Effect of *Azotobacter* and nitrogen levels on growth, yield and quality of broccoli (*Brassica oleracea* var. *italica*)

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Abstract: Two field experiments were carried out at the experimental station of the Faculty of Agriculture, Suez Canal University, Ismailia, Egypt during the winter seasons of 2006-2007 and 2007-2008.The study aimed to investigate the effect of four mineral nitrogen rates (0.0, 45, 90 and 120 kg N/fed.) and three levels of *Azotobacter* as bio-fertilizer (0.0, 2 and 4 kg/fed.) treatments on growth, photosynthetic pigments, yield and its quality components as well as mineral elements of broccoli. Results showed that increasing the supplied nitrogen fertilizer level up to 90 kg N/fed. significantly improved all tested vegetative growth parameters, yield and N content. However, vitamin C and carotenoids in curd as well as leaf and curd chlorophyll contents were the best at 45 kg N/fed. The highest nitrate and free amino acids were associated with the highest N-rate (120 kg/fed.). The addition of bio-fertilizer (*Azotobacter*) resulted in significant increases in vegetative growth and yield. In addition, the lowest content of nitrate in curd was found in plants inoculated with *Azotobacter*. However, other tested parameters i.e; vitamin C, SSC%, total carbohydrates, photosynthetic pigments and mineral elements were not affected by the inoculation with *Azotobacter*. Positive interaction between nitrogen fertilizer levels and *Azotobacter* was detected, regarding the vegetative growth, yield and reduction of nitrate accumulation.

Keywords: Broccoli, mineral and bio-N fertilizers and curds quality

INTRODUCTION

Broccoli is a widely cultivated vegetable crop in America and many European countries. In Egypt, broccoli is grown in very limited scattered areas and the total cultivated area is not exactly known (Tolba, 2005).

Broccoli is known as the "Crown Jewel of Nutrition" for its vitamin-rich, high in fiber, and low in calorie properties. Broccoli has multiple cancer-fighting properties including vitamin C, beta carotene, and fiber (American Dietetics Association, 1992). It is also, rich of phytochemicals which appear to offer human protection against certain cancers and heart disease (Nonnecke, 1989).

According to the above-mentioned nutritive value and the possibility of early exportation to Europe, efforts should be directed towards increasing the cultivated area and productivity of broccoli in Egypt, through encouraging growers, consumers and exporters to grow, consume and export broccoli, respectively. Increasing broccoli production per unit area can be achieved through the use of high yielding cultivars with good qualities and by the application of better cultural practices (Hegazi *et al.*, 2004).

Nutrient elements are among major factors affecting growth of broccoli plant, as well as its yield. Nitrogen is considered as one of the most important nutrients for vegetable crops, where it is a main constituent of many organic compounds in plants, such as protein, enzymes, pigments, hormones and vitamins (Babik *et al.*, 2002, Hegazi *et al.*, 2004 and Tolba, 2005).

Numerous investigations have been carried out to evaluate the effects of N-fertilizers on growth, yield and chemical constituents of broccoli plants (Tremblay, 1989; Singh and Singh, 2000; Kumar and Sharma, 2001; Karitonas, 2003; Hegazi *et al.*, 2004 and Moniruzzuman *et al.*, 2007), but results vary according to locations and doses.

In the last decade, great efforts have been directed to overcome the problem of excess of chemical fertilizers which caused soil pollution. Fortunately under such conditions, it has become essential to use the bio-fertilizers as a partial substitute or supplement for chemical fertilizers, reduce the costs of produced yield and environmental pollution as well as increase production and improve quality of vegetable crops. In this regard, investigators indicated that inoculation of broccoli plants with N_2-fixing bacteria, i.e., *Azospirillum* or *Azotobactar*, increased plant vegetative growth, head yield and its quality, as well as improving soil structure and increasing soil organic matter contents (Wange and Kale, 2004).

This study was done with the objective of testing the hypothesis that the interaction between mineral and bio-nitrogen fertilization will affect yield and its quality components of broccoli plants (*Brassica oleracea* L. var. *italica*) cv. Southern Star under the condition of Ismailia Governorate.

MATERIALS AND METHODS

Two field experiments were carried out at the Experimental Research Farm, Faculty of Agriculture, Suez Canal University, Ismailia Governorate, Egypt, during the two successive winter seasons of 2006-2007 and 2007-2008 to investigate the effects of nitrogen fertilizer, *Azotobacter* treatments and their interactions, on plant growth, yield, quality and chemical constituents of broccoli (*Brassica oleracea* L. var. *italica*) cv. Southern Star.

The experimental layout was split plot in a randomized complete blocks design with three replicates contained 12 treatments, which were the combination of four mineral nitrogen fertilizer levels (0, 45, 90 and 120 kg N/fed. Which equivalent to 0, 135, 270, 360 kg ammonium nitrate) arranged at random in

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the main plots and three biofertilizer treatments with Cerealine [(2 and 4 kg/fed. peat-based, *Azotobacter* inoculants containing 10^8 cells ml^{-1}), in addition to control without inoculation] assigned at random in subplots. Cerealine inoculum was mixed with wet soft dust at (1:10 ratio) and it was applied to the root absorption zone of plants, two weeks after transplanting, just before irrigation. Plots soil texture was sandy (85.21% sand, 3.29% clay and 11.5% silt), pH was 8.27, EC was 0.47 dSm^{-1}, Ca was 0.8 meq^{-1}, Mg was 0.6 meq^{-1}, K was 0.3 meq^{-1}, Na was 3.0 meq^{-1}, HCO_3 was 1.6 meq^{-1}, Cl was 3.0 meq^{-1} and SO_4 was 0.1 meq^{-1}.

Each subplot area was 10.8 m^2 contained 4 rows, each was 4.5 m in length and 0.6 m in width and planting distance was 0.4 m apart. Each two adjacent experiment units (sub plots) were separated, on each side, by 1.00 m to protect against border effects. Plots were enriched with farm yard manure at rate of 20 m^3/feddan and calcium super phosphate (15.5% P_2O_5) at uniform dose of 31 kg P_2O_5 /feddan during the soil preparation. Broccoli nurslings, four weeks old, were transplanted on the third top of slope ridges, from the first of October, 2006 to the mid of January, 2007 and the same planting date in 2007-2008. Growing plants were fertilized with potassium sulphate (48% K_2O) at a rate 48 kg K_2O/fed. which was divided and added in three equal portions at 2, 4 and 6 weeks after transplanting. Nitrogen fertilizer levels as ammonium nitrate (33.5%) were added at three equal doses 2, 4 and 6 weeks after transplanting. In the field, average air temperature, average daily relative humidity and average pan evaporation fluctuated between 10.95-27.24 °C, 55.86–60.24% and 1.09–7.1 mm/day, respectively, during the experiment.

Recorded data:

1- Foliage fresh, dry weight, number of leaves and leaves area per plant of three plants for each replicate were determined. Dry weight determined after fixing plant material at 90 °C and drying at 70 °C up to constant weight.

2- Primary, Secondary curd yield (g/plant and ton/fed.) and comulative total curd yield (ton/fed.) in addition to average primary or secondary curd diameter were determined.

3- Chemical constituents of leaves and, curds such as;

- Total chrophyll and carotenoids content of leaves and primary curds were determined, spectrophotometricaly, using acetone as a solvent, according to Lichenthaler and Wellburn (1983), and then calculated as mg/100 g fresh weight.
- Soluble solids content (SSC %) percentage of primary curds was determined handly by rafractometer (stalks) according to A.O.A.C. (1996).
- Ascorbic acid of primary curds, was determined by the titration method using 2,6 dichlorophenolindophenol according to Pearson (1970).
- Nitrate content of primary curds was determined spectrophotometricaly at 540 nm. and then calculated as mg/kg dry weight as described by Singh (1988).
- Total carbohydrates: Total carbohydrates hydrolyzed determination was carried out for leaves and primary curds. Samples taken from each treatment were washed and dried at 70 °C, then total carbohydrates were determined spectrophotometricaly at 630 nm by anthrone reagent according to Mazumdar and Majumder (2003).
- Total free amino acid, concentrations in primary curd was estimated, using the method of Rosen (1957) with ninhydrin reagent, spectrophotometricaly at 650 nm.
- Nitrogen, phosphorus, potassium and calcium contents were determined via grounding of 0.5 g dried material taken from leaves and primary curds, which were digested with H_2SO_4 and H_2O_2 and then brought to a final volume of 50 ml with distilled water. Nitrogen was estimated using semimicro-kjeldahl method as described by Ling (1963). Phosphorus was analyzed by a vanadate-molybdate method at 660 nm using a Spectro 22 spectrophotometer (Chapman and Pratt, 1982). Potassium and Calcium concentrations (%) were determined using a Perkin-elmer, Flame photometer (Page *et al.*, 1982).

Data statistical analysis:

Data were statistically analyzed using ANOVA/MANOVA of Statistica 6 software (Statsoft, 2001) with mean values compared using Duncanś multiple range test with a significance level of at least $p \leq 0.05$.

RESULTS AND DISCUSSION

The results of the two conducted experiments will be presented and discussed as follows:

Vegetative growth characters and dry weight:

Main effect of N rate. The increasing of nitrogen fertilizer up to 90 kg/fed. generally produced the highest values of number of leaves, leaf area, foliage fresh and dry weight, except only foliage dry weight in the second season which increased with N levels up to 120 kg/fadden. Higher nitrogen level (120 kg/fed.) resulted, mostly, in non-significant changes in all studied parameters in both seasons, except only leaf area which significantly decreased in the first season, due to increase nitrogen fertilizer from 90 kg N/fed. to 120 kg N/fed. (Table1). The obtained results indicated that vegetative growth increased with increasing nitrogen fertilizer up to 90 kg N/fed. and this may be due to the beneficial effect of nitrogen on stimulating the meristmatic activity for producing more tissues and organs, since N plays major roles in the synthesis of structural proteins and other several macro-molecules, in addition to its vital contribution in several biochemical processes in plant related to growth (Marschner, 1995). The obtained results are in harmony with those of Farrag *et al.* (2000) who found that increasing rate of N up to a certain level (70 kg n/fed.) increased vegetative growth of cauliflower plants, then non-significant enhancement was achieved when N rate increased up to 105 kg N/fed. Also, Hegazi *et al.* (2004) examined the effect of N-rates up to 90 kg/fed. on broccoli plants. They found that 90 kg N/fed. produced the highest plant fresh weight and leaf area of plant, but they did not examine nitrogen levels higher than 90 kg/fed.

An obvious trend was observed, where as increasing bio-N-fertilizer level, tested vegetative characters increased, so the highest level (4 kg/fed.) formed the highest values of number of leaves, leaf area, foliage fresh and dry weight compared with control without inoculation (Table 1). Higher *Azotobacter* level (4 kg/fed.) resulted in non-significant increase compared with low level (2 kg/fed.),in some cases of terms of number of leaves and leaf area, in both seasons. These results show good effect of treatment with *Azotobacter* which have the capability for fixing nitrogen and reduce pH level in root zone through producing organic acids, which in turn increase macro and micro-nutrients availability to plants (Awasthi *et al.*, 1998) as well as stimulation of disease resistance mechanisms (Zdor and Anderson, 1992), where all together may promote the vegetative growth to go forward. Similar results were reported by Manivanna and Singh (2004) who showed that the use of *Rhizobium* as bio-fertilizer in broccoli plants significantly increased plant height, compared with untreated plants. Similarly, Sharma (2002) and Kashyap *et al.* (2005) found that application of bio-fertilizers such as *Rhizobium*, *Azospirillum* and *Azotobacter* to cabbage and cauliflower plants significantly increased plant growth.

Concerning the interactions between N-mineral and bio-fertilizer levels, results in Table (1) showed that number of leaves, leaf area, foliage fresh and dry weight were significantly affected by such interaction treatments. The addition of bio-fertilization non-significantly affected the studied vegetative parameters in the absence of mineral fertilization of nitrogen. However, at 45 kg N/fed. the inoculation of *Azotobacter* as N-biofertilizer significantly improved foliage fresh and dry weight, in addition to number of leaves in the first season. Meanwhile other tested parameters were not significantly affected. At 90 kg N/fed., the application of *Azotobacter* as bio-fertilizer significantly increased leaf area, foliage fresh and dry weight, especially in the second season. It is clear from the data that the high rate of bio-fertilizer was more efficient in producing higher vegetative growth, but in most cases, no significant differences were found between low and high rate of *Azotobacter* (2 and 4 kg/fed.) under 90 kg N/fed. At the combination of 120 kg N/fed. and high rate of bio-fertilizer (4 kg/fed.), all measured vegetative characters were not-significantly affected in comparison with the treatment containing 90 kg N/fed. interacted with 4 kg/fed. *Azotobacter,* in both seasons. These results might be explained on the basis that the interactive effects of the two studied factors were additive. A large number of reports emphasized the beneficial effects of the interaction between mineral N fertilizer and inoculation with bio-fertilizer on plant growth of different vegetable crops. In this regard, Ibrahim *et al.* (2004) showed that nitrogen fertilization was more effective in stimulating growth of cauliflower in the presence of Nitrobin. Furthermore, Sharma (2002) mentioned that the use of mineral nitrogen fertilizer in combination with *Azotobacter* or *Azospirillum* may activate N-fixation bacteria in soil, and consequently increases available nitrogen and plant growth promoting substances for cabbage plants.

Curd Yield and its components:

Data in Table (2) show that various comparisons among the over-all means of nitrogen levels up to 90 kg N/fed., reflected significant effects on the curds yield and its components, in both seasons. The increases were about 362.9% and 422.23% in yield of primary curds, 513.13% and 476% in yield of secondary curds and 404 % and 444.6% in the yield of total curds, in both seasons, respectively. The data reflected also, when nitrogen fertilizer increased from 90 to 120 kg/fed., the yield of primary curds decreased by 20.58 and 28.00%, secondary curds decreased by 88.7% and 73.23% and total yield decreased by 43.82 and 52%, in both seasons, respectively. These results might be due to the increase in vegetative growth characters (Table 1). Positive yield response is the result of different individual processes, such as an increase in leaf area and net photosynthesis per unit of leaf area (i.e., effects at the source) or an increase in the yield (i.e., effects at the sink). The obtained results are in harmony with the results indicated that the yield was increase as a result of increasing nitrogen fertilization rates up to 70 kg N/fed. on cauliflower (Farrage *et al.,* 2000) and the results of Hegazi *et al.* (2004) on broccoli. Results of Yildirim *et al.* (2007), Mellgren (2008) and Yoldas *et al.* (2008) on broccoli showed similar trend. However, they examined other nitrogen levels up to 600 kg N/ha.

Concerning the effect of *Azotobacter* levels, the results in Table (2) showed that the most significant primary, secondary and total yields of curds were obtained when *Azotobacter* was applied at 4 kg/fed. in comparison with untreated plants, in both seasons. Also, the results showed a non-significant increases in secondary yield of curds in both seasons and total yield of curds in the first season, when broccoli plants inoculated with high level (4 kg/fed.) in comparison with low level (2 kg/fed.). The enhancing effect of the bio-fertilizer application have been attributed to several mechanisms, including biological nitrogen fixation and producing plant growth promoting substances (Okan and Labandera-Gonzalez, 1994).

The differences detected among the interaction treatments, generally, appeared significant (Table 2). It was noticed that the primary curd yield, secondry curd yield and total curd yield were increased as a result of increasing *Azotobacter* level up to 4 kg/fed. combined with increasing the nitrogen level up to 90 kg N/fed. and this increasing was significant in the second season of cultivation. The increasing in yield was more than four and eight times in both seasons, respectively, compared with the respective control treatment. This big differences between both seasons might be due to the fluctuation in environmental conditions such as; temperature. However, the highest nitrogen level (120 kg/fed.) combined with *Azotobacter* significantly decreased total yield of curd in comparison with 120 kg N/fed. in the absence of *Azotobacter*. The obtained results could be explained by the results of Sharma (2002) who showed that nitrogen fertilization was more effective in stimulating growth of cabbage plants in the presence of *Azotobacter* or *Azospirillum* as bio-fertilizer. Furthermore, El-Morsy and Shokr (2005) mentioned that use of mineral nitrogen fertilizer at level of 90 kg/fed. in combination with rhizobacterin may activate N-fixtion bacteria in soil and consequently increase plant growth promoting substances and subsequently the yield of garlic.

Table (1): Effect of nitrogen, *Azotobacter* and their interactions on number of leaves, leaf area and foliage fresh and dry weight of "Southern Star" broccoli cultivar, during the two successive winter seasons of 2006-2007 and 2007-2008.

N (kg/Fed.)	Cerea.*	No. of leaves/plant		Leaf area (cm^2/plant)		Foliage Fresh Weight (g/plant)		Foliage Dry Weight (g/plant)	
		2006-07	2007-08	2006-07	2007-08	2006-07	2007-08	2006-07	2007-08
0		10.89 ^{ab}	11.89 ^{c}	1226.3 ^{d}	2060.1 ^{c}	88.91 ^{c}	206.67 ^{d}	14.40 ^{c}	31.26 ^{c}
45		11.44 ^{ab}	13.33 ^{b}	1780.0 ^{c}	3328.2 ^{b}	117.50 ^{b}	320.47 ^{c}	16.68 ^{bc}	45.77 ^{b}
90		12.44 ^{a}	15.11 ^{a}	3538.5 ^{a}	6489.5 ^{a}	169.10 ^{a}	582.40 ^{b}	24.04 ^{a}	71.04 ^{a}
120		11.78 ^{ab}	15.67 ^{a}	2886.5 ^{b}	7173.6 ^{a}	157.16 ^{a}	608.18 ^{a}	20.89 ^{ab}	70.85 ^{a}
	0	10.83 ^{b}	13.17 ^{b}	1660.7 ^{b}	3587.2 ^{b}	114.53 ^{b}	335.94 ^{c}	16.81 ^{b}	47.25 ^{c}
	2	11.83 ^{ab}	13.92 ^{ab}	2528.3 ^{a}	5088.6 ^{a}	111.42 ^{b}	406.73 ^{b}	15.92 ^{b}	57.02 ^{b}
	4	12.25 ^{a}	14.92 ^{a}	2884.5 ^{a}	5612.8 ^{a}	158.80 ^{a}	443.52 ^{a}	23.12 ^{a}	59.91 ^{a}
	0	10.67 ^{b}	11.33 ^{c}	1305.1 ^{d}	1761.0 ^{f}	85.08 ^{e}	171.40 ^{i}	14.04 ^{c}	25.53 ^{f}
0	2	11.00 ^{ab}	11.33 ^{c}	1074.1 ^{d}	2097.3 ^{ef}	80.31 ^{e}	190.90 ^{h}	13.09 ^{c}	2.23 ^{f}
	4	10.00 ^{ab}	13.00 ^{bc}	1299.8 ^{d}	2322.0 ^{ef}	103.72 ^{cde}	257.70 ^{f}	16.54 ^{bc}	41.00 ^{d}
	0	10.00 ^{ab}	12.00 ^{c}	1668.7 ^{d}	2449.3 ^{ef}	94.20 ^{de}	236.01 ^{g}	12.69 ^{c}	34.19 ^{e}
45	2	11.67 ^{ab}	14.00 ^{b}	1828.3 ^{cd}	3666.1 ^{ef}	118.44 ^{cde}	356.11 ^{e}	13.95 ^{c}	51.73 ^{c}
	4	11.67 ^{ab}	14.00 ^{b}	1843.1 ^{cd}	3869.1 ^{ef}	128.67 ^{bcd}	369.28 ^{e}	21.40 ^{bc}	51.39 ^{c}
	0	11.00 ^{ab}	15.00 ^{b}	2339.2 ^{c}	5987.3 ^{cd}	130.42 ^{bcd}	538.34 ^{d}	19.38 ^{bc}	66.11 ^{b}
90	2	13.00 ^{ab}	15.00 ^{b}	3871.1 ^{b}	6342.8 ^{bc}	169.27 ^{b}	599.78 ^{c}	21.37 ^{bc}	74.60 ^{a}
	4	13.33 ^{a}	15.33 ^{ab}	4405.1 ^{a}	7138.4 ^{abc}	246.29 ^{a}	631.10 ^{ab}	36.03 ^{a}	72.42 ^{a}
	0	10.67 ^{ab}	14.33 ^{b}	1329.8 ^{d}	4151.0 ^{de}	123.53 ^{cde}	522.12 ^{d}	18.55 ^{bc}	63.18 ^{b}
120	2	11.67 ^{ab}	15.33 ^{ab}	3339.6 ^{b}	8248.1 ^{ab}	139.86 ^{bc}	613.34 ^{bc}	19.41 ^{bc}	74.52 ^{a}
	4	13.00 ^{ab}	17.33 ^{a}	3990.2 ^{ab}	9121.7 ^{a}	241.74 ^{a}	646.06 ^{a}	26.20 ^{ab}	74.83 ^{a}

Values are the means of three replicates. Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple range test
Cerea.* (Cerealine) is the commercial name of azotobacter containing biofertilizer.

Quality characters.

Results in Table (3) show that vitamin C increased significantly with the application of nitrogen at level of 45 kg/fed., however, no significant difference was found with increasing nitrogen level up to 120 kg/fed., in the first season of cultivation. While in the second season of cultivation, vitamin C did not significantly affect by the application of nitrogen fertilization in comparison with control treatment. A similar relationship was found by Albrecht *et al.* (1991). Also, Sorensen (1999) and Lee and Kader (2000) reported that nitrogen fertilization did not affect the content of vitamin C in different vegetable crops such as broccoli and cauliflower. Meanwhile, significantly the highest nitrate and free amino acids contents were obtained at the highest nitrogen fertilization rate (120 kg/fed.). This increase was linear in the second season of cultivation. With an increase in the fertilization dose from 0.0 to 120 kg N/fed., the content of nitrates increased by 53.5% and 114.09%, in both seasons, respectively, confirming the role of nitrogen fertilization in the accumulation of this compound (Greenwood and Hunt, 1986). Regarding total carbohydrates and SSC%, the results indicated that carbohydrates were slightly increased when broccoli plants received 45 kg N/fed., and then, decreased with increasing nitrogen fertilizer from 45 kg/fed. to 120 kg N/fed. in the first season of cultivation. However, in the second season, the unfertilized plants with mineral nitrogen had the highest carbohydrate content and SSC% in comparison with plants fertilized at any level of mineral nitrogen fertilization. These results are in agreement with those of Babik *et al.* (1996) who found a negative relation between nitrogen fertilization and total sugars in brussels sprouts.

As for the comparison among the means of *Azotobacter* levels and control treatment on quality characteristics, results in Table (3) indicated that vitamin C, SSC% and total carbohydrates did not significantly change by the inoculation of broccoli plants with bio-fertilizer (*Azotobacter*) compared with un-inoculated ones. In contrast, nitrate and free amino acids contents were significantly decreased in inoculated plants with bio-fertilizer (*Azotobacter*) in comparison with untreated plants (control). Similarly, Hammad and Abdel-Ati (1998) and Hanafy *et al.* (2000) found a negative correlation between application of bio-fertilization and accumulation of nitrate in potato tubers and lettuce, respectively.

Table (2): Effect of mineral nitrogen, *Azotobacter* and their interaction on curd yield of "Southern Star" broccoli cultivar, in 2006-2007 and 2007-2008 seasons.

N (kg/Fed.)	*Cerea*	Primary Curd: Weight/Plant (g) 2006-2007	Primary Curd: Weight/Plant (g) 2007-2008	Primary Curd: Diameter (cm) 2006-2007	Primary Curd: Diameter (cm) 2007-2008	Primary Curd: Yield (Ton/fed.) 2006-2007	Primary Curd: Yield (Ton/fed.) 2007-2008	Secondary Curd: Weight/Plant (g) 2006-2007	Secondary Curd: Weight/Plant (g) 2007-2008	Secondary Curd: Diameter (cm) 2006-2007	Secondary Curd: Diameter (cm) 2007-2008	Secondary Curd: Yield (Ton/fed.) 2006-2007	Secondary Curd: Yield (Ton/fed.) 2007-2008	Total Yield (Ton/Fed.) 2006-2007	Total Yield (Ton/Fed.) 2007-2008
0		88.00^{d}	108.08^{d}	11.70^{c}	12.08^{c}	1.47^{d}	1.80^{d}	3.16^{d}	73.85^{d}	3.67^{c}	4.33^{b}	0.62^{d}	1.23^{d}	2.09^{d}	3.03^{d}
45		207.7^{c}	269.39^{c}	15.89^{b}	18.60^{c}	3.46^{c}	4.49^{c}	72.01^{c}	174.35^{c}	5.00^{b}	5.78^{a}	1.20^{c}	2.91^{c}	4.66^{c}	7.40^{c}
90		319.25^{a}	456.35^{a}	19.28^{a}	21.30^{a}	5.32^{a}	7.61^{a}	190.69^{a}	351.53^{a}	7.00^{a}	6.22^{a}	3.18^{a}	5.86^{a}	8.50^{a}	13.47^{a}
120		253.55^{b}	328.56^{b}	16.31^{b}	19.11^{b}	4.23^{b}	5.48^{b}	101.06^{b}	202.93^{b}	5.00^{b}	5.67^{a}	1.68^{b}	3.38^{b}	5.91^{b}	8.86^{b}
	0	212.97^{a}	246.81^{c}	15.63^{a}	16.63^{b}	3.55^{a}	4.11^{c}	89.36^{b}	164.36^{b}	4.92^{a}	5.33^{a}	1.46^{b}	2.74^{b}	5.01^{b}	6.85^{c}
	2	218.30^{a}	279.06^{b}	15.57^{a}	17.97^{a}	3.64^{a}	4.65^{b}	107.45^{a}	212.37^{a}	5.17^{a}	5.42^{a}	1.79^{a}	3.54^{a}	5.43^{a}	8.19^{b}
	4	220.15^{a}	345.92^{a}	16.19^{a}	18.73^{a}	3.67^{a}	5.77^{a}	105.88^{a}	225.26^{a}	5.42^{a}	5.75^{a}	1.76^{a}	3.75^{a}	5.43^{a}	9.52^{a}
	0	76.65^{e}	68.26^{i}	11.33^{c}	9.67^{e}	1.23^{e}	1.14^{i}	30.04^{f}	34.76^{h}	3.33^{d}	4.00^{cd}	0.50^{f}	0.58^{g}	1.78^{e}	1.72^{i}
0	2	92.02^{e}	106.49^{h}	11.67^{c}	12.23^{d}	1.53^{e}	1.77^{h}	40.70^{ef}	95.35^{g}	3.67^{cd}	4.33^{cd}	0.68^{ef}	1.59^{f}	2.21^{e}	3.36^{h}
	4	95.33^{e}	149.5^{g}	12.10^{c}	14.33^{d}	1.59^{e}	2.49^{g}	40.74^{ef}	91.43^{g}	4.00^{cd}	4.67^{bcd}	0.68^{ef}	1.52^{f}	2.2^{e}	4.02^{h}
	0	168.58^{d}	220.92^{f}	15.33^{abc}	17.57^{c}	2.81^{d}	3.68^{f}	55.03^{def}	106.06^{g}	4.67^{cd}	5.33^{abcd}	0.92^{def}	1.77^{f}	3.73^{d}	5.45^{g}
45	2	224.03^{c}	268.81^{e}	15.50^{abc}	18.57^{c}	3.73^{c}	4.48^{e}	66.17^{cd}e	183.32^{ef}	5.00^{bcd}	5.67^{abcd}	1.10^{cde}	3.06^{de}	4.84^{c}	7.54^{f}
	4	230.67^{c}	318.44^{d}	16.83^{ab}	19.67^{bc}	3.84^{c}	5.31^{d}	94.83^{c}	233.69^{d}	5.33^{abcd}	6.33^{abc}	1.58^{c}	3.89^{c}	5.43^{c}	9.20^{de}
	0	313.31^{ab}	365.67^{c}	18.50^{a}	19.57^{bc}	5.22^{ab}	6.09^{c}	137.84^{b}	293.01^{c}	6.00^{abc}	6.00^{abc}	2.30^{b}	4.88^{c}	7.52^{b}	10.98^{c}
90	2	316.53^{ab}	412.11^{b}	19.17^{a}	21.43^{ab}	5.28^{ab}	6.87^{b}	226.86^{a}	353.72^{b}	7.33^{ab}	6.00^{abc}	3.8^{a}	5.89^{b}	9.06^{a}	12.76^{b}
	4	327.92^{a}	591.27^{a}	20.17^{a}	22.90^{a}	5.47^{a}	9.85^{a}	207.38^{a}	407.85^{a}	7.67^{a}	6.67^{a}	3.46^{a}	6.80^{a}	8.92^{a}	16.65^{a}
	0	293.35^{b}	332.39^{cd}	17.33^{a}	19.70^{bc}	4.89^{b}	5.54^{cd}	126.53^{b}	223.61^{de}	5.67^{abcd}	6.00^{abc}	2.11^{b}	3.73^{cd}	6.99^{b}	9.27^{d}
120	2	240.62^{c}	328.81^{cd}	15.93^{abc}	19.63^{bc}	4.01^{c}	5.48^{cd}	96.07^{c}	217.11^{de}	4.67^{cd}	5.67^{abcd}	1.60^{c}	3.62^{cd}	5.61^{c}	9.10^{de}
	4	226.68^{c}	324.49^{d}	15.67^{abc}	18.00^{c}	3.78^{c}	5.41^{d}	80.58^{cd}	168.06^{f}	4.67^{cd}	5.33^{abcd}	1.34^{cd}	2.80^{e}	5.12^{c}	8.21^{ef}

Values are the means of three replicates. Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple range test

Concerning the interaction between nitrogen and *Azotobacter* levels, the results in Table (3) show that the lowest vitamin C content was found in broccoli plants that did not receive nitrogen fertilization, with or without the inoculation of plants by *Azotobacter* at 2 kg/fed., in the first season and only broccoli plants which did not fertilize with mineral or bio-Nitrogen, in the second season. The highest vitamin C was found when nitrogen fertilization at 45 kg N/fed. accompanied with *Azotobacter* at 4 kg/fed., in the second season and only plants received nitrogen at 90 kg/fed. in the first season. These results are in agreement with the results of El-Shimi *et al.* (2002) who found that the application of mineral, bio-N or their combination did not affect vitamin C content of strawberry fruit. However, nitrate accumulation and free amino acids content were increased with increasing nitrogen fertilization up to 120 kg N/fed., and this effect was clear in the second season. However, application of *Azotobacter* to the broccoli plants reduced the accumulation of nitrate and free amino acids content in primary curd of broccoli. Also, data showed that the highest concentration of *Azotobacter* (4 kg/fed.) was more effective in reducing the accumulation of nitrate in comparison with 2 kg/fed. *Azotobacter*. With regard to total carbohydrates content and SSC%, the results indicated that the heads of broccoli plants without mineral and bio-nitrogen fertilization (control) had the highest total carbohydrates content and SSC%.

Chemical constitutes.

The results indicated that increasing nitrogen fertilization levels significantly increased nitrogen content in leaves and curds, in addition to total chlorophyll in leaves and carotenoids content in curds of broccoli, in both seasons, where the highest nitrogen, chlorophyll and carotenoids were associated with 90 and 120 kg N/fed. (Figure 1 and Table 4). While, increasing nitrogen fertilization in most cases did not significantly affect the contents of leaf caroteinoids, chlorophyll in curds, in addition to potassium, phosphorus and calcium in leaves and curds of broccoli, except, curds chlorophyll and leaf calcium contents, in the second season, in addition to potassium and phoshorus contents in primary curds, in the first and second seasons, respectively. Although, the highest chlorophyll in curds and leaf calcium contents were associated with 90 and 120 kg N/fed., however, high potassium and phosphorus contents in primary curds were found at any nitrogen fertilizer levels (45, 90 and 120 kg/fed.) in comparison with control treatment (Table 4). It is reported that mineral nutrition can influence net photosynthesis in various ways (Barker, 1979), where it is required for chloroplast formation, either for the synthesis of proteins, thylakoid membranes or for chloroplast pigments. The obtained results in Table (4) indicated that broccoli plants which did not receive mineral nitrogen fertilizer characterized with low leaf chlorophyll and nitrogen contents. This finding is supported by the results of Spencer and Possingham, (1960) who found that a deficiency of mineral nutrition in plants may be directly involved in low synthesis of protein or low formation of chloroplast pigments which subsequantely can depress net photosynthesis by influencing the CO_2 fixation reaction and entry of CO_2 through the stroma. Also, it is clear from the indicated results that the increasing of chlorophyll content was very low (14% and 23.9%) with the increase of nitrogen fertilization, in comparison with the increasing of total yield (more than 300%, in both seasons) and vegetative characters, as fresh weight of leaves (74.13% and 182.84%). This finding could be explained by a decreasing in source-sink ratio, as reported by Khamis *et al.* (1990). Such results show that the effect of mineral nitrogen fertilizer, as illustrated in Table (4), are similar to those reported by Hegazi *et al.* (2004), Yildirim *et al.* (2007) and Yoldas *et at.* (2008) who found a linear increase in chlorophyll and nitrogen contents of broccoli leaves with the increasing of nitrogen fertilizer level.

The effect of bio-fertilizer alone or in combination with N-rate on mineral and photosynthetic pigments content of broccoli indicated that, generally, these parameters were not significantly affected by the inoculation of broccoli plants with *Azotobacter* as presented in the analysis of variance (Table 4). The obtained results are in agreement with those of Mansour (2002) and El-Sharkawy *et al.* (2003) who reported that the content of N, P and K did not significantly affect by the application of Nitrobein as bio-fertilizer in sweet potato and taro, respectively. However, These results were in contradiction with the results of Chaterjee *et al.* (2005), who revealed that the pigments in broccoli plants were increased by the application of bio-fertilizers. During the last decade many field experiments have conducted on the effect of inoculation, particularly with *Azospirillum* and *Azotobacter* on the growth and yield of several temperate and tropical crop species. The results are highly variable, but increases in the yield of the different crops may be achieved by inoculation. Whether this yield increase is attributable to N_2 fixation, hormonal or other effects on growth and development, remains unclear. It is well known that, *Azotobacter* forms the free-living N2-fixing soil bacteria which are mostely heterotrophic and thus often restricted in their N2-fixation capacity by substrate limitation. Because of carbon-limitation, the actual contribution of this bacteria to the nitrogen input is considered to be very small, and on average less than 1 kg N/ha. per year (Marschner, 1995). These statements may explain why vegetative and yield parameters were increased by the application of *Azotobacter*, however in general, the chemicals components (quality and mineral components) were not affected, except only reducing nitrate content.

Table (3): Effect of mineral nitrogen, *Azotobacter* levels and their interactions on primary curd quality characters of Southern Star broccoli cultivar, during the two successive winter seasons of 2006-2007 and 2007-2008.

Treatments		Vitamin C (mg/100 g FW)		SSC %		Total Carbohydrates %		Free amino acids (mg/g FW)		Nitrate (mg/kg DW)	
N (kg/Fed.)	Cerea	2006-07	2007-08	2006-07	2007-08	2006-07	2007-08	2006-07	2007-08	2006-07	2007-08
0		70.49 b	90.28 a	-	6.02 a	11.98 ab	23.29 a	45.00 c	50.11 c	95.15 b	111.28 d
45		86.33 a	98.67 a	-	5.42 b	12.25 a	20.84 b	40.78 c	45.67 c	104.60 b	129.71 c
90		91.76 a	95.70 a	-	4.9 b	10.83 ab	19.44 b	79.81 b	84.00 b	93.67 b	196.52 b
120		89.29 a	91.76 a	-	5.11 b	9.51 b	19.10 b	96.76 a	104.86 a	146.09 a	238.24 a
	0	84.36 a	91.76 a	-	5.20 a	11.17 a	19.79 a	86.40 a	94.13 a	170.66 a	218.94 a
	2	82.84 a	94.35 a	-	5.47 a	10.09 a	21.38 a	52.00 b	55.50 b	93.52 b	163.57 b
	4	86.21 a	96.20 a	-	5.43 a	12.14 a	20.88 a	58.83 b	63.92 b	71.87 c	115.26 c
0	0	69.56 d	82.88 b	-	6.33 a	14.10 a	24.03 a	60.00 cd	63.33 bcde	156.40 b	120.97 e
	2	59.04 d	91.76 ab	-	5.63 abcd	9.60 ab	23.32 ab	36.33 e	42.33 de	73.34 cd	123.64 e
	4	82.88 bc	96.20 ab	-	6.10 ab	13.30 ab	22.14 abc	38.67 e	44.67 cde	55.70 d	89.22 fg
45	0	82.88 bc	100.62 a	-	5.13 bcde	13.41 ab	17.46 c	57.00 d	62.67 bcde	162.85 b	233.6 bc
	2	90.28 abc	93.24 ab	-	5.17 bcde	11.59 ab	22.13 abc	30.67 e	33.67e	79.65 cd	113.85 ef
	4	85.84 abc	102.12 a	-	5.97 abc	12.07 ab	21.80 abc	34.67 e	40.67de	71.29 cd	76.30 g
90	0	94.72 a	91.76 ab	-	4.33 e	9.27 ab	18.65 bc	88.22 b	92.67 b	158.26 b	252.90 ab
	2	93.24 ab	99.16 ab	-	5.40 abcde	10.24 ab	19.90 abc	69.67 bcd	75.67 bcd	80.84 cd	217.01 cd
	4	87.32 abc	96.20 ab	-	4.97 cde	13.25 ab	19.76 abc	77.33 bc	79.33 bcd	63.43 d	119.65 e
120	0	90.28 abc	91.76 ab	-	5.00 bcde	9.97 ab	18.23 c	121.78 a	137.11 a	200.98 a	273.18 a
	2	88.80 abc	93.24 ab	-	5.67 abcde	8.76 b	19.59 abc	71.33 bcd	70.33 bcd	140.24 b	217.89 cd
	4	88.80 abc	90.28 ab	-	4.67 e	9.93 ab	19.90 abc	84.67 b	91.00 b	97.05 c	206.18 d

Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple range test

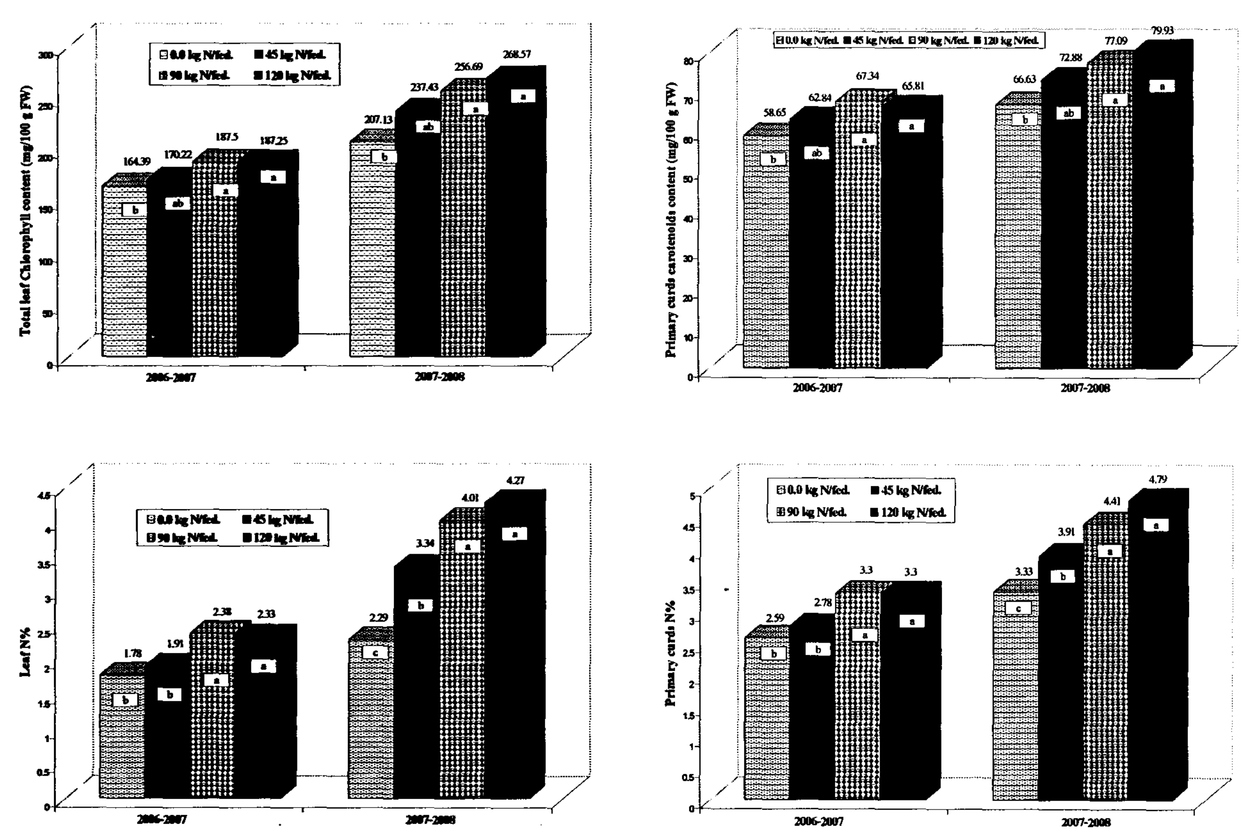

Figure (1): Effect of mineral nitrogen on leaf chlorophyll, curds' carotenoids, leaf N and curds' N contents of Southern Star broccoli cultivar, during the two successive winter seasons of 2006-2007 and 2007-2008.

Table (4): Analysis of variance summaries (p values) of data for mineral elements and pigment contents of Southern Star cultivar, during the two successive winter seasons of 2006-2007 and 2007-2008.

Source of variation		Mineral elements				Photosynthetic pigments	
		N%	P%	K%	Ca%	Total Chlorophyll (mg/100 g FW)	Carotenoid (mg/100 G FW)
Leaves							
2006-2007	N	0.01*	0.41 ns	0.59 ns	0.15 ns	0.033*	0.21 ns
	Cerea	0.062 ns	0.33 ns	0.25 ns	0.50 ns	0.47 ns	0.89 ns
	N*Cerea	0.34 ns	0.63 ns	0.77 ns	0.45 ns	0.89 ns	0.97 ns
2007-2008	N	0.000***	0.18 ns	0.18 ns	0.000**	0.01*	0.38 ns
	Cerea	0.76 ns	0.94 ns	0.34 ns	0.79 ns	0.76 ns	0.49 ns
	N*Cerea	0.56 ns	0.41 ns	0.77 ns	0.74 ns	0.72 ns	0.91 ns
Primary Curds							
2006-2007	N	0.002**	0.18 ns	0.004**	0.59 ns	0.07 ns	0.045*
	Cerea	0.68 ns	0.94 ns	0.37 ns	0.52 ns	0.79 ns	0.92 ns
	N*Cerea	0.63 ns	0.41 ns	0.40 ns	0.20 ns	0.37 ns	0.48 ns
2007-2008	N	0.000***	0.03*	0.12 ns	0.25 ns	0.00***	0.003**
	Cerea	0.79 ns	0.66 ns	0.16 ns	0.18 ns	0.031*	0.074 ns
	N*Cerea	0.74 ns	0.35 ns	0.60 ns	0.77 ns	0.36 ns	0.71 ns

Ns, *, **, *** Nonsignificant, significant at 5%, 1%, or 0.1% levels, respectively.

CONCLUSION AND RECOMMENDATION

The obtained results indicated generally that combination of mineral nitrogen fertilization level at 90 kg/fed. with inoculation of broccoli plants with *Azotobacter* at 4 kg/fed might be considered as an economical yield treatment for the production of high yield of broccoli and consequently, raising net profit for farmers under the prevailing environmental conditions of Ismailia Governorate and other similar regions. Also, results showed that the florets of broccoli plants inoculated with *Azotobacter* had the lowest nitrate content which considered as a safety foods.

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تأثير الأزوتوباكتر و مستويات النيتروجين على نمو و محصول و جودة البروكلى

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نفذت تجربتان حقليتان على محصول البروكولى (صنف سوزرن ستار) في مزرعة كلية الزراعة جامعة قناة السويس بالإسماعيلية خلال موسمي الزراعة ٢٠٠٦-٢٠٠٧ ، ٢٠٠٧-٢٠٠٨ بهدف دراسة تأثير المعاملات المختلفة من التسميد النيتروجينى المعدني والحيوي وكذلك التفاعل بينهم على النمو الخضري والمحصول ومكوناتة وكذا مكونات الجودة بالإضافة لصبغات التمثيل والعناصر المعدنية.

أحتوت الدراسة على ١٢ معاملة عبارة عن اتحاد أربع مستويات من التسميد النيتروجينى (نترات النشادر ٣٣,٥ %) وهى صفر، ٤٥، ٩٠ و ١٢٠ كجم ن/فدان مع ثلاث مستويات من التسميد الحيوي الازوتى بأستخدام الازوتوباكتر وهى صفر، ٢ و ٤ كجم/للفدان. وقد وزعت المعاملات في قطع منشقة مرة واحدة في ثلاث مكررات. ويمكن تلخيص النتائج المتحصل عليها فيما يلي:

زيادة معدلات التسميد النيتروجينى حتى ٩٠ كجم ن/فدان أدت إلى حدوث زيادة معنوية في كل النموات الخضرية المدروسة والمحصول وكذلك المحتوى النيتروجينى للأوراق والرؤوس. في نفس الوقت وجد أن أعلى قيم لفيتامين ج ، الكاروتينيدات والكلوروفيل ظهرت عند التسميد النيتروجينى بمستوى ٤٥ كجم ن/فدان. في حين أن أعلى مستوى من التسميد النيتروجينى أعطى أعلى قيم من الأحماض الامينية والنترات.

أدى تلقيح نباتات البروكولى بالسماد الحيوي إلى زيادة معنوية في معظم الصفات الخضرية والمحصول بالإضافة إلى انخفاض معنوي في محتوى رؤوس البروكولى من النترات ولم تتأثر الصفات المدروسة الأخرى بتلقيح النباتات بالسماد الحيوي. أوضحت النتائج أيضا وجود تفاعل معنوي بين التسميد المعدني والحيوي فيما يتعلق بالنمو الخضري والمحصول. وبناء علية يمكن القول أن استخدام التسميد الحيوي يقلل من تلوث البيئة ويزيد من صافى ربح المزارع بالإضافة إلى إنتاج محصول آمن لصحة الإنسان.