Response of Okra Growth and Yield to Plant Density Management and Bio-Stimulants (Vitamin E and Humic Acid)

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Abstract

A field experiment was conducted during the spring growing season of 2025 at the Vegetable Research Farm, Department of Horticulture and Landscape Gardening, College of Agriculture, University of Diyala, Baqubah, Diyala, Iraq (33°40′49″ N, 44°35′39″ E), to investigate the individual and interactive effects of plant density, soil-applied humic acid, and foliar-applied α-tocopherol on the growth, biochemical characteristics, yield components, and pod quality of okra. The experiment followed a split-split plot arrangement within a randomized complete block design (RCBD) with three replications. Treatments comprised two plant densities (one and two plants hill^{-1}), two humic acid levels (0 and 10 kg ha^{-1}), and four α-tocopherol concentrations (0, 50, 100, and 200 mg L^{-1}). Low plant density (D_1), humic acid at 10 kg ha^{-1} (H_1), and α-tocopherol at 200 mg L^{-1} (F_3) significantly increased leaf carbohydrate, N, P, and K contents, individual plant yield, and fruit diameter compared with their respective controls. The highest values were consistently obtained with the combined treatment D_1 H_1 F_3, which recorded 29.26% carbohydrates, 4.010% N, 0.578% P, 4.500% K, 554.00 g plant^{-1} yield, and 2.15 cm fruit diameter, whereas the untreated high-density treatment (D_2 H_0 F_0) produced the lowest values. Overall, the combined application of reduced plant density, humic acid, and α-tocopherol was more effective than individual treatments in enhancing the biochemical status, productivity, and fruit development of okra.

Keywords: α-Tocopherol, Foliar application, Humic acid, Okra, Plant density, Yield

Introduction

Okra *Abelmoschus esculentus* L. a member of the family Malvaceae, is an economically important vegetable crop widely cultivated in tropical and subtropical regions as well as under protected cultivation.

Its adaptability to diverse environmental conditions, together with its high nutritional value, makes it an important crop for sustainable agricultural production [1]. Okra pods are rich in dietary fiber, essential vitamins, minerals, phenolic compounds, and natural antioxidants, which contribute to their nutritional and functional value. In addition, okra mucilage is widely utilized as a natural stabilizer and thickening agent in the food and pharmaceutical industries because of its distinctive physicochemical properties [2, 3].

Plant density is an important agronomic factor affecting crop growth, productivity,

ISSN 2072-3857

and fruit development through its influence on the availability and distribution of light, water, and mineral nutrients among plants. Excessive plant density increases interplant competition and may restrict canopy development, photosynthetic activity, and nutrient acquisition, whereas an appropriate density can improve resource utilization, assimilate production, and the allocation of photosynthates toward developing fruits. Therefore, optimizing plant density is an important management strategy for improving individual plant productivity and fruit characteristics of okra. In addition to plant density, the use of soil amendments such as humic acid has received considerable attention as an environmentally friendly approach for improving plant performance. Humic acid can improve soil properties, stimulate root development, enhance nutrient availability and uptake, and facilitate the translocation of mineral nutrients within the plant. These effects can improve plant nutritional status and strengthen antioxidant defense mechanisms, thereby contributing to greater tolerance to environmental stresses and improving growth, yield, and fruit quality [4]. The beneficial effects of humic acid are therefore particularly relevant to improving the efficiency of nutrient acquisition and utilization under different growing conditions.

Alpha-tocopherol (vitamin E) is another important biostimulant and lipid-soluble antioxidant involved in protecting plant cells against oxidative damage associated with reactive oxygen species (ROS). Foliar application of α-tocopherol can contribute to maintaining the integrity of cellular membranes and chloroplast structures, protecting photosynthetic pigments, delaying leaf senescence, and sustaining photosynthetic activity. These functions may enhance carbon assimilation, nutrient translocation, and assimilate accumulation, thereby supporting vegetative growth, yield formation, and fruit development [5]. The use of α-tocopherol as a foliar treatment may consequently complement improvements in soil nutrient availability and plant resource utilization achieved through appropriate plant density and humic acid application. Although plant density, humic acid, and α-tocopherol have individually been associated with improved plant growth and productivity, their combined effects on biochemical attributes, nutrient accumulation, individual plant yield, and fruit characteristics of okra require further evaluation. Therefore, this study aimed to evaluate the individual and interactive effects of two plant densities, two levels of soil-applied humic acid, and four concentrations of foliar-applied α-tocopherol on leaf carbohydrate, nitrogen, phosphorus, and potassium contents, individual plant yield, and fruit diameter of okra.

Materials and Methods

A field experiment was conducted during the spring growing season of 2025 at the Vegetable Research Farm, Department of Horticulture and Landscape Gardening, College of Agriculture, University of Diyala, Iraq, to investigate the individual and interactive effects of plant density, soil-applied Humic acid, and foliar application of alpha-tocopherol on the growth, biochemical characteristics, yield components, and pod quality of okra . The experiment was arranged in a randomized complete block design (RCBD) using a split-split plot layout with three replications. Alpha-tocopherol treatments were assigned to the main plots,

ISSN 2072-3857

plant density to the subplots, and Humic acid application to the sub-subplots. Two planting densities were evaluated: one plant per hill (D_1 ; 44,444 plants ha^{-1}) and two plants per hill (D_2 ; 88,888 plants ha^{-1}), maintaining a spacing of 0.75 × 0.30 m. Humic acid was applied at two rates, 0 (H_0) and 10 kg ha^{-1} (H_1), whereas alpha-tocopherol was foliar-applied at four concentrations: 0 (F_0), 50 (F_1), 100 (F_2), and 200 mg L^{-1} (F_3)

Measured Traits

Data were collected from five randomly selected plants in each experimental unit. The following parameters were determined:-

1. **Total leaf carbohydrate content (%)** was determined using the phenol–sulfuric acid method described by [6] and the absorbance was measured at 490 nm using a UV–Vis spectrophotometer
2. **Leaf nitrogen content (%)** Leaf nitrogen concentration was determined using the Kjeldahl method following the procedure described by [7].
3. **Leaf phosphorus content (%)** Leaf phosphorus concentration was determined colorimetrically according to the method of [8], with absorbance measured at 880 nm using a UV–Vis spectrophotometer.
4. **Leaf potassium content (%)** Potassium concentration was determined using a flame photometer according to the method described by [9] .
5. **Plant yield (g $plant^{-1}$)** Plant yield was calculated by dividing the cumulative pod yield per experimental unit by the total number of plants within the corresponding experimental unit.
6. **Fruit diameter (mm)** Fruit diameter was measured at the midpoint of ten randomly selected pods from each experimental unit using a digital Vernier caliper, and the mean value was recorded.

Results and Discussion

1- Total leaf carbohydrate content (%)

As shown in Table 1, leaf total carbohydrate content was significantly affected by plant density, humic acid, α-tocopherol, and their interactions. D_1 recorded a higher carbohydrate content (22.55%) than D_2 (20.22%), which may be attributed to reduced competition for light, water, and nutrients. Humic acid at 10 kg ha^{-1} (H_1) increased carbohydrate content to 23.12% compared with 19.65% under H_0 , possibly due to improved root development and nutrient uptake. Foliar α-tocopherol application progressively increased carbohydrate accumulation, with F_3 recording the highest value (24.69%) compared with 18.54% under F_0 , likely reflecting its antioxidant protection of photosynthetic tissues. The two-way interactions showed that D_1 H_1 , D_1 F_3 , and H_1 F_3 produced the highest

ISSN 2072-3857

carbohydrate contents (24.51%, 26.40%, and 27.20%, respectively), compared with 18.71%, 17.79%, and 17.55% under D_2 H_0 , D_2 F_0 , and H_0 F_0 , respectively. This indicates a complementary effect of lower plant competition, improved nutrient availability, and antioxidant protection on carbohydrate accumulation. The three-way interaction D_1 H_1 F_3 recorded the highest value (29.26%), whereas D_2 H_0 F_0 recorded the lowest (16.84%), confirming that the combined application of the favorable treatment levels maximized carbohydrate accumulation.

Table 1. Effects of plant density, Humic acid application, alpha-tocopherol foliar spray, and their interactions on total leaf carbohydrate content (%) of okra.

Alpha-tocopherol mg L^{-1}	plant density	Humic acid kg ha^{-1}		Interaction (Alpha * density)
		(H_0) 0	(H_1) 10	
(F_0) 0	D1	18.25 n	20.34 j	19.30 f
	D2	16.84 p	18.74 m	17.79 h
(F_1) 50	D1	19.43 k	22.82 e	21.13 d
	D2	17.94 o	20.54 i	19.24 g
(F_2) 100	D1	21.13 g	25.63 b	23.38 b
	D2	19.25 l	22.46 f	20.85 e
(F_3) 200	D1	23.53 d	29.26 a	26.40 a
	D2	20.83 h	25.14 b	22.98 c
				Mean Alpha
Interaction (Alpha * Humic acid	0	17.55 h	19.54 f	18.54 D
	50	18.69 g	21.68 d	20.19 C
	100	20.19 e	24.05 b	22.12 B
	200	22.18 c	27.20 a	24.69 A
				Mean density
Interaction density * Humic acid	D1	20.59 c	24.51 a	22.55 A
	D2	18.71 d	21.72 b	20.22 B
Mean Humic acid		19.65 B	23.12 A	

Means followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

2- Leaf nitrogen content (%)

As shown in table 2, leaf nitrogen content was significantly influenced by plant density, Humic acid application, alpha-tocopherol foliar spray, and their interactions. The lower plant density (D_1) resulted in a significantly higher nitrogen content (3.149%) than D_2 (2.490%). Likewise, Humic acid application (H_1) significantly increased leaf nitrogen concentration to 3.158%, compared with 2.480% in control (H_0). Foliar application of alpha-tocopherol also significantly

ISSN 2072-3857

enhanced nitrogen accumulation, with highest concentration (F_3) producing greatest value (3.232%), whereas control treatment (F_0) recorded lowest value (2.452%). Significant interaction effects were also observed among studied factors. The D_1 H_1, D_1 F_3, and H_1 F_3 combinations recorded highest leaf nitrogen contents of 3.476%, 3.576%, and 3.691%, respectively, while corresponding lowest values were obtained under D_2 H_0 (2.139%), D_2 F_0 (2.111%), and H_0 F_0 (2.320%), respectively. Furthermore, the three-way interaction significantly affected leaf nitrogen content, with D_1 H_1 F_3 treatment producing highest value (4.010%), whereas lowest value (1.880%) was recorded under D_2 H_0 F_0 .

Table 2. Effects of plant density, Humic acid application, alpha-tocopherol foliar spray, and their interactions on leaf nitrogen content (%) of okra

Alpha-tocopherol mg L^{-1}	plant density	Humic acid kg ha^{-1}		Interaction (Alpha * density)
		(H_0) 0	(H_1) 10	
(F_0) 0	D1	2.526 j	2.950 fg	2.738 e
	D2	1.880 o	2.343 l	2.111 h
(F_1) 50	D1	2.710 h	3.303 d	3.006 c
	D2	2.050 n	2.643 i	2.346 g
(F_2) 100	D1	2.906 g	3.643 b	3.275 b
	D2	2.223 m	3.003 f	2.613 f
(F_3) 200	D1	3.143 e	4.010 a	3.576 a
	D2	2.403 k	3.373 c	2.888 d
				Mean Alpha
Interaction (Alpha * Humic acid	0	2.203 h	2.646 e	2.425 D
	50	2.380 g	2.973 c	2.676 C
	100	2.565 f	3.323 b	2.944 B
	200	2.773 d	3.691 a	3.232 A
				Mean density
Interaction density * Humic acid	D1	2.821 b	3.476 a	3.149 A
	D2	2.139 c	2.840 b	2.490 B
Mean Humic acid		2.480 B	3.158 A	

Means followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

3- Leaf phosphorus content (%)

As presented in table 3, leaf phosphorus content was significantly affected by plant density, Humic acid application, alpha-tocopherol foliar spray, and their interactions. The lower plant density (D_1) resulted in a significantly higher phosphorus content (0.428%) than D_2 (0.379%). Likewise, Humic acid application (H_1) significantly increased leaf phosphorus content to 0.453%, compared with 0.353%

ISSN 2072-3857

in control (H_0). Foliar application of alpha-tocopherol also significantly enhanced phosphorus accumulation, with highest concentration (F_3) recording greatest value (0.479%), whereas control treatment (F_0) produced lowest value (0.331%). Significant interaction effects were also observed. The D_1 H_1 , D_1 F_3 , and H_1 F_3 combinations recorded highest phosphorus contents of 0.480%, 0.505%, and 0.551%, respectively, while corresponding lowest values were obtained under D_2 H_0 (0.331%), D_2 F_0 (0.309%), and H_0 F_0 (0.304%), respectively. Furthermore, three-way interaction significantly affected leaf phosphorus content, with D_1 H_1 F_3 treatment producing highest value (0.578%), whereas lowest value (0.284%) was recorded under D_2 H_0 F_0 .

Table 3. Effects of plant density, Humic acid application, alpha-tocopherol foliar spray, and their interactions on leaf phosphorus content (%) of okra.

Alpha-tocopherol mg L^{-1}	plant density	Humic acid kg ha^{-1}		Interaction (Alpha * density)
		(H_0) 0	(H_1) 10	
(F_0) 0	D1	0.324 l	0.384 h	0.354 d
	D2	0.284 n	0.334 k	0.309 e
(F_1) 50	D1	0.354 i	0.444 e	0.399 c
	D2	0.312 m	0.394 g	0.353 d
(F_2) 100	D1	0.393 g	0.516 c	0.454 b
	D2	0.346 j	0.456 d	0.401 c
(F_3) 200	D1	0.433 f	0.578 a	0.505 a
	D2	0.382 h	0.524 b	0.453 b
				Mean Alpha
Interaction (Alpha * Humic acid	0	0.304 h	0.359 f	0.331 D
	50	0.333 g	0.419 c	0.376 C
	100	0.369 e	0.486 b	0.427 B
	200	0.407 d	0.551 a	0.479 A
				Mean density
Interaction density * Humic acid	D1	0.376 c	0.480 a	0.428 A
	D2	0.331 d	0.427 b	0.379 B
Mean Humic acid		0.353 B	0.453 A	

Means followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

4- Leaf potassium content (%)

Table 4 showed that plant density significantly affected leaf potassium concentration. lower density (D1) produced highest mean (3.665%), whereas higher density (D2) recorded 3.262%. Humic acid application also had a significant effect, with H1 producing highest potassium concentration (3.794%) compared with 3.132% for H0. Foliar application of α-tocopherol significantly increased leaf potassium concentration, with F3 recording highest mean (3.862%), while the control

ISSN 2072-3857

(F0) gave lowest value (3.062%). The interaction between plant density and Humic acid was significant, with D1H1 producing highest potassium concentration (4.000%) and D2H0 the lowest (2.935%). Likewise, interaction between plant density and α-tocopherol significantly influenced trait, as D1F3 recorded highest value (4.077%), whereas D2F0 produced lowest (2.864%). The interaction between Humic acid and α-tocopherol was also significant, with H1F3 giving highest potassium concentration (4.274%) compared with 2.813% in H0F0. Furthermore, three-way interaction among plant density, Humic acid, and α-tocopherol significantly affected leaf potassium concentration, with D1H1F3 producing highest value (4.500%), while D2H0F0 recorded lowest (2.614%).

Table 4. Effects of plant density, Humic acid application, alpha-tocopherol foliar spray, and their interactions on leaf potassium content (%) of okra.

Alpha-tocopherol mg L^{-1}	plant density	Humic acid kg ha^{-1}		Interaction (Alpha * density)
		(H_0) 0	(H_1) 10	
(F_0) 0	D1	3.012 l	3.508 g	3.260 f
	D2	2.614 n	3.114 k	2.864 h
(F_1) 50	D1	3.210 j	3.838 d	3.524 d
	D2	2.838 m	3.438 h	3.138 g
(F_2) 100	D1	3.444 h	4.154 b	3.799 b
	D2	3.042 l	3.754 e	3.398 e
(F_3) 200	D1	3.654 f	4.500 a	4.077 a
	D2	3.249 i	4.048 c	3.648 c
				Mean Alpha
Interaction (Alpha * Humic acid	0	2.813 h	2.813 h	3.062 D
	50	3.024 g	3.024 g	3.331 C
	100	3.243 f	3.243 f	3.598 B
	200	3.451 d	3.451 d	3.862 A
				Mean density
Interaction density * Humic acid	D1	3.330 c	4.000 a	
	D2	2.935 d	3.588 b	
Mean Humic acid		3.132 B	3.794 A	

Means followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

5- Plant yield (g $plant^{-1}$)

ISSN 2072-3857

The results in table (5) showed significant effects of plant density, humic acid, and α-tocopherol on individual plant yield. D1, H1, and F3 recorded highest yields of 406.33, 395.25, and 423.33 g plant^{-1}, respectively, compared with 334.37, 345.45, and 339.16 g plant^{-1} under D2, H0, and F0, respectively. Significant two-way interactions were also observed, with D1H1, D1F3, and H1F3 producing highest yields of 439.00, 471.83, and 476.83 g plant^{-1}, respectively. The corresponding lowest values were 317.25 g plant^{-1} for D2H0, 307.83 g plant^{-1} for D2F0, and 318.16 g plant^{-1} for H0F0. Moreover, the three-way interaction significantly affected individual plant yield, with D1H1F3 achieving highest value (554.00 g plant^{-1}), compared with 288.30 g plant^{-1} under D2H0F0.

Table 5. Effects of plant density, Humic acid application, alpha-tocopherol foliar spray, and their interactions on plant yield (g plant^{-1}) of okra.

Alpha-tocopherol mg L^{-1}	plant density	Humic acid kg ha^{-1}		Interaction (Alpha * density)
		(H_0) 0	(H_1) 10	
(F_0) 0	D1	348.0 h	393.0 d	370.5 d
	D2	288.3 k	327.3 i	307.8 g
(F_1) 50	D1	370.0 g	402.0 c	386.0 c
	D2	302.7 j	329.0 i	315.8 f
(F_2) 100	D1	387.0 f	407.0 b	397.0 b
	D2	328.0 i	350.0 h	339.0 e
(F_3) 200	D1	389.7 e	554.0 a	471.8 a
	D2	350.0 h	399.7 c	374.8 d
				Mean Alpha
Interaction (Alpha * Humic acid	0	318.2 h	360.2 e	339.2 D
	50	336.3 g	365.5 d	350.9 C
	100	357.5 f	378.5 b	368.0 B
	200	369.8 c	476.8 a	423.3 A
				Mean density
Interaction density * Humic acid	D1	373.7 b	439.0 a	406.3 A
	D2	317.3 d	351.5 c	334.4 B
Mean Humic acid		345.5 B	395.3 A	

Means followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

6- Fruit diameter (mm)

The results in table (6) revealed significant effects of plant density, Humic acid, and α-tocopherol, as well as their interactions, on fruit diameter. D1 produced a greater fruit diameter (1.800 cm) than D2 (1.696 cm), while Humic acid application (H1) increased

ISSN 2072-3857

fruit diameter to 1.850 cm compared with 1.646 cm in H0. Foliar application of α-tocopherol was also effective, with F3 recording the highest value (1.942 cm) versus 1.565 cm in control (F0).

Significant two-way interactions were observed among studied factors. The combinations D1H1, D1F3, and H1F3 recorded fruit diameters of 1.900, 2.000, and 2.100 cm, respectively, compared with 1.593, 1.515, and 1.500 cm for D2H0, D2F0, and H0F0, respectively. The three-way interaction was also significant, with D1H1F3 producing highest fruit diameter (2.150 cm), whereas D2H0F0 recorded lowest value (1.450 cm).

Table 6. Effects of plant density, Humic acid application, alpha-tocopherol foliar spray, and their interactions on fruit diameter (mm) of okra.

Alpha-tocopherol mg L^{-1}	plant density	Humic acid kg ha^{-1}		Interaction (Alpha * density)
		(H_0) 0	(H_1) 10	
(F_0) 0	D1	1.550 k	1.680 h	1.615 g
	D2	1.450 l	1.580 j	1.515 h
(F_1) 50	D1	1.650 i	1.820 e	1.735 e
	D2	1.550 k	1.720 g	1.635 f
(F_2) 100	D1	1.750 f	1.950 d	1.850 c
	D2	1.650 i	1.850 d	1.750 d
(F_3) 200	D1	1.850 d	2.150 a	2.000 a
	D2	1.720 g	2.050 b	1.885 b
				Mean Alpha
Interaction (Alpha * Humic acid	0	1.500 h	1.630 f	1.565 D
	50	1.600 g	1.770 d	1.685 C
	100	1.700 e	1.900 b	1.800 B
	200	1.785 c	2.100 a	1.942 A
				Mean density
Interaction density * Humic acid	D1	1.700 c	1.900 a	
	D2	1.593 d	1.800 b	
Mean Humic acid		1.646 B	1.850 A	

Means followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

The results presented in tables 1–4 revealed significant differences among the treatments. The combination of low plant density (one plant per hole), Humic acid application, and foliar α-tocopherol at 200 mg L^{-1} (D1H1F3) produced the highest leaf biochemical attributes, including carbohydrate, nitrogen, phosphorus, and potassium contents. In contrast, the untreated plants grown at high density (D2H0F0) recorded the lowest values. The superior performance under low density may be attributed to reduced interplant competition for water and nutrients, together with improved light interception and photosynthetic efficiency, which enhanced

ISSN 2072-3857

carbohydrate synthesis and accumulation [10]. Humic acid improves nutrient availability and mobility, stimulates root development, and consequently enhances the uptake and translocation of N, P, and K to the leaves [11]. Moreover, α-tocopherol, as an effective antioxidant, protects chlorophyll, cellular membranes, and chloroplast structures from oxidative damage, thereby maintaining photosynthetic activity and supporting carbon assimilation and vegetative growth [12].

Similarly, tables 5 and 6 showed significant differences in yield and its components. The D1H1F3 treatment resulted in the highest individual plant yield and fruit diameter, whereas D2H0F0 recorded the lowest values. The increased yield under low plant density was likely associated with reduced competition for light, water, and nutrients, allowing greater canopy development and photosynthetic capacity. This enhanced dry matter production, reduced flower and fruit abscission, and promoted the allocation of assimilates toward developing fruits, thereby increasing fruit size and individual plant yield [13]. Humic acid further enhanced nutrient uptake and cell expansion, while α-tocopherol protected chloroplasts and structural proteins against oxidative damage. Their combined effects may have improved the source–sink relationship, facilitating the translocation of water, nutrients, and photoassimilates from leaves to developing fruits and consequently enhancing cell division, elongation, fruit diameter, and yield [14]. It is recommended to adopt the combination of one plant per hill, humic acid at 10 kg ha^{-1}, and foliar α-tocopherol at 200 mg L^{-1} to improve the biochemical attributes, individual plant yield, and pod quality of okra under the conditions of the present study.

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ISSN 2072-3857