The Role of Nitrogen, Organic and Biofertilizers in some Chemical and Qualitative Characteristics of Spinach *Spinacea oleracea* L.

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Abstract. The experiment was conducted in the fields of the Department of Horticulture and Landscape / College of Agriculture / University of Karbala in Al-Hussainiya district of Karbala governorate during the autumn season 2021-2022 to study the response of some chemical and qualitative characteristics of spinach variety Viroflay, with 2 factors,the levels of nitrogen fertilizer (urea) represent the main plots and the sub plots the fertilization treatments of the secondary panels (Sub plot) and the experiment included 54 experimental units, and the differences were compared using the least significant difference test (L.S.D) at a probability level of 0.05. The most important results that were reached can be summarized as follows The results of the fertilizer addition biological fertilizer + poultry manure (T6 treatment) showed a significant superiority in the leaves' content of nutrients (N, P, K)in leaves, the leaf area(86.68 cm2.plant-1) , the total chlorophyll content of leaves (212.8 mg.100g) and the total yield (44.81 tons.ha-1). The addition of nitrogen fertilizer (urea) in treatment with N100% led to significant differences in the leaves in most of study indicators, The binary interactions between the fertilizer treatments and the nitrogen fertilization (urea) significantly affected the studied traits, as the interaction treatment N100×T6 achieved the highest values in the leaf nutrients content (N 6.217%, P 2.202% and K 4.480%), The same interaction treatment showed a significant increase between the levels of fertilization, as it gave the highest rates of vegetative characteristics and yield, and the leaf area was 104.0 cm2.plant-1 and the total chlorophyll content of leaves was 2016.0 mg.100gm and the total yield was 48.86 tons. ha-1.

Keywords. Spinach, Sheep manure, Bio fertilization, Azotobacter, Nitrogen fertilizer.

.Introduction

Spinach is a plant that belongs to the family Chenopodiaceae, one of the most important vegetable crops in the world, and the region of southwest Asia (Iran) is its original home and from there it spread to the rest of the world [1]. The cultivated area in the world is estimated at about 980,000 hectares, while in Iraq the cultivated area is about 250 hectares, with a production rate of about 17000 tons annually [2]. The spinach plant contains many vitamins, including vitamin C, vitamin E and vitamin A, and various nutrients such as calcium, iron, phosphorous and zinc [3]. It also contains beta-carotene, which protects against heart and blood vessel diseases and cancerous diseases [4]. However, it is considered one of the plants with a high content of oxalic acid, which is attributed to the pungent taste in the plant. Its quantity is estimated at 400-100 mg. 100 g-1 Fresh weight, while in some European varieties it may reach more than 930 mg. 100 gm-1 [5.[

The global trend is to words the use of organic materials with animal or plant origin as a source of fertilizer for the purpose of reducing

ISSN 2072-3857

the pollution of the environment and agricultural soils with chemical, and the production of agricultural crops safe for humans and animals and compensation of organic matter, which loses soil as a result of intensive agriculture. Organic fertilizers additionally enhance soil physical and compound properties and decrease the requirements for mineral composts, which is reflected increase of vegetative growth and yield of plants[6.[

Also, the accumulation of nitrate in vegetables depends on many endogenous and exogenous factors for which mineral nutrition is of critical importance given the positive response of spinach to the use of organic fertilizers.It appears that the use of these organic fertilizers will significantly reduce the input of chemical fertilizers as well as reduce the environmental side effects of the chemicals and actually go into the production of safe organic products [7,8[

As for bio-fertilizers, they are one of the best modern technologies in agriculture and were used as an alternative to traditional fertilizers [9]. They are micro-organisms that improve the growth of host plants and their important role in maintaining soil fertility.It is used with organic and mineral fertilizers side by side, and reduces the use of chemical fertilizers, which is less costly and environmentally friendly. Rather, it is one of the means of preserving the environment, Therefore, its use has been directed [10,11]. Therefore, this research aims to study aims to rely on environmentally friendly fertilizers such as bio and organic fertilizers to reduce the amount of chemical fertilizer (urea) by reaching the appropriate mixture in the soil of biological, organic and chemical fertilizer (urea) to improve production in quantity and quality.

.2Material and Method

The experiment was carried out in the open field of the Department of Horticulture and Landscape Engineering/ College of Agriculture/ University of Karbala, Al-Hussainiya district, for the agricultural season 2021-2022 to know the role of nitrogen, organic and biological fertilizers in the chemical and qualitative growth indicators of spinach, The experiment was carried out according to a split plot system in a randomized complete block design with three replications in each replicate the levels of chemical fertilizer (urea) represent the main plot and six fertilizer treatments are without fertilizer (T1), biofertilizer (T2), sheep manure (T3), poultry manure (T4), biofertilizer + sheep manure (T5) and biofertilizer + Poultry manure (T6) represents the sub plot, The averages of the treatment were compared with the LSD test at a probability level of 5% [12]. and the experiment included 54 experimental units (with three replications), each replicate (18 experimental units) in which the following treatment are distributed:

- The levels of three urea fertilizers were without fertilization (N0%), 30 kg ha-1 (N50%), and 60 kg ha-1 (N100%) were added to the soil based on the fertilizer recommendation 60 kg/ha-1 [13]., which was returned to a level of 100%.

- Fertilization was carried out using special isolates of . (Azotobacter SP, Azospirillum SP.(

The land was divided into 6 terraces, the length of which is 18 m, and the distance between them is 1 m, with two terraces for each duplicate, the width of each terrace is 1 m. Each terrace contains 9 experimental units, each area is 2 m2 (length 2 m x width 1 m). A fertilizer recommendation was adopted to add nitrogen fertilizer at a rate of 60 kg.h-1 N in the form of urea added in two batches, the first after germination and the second after the first application [13]. Spinach seeds, Viroflay variety, produced by Elite seeds of the Netherlands, were planted in planting lines on 10/28/2021, knowing that each experimental unit contained 6 lines, the distance between one line and another was 15 cm and a distance

ISSN 2072-3857

of 10 cm between one plant and another. The number of plants was 120 plants per experimental unit. Drip irrigation was adopted as The distance between the dots is 30 cm. Agricultural service operations were carried out as recommended, Two types of animal manure (decomposed waste) were added according to the fertilizer recommendation of 10 m3 dunams [14]. the first is poultry manure and its symbol is p, and the second is sheep manure (Sheep) and its symbol is S as well as the non-fertilization and its symbol 0. Then the samples were analyzed from Animal manure.

.2.1Studied Traits

- Percentage of nitrogen in leaves.%
- Percentage of phosphorous in leaves .%
- Percentage of potassium in leaves.%
- Quantity of yield (tons. h-1.(
- Leaf area (cm2. Plant-1 .(
- Chlorophyll content in leaves (mg.100g fresh weight.(

.3Results

.3.1Percentage of Nitrogen in Leaves%

The results of Table (1) showed that the percentage of nitrogen in spinach leaves increased significantly when using organic and biological fertilizers, reaching the highest percentage in treatment T6 (6.061) %, followed by a significant difference in treatment T5, which gave a nitrogen percentage of 5.332% compared to the lowest percentage when treated Comparator T1 (2.747) %, Also, the percentage of nitrogen was significantly affected by the level of urea, and it was found that the two treatments N100% and N50% were similar to each other and without significant difference, and they outperformed the treatment N0% by increasing the percentage of nitrogen to 4.586 and 4.342%, respectively, As for the effect of the interaction between fertilizer treatments and urea levels, it was significant in the percentage of nitrogen, and the highest value found in the interaction treatment was N100%×T6, which amounted to 6.217%, followed by a significant difference of N50%×T5 and N0%×T5, where the ratio reached 5.390 and 5.037%, respectively, compared to the lowest A value of 2.657% when nesting the transaction N0%×T1.

3.2 **Table 1.** The effect of organic and biological fertilizers and the reduction of urea fertilizer in estimating the percentage of nitrogen (%) for spinach plants.

Composting Treatment	Treatment code	urea levels			Average composting Treatment
		N0%	N50%	N100%	
Comparison	T1	2.657	2.757	2.827	2.747
bio fertilizer	T2	2.940	3.167	3.567	3.224
sheep manure	T3	3.823	4.003	4.283	4.037
poultry manure	T4	4.487	4.663	4.947	4.699
Bio fertilizer + sheep manure	T5	5.037	5.390	5.570	5.332
Bio fertilizer + poultry manure	T6	5.897	6.070	6.217	6.061
Average urea levels		4.140	4.342	4.568	
L.S. D	Composting Treatment 0.757		Transaction 0.677		urea levels 0.371

.Percentage of Phosphorous in Leaves%

ISSN 2072-3857

The results of Table (2) show that there are significant differences between the averages of fertilizer treatments in the percentage of phosphorous in spinach leaves. The results show that treatment T6 is significantly superior to the rest of the fertilizer treatments and it gave the highest percentage of phosphorous in leaves that amounted to 2.060% compared to the lowest percentage of 0.548% in the comparison treatment. T1, The treatment of urea levels had a moral effect in increasing this percentage, as it reached 1.395% at the N100% level, while the lowest percentage was found at the N0% level, which amounted to 1.070%. The interaction between fertilizer treatments and urea levels showed a significant effect on the percentage of phosphorous in the leaves, as the N100% × T6 interaction was superior by giving it the highest percentage of phosphorus in the leaves (2.202%), while the lowest percentage of phosphorus in the N0% × T1 interaction treatment amounted to 0.433%.

3 **Table 2.** The effect of organic and biological fertilizers and the reduction of urea fertilizer in estimating the percentage of phosphorous element (%) of spinach.

Composting Treatment	Treatment code	urea levels			Average composting Treatment
		N0%	N50%	N100%	
Comparison	T1	0.433	0.571	0.640	0.548
bio fertilizer	T2	0.579	0.713	0.827	0.706
sheep manure	T3	0.716	0.914	1.044	0.891
poultry manure	T4	1.149	1.397	1.607	1.384
Bio fertilizer + sheep manure	T5	1.634	1.894	2.048	1.859
Bio fertilizer + poultry manure	T6	1.910	2.069	2.202	2.060
Average urea levels		1.070	1.260	1.395	
L.S. D	Composting Treatment		Transaction		urea levels
	0.073		0.122		0.053

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3

.Percentage of Potassium in Leaves%

The results presented in Table (3) showed that there were significant differences between the averages of fertilizer treatments in the percentage of potassium in the leaves, and the T6 treatment significantly outperformed the rest of the treatments by giving it the highest percentage of potassium that amounted to 4.071%, while the lowest percentage of potassium was 3.437% in the comparison treatment. (T1), The results also show that there are significant differences between the average levels of urea, and the level N100% gave the highest percentage of potassium, which amounted to 4.199%, while the lowest percentage was found at the level of N0%, which amounted to 3.418%, As it turns out from the interaction between fertilizer treatments and urea levels, there were significant differences between the interactions, the highest percentage was 4.480% in the treatment N100% x T6, while the lowest percentage was in the interaction treatment N0% x T1 amounted to 3.110%.

ISSN 2072-3857

Table 3. The effect of organic and biological fertilizers and the reduction of urea fertilizer in estimating the percentage of potassium (%) for spinach.

Composting Treatment	Treatment code	urea levels			Average composting Treatment
		N0%	N50%	N100%	
Comparison	T1	3.110	3.280	3.923	3.437
bio fertilizer	T2	3.263	3.516	4.043	3.607
sheep manure	T3	3.346	3.636	4.113	3.698
poultry manure	T4	3.453	3.753	4.283	3.830
Bio fertilizer + sheep manure	T5	3.630	3.910	4.353	3.964
Bio fertilizer + poultry manure	T6	3.710	4.023	4.480	4.071
Average urea levels		3.418	3.686	4.199	
L.S. D	Composting Treatment		Transaction		urea levels
.3.4					

Quantity of Yield (tons. h-1(

It is noted from the results of Table (4) that there are significant differences between the average fertilizer treatments in the total yield of spinach, as treatment T6 excelled and gave 44.81 tons. hectares -1, while the lowest total yield was in the comparison treatment T1 and it amounted to 11.36 tons. hectares-1, The results of the same table showed that there were significant differences between urea levels in the plant yield. The treatment N100% achieved the highest yield of the plant, which amounted to 28.00 tons. ha-1, and the lowest total yield was found in the treatment N0%, which amounted to 23.73 tons. ha-1. The results also show that there are significant differences in the interaction between fertilizer treatments and different levels of urea in increasing the yield. The interaction treatment N100%×T6 gave the highest total yield amounting to 48.86 tons. ha-1, while the lowest total yield was when the interaction treatment N0%×T1 reached 9.80 tons. ha -1.

Table 4. The effect of organic and biological fertilizers and the reduction of urea fertilizer on the total yield (ton. ha^{-1}) of spinach.

Composting Treatment	Treatment code	urea levels			Average composting Treatment
		N0%	N50%	N100%	
Comparison	T1	9.80	11.73	12.55	11.36
bio fertilizer	T2	15.86	16.11	17.27	16.41
sheep manure	T3	18.62	19.71	21.57	19.97
poultry manure	T4	24.05	26.18	30.43	26.89
Bio fertilizer + sheep manure	T5	32.22	34.81	37.30	34.77
Bio fertilizer + poultry manure	T6	41.85	43.71	48.86	44.81
Average urea levels		23.73	25.37	28.00	
L.S. D	Composting Treatment		Transaction		urea levels
	5.601		6.107		3.5
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ISSN 2072-3857

.Leaf Area (cm2. Plant-1 (

It is clear from the results of Table (5) the moral effect of the fertilizer treatments on the leaf area, as treatment T6 was distinguished by giving it the highest leaf area amounting to 86.68 cm2.plant-1, and the lowest leaf area in non-treated plants T1 was 35.02 cm2.plant-1 ,

As for the effect of urea levels, it was significant as the leaf area increased with the increase of urea levels, as it gave N100%, followed by N50%, which gave 57.92 cm2.plant-1, followed by N0%, which gave 48.32 cm2.plant-1, It was noticed from the effect of the interaction between the fertilizer treatments and urea levels that there was a significant effect on the leaf area, and the interaction treatment N100%×T6 gave the highest value of 104.08 cm2.plant-1, while the lowest value was found in plants of the interaction treatmentN100%×T1 which amounted to 32.30 cm2.plant-1.

Table 5. The effect of organic and biological fertilizers and the reduction of urea fertilizer on the leaf area ($cm^2.plant^{-1}$) of spinach.

Composting Treatment	Treatment code	urea levels			Average composting Treatment
		N0%	N50%	N100%	
Comparison	T1	32.30	34.52	38.23	35.02
bio fertilizer	T2	37.19	41.60	45.97	41.59
sheep manure	T3	42.43	46.87	52.43	47.24
poultry manure	T4	51.59	58.24	72.93	60.92
Bio fertilizer + sheep manure	T5	60.29	76.46	88.15	74.97
Bio fertilizer + poultry manure	T6	66.12	89.84	104.0	86.68
Average urea levels		48.32	57.92	66.96	
L.S. D	Composting Treatment 6.542		Transaction 11.331		urea levels 4.626

3.6

.Chlorophyll Content in Leaves (mg.100g fresh weight(

The results in Table (6) showed a significant effect on the chlorophyll content of the leaves among the fertilizer treatments for spinach. It is clear from the table that treatment T3 (201.8 mg.100g) outperformed the two treatments T2 and T1 and that treatment T6 gave the highest value of 212.8 mg.100g, while the T1treatment recorded the lowest content of chlorophyll was 185.8 mg.100 g, The results also showed a significant effect between urea levels, where the N100% level outperformed the N0% level by giving it the highest content of 207.0 mg.100 g, and it did not differ significantly from the N50% level, It was noticed from the interaction between the fertilizer treatments and urea levels that there was a significant difference that was clear in the treatment T4×N0% that amounted to 200.4 mg.100g, while the comparison treatment T1×%N0 gave the lowest chlorophyll content of 170.0 mg.100g.

ISSN 2072-3857

Table 6. The effect of organic and biological fertilizers and the reduction of urea fertilizer on the estimation of chlorophyll concentration (mg.100g) for spinach plants.

Composting Treatment	Treatment code	urea levels			Average composting Treatment
		N0%	N50%	N100%	
Comparison	T1	170.0	189.1	198.4	185.8
bio fertilizer	T2	186.9	196.9	201.1	194.9
sheep manure	T3	197.0	202.2	206.2	201.8
poultry manure	T4	200.4	207.5	209.0	205.6
Bio fertilizer + sheep manure	T5	206.4	209.1	211.2	208.9
Bio fertilizer + poultry manure	T6	209.8	212.6	216.0	212.8
Average urea levels		195.1	202.9	207.0	
L.S. D	Composting Treatment 6.829		Transaction 11.82		urea levels 4.829

.Discussion

It is noted from the above tables (1, 2 and 3) that there are significant differences in the content of the leaves of nutrients as a result of adding organic, biological and nitrogen fertilizers, The reason for the increase in the proportion of these elements (, K, P, N) may be attributed to in spinach leaves to increase nitrogen fertilizer levels in general, as it led to an increase in the content of spinach leaves of N, P and K [15], And that the integrated supply of organic fertilizers with chemical and biological fertilizers (Azotobacter sp.) led to an increase in the availability and absorption of nutrients N, P and K that lead to increased growth this is because nitrogen fertilization enhances nitrogen absorption and efficiency of use, and the organic matter works to modify the PH of the soil and thus increases the readiness of nutrients close to the root area, especially that biofertilizers that contain beneficial bacteria stabilize nutrients in the soil and increase the efficiency of their absorption [16], The nitrogen that enters into the composition of the amino acids contained within the components of these fertilizers is ready for absorption and assimilation directly [17.[

The above results indicate that there are significant differences in the vegetative characteristics Table (5) leaf area, Table (6) leaf content of chlorophyll as a result of adding nitrogen, organic and bio fertilizers to spinach plant ,

The reason for this increase may be due to the role of nitrogen, which stimulates the different growth characteristics of the plant, because nitrogen is an essential element for building protoplasm, amino acids and protein that promote cell division and thus increase vegetative growth, which is reflected in the height of the plant and the number of leaves and leaf area that increases the manufactured materials inside the plant as a result Because of the high content of chlorophyll in the leaves, which is reflected in the increase in the amount of yield, and nitrogen plays a vital contribution in many biochemical processes related to plant growth, adding that the use of nitrogen greatly enhances the characteristics of vegetative growth [18,19], The reason for the increase in vegetative growth may be due to the role of these fertilizers in increasing the solubility of phosphorous and other nutrients, raising the efficiency of their absorption and increasing the content of organic matter, The reason for the increase in the yield Table (4) is also attributed to the biological fertilizers

ISSN 2072-3857

containing the vital stimulants that increase the plant's metabolic and nutritional activity, It increases the vital activities of the plant, thus increasing the vegetative growth, such as the number of leaves and leaf area, which positively reflects on the quantity and quality of the yield [20.[

Conclusions

- The effect of adding nitrogen fertilizer (urea) at a level of 100% showed an increase in all indicators of vegetative growth, yield and leaf nutrient content over the rest of the other levels.

- The results of the experiment indicate that the use of a fertilizer combination of organic and biological fertilizers (poultry with Azotobacter and Azospirillium bacteria) to the superiority of all measured traits of vegetative growth, yield and chemical indicators.

- The binary interaction between nitrogen fertilization (100%) and organic fertilization (poultry) and biological fertilization (Azotobacter and Azospirillium) had significant effects on the other levels in all vegetative characteristics, yield and qualitative indicators.

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ISSN 2072-3857