Studying bearing of some cultivars of clove plant (*Dianbhus caryophyllus* L.) for salt stress under Iraqi conditions

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ABSTRACT

The experiment was conducted in the unheated greenhouse belonging to the Department of Horticulture and Landscape Gardening, College of Agriculture, Al-Qasim Green University during the growth season (2017-2018) in order to study bearing of some cultivars of clove plant (*Dianbhus caryophyllus* L.) for salt stress under Iraqi conditions. The addition of the bio-vaccine led to a significant increase in the averages of traits of plant height, leaf area, reducing the number of days required to open the first flower on the plant, vase life of flowers, length of the main root, number of main roots, the leaves content of total soluble carbohydrates, decreasing the effectiveness of enzymatic antioxidants ((Superoxide dismutase(SOD) and Catalase (CAT)), which amounted to (59.81 cm, 248.44 cm^2, 159.46 days, 6.55 days, 20.80 cm, 17.18 $roots.plant^{-1}$, 11.43 mg. 100 g^{-1} dry weight, 31.45 $units.mol^{-1}$, and 8.74 $units.mol^{-1}$), respectively. Salt stress reduced the averages of vegetative and root traits and The lowest values were recorded in plants growing under the S4 salt level (9 $ds.m^{-1}$), which led to un-flowering the plants. Triple interaction treatments between the study factors worked to achieve the best values for the averages of all studied traits represented by the plant height, leaf area, the number of days required to open the first flower on the plant, the vase life of the flowers, the length of the main root, the number of main roots, the leaves content of total soluble carbohydrates and the effectiveness of enzymatic antioxidants (Superoxide dismutase and Catalase), which amounted to (80.90 cm, 472.08 cm^2, 178.22 days, 10.16 days, 37.21 cm, 30.29 $roots.plant^{-1}$, 16.64 mg.100 g^{-1} dry weight, 17.32 $units.mol^{-1}$, 1.67 $units.mol^{-1}$), respectively.

دراسة تحمل بعض أصناف القرنفل *Dianbhus caryophyllus L.* للإجهاد الملحي تحت ظروف العراق

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الخلاصة :

نفذت التجربة في البيت الزجاجي غير المدفئ التابع لقسم البستنة وهندسة الحدائق - كلية الزراعة - جامعة القاسم الخضراء خلال موسم النمو2017 - 2018 بهدف دراسة تحمل بعض أصناف القرنفل للإجهاد الملحي تحت ظروف العراق. أدت إضافة اللقاح الإحيائي إلى زيادة معنوية في معدلات صفات ارتفاع النبات, المساحة الورقية , تقليل عدد الأيام اللازمة لتفتح أول زهرة على النبات, العمر ألمزهري للأزهار, طول الجذر الرئيس , عدد الجذور الرئيسة, محتوى الأوراق من الكاربوهيدرات الذائبة الكلية, انخفاض فاعلية مضادات الأكسدة الأنزيمية (Superoxide dismutase (SOD و Catalase (CAT) والتي سجلت 59.81سم, 248.44سم^2, 159.46يوم, 6.55يوم, 20.80سم, 17.18جذر.نبات$^{-1}$, 11.43ملغم.100غم$^{-1}$ وزن جاف, 31.45وحدة.مول$^{-1}$, 8.74وحدة.مول$^{-1}$ على التوالي. أدى الإجهاد الملحي إلى خفض معدلات الصفات الخضرية والجذرية وسجلت اقل القيم في النباتات النامية تحت المستوى الملحي S_4(9 $dS.m^{-1}$) الذي سبب عدم ازهار النباتات. عملت معاملات التداخل الثلاثي بين عوامل الدراسة على تحقيق أفضل القيم لمعدلات جميع الصفات المدروسة والمتمثلة بارتفاع النبات و المساحة الورقية وعدد الأيام اللازمة لتفتح أول زهرة على النبات والعمر ألمزهري للأزهار وطول الجذر الرئيس وعدد الجذور الرئيسة ومحتوى الأوراق من الكاربوهيدرات الذائبة الكلية وفاعلية مضادات الأكسدة الأنزيمية (CAT,SOD) والتي بلغت 80.90سم, 472.08سم^2, 178.22يوم, 10.16يوم, 37.21سم, 30.29جذر.نبات$^{-1}$, 16.64ملغم.100غم$^{-1}$ وزن جاف, 17.32وحدة.مول$^{-1}$, 1.67وحدة.مول$^{-1}$ على التوالي.

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1. INTRODUCTION

Clove plant (*Dianthus caryophyllus* L.) is one of Caryophyllaceae family plants, which their plants grow in the temperate zone of the northern hemisphere, and this family includes 2100 species and 89 genera, one of which is Dianthus genus which includes about 300 species, most of them are annual or perennial herbs, and few are shrubs (15). It is one of the perennial herbaceous plants with special education, where it requires high technical expertise in order to produce its flowers with good commercial quality. Clove is considered one of the internationally and economically important cut flowers, with great coordination and aesthetic value. Despite the importance of cloves and the desire to produce its flowers with high-quality specifications, but we note that environmental factors often stand in the way of this desire, especially the problem of salinity of irrigation water, which limits its cultivation in areas prone to a lack of irrigation water suitable for agricultural irrigation, where high salinity causes deadly effects for the plant such as the decrease in the water potential for plant cells and the ionic toxicity of sodium and chloride (5). The use of biotechnology and its various applications is considered a scientific and civilized revolution initiated by developed countries and made significant scientific innovations and remarkable achievements, Which prompted other countries to follow the example of those developed countries and benefit from them in the development and advancement of society. Therefore, many studies were conducted by researchers in order to improve the tolerance of plants to salt stress through several traditional methods such as the production of salt-tolerant cultivars through breeding and selection, but these methods have become uneconomical nowadays because they require a long time and great effort (2). In addition to the previous capabilities, there are serious attempts in recent decades to use agricultural, physiological and biological methods such as the use of bacterial and fungal microorganisms that encourage growth and raise the plant's ability to withstand salt stress by secreting Globin that works to hold soil particles and increases the soil's ability to retain water and increase plant resistance to biotic and abiotic stresses (10). In addition to its role in increasing the availability of the essential elements for plant growth, such as nitrogen, which is fixed by Alazosperlum bacteria nd phosphorus, which prepares mycorrhizal fungi and their work to reduce the number of soil interaction (pH), which increases the availability of micronutrients required by the plant and enrich the root environment with many compounds, as well as the production of Growth regulators such as Auxins, gibberellins, and cytokines (9, 26). Sonneveld et al., (24) observed that when growing gerbera in nutritional solutions with an electrical conductivity of (1.8, 2.2, 2.7, 3.2 and 3.7 $ds.m^{-1}$), Increasing the concentration of sodium chloride salts in the nutrient solution reduces the diameter of the flower for the plant. Sahi, (6) mentioned when studying the effect of the salinity of irrigation water on the growth of the plant that the number of flowers, their diameter, and the length of the flower stalk for the plant decreased with increasing levels of salinity of irrigation water. Narayana Gowda, (18) also mentioned that inoculation the roots of the Gerbera plant with Azospirillum bacteria and mycorrhizal fungi had a positive effect in prolonging the vase life of the cut flowers. The use of mycorrhizal fungi had a positive effect on prolonging the vase life of the cut flowers. The use of mycorrhizal fungi and Phosphorus-solubilizing bacteria in Azospirillum bacteria in the inoculation of the clove plant led to improve the plant growth and increased the number of flowers buds and the size of the formed flowers, in addition to prolonging the vase life of the cut flowers (12). Due to the great economic importance of clove plants and the lack of interest in cultivating them for cut flowers in Iraq and the lack of studies on introducing technologies to raise the ability of cloves to tolerate salt stress, The intention was to conduct this study, which aims to introduce new cultivars of cloves suitable for commercial picking into Iraq and studying their vegetative and flowering traits in the conditions of the

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region and evaluate the tolerance of these cultivars for salt stress resulting from the salinity of irrigation water with the help of some bio-fertilizers.

2. MATERIALS AND METHODS

The study was conducted in the unheated greenhouse belonging to the Department of Horticulture and Landscape Gardening, College of Agriculture, Al-Qasim Green University during the growth season (2017-2018) in order to study bearing of some cultivars of clove plant (*Dianbhus caryophyllus* L.) for salt stress under Iraqi conditions. Small seedlings with a length of (8-10 cm) were used not exceeding four nodes for the five vegetative cultivars of cloves produced by Hilverda Kooij company, which were propagated by tissue culture. These seedlings were planted in the autumn season on November 1, 2017, in plastic flowerpots with a capacity of (3.500 kg), a diameter of (25 cm), and a height of (24 cm) after filling it with an agricultural media consisting of river soil and peat moss with a ratio of 2: 1, respectively, by one plant per pot. Before starting the agricultural operations, a random sample of the study soil was taken before mixing it with peat moss and chemical and physical analyzes were conducted as shown in Table (1) in the laboratories of the Department of Soil Science and Water Resources, College of Agriculture, Al Qasim Green University. As for the peat moss, its traits were fixed on the bags. Table (2) shows the chemical traits of the peat moss (German origin) producing from the Sab-Germany company.

Table 1: Some chemical and physical traits for study soil before cultivating

Trait		Value	Unit
Degree of soil reaction (pH)		7.4	
Electrical conductivity (EC)		1.7	$dS.m^{-1}$
Organic matter		0.83	%
Available ions	Nitrogen	16.8	$mg.kg^{-1}$ soil
	Phosphorus	10.5	
	Potassium	168.3	
Soil separates	Sand	322	$g.kg^{-1}$ soil
	Silt	345	
	Clay	333	
Soil texture		**sandy loam**	

Table 2: The chemical traits of the used peat moss in agriculture.

Salt content $g.L^{-1}$	pH	N $mg.L^{-1}$	P_2O_5 $mg.L^{-1}$	K_2O $mg.L^{-1}$
0.9-0.7	6.5-5.7	160-70	180-70	190-80

All agricultural operations used in the breeding of these plants were conducted by commercial harvesters for all plants in experimental units and whenever needed. The second is four salt levels of irrigation water. The third factor is the bio-vaccine, which is two levels without the bio-vaccine, and it is symbolized by (M1). Treatment of the bio-vaccine, which is symbolized by (M2), where 10 g of the bio-vaccine loaded on the peat moss, consisting of the mixture of mycorrhiza fungus (Glomus mosseae) (50 spores/ g soil) and Azospirilum brasilense produced in the Agricultural Research Department belonging to the Ministry of Science and Technology. The mixture was placed in the pit prepared for cultivating seedlings. The bio-vaccine must be in contact with the roots of cultivated clove seedlings.

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Table 3: shows the salt levels used in irrigating clove plants

Quality of used irrigation water	Salt level	Concentration ($dS.m^{-1}$)
Tap water only	S1	1.12
Mixing the drainage water with the Tap water	S2	3
	S3	6
	S4	9

The four salt levels were prepared by calculating the amount of drainage water mixed with tap water using the mixing equation proposed by workers in the American Salinity Laboratory (20) for the purpose of reaching the concentrations shown in Table (3).

Mixing treatment = $\frac{v_g}{v_p} = \frac{s_p - s_b}{s_p - s_g}$

where: vg: volume of good water (Tap water), vp: volume of saltwater (drainage water)

sp: salinity of saline water (drainage water), sb: The required salinity.

SG: Good Water Salinity (Tap Water).

The irrigation was done manually at the level of drain 50% of the available water for absorption.

Table 4: shows the most important chemical traits for tap water and drainage water used in the study.

Traits	Value		Unit
	Tap water	Drainage water	
pH	7.24	7.85	
EC	1.12	24.3	$dS.m^{-1}$
Ca	5.22	91.55	$Meq.L^{-1}$
Mg	3.45	74.20	
Na	3.70	27.80	
K	0.25	3.00	
Cl	8.90	179.5	

The water was analyzed in the Laboratory of Soil Science and Water Resources Department, College of Agriculture, Al-Qasim Green University

The experiment was conducted according to The randomized complete block design (RCBD), with a Factorial Experiment (5 x 4 x 2). It included 40 Factorial treatments and three replicates per treatment, thus the number of experimental units will be 120 experimental units and with a rate of 5 plants per experimental unit. Thus, the number of the study plants was 600 plants and the averages were compared for all study indicators according to the least significant difference test (L.S.D) at the probability level of 0.05 (2).

3. RESULTS AND DISCUSSION

Plant height (cm)

Table (5) shows that the M1 vaccine treatment was significantly excelled in the height of the clove plant. The bio-vaccine treatment with the mycorrhizal fungi (Glomus mosseae) and Azospirilum brasilense gave the highest value of the plant height amounted to (59.81 cm), with an increase of 21.31% from the non-vaccine treatment (M0), which recorded the lowest average of plant height amounted to (49.30 cm). The results of the same table also show that there is a negative significant effect on plant height whenever the salinity of irrigation water increases. where the irrigation treatment (S1) (tap water) has excelled the rest of the treatments by recording the highest average plant height amounted to (65.23 cm) while the irrigation treatment S4 (9 $dS.m^{-1}$)

recorded the lowest average trait amounted to (43.99 cm). The results of the same table also showed the significant effect for the plant cultivar on the plant height, where the plant cultivar (V5) achieved the best results for this trait amounted to (60.48 cm) and without a significant difference from the plant cultivar V1 (Orange) which recorded (59.46 cm), while the lowest average of plant height amounted to (47.79 cm) which recorded by the plant cultivar (V2)(Viana). As for the triple interaction between the study factors, the V5S1M1 treatment recorded the best results for the trait of plant height amounted to (80.90 cm), without a statistical difference with the factors (V1S1M1 and V5S2M1 and V1S2M1 and V4S1M1) which recorded (78.76, 76.90, 74.54 and 71.21 cm), respectively, while the V2S4M0 treatment recorded the lowest values for this trait amounted to (37.67 cm).

Table 5: The effect of the bio-vaccine, the salinity of irrigation water, and plant cultivar on the height of the clove plant (cm)

Cultivar (V)	Bio-vaccine (M)	The salinity of irrigation water (dS.m^{-1})				Interaction
		S1	**S2**	**S3**	**S4**	**M × V**
		1.12 Tap water	**3**	**6**	**9**	
V1 Orange	M0	62.79	56.04	50.04	47.73	54.15
	M1	78.76	74.54	56.89	48.91	64.77
V2 Viana	M0	50.47	44.94	37.18	37.67	42.56
	M1	65.77	61.27	46.27	38.77	53.02
V3 Liberty	M0	54.34	49.99	42.18	40.94	46.86
	M1	68.87	64.90	49.88	41.88	56.38
V4 Bizet	M0	56.30	51.00	43.87	42.65	48.45
	M1	71.21	67.64	51.57	43.19	58.40
V5 Mariposa	M0	62.93	56.77	50.47	47.77	54.48
	M1	80.90	76.90	57.70	50.44	66.48
L.S.D 0.05		**11.76**				**5.88**
						V
Interaction S × V	V1	70.77	65.29	53.46	48.32	59.46
	V2	58.12	53.10	41.72	38.22	47.79
	V3	61.60	57.44	46.03	41.41	51.62
	V4	63.75	59.32	47.72	42.92	53.43
	V5	71.91	66.83	54.08	49.10	60.48
L.S.D 0.05		**8.31**				**4.16**
						M
Interaction S × M	M0	57.36	51.74	44.75	43.35	49.30
	M1	73.10	69.05	52.46	44.63	59.81
L.S.D 0.05		**5.26**				**2.63**
The salinity of irrigation water S		65.23	60.40	48.60	43.99	
L.S.D 0.05		**3.72**				

Leaf area (cm^2)

Table (6) shows that the plants treated with the bio-vaccine were significantly excelled on the untreated plants, where the leaf area in plants treated with the bio-vaccine amounted to (248.44 cm^2), with an increase of (48.76%) over untreated plants, which gave the lowest average of leaf area amounted to (167.00 cm^2). It is also noted from the results of the same table that the salt stress led to a significant decrease in the value of the leaf area for the plant, where the salt level S1 (tap water) achieved the highest average of this trait amounted to (313.99 cm^2) compared to the S4 treatment (9 $dS.m^{-1}$), which gave the lowest average of the leaf area amounted to (110.17 cm^2). The results of the same table also showed the significant effect for the plant cultivar on the leaf area, where the plant cultivar (V5) achieved the best results for this trait amounted to (239.78 cm^2) and without a significant difference from the plant cultivar V1 (Orange) which gave (220.26 cm^2), while the lowest average of leaf area amounted to (183.38 cm^2) which recorded by the plant cultivar (V2)(Viana). As for the triple interaction between the study factors, the results of the same table showed that the bio-vaccine and plant cultivar reduced the effects of salinity of irrigation water, where the V5S1M1 treatment recorded the best results for the trait of leaf area amounted to (472.08 cm^2), without significant difference with the V1S1M1 treatment which gave (427.89 cm^2) while the V2S4M0 treatment recorded the lowest average for this trait amounted to (90.44 cm^2).

Table 6: The effect of the bio-vaccine, the salinity of irrigation water, and plant cultivar on the leaf area for the clove plant (cm^2)

Cultivar (V)	Bio-vaccine (M)	The salinity of irrigation water ($dS.m^{-1}$)				Interaction
		S1	S2	S3	S4	M × V
		1.12 Tap water	3	6	9	
V1 Orange	M0	256.70	189.90	141.99	108.50	174.28
	M1	427.89	357.56	154.74	124.79	266.25
V2 Viana	M0	220.59	146.02	119.79	90.44	144.21
	M1	318.49	316.93	144.84	109.88	222.54
V3 Liberty	M0	235.43	179.01	133.57	94.15	160.54
	M1	321.02	323.38	145.41	114.88	226.18
V4 Bizet	M0	251.37	184.36	133.78	96.50	166.51
	M1	341.23	333.83	151.17	122.30	237.14
V5 Mariposa	M0	295.11	209.99	144.15	108.51	189.44
	M1	472.08	395.19	161.47	131.73	290.12
L.S.D 0.05		**56.84**				**28.42**
						V
Interaction S × V	V1	342.30	273.73	148.37	116.65	220.26
	V2	269.54	231.47	132.32	100.16	183.38
	V3	278.23	251.20	139.49	104.52	193.36
	V4	296.30	259.10	142.49	109.40	201.82
	V5	383.60	302.59	152.81	120.12	239.78
L.S.D 0.05		**40.19**				**20.10**
						M
Interaction S × M	M0	251.84	181.86	134.66	99.62	167.00
	M1	376.15	345.38	151.53	120.72	248.44
L.S.D 0.05		**25.42**				**12.71**
The salinity of irrigation water S		313.99	263.62	143.09	110.17	
L.S.D 0.05		**17.98**				

Number of days required to open the first flower on the plant (day)

Table (7) shows that the significant excelling for the bio-vaccine treatment consisting of mycorrhizal fungi and azospirillum bacteria by giving them the shortest period required for the flowers of the clove plant amounted to (195.51 days), with a decrease of 7.73% compared to the non-vaccine treatment that recorded the longest period for this trait amounted to (211.89 days). The number of days required to open the first flower on the plant decreased significantly with increasing salinity levels of irrigation water which amounted to (191.10 days) in the treatment of salinity level S1 (tap water), with a percentage of decrease of (13.32%) compared to the S3 treatment (6 $dS.m^{-1}$) which gave the highest number of days for this trait amounted to (220.48 days). The plant cultivar achieved a significant increase in reducing the number of days required to open the first flower on the plant, where the V5 cultivar recorded the lowest number of days amounted to (199.46 days), without a statistical difference with the cultivars (V1, V4, V3) that gave averages amounted to (202.13, 203.33 and 205.53 days), respectively compared to the Viana cultivar which gave the highest number of days amounted to (208.05 days). As for triple interaction between the research factors, the V5S1M1 treatment was significantly increased by giving it the lowest number of days amounted to (178.22 days), while the highest number of days amounted to (236.11 days) for the plants of the V2S3M0 treatment.

The vase life of flower (day)

The use of the bio-vaccine achieved a significant increase in the vase life of the cut flowers, where the results of Table (8) show that the bio-vaccine treatment gave the flowers were the longest life after the cutting amounted to (6.55 days) with an increase of (63.75%) compared to the non-vaccine treatment that amounted to (4.00 days). The table also shows that the saline stress caused by increased salt concentrations in irrigation water led to a significant decrease in the vase life after cut flowers, where it decreased from 6.87 days in the treatment of saline level S1 (tap water), with a decrease of 50.21% to amounted to (3.42 days) in the treatment of the Saline level S3 (6 $dS.m^{-1}$). The results of the same table also showed the significant effect of the plant cultivar on prolonging the vase life of the flowers after the cut flowers, where the V5 cultivar gave the highest average vase life after cut flowers amounted to (5.93), without a statistical difference with the cultivars (V1, V4) which recorded an average amounted (5.77, 5.15 days), respectively, while the cultivar (V2) gave the shortest vase life for flowers after the harvest amounted to (4.52 days). The results of the same table indicated that the triple interaction between the factors of the experiment had a significant effect on this trait, where the V5S1M1 treatment recorded the best results for the trait of vase life amounted to (10.16 day), without a statistical difference with the treatments (V1S1M1 and V5S2M1 and V1S2M1 and V4S1M1) which recorded (9.60, 8.28 and 8.23), respectively, while the V2S4M0 treatment recorded the lowest values for this trait amounted to (2.55 cm).

Length of the main root (cm)

Table (9) shows that there is a significant response to adding the bio-vaccine in the trait of the length of the main root for the clove plant, where the treatment of the bio-vaccine has excelled by giving it the highest values for this trait amounted to (20.80 cm), with an increase of 65.07% compared to the non-vaccination treatment that recorded the lowest values for this trait amounted to (12.60 cm). The results of the same table showed a significant decrease in the averages length of the main root with increasing the salinity levels of irrigation water, where the lowest average amounted to (8.73 cm) in the treatment of saline level S4, where the percentage of decline was 67.04% from the treatment of the saline level S1, which achieved the highest average amounted to (26.49 cm). The plant cultivar showed its significant effect on the trait of the length of the main root, where the plant cultivar V5 (Mariposa) has excelled by giving it the highest length of the main root amounted to (19.73 cm), without a statistical difference from the plant cultivars (V1 (Orange) and V4 (Bizet)) which gave (8,851 and 17.50 cm), respectively, while the plant cultivar V2

(Viana) recorded the lowest root length amounted to (11.51 cm). The effects of the salinity of irrigation water decreased when all study factors interacted. The V5S1M1 treatment showed the highest averages for the length of the main root amounted to (37.21 cm) compared to the V2S4M0 treatment that recorded the lowest averages for this trait amounted to (2.80 cm).

Table 7: The effect of the bio-vaccine, the salinity of irrigation water, and plant cultivar on the number of days required to open the first flower on the plant (day)

Cultivar (V)	**Bio-vaccine (M)**	**The salinity of irrigation water ($dS.m^{-1}$)**			**Interaction**
		S1	**S2**	**S3**	**M × V**
		1.12 Tap water	**3**	**6**	
V1 Orange	M0	195.66	207.00	223.55	208.74
	M1	185.39	191.66	209.54	195.53
V2 Viana	M0	199.66	218.55	236.11	218.11
	M1	188.52	188.89	216.55	197.99
V3 Liberty	M0	196.78	211.00	232.55	213.44
	M1	187.77	188.88	216.22	197.62
V4 Bizet	M0	196.66	208.66	228.00	211.11
	M1	186.78	186.78	211.55	195.55
V5 Mariposa	M0	195.55	206.99	221.66	208.07
	M1	178.22	185.22	209.11	190.85
L.S.D 0.05		**20.66**			**11.93**
					V
Interaction S × V	V1	190.52	199.33	216.55	202.13
	V2	194.09	203.72	226.33	208.05
	V3	192.27	199.94	224.39	205.53
	V4	191.72	198.50	219.78	203.33
	V5	186.89	196.11	215.38	199.46
L.S.D 0.05		**14.61**			**8.44**
					M
Interaction S × M	M0	196.86	210.44	228.37	211.89
	M1	185.33	188.60	212.59	195.51
L.S.D 0.05		**9.24**			**5.34**
The salinity of irrigation water S		191.10	199.52	220.48	
L.S.D 0.05		**6.53**			

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Table 8: The effect of the bio-vaccine, the salinity of irrigation water, and plant cultivar on the vase life for the flowers (day)

Cultivar (V)	Bio-vaccine (M)	The salinity of irrigation water (dS.m^{-1})			Interaction
		S1	S2	S3	M × V
		1.12 Tap water	3	6	
V1 Orange	M0	5.89	3.77	3.05	4.24
	M1	9.60	8.23	4.05	7.29
V2 Viana	M0	4.74	3.27	2.55	3.52
	M1	6.56	6.29	3.71	5.52
V3 Liberty	M0	5.15	3.66	2.82	3.88
	M1	7.47	6.99	3.97	6.14
V4 Bizet	M0	5.29	3.77	2.95	4.00
	M1	7.86	7.05	4.00	6.30
V5 Mariposa	M0	6.05	3.99	3.05	4.36
	M1	10.16	8.28	4.05	7.50
L.S.D 0.05		**1.98**			**1.98**
					V
Interaction S × V	V1	7.74	6.00	3.55	5.77
	V2	5.65	4.78	3.13	4.52
	V3	6.31	5.33	3.40	5.01
	V4	6.58	5.41	3.48	5.15
	V5	8.11	6.14	3.55	5.93
L.S.D 0.05		**1.41**			**0.81**
					M
Interaction S × M	M0	5.43	3.69	2.88	4.00
	M1	8.33	7.37	3.96	6.55
L.S.D 0.05		**0.89**			**0.51**
The salinity of irrigation water S		6.87	5.53	3.42	
L.S.D 0.05		**0.63**			

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Table 9: The effect of the bio-vaccine, the salinity of irrigation water, and plant cultivar on the length of the main root (cm)

Cultivar (V)	Bio-vaccine (M)	The salinity of irrigation water (dS.m^{-1})				Interaction
		S1	S2	S3	S4	M × V
		1.12 Tap water	3	6	9	
V1 Orange	M0	23.21	15.34	10.20	8.50	14.31
	M1	35.52	31.37	14.80	11.84	23.38
V2 Viana	M0	15.04	7.80	6.20	2.80	7.96
	M1	23.60	19.24	9.60	7.80	15.06
V3 Liberty	M0	19.80	13.11	9.80	6.50	12.30
	M1	29.34	25.38	12.80	10.90	19.61
V4 Bizet	M0	22.40	13.80	10.00	7.00	13.30
	M1	33.01	29.10	13.20	11.50	21.70
V5 Mariposa	M0	25.82	15.80	10.24	8.64	15.12
	M1	37.21	33.37	14.93	11.87	24.34
L.S.D 0.05		**9.94**				**4.97**
						V
Interaction S × V	V1	29.36	23.35	12.50	10.17	18.85
	V2	19.32	13.52	7.90	5.30	11.51
	V3	24.57	19.25	11.30	8.25	15.95
	V4	27.70	21.45	11.60	9.25	17.50
	V5	31.51	24.58	12.58	10.25	19.73
L.S.D 0.05		**7.03**				**3.52**
						M
Interaction S × M	M0	21.25	13.17	9.29	6.69	12.60
	M1	31.74	27.69	13.07	10.78	20.80
L.S.D 0.05		**4.45**				**2.22**
The salinity of irrigation water S		26.49	20.43	11.18	8.73	
L.S.D 0.05		**3.15**				

Number of main roots (root.plant^{-1})

Table (10) shows that the number of main roots increased significantly when vaccinating clove plants with the bio-vaccine, which gave the highest average of this trait amounted to (17.18 roots.plant^{-1}), with an increase of (40.16%) to excelling on the non-vaccination treatment that gave the lowest average amounted to (10.28 root.plant^{-1}). The results also indicated that the increase in the salinity levels of irrigation water was accompanied by a significant decrease in the trait of the number of main roots from (20.06 root.plant^{-1}) in treatment of salt level S1 (tap water) to reach (7.74 root.plant^{-1}) in treatment of salt level S4 (9 ds.m^{-1}) with a significant decrease of 61.41%. The results of the same table also showed a significant effect for the plant cultivar in this trait, which reflected positively on the number of main roots, where the plant cultivar V5 (Mariposa) achieved the highest averages for the number of main roots amounted to (15.49 roots.plant^{-1}) which in turn did not differ significantly from the two plant cultivars (V1 (Orange) and V4 (Bizet)) which amounted to (15.03 and 14.25 roots.plant^{-1}), respectively, compared to the plant cultivar V2 (Viana), which recorded the lowest average for this trait amounted to (11 roots.plant^{-1}). The triple interaction between the factors of the experiment led to a significant effect on this trait, where the V5S1M1 treatment gave the largest number of main roots amounted to (30.29 roots.plant^{-1}) without a statistical difference on the treatments (V1S1M1, V5S2M1, and V4S1M1) which gave averages amounted to (29.15, 27.30 and 26.92 roots.plant^{-1}), respectively, while the V2S4M0 treatment gave the lowest average for this trait amounted to (5.37 root.plant^{-1}).

Table 10: The effect of the bio-vaccine, the salinity of irrigation water, and plant cultivar on the number of main roots (root.plant^{-1})

Cultivar (V)	Bio-vaccine (M)	The salinity of irrigation water (dS.m^{-1})				Interaction
		S1	S2	S3	S4	M × V
		1.12 Tap water	3	6	9	
V1 Orange	M0	15.15	11.89	9.47	7.15	10.92
	M1	29.15	26.42	11.87	9.15	19.15
V2 Viana	M0	12.5	9.23	7.15	5.37	8.50
	M1	20.15	16.82	10.15	7.26	13.59
V3 Liberty	M0	14.25	11.15	9.32	6.47	10.30
	M1	2.372	19.25	11.15	8.69	15.36
V4 Bizet	M0	14.76	11.26	9.43	7.06	10.63
	M1	26.92	23.66	11.77	9.15	17.88
V5 Mariposa	M0	15.29	11.94	9.79	7.15	11.04
	M1	30.29	27.30	12.15	9.99	19.93
L.S.D 0.05		**3.55**				**1.77**
						V
Interaction S × V	V1	22.15	19.15	10.67	8.15	15.03
	V2	16.20	13.02	8.65	6.32	11.05
	V3	18.31	15.20	10.24	7.58	12.83
	V4	20.84	17.46	10.60	8.11	14.25
	V5	22.79	19.62	10.97	8.57	15.49
L.S.D 0.05		**2.51**				**1.25**
						M
Interaction S × M	M0	14.34	11.09	9.03	6.64	10.28
	M1	25.78	22.69	11.42	8.85	17.18
L.S.D 0.05		**1.59**				**0.79**
The salinity of irrigation water S		20.06	16.89	10.23	7.74	
L.S.D 0.05		**1.12**				

The leaves content of total soluble carbohydrates (mg. 100 g^{-1} dry weight)

Table (11) shows the significant effect for the leaves content of total soluble carbohydrates when using the bio-vaccine, where the carbohydrate content increased from (8.56 mg. 100 g^{-1} dry weight) in non-vaccination treatment, with an increase of (33.52%) to (11.43 mg.100 g^{-1} dry weight) in the vaccination treatment. The same table also shows that the saline stress resulting from increased salt concentrations in irrigation water

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led to a significant decrease in the carbohydrate content, where it decreased from (13.78 mg.100 g^{-1} dry weight) in the treatment of saline level S1 (Tap water), without a statistical difference with the salt level S2 (3 $dS.m^{-1}$) to (6.60 mg. 100 g^{-1} dry weight). It is clear from the same table that the leaves content of total dissolved carbohydrates did not reach the differences in them to the significant degree, although the Mariposa cultivar (M5) achieved the highest content of this trait compared to other cultivars. The results of the triple interaction between the study factors showed significant differences between the treatments, where the V5S1M1 treatment gave the highest carbohydrate content amounted to (16.64 mg. 100 g^{-1} dry weight) which in turn did not differ significantly from most of the treatments, while the V2S4M0 treatment recorded the lowest average for this trait amounted to (3.09 mg. 100 g^{-1} dry weight).

Table 11: The effect of the bio-vaccine, the salinity of irrigation water, and plant cultivar on the leaves content of total soluble carbohydrates (mg. 100 g^{-1} dry weight)

Cultivar (V)	Bio-vaccine (M)	The salinity of irrigation water ($dS.m^{-1}$)				Interaction
		S1	S2	S3	S4	M × V
		1.12 Tap water	3	6	9	
V1 Orange	M0	13.39	9.34	7.34	6.89	9.24
	M1	16.49	15.52	9.64	7.79	12.36
V2 Viana	M0	10.59	7.49	5.34	3.09	6.63
	M1	12.84	11.73	8.39	6.29	9.81
V3 Liberty	M0	12.34	8.94	7.19	6.04	8.63
	M1	13.99	13.09	9.39	7..34	10.95
V4 Bizet	M0	13.14	9.04	7.34	5.93	8.86
	M1	14.94	13.96	9.54	7.49	11.48
V5 Mariposa	M0	13.39	9.54	7.79	7.09	9.46
	M1	16.64	15.56	9.79	8.04	12.51
L.S.D 0.05		**9.70**				**4.85**
						V
Interaction S × V	V1	14.94	12.43	8.49	7.34	10.80
	V2	11.72	9.61	6.87	4.69	8.22
	V3	13.17	11.02	8.29	6.69	9.79
	V4	14.04	11.50	8.44	6.71	10.17
	V5	15.02	12.55	8.79	7.57	10.98
L.S.D 0.05		**6.86**				**3.43**
						M
Interaction S × M	M0	12.57	8.87	7.00	5.81	8.56
	M1	14.98	13.97	9.35	7.39	11.43
L.S.D 0.05		**4.34**				**2.17**
The salinity of irrigation water S		13.78	11.42	8.18	6.60	
L.S.D 0.05		**3.07**				

Estimating the effectiveness of the Superoxide dismutase enzyme (SOD) ($unit.mol^{-1}$)

Table (12) shows that the use of the bio-vaccine had a significant effect in reducing the effectiveness of the superoxide dioxide (SOD) enzyme, where the treatment of the bio-vaccine gave the lowest effectiveness for this enzyme amounted to (31.45 $units.mol^{-1}$), with a decrease of (21.64%) compared to the non-vaccination treatment that gave (40.14 $units.mol^{-1}$). The same table also showed the significant effect of saline stress resulting from the salinity of irrigation water in increasing the effectiveness of SOD where it increased from (22.88 units.mol^{-1}) in the treatment of salt level S1 (Tap water) to (54.05 units.mol^{-1}) in the treatment of the salt level S4 (9 dS.m^{-1}). The effectiveness of the superoxide dioxide (SOD) enzyme was affected by the plant cultivar, where the effectiveness of the enzyme decreased significantly from (41.24 units.mol^{-1}) in the plant cultivar V2 (Viana), with a significant difference of (32.74 units.mol^{-1}) in the plant cultivar V5 (Mariposa), which in turn did not differ significantly from the plant cultivars (V1 and V4) which recorded (33.50 and 34.49 units.mol^{-1}), respectively. The results of triple interaction between the experiment factors showed that the V5S1M1 treatment achieved the lowest effectiveness of SOD amounted to (17.32 units.mol^{-1}) compared to the V2S4M0 treatment that gave the highest average for this effectiveness amounted to (62.32 units.mol^{-1}).

Table 12: The effect of the bio-vaccine, the salinity of irrigation water, and plant cultivar on the effectiveness of the Superoxide dismutase enzyme (SOD) (unit.mol^{-1})

Cultivar (V)	Bio-vaccine (M)	The salinity of irrigation water (dS.m^{-1})				Interaction
		S1	S2	S3	S4	M × V
		1.12 Tap water	3	6	9	
V1 Orange	M0	24.21	27.94	42.91	56.03	37.77
	M1	17.94	18.46	33.50	47.04	29.23
V2 Viana	M0	30.32	38.44	53.54	62.32	46.16
	M1	22.96	22.84	41.96	57.52	36.32
V3 Liberty	M0	27.14	30.00	46.36	59.24	40.69
	M1	20.00	22.00	39.50	51.71	33.30
V4 Bizet	M0	26.04	28.27	43.27	57.38	38.74
	M1	18.98	20.74	34.00	47.26	30.25
V5 Mariposa	M0	23.86	27.55	42.32	55.72	37.36
	M1	17.32	18.72	30.21	46.23	28.12
L.S.D 0.05		**9.8**				**4.91**
						V
Interaction S × V	V1	21.08	23.20	38.21	51.54	33.50
	V2	26.64	30.64	47.75	59.92	41.24
	V3	23.57	26.00	42.93	55.48	36.99
	V4	22.51	24.51	38.64	52.32	34.49
	V5	20.59	23.13	36.27	50.97	32.74
L.S.D 0.05		**6.94**				**3.47**
						M
Interaction S × M	M0	26.31	30.44	45.68	58.14	40.14
	M1	19.44	20.55	35.83	49.95	31.45
L.S.D 0.05		**4.39**				**2.20**
The salinity of irrigation water S		22.88	25.50	40.76	54.05	
L.S.D 0.05		**3.10**				

Estimating the effectiveness of Catalase enzyme (CAT) (unit.mol^{-1})

Table (13) shows that the bio-vaccine managed to significantly reduce the effectiveness of the catalase enzyme (CAT) from (12.33 units.mol^{-1}) when absence bio-vaccine which amounted to (8.74 units.mol^{-1}) with the presence of bio-vaccine, with a significant decrease amounted to (29.11%). All salinity treatments differed significantly between them, where the S1 treatment (Tap water) has excelled on the treatments by recording it the lowest effectiveness of the catalase enzyme amounted to (4.78 units.mol^{-1}) followed by the treatments (S2 and S3), while the effectiveness enzyme increased to (18.41 units.mol^{-1}) in the S4 treatment (9 dS.m^{-1}). The results of the statistical analysis showed a significant effect for the plant cultivar on the effectiveness of the catalase enzyme, where the plant cultivar V5 (Mariposa) recorded the lowest averages of enzyme activity amounted to (7.79 units.mol^{-1}) without a statistical difference from the plant cultivar V1 (Orange) which gave (8.06 units.mol^{-1}), while plant cultivar V2 (Viana) gave the highest average of catalase enzyme activity amounted to (14.09 units.mol^{-1}). Among the results of the triple interaction of all the factors of the experiment, the significant effect is shown in this trait, where the role of experiment factors showed the bio-vaccine and the plant cultivar in increasing the ability of the plant cultivar to tolerate salinity damage and reducing the effectiveness of the catalytic enzyme, where the V5S1M1 treatment recorded the highest decrease in effectiveness amounted to (1.67 units.mol^{-1}) compared to the V2S4M0 treatment, which gave the lowest decrease in the effectiveness amounted to (26.12 units.mol^{-1}).

Table 13: The effect of the bio-vaccine, the salinity of irrigation water, and plant cultivar on the effectiveness of the Catalase enzyme (CAT) (unit.mol^{-1})

Cultivar (V)	**Bio-vaccine (M)**	**The salinity of irrigation water (dS.m^{-1})**				**Interaction**
		S1	**S2**	**S3**	**S4**	**M × V**
		1.12 Tap water	**3**	**6**	**9**	
V1 Orange	M0	4.81	6.54	10.16	17.39	9.72
	M1	1.72	2.95	7.73	13.16	6.39
V2 Viana	M0	8.34	12.99	18.05	26.12	16.37
	M1	5.89	8.41	13.79	19.16	11.81
V3 Liberty	M0	6.99	9.59	15.01	23.46	13.76
	M1	4.88	7.27	12.66	17.77	10.64
V4 Bizet	M0	4.88	8.56	13.38	21.75	12.14
	M1	3.84	6.12	10.22	15.51	8.92
V5 Mariposa	M0	4.79	6.46	10.06	17.36	9.67
	M1	1.67	2.86	6.66	12.46	5.91
L.S.D 0.05		**5.07**				**2.53**
						V
Interaction S × V	V1	3.26	4.74	8.94	15.27	8.06
	V2	7.11	10.70	15.92	22.64	14.09
	V3	5.93	8.43	13.83	20.61	12.20
	V4	4.36	7.34	11.80	18.63	10.53
	V5	3.23	4.66	8.36	14.91	7.79
L.S.D 0.05		**3.58**				**1.79**
						M
Interaction S × M	M0	5.96	8.83	13.33	21.22	12.33
	M1	3.60	5.52	10.21	15.61	8.74
L.S.D 0.05		**2.27**				**1.13**
The salinity of irrigation water S		4.78	7.17	11.77	18.41	
L.S.D 0.05		**1.60**				

The general framework of the results shows the significant response for the bio-vaccine consisting (Glomus mosseae and Azospirilum brasilense) for cloves, which reflected positively on plant growth. The reason may be due to the role of these organisms in increasing the availability of nutrients and their average of absorption, as well as the secretion of plant hormones such as Auxins and gibberellins that stimulate cell division and its elongation (25), thus increasing the leaf area of the plant, which leads to an increase in nutrients and carbohydrate production that achieves adequate and balanced nutrition for the plant and improving the traits of vegetative growth and development, which reflects positively on the plant height, the flowering date, and prolonged vase life of flowers (1, 22, 23). The results of increasing the averages of the studied root traits through the ability of these organisms to increase the physiological and metabolic activities in the plant roots, especially the secretion of some plant hormones (indole acetic acid), which is the most active hormone in elongation and division of plant cells and their differentiation (7, 17), which may enhance the growth of root system, which increases its ability to absorb water and nutrients and sustain root growth (8). The reduction in the averages of the effectiveness of the antioxidant enzymes under study (SOD and CAT) may be attributed to the ability of these organisms to activate the enzymatic and non-enzymatic defense system for the plant, thus reduce the Reactive oxygen species (ROS) (11). As for the effect of salt stress, it is noted that the values of vegetative, flower, and root growth indicators have decreased with increasing salinity levels of irrigation water. This may be due to the effect of salt stress on plant growth, where the high concentration of salinity in the culture media causes confusion in the ionic balance in plants as well as the imbalance of nutrient absorption and then its effect on the effectiveness of enzymes, especially enzymes related to bio-activities and plant metabolism, and this reflects negatively on the processes of dividing and elongating plant cells, thus inhibiting the indicators of plant growth (13, 27). On the other hand, the results indicate that saline stress led to an increase in the effectiveness of antioxidant enzymes, this result can be explained according to (Sakir et al., 21; Al-Mamouri, 3; Al Nassirawi, 4) that salinity works to form Reactive oxygen species (ROS) and then increase the oxidative stress so the plant defends itself against these free radicals by developing an antioxidant complex system by producing some antioxidant enzymes like superoxide dismutase, Catalase, peroxidase and other important enzymes in removing the hydrogen peroxidase H_2O_2 (16) and then converting it to H_2O and O_2 (14). The results also showed that the plant cultivars under study differed in the indicators of the studied traits and this may be due to the influence of genotypes between the cultivars in addition to the variation of the sensitivity of these cultivars to tolerate the salinity levels of irrigation water.

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