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Effect of Biofertilizers and Spraying with Vitamins on Two Cultivars of Iris spp.

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Abstract. *The study was conducted in the plastic house belonging to Al-Mussaib Technical College, Al-Furat Al-Awsat Technical University during the growth seasone2020-2021, to study the effect of bio-fertilizers and spraying with vitamins on the growth and flowering of two cultivars of Iris sp. The experiment included studying the effect of two cultivars(Sapphire Beauty and white magic) which are symbolized by (C1, C2), respectively , bio fertilizer spraying with distilled water (the control), Azotobacter spp, and Bacillus spp), which are symbolized by (S1, S2, S3), and spraying of vitamins (control) 0 , ascorbic acid (vitamin C) (200 mg.L*$^{-1}$ *and thymine (B1) (200 mg.L*$^{-1}$*), which symbolized by (F1, F2, F3), respectively. The study was conducted as a factorial experiment according to the Randomized Complete Blocks Design (R.C.B.D), with three replications, and the averages were compared according to the least significant difference (L.S.D) test under the probability level of 5%. The results showed that the blue cultivar was significantly excelled and gave the highest values for all the studied traits, The Azotobacter treatment has significantly excelled on the rest of the other treatments in plant height 49 cm, chlorophyll content 52.94 spad, percentage of carbohydrate 10.69%, vase life 10.73 days, anthocyanin in petals 15.93 mg 100.g*$^{-1}$ *and carotenoids in petals 7.53.The spraying treatment with Ascorbic acid at a concentration of (200 mg.L*$^{-1}$*) has significantly excelled and gave the highest values for all the studied traits,The triple interaction treatment consisting of (blue cultivar + Azotobacter bacteria + spraying with ascorbic acid at a concentration of (200 mg.L*$^{-1}$*) has excelled in the traits of plant height 82.88 cm, chlorophyll content 58 44Spad, percentage of carbohydrate 11.86%, vase life 11.85 days, and anthocyanins in petals 29.69 mg 100.g*$^{-1}$*.*

Keywords: *Azotobacter, Ascorbic acid, Bio-fertilizers, Iris, thymine.*

1.INTRODUCTION

Iris spp. belongs to the Iridaceae family, which includes more than 66 genera. Iris bulbs are true bulbs with Monocotyledon, some of which are annuals in summer or winter, and others are perennials that form a ground stem that buds emerge from the axils of the leaves. Its Linear shape leaves on both sides of the stem to form a palmate growth and end with a beautiful inflorescence with a strong flower stalk suitable for cutting flower [1]. some of them are used as ornamental plants, and the other are used in the manufacture of medicinal drugs [2]. The Mediterranean Basin and Japan are its origin country, and wild Iris is found in northern Iraq [3], with the addition of bio-bacterial fertilizers, which is one of the modern techniques that were used to reduce the excessive use of chemical fertilizers, Azotobacter and Bacillus bacteria have the ability to Nitrogen fixation and phosphorous and secrete stimulants that help root growth such as gibberellins, cytokines, and auxins [4]. The spraying of vitamins on the vegetative system has an important role in many metabolic processes and is even necessary to make these processes normal. As well as to ensure the work of many enzymes, as adding them to the plant leads to stimulating growth by activating some enzymatic reactions, where vitamins B and C act as coenzymes for some enzymes that contribute to stimulating processes or biological activities as they have a positive effect on carbon dioxide absorption and protein synthesis, and the external addition of vitamins Exogenous application lead to increased production by stimulating the formation of flowers and roots [5]. In view of the difficulty of production in Iraq for cut flowers, this research was conducted for the purpose of obtaining flowers with high specifications suitable for commercial cutting.In view of the importance of the Iris plant as one of the important cut flowers globally and in Iraq, the study was conducted with the aim of studying the response of the two cultivars of Iris to biofertilizers and vitamins, Determining the best biological fertilizer for the growth and flowering of the two cultivars of Iris bulbs and Determine which vitamins are best for the growth and flowering of the two Iris cultivars.

2. MATERIALS AND METHODS

The factorial experiment was conducted according to Randomized Complete Blocks Design (R.C.B.D) in the plastic house belonging to the Al-Mussaib Technical College, AL-Furat Al-Awsat Technical University during the growth season 2020-2021. To study the effect of bio-fertilizers (control), Azotobacter spp, Bacillus spp) and which are symbolized by(S1, S2,S3) , spraying vitamins(spraying with distilled water (the control treatment ,200 $mg.L^{-1}$ of Ascorbic acid (vitamin C), 200 $mg.L^{-1}$ of thymine (B1) and which are symbolized by (F1, F2, F3) on the growth and flowering of two cultivars of Iris (Sapphire Beauty and white magic) (C1, C2),. The experiment included studying the Bio fertilizers were loaded on peat moss in the laboratories of the Agricultural Research Department, Ministry of Science and Technology, as they were added, according to the experiment's treatments, at an average of 10 g. Pot-1 into the pit prepared for growing bulbs. It was taken into account that the biological vaccine was in direct contact with the bulbs [6].The process of spraying plants with vitamin C and B1 began after the appearance of two real leaves on the plant, then the spraying process was repeated every ten days, with a difference of two days between spraying one vitamin and another until the time of flowering of the plants.

Spraying operations were conducted by backpack sprayer in the early morning until the plant was completely wet with the addition of a few drops of liquid soap as a diffuser to reduce the surface tension of the solutions. Bulbs produced by the Dutch company (Stoop flower bulb) were planted on 01/29/2020 with a diameter of (3 ± 5) cm in plastic pots with a diameter of 27 cm filled with an agricultural medium consisting of (peat moss + river soil + sheep manure) at an average of 1: 1:2) respectively. Random samples were taken from river soil, sheep manure, and peat moss for analysis in the laboratories of Al-Qasim Green University, College of Agriculture as shown in Table (1, 2). The study was conducted as a factorial experiment (3 x 3 x 2) according to the Randomized Complete Blocks Design (R.C.B.D) with three replicates, each replicate containing 18 treatments as shown in Table (3), five plants for each experimental unit. The averages were compared according to the least significant difference (L.S.D) test at a probability level of 5% [7]. The data were analyzed using available statistical program Genstat.

Table 1. Chemical and physical traits of the experimental soil.

Traits	Values	units
pH	**7.60**	**-**
Organic matter	2.17	g.kg/soil
Electrical conductivity (EC)	**3.08**	dsm^{-1}
total nitrogen	**7.89**	$g.kg^{-1}$
availability phosphorous	**17.41**	$mg.kg^{-1}$
soluble potassium	**31.25**	
Soil Separators		
sand	**698.39**	$g.kg^{-1}$
silt	**186.25**	
Clay	**115.36**	
texture	sandy loam	

Table 2. The chemical and physical traits of sheep manure and peat moss.

Traits	**peat moss**	**sheep manure**	**Units**
EC . electrical conductivity	3.10	1.45	$d.S.m^{-1}$
pH	7.10	6.80	---
Organic matter	56.57	410	$g.kg^{-1}$
C / N Ratio	17.74	14.36	---
total nitrogen	12.02	36.54	$g.kg^{-1}$
total phosphorous	6.25	10.30	$g.kg^{-1}$
total potassium	11.87	24.35	$g.kg^{-1}$

2.1. STUDIED TRAITS :

Plant Height *(cm)*The height of the plant was calculated from the soil surface of the pot to the top of the flower using the metric tape for all plants of the experimental unit and then according to the average for each treatment and for the two cultivars, Chlorophyll Content In Leaves (Spad Unit)The content of chlorophyll in leaves was estimated by using a device ((Chlorophyll meter Model SPAD-502)), which was supplied by the Japanese company Minolta. By taking the reading of three leaves for each plant and extracting the average for both cultivars. the percentage of carbohydrates in the leaves The total carbohydrate content in the leaves was estimated according to the method mentioned by [8]. Vas life (day) The flowering age was calculated by choosing three plants at random from each treatment and for both cultivars. The flowers were cut at the stage of the closed (unopened) flower bud and the length of the flower stalk was standardized to 25 cm and placed in homogeneous containers containing 250 ml of distilled water, and the containers were placed at a temperature the room and the number of days that it remained valid in coordination was calculated and the average was extracted for each treatments and for both cultivars [9].Content of total carotenoids in iris flower petals(mg.100.g^{-1} dry weight)The total carotenoid content of Iris white flowers was estimated according to the method [10] by following the following equation:

$$\text{Total carotenoids} = \frac{(\text{Supplementary volume (50 ml)} \times \text{spectrophotometric reading} \times 3.856)}{(\text{Sample weight} \times 1000)} \times 100$$

Determination of anthocyanin pigment in iris flower petals (mg 100.g^{-1} dry weight)the anthocyanin content of Iris blue flowers was estimated by taking 1 gm of dry plant tissue of flower petals. It was crushed in acidified ethanol (HCL, standard 1.5 and 95% ethyl alcohol), filtered the mixture, took 1 ml of it and completed the volume to 10 ml, then measured by a Spectrophotometer at a wavelength of 535 nm [10] according to the equation:

$$\text{Anthocyanin pigment} = \frac{(\text{The solution used in the extraction} \times \text{dilutions} \times \text{optical density at a wavelength of 535})}{(98.20 \text{ x sample weight})} \times 100$$

3. RESULTS AND DISCUSSION

3.1.RESULTS

3.1.1.PLANT HEIGHT (cm)

Table (3) shows that the blue cultivar (C1) has significantly excelled by achieving it the highest plant height amounted to (68.97 cm), while the white cultivar (C2) gave the lowest plant height amounted to (62.71 cm). The treatment of Azotobacter (S2) has significantly excelled over the rest of the other treatments and by achieving it the highest average of plant height amounted to (75.49 cm), followed by the treatment of (S3) which gave a plant height amounted to (65.63 cm), while the control treatment (S1) recorded the lowest plant height amounted to (56.40 cm). The results of the same table also show that the treatment of Ascorbic acid (F2) at a concentration of (200 mg.L^{-1}) has significantly excelled over the rest of the other treatments by achieving it the highest average of plant height amounted to (69.43 cm), followed by the spraying treatment with Thymine (F3) at a concentration of (200 mg.L^{-1}) by achieving it a plant height amounted to (65.86 cm). While the control treatment without spraying (F1) gave the lowest plant height amounted to (62.23 cm). The bi-interaction between cultivars and fertilizers had a significant effect on increasing plant height. The interaction treatment (blue cultivar + Azotobacter) has significantly excelled on the rest of the other interaction treatments by achieving it the highest average of plant height amounted to (78.73 cm). As for the interaction treatment (white cultivar C2 + without adding S1). It gave the lowest plant height amounted to (53.41 cm). The interaction treatment between (blue cultivar C1 + spraying with Ascorbic (F2) at a concentration of (200 $mg.L^{-1}$)) has significantly excelled in the trait of plant height by achieving it the highest average of plant height amounted to (72.70 cm), while the interaction treatment (white cultivar C2 + without spraying vitamins F1) recorded the lowest average of plant height amounted to (59.69 cm). The triple interaction between the factors of the experiment had a significant effect on increasing plant height. The triple interaction treatment consisting of (blue cultivar C1 + Azotobacter S2 + spraying with Ascorbic acid F2 at a concentration of (200 $mg.L^{-1}$)) was significantly excelled on the other interaction treatments by achieving it the highest plant height amounted to (82.88 cm), while the interaction treatment (white cultivar C2 + without adding bio-fertilizer S1 + without spraying with vitamins F1) gave the lowest plant height amounted to (50.21 cm).

Table 3. Effect of bio-fertilizers and spraying with vitamins and their interactions on the plant height (cm) for the two cultivars of Iris sp.

Cultivars (C)	**bio-fertilizers (S)**	**Spraying with vitamins (F)**			**S x C**
		Without spraying (F1)	**Spray ing with Ascorbic (200 $mg.L^{-1}$) (F2)**	**Spraying with Thymine at a concentration of (200 $mg.L^{-1}$) (F3)**	
Blue cultivar (C1)	Control (S1)	53.68	64.63	59.90	59.40
	Azotobacter (S2)	73.51	82.88	79.80	78.73
	Bacillus (S3)	67.12	70.59	68.62	68.78
White cultivar (C2)	Control (S1)	50.21	57.34	52.67	53.41
	Azotobacter (S2)	68.46	76.01	72.24	72.24
	Bacillus (S3)	60.38	65.11	61.93	62.47
L.S.D 0.05		**2.12**			**1.22**
Interaction between cultivars and spraying with vitamins					**Cultivars (C)**
Blue cultivar (C1)		64.77	72.70	69.44	68.97
White cultivar (C2)		59.69	66.15	62.28	62.71
L.S.D 0.05		**1.22**			**0.71**
The interaction between fertilizers and spraying with vitamins					**bio-fertilizers (S)**
Control (S1)		51.95	60.98	56.29	56.40
Azotobacter (S2)		70.99	79.45	76.02	75.49
Bacillus (S3)		63.75	67.85	65.27	65.63
L.S.D 0.05		**1.50**			**0.87**
Spraying with vitamins (F)		62.23	69.43	65.86	
L.S.D 0.05		**0.87**			

3.1.2.THE LEAVES CONTENT OF CHLOROPHYLL (SPAD UNIT)

Table (4) shows that the blue cultivar (C1) has significantly excelled by achieving it the highest content of chlorophyll in leaves amounted to (44.98 spad unit), while the white cultivar (C2) achieved the lowest highest content of chlorophyll in leaves amounted to (39.58 spad unit). The results also showed that the addition of bio-fertilizers had a significant effect on the highest content of chlorophyll in leaves. The Azotobacter (S2) treatment has significantly excelled over the rest of the other treatments by achieving it the highest content of chlorophyll in leaves, which amounted to (52.94 spad), followed by the treatment of adding Bacillus bacteria (S3), which amounted to (41.32 spad), while the treatment of Bacillus (S3) amounted to (41.32 spad). As for the control treatment (without addition) achieved the lowest content of chlorophyll in leaves amounted to (32.58 spad), and the spraying treatment with Ascorbic acid (F2) at concentration of (200 mg.L^{-1}) achieved the highest average content of chlorophyll in leaves amounted to (45.50 spad), while the treatment without spraying gave the lowest chlorophyll content amounted to (39.30 spad). The results of the two-interaction also showed a significant effect of the chlorophyll content in the leaves, where the interaction treatment (blue cultivar C1 + Azotobacter bacteria) has significantly excelled over the rest of the other interaction treatments by achieving it the highest average content of chlorophyll amounted to (56.01 spad), while the lowest content of chlorophyll in leaves recorded at the interaction treatment (White cultivar C2 + without adding S1) by giving it the lowest average content of chlorophyll amounted to (30.54 spad), and the interaction treatment (blue cultivar C1 + spraying with Ascorbic F2 at a concentration of (200 mg.L^{-1})) achieved the highest average of chlorophyll content amounted to (48.39 spad). While the interaction treatment (white cultivar C2 + without spraying F1) recorded the lowest average of chlorophyll content in the leaves amounted to (36.41 spad), while the interaction treatment (Azotobacter S2 + spraying with ascorbic acid F2 (200 mg.L^{-1}) achieved the highest average content of chlorophyll in leaves amounted to (56.38 spad), while the content of chlorophyll in leaves decreased and amounted to (29.88 spad) at the interaction treatment (without adding fertilizers S1 + without spraying F1). The triple interaction treatment (blue cultivar C1 + Azotobacter S2 + spraying with Ascorbic acid F2 at a concentration of (200 mg.L-1) has significantly excelled over the rest of the other treatments by achieving it the highest average of chlorophyll content in leaves amounted to (58.44 spad), while the interaction treatment (white cultivar C2 +Without adding bio-fertilizers S1 + without spraying with vitamins F1) achieved the lowest chlorophyll content in the leaves amounted to (28.59 spad).

Table 4. Effect of bio-fertilizers and spraying with vitamins and their interactions on the leaves content of chlorophyll (spad unit) for the two cultivars of Iris sp.

Cultivars (C)	bio-fertilizers (S)	Spraying with vitamins (F)			S x C
		Without spraying (F1)	Spray ing with Ascorbic (200 mg.L^{-1}) (F2)	Spraying with Thymine at a concentration of (200 mg.L^{-1}) (F3)	
Blue cultivar (C1)	Control (S1)	31.17	39.02	33.65	34.61
	Azotobacter (S2)	53.60	58.44	56.01	56.01

	Bacillus (S3)	41.82	47.70	43.42	44.31
White cultivar (C2)	Control (S1)	28.59	33.02	30.00	30.54
	Azotobacter (S2)	45.11	54.32	50.14	49.86
	Bacillus (S3)	35.51	40.48	39.02	38.34
L.S.D 0.05		**1.86**			**1.07**
Interaction between cultivars and spraying with vitamins					**Cultivars (C)**
Blue cultivar (C1)		42.19	48.39	44.36	44.98
White cultivar (C2)		36.41	42.61	39.72	39.58
L.S.D 0.05		**1.31**			**0.62**
The interaction between fertilizers and spraying with vitamins					**bio-fertilizers (S)**
Control (S1)		29.88	36.02	31.83	32.58
Azotobacter (S2)		49.35	56.38	53.07	52.94
Bacillus (S3)		39.66	44.09	41.22	41.32
L.S.D 0.05		**1.07**			**0.76**
Spraying with vitamins (F)		39.30	45.50	42.04	
L.S.D 0.05		**0.76**			

3.1.3.PERCENTAGE OF CARBOHYDRATES IN LEAVES (%)

Table (5) shows that the blue cultivar (C1) was significantly excelled by recording it the highest percentage of carbohydrates in the leaves, which amounted to (9.35%) compared to the white cultivar (C2), which recorded the lowest percentage of carbohydrates in the leaves amounted to (8.05%).The results also showed that adding bio-fertilizers had a significant effect on the average percentage of carbohydrates in the leaves, where the Azotobacter treatment (S2) has significantly excelled over the rest of the other treatments by achieving it the highest average amounted to (10.69%), followed by the treatment of adding Bacillus (S3), which amounted to (8.86%), compared to the control treatment Without addition (S1) which achieved (6.56%).The spraying treatment with Ascorbic acid (F2) (200 mg.L^{-1}) achieved the highest average of carbohydrates in the leaves amounted to (9.45%), while the contol treatment without spraying gave the lowest values amounted to (8.05%).The bi-interaction also had a significant effect on the percentage of carbohydrates in leaves. The interaction treatment (blue cultivar C1 + Azotobacter bacteria) was significantly excelled on the rest of the treatments by giving it a value amounted to (11.13%0, compared to the interaction treatment (white cultivar C2 + without adding S1), which gave a value amounted to 5.91%. The interaction treatment (blue cultivar C1 + spraying with Ascorbic (F2) at a concentration of (200 mg.L^{-1})) had the highest percentage of carbohydrates in leaves amounted to (10.11%), while the interaction treatment (white cultivar C2 + spraying with without F1) recorded the lowest percentage of carbohydrates in leaves amounted to (7.49%), while the treatment of (Azotobacter bacteria + spraying with Ascorbic acid F2 (200 mg.L^{-1}) gave the highest values amounted to (11.32%), while the percentage of carbohydrates decreased in the leaves which amounted to (5.65%) at the interaction treatment (without adding fertilizers S1 + without spraying F1). The results of the same table also showed that the triple interaction between the factors of the experiment had a significant effect on the percentage of carbohydrates in the leaves. The interaction treatment (blue cultivar C1 + Azotobacter S2 + spraying with ascorbic acid F2 at a concentration of (200 mg.L^{-1})) has significantly excelled on the rest of the other treatments by achieving it the highest average amounted to (11.86%), while the interaction treatment (white cultivar C2 + without adding bio-fertilizers S1 + without spraying with vitamins F1) gave the lowest percentage of carbohydrates in leaves amounted to (5.15%). The interaction treatment (blue cultivar C1 + spraying with Ascorbic F2 at a concentration of (200 mg.L^{-1}) gave the highest percentage of carbohydrates in leaves, which amounted to (10.11%), while the interaction treatment (white cultivar C2 + without spraying F1) recorded the lowest percentage of carbohydrates in leaves, which amounted to (7.49%), while the treatment of interaction (white cultivar C2 + without spraying F1) recorded the lowest percentage of carbohydrates in leaves amounted to (7.49%), while the interaction treatment (Azotobacter bacteria + spraying with Ascorbic acid F2 (200 mg.L^{-1})) gave the highest values amounted to (11.32%), while the percentage of carbohydrates in the leaves decreased amounted to (5.65)% at the interaction treatment (without adding fertilizers S1 + without spraying F1).

Table 5. Effect of bio-fertilizers and spraying with vitamins and their interactions on the percentage of carbohydrates in the leaves (%) for the two cultivars of Iris sp.

Cultivars (C)	**bio-fertilizers (S)**	**Spraying with vitamins (F)**			**S x C**
		Without spraying (F1)	**Spray ing with Ascorbic (200 mg.L^{-1}) (F2)**	**Spraying with Thymine at a concentration of (200 mg.L^{-1}) (F3)**	
Blue cultivar (C1)	Control (S1)	6.15	8.22	7.24	7.20
	Azotobacter (S2)	10.37	11.86	11.15	11.13
	Bacillus (S3)	9.35	10.27	9.58	9.73
White cultivar (C2)	Control (S1)	5.15	6.85	5.72	5.91
	Azotobacter (S2)	9.88	10.79	10.11	10.26
	Bacillus (S3)	7.43	8.75	7.77	7.98
L.S.D 0.05		**0.23**			**0.14**
Interaction between cultivars and spraying with vitamins					**Cultivars (C)**
Blue cultivar (C1)		8.62	10.11	9.32	9.35
White cultivar (C2)		7.49	8.79	7.89	8.05
L.S.D 0.05		**0.14**			**0.08**
The interaction between fertilizers and spraying with vitamins					**bio-fertilizers (S)**
Control (S1)		5.65	7.53	6.48	6.56
Azotobacter (S2)		10.13	11.32	10.63	10.69
Bacillus (S3)		8.39	9.51	8.67	8.86
L.S.D 0.05		**0.17**			**0.10**
Spraying with vitamins (F)		8.05	9.45	8.60	
L.S.D 0.05		**0.10**			

3.1.4.VASE LIFE (DAY)

Table (6), as in the previous tables, showed that the blue cultivar (C1) has significantly excelled by achieving it the highest average of vase life amounted to (10.23 days) compared to the white cultivar (C2), which amounted to (9.46 days). The results also showed that adding bio-fertilizers had a significant effect on vase life. The Azotobacter treatment (S2) has significantly excelled over the rest of the other treatments by achieving it the highest average of vase life (10.73 days), followed by the treatment of adding Bacillus (S3) bacteria, which gave an average of vase life amounted to (9.94 days). while a treatment without adding fertilizers gave the lowest average of vase life amounted to (8.89 days). The spraying treatment of Ascorbic acid (F2) at a concentration of (200 $mg.L^{-1}$) gave the highest average of vase life amounted to (10.32 days), followed by a treatment of spraying with Thymine (F3) acid) at a concentration of (200 $mg.L^{-1}$), which gave a vase life amounted to (9.83 days), while a treatment without spraying gave the lowest average of vase life amounted to (9.42 days). The results of the bi-interaction had a significant effect on the average of vase life, where the interaction treatment (blue cultivar C1 + Azotobacter bacteria) has significantly excelled over the rest of the other treatments by achieving it the highest average of vase life amounted to (11.11 days), while the interaction treatment (white cultivar C2 + without adding) achieved the lowest average of vase life amounted to (8.63 days). The interaction treatment (blue cultivar C1 + spraying with Ascorbic (200 $mg.L^{-1}$)) achieved the highest average of vase life amounted to (10.71 days), while the interaction treatment (white cultivar C2 + without spraying) gave the lowest average of vase life amounted to (9.06 days), while the interaction treatment (Azotobacter bacteria + spraying with Ascorbic acid F2 at a concentration of (200 $mg.L^{-1}$)) gave the highest average of vase life amounted to (11.42 days), while The interaction treatment (without adding bio-fertilizers + without spraying with vitamins F1) gave the lowest average of vase life amounted to (8.54 days). The results of the same table also show that the triple interaction between the factors of the experiment had a significant effect on the vase life. The treatment (blue cultivar C1 + Azotobacter bacteria + spraying with Ascorbic acid F2 at a concentration of (200 $mg.L^{-1}$)) has significantly excelled over the rest of the other treatments by achieving it the highest average of vase life amounted to (11.85 days) compared to the control treatment, which recorded the lowest average amounted to (8.16 days).

Table 6. Effect of bio-fertilizers and spraying with vitamins and their interactions on the vase life (day) for the two cultivars of Iris sp.

Cultivars (C)	bio-fertilizers (S)	Spraying with vitamins (F)			S x C
		Without spraying (F1)	Spray ing with Ascorbic (200 $mg.L^{-1}$) (F2)	Spraying with Thymine at a concentration of (200 $mg.L^{-1}$) (F3)	
Blue cultivar (C1)	Control (S1)	8.92	9.48	9.04	9.15
	Azotobacter (S2)	10.42	11.85	11.07	11.11
	Bacillus (S3)	9.98	10.80	10.65	10.48
White cultivar (C2)	Control (S1)	8.16	9.14	8.59	8.63
	Azotobacter (S2)	9.80	11.00	10.25	10.35
	Bacillus (S3)	9.24	9.63	9.35	9.41

L.S.D 0.05	**0.12**			**0.07**
Interaction between cultivars and spraying with vitamins				**Cultivars (C)**
Blue cultivar (C1)	9.78	10.71	10.26	10.23
White cultivar (C2)	9.06	9.92	9.40	9.46
L.S.D 0.05	**0.07**			**0.04**
The interaction between fertilizers and spraying with vitamins				**bio-fertilizers (S)**
Control (S1)	8.54	9.31	8.82	8.89
Azotobacter (S2)	10.11	11.42	10.66	10.73
Bacillus (S3)	9.61	10.22	10.00	9.94
L.S.D 0.05	**0.09**			**0.05**
Spraying with vitamins (F)	9.42	10.32	9.83	
L.S.D 0.05	**0.05**			

3.1.5. ANTHOCYANIN CONTENT IN PETALS (mg.100 g^{-1} DRY WEIGHT)

Table (7) shows that the blue cultivar (C1) has significantly excelled by achieving it the highest content of anthocyanins in the petals, which amounted to (21.30 mg.100 g^{-1} dry weight) compared to the white cultivar (C2), which achieved a value amounted to (6.60 mg.100 g^{-1} dry weight). The results also showed that adding bio-fertilizers had a significant effect on the anthocyanin content in the petals. The Azotobacter (S2) treatment has excelled by recording the highest values amounted to (15.93 mg. 100 g^{-1} dry weight), followed by the treatment of adding Bacillus (S3), which gave an average amounted to (13.69 mg. 100 g^{-1} dry weight). As for the control treatment (without adding S1), it achieved the lowest values amounted to (12.23 mg. 100 mg^{-1} dry weight). The spraying treatment with Ascorbic acid (F2) at a concentration (200 mg.L^{-1}) achieved the highest content of anthocyanins in the petals, which amounted to (17.48 mg.100 g^{-1} dry weight), compared to the control treatment without spraying, which amounted to (11.10 mg.100 g^{-1} dry weight). The bi-interactions had a significant effect on the anthocyanin content in the petals. The interaction treatment (blue cultivar C1 + Azotobacter) was significantly excelled by giving it the highest average amounted to (24.62 mg.100 g^{-1} dry weight). While the lowest content of anthocyanins was recorded at the treatment (white cultivar C2 + without adding S1), which gave an average amounted to (5.95 mg. 100 g^{-1} dry weight). The interaction treatment (blue cultivar C1 + spraying with Ascorbic F2 at a concentration of (200 mg.L^{-1}) recorded the highest average amounted to (27.45 mg. 100 g^{-1} dry weight). As for the interaction treatment (Azotobacter bacteria + spraying with Ascorbic acid (200 mg.L^{-1})), it achieved the highest average of anthocyanin content in the petals amounted to (19.00 mg.100 g^{-1} day weight), while its percentage decreased to (9.72 mg.100 g^{-1} dry weight) at the treatment (without adding fertilizers S1 + without spraying F1). The triple interaction between the factors of experiment had a significant effect on the anthocyanin content in the petals, the treatment (blue cultivar C1 + Azotobacter S2 + spraying with Ascorbic F2 at a concentration of (200 mg.L^{-1})) was significantly excelled by giving it the highest average amounted to (29.69 mg.100 g^{-1} dry weight) compared to the control treatment that achieved the lowest average amounted to (5.07 mg.100 g^{-1} dry weight).

Table 7. Effect of bio-fertilizers and spraying with vitamins and their interactions on the Anthocyanin content in petals (mg.100 g^{-1} dry weight) for the two cultivars of Iris sp.

Cultivars (C)	bio-fertilizers (S)	Spraying with vitamins (F)			S x C
		Without spraying (F1)	Spray ing with Ascorbic (200 mg.L^{-1}) (F2)	Spraying with Thymine at a concentration of (200 mg.L^{-1}) (F3)	
Blue cultivar (C1)	Control (S1)	14.37	25.24	15.91	18.51
	Azotobacter (S2)	20.80	29.69	23.21	24.62
	Bacillus (S3)	15.69	27.41	19.21	20.77
White cultivar (C2)	Control (S1)	5.07	6.71	6.07	5.95
	Azotobacter (S2)	5.45	8.31	7.97	7.24
	Bacillus (S3)	5.20	7.49	7.11	6.60
L.S.D 0.05		**0.80**			**0.46**
Interaction between cultivars and spraying with vitamins					**Cultivars (C)**
Blue cultivar (C1)		16.95	27.45	19.50	21.30
White cultivar (C2)		5.24	7.50	7.05	6.60
L.S.D 0.05		**0.46**			**0.27**
The interaction between fertilizers and spraying with vitamins					**bio-fertilizers (S)**
Control (S1)		9.72	15.98	10.99	12.23
Azotobacter (S2)		13.13	19.00	15.68	15.93
Bacillus (S3)		10.44	17.45	13.16	13.69
L.S.D 0.05		**0.57**			**0.33**
Spraying with vitamins (F)		11.10	17.48	13.28	
L.S.D 0.05		**0.33**			

3.1.6.TOTAL CAROTENOID CONTENT IN PETALS (mg.100 g^{-1} DRY WEIGHT)

Table (8) shows that the white cultivar (C2) has excelled by giving it the highest content of total carotenoids in the petals amounted to (8.17 mg.100 g^{-1} dry weight) compared to the blue cultivar (C1) which achieved the lowest average of carotenoids amounted to (5.89 mg.100 g^{-1} dry weight). Bacterial fertilizers have an important role in increasing the content of carotenoids in petals, where Azotobacter bacteria (S2) were significantly excelled by achieving it the highest average amounted to (7.53 mg.100 g^{-1} dry weight), followed by the treatment of Bacillus bacteria (S3) which gave (7.00 mg.100 g^{-1} dry weight), while the control treatment (without adding (S1) recorded the lowest average amounted to (6.56 mg.100 g^{-1} dry weight). As for the spraying of vitamins, the treatment of ascorbic acid (F2) at a concentration of (200 mg.L^{-1}) has significantly excelled on the rest of the other treatments by achieving it the highest content in the petals amounted to (7.67 mg.100 g^{-1} dry weight), followed by the treatment of spraying with Thymine (F3) at a concentration of (200 mg.L^{-1}) amounted to (7.13 mg.100 g^{-1} dry weight), while the control treatment (without spraying) F1 achieved the lowest content amounted to (6.29 mg.100 g^{-1} dry weight). The results also showed that the bi-interaction between cultivars and fertilizers had a significant effect on this trait, where the treatment of (white cultivar C2 + Azotobacter bacteria) has excelled by giving it the highest average amounted to (8.65 mg.100 g^{-1} dry weight). As for the intervention treatment (blue cultivar C1 + without adding S1), it achieved the lowest average amounted to (5.41 mg. 100 g^{-1} dry weight). The results of the same table also showed that the interaction treatment between (white cultivar C2 + spraying with Ascorbic at a concentration of (200 $mg.L^{-1}$)) had a significant effect on the content of total carotenoids in the petals by giving it an averge amounted to (8.74 mg.100 g^{-1} dry weight), while the treatment of (Blue cultivar C1 + without spraying with vitamins F1) achieved the lowest average amounted to (5.14 mg. 100 g^{-1} dry weight). The treatment of (Azotobacter bacteria + spraying with Ascorbic acid at a concentration of (200 $mg.L^{-1}$)) achieved the highest content of total carotenoid in the petals, which amounted to (8.14 mg.100 g^{-1} dry weight), while treatment (without adding fertilizer S1 + without spraying with vitamins F1) gave the lowest average amounted to (5.69 mg.100 g^{-1} dry weight). The same table also shows the positive role of the triple interaction between the factors of the experiment, where the treatment of (white cultivar C2 + Azotobacter bacteria + spraying with Ascorbic acid at a concentration of (200 $mg.L^{-1}$)) has significantly excelled by giving an average amounted to (9.50 mg.100 g^{-1} dry weight), While the treatment (blue cultivar C1+ without adding bio-fertilizer S1 + without spraying with vitamins F1) gave the lowest content of total carotenoid in the petals, which amounted to (4.28 mg.100 g^{-1} dry weight).

Table 8: Effect of bio-fertilizers and spraying with vitamins and their interactions on the Total carotenoid content in petals (mg.100 g^{-1} dry weight) for the two cultivars of Iris sp.

Cultivars (C)	bio-fertilizers (S)	Spraying with vitamins (F)			S x C
		Without spraying (F1)	Spray ing with Ascorbic (200 $mg.L^{-1}$) (F2)	Spraying with Thymine at a concentration of (200 $mg.L^{-1}$) (F3)	
Blue cultivar (C1)	Control (S1)	4.28	6.46	5.50	5.41
	Azotobacter (S2)	6.07	6.78	6.37	6.40
	Bacillus (S3)	5.08	6.58	5.85	5.84
White cultivar (C2)	Control (S1)	7.10	8.09	7.95	7.71
	Azotobacter (S2)	7.69	9.50	8.76	8.65
	Bacillus (S3)	7.51	8.62	8.33	8.15
L.S.D 0.05		**0.22**			**0.13**
Interaction between cultivars and spraying with vitamins					**Cultivars (C)**
Blue cultivar (C1)		5.14	6.61	5.91	5.89
White cultivar (C2)		7.43	8.74	8.35	8.17
L.S.D 0.05		**0.13**			**0.07**
The interaction between fertilizers and spraying with vitamins					**bio-fertilizers (S)**
Control (S1)		5.69	7.28	6.73	6.56
Azotobacter (S2)		6.88	8.14	7.57	7.53
Bacillus (S3)		6.30	7.60	7.09	7.00
L.S.D 0.05		**0.16**			**0.09**

Spraying with vitamins (F)	6.29	7.67	7.13	
L.S.D 0.05	**0.09**			

3.2. DISCUSSION

The results of tables (3-8) showed that the cultivars had a significant effect on the vegetative traits. The blue cultivar C1 was significantly excelled in most of the studied traits, and this is mainly due to the genetic differences between the cultivars and the nature of the interaction of the genotypes with the environmental conditions and the extent of their genetic expressions for the traits of the cultivar. Each cultivar has special traits that lead to an increase in the absorption of materials involved in photosynthesis, which was reflected in an increase in metabolic products [11]. These results agree with [12] who observed during their study on two cultivars of Gladiolus (Summer Rose and Friendship) and [13] on two tulip cultivars (Burgundy lace and Judith leyster). It also agrees with [14] in their study to evaluate five cultivars of Lilium. The bio-fertilizers had a significant effect on the traits of vegetative growth, the results showed that the Azotobacter (S2) treatment has significantly excelled over the rest of the other treatments. This is attributed to the role of Azotobacter bacteria in fixing nitrogen [15] as well as its role in secreting plant hormones such as auxin and gibberellins that increase cell division and their expansion [16], thus increase plant height as shown in Table (3). Azotobacter also plays an important and positive role in the bio-nitrogen fixation process, where it converts nitrogen from the inactive form (N2) to the active form (ammonium) with the help of the enzyme Nitrogenase. Nitrogen is an important element and is directly involved in the formation of the chlorophyll molecule [17], so the chlorophyll content increases in the leaves as shown in Table (4), and since Azotobacter works to improve root growth and stimulate the formation of secondary roots and increase absorption, thus increase the process of photosynthesis and the accumulation of carbon-building products inside the plant [18], thus leads to an increase in carbohydrates in leaves as shown in Table (5) and an increase in the percentage of dry matter in leaves as shown in Table 10). These results agree with [19] on the ranunculus, [20] on the dahlia plant (Kenya orange cultivar), and [21] in his experiment on the ranunculus bulbs and gladiolus. Vitamins lead to an increase in the studied traits, This may be attributed to the role of ascorbic acid, which protects cells for its action as an antioxidant and is considered a regulator of plant growth and development and affects cell division and differentiation [22], thus increases plant height as shown in Tables (3). The speed of movement of the formed carbohydrates and their lack of accumulation leads to an increase in the speed and efficiency of the carbon building processes for the manufacture of new carbohydrate materials, and this, in turn, requires the expenditure of energy that comes from respiration processes. This is due to the spraying of Ascorbic, which acts as a coenzyme [23], thus increases the accumulation of processed and stored carbohydrates in the leaves as shown in Table (5). Ascorbic prevents the decomposition of the anthocyanin pigment by working as an enzymatic co-enzyme of the antioxidant enzymes, especially (Peroxidase and Catalase), which keep the anthocyanin pigment from being destroyed, thus increasing the flower content of this pigment in the petals [24]. Ascorbic also plays an important role in delaying the senescence of petals by blocking the effect of ethylene through a cycle in stimulating the special gene in the production of carotenoids [25]. These results agree with [26] in her experiment on the dahlia plant (Arizona cultivar),

[27] on the Gladiolus plant, [28] on the Iris plant (Gemengd cultivar), and [29] in their experiment on Gladiolus plant.

4. CONCLUSIONS

The blue cultivar (Sapphire Beauty) was spraying with Ascorbic acid and Thiamine together increased in all student traits. The addition of the bio-fertilizer treatment (Azotobacter) caused a significant increase in some traits plant height , chlorophyll content , percentage of carbohydrate , vase life , anthocyanin in petals and carotenoids in petals .

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