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Productivity of Squash plant to Mineral and Bio-Nitrogen Fertilizers on plant Growth, Total fruit Yield and leaves mineral content on a Sandy Soil.

Shafeek M.R.; Y.I. Helmy and A.A. Ahmed

Vegetable Research Dept., National Research Centre, Dokki, Cairo, Egypt

Abstract: Two experiments were conducted during summer season of 2013 and 2014 at the experimental station of National Research Centre at Nubaria region to study the effect of three rates of mineral N-fertilizer (50, 100 and 150 kg N/fed.) With or without inoculation of bio-fertilizers (Biogein + Nitrobein) and their interaction treatments on summer squash plants (*Cucurbita peop* cv. Eskandarani) to influence plant growth, total fruits yield and mineral leaves contents as well as leaf chlorophyll content. These treatments were laid out in split plot design arrangement with 3 replications. N rates were randomly distributed in main plots, while the bio-fertilizers treatment was randomly arranged in sub plots. The summarized results obtained from this field study that:

1- Increasing the rates of N-fertilizer significantly improved all plant growth characters expressed as (plant length, No. of leaves/plant, dry weight of whole plant and leaf area/plant) and total yield of fruits, physical fruit characters as well as leaf contents of N, K and total chlorophyll.

2- The trend was 2 kg/fed. inoculation bio-fertilizer (piogen+ nitrobein) > 1 kg/fed. bio-fertilizer > control. However, inoculation by the highest level of bio fertilizer (2 kg/fed.) significantly increased squash plant growth characters, total yield (ton/fed.) and its components as well as the leaves contents of N, P, K and total chlorophyll as compared inoculation by low level (1 kg/fed.) and without inoculation of bio-fertilizer.

3- Using nitrogen fertilizer at a higher rate (150 kg N /fed.) with inoculation by high level of bio fertilizer (2 kg /fed. piogen+ nitrobein) gave the highest plant growth characters, total fruit yield and total leaves content of N, K and total chlorophyll of squash leaves but these increase did not reach the significant levels.

Key words: Summer squash plants- N-fertilizer rate – bio-fertilizer levels- Plant growth- Total fruits yield- Total leaves contents.

Introduction

Squash plants (*Cucurbita pepo* L.) belong to the family Cucurbitaceae, grown in summer season in tropical and subtropical conditions it should be harvested when the fruits are physiologically immature [1]. It is has various health and medicinal benefits to human [2]. Squash is very rich in nutrients and bioactive compounds such as flavonoids, vitamins, amino acids, carbohydrates and minerals (especially potassium), and it is also low in calories and with large amount of fiber [3]. It has various medicinal effects such as comprising antidiabetic, antitumor, antimutagenic, antibacterial, and antiinflammation affects [4].

Many farmers have resorted to the use of subsidized inorganic fertilizers such as (Urea- 46%N) since N fertilizers are essential for the growth due to its immediate effect on plant roots.[5] However, the use of excess nitrogen fertilizers in production of vegetables leads to accumulation of nitrates beyond safe limits which is very dangerous on human health [6]. Nitrogen also plays a role in chlorophyll synthesis and hence the process of photosynthesis and carbon dioxide assimilation [7]. However, nitrogen is an essential nutrient in crop production that is absorbed primarily in the form of nitrate [8]. It constitutes about 1.5 - 6 % of the dry weight of many crops beside the constituent of many organic compounds, nucleic acids and protein compounds [9]. Plants absorb nitrogen mainly in the nitrate (NO_{3-}) and ammonium (NH_{4+}) forms that are essential for metabolism. It stimulates vegetative growth and it also influences the quality of crop. Nitrogen also mediates the utilization of potassium, phosphorus and other elements in plants and the optimum amounts of these elements in the soil cannot be utilized efficiency if nitrogen is deficient in plants [10]. Plants under low levels of nitrogen develop an elevated root: shoot ratio with short branches. Higher levels of NO_{3-} inhibit plant root and leads to a decrease in the root: shoot ratio [11]. However, increasing the rate of N-fertilization generally increases cucurbit yield [12]**.** Plants supplied with 150 kg N/ fed. produced heavier dry weight of the aerial vegetative parts, more number and larger area of leaves plant than those supplied with lower N levels [13]. In addition, [14] found that all rates of nitrogen fertilizers significantly improved plant growth characters (i.e. No. of leaves per plant, stem length, total leaf area plant and dry weight plant) as well as fruit yield and its components (i.e. No. of fruits per plant, yield/ plant and total yield per fed.). Moreover, the concentrations of N, P and K % in leaves were increased. The same results were obtained with nitrogen fertilizer rates; 200, 250 and 300 kg ammonium nitrate/ fed. as compared to the 150 kg/ fed. with some exceptions; P % was not affected and K % was decreased with increasing the rate of nitrogen. In the same respect, [15] on pepper and [16] on spinach reported that, increments in the fertilizer of nitrogen rate increased the growth characters and total yield as well as the nutritional values of plants.

Inoculation of bio-fertilizers is important to substitute of chemical fertilizers for healthy and cheap production. It is are formulations of beneficial microorganisms, which upon application can increase the availability of nutrients by their biological activity and help to improve the soil health and increase soil fertility by increasing the number of such microorganisms and accelerate certain microbial processes. [17]. In addition, inoculation of bio fertilizers can act as bio control agents in the rhizosphere. Bio-fertilization is generally based on altering the rhizosphere flora, by seed or soil inoculation with certain organisms (microbial inoculants), capable of inducing beneficial effects on a compatible host [18]. Azotobacter species are free living bacteria grows well on a nitrogen free medium. These bacteria utilize atmospheric nitrogen gas for their cell protein synthesis. This cell protein is then mineralized in soil after the death of the Azotobacter cells thereby contributing towards the nitrogen availability of the crop plants thus resulting in a strong symbiotic relationship [19]. However, the results of [20] indicated a positive effect of Azotobacter on growth and yield of potato plants. There is some micro-organism which stimulates the Azotobacter population in soil thereby increasing the nitrogen fixation by Azotobacter. However, [21] results showed that the maximum increments of vine length and leaf number as well as fresh and dry weight of shoots were recorded by the inoculation of seeds with Azotobacter. Moreover, Azotobacter at both levels significantly enhanced the induction of female flowers of squash plants. All bio-fertilizer treatments increased N percentage in plant leaves compared to check untreated seeds, but it was only significant for Biogein treatment. Treating the seeds with Azotobacter at both concentrations was found the best treatment, since it increased the number of fruits, early and total fruit yield. On the other hand, check plants gave the lowest values, while plants from inoculated from seeds with local media, Biogein and Microbein produced the intermediate values. In the same respect,[22] results revealed that Azotobacter alone significant effects on vegetative (shoot) parameters, and substantially improved the quantitative and qualitative traits of fruit yield of summer squash plants including plant height (cm), number of branches plant, number of leaves plant, fresh weight of the plants, dry weight of the plants, and total chlorophyll also on yield characteristics such as number of fruits plant, fruit length (cm), fruit diameter (cm), percent of TSS, early yield (kg plant) and total yield (ton \fed.). Moreover, [23] on spinach and [24] on pea found that inoculation of high rate of bio fertilizer piogen (2 kg/fed.) resulted significant increase in most growth characters, i.e. number of leaves/plant, fresh and dry weight of whole plant, leaf area/plant and total chlorophyll contents as well as total yield of leaves (ton/fed.). Also gave the highest percentage of protein, N, P, K and No_3 content.

In view of limited information on the nutrient requirement of squash plants and the importance of nitrogen fertilizer with or without inoculation of bio-fertilizers and their interaction treatments for getting higher yield in crop production of squash plants (*Cucurbita pepo* cv. Eskandarani).

Materials and Methods

Two experiments were conducted at Experimental Station of National Research Centre at Nubaria region, North Egypt under plastic house during of two successive seasons 2012/2013 and 2013/2014 under newly reclaimed sandy lands to study the effect of three rates of mineral N fertilizer (Urea - 46%N) (50, 100 and 150 kg N/fed.) with or without bio fertilizer (Biogein + Nitrobein) on the vegetative growth, total yield and its nutrition values of fruit tissues of squash plants. Physical and chemical properties of the soil are presented in Table (1). All agricultural practices for summer squash crop production were carried out as recommended by Egyptian Ministry of Agriculture. Date of sowing was June, 1st in both growing seasons. Biogein contains *Azotobacter* sp. a nitrogen fixing bacteria, and Nitrobein contains Azospirillum, nitrogen fixing bacteria, were obtained from General Organization for Agriculture Equalization Fund (G.O.A.E.F.) Ministry of Agriculture, Egypt. Urea - 46%N was applied as a top dress 30 days after planting as the 1st split (50, 100 and 150 kg/fed.) while the second split was applied 2 weeks later at the same rate. To avoid direct contact of urea with the plants, fertilizer was applied at a distance of 10 cm radius in a furrow around the plant and then covered with sufficient soil to prevent volatilization. All plots received uniform dose of calcium super phosphate (15.5% P_2O_5) at 200 kg fed. during land preparation, while an identical rate of K fertilizer in the form of potassium sulphate (48% K_2O) at 100 kg fed. was side banded at two equal portions; 3 and 6 weeks from sowing.

Table 1: Physical and chemical properties of the experimental soil.

Physical properties							
Sand	Clay	Silt	Texture	F.C. %	W.P. %		
90.08	9.26	0.66	Sandy	16.57	5.25		
Chemical analysis							
EC	pH	Meq/ L					
1.7	8.2	Ca	Mg	Na	K	HCo_3	Cl
		7.02	0.527	0.982	0.31	1.30	0.566

Drip irrigation system was used. cv Eskandarani squash seeds were sown on ridges of 1.5 m width, 4 m length on one side of ridge and 30 cm apart. The experiment included 9 treatments which were the combination of three nitrogen levels i.e., (50, 100 and 150 kg N\ fed.) and three bio-fertilizers levels (control, biogein at 2 kg/fad.+ nitrobein at 2 kg /fad., and biogein at 1 kg /fed + nitrobein at 1 kg /fad.). These treatments were arranged in a split plot design with three replicates. Nitrogen rates were randomly in distributed in main plots, while the bio-fertilizers were randomly arranged in the sub main plots. The used bio-fertilizers added as inoculated the seeds and sowing. The bio-fertilizers, biogein and nitrobein was supplied for both mixed with wet seeds by adding 20 % Arabic gum solution before planting at the rate of one kg/100 seeds.

Experimental measurements:

Five plants were selected randomly from each experimental unit to measure: vegetative growth characteristics:

1. Plant height (cm) was measured starting from the ground level to the epical meristem of the stem.
2. Number of leaves plant was counted.
3. Total leaf area plant (cm^2) using leaf area-leaf weight relationship as illustrated by [25].
4. Leaves and stems dry weights plant (g) were gained by drying at 70°C in a forced-air oven till the constant weight.

Total fruits yield: Squash fruits were harvest at 3 days intervals, upon reaching 12-15 cm length in both seasons. The fruits were picked from each single plant separately and the following data were recorded:

1. Number of fruits plant; average number of harvested fruits through the entire harvesting period.
2. Yield plant; average weight of fruits during the whole harvesting period.
3. Fruit weight average; calculated by dividing weight of fruits plant by number of fruits plant.
4. Total yield: recorded as the total weight of harvested fruits from all plants of the three middle rows, and then theoretically calculated as (tones / fed.).

Leaf samples for chemical determination, from four randomly selected plants in each experimental unit, after 45 days of seed sowing, were collected, washed with tap water, rinsed three times with distilled water and dried at 70°C in a forced-air oven till constant weight. The dried samples of leaves were finely grounded and weights of 0.2 g of the fine powder were digested using a mixture of sulphoric and perchloric acids. The following determinations were performed:

1. Leaf N% was estimated using the Microkjeldahal apparatus as described in [26].
2. Leaf P % was colourimetrically estimated according to the Stannous molybdate chloride method as illustrated in [26].
3. Leaf K was photometrically measured using flam photometer as mentioned by [27].
4. Total chlorophyll content of leaves, using a TYS-A chlorophyll Meter (Zhe Jang Top Instrument Co. LTD., China) was measured.

The obtained data of experiments were subjected to the statistically analysis of variance procedure and means were compared using the LSD method at 5% level of significance according to [28]. The combined analysis of the data in both seasons was used, since the same trend of the result was obtained.

Results and Discussion

Plant growth characters:

1- Effect of N-fertilizer rates:

The comparisons among N fertilizer treatments reported that increasing nitrogen rate from 50 kg N/fed. to 100 kg N/ fed and from 100 to 150 kg N/ fed. progressive improve squash growth characters i.e. (stem length, No. of leaves/plant, total dry weight of whole plant and leaf area/plant). However, soil application of nitrogen fertilizer at 150 kg N/fed. gave heavier plant growth characters than 100 kg N/fed of squash plant to go forward in both seasons (Table 2).

Table (2): Effect of mineral, bio nitrogen fertilizers and there interaction on the growth characters of squash plants during 2013 and 2014 seasons.

Mineral N fertilizer (kg/fed.)	Bio N fertilizer (kg/fed.)	2013 season				2014 season			
		Plant length (cm)	No. of leaves/plant	Dry W. of whole plant	Leaf area (cm^2)	Plant length (cm)	No. of leaves/plant	Dry W. of whole plant	Leaf area (cm^2)
50	0	56.30	19.81	17.38	1500.00	57.21	20.38	17.79	1543.33
	1 kg/fed.	57.60	20.97	19.89	1547.67	58.66	21.40	20.50	1599.00
	2kg/fed.	59.60	22.19	21.85	1595.00	60.44	22.63	22.49	1628.33
mean		**57.83**	**20.99**	**19.71**	**1547.56**	**58.77**	**21.47**	**20.26**	**1590.22**
100	0	60.32	20.63	17.72	1579.33	61.17	21.03	18.33	1623.00
	1 kg/fed.	61.47	22.14	20.31	1657.67	61.93	22.48	20.97	1691.33
	2kg/fed.	63.51	22.92	23.21	1700.33	64.36	23.47	23.85	1733.00
mean		**61.77***	**21.90***	**20.41***	**1645.78***	**62.48***	**22.33***	**21.05***	**1682.44***
150	0	64.17	21.68	19.99	1546.33	65.00	22.02	20.77	1602.67
	1 kg/fed.	66.09	23.42	22.41	1654.00	66.92	23.95	22.89	1695.00
	2kg/fed.	68.16	24.59	25.68	1737.33	68.87	25.21	26.35	1783.67
mean		**66.14****	**23.23****	**22.69****	**1645.89***	**66.93****	**23.73****	**23.34****	**1693.78***
Average	0	60.26	20.71	18.36	1541.89	61.13	21.14	18.96	1589.67
	1 kg/fed.	61.72*	22.18*	20.87*	1619.78*	62.50*	22.61*	21.45*	1661.78*
	2kg/fed.	63.76**	23.24**	23.58**	1677.56**	64.56**	23.77**	24.23**	1715.00**
LSD at 5% levels	Mineral N	**0.86**	**0.40**	**0.62**	**18.61**	**0.92**	**0.35**	**0.68**	**24.13**
	Bio- N	**0.75**	**0.30**	**0.54**	**17.85**	**0.78**	**0.32**	**0.47**	**17.91**
	interaction	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

These results same with those obtained by ([5], [12], [13], [14], [16 and 30]). In the same effect, [31] found that the high level of NPK fertilizers (100%) application gave significantly the best growth characters of cantaloupe. In addition, nitrogen is most recognized in plants for its presence in the structure of the protein molecule. However, nitrogen

is found in such important molecules as purines, pyrimidines, porphyrines (found in the nucleic acids RNA and DNA) porphyrines found in chlorophyll pigments and the cytochromes, which are essential in photosynthesis and respiration. Also, coenzymes that is essential to the function of many enzymes. Accordingly, nitrogen plays an important role in synthesis of the plant constituents through the action of different enzymes [32].

2- Effect of inoculation of bio fertilizer:

The results illustrated in Table (2) for influence of the studied bio-fertilizer treatments on squash plant growth characters i.e., plant length (cm), No. of leaves/plant and dry weight of whole plant as well as leaf area/ plant (cm^2). However, increasing levels of bio-fertilizer inoculation significantly increased all plant growth characters of squash plants. These results were same in both seasons. These superiority may be attributed to the microorganisms inoculation, in the first place, enriched the rhizosphere with these bacteria. However, these microbial inoculation increased plant growth either directly, by producing plant hormones and improving nutrient uptake, or indirectly, by changing the microbial balance in rhizosphere in favor of the beneficial microorganisms [33]. Furthermore, [34] found that N bio-fertilizer bacteria (Piogen) enhanced the plant growth by N-fixing in the cultivated soil and /or contributing some growth hormones such as gibberellins, auxins and cytokinins. Recently, this beneficial effect was concordant with those obtained with ([24, 25 and 36]). The result showed that, not only can the bio- fertilizer inoculation promote the stems and leaves of plant growth, increase the chlorophyll content and the photosynthesis of leaves, improve the physiological metabolism of plants promote the photosynthetic organism to transfer to the plant root and increase the yield and starch content in the seeds but also decrease the soil unit weight, promote microbe activity in the soil, promote the availability of nutrients, increase content of organic matter, available nitrogen, phosphorus and potassium, and increase the ratio of utilization of fertilizer. It was effective way to apply the bio-fertilizer to plants for increasing the growth, yield and fruits content, improving the soil physical and chemical character and increasing the soil fertility. However, the significant effect of bio-fertilizers may be due to the effect of strain groups of nitrogen fixer, nutrient microorganisms which help in increasing the availability of minerals and increase levels of extractable of macro or micronutrients [37]. Application of Biogein reduced the required mineral nitrogen by 25%, increased the availability of various nutrients, enhances the resistance of plants to root disease and reduces the environmental pollution from chemical fertilizer application [38] on *Vicia faba).* Inoculation by bio-fertilizer encouraged plant growth and productivity of many crops was studied by some investigators [39] on Cantaloupe [40]) and on squash indicated that bio-fertilizer treatment (Halex2) significantly enhanced the induction of female flowers, which was reflected afterward on the increase of fruit yield, and reduced the male, and consequently reduced the sex ratio of squash plants. Utilization of bio-fertilizers in the form of Microbein is very successful in minimizing chemical fertilizer to half of the recommended dose.

3- Effect of the interaction between mineral and bio N-fertilizer:

Highest vegetative growth i.e. plant length, No. of leaves, total dry weight of whole plant as well as leaf area plant were obtained by adding 150 kg N/fed. with inoculation by high level of bio fertilizer (2 kg/fed.). However, the lower values were recorded when N was applied at 50 kg\fed. without bio fertilizer (Table 2). The statistical analysis of the obtained data reported that these increased on plant growth characters were not enough to reach the 5% levels. The obtained results in the two seasons were similar.

Total fruits yield:

1- Effect of N-fertilizer rates:

Table (3) illustrates influence of N-fertilizer levels on yield / plant and declared that application of either 100 or 150 kg N/ fed. produced pronounced increase over the 50 kg N/fed. by 7.82 % and 16.85 % and by 8.11 % and 17.34 % in 2013 and 2014 seasons, respectively. The influence of N-fertilizer rates on total yield (ton / fed.) was significant, in both 2013 and 2014 seasons. Soil application of nitrogen fertilizer at 100 and 150 kg N/ fed increased total yield per ton/fed. over 50 kg N/ fed. rate by 3.61 % and 8.58 %, in 2013 season and by 4.03 % and 10.07 % in 2014 season, respectively. However, the high level of N fertilizer (150 kg/fed.) significantly increased total yield and its components compared the medium level (100 kg N/ fed.). These results were similar in both seasons. [40] on squash stated that increasing the fruits yield by the application of N levels may be due to the enhancement effect of nitrogen to vegetative growth and leaves area/plant which create a large surface available for photosynthesis. Nitrogen is an essential nutrient in creating the plant dry matter and

many energy-rich compounds which regulates photosynthesis and plant production [41]. In the same respect, nitrogen fertilization, markedly, enhanced N concentration in fruit of squash with increasing N fertilization up to 150 kg N /fed. and the trend was the same in both seasons. It could be concluded that, adequate supply of nitrogen (N) can promote plant growth and increase crop production. in the same respect, increased the nitrogen level has important role as a basic element of protein, nucleic acids, chlorophyll and growth hormones [42] and is essential in periods of rapid growth. These results agreed with those obtained by ([5], [12], [13], [14], [16 and 30]). They reported that higher N levels enhanced vegetative growth of vegetable plants.

Table (3): Effect of mineral, bio nitrogen fertilizers and there interaction on total yield and its component of squash plants during 2013 and 2014 seasons.

Mineral N fertilizer (kg/fed.)	Bio N fertilizer (kg/fed.)	2013 season				2014 season			
		Number of fruits/plant	W. of fruits/plant (g)	Average fruit W. (g)	Total yield (ton/fed.)	Number of fruits/plant	W. of fruits/plant (g)	Average fruit W (g)	Total yield (ton/fed.)
50	0	5.95	284.33	57.30	3.95	6.00	289.00	58.67	3.96
	1 kg/fed.	6.19	299.67	58.97	4.21	6.33	303.67	59.00	4.25
	2kg/fed.	7.34	349.67	58.70	5.12	7.45	357.00	59.37	5.19
mean		**6.50**	**311.22**	**58.32**	**4.43**	**6.59**	**316.56**	**59.01**	**4.47**
100	0	6.08	292.33	58.40	4.04	6.20	301.00	59.77	4.08
	1 kg/fed.	6.43	333.33	60.33	4.68	6.58	340.67	60.67	4.73
	2kg/fed.	7.34	381.00	42.33	5.06	7.44	385.00	42.00	5.15
mean		**6.62***	**335.56***	**53.69**	**4.59***	**6.74**	**342.22***	**54.14**	**4.65***
150	0	6.77	323.67	58.00	4.36	6.85	331.67	58.33	4.47
	1 kg/fed.	7.01	367.33	59.67	4.66	7.00	375.33	60.00	4.77
	2kg/fed.	7.45	400.00	60.20	5.40	7.60	407.33	60.83	5.52
mean		**7.08****	**363.67****	**59.29**	**4.81****	**7.15****	**371.44****	**59.72**	**4.92****
Average	0	6.27	300.11	57.90	4.12	6.35	307.22	58.92	4.17
	1 kg/fed.	6.54*	333.44*	59.65	4.52*	6.64*	339.89*	59.89	4.58*
	2kg/fed.	7.38**	376.89**	53.75	5.19**	7.49**	383.11**	54.08	5.29**
LSD at 5% levels	Mineral N	**0.12**	**10.15**	**NS**	**0.07**	**0.17**	**11.37**	**NS**	**0.13**
	Bio- N	**0.16**	**11.47**	**NS**	**0.14**	**0.16**	**11.03**	**NS**	**0.14**
	interaction	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

2- Effect of inoculation of bio fertilizer:

The trend was 2 kg/fed. bio fertilizer (piogen+ nitrobein) > 1 kg/fed. bio fertilizer > control. However, by increasing the level of inoculation of bio fertilizer up to (2 kg/fed.) significantly increased total yield (ton/fed.) compared to low level (1 kg/fed.) and control. These results were true in both seasons. Total fruits yield as (ton/fed.) recorded its heaviest values (5.19 and 5.29 ton/fed. for 1st and 2nd seasons respectively with inoculation by bio fertilizer (piogen + nitrobein) at rate of 2 kg/fed compared (4.12 and 4.17 ton/fed. for 1st and 2nd seasons respectively by without bio fertilizer control (Table 2). Moreover, the inoculation of highest level of bio fertilizer 2 kg/fed. (piogen + nitrobein) significantly increased total fruits yield and its components compared low level (1 kg/fed.). It could be concluded that, the heaviest fruits yield and its best values of which resulted may be attributed to increasing the availability of various nutrients by plant and increasing the resistance of plant to root diseases and reducing the environment pollution by the application of chemical fertilizers [43]. The obtained results the effect of inoculation of bio-fertilization on growth and yield of squash plants were in agreement with that obtained by ([33, 34, 35 and 36]).

3- Effect of the interaction between mineral and bio N-fertilizer:

The interaction effect between nitrogen rates and bio-fertilizer inoculation recorded no significant differences on total fruit yield and its components as shown in Table (3). Generally it could be declared that using nitrogen fertilizer at a higher rate (150 kg N /fed.) with the highest inoculation of bio-fertilizer (2 kg /fed. piogen+ nitrobein) gave high values of total fruit yield and its components of squash fruits.

Leaf mineral contents and chlorophyll content:

Table (4): Effect of mineral, bio nitrogen fertilizers and there interaction on leaf contents of squash plants during 2013 and 2014 seasons.

Mineral N fertilizer (kg/fed.)	Bio N fertilizer (kg/fed.)	2013 season				2014 season			
		%			Total chlorophyll	%			Total chlorophyll
		N	P	K		N	P	K	
50	0	2.31	0.26	2.87	40.59	2.34	0.26	2.94	40.23
	1 kg/fed.	2.70	0.33	2.95	44.68	2.73	0.32	3.00	44.36
	2kg/fed.	2.84	0.35	3.17	46.80	2.87	0.35	3.44	46.47
mean		**2.62**	**0.31**	**3.00**	**44.02**	**2.65**	**0.31**	**3.13**	**43.69**
100	0	2.59	0.28	2.92	41.08	2.63	0.28	3.05	40.95
	1 kg/fed.	2.84	0.33	3.04	45.15	2.87	0.33	3.33	45.45
	2kg/fed.	3.00	0.38	3.45	47.12	3.01	0.37	3.59	46.45
mean		**2.81***	**0.33**	**3.14***	**44.45***	**2.84***	**0.33**	**3.33***	**44.28***
150	0	2.66	0.32	3.03	41.47	2.74	0.32	3.08	41.26
	1 kg/fed.	2.87	0.36	3.44	45.66	2.91	0.35	3.55	45.77
	2kg/fed.	3.18	0.43	3.52	47.56	3.21	0.42	3.60	47.71
mean		**2.91****	**0.37**	**3.33****	**44.90****	**2.95****	**0.36**	**3.41***	**44.91****
Average	0	2.52	0.29	2.94	41.05	2.57	0.29	3.02	40.81
	1 kg/fed.	2.80*	0.34*	3.14*	45.16*	2.84*	0.33*	3.29*	45.19*
	2kg/fed.	3.01**	0.39**	3.38**	47.16**	3.03**	0.38**	3.55**	46.87**
LSD at 5% level	Mineral N	**0.07**	**NS**	**0.12**	**0.22**	**0.06**	**NS**	**0.12**	**0.50**
	Bio- N	**0.14**	**0.02**	**0.15**	**0.17**	**0.14**	**0.02**	**0.19**	**0.42**
	interaction	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

1- Effect of N-fertilizer rates:

The effect of N rates on leaf contents of N, K and total chlorophyll contents showed a significant increase (Table 4). However, the increased of the N-fertilizers level (150 kg N/fed.) significantly improved chemical characters of squash leaves compared to the intermediate and the lowest levels of N (50 kg N/fed.). The highest significant values of the percentage of N, K and total chlorophyll contents were recorded with the highest rate of N fertilizers. The superiority contents of squash leaves tissues may be attributed to increase the availability of N and K in the soil solution. Consequently absorption would be higher and nutrient accumulation in leaves tissue increased. These results are in good accordance with those of [40] on squash and [29] and [44] on cantaloupe.

2- Effect of inoculation of bio fertilizer:

The data reported in Table (4) show the response of some chemical consistent N, P, K and total chlorophyll contents of squash leaves to the application of bio fertilizer (piogen+ nitrobein) during the experimental seasons of 2013 and 2014. Whereas, the plants which treated with inoculated by pioge+ nitrobein at high rates (2 kg/fed.) resulted in the high percentage of Nitrogen (3.01 and 3.03), phosphorus (0.39 and 0.38) and potassium (3.38 and 3.55) in the two seasons respectively compared to the low level (1 kg/fed.) and control treatment. However, the two levels of bio fertilizer application significantly increased the percentage of nitrogen, phosphorus and potassium content in dry leaves tissues compared (control). These results were similar in both seasons. These results were same with those reported by ([33, 35 and 36]). They reported that there were considerable increases in nitrogen potassium and phosphorus contents of crop leaves which inoculation with bio fertilizers N fixing.

3- Effect of the interaction between mineral and bio N-fertilizer:

In spite of non significant effect of the interaction of N fertilizer and three levels of bio fertilizer on the leaves tissues of squash plants in the two seasons (Table 4). It could be concluded that, the highest nutrient values of the percentage of N, K and total chlorophyll content in squash leaves tissues were associated with that

plants received higher N rate (150 kg/ fed.) with higher level of inoculated by bio-fertilizer (2 kg piogen + nitrobein /fed.). But the increased did not reach the significant levels. On the contrary, the little values from the above mentioned that were obtained by low N fertilizer rate (50 kg N/fed.) without bio fertilizer inoculation. These findings are in good accordance in both seasons.

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