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EFFECT OF *AZOSPIRILLUM BRASILENSE*, WATER TYPE AND POTASSIUM IN THE GROWTH AND FLOWERING OF THE GLADIOLUS PLANT

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Abstract

An experiment was conducted in the Lath House belonging to Department of Horticulture and Landscaper, Abu Ghraib, Alternative location of College of Agriculture, University of Anbar during the period from 21/3/2017 to 20/9/2017 to study the effect of bio-fertilization of *Azospirillum brasilense* and three sources of irrigation is the tap water and well water and irrigation alternately, Three levels of foliar spraying by (0, 5, 10 g.L^{-1} of potassium sulphate (K_2SO_4)) on the Gladiolus SP plant (Amsterdam cultivar) for some vegetative and biochemical flowering traits and corm yield of plant. A factorial experiment was conducted according to the Randomized Complete Block Design (RCBD), with three replicates, the results showed that the plants inoculation with Azospirillum was excelled in the traits of plant height, leaf area, relative content chlorophyll in leaves, percentage of dry matter in leaves and leaves content of N, P, K, Reducing the effectiveness of POD, increasing the diameter first base rosette, the vase life and the percentage of the dry matter of the corms. The results showed that the alternation irrigation was not significantly different from the irrigation treatment with tap water in the traits of the percentage of the dry matter of leaves and the diameter first base rosette, while the irrigation treatment with tap water was excelled on the rest of the traits. The spraying with potassium sulfate (10 g.L^{-1}) showed a significant excelling in all of the above mentioned traits. The irrigation with well water led to a significant decrease in the above mentioned traits. Potassium spraying and alternation irrigation showed significant effect in reducing the harmful effects of well water salinity. The triple interaction of the experimental factors, The results have showed that the irrigation treatment with tap water and spraying with potassium sulfate (10 g.L^{-1}) and the bacterial inoculation was excelled in all the samples was not significantly different from the interaction treatment between alternation irrigation and spraying with potassium sulfate (10 g.L^{-1}) and the bacterial inoculation in the traits of plant height, the percentage of dry matter for leaves, leaves content of N, P, K and the diameter first base rosette.

Introduction

As a result of the increasing demand for water resources in the world, especially in dry and semi-dry areas, in order to preserve water resources and reduce water deficit, emphasis should be placed on the use of technologies that preserve limited freshwater resources through the use of non-conventional water resources and adaptation to climate change, Iraq is characterized by a rainfall rate of (150 mm / year), and evaporation rate is more than (2400 mm / year) (Hayani, 2003), and because the sources of surface resources are located in the neighboring countries which have created dozens of dams on it, which led to reduce the amount of water to one third of what was previously reached and was estimated about (45.8 billion m^3.year^{-1}), This constitutes 40% of the minimum water requirement required for existing irrigation projects (Al-Halabi, 2001), Therefore, Iraq suffers from a large water deficit for agricultural purposes as well as for human consumption. So basis on this, interest was directed towards the use of alternative water resources for fresh surface water and it was necessary to use low quality water such as brackish water (wells and drainage) (Fahd *et al.,* 2003). The choice of suitable irrigation method and good water and soil management by using the requirements of washing or mixing or alternating or supplementary irrigation increases the efficiency of irrigation and allows to keep the water salinity below the salinity threshold of the selected agricultural crops and the failure to manage this water ideally will lead to the accumulation of salts in the soil (Subba and

John, 2005; Esmail *et al.,* 2007; Salih, 2008). The method of irrigation alternately is a good way to cope with water scarcity, easy to use, do not need tanks and complex techniques, must take into account the sensitive stages of the plant life, so should be irrigated with fresh water during this period and often at the beginning of growth, can benefit from salt water to meet the need of the plant Areas less affected by the salinity of irrigation water to cover the gap of water shortage required. In order to mitigate the adverse effects of irrigation water salinity, appropriate irrigation strategies should be adopted taking into account the type of crop, soil type and its nature (Sharma *et al.,* 2005; 2010, Baba and Zaidi, 2011). Gladiolus are used in good soils and need to be irrigated at regular intervals. It is one of the most important cut flowers plants, are widely distributed among the world markets, ranking fourth in the world trade of cut flowers (Singh, 2006). Bacterial Bio-fertilizers are considered as a bio-fertilizers that contain a bacterial cells that vary in their species Which are included in sustainable clean agriculture. The purpose of these projects is to produce plants that are free of pollutants and contribute to increasing production and protecting the environment, which adding to the soil in a vaccine and supplying the plant with part of its nutrient needs. Through its vital activity, The transformation of elements from Non-absorbable form by the plant into Absorbable form as well as its production of stimulant and growth-promoting substances such as growth regulators and hormonal (Al-Badawi, 2008; Ahsan *et al.,* 2012; Allawi, 2013). It also has the ability to stabilize atmospheric nitrogen and protects the plant host from certain pathogens due to its competition to revive other soil. These organisms contribute about 25% of its nutrient needs of plant, especially N, P, K and thus reduce production costs (Smith, 2003, 2008). *Azotobacter* and *Azospirllium sp*are considered from more organisms of nitrogen-fixing as well as its ability to reduce the water stress by regulating the water stress and water content of the plant while reducing structural stress of the soil Because these organisms are able to secrete Auxins which leading to the formation of a good radical system and improves the absorption of water and nutrient materials (El-Komy, 2003; Arzasesh *et al.,* 2011). Fertilizing has an important role in improving plant resistance to various stresses where potassium is considered one of the most important fertilizers that contribute to salinity tolerance and increase plant efficiency to drought resist. It also plays an important role in photosynthesis, transfer of photosynthesis products, preservation of plant cell production, enzymatic activity, protein synthesis and sap transfer within the plant and controlling transpiration Through its role in the mechanism of open and close of stoma and works to increase the availability of nutrient elements by reducing the absorption of elements with the toxic effect on the plant, such as sodium in saline conditions and the use of bio-fertilizer technologies and alternation irrigation.

Materials and Methods

The study was conducted in the Lath House belonging to Department of Horticulture and Landscaper, Abu Ghraib, Alternative location of College of Agriculture, University of Anbar for the spring season 2017 to study the effect of *Azospirillum brasilense*, the type of irrigation water and spraying (K_2SO_4) in the growth and plant yield of cut flowers and corms of *Gladiolus hybrida*. The homogeneous corms were taken in their diameter (3-4 cm) and size. The corms were cultivated in black plastic flowerpot of polyethylene with a capacity of 12 kg, 33 cm height and 22 cm diameter which contained of on loam and peatmoss 3: 1 and organic fertilizer with 3% soil weight. Soil analysis was conducted in the laboratories of College of Agriculture, University of Baghdad.

Study factors

The first factor: Bio-fertilizer: It was used in two levels: non-inoculation with Azospirillum bacteria (it is symbolized by B0) and inoculation with Azospirillum bacteria (it is symbolized by B1). The second factor is the type of irrigation water: it was used with three levels of irrigation water from the tap (0.8 $ds.m^{-1}$), it is symbolized by (W0), it is the control treatment for irrigation water level, well water levels at the location of the study (3.6 $ds.m^{-1}$), it is symbolized by (W1) and the alternation irrigation between the well water and the tap water (it is symbolized by W2). The third factor: spraying with Potassium Fertilizer (K_2SO_4), three concentrations of Potassium Fertilizer (K_2SO_4) were used spraying on the leaves are 0 $g.L^{-1}$ (it is symbolized by K0), 5 $g.L^{-1}$ (it is symbolized by K1) and 10 $g.L^{-1}$ (it is symbolized by K2). The plants were sprayed with 5 spraying, the first on 20/4/2017 after formation of four real leaves followed by other 4 spraying between the one spraying and the other 10 days. spraying was conducted in the early morning until full wetness, Tween 20 was used as a diffuser materials to reduce surface tension. One half of the fertilizer recommendation was used for nitrogen fertilizers and the full recommended fertilizer for phosphorus. A factorial experiment was conducted according to the Randomized Complete Block Design (RCBD), with three replicates, the single replicate contained on 18 treatments, so the number of experimental units became 54 experimental units and 6 plants per experimental unit. A number of traits were measured as indicators of growth and yield and recorded all the traits of vegetative growth at the beginning of the flowering (the appearance of inflorescence and the beginning of emergence of buds), included: plant height (cm), the leaf area (dm.plant^{-1}), the relative content of the chlorophyll in the leaves (SPAD UNIT), the percentage of dry matter in the leaves (%).The biochemical traits of N, P, K elements and the effectiveness of POD enzyme, the flowering growth traits, which included the diameter first base rosette (mm), Vase Life and the percentage of the dry matter of the corms were measured. The data was collected and Statistically analyzed according to the statistical program (Gen stat 2012), The averages treatments were compared at a probability level of 0.05.

Table 1: Represents some of the physical and chemical properties of the agriculture soil.

Analysis	Unit	value
EC	ds.m^{-1}	0.3
PH		7.18
N	%	0.002
P	mg.kg^{-1}	8.22
K availability		98.78
Ca	meq.L^{-1}	1.28
Mg		0.79
Cl		1.9
HCO_3		0.73
CO_3		Nill
Na		1.13
O.M	%	0.78
Dissolved K	Meq.L^{-1}	0.1
$CaCO_3$	%	26.13
Gypsum		0.13
SO_4		0.32
SAR1.		1.14
CEC		1.15
Number of Bacterial	Cfu	22×10^3
Number of fungi		11×10^3
Field capacity	cm^3.cm^3	0.36
Permanent wilt point	cm^3.cm^3	0.12
Texture		Sandy loam
Sand		75.2
Silt		8
Clay		16.8

Table 2: Represents some of the physical and chemical properties of well and tap water.

Analysis	Unit	Well water value	Tap water value
EC	ds.m^{-1}	3.2	0.8
PH		7.03	8.0
N	mg.kg^{-1}	21	
Ca	meq.L^{-1}	12.2	4.0
Mg		9.81	3.6
Cl		15.3	
HCO_3		1.53	
CO_3		nill	
Na		7.03	1.3
Number of Bacterial	Cfu	2.2×10^7	
Number of fungi		1.1×10^4	

Analyzes were conducted in the laboratories of College of Agriculture, Department of Horticulture, University of Baghdad.

Results

Plant height (cm)

Table 3 shows the significant effect of the three experimental treatments. In the first factor (bio-inoculation), the inoculation treatment with (*Azospirillum brasilense*) was significantly excelled in the traits of plant height which recorded the highest average of (113.24 cm), while the non-inoculation treatment recorded of 101.81 cm. The results showed that the effect of the type of irrigation water on the variation of plant height averages where that the treatment of W0 (the tap water) gave the highest average of 120.84 cm significantly excelling on the rest of the treatments while the lowest average of plant height at the treatment of w1 (well water) which amounted to 91.18 cm. It was noted that spraying with potassium (potassium sulfate K_2SO_4) gave a significant effect on the plant height. The K2 treatment with a concentration of 10 g.L^{-1} was excelled giving it an average of 112.94 cm which did not differ significantly with K1 treatment. While the treatment of K0 (spraying with distilled water only) gave the lowest average of plant height of 101.38 cm. As for the bi-interaction between Azospirillum bacteria and the type of irrigation water. The W0B1 treatment has significantly excelled by giving it a height of 125.67 cm, followed by the alternation irrigation treatment and the biological factor W2B1, while W1B0 treatment gave 84.99 by recording it the lowest average of plant height. In the bi-interaction between spraying with potassium and biological factor, it was found that the inoculation with bacteria and spraying with potassium contributed to the reduction of salinity damage. The K2B1 treatment gave the highest average of plant height of 118.44 cm which did not differ significantly from the K1B1 treatment and thus significantly excelled on the other treatments which reached 96.01 cm. For the bi-interaction between the type of irrigation water and the spraying with potassium, W0K2 treatment recorded the highest average of plant height of 126.15 cm, which

Table 3: Effect of bio-fertilization, water type and potassium in plant height (cm).

The type of irrigation water W	Spraying with potassium K	Bacterial inoculation B		W×K
		Non-inoculation B0	Inoculation B1	
Tap water W0	K0	109.92	119.00	114.46
	K1	117.00	126.83	121.92
	K2	121.13	131.17	126.15
Well water W1	K0	79.00	90.77	84.88
	K1	84.60	98.00	91.30
	K2	91.37	103.33	97.35
Alternation irrigation W2	K0	99.10	110.50	104.80
	K1	104.33	118.77	111.55
	K2	109.83	120.83	115.33
L.S.D		11.50		8.13
W×B		B0	B1	W
	W0	116.02	125.67	120.84
	W1	84.99	97.37	91.18
	W2	104.42	116.70	110.56
L.S.D		11.50		4.69
B×K		B0	B1	K
	K0 (0 $g.L^{-1}$)	96.01	106.76	101.38
	K1 (5 $g.L^{-1}$)	101.98	114.53	108.26
	K2 (10 $g.L^{-1}$)	107.44	118.44	112.94
L.S.D		6.64		4.69
B		101.81	113.24	
L.S.D		3.83		

was not significantly different from W0K1.W1K0 treatment recorded the lowest average of plant height of 84.88 cm. In the triple interaction between the study factors, W0K2B1 treatment gave the highest average of 131.17 cm, which did not differ significantly from W0K1B1 (W0K2B0, W2K2B1), while W1K0B0 gave the lowest average of 79.00 cm.

Relative content of chlorophyll in leaves (Spad unit)

Table 4 shows the superiority of biologically fertilized plants under the treatment of B1 significantly where the content of chlorophyll pigment (50.37 spad unit) while the non-fertilized plants gave B0 (48.05 Spad unit). The salinity stress from the different type of irrigation water caused a significant reducing in this traits where the irrigation treatment with well water W1 recorded (45.20 Spad unit), while the W0 treatment gave the highest average of (52.66 spad unit) significantly excelling on the rest of the treatments. The spraying treatment with potassium sulfate (K_2SO_4) at concentration of (10 $g.L^{-1}$) was significantly excelled by giving it an average of (50.59 Spad unit) while the spraying treatment with water K0 gave an average of (47.89 Spad unit). The interaction between the biological fertilizer with the type of irrigation water, The table shows a significant superiority of W0B1 treatment by giving it an average of (53.94 spad unit), while W1B0 treatment showed the lowest content of chlorophyll (44.24 spad unit). The interaction between bio-fertilizer with potassium, The table showed a significant superiority of K2B1 by giving it an average of (51.69 Spad unit) while K0B0 showed the lowest content of chlorophyll of (46.61 spad unit), and when the type of irrigation water and potassium were interacted, the effect of salinity was reduced than when spraying with potassium. The treatment of W0K2 gave the highest average of 53.88 and did not differ significantly with W0K1 treatment while the W1K0 treatment gave the lowest average of 44.04. As for Triple interaction, The results showed the excelling of W0K2B1 treatment, which gave 55.50, while the treatment of W1K0B0 recorded the lowest average of 42.60.

Percentage of dry matter of leaves

Table 5 shows that the treatment of bio-fertilization with bacterium Azospirillum (B1) was significantly excelled which amounted of 24.10% and while B0 treatment gave of (22.31%). The negative effect of salinity of irrigation water, where W1 (water well) treatment recorded the lowest average of 21.36%, while the W0 (tap water) treatment was significantly excelled

Table 4: Effect of bio-fertilization, water type and potassium in the relative content of chlorophyll in leaves (Spad unit).

The type of irrigation water W	Spraying with potassium K	Bacterial inoculation B		W×K
		Non-inoculation B0	Inoculation	
Tap water W0	K0	50.03	52.97	51.50
	K1	51.83	53.37	52.60
	K2	52.27	55.50	53.88
Well water W1	K0	42.60	45.57	44.08
	K1	45.03	45.77	45.40
	K2	45.10	47.13	46.12
Alternation irrigation W2	K0	47.20	48.97	48.09
	K1	47.27	51.60	49.43
	K2	51.13	52.43	51.78
L.S.D		2.04		1.44
W×B		B0	B1	W
	W0	51.38	53.94	52.66
	W1	44.24	46.16	45.20
	W2	48.53	51.00	49.77
L.S.D		1.18		0.83
B×K		B0	B1	K
	K0 (0 g.L^{-1})	46.61	49.17	47.89
	K1 (5 g.L^{-1})	48.04	50.24	49.14
	K2 (10 g.L^{-1})	49.50	51.69	50.59
L.S.D		1.18		0.83
B		48.05	50.37	
L.S.D		0.68		

by giving it an average of (24.21%) which did not differ significantly from the alteration irrigation treatment which amounted of 24.04%. While the treatment of (K0) gave 22.04%. The effect of the biological factor and the alteration irrigation show in the bi-interaction between the Azospirillum bacteria and the type of irrigation water where the treatment of W0B1 was excelled by recording it a value of (25.19%) and did not differ significantly from W2B1 treatment. While the W1B0 treatment gave the lowest average of 20.40%. In the bi-interaction between potassium and the bio-factor, the K2B1 treatment recorded the highest value of 25.21% while the lowest value was for K0B0 treatment. The results of the interaction between potassium and irrigation water showed the effect of potassium and the alteration irrigation in reducing the salinity of irrigation water. The W0K2 treatment recorded the highest value of 25.79% and without significant difference from the W2K2 treatment, while the W1K0 treatment recorded the lowest value of 20.25%. In the triple interaction for the study factors, The treatment of W0K2B1 was excelled by giving it a value of 26.49% and did not differ significantly from W2K2B1 and W0K2B0. While W1K0B0 treatment gave the lowest value for the average percentage of dry matter of leaves was 18.96%.

The diameter of first base rosette

Table 6 shows that the superiority of the bio-fertilizer treatment (Azospirillum bacteria) significantly in the trait of the diameter of first base rosette of 73.29 mm compared to the non-fertilized plants which gave 68.43 mm. The salt stress effect represented by the type of irrigation water was significantly decreased in the diameter of first base rosette which gave of 62.8 mm in W1 treatment compared to W0 treatment, which gave 75.84 mm, which did not differ significantly from W2 treatment. The spraying with potassium (potassium sulphate K_2SO_4) showed a significant effect in this trait. The K2 treatment (10 g.L^{-1}) was excelled by giving it an average of 74.65 mm compared to the K0 treatment, which gave the lowest average of the diameter of first base rosette, which recorded (67.77 mm). The bi-interaction between Azospirillum and the type of irrigation water showed that the bacteria contributed to the reduction of salinity damage as well as alteration irrigation. Where the treatment of W0B1 was significantly excelled by giving it an average of 78.51 mm, which did not differ

Table 5: Effect of bio-fertilization, water type and potassium in the percentage of dry matter for leaves (%).

The type of irrigation water W	Spraying with potassium K	Bacterial inoculation B		W×K
		Non-inoculation B0	Inoculation	
Tap water W0	K0	21.59	24.28	22.94
	K1	23.03	24.79	23.91
	K2	25.09	26.49	25.79
Well water W1	K0	18.96	21.54	20.25
	K1	20.80	22.27	21.53
	K2	21.44	23.16	22.30
Alternation irrigation W2	K0	22.23	23.63	22.93
	K1	23.49	24.74	24.11
	K2	24.17	25.99	25.08
L.S.D		1.58		1.12
W×B		B0	B1	W
	W0	23.24	25.19	24.21
	W1	20.40	22.32	21.36
	W2	23.30	24.79	24.04
L.S.D		0.91		0.65
B×K		B0	B1	K
	K0 (0 g.L^{-1})	20.93	23.15	22.04
	K1 (5 g.L^{-1})	22.44	23.93	23.19
	K2 (10 g.L^{-1})	23.56	25.21	24.39
L.S.D		0.91		0.65
B			22.31	24.10
L.S.D		0.53		

significantly with alteration irrigation treatment W2B1 while W1B0 treatment gave 60.48 mm.In the bi-interaction between the spraying with potassium and bio-factor, K2B1 treatment gave the highest average of the diameter of first base rosette of 78.93 mm, while K0B0 gave the lowest average of the diameter of first base rosette which amounted of 66.18 mm. The effect of potassium and alternation in irrigation was shown to reduce the adverse effect of salinity and increase in of the diameter of first base rosette. W0K2 treatment recorded the highest average of the diameter of first base rosette was 80.52 mm, which did not differ significantly from W2K2 treatment while W1K0 recorded the lowest average of 59.88. In the triple interaction between the study factors, W0K2B0 treatment gave the highest average of 85.90 mm, which did not differ significantly from W2K2B1 treatment, while W1K0B0 treatment gave the lowest average of 57.59 mm.

Vase life using distilled water (day)

The period of survival of the flowers in the vase is a significant trait, depending on the amount of nutrient absorbed by the plant and on the environmental envelope prior to cut flowers. Table 7 shows the superiority of the bio-fertilized plants under the treatment of B1 significantly which gave of 8.02 days while the non-fertilized plants B0gave 6.67 days. The saline stress due to the different type of irrigation water was significantly reduced in this trait. Where the treatment of irrigation with well water W1 recorded (5.60 days), While the W0 treatment gave the highest average of 8.60 days. Spraying with a concentration of (10 g.L^{-1}) of potassium sulphate (K_2SO_4) gave a significant increase in vase life which amounted of 8.04 days, which did not differ significantly from K1 treatment, while the spraying treatment with water K0 gave the lowest average of 6.79 days. From the interaction of the bio-fertilizer with the type of irrigation water, the table shows significant excelling of W0B1 treatment by recording it an average of 9.46 days, while the treatment of W1B0 gave the lowest average, which amounted to 5.08 days. From the interaction between bio-fertilizer and potassium. The table showed a significant superiority of K2B1 treatment by giving it an average of 8.85 days, while K0B0 gave the lowest average for vase life of 6.14 days. When the type of irrigation water was interacted with potassium, it was observed that the salinity damage was decreased when spraying with potassium and the alteration irrigation. The treatment of W0K2 gave the highest average of 9.31 days, which did not differ

Table 6: Effect of bio-fertilization, water type and potassium in the diameter of first base rosette (mm).

The type of irrigation water W	Spraying with potassium K	Bacterial inoculation B		W×K
		Non-inoculation B0	Inoculation	
Tap water W0	K0	70.93	73.32	72.13
	K1	73.44	76.31	74.87
	K2	75.14	85.90	80.52
Well water W1	K0	57.59	62.17	59.88
	K1	60.85	64.44	62.64
	K2	63.00	68.77	65.88
Alternation irrigation W2	K0	70.03	72.60	71.31
	K1	71.93	73.96	72.94
	K2	72.97	82.12	77.54
L.S.D		6.73		4.76
W×B		B0	B1	W
	W0	73.17	78.51	75.84
	W1	60.48	65.13	62.80
	W2	71.64	76.23	73.93
L.S.D		3.89		2.75
B×K		B0	B1	K
	K0 (0 g.L^{-1})	66.18	69.37	67.77
	K1 (5 g.L^{-1})	68.74	71.57	70.15
	K2 (10 g.L^{-1})	70.37	78.93	74.65
L.S.D		3.89		2.75
B		68.43	73.29	
L.S.D		2.24		

significantly from the W2K2 and W0K1 treatments, while the W1K0 treatment gave the lowest average of vase life of 5.02 days. W0K2B1 treatment was significantly excelled by recording it the highest average of vase life of 10.51 days, which was not significantly different from W2K2B1 and W0K1B1, while W1K0B0 gave the lowest average of 4.55 days.

Percentage of nitrogen in leaves%

Table 8 shows that the using of bio-fertilizer gave a significant increase in the percentage of nitrogen where the percentage of nitrogen increased from 1.96 to non-fertilized treatment B0 to amounted of 2.48 at B1 treatment. Saline stress contributed to a decrease in the percentage of nitrogen in the leaves. A well water treatment W1 recorded of 1.48% while W0 treatment gave the highest percentage of nitrogen 2.64%. The spraying with potassium affected by increasing the percentage of nitrogen. Where the treatment of K2 recorded (2.52%) the highest average for the percentage of nitrogen, with a significant increase of 34.04% compared to K0 treatment which gave 1.88%. The interaction of the Azospirillum and the type of irrigation water showed that the W0B1 treatment was significantly excelled by giving it an average of 2.97%. While the lowest average were 1.33% for W1B0 treatment. The interaction treatment K2B1 has excelled by recording it an average of 2.75 % compared to K0B0 treatment which gave 1.62%. The role of potassium and the type of irrigation water by increasing the percentage nitrogen and reducing the negative salinity effect in the absorption of this element, The treatment of W0K2 gave 2.93%, which was not significantly different from the W2K2 treatment, while the lowest percentage of nitrogen for the treatment of W1K0 was 1.20%. triple interaction of experimental factors showed that W0K2B1 was excelled by recording of 3.25% and did not differ significantly from W2K2B1 and W0K1B1. W1K0B0 treatment gave the lowest value of 1.04%.

Percentage of phosphorus in leaves%

Table 9 shows the positive effect of Azospirillum bacteria, which increased the number of root hairs and thus increase the surface area of the roots, which led to increased absorption of water and nutrient elements, including phosphorus availability, Where the bio-fertilizer treatment B1 was excelled by giving it the highest average of 10.41%, while B0 treatment amounted to 0.358%. It also shows the negative impact of irrigation water salinity, W1 (water well) treatment recorded the lowest average

Table 7: Effect of bio-fertilization, water type and potassium in the vase life (day).

The type of irrigation water W	Spraying with potassium K	Bacterial inoculation B		W×K
		Non-inoculation B0	Inoculation	
Tap water W0	K0	7.16	8.72	7.94
	K1	7.93	9.15	8.54
	K2	8.12	10.51	9.31
Well water W1	K0	4.55	5.50	5.02
	K1	4.70	5.87	5.29
	K2	6.00	6.99	6.49
Alternation irrigation W2	K0	6.72	8.07	7.39
	K1	7.33	8.32	7.83
	K2	7.57	9.04	8.31
L.S.D		1.68		1.19
W×B		B0	B1	W
	W0	7.73	9.46	8.60
	W1	5.08	6.12	5.60
	W2	7.21	8.48	7.84
L.S.D		0.97		0.69
B×K		B0	B1	K
	K0 (0 g.L^{-1})	6.14	7.43	6.79
	K1 (5 g.L^{-1})	6.65	7.78	7.22
	K2 (10 g.L^{-1})	7.23	8.85	8.04
L.S.D		0.97		0.69
B		6.67	8.02	
L.S.D		0.56		

of 0.322, while the W0 (tap water) treatment was significantly excelled by giving it an average of 0.438% followed by W2 treatment which reached 0.394%. The spraying treatment with potassium (10 g.L-1) (K2) recorded the highest average of (0.421%), while the treatment of K0 gave 10.35%, W0B1 treatment was excelled by giving it an average of 0.666%, while W1B0 treatment recorded the lowest average of 0.290%. In the bi-interaction between potassium and the bio-fertilizer, K2B1 treatment recorded the highest value of 20.45% while the lowest value was for K0B0 treatment. Results of the interaction between potassium and the type of irrigation water showed significant increase in the percentage of phosphorus. The treatment of W0K2 was excelled by giving it an average of 0.477%, which was not significantly differed from W0K1 and W2K2. W1K0 treatment recorded 0.280%. In the triple interaction of the study factors, the effect of the bio-factor, potassium and alternation decreased the negative effect of salinity of irrigation water. The treatment of W0K2B1 was excelled by giving it the highest value of 30.51%, which did not differ significantly from the W0K1B1, W2K2B1 and W0K2B0 treatment, while W1K0B0 gave the lowest value of 0.557 %.

Percentage of potassium in leaves%

Table 10 shows that the treatment of bio-fertilizer with Azospirillum bacterium (B1) has excelled in the increase the percentage of potassium which amounted of 3.38% while B0 treatment gave 3.25%. The negative effect of irrigation water salinity, W1 (well water) treatment recorded the lowest average of 3.22%, while the W0 (tap water) treatment was significantly excelled by giving it an average of 3.39%. The spraying treatment with potassium (10 g.L^{-1}) (K2) achieved the highest average was 3.47%, while the treatment of K0 gave 3.14%, The bi-interaction treatment W0B1 was excelled by giving it 3.45%, which did not differ significantly from W2B1, while W1B0 gave the lowest average of 3.15%. In the bi-interaction between potassium and bio-factor, K2B1 treatment recorded the highest average of 3.47% and the lowest value for K0B0 treatment was 3.03%. Results of the interaction between potassium and irrigation water were significantly excelled. where the two interaction treatment of (W0K2 and W2K2) recorded the highest values of (3.56%, 3.49%), respectively. W1K0 treatment gave the lowest value of 3.05%. In the triple interaction of the study factors, the treatment of W0K2B1 was excelled by giving it the highest value of 3.61% with

Table 8: Effect of bio-fertilization, water type and potassium in the percentage of nitrogen in leaves (%).

The type of irrigation water W	Spraying with potassium K	Bacterial inoculation B		W×K
		Non-inoculation B0	Inoculation	
Tap water W0	K0	2.01	2.60	2.31
	K1	2.30	3.06	2.68
	K2	2.60	3.25	2.93
Well water W1	K0	1.04	1.37	1.20
	K1	1.33	1.64	1.48
	K2	1.62	1.92	1.77
Alternation irrigation W2	K0	1.80	2.49	2.15
	K1	2.33	2.86	2.59
	K2	2.65	3.10	2.88
L.S.D		0.22		0.16
W×B		B0	B1	W
	W0	2.30	2.97	2.64
	W1	1.33	1.64	1.48
	W2	2.26	2.82	2.54
L.S.D		0.13		0.09
B×K		B0	B1	K
	K0 (0 g.L^{-1})	1.62	2.15	1.88
	K1 (5 g.L^{-1})	1.98	2.52	2.25
	K2 (10 g.L^{-1})	2.29	2.75	2.52
L.S.D		0.13		0.09
B		1.96	2.48	
L.S.D		0.07		

no significant difference with two treatments of W2K2B1 and W0K2B0, while W1K0B0 gave the lowest value of the percentage of potassium of 2.92%.

Effectiveness of peroxidase enzyme (POD) (Absorption unit. mg^{-1} protein)

Table 11 shows that the bio-fertilization with Azospirillum achieved a significant effect in reducing the effectiveness of the POD enzyme in the leaves of the plant where it reached (2.24 µg.g^{-1} dry weight protein) compared to the non-fertilized plants which reached (2.90 µg.g^{-1}dry weight protein). The results showed that the effect of the POD enzyme on the leaves was affected by the type of irrigation water were it increased from (0.51 unit.mg^{-1}) in the treatment of W0 (tap water) to 1.55 in W1 treatment (well water). The spraying with potassium significantly reduced the effectiveness of POD enzyme in the leaves from (3.02 units.mg^{-1}) in the treatment of K0 to (2.24 units.mg^{-1}) in the K2 treatment. In the interaction between the bacteria and the type of irrigation water, this interaction contributed in reducing the effectiveness of POD enzyme in the leaves. Where the treatment of W0B1 gave the lowest average of effectiveness of the POD (0.38 units. mg^{-1}) while the treatment of W1B0 gave the highest average of (6.33 units. mg^{-1}). The bi-interaction between the bio-fertilizer and potassium was also significantly affected. The K2B1 treatment recorded the lowest value of (2.01 units. mg^{-1}). While the treatment of K0B0 gave the highest average of (3.57 units. mg^{-1}). The results showed the effect of potassium when it was interacted with the type of irrigation water. W0K2 treatment recorded the lowest average of 0.39 while W1K0 gave the highest average of (6.62 units. mg^{-1}). In the triple interaction, the effect of bacteria, potassium and the type of irrigation water showed to reduce the effectiveness of the POD enzyme. W0K2B1 treatment recorded the lowest average of the effectiveness of the POD enzyme of (0.27 units.mg^{-1}), while W1K0B0 recorded the highest average of (7.91 units.mg^{-1}).

Discussion

The inoculation with Azospirillum recorded an increase in all traits of vegetative growth represented by plant height, the relative content of chlorophyll, the percentage of dry matter for leaves of Gladiolus plant as shown in tables (3-5). This may be due to the ability of Azospirillum bacteria to secrete many compounds that improve physiological processes such as Auxins, cytokinines and Gibberellins rather than to its stabilizing

Table 9: Effect of bio-fertilization, water type and potassium in the percentage of phosphorus in leaves (%).

The type of irrigation water W	Spraying with potassium K	Bacterial inoculation B		W×K
		Non-inoculation B0	Inoculation	
Tap water W0	K0	0.380	0.420	0.400
	K1	0.410	0.463	0.437
	K2	0.440	0.513	0.477
Well water W1	K0	0.257	0.303	0.280
	K1	0.283	0.363	0.323
	K2	0.330	0.393	0.362
Alternation irrigation W2	K0	0.353	0.390	0.372
	K1	0.370	0.403	0.387
	K2	0.397	0.450	0.423
L.S.D		0.079		0.056
W×B		B0	B1	W
	W0	0.410	0.466	0.438
	W1	0.290	0.353	0.322
	W2	0.373	0.414	0.394
L.S.D		0.046		0.033
B×K		B0	B1	K
	K0 (0 g.L^{-1})	0.330	0.371	0.351
	K1 (5 g.L^{-1})	0.354	0.410	0.382
	K2 (10 g.L^{-1})	0.389	0.452	0.421
L.S.D		0.046		0.033
B		0.358	0.411	
L.S.D		0.027		

of atmospheric nitrogen and thereby increase the absorption of nutrient elements, including nitrogen (Samurai and Rahi, 2006; Allawi, 2013). This in turn improves plant growth as nitrogen stimulates the plant to build proteins. The nitrogen fixation and associated Nitrogenase secretion increases the effectiveness of gibberellins and cytokinines in plant tissues, which stimulate cell division, differentiation and elongation, increase chlorophyll production and thus obtain more energy which contributes to increased vegetative growth (Bashan and de-Bashan, 2010). The reason for the decrease in the indices of vegetative growth in this study for Gladiolus plant (Ammsterdam cultivar) when irrigation with well water may be due to the direct effect of salinity in irrigation water in inhibiting the work of enzymes, which leads to imbalance in food and cellular membrane functions and metabolism of the plant, This in turn affects carbon construction, transfer of energy and respiration. The effect may be indirectly caused by the salinity effect on the physical and chemical properties of the soil as in Table 2, which leads to an increase in the Osmotic pressure and decrease the ability of the plant to absorb the water. The shrinkage of the cells decreases. The amount of CO_2 is reduced by the Stoma. The production of active oxygen is increased, An ionic imbalance occurs, The leaves content of chlorophyll decreases, and thus decrease of carbohydrates, proteins and hormones leading to reduction of surface area of leaves and decrease in plant height and plant diameter as shown in tables 3-5, The cause may be due to the toxicity of Na and Cl ions, which cause low the leaves content of nitrogen and magnesium. Dry weight gain may be due to the fact that the addition of potassium has a role in most vital activities related to growth, cell division and increasing the growth. Which encourages the formation of a radical total that increases the efficiency of absorption of water and nutrients, which are used efficiently in the manufacture of carbohydrates in the process of photosynthesis and then converted to protein substances involved in the formation of tissues and members of the plant, which leads to increased dry matter of the plant (Yassin, 2001). Potassium contributes in the stimulate of photosynthesis process by its effect on the construction of the ATP energy compound, which is considered a major energy carrier in the plant and its reservoir for the stabilization of CO_2 as well as its effect on the construction of sugars (Cakmak, 2005; Al-Samurai *et al.,* 2014). Potassium has a role in reducing the effect of salt stress by excluding the sodium and chlorine elements and maintaining a high percentage of potassium in the leaves and by controlling the process

Table 10: Effect of bio-fertilization, water type and potassium in the percentage of potassium in leaves (%).

The type of irrigation water W	Spraying with potassium K	Bacterial inoculation B		W×K
		Non-inoculation B0	Inoculation	
Tap water W0	K0	3.12	3.29	3.21
	K1	3.36	3.45	3.41
	K2	3.50	3.61	3.56
Well water W1	K0	2.92	3.17	3.05
	K1	3.21	3.29	3.25
	K2	3.31	3.40	3.35
Alternation irrigation W2	K0	3.04	3.28	3.16
	K1	3.29	3.41	3.35
	K2	3.45	3.53	3.49
L.S.D		0.13		0.09
W×B		B0	B1	W
	W0	3.33	3.45	3.39
	W1	3.15	3.29	3.22
	W2	3.26	3.41	3.33
L.S.D		0.08		0.05
B×K		B0	B1	K
	K0 (0 g.L^{-1})	3.03	3.25	3.14
	K1 (5 g.L^{-1})	3.29	3.39	3.34
	K2 (10 g.L^{-1})	3.42	3.51	3.47
L.S.D		0.08	0.05	
B		3.25	3.38	
L.S.D		0.04		

of opening and closing the Stoma through the swelling and shrinkage of the Stoma and helps in the regulation of the Osmotic pressure. Alternation Irrigation led to reduce salts in the growth media, which stimulated plant growth. These results agree with (Yue *et al.,* 2012; Al-Taher *et al.,* 2014) showed that the well-irrigated land with a low salinity level would stimulate the plant to grow. As is known, one third of the preservation capacity of flowers is due to pre-cut conditions (Masar, 2003; Abdullatif, 2006; Khan *et al.,* 2009).The results also showed that the spraying with Potassium led to an increase in all flowering traits, which can be attributed to the role of potassium in all enzymatic processes, carbonate construction and biotic events, which increases vegetative growth and leaf area (Sadak *et al.,* 2010). Tables 6-9 show the increase in the levels of elements N, P and K. This is due to the ability of Azospirillum bacteria to increase nutrient processing of the plant. These bacteria are capable of stabilizing atmospheric nitrogen and raising the efficiency of the plant by nitrogen absorption (Bashan and De-Bashan, 2010). As well as the secretion of these bacteria to Auxins, which is a major development in the root system and is due to the absorption of nitrates, phosphates and potassium (Bashir, 2003) as well as their production of growth regulators that increase the concentration of potassium and improve its functionality, For a positive correlation between nitrogen and potassium, the increase in nitrogen is accompanied by increased potassium (Krishna, 2002; Singh *et al.,* 2015). The increase in the concentration of potassium to the spraying treatments with potassium may be due to increased salinity of irrigation water led to decrease in plant content of nutrient elements, The concentration of NaCl in the soil solution results in an increase in the Osmosic pressure of the soil solution, making it difficult for the plant to absorb water and ions, Inhibition of cell growth and division occurs, in the cellular membranes also occurs, which negatively affects their elective permeability. As well as the state of contrast between chlorine ion and nitrate NO_3- leading to reduced absorption of nitrates and also reduce the absorption of 2-HPO, which precipitate with calcium, magnesium and zinc and transformation to the non-available state for plant (Ashraf, Harris, 2004; Abdul Latif and chaoxing, 2011). A contrast between sodium and potassium occurs, leading to a sharp decrease in the concentration of potassium in the leaves due to the high concentration of sodium around the roots (Cuin *et al.,* 2009). The results of this study indicate a decrease in the effectiveness of the peroxidase enzyme, because these bacteria are able to produce hormones, Auxins and

Table 11: Effect of bio-fertilization, water type and potassium in the effectiveness of peroxidase enzyme (POD) (Absorption unit. mg-1 protein).

The type of irrigation water W	Spraying with potassium K	Bacterial inoculation B		W×K
		Non-inoculation B0	Inoculation	
Tap water W0	K0	0.87	0.46	0.67
	K1	0.54	0.41	0.47
	K2	0.52	0.27	0.39
Well water W1	K0	7.91	5.32	6.62
	K1	5.68	4.70	5.19
	K2	5.42	4.33	4.87
Alternation irrigation W2	K0	1.93	1.65	1.79
	K1	1.78	1.60	1.69
	K2	1.50	1.43	1.47
L.S.D		0.45		0.32
WB		B0	B1	W
	W0	0.64	0.38	0.51
	W1	6.33	4.78	5.56
	W2	1.73	1.56	1.65
L.S.D		0.26		0.18
BK		B0	B1	K
	K0 (0 $g.L^{-1}$)	3.57	2.48	3.02
	K1 (5 $g.L^{-1}$)	2.66	2.24	2.45
	K2 (10 $g.L^{-1}$)	2.48	2.01	2.24
L.S.D		0.08		0.26
B		2.90	2.24	
L.S.D		0.15		

Gibberellins and cytokinines that increase the efficiency of water absorption by the roots and thus increase the transpiration process and thus increase the absorption and transfer of metal ions (Haroun *et al.,* 2003). This may be due to the ability of these bacteria to improve plant growth under conditions of salt stress and thus decrease the production of effective Oxygen and thus decrease the effectiveness of antioxidant enzymes, including POD and perhaps the reason for the decline of POD to the role of potassium in the improvement of plant metabolism under conditions of salt stress.

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