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Effect of Bio-Nitrogen as a Partial Alternative to Mineral-nitrogen Fertilizer on Growth, Yield and Head Quality of Broccoli (*Brassica oleracea* L. Var. *Italica*)

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Abstract: Two field experiments were conducted in an area of newly reclaimed land during two winter seasons of 2010/2011 and 2011/2012 at El-Nuberia Province, El-Beheira Governorate, Egypt to study the effect of bio-nitrogen and different levels of mineral nitrogen on vegetative growth, yield and head quality of broccoli (cv. Hybrid Decathlon). The treatments included three bio-nitrogen treatments i.e. without bio-fertilization, *Azospirillum brasilense* and *Azotobacter chroococcum* were combined with three levels of mineral nitrogen, i.e. 60, 90 and 120 kg N per faddan (one faddan = 0.42 ha). Plants treated with bio-nitrogen had higher vegetative growth, i.e. plant length, leaves number, fresh weight of leaves, stems and total plant, dry weight of leaves and heads, main head yield and physical heads quality (weight and diameter) as well as N, P and K content of leaves and heads of broccoli than the untreated (control). *Azotobacter chroococcum* treatment is superior in these characters of vegetative growth, main head yield and physical heads quality (weight and diameter), as well as, N, P and K content of leaves and heads of broccoli followed by the treatment of *Azospirillum brasilense* compared with non-inoculated plants (control). Nitrogen levels differed statistically in their effects on the vegetative growth of broccoli plants (plant height, leaves number per plant, fresh weight of leaves, stems and spears, dry weight of leaves and spears, main spear yield and physical head quality (weight and diameter), as well as, N, P and K content of leaves and heads. The highest vegetative growth was obtained by adding 120 kg N/faddan, followed by 90 kg N/faddan, which came in the second order. The lowest values of vegetative growth, main head yield, physical heads quality and N, P and K of broccoli leaves and heads were obtained by 60 kg N/faddan. The results indicated that combined effect of bio-fertilization and mineral nitrogen levels caused statistical increases in vegetative growth, yield, N, P and K content of broccoli leaves and heads. The highest vegetative growth, yield and chemical contents were obtained by the combined effect of 120 kg N/faddan with bio-nitrogen *Azospirillum brasilense*.

Key words: *Azospirilum brasilense* · *Azotobacter chroococcum* · Bio-fertilization · Broccoli · Mineral fertilizer · Nitrogen content · Yield and quality

INTRODUCTION

Cruciferous vegetables are a large and increasingly important crop group. Broccoli is a member of the cruciferae family which found along the Mediterranean region [1]. Broccoli cultivated in Italy in ancient roman times and about 1720 in England. It is appeared in 1806 in USA but it was commercially cultivated around 1923 [1]. However in Egypt, broccoli due to increase in its popularity, there is a trend to increase its cultivation by farmers as well as consumption by consumers. Broccoli in Egypt is considered as a promising vegetable that could be developed for local and export purposes. Recently, an attention has focused on the increase in the production of some untraditional vegetable crops, including broccoli, because of their great importance. Broccoli has enormous nutritional and medicinal values due to its high content of vitamins (A, B1, B2, B5, B6 and E), minerals (Ca, Mg, Zn and Fe) and a number of antioxidants [2, 3], which prevent the formation of cancer-causing agents [4]. Yoldas *et al*.

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[5] added that broccoli is an important vegetable crop and has high nutritional and good commercial value. It is low in sodium food, fat free and calories, high in vitamin C and good source of vitamin A, vitamin B2 and calcium [1]. Nowadays, broccoli attracted more attention due to its multifarious use and great nutritional value [6, 7]. Broccoli is a rich source of sulphoraphane, which has been shown to display potent anti carcinogenic properties. However, over half of the national population fails to benefit from this because they lack a specific gene (GSTM1) that helps retain the compound in the body. Eating a few portions of broccoli each week may help to reduce the risk of cancer. The main isothiocynate from broccoli is sulforaphane. A broccoli seed extract from a plant strain that is reported to be unusually high in sulforaphane glucosinolate (SGS). Eating more than one serving of broccoli a week may reduce the risk of prostate cancer by up to 45 per cent, says a new study. Eating larger portions may have additional benefits, since broccoli is also a rich source of other vitamins and minerals. The tissue of crucifer vegetables, which also include cauliflower, cabbage and Brussels sprouts, contain high levels of the active plant chemicals glucosinolates. These are metabolized by the body into isothiocynates, which are known to be powerful anti-carcinogens. It is, therefore, widely cultivated in many European and American countries, but in Egypt it still grown in limited areas. The total cultivated area is not exactly known [8]. Growing broccoli in the newly reclaimed soils is faced by various problems, such as, low amounts of available nutrients and low organic matter content as well as poor hydrophilic, chemical and biological properties. The best means of maintaining soil fertility and productivity could be through addition of the best level of mineral nitrogen fertilizer and bio-fertilization such as *Azospirilum* and *Azotobacter*.

Bio-fertilizers, microbial inoculants that can promote plant growth and productivity, are internationally accepted as an alternative source of N-fertilizer. In the bio-fertilizer technology, new systems are being developed to increase the biological N_2-fixation with cereals and other non-legumes by establishing N_2-fixing bacteria within the roots [9]. The mechanism by which bio-fertilizers can exert a positive effect on plant growth can be through the synthesis of phytohormones, N2-fixation, reduction in membrane potential of roots, synthesis of some enzymes (such as ACC deaminase) that modulate the level of plant hormones. Free living nitrogen-fixing bacteria such as *Azotobacter* and *Azospirilum* have the ability not only to fix nitrogen but also to release certain phytohormones i.e. GA3, IAA and cytokinins which could stimulate plant growth and increase the availability of nutrients for plant roots by the increase in their dissolution. In addition, the increase in the capacity of photosynthesis process [10]. Several reports indicated that the inoculation of some plants with bio-fertilizers singly or in combination with mineral fertilizers improved plant growth, yield and chemical composition [11, 12]. Inoculation of potato tuber seeds with bio-fertilizer [*Azotobacter chroococcum* (AT) + *Azospirilum brasilense* (AZ)] significantly increased growth and yield and its components [12]. The application of bio-fertilizer increased the ability to convert N_2 to NH_4 and thus make it available to plants and enhanced the concentration of nitrogen, phosphorus and potassium in onion [13, 14].

Mineral nutrition is one of the most important factors for plant growth and yield. Mineral fertilizers, particularly mineral-nitrogen, are important means of plant nutrition; however, they are also a potential source of environmental pollution [15]. An attention has therefore focused on alternative fertilizers, including bio-fertilizers in Middle East. Nowadays, there is renewed interest in bio-fertilizers for nutrient supply and improve soil fertility and productivity in this region. The integrated use of bio-fertilizers and mineral fertilizers is considered as the best option not only to reduce the intensive consumption of chemical fertilizers, but also to sustain the soil with minimum undesirable impacts and to maximize fertilizer use efficiency in soil [16, 17]. Bio-fertilizers are considered as eco-friendly way to sustainable agriculture. They positively affect plant growth and yield, reduce the negative effects of chemical fertilizers and minimize. Some chemicals such as NO_3^- and NO_2^- ions in the soil and consequently in plants. Therefore, the way to a healthy agriculture with a minimum pollution requires a conjunctive use of bio-nitrogen and mineral-nitrogen fertilizers. Some investigators reported that bio-fertilization increased yield of broccoli and cruciferous vegetables [18, 19], other investigators studied the effect of bio-fertilization on cabbage and cauliflower [20, 21]. Also, many investigators reported that bio-fertilization increased yield of cruciferous vegetables [20, 22-26]. Other investigators studied the effect of mineral nitrogen levels on broccoli plant [25-29].

Therefore, this work was performed to evaluate vegetative growth, heads yield and chemical content of broccoli plants under different levels of mineral nitrogen

fertilizer in combination with or without bio-nitrogen fertilizer (*Azotobacter chroococcum* or *Azospirillium brasilense*) doses in broccoli plants grown under the Egyptian conditions.

MATERIALS AND METHODS

Two field experiments were carried out on broccoli *(Broccoli oleraceae L.* var. *italica* cv. Hybrid Decathlon), family Cruciferae in an area of newly reclaimed land at El-Nuberia, El-Beheira Governorate, Egypt, during the two successive winter seasons of 2010/2011 and 2011/2012. The aim of this work was to study the effect of bio-nitrogen and mineral nitrogen fertilizer on growth, yield, quality and chemical content of broccoli heads. Soil samples were collected at random before planting from the top layer (0-30 cm depth) for physical and chemical analysis. Soil analysis is presented in Table 1. Soil physical properties were analyzed using the procedures described by Black *et al.* [30] for particle size distribution and soil texture, while soil chemical analysis was determined according to the procedures described by Jackson [31]. Seeds of broccoli (cv. Hybrid Decathlon) were imported from Holland. Seeds were sown in the nursery in foam trays filled with a mixture of peat moss and vermiculite (1:1 volume) on 1st of September in 2010 season and 3th of September in 2011 season. Seedlings were transplanted in the open field on 19th of October and 18th of October in 2010 and 2011, respectively.

Treatments Were as Follows:

Bio-fertilization Treatments: The experiment included three treatments:

- Without bio-fertilizer (control).
- With bio-nitrogen culture of *Azospirilum brasilense*.
- With bio-nitrogen culture of *Azotobacter chroococcum*.

The bio-nitrogen fertilizers were kindly supported by the Agricultural Microbiology Department, National Research Centre, Dokki, Giza, Egypt. The bio-N fertilizers were grown separately in Bach cultures to the late exponential phase of each microorganism [32] to get a cell suspension of 4 x 10^4 and 2 x 10^4 cell/g soil), respectively for *Azospirilum* and *Azotobacter* after 6 days incubation period at 28-30°C. These cultures were mixed on the site just before inoculation. The bacterial suspension on the rate of 50 liter/faddan. The bio-Nitrogen was applied as soil application 50 ml besides every plant once after two weeks from transplanting.

Table 1: Physical and chemical properties of the experimental soil during the two seasons of 2010/2011 and 2011/2012.

Soil properties	2010/2011	2011/2012
Physical properties		
Sand %	55.85	56.72
Clay %	3.45	3.72
Silt %	40.70	39.56
Texture	Sandy	Sandy
Chemical properties		
EC (dSm-1)	2.18	1.26
pH	7.81	7.76
Cations (meq/l)		
Ca++	8.61	7.60
Mg++	4.80	2.20
Na+	4.64	3.79
K+	1.59	0.91
Anions (meq/l)		
CO3--	Nil	Nil
HCO3-	1.40	1.40
Cl-	1.80	1.60
SO4--	17.40	12.20

Nitrogen Levels: Three levels of mineral nitrogen fertilizer: 60, 90 and 120 N kg/faddan (one faddan= 0.42 ha). Ammonium nitrate (33.5% N) was used as a source of nitrogen fertilizer.

Organic fertilizer and calcium super phosphate were added through the ditches before transplanting and ditches were covered by soil. Drip irrigation lines were spread over the ditches. Seedlings were transplanted one on the eastern side of each line in 75 cm width and 50 cm apart. Other agricultural practices of growing broccoli crop were followed. Each plot included three rows, plot area was 10.5 m^2.

Experimental Design: Split-plot design with four replicates was used. Bio-N treatments were located in the main plots, whereas the nitrogen levels were assigned in the sub-plots.

Data Recorded:

Vegetative Growth Characters: A random sample of five plants was taken from each experimental treatment at 70 days after transplanting and the following data were recorded during the two seasons:

- Plant length (cm).
- Leaves number/plant.
- Leaves fresh weight (g/plant).
- Stems fresh weight (g/plant).
- Main head fresh weight (g/plant).

- Total plant fresh weight (g/plant).
- Leaves dry weight (%).
- Heads dry weight (%).

Heads Yield and Physical Head Quality: Three harvests of broccoli heads of each plot were harvested at maturing in order to record the following data:

- Main heads yield (ton/ faddan).
- Yield increase (ton/faddan).
- Yield increase (%).
- Average head weight (g/plant).
- Head diameter (cm).
- Stem diameter (cm).

Chemical Composition: Samples of leaves and heads of broccoli plants were oven dried at 70°C till constant weight. The dry samples were pulverized separately and then acid digested. The percentages of nitrogen, phosphorus and potassium in the acid digested samples of dry leaves and heads were determined as follows: Total nitrogen was determined as a percentage using Nesslar method according to the procedure described by Koch and McMeekin [33]. Phosphorus percentage was determined colorimetrically according to the method described by Trough and Meyer [34]. Potassium percentage was determined using Flame Photometer according to Brown and Lilleland [35].

Statistical Analysis: The obtained data of the experiments was tabulated and were subjected to statistical analysis according to Snedecor and Cochran [36].

RESULTS AND DISCUSSION

Vegetative Growth Characteristics

Effect of Bio-Nitrogen: Vegetative growth of broccoli plants expressed as plant length, leaves number/ plant and fresh weight of leaves, stems and total plant as well as dry weight of leaves and heads was affected by bio-nitrogen fertilization (Table 2). Bio-nitrogen treatments enhanced vegetative growth characteristics as compared with the untreated plants. *Azotobacter* treatment exceeded in these characters of vegetative growth compared with *Azospirilum* treatment. *Azotobacter* recorded the highest values of vegetative growth characters compared with *Azospirillum* except for leaves number. These results were similar in the two seasons. Many investigators came to similar. Wang and Kale [37] on broccoli, studied the growth of broccoli response to 12 different treatments of bio-fertilizers (*Azotobacter* and *Azospirillum*) and N levels (100, 125 and 150 kg/ha). The treatment of *Azotobacter* + *Azospirillum* with 150 kg/ha was significantly superior over the recommended fertilizer rate alone. Manivannan and Singh [18] studied the effect of bio-fertilizers namely *Azospirillum sp*. and *Azotobacter sp*., applied at 5 and 10% on the growth and yield of sprouting broccoli. They found that the maximum vegetative growth was recorded in plants applied with 5% *Azotobacter sp*. In addition, Zaki *et al*. [25] on broccoli reported that inoculated plants with bio-fertilizer showed higher vegetative growth parameters (plant height, leaves number, branches number as well as fresh and dry weight of leaves and stems) than the untreated plants. In addition, Osman [12] found that inoculation of bacteria (Bio-N) singly or in combination with chemical fertilizers positively affected growth characters of potato plants. He added that, this may be attributed to the increased activity and efficiency of bacteria in reduction of soil pH by secreting organic acids i.e. acetic, propionic, fumaric and succinic and consequently more solubility and availability of nutrients for plants. Furthermore, bio-fertilizers can exert a positive effect on plant growth through the enhanced levels of phytohormones (GA_3, IAA and cytokinins) that modulated by ACC deaminase enzyme, N_2-fixation and the reduction in root membrane potential. The noticeable increases of growth traits of broccoli plants by the increase in the applied bio-fertilizer dose may be confirmed by the progressively increase in the nutritional elements in the tested soil and in plants. Our results indicated that, bio-N is beneficial for sustainable agriculture and human healthy nutrition as a partial alternative to mineral-N fertilizer. Gadalla and El-Masry [13] and Hanafy *et al*. [14] reported that application of bio-fertilizer increased the ability to convert N_2 to NH_4 and thus make it available to plants and enhanced the concentration of N, P and K in onion. In addition, the mechanism by which bio-fertilization can exert a positive effect on plant growth can be through the synthesis of phytohormones, N_2 fixation, reduction in membrane potential of roots, synthesis of some enzymes (such as ACC deaminase) that modulate the level of plant hormones. Free living N-fixing bacteria such as *Azotobacter* and *Azospirillum* have the ability not only to fix nitrogen but also to release certain phytohormones, i.e. GA_3, IAA and cytokinins which could stimulate plant growth and increase the availability of nutrients for plant roots by the increase in their dissolutions. In addition, the increase in the capacity of photosynthesis process [10].

Table 2: Effect of bio-nitrogen on vegetative growth and dry weight of broccoli under newly reclaimed soils during two seasons (2010/2011 and 2011/2012).

			Fresh weight (g/plant)			Dry weight (%)	
Bio-nitrogen	Plant length (cm)	No. of leaves/plant	Leaves	Stem	Main head	Leaves	Heads
First season (2010/2011)							
Without	48.87	13.33	329.68	171.23	320.39	12.34	11.97
Azospirilum	52.50	15.33	406.02	182.29	335.34	10.51	13.01
Azotobacter	53.28	14.75	418.17	203.76	368.57	10.91	12.68
LSD 0.05	0.52	0.67	15.77	8.78	6.76	0.20	0.29
Second season (2011/2012)							
Without	48.52	13.64	322.69	164.13	318.30	12.26	12.05
Azospirilum	52.85	16.02	406.86	177.29	336.09	10.07	13.12
Azotobacter	53.78	15.32	421.32	202.84	368.96	10.55	12.90
LSD 0.05	1.61	1.20	12.67	11.96	21.36	0.37	0.16

Table 3: Effect of different levels of mineral nitrogen on vegetative growth and dry weight of broccoli under newly reclaimed soils during two seasons (2010/2011 and 2011/2012).

			Fresh weight(g/plant)			Dry weight (%)	
Mineral-nitrogen levels (kg/faddan)	Plant length (cm)	No. of leaves/plant	Leaves	Stem	Main head	Leaves	Heads
First season (2010/2011)							
60	52.17	13.58	362.35	170.18	270.26	11.17	12.11
90	49.40	14.67	375.13	195.58	359.75	11.22	13.14
120	53.08	15.17	416.38	191.52	394.29	11.38	12.40
LSD 0.05	0.26	0.10	12.14	2.87	9.01	NS	NS
Second season (2011/2012)							
60	52.45	13.93	361.57	162.88	265.32	10.86	12.22
90	49.16	15.22	376.77	193.11	358.47	10.92	13.28
120	53.54	15.82	412.53	188.27	399.57	11.11	12.57
LSD 0.05	0.66	0.57	18.23	3.26	63.12	NS	0.17

Effect of Nitrogen Levels: Data in Table 3 indicated that nitrogen fertilization enhanced vegetative growth of broccoli plants. Vegetative growth of broccoli plants was activated by the increased nitrogen levels up to its highest level, i.e. 120kg N/faddan. The highest vegetative growth of broccoli plants expressed as plant length leaves number and fresh weight of leaves, stems and total plant was obtained by the application of 120kg N/faddan followed by plants which received 90kg N/faddan which came in the second order (Table 3). In general, lower values of plant growth were obtained by 60kg N/faddan. The same trend was observed over both growing seasons. Many investigators reported generally that, mineral fertilizer increase vegetative growth [25, 26]. It is obvious to mention that nitrogen is an important constituent of protein and plays an important role in the cell enlargement and cell division and consequently on plant growth. Nitrogen also plays an important role in photosynthesis and in turn in the condensation of its products through plant tissues [38].

Effect of Interaction: The obtained data revealed that the interaction treatments (Table 4) significantly enhanced all growth parameters except leaves number and stem fresh weight as well as leaves fresh weight in the first season. These results were nearly similar in both seasons of the experiment. In general, data in Table 4 indicated that the most efficient interaction treatments were those of both *Azotobacter sp*. and/or *Azospirillum sp.* combined with the highest N level. Plant length, leaves number and fresh weight of leaves, stems, heads and total plant recorded their highest values by the combined effect of any of *Azospirillum sp*. or *Azotobacter sp*. with high mineral N level, i.e. 120kg N/faddan. Results of the interaction indicate that the mineral nitrogen and bio-nitrogen act in a complementary role.

Table 4: Effect of the interaction between bio-nitrogen and different levels of mineral nitrogen on growth and yield of broccoli under newly reclaimed soils during two seasons (2010/2011 and 2011/2012).

Bio-nitrogen	Mineral nitrogen levels(kg N/faddan)	Plant length (cm)	No. of leaves /plant	Fresh weight(g/plant) Leaves	Stem	Main head	Dry weight (%) Leaves	Heads
			First season (2010/2011)					
Without	60	50.50	12.50	309.51	149.65	242.40	12.83	11.66
	90	44.60	14.00	335.85	187.83	345.35	11.89	12.84
	120	51.50	13.50	343.68	176.22	373.42	12.31	11.42
Azospirilum	60	52.00	14.00	353.53	158.75	246.44	9.64	12.63
	90	51.50	15.50	407.05	201.86	346.34	11.73	13.70
	120	54.00	16.50	457.47	186.25	413.25	10.15	12.70
Azotobacter	60	54.00	14.25	424.02	202.13	321.95	11.03	12.05
	90	52.10	14.50	382.50	197.05	387.55	10.03	12.90
	120	53.75	15.50	447.99	212.09	396.21	11.67	13.09
LSD 0.05	--	0.91	NS	NS	NS	11.71	0.35	0.17
			Second season (2011/2012)					
Without	60	50.47	12.65	298.69	138.45	238.83	12.84	11.68
	90	43.44	14.43	330.03	183.88	341.34	11.71	13.08
	120	51.66	13.84	339.35	170.07	374.73	12.22	11.39
Azospirilum	60	52.25	14.43	351.07	149.28	243.63	9.04	12.83
	90	51.66	16.22	414.76	200.58	342.51	11.53	13.61
	120	54.63	17.41	454.76	182.01	422.13	9.65	12.92
Azotobacter	60	54.63	14.73	434.95	200.90	313.49	10.70	12.14
	90	52.37	15.03	385.54	194.86	391.55	9.51	13.16
	120	54.33	16.22	443.48	212.75	401.85	11.46	13.39
LSD 0.05		1.02	NS	29.15	NS	35.11	0.77	0.69

Table 5: Effect of bio-nitrogen on main head yield (ton/faddan), yield increase and heads quality of broccoli during two seasons (2010/2011 and 2011/2012).

Bio-nitrogen	Main head yield(ton/faddan)	Yield Increase (ton/faddan)	(%)	Head quality Diameter (cm)	Weight (g)
		First season (2010/2011)			
Without	3.840	0.000	0.00	13.10	320.39
Azospirilum	4.020	0.180	4.69	13.62	335.34
Azotobacter	4.420	0.580	15.10	14.40	368.57
LSD 0.05	0.080	--	--	0.29	6.76
		Second season (2011/2012)			
Without	3.820	0.000	0.00	13.20	318.3
Azospirilum	4.030	0.210	5.50	13.81	336.09
Azotobacter	4.430	0.610	15.97	14.75	368.96
LSD 0.05	0.600	---	---	0.57	21.36

These two factors act dependently on the vegetative growth of broccoli plants. Higher values of leaves number and leaves fresh weight were obtained by the high mineral level combined with *Azospirillum sp*., but the differences were not statistically significant. Data of the dry weight percentage were without clear trend as affected by interaction. These results emphasized that the bio-N + mineral-N treatment was enough to reach the highest levels of N, P and K content. This may be attributed to the increased availability of these nutrients because of the beneficial effects of bacteria (*Azotobacter chroococcum* and *Azospirillium brasilense*) on the soil. They reduced soil pH by secreting some organic acids (e.g. acetic, propionic, fumaric and succinic) and maintaining a suitable air-moisture regime. In addition, Azospirilum-inoculated plants exhibited higher foliar N, P and K contents in marigold [39]. Plants also showed increased growth of root system that enables them to absorb more nutrients from soil [40].

Total Yield and Head quality:

Effect of Bio-Nitrogen: Results in Table 5 showed that bio-N significantly affected in the total yield and physical and chemical quality of main heads of broccoli in the two seasons of this study. Bio-N reflected increases in the total yield of broccoli crop, yield increases and their percentage as well as head quality (weight and diameter). Main spears yield, yield increase (ton/faddan) and yield increase (%) of the plants which inoculated by *Azotobacter sp*. recorded the highest values compared with plants which inoculated by *Azospirilum sp*. and the control treatment. Increases in the total green yield due to *Azotobacter* amounted to 0.580 and 0.610 ton/fed which equals 13.12 and 13.77 % of the control in the two

seasons, respectively. Moreover, the highest values of heads physical quality, i.e. weight, diameter and height were recorded by *Azotobacter* treatment and the lowest values were found by without bio-N treatment. *Azospirillum sp.* recorded lower values of total head yield, yield increases and their percentage and head quality. These values lying in the second order following *Azotobacter* treatment. The lowest values of total yield, yield increases and their percentage, as well as head quality were recorded by the control treatment. Bio-N caused increases in mineral content, vegetative growth of broccoli plants. Increases in head yield and quality by *Azospirillum* and *Azotobacter* treatments might be due to their positive effect on nutrients absorption, higher photosynthetic rate, higher dry matter accumulation and higher vegetative growth. Singh and Sinsinwar [41] reported that *Azotobacter chroococcum* have the capability for contributing nitrogen to a number of non-legumes by trapping the nitrogen from aerial nitrogen reservoir.

Effect of Nitrogen Levels: Data presented in Table 6 indicated that mineral nitrogen fertilization reflected significant increases in the total head yield, yield increases and their percentage and head quality of broccoli crop. Application of 120kg N/faddan of mineral-N fertilizer recorded the highest values of the total main head yield, yield increase (ton/faddan), yield increase (%) and heads quality of broccoli. However, the highest total yield and heads quality were recorded by 120kg N/faddan as mineral-N fertilizer treatments. Plants which received 90kg N/faddan came in the second order in the two seasons of this study. The lowest values of total yield and heads quality were obtained by application 60kg N/faddan. The data in Table 6 show clearly that, the best quality of broccoli plants (mean head weight, head and stem diameters) was recorded by 120kg N/faddan in the two seasons of this study. The lowest values of head yield, yield increases and percentage as well as head quality were obtained by 60kg N/faddan. Results of yield and quality followed the same trend of the vegetative growth and chemical content of broccoli plants. It is obvious that, the total yield is the direct reflection of the vegetative growth, mineral absorption and photosynthetic activity. So that, higher mineral N levels reflected higher mineral absorption, higher vegetative growth, higher dry matter accumulation, which in turn produce higher yield with higher quality. These results are in agreement with those obtained by Zaki *et al*. [25] and Saad *et al*. [26] on broccoli.

Effect of Interaction: Interaction between bio-N and mineral nitrogen levels statistically affected yield and quality of broccoli as shown in Table 7. The obtained data revealed that, the interaction treatment significantly affected total head yield (ton/faddan), yield increase (ton/faddan), yield increase (%) and quality of broccoli heads. These results held well in the two experimental seasons. Generally, it could be concluded that, the highest total yield of broccoli plants was recorded by the combined effect of *Azospirilum* with 120kg N/faddan in the two seasons of this study. On the contrary, the lowest total yield of broccoli plants was recorded by 60kg N/faddan without bio-fertilizer in the two seasons of this study. However, the highest heads quality (weight and diameter) were recorded by the combined effect of *Azospirilum sp*. with 120kg N/faddan treatment followed by plants which received *Azotobacter sp*. and 120kg N/faddan in the two seasons of this study.

Table 6: Effect of different mineral nitrogen levels on main head yield (ton/faddan), yield increase and heads quality of broccoli during two seasons (2010/2011 and 2011/2012).

		yield Increase		Head quality	
Mineral nitrogen levels (kg N/faddan)	Main head yield(ton/faddan)	(ton/faddan)	(%)	Diameter (cm)	Weight (g)
First season (2010/2011)					
60	3.240	0.000	0.00	11.95	270.26
90	4.320	1.080	33.33	14.30	359.75
120	4.730	1.490	45.99	14.87	394.29
LSD 0.05	0.110	--	--	0.28	9.01
Second season (2011/2012)					
60	3.180	0.000	0.00	11.83	265.32
90	4.300	1.060	33.33	14.63	358.47
120	4.790	1.550	48.74	15.30	399.57
LSD 0.05	0.280	--	--	0.72	63.12

Table 7: Effect of the interaction between bio-nitrogen and mineral nitrogen levels on main head yield (ton/faddan), yield increase and heads quality of broccoli during two seasons (2010/2011 and 2011/2012).

Bio-nitrogen	Mineral nitrogen levels (kg/faddan)	Main head yield (ton/faddan)	yield Increase (ton/faddan)	yield Increase (%)	Head quality Diameter (cm)	Head quality Weight (g)
First season (2010/2011)						
Without	60	2.910	0.000	2.910	10.85	242.40
	90	4.140	1.230	4.140	13.75	345.35
	120	4.480	0.340	4.480	14.10	373.42
Azospirilum	60	2.960	0.000	2.960	13.55	246.44
	90	4.160	1.200	4.160	14.45	346.34
	120	4.960	0.800	4.960	15.40	413.25
Azotobacter	60	3.860	0.000	3.860	11.44	321.95
	90	4.650	0.790	4.650	14.25	387.55
	120	4.750	0.100	4.750	14.55	396.21
LSD 0.05	--	0.140	--	--	0.51	11.71
Second season (2011/2012)						
Without	60	2.870	0.000	2.870	10.52	238.83
	90	4.100	1.230	4.100	13.97	341.34
	120	4.500	0.400	4.500	14.39	374.73
Azospirilum	60	2.920	0.000	2.920	11.22	243.63
	90	4.110	1.190	4.110	14.92	342.51
	120	5.070	0.960	5.070	16.00	422.13
Azotobacter	60	3.760	0.000	3.760	13.73	313.49
	90	4.700	0.940	4.700	14.57	391.55
	120	4.820	0.120	4.820	15.94	401.85
LSD 0.05	--	0.360	--	--	1.25	35.11

Bio-N fertilization acts as a nutrient reservoir through N_2-fixation and N ions are released slowly over the entire growth period leading to higher yields and their quality. The favorable conditions of soil nutrients status (Table 1) after inoculation were positively reflected in the nutritional status of broccoli plants (Table 3) and consequently reflected in the increased growth, yields and their quality components. These results may be explained by the role of *Azospirilum* in atmospheric nitrogen fixation, better root proliferation and uptake of nutrients and water [42]. These results are in agreement with those obtained by Osman [12], who pointed out that total yield was highly correlated with the development of vegetative growth as well as dry matter accumulation.

Chemical Composition of Broccoli

Effect of Bio-Nitrogen: Data in Table 8 indicated that there were significant differences in N, P and K percentages by the different bio-N treatments in the two seasons of study. However, the highest N, P and K percentages in leaves and heads of broccoli were produced by *Azotobacter sp* treatment followed by *Azospirilum sp*. treatment. In addition, the lowest values of N, P and K percentage in leaves and heads of broccoli were obtained by the control. These results are in accordance with those reported by Zaki *et al*. [25] and Saad *et al*. [26] on broccoli. These results might be due to that *Azotobacter* and *Azospirillum* species increased the solubility of soil nutrients. This might be attributed to the increased activity and efficiency of bacteria in reduction of soil pH by secreting organic acid, i.e. acetic, propionic, fumaric and succinic and consequently more solubility and availability of nutrients for plants [12]. Gadallah and El-Masry [13] and Hanafy *et al*. [14] reported that the application of bio-fertilizer increased the ability to convert N_2 to NH_4 and thus make it available to plants and enhanced the concentration of N, P and K in onion.

Effect of Nitrogen Levels: Data in Table 9 showed that there were significant differences in N, P and K percentages by the different nitrogen levels in the two seasons of study. However, the highest N, P and K percentages in leaves and heads of broccoli were obtained by applied of 120KgN/faddan, while the lowest amount of N, P and K percentage in leaves and heads of broccoli were obtained by control plants in both seasons. These results are in accordance with those reported by El-Shakry [38] and Abou El-Magd *et al*. [43].

Table 8: Effect of bio-nitrogen on N, P and K contents in leaves and heads of broccoli during two seasons (2010/2011 and 2011/2012).

	Mineral content %					
	N		P		K	
Bio-nitrogen	Leaves	Heads	Leaves	Heads	Leaves	Heads
			First season (2010/2011)			
Without	1.47	1.67	0.05	0.07	0.65	0.88
Azospirilum	1.57	1.94	0.07	0.08	0.73	0.95
Azotobacter	1.77	2.02	0.05	0.09	0.76	0.63
LSD 0.05	0.08	0.07	N.S.	N.S.	N.S.	0.04
			Second season (2011/2012)			
Without	1.52	1.76	0.05	0.07	0.63	0.90
Azospirilum	1.63	2.07	0.07	0.09	0.72	0.98
Azotobacter	1.88	2.17	0.06	0.10	0.75	0.60
LSD 0.05	0.09	0.07	N.S.	N.S.	N.S.	0.06

Table 9: Effect of mineral-nitrogen levels on N, P and K contents in leaves and heads of broccoli during two seasons (2010/2011 and 2011/2012).

	Mineral content %					
	N		P		K	
Mineral-nitrogen levels (kg/faddan)	Leaves	Heads	Leaves	Heads	Leaves	Heads
			First season (2010/2011)			
60	1.20	1.82	0.06	0.07	0..67	0.64
90	1.73	1.86	0.05	0.08	0.72	0.88
120	1.88	1.94	0.06	0.10	0.74	0.94
LSD 0.05	0.09	0.03	NS	NS	NS	0.05
			Second season (2011/2012)			
60	1.20	1.94	0.06	0.07	0.65	0.62
90	1.83	1.99	0.06	0.08	0.71	0.90
120	2.01	2.08	0.06	0.11	0.73	0.97
LSD 0.05	0.13	0.05	NS	NS	NS	0.06

Table 10: Effect of the interaction between bio-nitrogen and mineral-nitrogen levels on N, P and K contents in leaves and heads of broccoli during two seasons (2010/2011 and 2011/2012).

		Mineral content %					
		N		P		K	
Bio-nitrogen	Mineral-nitrogen levels (kg/faddan)	Leaves	Heads	Leaves	Heads	Leaves	Heads
			First season (2010/2011)				
Without	60	1.01	1.68	0.05	0.07	0.71	0.93
	90	1.12	1.87	0.07	0.12	0.76	0.92
	120	1.47	1.91	0.07	0.11	0.76	0.97
Azospirilum	60	1.65	1.47	0.04	0.08	0.56	0.90
	90	1.72	1.98	0.09	0.07	0.74	0.91
	120	1.82	2.14	0.03	0.06	0.72	0.83
Azotobacter	60	1.75	1.86	0.07	0.06	0.69	0.82
	90	1.86	1.96	0.04	0.06	0.69	1.01
	120	2.03	2.00	0.06	0.11	0.79	1.10
LSD 0.05	--	0.08	0.13	NS	NS	NS	NS
			Second season (2011/2012)				
Without	60	0.97	1.77	0.05	0.07	0.69	0.96
	90	1.10	2.00	0.06	0.13	0.75	0.94
	120	1.52	2.04	0.07	0.12	0.75	1.00
Azospirilum	60	1.73	1.52	0.04	0.09	0.52	0.92
	90	1.82	2.13	0.10	0.07	0.73	0.93
	120	1.94	2.32	0.06	0.06	0.71	0.84
Azotobacter	60	1.85	1.98	0.07	0.06	0.67	0.83
	90	1.98	2.10	0.05	0.06	0.67	1.05
	120	2.19	2.15	0.06	0.12	0.79	1.03
LSD 0.05	--	0.12	0.08	NS	NS	NS	NS

These results might be attributed to the positive effect of nitrogen in increasing the solubility of soil phosphorus and potassium. Consequently, higher plant absorption of N, P and K and higher content of these elements in the plant organs.

Effect of Interaction: Interaction between bio-N and mineral-N levels statistically affected on the N, P and K% in tissues of broccoli leaves and heads (Table 10). The obtained data revealed that, the interaction treatments significantly affected N, P and K percentage in tissues of broccoli leaves and spears. These results held well in the two seasons. Generally, it could be summarized that, the highest amount of N, P and K percentages in tissues recorded when plants fertilized with bio-N *Azospirillum* combined with 120kg N/faddan. On the contrary, the lowest amount of N, P and K percentages was recorded by 60kg N/faddan without bio-fertilization. These results were completely similar in both seasons. Since the effect of bio and mineral fertilization is with complementary action. In addition, both the bio and mineral N fertilization increased N, P and K content in leaves and heads of broccoli. It is obvious that the combined effect of both factors is the outcome of their effect. So that, the combined effect of bio and mineral N fertilization caused increases in N, P and K content of leaves and heads of broccoli plants.

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