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The effect of bio-fertilization and nitrogen fertilizer spraying on some growth traits and yield of two hybrids of chilli pepper (*Capsicum annuum* L.)

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Abstract

This study was conducted to determine the role of azotobacter bacteria and nitrogen fertilizer in the growth and yield of two hybrids of chilli pepper. The experiment included two factors: the first factor was two hybrids of chilli pepper (Barbarian and Hyffa), while the second factor was the fertilizer treatments, which consisted of eight treatments: the controle treatment, spraying with distilled water (T1), inoculation with the Azotobacter biofertilizer (T2), spraying urea fertilizer at a concentration of 150 mgl^{-1} (T3), spray urea fertilizer at a concentration of 300 mgl^{-1} (T4), spray urea fertilizer at a concentration of 450 mgl^{-1} (T5), Azotobacter + spraying urea fertilizer at a concentration of 150 mgl^{-1} (T6), Azotobacter + spraying urea fertilizer at a concentration of 300 mgl^{-1} (T7), and Azotobacter + spraying urea fertilizer at a concentration of 450 mgl^{-1} (T8). The experiment carried out according to a split plot system design within a randomized complete block design(RCBD) with three replications. The first factor (hybrid) placed in the main plots and the second factor (fertilizer treatments) was placed in the sub-plots. The means compared according to the least significant difference test. (L.S.D) at a probability level of 5%. The results can summarized as follows: The interaction treatment between the fertilizer treatments and the chilli pepper hybrid (T8×Barbarian) showed significant superiority in most of the studied indicators compared to the other interaction treatments and gave the highest values in the percentage of elements in the leaves: Nitrogen (3.021%), Phosphorus (0.567%), Potassium (3.791%). It excelled in indicators of vegetative growth and chlorophyll content in leaves (362.1 mg 100 g^{-1} fresh weight). It also excelled in quantitative indicators of yield represented by fruit weight (33.66 g), number of fruits (34.16 fruits $Plant^{-1}$), and plant yield (1.149 kg $plant^{-1}$), and it also excelled in the qualitative indicators of the fruits and their vitamin C content (121.3 mg 100 g^{-1}).

Keywords: chilli, Barbarian, Azotobacter , urea spray, vitamin C

Introduction

Chilli pepper(*Capsicum annuum* L.) is one of the most important summer crops belonging to the Solanaceae family [1], many previous studies have confirmed its economic, nutritional and medical importance, as it is used fresh, cooked or in the form of hot spices [2], and it is consumed by half the earth's population and is one of the most consumed spices in the world with an average annual consumption of 3.5 million tons of dried fruits [3].

The cultivated area of the pepper plant in 2020 around the world reached 2,069,990 hectares, with a total production of 32,776,260 tons [4]. As for Iraq, the pepper plant is grown in most Iraqi governorates [5], and according to the statistics of the Iraqi Ministry of Planning, the total production of the chilli pepper crop reached 7.041 tons. hectare^{-1} [6].

A balanced supply of nutrients and scientific management practices has the potential to increase the yield of chilli peppers. Using chemical fertilizers alone can provide only one or two nutrients to the crop. On the other hand, providing only organic inputs can improve the physical and biological environment of the soil but is low in nutrient content.

The integrated use of chemical fertilizers, organic fertilizers and biofertilizers leads to obtaining a large yield of crops of good quality and also preserves the soil from degradation and pollution. Therefore, there is a need to develop appropriate technology for integrated nutrient management that will go a long way in building soil fertility and chilli pepper productivity [7].

The application of biofertilizers helps to improve the biological activities of the desired soil microorganisms and helps to improve plant growth, yield and quality of fruits and seeds. Furthermore, biofertilizers are cheap sources of nutrients, environmentally friendly and have the potential to increase the productivity of vegetable crops by 2 to 45% [8].

Among biofertilizers, Azotobacter has beneficial effects not only on nitrogen fixation efficiency but also with its ability to produce antibacterial and antifungal components, and growth regulators [9]. To obtain good quality fruits, hybrids with good specifications must be chosen that have the ability to respond to growth indicators in different environmental conditions and that have a clear effect on the plant, whether vegetative or fruitful. Fertilization factors, especially organic and biological fertilization, are one of the important environmental factors in growth [10].

Bhuvaneswari [11] found, when fertilizing pepper plants with urea, a significant increase in the chlorophyll content of the leaves during the vegetative growth stage, as the concentration of 50 kg nitrogen ha^{-1} gave the highest value of 50.12 SPAD compared to the comparison treatment. Ayodele [12] found, when fertilizing chilli pepper with urea, a significant increase in leaf area and vitamin C content of fruits in the sixth week after planting, as the concentration of 75 kg N ha^{-1} gave the highest value of 37.40 cm^{2} and 34.04 mg 100gm^{-1}, respectively. Sharma and Singh [13] found that when fertilizing chilli pepper with Azotobacter, there was a significant increase in fruit weight and yield per plant, and gave the highest value of 8.43 gm Fruit $^{-1}$ and 153.47 gm plant^{-1}, respectively, compared to the comparison treatment.

Therefore, the present study designed to study the integrated use of biofertilizers and chemical fertilizers and their impact on growth two hybrids of chilli peppers.

Materials and Methods

The experiment was carried out in the open vegetable field of the Department of Horticulture and Garden Engineering/ Faculty of the Agriculture/University of Karbala, Husseiniya district for the 2022-2023 agricultural season to see the role of Azotobacter and nitrogen fertilizer in the growth and product of two hybrids of chilli.

Table (1): Properties soil of the experiment physical and chemical

Property	pH	EC ds.m^{-1}	Total N mg.Kg^{-1}	CaCo$_3$ mg.Kg^{-1}	Organic matter %	Availaple P mg.Kg^{-1}	Clay-silt-sand %
Value	7.42	3.52	22	21.43	3	4.1	43.6-20.42-36

The experiment was carried out according to the split plot system within the design of the Randomized Complete Block Design with three replications, each replication includes two pepper hybrids: Barbarian and Hyffa The experiment consisted of the following treatments: T1 = Control (Without fertilization); T2 = Azotobacter ; T3= Spraying urea 150 mgl^{-1}; T4= Spraying urea 300 mgl^{-1}; T5= Spraying urea 450 mgl^{-1}; T6= Azotobacter + spraying urea 150 mgl^{-1}; T7= Azotobacter + spraying urea 300 mgl^{-1}; T8= Azotobacter + spraying urea 450 mgl^{-1}.

The experiment includes 48 experimental units, and the area of each experimental unit is 3 m^2. The number of plants in each experimental unit is (8 plants), and the distance between the plants is (40 cm).

Seedlings of two hybrid chilli (*Capsicum annuum* L.) were planted on March 25, 2022. The first was Barbarian, belonging to the Indian company United Genetics, and the second was Hyffa, of Dutch origin, produced by the West Frisian Seeds Company, in Raised Bed with the use of a drip irrigation system. Nitrogen fertilizer (urea) sprayed in three batches. The first 4/30/2022, the second 5/14/2022, and the third 5/29/2022. The compound chemical fertilizer PK (144 kg K2O + 160 kg P2O5)/ ha [14], and organic fertilizer (sheep manure) added to the soil according to the fertilizer recommendation of 5% of Soil volume [15]. Field service operations e carried out, including irrigation, hoeing, weed removal, and pest control, as needed.

Azotobacter added at concentrations of 1 x 18 CFU/ ml at a rate of 10 ml/ hill to treatments that required addition in the field with planted seeds.

Five plants randomly selected from each experimental unit for the purpose of conducting the required measurements, which included estimating the percentage of nutrients in the leaves such as Nitrogen [16], Phosphorus [17], and Potassium [18], and the leaf area of the plant (dm2), the leaf content of total chlorophyll (mg. 100 g^{-1} fresh weight) [19], weight of the fruit (g), number of fruits per plant, plant yield (kg. plant^{-1}), and the vitamin C content of the fruits (mg. 100 gm^{-1} fresh weight) [20].

Statistical analysis

After collecting and tabulating the data related to the study, it was statistically analyzed according to the factorial experiment system applied by randomized complete blocks designing (R.C.B.D), and using the GenStat15 program, the least significant difference ($L.S.D_{0.05}$) test was used to compare and separate the means [21].

Results and Discussion

Characteristics of vegetative growth

The results presented in Table 2 show that there is a significant effect of the interaction between hybrids and fertilizer treatments on the leaf content of nutrients represented by N, P, and K. The T8 × Barbarian interaction treatment excelled, with rates reaching 3.21%, 0.567%, and 3.791%, respectively, and did not differ significantly from the results. Achieved by the T8 × Hyffa**.** The lowest percentage of nutrients was in the leaves of plants in the interaction treatment (T1 × Hyffa) for Nitrogen and Potassium, the treatment (T1 × Barbarian) for phosphorus. This is attribute to the role of growth-stimulating bacteria (Azotobacter) in fixing atmospheric Nitrogen in the soil and increasing the availability of elements by lowering the pH, which in turn leads to the release of elements, including Phosphorus and Potassium [22], if it secretes organic acids such as citric, lactic, oxalic and succinic acids, as well as its release of CO_2 gas and its solubility in water produces carbonic acid, which contributes to the dissolution of phosphate compounds and some potassium-containing minerals [23], It also secretes chelated compounds such as hydroxyl and carboxyl groups that chelate positive ions, including Ca+2 of calcium phosphate converted phosphorus into dissolved forms [24. In addition, bacteria secrete growth regulators and enzymes that increase vegetative, root activity, and thus increase nutrients from the soil, which has led to an increase in their concentration in the plant [25].

Spraying urea fertilizer, which is characterized by its high nitrogen content (46%), is a direct source of nitrogen in the leaves, and its entry into cellular metabolism contributes to the formation of the amino acid Tryptophan, which is the initiator of IAA, which encourages vegetative and root growth [26], and increases the absorption capacity of the root, which in turn increases nutrients such as N, P, K [27].

The results presented in Table 2 show that there is a significant effect of the interaction between hybrids and fertilizer treatments on the leaves’ chlorophyll content and leaf area. The T8×Barbarian interaction treatment achieved the highest results, reaching (362.1 mg. 100 gm^{-1} wet weight, 74.15 dm2. plant^{-1}), respectively. While the T1 × Hyffa intervention treatment gave the lowest chlorophyll content, amounting to 129.8 mg 100 gm^{-1} fresh weight, while the lowest leaf area was in T1 × Barbarian, amounting to 52.25 dm² plant^{-1}. This is due to the increase in the content of nutrients of the leaves, especially nitrogen, in the T8 and T7 treatments, which had a clear effect on increasing the leaf area and the total chlorophyll content of the leaves. The reason may due to the azotobacter secreting biochemical compounds in the root growth area (Rhizospher), which are substances similar to Auxins, Gibberellins, Cytokinins, and Vitamins, which increase the root hairs and their growth, which increases the surface area of the roots and increases absorption, which contributes to encouraging vegetative growth, and this is consistent with what Singh and Sharma [13], Dhopavakar et al. [28] found on the chilli pepper plant, and with what Kazem and Mutar [29] found.

It seems that the increase in the total chlorophyll content is due to the role of nitrogen in building the four groups of prophyrins that are involved in the formation of chlorophylls (chlorophyll A and b) and the construction of cytokinins, each of which is the basis for the processes of carbon metabolism and respiration [30], which increases the accumulation of carbohydrates, thus increasing the activity of cell division and elongation in the plant and then increasing vegetative growth, especially the leaf area, and perhaps the role played by nitrogen in building growth hormones, especially Auxins and Cytokinins, which stimulate cell division and elongation and then increase Vegetative growth, especially leaf area [31].

Table (2): Effect of Azotobacter and urea spraying in some chemical components of leaves and growth of two Chilli Pepper hybrids

Fertilizer treatments	Hybrid	N(%)	P(%)	K(%)	Chlorophyll (mg 100g wet weight^{-1})	Leaf area (dm^2 plant^{-1})
T1	Barbarian	1.042	0.324	2.956	132.6	54.61
T2		2.232	0.392	2.961	143.7	60.23
T3		2.221	0.413	2.964	140.6	63.11
T4		2.431	0.442	2.960	194.9	69.78
T5		2.693	0.456	3.080	223.6	70.81
T6		2.762	0.481	3.396	286.7	73.22
T7		2.881	0.507	3.425	323.3	73.61
T8		3.021	0.567	3.791	362.1	74.15
T1	Hyffa	1.006	0.339	2.631	129.8	52.25
T2		2.172	0.410	2.701	133.3	59.21
T3		2.206	0.447	2.796	146.2	63.15
T4		2.513	0.461	3.022	170.6	66.17
T5		2.701	0.467	3.038	206.4	69.64
T6		2.863	0.477	3.271	239.8	70.10
T7		2.981	0.489	3.441	281.6	72.18
T8		2.997	0.521	3.564	309.8	73.19

L.S.D(0.05)	0.182	**0.049**	**0.381**	**42.02**	**11.31**

T1= Control , T2= Azotobacter, T3= Spraying urea 150 mg L^{-1}, T4= Spraying urea 300 mg L^{-1}, T5= Spraying urea 450 mg L^{-1}, T6= Azotobacter + Spraying urea 150 mg L^{-1}, T7= Azotobacter + Spraying urea 300 mg L^{-1}, T8= Azotobacter + Spraying urea 450 mg L^{-1}

Characteristics of fruit growth

The results presented in Table 3 show that there is a significant effect of the interaction between hybrids and fertilizer treatments on most of the quantitative and qualitative characteristics of the fruit, represented by the number of fruits, fruit weight, plant yield, and vitamin C content of the fruits. The T8 × Barbarian interaction treatment achieved the highest results and reached (31.60 fruits. $Plant^{-1}$, 32.51 gm , 1.027 $kg.plant^{-1}$, 120.3 mg.100 g^{-1} fresh weight) respectively. While the T1 × Hyffa interaction treatment gave the lowest results and reached (23.66 fruits. $plant^{-1}$, 11.33 g, 0.268 kg. $plant^{-1}$, 82.78 mg. 100 g^{-1} fresh weight). The superiority of treatments T6, T7, and T8 in yield indicators may be due to their role in increasing vegetative growth indicators such as leaf area and leaf content of total chlorophyll (Table 2), which in turn led to an increase in the accumulation of carbohydrates, which is associated with improving the nutritional status of the plant, especially potassium (Table 2) and its important role in the transport of nutrients and storage in the fruits, which reduced the competition between the fruits on the accumulated nutrients and this explains the increase in the average weight of the fruit (Table 3) In T8 treatment [32]. Also, the role of Azotobacter and spraying urea contributes to increasing nitrogen in the leaves, which has a role in the formation of growth hormones, as well as hormone-like substances (such as Auxins and Cytokinins) secreted by Azotobacter, which act as chemical vectors in the transfer of nutrients from the places of production (leaves) to the fruits, which leads to Increased fruit growth and weight [32], which was reflected in the increase in the yield of one plant.

The reason for the significant superiority of the vitamin C content of fruits in treatments T7 and T8 is attributed to the role of Azotobacter and urea fertilizer spraying in the nitrogen supply to the plant, which increased the content of leaves from it (Table 2), which was reflected in the increase in vegetative growth indicators, especially leaf area and total chlorophyll (Table 2), which led to an increase in the interception of the leaves and their absorption of sunlight and its positive impact on activating the process of photosynthesis, and as a result increasing the production and accumulation of manufactured materials, which was reflected in an increase in the vitamin C content of the fruits.

Table (3): Effect of Azotobacter and urea spraying in some yield and Quality traits of two Chilli Pepper hybrids.

Fertilizer treatments	Hybrid	Fruits number	Fruit weight (g)	Plant yield (Kg $plant^{-1}$)	Vitamin C (mg 100g wet $weight^{-1}$)
T1	Barbarian	26.13	12.08	0.315	81.63
T2		27.66	15.13	0.418	82.60

T3		27.81	15.03	0.417	81.67
T4		29.33	17.10	0.501	88.96
T5		31.22	20.05	0.625	93.74
T6		33.15	27.82	0.922	102.8
T7		33.69	32.44	1.089	111.7
T8		34.16	33.66	1.149	121.3
T1	Hyffa	23.66	11.33	0.268	82.78
T2		26.81	13.07	0.350	82.94
T3		26.84	13.12	0.352	82.76
T4		27.98	16.87	0.472	88.01
T5		28.13	19.63	0.552	92.62
T6		29.16	23.16	0.675	101.6
T7		29.81	28.07	0.836	109.6
T8		31.60	32.51	1.027	120.0
L.S.D(0.05)		3.061	2.063	0.051	13.02

T1= Control , T2= Azotobacter, T3= Spraying urea 150 mg L^{-1}, T4= Spraying urea 300 mg L^{-1}, T5= Spraying urea 450 mg L^{-1}, T6= Azotobacter + Spraying urea 150 mg L^{-1}, T7= Azotobacter + Spraying urea 300 mg L^{-1}, T8= Azotobacter + Spraying urea 450 mg L^{-1}

The differences between hybrids in most of the growth and yield traits studied may be due to variation in genetic nature and the control of genes and their influence on physiological processes and metabolism [33]. The difference in genetic nature may affected by environmental conditions, which is reflected in the behavior and response of the plant depending on the prevailing environmental conditions, which affects the qualitative yield indicators of the fruits [34].

It is clear from the previous results that the combination between biofertilizers (Azotobacter) and nitrogen fertilizers (Urea) has led to an increase in most of the vegetative growth indicators and yield, in addition to improving the Capsaicin content of fruits. Therefore, it recommended to use a fertilizer treatment consisting of adding Azotobacter and spraying urea fertilizer at a concentration of 450. mg. L^{-1} because of its major role in improving growth and yield, in addition to reducing excessive use of chemical fertilizers and reducing their harmful impact on the environment. The difference in the genetic compositions of the studied hybrids has affected the quantitative and qualitative yield indicators. The Barbarian hybrid showed significant superiority in increasing the studied quantitative and qualitative yield indicators compared to the Hyffa hybrid. Therefore, we recommend the possibility of planting the hybrid Barbarian in the holy governorate of Karbala due to the suitability of climatic conditions with the requirements of its growth.

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