



EFFECT OF FOLIC ACID ON GROWTH TRAITS OF FOUR SUNFLOWER *HELIANTHUS ANNUUS* L. GENOTYPES

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Abstract

A field experiment was carried out in the fields of College of Agricultural Engineering Sciences - the University of Baghdad - Jadriya in the spring season 2019, using randomized complete block design RCBD with the factorial experiments arrangement. The current study goals were to identify the response of the growth traits of four genotypes from the sunflower to spraying with folic acid (vitamin B9). The experiment included two factors, the first one included four genotypes of the sunflower of Esihaqi 1, Esihaqi 2, Tarzan and Aqmar, as for the second factor, it included spraying folic acid (vitamin B9) with three concentrations 1, 2 and 3 g L⁻¹, as well as the comparison treatment (without spraying). Folic acid was sprayed in two stages, the first in the stage of four real leaves (75% of plants), and the second at the beginning of the appearance of flower buds (75% of plants). However, the study results showed that Aqmar variety exceeded significantly in all studied growth traits except the leaves content of total chlorophyll, as genotype Esihaqi 2 exceeded in this trait. Finally, the studied growth traits increased with increasing concentrations of acid spraying and reached its maximum average at spraying in a concentration of 2 g L⁻¹ except for the leaf area and its index, as the concentration 3 g/l exceeded significantly in this trait.

Key words : Genotypes, Sunflower, Folic acid, Growth traits.

Introduction

The sunflower crop *Helianthus annuus* L. is one of the important economic crops in the world because of its short growth period and its high economic returns, where its seeds contain a high percentage of oil that may reach 55%. Furthermore, it is characterized by containing Omega3 fatty acids as well as unsaturated fatty acids such as Oleic and Linoleic with less sensitivity to oxidation during the filling and storage period (Srivestance and Pande, 1988). The time for planting this crop in Iraq is mostly limited in the spring season, where this date is characterized by its imperfect environmental conditions, especially when it coincides with the date of flowering and pollination, which leads to less fertility then the productivity per unit area decreases compared to global production. However, crop management and the follow-up of modern field agricultural practices greatly affect the increase in production, and one of these practices is the use of safe and environmentally friendly substances.

Recent attention in the world has focused on the use of these substances to improve plant growth as a result of the major problems caused by the excessive use of chemical fertilizers that reflected negatively on animal and human health. Among these substances is the use of vitamins, including vitamin B9 (folic acid), which is from the group of the vitamin B complex, which has an important role in the metabolism of amino acids. Moreover, the formation of nucleic acids and the Reactive Oxygen Species (ROS) that are produced in the plant during the process of carbon assimilation and respiration and when the plant exposed to stress. As well as, its auxins role that regulates cell division and elongation and is an enzymatic companion in many metabolic pathways (Samihllah *et al.*, 1988 and Foyer *et al.*, 1991; Andrew *et al.*, 2000; Fardet *et al.*, 2008; Naheif and Mohamed, 2013). Also, the synergistic effect in growth, yield, and quality of several plant species (Emam *et al.*, 2011, Zewail *et al.*, 2011, Vician and Kovacik, 2013). Genotypes differ

in their ability to exploit the available growth resources and understanding the performance and behavior of each variety and its response to growth factors and field practices are one of the important things to increase production. Therefore this study was carried out with the aim of identifying the response of growth characteristics of four sunflower genotypes to spraying with concentrations of folic acid.

Materials and Methods

A field experiment was carried out in the fields of College of Agricultural Engineering Sciences - the University of Baghdad - Jadriya in the spring season 2019, which was conducted using randomized complete block design RCBD with the factorial experiments arraignment. This study aimed to identify the response of the growth characteristics of four genotypes from the sunflower to spraying with folic acid (vitamin B9). The land of the experiment plowed by two vertical plows using the moldboard plow and harrowing by Rotovater and then leveled and divided into three replicates by 16 experimental units per replicate. The number of experimental units reached 48 units with an area of 9 m² (3 * 3 m), and the experimental unit included 5 lines, where the distance between them 75 cm and between a plant and another 20 cm to obtain a plant density of 66,666 plant.ha⁻¹. Finally, the experiment included two factors, the first factor included four genotypes of the sunflower of Esihaqi 1, Esihaqi 2, Tarzan and Aqmar, as for the second factor, it included spraying folic acid (vitamin B9) with three concentrations 1, 2 and 3 g/l as well as the comparison treatment (without spraying). Furthermore, Folic acid was sprayed in two stages, the first in the stage of four real leaves (75% of the plants), and the second at the beginning of appearance the flower buds (75% of the plants). The spraying was done in the evening, used the knapsack sprayer for this purpose, where the liquid soap as a surfactant, and flatten the refracting surface. The experiment land was planted on 27/2/2019 by placing 3-5 seeds in one hole at a depth of 4-5 cm and the thinning was carried out to one plant after two weeks of the emergence. Nitrogen fertilizer was added in the form of urea (46% N) at a rate of 360 kg N.ha⁻¹ in two batches, the first is in the stage of the appearance of four real leaves and the second batch at the beginning of appearance the flower buds (AL-Rawie, 2001). The Cutworm was controlled by insecticide Morisban4 at a rate of 50 ml per 50 liters of water, and all crop service operations were carried out according to the plant need. Folic acid from the Indian CDH company was used and prepared three concentrations of it 1, 2, and 3 g L⁻¹, and each of them was added to one liter of water.

Studied traits

Upon completion of flowering, five plants were randomly selected from the middle three lines of each experimental unit for studying the following vegetative growth traits: -

1. Plant height (cm); the plant height was calculated from the soil surface to the base of the disk as an average of five plants.
2. Stem diameter: - The stem circumference was measured from the center of the stem using the Vernier device.
3. The number of leaves; the total number of leaves per plant was calculated from the first green leaf at the soil surface to the last leaf on the plant.

Leaf area (m²): - it was calculated from the following equation: (EL-Sahooki and EL-Dabas, 1982).

$$\text{Leaf area} = \text{sum squares of maximum width of plant leaves} \times 0.65$$

4. Leaf area index; was calculated from the division of the leaf area of a plant by the area it occupies.
5. Chlorophyll content $\mu\text{g g fresh weight}^{-1}$; Total chlorophyll was estimated by Goodwin method (1976), as 1 g of fresh sunflower leaves was taken and cut into small pieces and crushed in a ceramic mill with adding 20 ml acetone (80%) to extract the dye and left a full day in the refrigerator. Then the volume was complete to 50 ml of distilled water and then it was separated in the centrifuge for 5 minutes at a speed of 1000 rpm, then the supernatant was taken, then the reading was recorded at the spectrometer by wavelengths of (633 and 645 nm). Chlorophyll was estimated using the following equation:

Total chlorophyll =

$$(20.2 \times D_{645} + 8.02 \times D_{633}) \times \frac{V}{1000} \times W$$

Where:

D = Optical density.

D₆₃₃ = Optical density of 633 nm wavelength.

D₆₄₅ = Optical density of 645 nm wavelength.

V = final volume of the extract (50 ml).

W = leaf tissue weight (1 g).

Results and Discussion

Plant height (cm)

The data in table 1 indicate that there were significant differences between the genotypes and folic acid spray

concentrations and the interaction between them in the plant height. The genotype Aqmar exceeded by giving the highest average of this trait reached 200.03 cm compared to the genotypes Esihaqi 1, Esihaqi 2, and Tarzan that their average plant height 157.87, 177.80 and 159.21 cm, with an increasing percentage of (26.70, 12.50, and 25.63%), respectively. However, the genotypes Esihaqi 1 and Tarzan did not differ between them in this trait, possibly the reason for the genotypes difference is due to the genetic nature of these genotypes, as this trait is highly covariance in the sunflower varieties. These results are consistent with (Ozturk *et al.*, 2017; AL-haidary 2018) which they pointed out that the sunflower genotypes differ between each other in this trait. As for the folic acid concentrations, it was observed from the same Table the sprayed plants exceeded at a concentration of 2 g L⁻¹ of the acid by giving the highest average for this trait reached 179.80 cm compared to the comparison concentrations 0, 1 and 3 g L⁻¹, which gave averages of 164.51, 171.60 and 179.01 cm, respectively. The concentration of 2 and 3 g L⁻¹ of folic acid did not differ significantly between them; the reason for the significant effect of the acid in this trait may be due to its auxins role in regulating the process of cell division and elongation and then increasing the plant height (De-Tullio *et al.*, 1999; Naheif and Mohamed, 2013). These results are consistent with (Emam *et al.*, 2011; Ibrahim *et al.*, 2015) that indicated the significant effect of this acid on increasing plant height. Furthermore, the interaction between the two factors was significant, and it was observed from the same Table the difference of the trait response to the genotypes with the different concentrations of acid spraying. These plant height increased for the two genotypes Esihaqi 1 and Aqmar a directly with the concentrations increasing of acid spraying until the concentration 2 g L⁻¹. Then, there was a decrease in this trait during the spray concentration increased to 3 g L⁻¹ from the acid, but it was not significant in the

genotype Esihaqi 1 and significant in genotype Aqmar. The trait behavior was not clear between the increase and decrease in genotype Esihaqi 2, while for the behavior of the genotype Tarzan was different from the rest of other genotypes, as the direct increase in plant height continued by increasing the spray concentrations to 3 g L⁻¹.

Stem diameter (thickness) (cm)

It was observed from the data in table 2 that there were significant differences between genotypes and folic acid spray concentrations in the stem diameter. The variety Aqmar exceeded of having the highest average for this trait of 3.45 cm compared to 3.02, 3.18, and 3.18 cm that recorded by the genotypes of Esihaqi 1, Esihaqi 2 and Tarzan respectively. The two genotypes Esihaqi 2 and Tarzan did not differ significantly between them, possibly the reason for this is due to that the genotypes different genetically among themselves in this trait, as well as their different influence by the surrounding environmental conditions. These results are consistent with (Nasralla *et al.*, 2014; Hassan and Halos 2017). As for the folic acid spray concentrations, it can be observed from the same Table the sprayed plants superiority at a concentration of 2 g L⁻¹ by giving the highest average of the stem diameter reached 3.33 mm compared to the concentrations 0, 1 and 3 g L⁻¹ which their average stem thicknesses were 3.14, 3.24 and 3.10 cm respectively. The reason for the folic acid influence may be attributed to the fact that the growth process requires protein assimilation, where this acid has an important role in the process of transporting amino acids to their appropriate location in the protein synthesis chain (Kelly, 1998). Besides, its role in the process of cell division and elongation and the synthesis of nucleic acids and its regulation for the process of carbon assimilation (Andrew *et al.*, 2000). This may have led to an increase in the products of this process, which contributed to increase growth (stem diameter), as for the interaction between

Table 1: The genotypes difference and the effect of spraying with folic acid on plant height (cm) for the spring season 2019.

Varieties	Folic acid concentrations				Average
	0	1	2	3	
Esihaqi 1	149.73	152.39	165.02	164.33	157.87
Esihaqi 2	169.94	197.90	180.02	181.33	177.80
Tarzan	150.83	154.69	162.91	168.43	159.21
Aqmar	187.52	199.40	211.25	201.95	200.03
L.S.D	5.80				2.90
Average	164.51	171.60	179.80	179.01	
L.S.D	2.90				

Table 2: The genotypes difference and the effect of spraying with folic acid on stem diameter (cm) for the spring season 2019.

Varieties	Folic acid concentrations				Average
	0	1	2	3	
Esihaqi 1	2.95	3.04	3.13	2.96	3.02
Esihaqi 2	3.17	3.22	3.28	3.06	3.18
Tarzan	3.10	3.24	3.32	3.07	3.18
Aqmar	3.36	3.48	3.62	3.32	3.45
L.S.D	N.S				0.1234
Average	3.14	3.24	3.33	3.10	
L.S.D	0.1234				

the two factors, it was not significant in this trait.

Number of leaves (leaf.plant⁻¹)

The data in table 3 indicated that there were significant differences between the genotypes and folic acid spray concentrations in the number of leaves. The variety Aqmar characterized by having the highest number of leaves with an average of 23.95 leaves and an increase of 3.97, 3.17, and 4.17 leaves over the genotypes Esihaqi 1, Esihaqi 2 and Tarzan respectively. These genotypes did not differ significantly between them in this trait, and the reason for this may be due to the nature of the genotype of having the highest number of leaves or the reason may be due to that the variety Aqmar having the highest plant height as shown in Table. In other words, an increase in the number of internodes and the number of stems nodes may have led to an increase in the number of leaves. These results are consistent with (AL-Doori and AL-Dulamy, 2011; Dawood *et al.*, 2012) pointed out that the genotypes of the sunflower differed between them in the number leaves. As for the folic acid concentrations, it can be observed from the same table the sprayed plant's superiority at a concentration of 2 g/l of the acid by giving the highest average of this trait was 22.01 leaf.plant⁻¹. However, the lowest number of leaves recorded in plants that not sprayed with acid by 19.82 leaves, and the concentration of 2 g L⁻¹ did not differ significantly from the concentrations 1 and 3 g/l of the acid. As well as, the reason for the significant effect of spraying the acid in this trait maybe it's considered as one of the antioxidants that protect the cell from the damage of free radicals when the plant performs vital processes such as carbon assimilation, respiration, etc. Thus, the cell's condition improves for performing these operations and then reflects that in the plant's growth (increasing the number of its leaves). Possibly spraying was done in two stages, which are 4 leaves (75% of plants), and the stage of beginning the appearance of flower buds (75% plants), the first stage is the beginning of vegetative growth, which is characterized by the rapid and active division of the cell. As well as, the other stage in which the plant continues to grow vegetatively in the sense of prolonging the period for remain the leaves effective (green), and possibly that the concentration 2 g L⁻¹ was sufficient to cause this increase. These results are consistent with (Youssif^o, 2017) findings, which indicated that the number of leaves was affected by the spraying of folic acid, and the interaction between the two factors was not significant in this trait.

Leaf area (m²)

Table 3: The genotypes difference and the effect of spraying with folic acid on the number of leaves (leaf.plant⁻¹) for the spring season 2019.

Varieties	Folic acid concentrations				Average
	0	1	2	3	
Esihaqi 1	19.17	20.20	20.50	20.07	19.98
Esihaqi 2	20.20	20.73	22.07	20.13	20.78
Tarzan	18.73	20.47	20.40	19.53	19.78
Aqmar	21.20	24.47	25.07	25.07	23.95
L.S.D	N.S				1.316
Average	19.82	21.47	22.01	21.20	
L.S.D	1.316				

The data in table 4 showed that the size of leaves of the genotypes in this study differ significantly between each other, as well as the significant effect of folic acid spray concentrations in this trait, whereas this trait was not affected significantly in the interaction between the two factors. The Table also showed that the highest leaf area was recorded at the variety Aqmar with an average of 1.033 m², followed by genotype Tarzan, with an average of 0.895 m², then the hybrid Esihaqi 1 with an average of 0.886 m², while the lowest leaf area recorded at the hybrid Esihaqi 2 was 0.861 m². The genotypes Esihaqi 1 and Esihaqi 2 and Tarzan did not differ significantly in this trait, the reason for the superiority of variety Aqmar may be attributed to its superiority of having the highest number of leaves as shown in Table 3, and this may have led to an increase in its leaf area. These results are consistent with (Shaker and Mohamed 2011, AL-Jayashi, 2017; Hassan, 2019), which indicated that the sunflower genotypes differed in their leaf area. The leaf area has direct increased with the concentrations increasing of folic acid spray. The maximum average of this trait at sprayed plants at a concentration of 3 g L⁻¹ of acid reached 0.976 m² with an increase of 17.17% compared to the comparison treatment of acid which recorded the lowest average of this trait was 0.833 m². Besides, the concentration of 3 g L⁻¹ did not differ significantly from the concentration 1 and 2 g L⁻¹, where the reason for the significant effect of acid spraying on increasing the leaf area may be attributed to its role in stimulating the activation of the biosynthesis process of glycin. In addition, it contributes to the synthesis of porphyrins and chlorophylls in the membranes of chloroplasts and increases its products, which contribute to increasing the plant growth (its leaf area). Otherwise, the reason for the superiority of the sprayed plants with this concentration in this trait is that they did not differ significantly in the number of leaves from the concentration of 2 g L⁻¹ as shown in (Table 3). These results are consistent with (Hanson and Roje 2001, AL-

Jabrin *et al.*, 2003), which indicated the role of acid in this trait.

Leaf area index

Table 4: The genotypes difference and the effect of spraying with folic acid on the leaf area (m²) for the spring season 2019.

Varieties	Folic acid concentrations				Average
	0	1	2	3	
Esihaqi 1	0.804	0.833	0.864	1.042	0.886
Esihaqi 2	0.834	0.882	0.895	0.832	0.861
Tarzan	0.745	0.907	1.001	0.929	0.895
Aqmar	0.950	1.043	1.039	1.099	1.033
L.S.D	N.S				0.0867
Average	0.833	0.916	0.949	0.976	
L.S.D	0.0867				

The results in table 5 showed that there were significant differences between genotypes and folic acid spray concentrations in the leaf area index, while this trait was not significantly affected by the interaction between the two factors. The variety Aqmar exceeded by giving the highest average for this trait of 6.88 compared to the Esihaqi 1, Esihaqi 2 and Tarzan. Their leaf area index amounted to 5.90, 5.73, and 5.97 respectively, and the genotypes Esihaqi 1, Esihaqi 2 and Tarzan did not differ significantly between each other in this trait, and possibly the reason is that they did not differ significantly in the trait of the leaf area as shown in Table 4. The reason for the superiority of the variety Aqmar in this trait is due to the superiority of having the highest leaf area as shown in table 4, which was reflected in increasing its index. These results are consistent with (AL-Jayashi, 2017; Hassan 2019) pointed out that the sunflower genotypes differ between each other in the leaf area index. The leaf area index for plants sprayed with folic acid spray concentrations increased and the maximum average of this trait at a concentration of 3 g L⁻¹ of acid reached 6.50 with an increase of 17.11%, compared to the comparison treatment (0 g L⁻¹) that recorded the lowest average for this trait reached 5.55. In addition, the concentrations 0, 1, and 2 g L⁻¹ did not differ significantly between each other, and the concentration of 3 g L⁻¹ was not significantly different from the concentrations 1 and 2 g L⁻¹. The reason for the superiority of the sprayed plants with the concentration of 3 g L⁻¹ in this trait was due to their superiority of having the highest average leaf area as shown in Table 4, and then this was reflected in increasing the leaf area index.

Chlorophyll content (µg g fresh weight⁻¹)

Table 5: The genotypes difference and the effect of spraying with folic acid on the leaf area index for the spring season 2019.

Varieties	Folic acid concentrations				Average
	0	1	2	3	
Esihaqi 1	5.36	5.55	5.75	6.94	5.90
Esihaqi 2	5.55	5.87	5.96	5.54	5.73
Tarzan	4.96	6.04	6.67	6.19	5.97
Aqmar	6.33	6.95	6.92	7.32	6.88
L.S.D	N.S				0.577
Average	5.55	6.108	6.32	6.50	
L.S.D	0.577				

The data in table 6 indicated a significant difference in the study genotypes and the plants sprayed with folic acid between each other in the leaves content of total chlorophyll. The plants of genotype Esihaqi 2 exceeded by containing the highest total chlorophyll content with an average of 31.52 µg g fresh weight⁻¹ and without significant difference with the genotype Tarzan which recorded 30.16 µg g fresh weight⁻¹, followed by the genotype Esihaqi 1 with an average of 27.47 µg g fresh weight⁻¹. However, the leaves of variety Aqmar contained a minimum average for this trait reached 27.30 µg g fresh weight⁻¹, which in turn did not differ significantly with the genotype Esihaqi 1 in this trait. These results differed with (Al-Ahbab 2015) findings in that there was a non-significant difference between the sunflower genotypes in this trait, and agreed with the findings of (Alag, 2007; Dawood *et al.*, 2012), which they indicated the difference in the sunflower genotypes by their chlorophyll content. As for the effect of the concentrations, the same Table showed that plants sprayed with a concentration of 2 g L⁻¹, their plant leaves gave the highest average of 31.74 µg g fresh weight compared to the concentrations 0, 1 and 3 g L⁻¹. They gave averages of 28.26, 29.97, and 26.48 µg g fresh weight⁻¹ respectively, with an increase of (12.31, 5.90, and 19.86%), respectively, and the concentration of 2 g L⁻¹ did not differ significantly from the concentration of 1 g L⁻¹. Besides, the lowest average of this trait was recorded at the concentration of 3 g L⁻¹ reached 26.48 µg g fresh weight⁻¹, and possibly an increase in the acid concentration had an adverse effect on the chlorophyll content, and that the concentration was sufficient to cause this increase. The reason for the effect of folic acid in increasing the chlorophyll may be due to its important role as an enzymatic companion in many biological reactions, including stimulating the synthesis of the amino acid glycine that contributes to the synthesis of porphyrins and chlorophylls in the membranes of chloroplasts (Hanson and Roje, 2001 and Jabrin *et al.*, 2003). These results are consistent with (Burguières *et*

al., 2007; Emam *et al.*, 2011; Ibrahim *et al.*, 2015) findings. As for the interaction between the two factors, it was not significant in this trait.

Table 6: The genotypes difference and the effect of spraying with folic acid on the chlorophyll content ($\mu\text{g g fresh weight}^{-1}$) for the spring season 2019.

Varieties	Folic acid concentrations				Average
	0	1	2	3	
Esihaqi 1	26.99	29.60	29.78	23.52	27.47
Esihaqi 2	30.61	32.35	35.82	27.31	31.52
Tarzan	28.49	31.45	33.33	27.38	30.16
Aqmar	26.96	26.49	28.06	27.71	27.30
L.S.D	N.S				2.559
Average	28.26	29.97	31.74	26.48	
L.S.D	2.559				

Conclusions

From this study, it can be concluded the possibility of spraying sunflower plants with folic acid by a concentration of 2 g L^{-1} for its effect of the most growth traits as well as being a natural and environmentally safe substance.

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