (Tables 2, 3, and 4) at the expense of the lateral growth of the leaf area, which is known as plant architecture (21).

The interaction between humic acid and zinc led to an increase in the average leaf area of the plant, as the interaction treatment at the level of 12 g.L^{-1} of humic acid and zinc at a concentration of 1 g.L^{-1} gave the highest average leaf area of the plant, reaching 75.37 dm2, with an increase rate of 6.40%, while the control treatment gave 70.83 dm^2.

Table (5) The effect of foliar feeding with humic acid and zinc and the interaction between them on the average leaf area of the plant (dm^2)

Zinc concentrations gm.l^{-1}	**Humic acid concentrations gm.l^{-1}**			**Mean of zinc**
	0	6	12	
0	70.83 a	73.03 a	75.12 a	72.99 a
1	72.57 a	69.82 a	75.37 a	72.58 a
	70.04 a	70.62 a	72.26 a	70.97 a

2				
Mea.of Humic acid	71.15 a	71.15 a	74.25 a	

*Means that share similar letters do not have statistically significant differences at a probability level of 0.05, according to Duncan's multiple range test.

4. CONCLUSION

We conclude from the study that foliar fertilization with humic acid at the level of 12 g.L^{-1} outperformed the rest of the levels of humic addition in all the studied vegetative growth traits of pepper plants, and that spraying zinc at a concentration of 2 g.L^{-1} outperformed the traits of plant height and chlorophyll index, while the concentration of 1 g.L^{-1} outperformed the trait of number of branches only, and the interaction of the two study factors gave the highest averages for all study traits.

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