



## Effect of Humic acid, Cytokinin and Arginine on Qualitative Traits and Yield of Bean Plant *Phaseolus vulgaris* L. Under Salt Stress

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### Abstract

To Investigate optimum growth and production under salinity, some materials have been added in sufficient quantities to obtain an ideal crop of salt sensitive bean plants. This experiment was conducted during the spring growing season in 2022 in the agricultural fields in Abu Ghraib, Baghdad governorate, to study the effects of humic acid, cytokinin and arginine and their interaction on 6 parameters reflecting the total of quantitative and yield traits of bean plants var. Astrid (from MONARCH seeds, China). A factorial design with 3 replicates was used, the first factor included 3 groups of Humic acid; H0, H1 (6 Kg.h<sup>-1</sup>), and H2 (12 Kg.h<sup>-1</sup>). The second factor included 2 groups; C0 (spray distilled water), and C1 (100 mg.l<sup>-1</sup> benzel adenine), and the third factor included 3 groups of Arginine; A0 (spray distilled water), A1 (100 mg.l<sup>-1</sup>), and A2 (200 mg.l<sup>-1</sup>). For humic acid, the results showed that H2 treatment caused the significantly highest values in all the studied traits, except for proline. Results of cytokinin treatment showed that C1 treatment led to significantly higher values in all the studied traits, except for proline. For arginine treatment, the results indicated that there was no significant difference between A1, and A2. For the binary overlap among treatments, the results showed the highest values were H2C1, A2C1, and H2A2, except for chlorophyll content was H2A1. The H2A2C1 triple overlap treatment resulted in the highest values compared to all other treatments for all traits. It is evident that from the results, proline was the highest value in the control treatment of all traits. In conclusion, the present study found that humic acid, cytokinin, arginine and their interactions enhance significantly the quantitative traits and production of bean plants under salinity stress.

**Keywords:** Arginine, bean plant, cytokinin, humic acid, *Phaseolus vulgaris* L., salt stress.

### 1. Introduction

Leguminosae is a family of high flowering plants that are the second family in terms of economic importance after the Poaceae family, as it includes many benefits for agricultural and food sustainability. Comprised approx. 770 genera and more than 19,500 species [1], [2], thus it is considered one of the most important and richest plant families in terms of diversity and has a high nutritional value, especially proteins that can reach 40% [3]. The common bean belongs to the family Leguminosae, to the subfamily Papilionoidea, to genus *Phaseolus*, to species *P. vulgaris*.



The study of beans is important, as they are plants very rich in proteins, amino acids [4], and carbohydrates [5], It is also rich in vitamins [6], As well as various macro and microelements [7], also unsaturated fatty acids, and dietary fiber [8], also rich in many antioxidants, like anthocyanins, polyphenols, flavonoids, [9]. The stresses, such as salinity, reduce the world's crop quantity and quality, leading to significant food, social, and economic insecurity. This is a reality for poor and developing countries, and the data reveals that 36.9% of the population suffer from food insecurity [10], and with the world population reaching 10.4 billion in 2080, according to estimates by the United Nations [11], and increasing future expectations of food demand for all countries in general and developing countries in Asia and Africa in particular [12], Therefore, effective measures to increase crop yields must be adopted to overcome the issue of increasing world population and to mitigate the harmful effects of salinity stress. This is done through the effective use of sustainable agricultural practices, especially with regard to adding some materials that reduce the effect of salt stress resulting from the lack of fresh irrigation water and the use of salty well water without compromising the crop, including plants of the bean plant, it is very important to integrate into the diet that is important for human health. This is in line with an excellent strategy to achieve the United Nations Sustainable Development Goals for reducing malnutrition and achieving food security, [13]. According to the estimates of the FAO for the year 2021, the cultivated area of the green bean crop in Iraq is 669 hectares, and the production reached 5.9013 tons / hectare. The cultivated area of dry beans was 760 hectares and the productivity was 6.897 tons/ha. [14], while the cultivated areas of green beans in Iraq witnessed a clear fluctuation during the past years, and this fluctuation in the cultivated areas is due to many reasons, including the problems that agricultural lands suffer from, especially the problem of salinity, as well as the lack of use of practical techniques that lead to raising productivity through production per unit area. Beans are considered one of the vegetable crops that are sensitive to salinity in the irrigation water or the soil, as they cause a severe decrease in yield, and because of the rapidly increasing saline soil in dry and semi-arid regions of the globe as a result of various climatic changes, insufficient irrigation water or due to irrigation with saline-rich water [15]. Salt stress reduced growth factors, water content, and photosynthetic pigments of common bean plants [16], also causes a lot of physiological, phenotypic and biochemical changes in plants, and greatly affected the plant productivity. Salt-sensitive plants decrease their productivity after exposure to salt stress. Among the harmful effects of salinity on plants are reduced root growth, inhibition of flowering and reduced germination, seed and yield decline [17]. Proline is one of the most important amino acids that accumulate in lower and higher plants when exposed to salt stress (to modify the osmotic potential) [18]. It plays an effective osmotic protective role, as proline accumulates by stimulating its synthesis again and stopping its demolition process [19]. It is well known that nitrogen is the most important macronutrient for proper growth and development in plants. However, it is the main component of all amino acids and various nitrogen-forming compounds such as proline, which are the most important compounds in plants exposed to salinity and sub saline soils [20]. It was found that sufficient presence of potassium can mitigate the harmful effects of soil salinity in lentils [21]. Humic acid is a substance that contains elements that improve the fertility and increase the availability of nutrients, thus affecting plant growth and yield and mitigating the harmful effects of salt stress [22]. In an experiment to study the effect of humic acid on plant biological aspects such as biomass of plant and chlorophyll content of common bean plants under salinity stress, the results showed that the use of humic acid leads to a significant increase in vegetative growth

characteristics as well as the content of chlorophyll and humic acid is considered able to overcome salt stress [23]. Another experiment, found that adding humic acid reduced proline, increased the  $K^+$  accumulation and reduced  $Na^+$  accumulation in shoots and roots under salinity treatment, also increasing the chlorophyll content, total biomass and yield [24]. As well as a study showing The effect of humic acid on the growth and yield of pepper plants led to a significant increase in the N, P and K contents in shoots and roots of pepper plants, and the  $Na^+$  content in root decreased with increasing doses of humic acid. It can be concluded that higher doses of humic acid have positive effects on salt tolerance based on plant growth characteristics and nutrient content [25]. Cytokinins influence plant growth and development, where recent studies revealed complex physiological functions, such as the discovery of plant defense system and stress resistance [26]. A study showed that Plants treated with cytokinin enhanced plant adaptation against salt stress, and verified the ability of the role of benzyl adenine (BA) to improve the salinity tolerance of broad bean (*Vicia faba* L). Salt-stressed leaves sprayed with 200 ppm BA enhanced growth performance which was demonstrated by fresh and dry weight of shoots and roots, and a decrease in proline was strongly associated with soluble proteins and free amino acids, which protects the osmotic potential of the plant after treatment with BA in salt-stressed bean, and BA also works to completely balance mineral elements and improve mineral absorption and transfer from roots to shoots [27]. Another experiment showed that Cytokinins regulate the ability of plants to absorb several nutrients from the environment, including N and P, thus improving plant growth [28]. In one study, it was indicated that adding arginine to sunflower plants under salt stress increased K and P and reduced Na. It caused an increase in the concentration of photosynthetic pigments by 22 %, while it showed that salinity reduced all elements except Na [29]. In another experiment that included studying the effect of adding arginine at 3 levels of 0, 200, and 250 ppm for potato plants and 3 levels of irrigation water salinity 1.6, 3.2, and 4.3 dSi/m, the result showed, an increase in the salinity of irrigation water from 1.6 to 4.3 dSi/m led to reduced total chlorophyll and total yield, while the addition of arginine led to an increase total chlorophyll and total yield ton/ha. [30], another study indicated that exogenous arginine increased the N concentration resulting in increased fruit yield and quality. Arginine is considered the organic N storage sink and N conversion medium due to its high N/C ratio [31]. Also an experiment indicates that Arginine significantly increased the concentration of N in shoots and fruits of tomatoes, through 3 pathways, which are the use of arginine as N source, increased gene expression of SINRT1.1, and increased root growth and activity. It then greatly improved photosynthesis by increasing N levels, resulting in increased plant growth, fruit size, and plant yield. Moreover, spraying with arginine positively affected plant traits, by improving N accumulation [32].

The aim of the present study is to evaluate the influence of adding humic acid and foliar spray of cytokinin and arginine on the quantitative traits and yield of the bean plants under salinity in the local environment, taking into account especially the problems of deficiency of fresh water, Increasing the saline area and growing demand for this plant in the Iraqi market.

## 2. Materials and Methods

This experiment was carried out during the spring growing season in 2022 in one of the agricultural fields of Abu Ghraib district, 20 km west of Baghdad governorate. A factorial design with 3 replicates was used. The first factor, Humic acid included 3 groups; H0, H1 (6 Kg.h<sup>-1</sup>), and H2 (12 Kg.h<sup>-1</sup>). The second factor, Cytokinins (benzel adenine) included 2 groups; C0

(distill water spray), and C1 (100 mg.l<sup>-1</sup>), and the third factor, Arginine included 3 groups; A0 (distill water spray), A1 (100 mg.l<sup>-1</sup>), and A2 (200 mg.l<sup>-1</sup>) as a result, it became 18 treatments, identical to combinations of humic acid, Cytokinins and Arginine. Each treatment included 3 replicates, each containing 7 plants. The leaves were sprayed with solutions of distilled water containing 0.1 % Tween 20 (polysorbate 20, which is a polysorbate-type nonionic surfactant formed by the ethoxylation of sorbitan) as surfactant.

Where the cultivation field was divided into 3 main panels (replicates), and then each panel was divided into 18 secondary panels to represent the experimental unit for each treatment, as it became 3 transactions with 54 experimental units. The land was plowed and cleaned from all the growing bushes. Soil samples were taken from the field at a depth of (0-30 cm) randomly and mixed well, the sample was analyzed in the Department of Soil Sciences and Water Resources of the College of Agriculture / University of Baghdad for the purpose of conducting some chemical and physical analyzes of the soil. As in **Table 1**.

**Table 1.** illustrate some physical and chemical properties of field soil

Adjective		Unit	value
Water salinity			2.2
Soil pH			7.7
Ece		Desi Simmons -1	16.21
CEC		Centimoles of a charge / kg soil	1.87
ESP			3.48
Organic Maters			4.0
Gypsum		g/kg soil	1.25
Available ions			
	Nitrogen		63.0
	Phosphorous	PPM	14.4
	Potassium		278.0
	Calcium		12.54
Dissolved cations			
	Magnesium	mEq liters -1	9.40
	Potassium		1.02
	Sodium		2.14
	Carbonate		219.3
Dissolved anions			
	Bicarbonate	mEq liters -1	0.6
	Sulfites		2.87
	Chloride		19.47
Soil articulations			
	Sand		532
	Silt	g/kg	380
	Mud		88
soil texture grade		Sandy Loam	
bulk density		megagm m <sup>-3</sup>	1.17
Porosity			0.56

The experimental unit was represented by 1 m-apart and 3 m-long furrows, each containing 20 plants, and 45 cm apart. During the period of plant growth , furrow irrigation with drip pipe irrigation was used regularly and weeds were kept under control manually. Bean plants (sensitive to salinity), *Phaseolus vulgaris* L. Var. Astrid (from MONARCH seeds, China) cultivars, sterilized and circulated in local agriculture, were sowing in an open field on the first of March 2022. At curds collection (harvest), seven plants were randomly chosen for experimentation. The leaves of plants were sprayed with Cytokinin and Arginine, adding humic acid thrice, the first

time after the formation of the four leaves, the second time when the flowering began to form, third time two weeks after.

The following parameters for qualitative and yield characteristics were recorded on randomly selected plants: The percentage of NPK in leaves, proline in the leaves ( $\mu\text{mol/gm}$  fresh weight), In addition, Leaves content of total chlorophyll ( $\text{mg } 100\text{g fresh material}^{-1}$ ) and Total yield ( $\text{ton hectare}^{-1}$ ). For the statistical analysis, the analysis of variance (ANOVA) was performed on the analytical data were analyzed using the statistical program Genstat Twelfth Edition, year of release 2012, to evaluate the effects of humic acid, Cytokinin, Arginine and their interactions on quantitative traits and yield of bean plant under salinity stress.

### 3. Results

#### 3.1. Leaves content of total chlorophyll ( $\text{mg } 100\text{g fresh material}^{-1}$ )

**Table 2.** showed the results of the effect of humic acid, cytokinins and arginine and their interactions in measuring the total chlorophyll, where there was a significant difference in the treatments, with the highest rate at (2H) 27.7651 and the lowest rate at (H0) 23.5850. in the same way with cytokinin, where the highest mean at (C1) 26.2412 compared to (C0) 25.0992, also found differences for the treatment of arginine, with the highest rate at (A2) 25.9156 and the lowest rate at (A0) 25.2358.

**Table 2.** Effect of humic acid, cytokinin and arginine and their interactions on the leaves content of total chlorophyll ( $\text{mg } 100 \text{ gm fresh material}^{-1}$ ).

A	C	H 0	H 1	H 2	A*C	Average -A
A 0	C 0	21.9613	25.0880	27.1320	24.7271	25.2358
		H	DEF	ABC	A	
A 1	C 1	23.5387	25.7693	27.9253	25.7444	25.8592
		FGH	CDE	AB	A	
A 2	C 0	23.3221	25.0973	27.2813	25.2336	25.9156
		GH	DEF	ABC	A	
A 2	C 1	24.5560	26.2453	28.6533	26.4849	25.9156
		EFG	DE	A	A	
A 2	C 0	23.5200	25.2280	27.2627	25.3369	25.9156
		FGH	DE	ABC	A	
A 2	C 1	24.6120	26.5347	28.3360	26.4942	25.9156
		FGHIJ	BCD	A	A	
value LSD		LSD: A*C*H = 2.2094			LSD:A*C= 1.9078	LSD: A=0.6456
H x A		---				
A 0		22.7500	25.4287	27.5287		25.2358
		D	B	A		
A 1		23.9391	25.6713	27.9673		25.8592
		CD	B	A	LSD: A*H =1.9565	
A 2		24.0660	25.8813	27.7993		25.9156
		C	B	A		
H x C		average C				
C 0		22.9345	25.1378	27.2253		25.0992
		F	D	B		B
C 1		24.2356	26.1831	28.3049		26.2412
		E	C	A		A
value LSD		LSD: C*H =1.9078				LSD: C = 0.5272
average H	---	23.5850	25.6604	27.7651		---
value LSD		C	B	A		---
value LSD		LSD: H = 0.6456				

There were no differences in the leaves content of total chlorophyll in the overlap between cytokinin and arginine. But the significant differences were clear with the overlap between humic and cytokinin, where the highest content at (H2C1) was 28.3049 and the lowest content at (H0C0) 22.9345. Also with the overlap between humic and arginine, the highest content when treated (H2A1) 27.9673 and the lowest content when treated (H0C0) 22.7500. As for the triple interaction among humic acid, cytokinin and arginine, the highest content at (H2C1A2) was 28.3360, and the lowest content in control (H0C0A0) 219613.

**3.2.The percentage of nitrogen in the leaves**

**Table 3.** indicated that there was a significant effect of the plants treated with the experimental factors, where the highest rate of the N % in the leaves was at (H2) and the lowest rate at control (H0). On the same path the cytokinin treatment, has the highest average at (C1) compared to the control (C0). While there were no significant differences in the treatment of arginine compared to the control treatment.

**Table 3.** Effect of humic acid, cytokinin and argentine and their interactions on the N% in the leaves

A	C	H 0	H 1	H 2	A*C	average -A
A 0	C 0	2.7433	3.1667	3.1533	3.0211	3.06389
		F	ABCDE	ABCDE	A	
	C 1	2.8333	3.1600	3.3267	3.1067	
A 1	C 0	2.7533	3.0100	3.2533	3.0056	3.06722
		F	CDEF	ABCD	A	
	C 1	2.8933	3.0733	3.4200	3.1289	
A 2	C 0	2.8467	2.8233	3.2933	2.9878	3.08611
		EF	EF	ABC	A	
	C 1	2.9567	3.1033	3.4933	3.1844	
		CDEF	ABCDEF	A	A	
value LSD		LSD: A*C*H = 0.4904			LSD: A*C=	LSD:
					0.2874	A=0.1528
H x A						---
A 0		2.7883	3.1633	3.2400		
		D	ABC	AB		
	A 1		2.8233	3.0417	3.3367	
		D	BCD	A	LSD: A*H =0.2947	
A 2			2.9017	2.9633	3.3933	
		CD	CD	A		
	H x C				average C	
C 0		2.7811	3.0000	3.2333	3.00481	
		E	CD	AB	B	
	C 1		2.8944	3.1122	3.4133	3.10400
		DE	BC	A	A	
value LSD			LSD: C*H =0.2875		LSD: C =0.1248	
average H	---	2.83778	3.05611	3.32333		
		C	B	A		---
value LSD		LSD: H =0.1528				

The interaction between cytokinin and arginine, did not find any significant differences. While the differences were clear between humic and cytokinin, as it reached the highest value when treated (H2C1) and the lowest value when untreated (H0C0). And it went on with the interaction

between humic and arginine, at the highest percentage with (H2A2) and the lowest percentage with control (H0A0). The same goes for threesome interaction among humic, cytokinin, and arginine, there were very significant differences, as it reached the highest percentage when treated with (H2C1A2), and the lowest percentage at control (H0C0A0).

**3.3.The percentage of phosphorus in the leaves**

**Table 4.** indicate that the highest rate of the P % in the leaves at the (H2) was 0.406667 and the lowest rate at (H0) 0.326667. As well the cytokinin spray treatment, with highest rate at (C1) was 0.378519 compared to the (C0) was 0.353333. While there were no significant differences for spraying the arginine compared to the control treatment. In regards the interaction between cytokinin and arginine, did not show any significant differences. While the differences were clear with humic and cytokinin, where the highest value when treated (H2C1) 0.424444, and the lowest value at (H0C0) 0.315556. It continued with the interaction between humic and arginine, was the highest percentage at (H2A2) was 0.41500, and the lowest percentage at (H0A0) was 0.32333. As for the triple interaction, there were very significant differences among the experimental factors, as the highest leaves P % when treated with (H2C1A2) reached 0.43333. And the lowest percentage with (H0C0A0) 0.31000.

**Table 4.** Effect of humic acid, cytokinin and argentine and their interactions on the P% in the leaves

A	C	H 0	H 1	H 2	A*C	average -A
A 0	C 0	0.31000	0.35667	0.38333	0.35000	0.362222
		J	EFGHI	CDE	A	
A 1	C 1	0.33667	0.37000	0.41667	0.37444	A
		GHIJ	DEFGH	ABC	A	
A 1	C 0	0.31333	0.35333	0.38667	0.35111	0.364444
		J	EFGHI	BCDE	A	
A 2	C 1	0.33333	0.37667	0.42333	0.37778	A
		HIJ	DEFG	AB	A	
A 2	C 0	0.32333	0.35667	0.39667	0.35889	0.371111
		IJ	EFGHI	ABCD	A	
A 2	C 1	0.34333	0.37333	0.43333	0.38333	A
		FGHIJ	DEFG	A	A	
value LSD		LSD: A*C*H =0.0577			LSD: A*C=	LSD: A=
					0.0381	0.0156
H x A						---
A 0		0.32333	0.36333	0.40000		
		C	B	A		
A 1		0.32333	0.36500	0.40500		
		C	B	A	LSD: A*H =0.0391	
A 2		0.33333	0.36500	0.41500		
		C	B	A		
H x C					average C	
C 0		0.315556	0.355556	0.388889	0.353333	
		E	CD	B	B	
C 1		0.337778	0.373333	0.424444	0.378519	
		D	BC	A	A	
value LSD			LSD: C*H = 0.0381		LSD: C =0.0127	
average H	---	0.326667	0.364444	0.406667		
		C	B	A		---
value LSD			LSD: H = 0.0156			

**3.4. The percentage of potassium in the leaves**

**Table 5.** shows the results of the effect of treatments on the K % in the leaves, where there was a significant difference, Note the superiority of the humic treatment, as there was the highest average at (H2) 1.71278 and the lowest rate with the (H0) 1.37444. So for the cytokinin treatment, as the highest % at (C1) was 1.58556 compared to (C0) 1.50963. While there were no significant differences in the arginine treatment. The same result with overlap between arginine and cytokinin, where no significant differences. Whilst the interaction between humic acid and cytokinin, the results showed that (H2C1) treatment was significantly superior, as the % value of K was 1.76000. In same way with overlap between humic and arginine, where the treatment (H2A2) excelled in the K % when treated over the rest of the overlap treatments. As for the interaction among humic acid, cytokinin, and arginine, the significant differences were very clear. The treatment (H2C1A2) excelled over the rest of the interaction treatments, as it reached the highest value of 1.79333 compared to the control (H0C0A0) 1.27667.

**Table 5.** Effect of humic acid, cytokinin and argentine and their interactions on the K % in the leaves

A	C	H 0	H 1	H 2	A*C	average -A
A 0	C 0	1.27667 J	1.52000 EFG	1.63000 BCDE	1.47556 A	1.51833
	C 1	1.39000 HIJ	1.57000 DEF	1.72333 ABC	1.56111 A	A
A 1	C 0	1.31667 IJ	1.54333 DEFG	1.68000 ABCD	1.51333 A	1.55500
	C 1	1.42667 FHIG	1.60000 CDE	1.76333 AB	1.59667 A	A
A 2	C 0	1.41333 HIGJ	1.52000 EFGH	1.68667 ABCD	1.54000 A	1.56944
	C 1	1.42333 FGHIJ	1.58000 CDE	1.79333 A	1.59889 A	A
value LSD			LSD: A*C*H = 0.1895		LSD:A*C= 0.1589	LSD: A= 0.0623
H x A						---
A 0		1.33333 D	1.54500 C	1.67667 AB		
A 1		1.37167 D	1.57167 BC	1.72167 A	LSD: A*H = 0.1576	
A 2		1.41833 D	1.55000 C	1.74000 A		
H x C					average C	
C 0		1.33556 D	1.52778 C	1.66556 B	1.50963 B	
C 1		1.41333 D	1.58333 C	1.76000 A	1.58556 A	
value LSD			LSD: C*H = 0.1589		LSD: C =0.0508	
average H	---	1.37444 C	1.55556 B	1.71278 A		---
value LSD			LSD: H = 0.0623			

**3.5. Proline content in the leaves (µmol/gm fresh weight)**

**Table 6.** showed the effect of treatments on the proline content in the leaves, the results showed that the lowest average at (H2) 2.22667 compare with (H0) 2.51722, for cytokinin spray,



the lowest value at (C1) 2.32444, compared to (C0) 2.41593. On the contrary, there no significant differences between arginine treatments. The Interaction between arginine and cytokinin, didn't show any differences between them, but for the interaction between humic and arginine, the results showed lowest value at (H2A2) 2.21667 and highest value at (H0A0) 2.57167. When the overlap between humic and cytokinin. The lowest content were at (H2C1) 2.16667 compare with highest value at (H0A0) 2.56333. As for the triple interaction of humic, cytokinin, and arginine, there were very significant differences, as the lowest value reached at (H2C1A2) 2.1667, and the highest value at (H0C0A0) 2.6600.

**3.6. Total yield (ton hectare)**

**Table 7.** showed the effect of the experimental factors on the total yield, where the highest average for humic acid was at (H2), and for cytokinin, the highest value was at (C1) compared to (C0), although, there were no differences for the total yield in arginine treatment. When the interaction of humic and cytokinin was used, there were significant differences, as the highest value of the total yield was in the (H2C1) treatment, and the lowest value was in the control treatment (H0C0).

**Table 6.** Effect of humic acid, cytokinin and arginine and their interactions on proline content in leaves (µmol/gm fresh weight)

A	C	H 0	H 1	H 2	A*C	average -A
A 0	C 0	2.6600 A	2.4333 BCD	2.3167 CDE	2.47000 A	2.40167 A
	C 1	2.4833 ABC	2.3567 BCDE	2.1600 E	2.40667 A	
A 1	C 0	2.5400 AB	2.3967 BCD	2.2833 CDE	2.33333 A	2.36111 A
	C 1	2.4567 ABCD	2.3233 BCDE	2.1667 E	2.31556 A	
A 2	C 0	2.4900 ABC	2.3633 BCDE	2.2600 DE	2.37111 A	2.34778 A
	C 1	2.4733 ABCD	2.3267 BCDE	2.1667 E	2.32444 A	
value LSD			LSD: A*C*H =0.2189		LSD:A*C= 0.1625	LSD: A= 0.0898
H x A						---
A 0		2.57167 A	2.39500 BC	2.23833 D		
	A 1	2.49833 AB	2.36000 BCD	2.22500 D	LSD: A*H = 0.1725	
A 2	2.48167 ABC	2.34500 CD	2.21667 D			
H x C						average C
C 0		2.56333 A	2.39778 BC	2.28667 C		2.41593 B
	C 1	2.47111 AB	2.33556 C	2.16667 D		2.32444 A
value LSD			LSD: C *H = 0.1625			LSD: C =0.0733
average H	---	2.51722 A	2.36667 B	2.22667 C		---
value LSD			LSD: H = 0.0898			

In addition, the interaction between the humic and arginine (H2A2) was the best, and the lowest value was in the control treatment (H0A0). While, in the interaction between cytokinin and arginine, no significant differences were observed. The interactions of all treatments with humic acid, cytokinin, and arginine, it was found a significant differences with (H2C1A2), and less when compared to the control treatment (H0C0A0).

**Table 7.** Effect of humic acid, cytokinin, and arginine, and their interactions on the total yield (ton/ha)

A	C	H 0	H 1	H 2	A*C	average -A
A 0	C 0	0.52773	0.80674	0.97088	0.76845	0.80807
		G	CDEF	ABC	A	
A 1	C 1	0.66463	0.90416	0.97429	0.84769	A
		EFG	BCD	ABC	A	
A 1	C 0	0.56419	0.83146	1.00571	0.80046	0.85324
		G	BCDE	AB	A	
A 2	C 1	0.75964	0.92872	1.02971	0.90602	A
		DEF	A B CD	A	A	
A 2	C 0	0.63492	0.84571	0.99086	0.82383	0.86884
		FG	BCD	AB	A	
A 2	C 1	0.76944	0.91929	1.05285	0.91386	A
		G	ABCD	A	A	
value LSD		LSD: A*C*H =0.1995			LSD:A*C=	LSD: A=
					0.1732	0.0752
H x A						---
A 0		0.59618	0.85545	0.97258		
		C	B	AB		
A 1		0.66191	0.88009	1.01771		
		C	B	A	LSD: A*H =0.1819	
A 2		0.70218	0.88250	1.02185		
		C	B	A		
H x C					average C	
C 0		0.57562	0.82797	0.98915	0.79758	
		E	C	AB	B	
C 1		0.73124	0.91739	1.01895	0.88919	
		D	BC	A	A	
value LSD		LSD: C*H = 0.1732			LSD: C =0.0614	
average H	---	0.65343	0.87268	1.00405		
		C	B	A		---
value LSD		LSD: H = 0.0752				

#### 4. Discussion

The present study outcomes can be initially discussed in light of the clear effects of adding of humic acid, spraying of benzyl adenine and arginine and their interactions on bean plant, as shown to a tolerance of salinity stress via mitigate salinity damage and reduce its toxicity in the plant by the significant increase in the quantitative traits, i.e. percentages of NPK, total chlorophyll, proline content and the yield.

Salt stress is one of the main environmental factors that weaken physiological processes in plants, including vegetative and flowering growth factors and water content [16], by reducing metabolism such as photosynthesis efficiency, decomposition of its pigments [33]. As well as

deterioration of the chloroplast membrane and other organelles such as mitochondria and endoplasmic reticulum [34], disruption of the functions of plant hormones, alteration of basic metabolic pathways and manipulation of gene expression pattern [35], accumulation of proline [36], Salinity stress leads to an ionic imbalance, which leads to the accumulation of harmful ions in plants such as Na and Cl and hinder the uptake of macroelements such as N, P, K, Ca and Mg [37], and microelements such as B, Zn, Cu and Fe [38]. It leads to an increase in the production of reactive oxygen species (ROS) as it causes cellular imbalance, which mainly results in cell membrane damage and biomolecules disruption such as lipids, DNA, and proteins [34]. The Significant reduction of salinity damage and stress tolerance resulting from addition humic acid to the plant leads to improve the vegetative growth characteristics by enhancing the uptake of nutrients and increasing their transfer and accumulation in the shoot system in a significant way, such as macronutrients N, P, K, Ca, Mg, S, and micronutrients such as Fe, Zn, Mn and Cu [39], As well as reducing the accumulation of some toxic elements by reducing their uptake, such as Na [40], as well as elongating root cells, increasing O<sub>2</sub> uptake and respiration, and increasing chlorophyll pigments and thus the efficiency of photosynthesis, which leads to plant tolerance to high concentrations of salinity [24]. In general, the addition of humic acid led to an increase in the vegetative and qualitative traits, and the yield [41].

Cytokinin spraying mitigate the toxicity of saline water, it perhaps be through vegetation growth parameters and also reduced lipid peroxidation, improved oxidative defense in leaves and increased membrane permeability, also compensated for oxidative damage by enhancing antioxidant defense mechanisms such as increasing the enzymatic activity of superoxide, catalase, peroxidase and ascorbate peroxidase, and scavenging ROS [27], Cytokinins also regulate the ability of plants to uptake many nutrients from the environment, including N, P, S and Fe [42], thus work on mineral balance. The nutrient status of plants regulate plant growth [28]. Positive regulation of substances that protect against osmotic stress and ionic balance, antioxidant activity, and finally plant growth and yield [27].

Amino acids such as arginine led to protective effects on plants in alleviating salinity stress by improving vegetative growth qualities, enzymatic activities of antioxidant, like Catalase, Peroxidase, Superoxide Dismutase, and Ascorbate Peroxidase [43], the increase in phenolic substances and osmotic modification, which led to a good antioxidant defense system for the plant and osmolytes accumulation [44], when using arginine, the photosynthesis pigments increased significantly in plants under salinity stress, thus the efficiency of the photosynthesis process increased, as consequence, carbohydrates accumulated, which formed the structure for the plants [45], also a decrease in Na, while an increase in P, K, N, Ca and Mg, It must be mentioned that arginine are an essential nitrogen source in building proteins and enzymes in plants, Therefore, the metabolism of arginine to urea by the enzyme arginase is involved in the recycling of nitrogen to meet the metabolic requirements of growing plants [46], which led to increase in yield characteristics [29], Thus, the ratio of K<sup>+</sup> / Na<sup>+</sup> increased in the leaves, as increased K<sup>+</sup> uptake helps maintain ionic balance, regulate osmotic balance, maintain swelling, and regulate membrane potential [47]. Also N is vital to the photosynthesis process because it participates in the synthesis of chlorophyll, the stabilization of the structure of chloroplasts and the activation of related enzymes, clearly, the arginine is mainly provided by the gene families encoding nitrite transporter (NRT) and ammonium transporter (AMT) proteins [48], In addition, arginine decreased proline accumulation, improved chlorophyll pigments [45], improved

hydration status and reduced oxidative stress under stress conditions [33], which in turn play many important roles in plant growth processes and in alleviating salinity stress.

## 5. Conclusion

In conclusion, the present study draws the following: humic acid, cytokinin, arginine and their interaction enhance significantly the quantitative traits and production of bean plants under salt stress. Optimum yield of bean plants under salinity is obtained when humic acid is applied at 12 Kg.h<sup>-1</sup>, the best overlap treatment is the (H2C1A2) (12 Kg.h<sup>-1</sup> Humic acid), (100 mg.l<sup>-1</sup> Cytokinins (benzel adenine)) and (200 mg.l<sup>-1</sup> Arginine) concentration for the local agricultural climate.

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## Conflict of Interest

The authors declare no conflict of interest.

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