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Effect of Biofertilizer and Foliar Applied Nutritive Compound on Growth, Yield and Chemical Composition of Spinach (*Spinacia oleracea* L.) In Sandy Soil

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Abstract: In order to emphasise on sustainable agricultural practices, alternative fertilization methods have garnered attention, particularly bio-fertilizers known for their eco-friendly attributes and potential multifaceted benefits. Therefore, two field experiments were carried out at Experimental Farm, National Research Centre El-Behaira Governorate, Egypt to study the effect of nitrogen biofertilizer (Biogen), and Nutrin compound fertilizers on growth, yield and quality of spinach during two winter seasons of 2022/2023and 2023/2024. The results showed that inoculation of spinach seeds with Biogen had a positive effect of spinach plants by increasing the vegetative growth, as well as total fresh yield during the two growing seasons. Foliar fertilizer treatments revealed a notable trend; the higher dose of foliar application of Nutrin indicates significant effectiveness in promoting robust growth characteristics, as well as total yield during the two growing seasons. The results indicate that both biofertilizer treatments and foliar nutrient Nutrin act independently, therefore no evident interaction between the two treatments occurred. Highly significant effects due to Biogen or Nurtrin application to spinach were reported for N %, protein, P, K % significantly increased when Biogen was applied especially at the higher rate (2kg/fed)or Nutrin at (4 cm/l). Regarding the nitrate content of spinach leaves it is clear that the reported values were within the acceptable daily intake permitted. A highly significant positive correlation coefficient between total fresh yield and N%, Crude protein % P% K% and nitrate content(ppm) were reported. (Fed= Feddan=4200m^2)

Key words: Biofertilization · Chemical components fresh yield · Growth · Nitrate content

INTRODUCTION

Spinach (*Spinacia oleracea* L.) is as an annual leafy popular winter vegetable crop grown in Egypt, it is used as fresh, canned or frozen product. Spinach is considered to be as one of healthy vegetable crops for human consumption. It contains the major vitamins, i.e. vitamin C, A and E, minerals such as iron, calcium, potassium, manganese and zinc [1]. Spinach is a vegetable with a high biological value because it extremely rich in antioxidants compounds, especially Beta-carotene and lutein which both have antioxidant and anticancer properties. The disadvantage of this crop had capable to accumulate NO_3 and oxalates with a high quantity in different parts of plant [2, 3]. These compounds are unfavorable because they have hazard effect on human health [4].

Leafy vegetables yield and nutritive value could be affected by the agricultural practices. One of these leading factors is fertilization. A wrong fertilization for vegetables can produce a nitrate excess in the leaves, beyond the EU regulation limits. Nitrate itself non-toxic, but its metabolites may produce a number of negative health effects [5]. The acceptable daily intake is 0-3.7 mg of nitrate per kg b.w., corresponding to 222 mg for a person of 60 kg per day [6].

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Biofertilizers offer a sustainable and eco-friendly alternative to chemical fertilizers, as they help in reducing the use of synthetic fertilizers and pesticides while enhancing soil fertility and plant productivity [7]. Biofertilizers, as carrier-based microbial inoculants, contain sufficient cells of efficient strains of specific microorganisms to enhance soil fertility through various mechanisms such as nitrogen fixation, solubilization/ mineralization of phosphorus, or decomposition of organic wastes, ultimately augmenting plant growth promoting substances with their biological activities [8].

These microbial inoculants, often comprising algae, fungi, and bacteria either alone or in combination, offer cost-effective and environmentally friendly solutions for improving soil fertility and health [9]. They are highly recommended for sustainable agriculture practices, biofertilizers are easily applicable and do not require specialized expertise for their use, making them accessible to a wide range of farmers [10]. Furthermore, biofertilizers facilitate the enrichment of soil through the buildup of beneficial microflora, contributing to long-term soil health and fertility [11].

Compared to synthetic fertilizers, biofertilizers are used in lesser quantities, thereby reducing the environmental burden associated with chemical inputs [12].

Spanich is a leafy vegetable crop needs sustainable and ecofriendly practices, therefore this work aims at evaluating some agronomic practices and their impact on growth, yields and chemical composition of spinach plants grown in sandy soil.

MATERIALS AND METHODS

Two field experiments were carried out at Experimental Farm, National Research Centre El-Behaira Governorate, Egypt (30.30°N, 30.18°E and 21 m above sea level). The experimental soil was sandy with a pH and EC of 0.38 and 8.3 dS m^{-1}, respectively, during two winter seasons of 2022/2023and 2023/2024 to study the effect of biofertilizer (Biogen), and compound fertilizers Nutrin rates on growth, yield and quality of spinach (local variety). This experiment included 9 treatments which were the combinations between the three biofertilizer treatments biogen mainly contains bacteria (Azotobacter) which exist in the biofertilizer and other three integrated treatments : foliar spray of Nutrin as follows:

1. Control.
2. Biogen 1kg fed
3. Biogen 2kg fed
4. Nutrin 2 cm/l
5. Nutrin 4 cm/l
6. Biogen 1 kg fed + Nutrin 2 cm/l
7. Biogen 1 kg fed + Nutrin 4 cm/l
8. Biogen 2 kg fed + Nutrin 2 cm/l
9. Biogen 2 kg fed + Nutrin 4 cm/l

The chemical composition of Nutrin was (Fe 1.7%, Zn 1.5%, Mn 1.5%, Cu 0.5%, B 0.2%, Mo 0.2%, Citric Acid 1.0%, Folic Acid 1.0%, Acetic Acid 1.0%). Seeds of spinach were sown on 24th November in both growing seasons after inoculation with Biogen (1 and 2 kg/fad.), and space between hills 10 cm apart on both sides of ridges. Each experimental plot area was 9.6 m^2 consisted of 4ridges, 0.6 m width and 4 m length. The recommended rates of NPK mineral fertilizers were as follows phosphorus as 200 kg/fed., calcium superphosphate (15.5% P_2O_5), which was added during soil preparation, potassium as 70 kg/fad., potassium sulphate (48-50% K_2O) were added three weeks after seeding, whereas nitrogen as 250 kg/fad., of ammonium sulphate (20.5%N), 50 kg which added during soil preparing and the rest (200 kg) was added in two equal portions, three and five weeks after sowing. Nutrin was sprayed two times at 2 and 4 cm /l,(three and five weeks after planting). The other agricultural practices of spinach planting were carried out as commonly followed in the district.

Data Recorded: After 70 days from sowing, ten spinach plants were randomly taken from each experimental plot to record the vegetative growth parameters (plant height (cm), number of leaves, as well as fresh and dry weight per plant (g). Spinach plants were harvested after 70 days after sowing and stems up to 20 cm in length, fresh weight of plants were recorded as ton/fad.

Leaf Chemical Composition: N, P and K contents were determined according to the methods described by Bremner and Mulvaney [13], Olsen and Sommers [14] and Jackson [15], respectively. Nitrate and iron determined according to Chapman and Pratt [16].

Statistical Analysis: The obtained data were subjected to the proper statistical analysis according to MSTAT-C, [17]. For means comparison, the Least Significant Difference (LSD 5%) was applied.

RESULTS AND DISCUSSION

Data presented in Table (1) reveal that inoculation of spinach seeds with Biogen had a positive effect through

Table 1: Effect of biofertilizer application rates on Spinach growth ,yield and chemical composition of leaves

Biofertilizer	Plant height cm	No. of leaves /plant	FW,of leaves /plant(g)	D w.of leaves/plant	Total fresh yield /ton/fed	N %	Crude protein %	P %	K %	Nitrate ppm
					2023					
Control	41.80	5.80	11.60	2.20	1.817	3.11	19.40	0.45	1.880	2.70
Biogen 1 kg fed	44.30	6.30	15.80	2.60	2.391	3.37	21.00	0.50	1.970	3.30
Biogen 2 kg fed	46.70	7.30	21.10	3.20	2.800	6.17	23.80	0.55	2.090	3.80
	1.40	0.80	2.43	0.72	0.120	NS	2.00	0.05	0.075	0.55
					2024					
Control	40.20	5.30	13.10	2.10	2.411	3.01	18.81	0.39	1.91	2.54
Biogen 1 kg fed	43.40	6.40	14.40	3.00	3.311	3.38	21.13	0.44	1.98	3.19
Biogen 2 kg fed	45.10	6.60	16.60	3.80	3.500	3.59	22.48	0.52	2.07	3.80
	2.00	0.53	2.80	0.45	0.080	0.38	2.38	0.07	0.08	0.59

Table 2: Effect of foliar application nutrient compound rates on Spinach growth ,yield and chemical composition of leaves

Foliar application	Plant height cm	No. of leaves /plant	FW,of leaves /plant(g)	D w.of leaves/plant	Total fresh yield /ton/fed	N %	Crude protein %	P %	K %	Nitrate ppm
					2023					
Control	41.80	5.80	11.60	2.20	1.817	3.11	19.40	0.45	1.880	2.70
Nutrin 2 cm/l	44.30	6.30	15.80	2.60	2.391	3.37	21.00	0.50	1.970	3.30
4 cm Nutrin	46.70	7.30	21.10	3.20	2.800	6.17	23.80	0.55	2.090	3.80
LSD 0.05	1.40	0.80	2.43	0.72	0.120	NS	2.00	0.05	0.075	0.55
					2024					
Control	40.20	5.30	13.10	2.10	2.411	3.01	18.81	0.39	1.910	2.540
Nutrin 2 cm/l	43.40	6.40	14.40	3.00	3.311	3.38	21.13	0.44	1.980	3.190
Nutrin 4 cm/l	45.10	6.60	16.60	3.80	3.500	3.59	22.48	0.52	2.070	3.800
LSD 0.05	2.00	0.53	2.80	0.45	0.080	0.38	2.38	0.07	0.080	0.590

increasing the vegetative growth, as well as total yield during the two growing seasons. All plant growth parameters, i.e. plant height, the number of leaves, fresh and dry weight per plant, moreover, total yield were increased by the application with Biogen. There were significant differences in all characters compared to the treatment without inoculation with Biogen in both seasons of study. The superiority of inoculation with Biogen might be due to the role of the bacteria (Azotobacter) which exist in the biofertilizer to contributing with some hormone substances, i.e. gibberellins, auxins and cytokinins [18]. These phytohormones may stimulate cell division and elongation, as well as development and this consequently reflect on plant growth [19]. Moreover, Subba [20] reported that Azotobacter is an aerobic bacteria, free living in nature and it can capable to produce antibiotics which inhibits the growth of many pathogenic microorganisms that found in the root region thereby maintain the survival of plants. Azotobacter as a nitrogen biofertilizer play a positive role in soil fertility through fixing the atmospheric nitrogen and produces plant growth substances in the soil, beside promote a natural available biological system of nutrient mobilization [21]. These results were parallel with those reported by Abdel-Fattah *et al*. [22], El-Assiouty and Abo-Sedera [23], Alderfasi *et al*. [24] and Ali *et al*. [25] on spinach as well as Ahmed *et al*. [26] and Hosseny and Ahmed [27] on lettuce. Biofertilizers offer a sustainable and eco-friendly alternative to chemical fertilizers, as they help reduce the use of synthetic fertilizers and pesticides while enhancing soil fertility and plant productivity [28]. Biofertilizers, as carrier-based microbial inoculants, contain sufficient cells of efficient strains of specific microorganisms to enhance soil fertility through various mechanisms such as nitrogen fixation, solubilization/ mineralization of phosphorus, or decomposition of organic wastes, ultimately augmenting plant growth promoting substances with their biological activities [8].

The data in Table (2) and Figure (1) shows the results from foliar fertilizer treatments. It reveal a notable trend, The higer dose of foliar application of Nutrin indicate significantly effectiveness in promoting robust growth characters by increasing the vegetative growth, as well as total yield during the two growing seasons. All plant growth parameters, i.e. plant height, number of leaves, fresh and dry weight of plant, moreover, total and relative yield were increased by the application with the

Table 3: Effect of the interaction between biofertilizer application rates and foliar fertilization treatments on Spinach growth, yield and chemical composition of leaves

Biofertilizer	Foliar application of nutrin	Plant height cm	No. of leaves / plant	FW,of leaves /plant(g)	Dw.of leaves /plant	Total yeild/ton /feddan	N %	Total protien	P %	K %	Nitrate ppm
Control	Control	38.8	4.3	11.1	1.77	2.033	2.75	15.3	0.25	1.70	3.50
	2 cm nutrin	41.2	5.3	12.2	2.80	3.067	3.00	18.5	0.33	1.83	2.01
	4 cm Nutrin	41.8	5.3	14.4	3.10	3.333	3.51	20.2	0.37	1.95	3.61
Biogen 1kg fd-1	Control	40.0	5.0	12.1	2.07	2.533	3.13	20.2	0.41	1.97	3.33
	2cm nutrin	43.7	6.3	13.0	2.93	3.367	3.42	21.7	0.42	1.99	3.01
	4cm Nutrin	45.0	6.7	15.7	3.80	3.533	3.84	21.9	0.50	2.05	3.21
Biogen 2kg fd-1	Control	41.9	6.7	15.9	2.40	2.667	3.46	21.0	0.50	2.07	2.76
	2cm nutrin	45.3	7.7	18.0	3.30	3.500	3.69	23.2	0.58	2.13	2.61
	4cm	48.5	7.7	19.7	4.43	2.033	3.16	25.3	0.71	2.19	4.59
		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Control	Control	39.2	4.7	9.0	1.6	1.58	3.00	17.3	0.29	1.73	1.52
	2 cm nutrin	42.2	5.3	12.1	2.2	2.13	3.51	18.7	0.33	1.88	2.12
	4 cm Nutrin	44.2	5.7	16.2	2.5	2.33	3.13	22.0	0.38	1.94	2.12
Biogen 1kg fd-1	Control	42.0	6.3	11.3	2.2	1.85	3.42	19.4	0.48	1.90	2.25
	2cm nutrin	44.5	6.3	16.7	2.6	2.40	3.84	21.4	0.54	1.97	2.60
	4cm Nutrin	46.3	7.7	22.2	3.4	2.90	3.46	24.0	0.60	2.06	3.93
Biogen 2kg fd-1	Control	44.2	6.3	14.6	2.7	2.02	3.69	21.6	0.56	2.01	4.27
	2cm nutrin	46.3	7.3	18.6	2.9	2.64	11.16	23.0	0.62	2.07	5.03
	4cm	49.7	8.7	24.9	3.7	3.17	3.00	25.5	0.68	2.28	5.46
		NS	NS	NS	NS	0.119	NS	NS	NS	NS	0.82

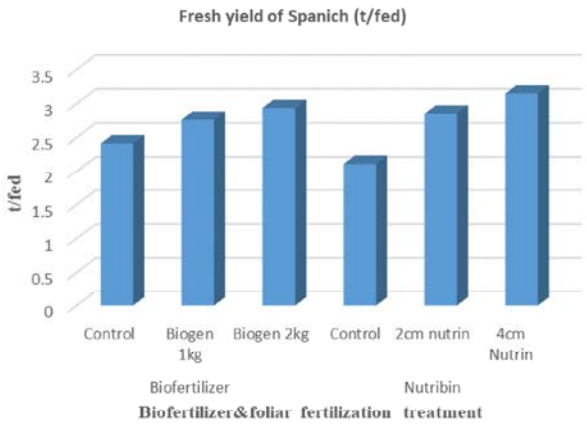

Fig. 1: Effect of biofertilizer application rates and foliar fertilization treatments on Spinach yield (Mean of two seasons)

foliar applied Nutrin. The vegetative growth of spinach was enhanced by amending soils with Biogen applied in the soil. However, the foliar spraying with Nutrin also clearly showed a short-term effect on yield. The plant showed visual health effects of direct foliar spray on the leaves. Foliar application produced the best yield. This result might be due to the high and easily accessible nutrients in the suggested fertilizer dose, which facilitated the sustaining of leafy vegetative growth in spinach (*Basella alba* L.). A group researchers found that applying compound fertilizers from various sources directly to soil increased fresh yield of spinach [29]. Other researchers suggested foliar nutrition as a supplement to soil application as it cannot meet plants' entire nutrition requirements, particularly when secondary and micronutrient deficiencies occur [30-32]. Haytova [33] observed foliar feeding is also more environmentally friendly because it prevents the accumulation of toxic concentrations of nutrients in the soil [33].

The Interaction Effect: The interaction between Biogen treatments and foliar fertilization with Nutrin fertilizer levels on some chemical contents of spinach leaves tissue are shown in Table (3). The recorded data shows that, the interaction effect between the application of Biogen treatments and foliar fertilization with Nutrin was insignificant in 2023 season. However in 2024 season It is clear that similar trend was recorded except total fresh yield and Nitrate content in spinach leaves. These results clearly indicate that both biofertilizer treatments and foliar nutrient Nutrin act independently, therefore no evident interaction between the two treatments occurred. The obtained results are in harmony with those obtained by Aisha *et al*. [34].

Chemical Composition of Spinach Leaves: Data presented in Tables 1 and 2, Fig. 1clearly show highely significant effects due to Biogen or nurtin application to

spinach. Except N%, protein, P, K % significantly increased when Biogen was applied especially at the higher rate (2 kg/fed). Similar trend was recorded for Nutrin application at 4 cm/l. The interaction was insignificant on the percentage of protein, nitrogen phosphorus and potassium contents on leaves tissues but they were marginally greater by adding the high level of bio fertilizer Biogen (2 kg/fed.) with application of foliar fertilizer (Nutrin). These results were true in both season, on the other hand, NO_3 content in leaves tissue was only significant in the 2nd season.

Regarding nitrate content of spinach leaves it is clear that the reported values were within the acceptable daily intake is 0-3.7 mg of nitrate per kg according to European Food Safety Authority[6]. The harmful effects of nitrate are related not so much to its toxicity, when it low, but to the dangerous compounds that are synthesized in the organism. Indeed, the most serious danger comes from nitrite which is produced by nitrate reduction and which can lead to methamoglobinemia as from nitrosamines and nitrosamides by reacting with amines and amides, whose carcinogenic action is well known Gangolli *et al*. [35]. Other claims have been advanced against dietary nitrate such as; an increased risk of congenital malformation, a tendency towards enlargement of the thyroid gland, an early onset of hypertension and enhanced incidence of childhood diabetes [36].

Correlation Studies: Correlation between Total fresh yield and chemical composition of spinach leaves. Data presentedin Table 4 indicte the simple correlation between Total fresh yield and chemical composition of spinach leaves in both seasons of study. Except for N% in 2023 season and nitrate content in 2024 season, highly significant positive correlation coefficient between total fresh yield and N%, Crude protein % P% K% and nitrate content(ppm) were reported. This means that as fresh yield of spinach increased these component will increase.

Table 4: Correlation between Total fresh yield and chemical composition of spinach leaves

2023				
N %	Crude protein %	P %	K %	Nitrate ppm
NS	0.75***	0.695***	0.81***	0.69***
2024				
0.64***	0.64***	- 0.58**	0.60***	NS

CONCUSION

It could be concluded that ecofriendly practices like biofertilizers and nutrient compounds could effectively produce and enhance leafy vegetables like spinach.

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