PARTIAL SUBSTITUTION OF MINERAL NITROGEN FERTILIZERS BY BIO-FERTILIZER TO ALLEVIATE THE POSSIBLE RISKS OF CHEMICAL POLLUTION FOR BROCCOLI PLANTS

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A field experiment was conducted on a clayey soil at Sonnuris district, El-Fayoum Governorate, Egypt during the two successive seasons of 2010-2011 and 2011-2012 to evaluate the response of vegetative growth, nutritional status and yield of broccoli (*Brassica oleracea L.* var. *Italica*) as well as head quality to a partial substitution of 50% and 25% N-mineral from the recommended fertilizer rate by the N-bio-fertilizer, *Azotobacter chroococcum* (AT) and *Azospirillium brasilense* (AZ) [1:1] at one or two or three doses as compared with applying 100 % recommended dose of N-mineral fertilizer (120 kg N fed^{-1}), hopping an alleviation of the possible reduces of chemical pollution for broccoli yield and environmental risks.

Data of the studied plant characters indicate that the greatest vegetative growth parameters of broccoli (*i.e.,* leaf area $plant^{-1}$, number of leaves $plant^{-1}$, dry weight of leaves $plant^{-1}$, dry weight of stem $plant^{-1}$, leaf sugar and leaf nutritional status) were achieved by plants supplied with the combined treatment of 75 % recommended dose of N-mineral fertilizer + three doses of (AT+AZ). Such favourable conditions were reflected positively on the followed growth stages and gave the greatest total yield fed^{-1} (*i.e.,* central head weight and lateral head weight), as well as better quality parameters of broccoli (*i.e.,* central head weight, lateral head weight, number of lateral heads $plant^{-1}$ and sugar head) during both two studied seasons. Such beneficial conditions were more attributed with the optimum soil case of the current experiment because of the renewed activity of bacteria in the soil by adding the three doses of (AT+AZ), which was achieved by ameliorated values of soil nutrients status.

Superiority of the applied N-mineral in combination with bio-fertilizer (AT+AZ) was mainly attributed to the later due to its ability to 1). Release some plant promoting substances, mainly IAA, gibberellic acid and cytokinin like substances which stimulate plant growth. 2) Increasing the ability to convert N_2 to NH_4 and thus make it available to plants. 3) Beneficial effects of (AT+AZ) bacteria on reducing soil pH by secreting organic acids (e.g. acetic, propionic, fumaric and succinic) and maintaining a suitable air-moisture regime, that finally leading to improve vegetative growth, chemical constituents and higher yield of broccoli with better quality heads. Moreover, the periodical application of such N-bio-fertilizer is considered the best option not only for reducing the harmful effects of using chemical fertilizers, but also for sustaining soil fertility status and help to alleviate the possible risks of environmental pollution on human health.

Key words: Bio-fertilization, N-mineral fertilizer, clayey soil, vegetative growth, yield and quality of broccoli.

INTRODUCTION

Humankind, particularly in the developed countries of World, faces a great problem either in the human health or in the environmental pollution due to the excessive uses of mineral or chemical fertilizers, especially those of nitrogenous ones. Interest in the N-excessive use, it could be partially attributed to the advent of high yielding crop cultivars under assured perennial irrigation. So that, there is a renewed interest in bio-fertilizer to nutrients supply as well as to improve soil fertility status and its productivity. Moreover, the integrated use of bio-fertilizer and mineral fertilizers is considered the best option not only for reducing the previous enormous consumption of chemical fertilizers, but also for sustaining soil fertility status and help to maximize fertilizer use efficiency in soil **(Singh *et al.,* 1999; Bhatia *et al.,* 2001 and Palm *et al.,* 2001)**. Thus, the way of clean agriculture with a minimum pollution should be include a conjunctive use of bio-fertilizer and N-mineral fertilizers.

Biofertilizers, microbial inoculants that can promote plant growth and productivity, are internationally accepted as an alternative source of N-fertilizer. They are environmentally friendly and can be used to ensure a sustainable cereal production. In the biofertilizer technology, new systems are being developed to increase the biological N_2 fixation with cereals and other nonlegumes by establishing N_2-fixing bacteria within the roots **(Cocking, 2000).**

The mechanisms by which bio-fertilizers can exert a positive effect on plant growth can be through the synthesis of phytohormones, N_2 fixation, reduction of membrane potential of the root, synthesis of some enzymes (such as ACC deaminase) that modulate the level of plant hormones. Free living nitrogen fixing bacteria such as Azotobacter and Azospirillum which have the ability not only to fix nitrogen but also to release certain phytohormons of GA3 and IAA nature which could stimulate plant growth, absorption of nutrients and photosynthesis process **(Fayez *et al.,* 1985 and Abdel-Latif *et al.,* 2001)**.

Several reports indicated that the inoculation of some plants with biofertilizers alone or in combination with mineral fertilizers improved plant growth, yield and chemical composition (**Abdel-Mouty *et al.*, 2002; Gadallah and El-Masry (2006) and Osman (2008).** The favourable soil conditions, which are associated with the applied bio-fertilizers, are ascribed by many investigators such as **Ibrahim and Abd El-Aziz, (1977)** explained the importance of biofertilizers in terms of reducing soil pH by secreting organic acids(e.g. acetic, propionic, fumaric and succinic), which bring about the dissolution of some bound nutrients and make them available for plants.

Inoculating potato tuber seeds with Nitrogen (bio-fertilizer) significantly increased total yield and average tuber weight plant^{-1} (**Ashour *et al.*, 1997**). **Osman (2008)** found that the favourable effect of bio-fertilizer treatments could be attributed to its enhancing effect on plant growth characters which could be reflected on potato yield and its components and inoculating potato tuber seeds with *Azotobacter chroococcum* [AT] + *Azospirillium brasilense* [AZ] significantly increased average yield plant^{-1}, total yield fed.$^{-1}$ and percentage of large and medium tuber size grades, while increasing level of (AT+AZ) decreased small sized tubers. **Hanafy *et al.*, (1997)** suggested that the addition of bio-fertilizers increases the ability to convert N_2 to NH_4 and thus make it available to plant. Also, many investigators showed that inoculation of bio-fertilizers increased N concentration in onion **(Gadallah and El-Masry, 2006).** Also, **Hanafy *et al.*, (1997)** mentioned that using *Azotobacters* increased root surface, root hairs and root elongation. Thus, factors caused by application of bio-fertilizers could improve P uptake on onion plant. With regard to K concentration, a significant increase of K concentration was obtained from combination between bio-fertilizer with the treatments of mineral-N fertilizer as compared to the treatment which only received mineral-N fertilizer in both seasons.

Recently, more attentions extending towards the devoted cultivated areas and increasing the production of some untraditional vegetable crops including broccoli, through the pathway of nutrition, for local consumption and early exportation to European countries, have been directed. So, the current work aimed at evaluating the partially N-mineral substitution by an alternative N-source supplied from some bio-fertilizers (*Azotobacter chroococcum* and *Azospirillium brasilense*) to achieve the new approaches of clean, bio-agriculture on maximizing the productivity and head quality of a newly vegetable crop such as Broccoli (*Brassica oleracea L.*var. *italica*) belongs to family Brassicacea and considers a number of cole vegetable crops; which includes cabbage, cauliflower, chinese cabbage, broccoli, brussels sprouts and kohlrabi. It is well known that, broccoli has enormous nutritional and medicinal values due to its high contents of vitamins (A, B1, B2, B5, B6 and E), besides minerals (Ca, Mg, Zn and Fe) and number of health supporting antioxidant substances **(Rozek and Wojciechowska, 2005 and Wojciechowska *et al.,* 2005)**, which prevent the formation of cancer causing agents **(Beecher, 1994)**. So that, it is widely cultivated in many European and American countries but in Egypt, broccoli still a grown in a very limited scattered areas and the total cultivated area is not exactly known **(Abou El-Magd *et al*., 2006)**.

MATERIALS AND MMETODS

A field experiment was carried out during the two successive seasons of 2010-2011 and 2011-2012 at a private farm in Sonnuris district, El-Fayoum Governorate, Egypt, characterized by clayey soil, to evaluate the response of vegetative growth and yield of broccoli (*Brassica oleracea* L. var. *italica*) as well as head quality to a partial substitution of 50 and 25 % N-mineral by an alternative N-source supplied from some bio-fertilizers (*Azotobacter chroococcum* and *Azospirillium brasilense* [1:1] (AT+AZ) at one or two or three doses) as compared with the 100% recommended dose of N-mineral fertilizer (120kg N fed^{-1}).

Disturbed and undisturbed soil samples were collected from the initial state of the experimental soil at the depth of 0-35cm for determining the main soil characteristics. The obtained data of the studied soil properties and nutrients status are presented in Table (1).

Experimental treatments were:

100 % of N-mineral fertilizer as a control treatment (120 kg N fed^{-1}, recommended N-mineral fertilizer for broccoli under El Fayoum Governorate conditions, **Tolba, 2005**) as ammonium nitrate.

50% of N-mineral fertilizer (60 kg N fed^{-1})	+ one dose of (AT+AZ). + two doses of (AT+AZ). + three doses of (AT+AZ).
75% of N-mineral fertilizer (90 kg N fed^{-1})	+ one dose of (AT+AZ). + two doses of (AT+AZ). + three doses of (AT+AZ).

Preparation of inocula

Modified Ashby's medium (**Hegazi and Niemela, 1976**) was used to grow the *Azotobacter chroococcum* and Dobereiner medium for *Azospirillium brasilense* (**Dobereiner *et al.,* 1976**). The strains (*A. chroococcum* FN 33 and *A. brasilense* FN 17) were isolated and identified in the microbiology laboratory, Faculty of Agriculture, Fayoum University from the soil in which the experiments were performed. Isolates and inoculates were prepared immediately before inoculation. At the logarithmic growth phase, the cultures were centrifuged at 1000 rpm and the cell pellets were washed three times with sterile phosphate buffer (100 mM, pH=7.0). The washed cells were resuspended in the same buffer to the final concentration of about $4x10^8$ cfu/ml.

Table (1) : The main soil characteristics of the experimental field.

Soil characteristics	Value	Soil characteristics	Value
Particle size distribution %:		*Soluble cations (soil paste, meq/l)*:	
Coarse sand	5.8	Ca^{2+}	14.8
Fine sand	23.3	Mg^{2+}	3.4
Silt	21.6	Na^{+}	10.9
Clay	49.3	K^{+}	0.8
Textural class	Clay		
Soil physical properties:		*Soluble anoions (soil paste, meq/l)*:	
Bulk density g cm^{-2}	1.3	CO_3^{2-}	0.00
Total porosity %	52.1	HCO_3^{-}	2.2
Available water %	17.9	Cl^{-}	15.6
Hydraulic conductivity cm h^{-1}	1.7	SO_4^{2-}	12.1
Soil structure factor %	79.5		
Soil chemical properties:		*Available macro and micronutrients (mg/kg)*:	
pH (1.25 soil water suspension)	7.9	N	81.3
$CaCO_3$ %	4.5	P	10.5
Gypsum %	0.7	K	476.9
Organic matter %	1.7	Fe	8.7
ESP	7.1	Mn	3.6
ECe (dS/m, soil paste extract).	2.9	Zn	0.8
		Cu	0.6

Critical levels of the studied available plant nutrients (mg/kg), after Lindsay and Norvell (1978)

Nutrient	N	P	K	Fe	Mn	Zn
Low	< 40.0	< 5.0	< 85.0	< 4.0	< 1.0	< 0.5
Medium	40.0-80.0	5.0-10.0	85.0-170.0	4.0-6.0	1.0	0.5-1.0
High	> 80.0	> 10.0	> 170.0	> 6.0	> 1.0	> 1.0

The Inoculation of Bio-fertilizer:

a. Seedlings inoculation

The roots of seedlings were dipping of *Azotobacter chroococcum* FN 33 and *Azospirillium brasilense* FN 17 mixed in equal quantities (1:1).

b. Injection of bio-fertilizer in soil:

A soil injection beside plant holes one or two times, i.e., four and seven weeks after transplanting, with a rate of 30 – 40 mL $hole^{-1}$ for each.

As for the applied N-mineral fertilizer, 30, 22.5 and 15 kg N /fed (ammonium nitrate, 33.5 % N) was broadcasted and incorporated during the soil preparation in cases of 100 %, 75 % and 50% N-mineral fertilizer, respectively while, after 3 and 6 weeks from transplanting; one third and two third of the remainder quantities of N-mineral fertilizer consecutively were side banded. Also, calcium superphosphate (15.5 % P_2O_5), as a source of phosphorus, was broadcasted and incorporated at a uniform dose during soil preparation at a rate of 31 kg P_2O_5/fed. Potassium sulphate (48 % K_2O), as a respective K source, was broadcasted and incorporated during the soil preparation at a rate of 12 kg K_2O/fed, then one third and two third of the remainder quantities of K_2O fertilizers consecutively were side banded after 3 and 6 weeks from transplanting.

The different treatments were arranged in complete randomized blocks design with four replications. Seeds of broccoli (*Brassica oleracea* L. var. *italica*) by Battistini Sementi Company, Italia were drilled in foam trays of 209 holes in a media consisting on peat moss and vermiculite 1:1 on September 14, 2010 and September 11, 2011. After 35 days seeds drilling, trays were brought as well as transplants were planted on rows of 6 m in length and 0.60 m in width as well as interplant spacing was 40 cm along each row (about 16000 plants/fed). Each plot was planned to consist of 5 rows (the experimental plot area was 18 m^2) and every two plots were separated from each other with one row. The irrigation water was applied through a furrow irrigation system and all other recommended agro-managements required for broccoli production as irrigation, cultivation and protection against pests and diseases were practiced whenever it was necessary.

Recorded Data:

The observations on vegetative growth features were taken using five randomly selected plants from the first row, in each experimental unit at nine weeks after transplanting. The aerial parts of the chosen plants were cut off at the ground level and sub-divided into leaves and stem. The following vegetative parameters were recorded: number of leaves $plant^{-1}$, leaf area $plant^{-1}$ (using leaf area-leaf weight relationship from leaf disks obtained by a cork borer **Wallacce and Munger, 1965**), dry weight of leaves and stem $plant^{-1}$ by drying in a forced–air oven at 70 ^{o}C till the weight became constant.

In each experimental plot, plants of the two middle rows were allocated to record observations on total head yield and its components. At harvest, total yield in kg/fed (*i.e.*, central and lateral heads having closed flowers buds, dark green colour and good compactness), and stalks, then were trimmed. The following parameters were considered: central head weight $plant^{-1}$, number and weight of lateral heads $plant^{-1}$ and total central and lateral heads yield fed^{-1}.

A plant sample consists of five plants was randomly chosen from the fourth row, in each experimental unit, for chemical determinations. At sampling time, 70 days after transplanting, plant samples were cut off at the ground level, sub-divided into leaves and heads. Total N in leaves and heads was estimated using the Microkjeldahal apparatus as described in **A.O.A.C. (1995)**, phosphorus was determined using spectrophotometer apparatus according to **(Page *et al.,* 1982)**, potassium was determined using Flame photometer (Perkin-Elmer, model 52) with acetylene burner as outlined by **(Page *et al.,* 1982)**, Fe, Mn, Zn and Cu (Inductively Coupled Plasma Spectrometry instrument, Plasma JY Ultima) and leaf or head sugar **(A.O.A.C., 1995).**

Also, disturbed and undisturbed soil samples were collected from the treated experimental plots at 70 days after transplanting to monitor the changes in soil physico-chemical properties and the nutrients status, *i.e.,* soil bulk density, total porosity **(Black and Hartge, 1986)**, structure factor, hydraulic conductivity, available water range **(Klute, 1986)**, soil organic matter (Walkely and Black method), pH, ECe and ESP **(Page *et al.,* 1982)**. Available macronutrients of N, P and K were extracted by 1 % potassium sulphate, 0.5 M sodium bicarbonate and 1 N ammonium acetate, respectively **(Soltanpour and Schwab, 1977)** and their contents in soil were determined according to **Page *et al.,* (1982)**. Available micronutrients of Fe, Mn, Zn, and Cu in soil were extracted using ammonium bicarbonate-DTPA extract according to **Soltanpour and Schwab (1977)**, and their contents in soil were measured by using Inductively Coupled Plasma Spectrometry instrument (Plasma JY Ultima).

Statistical analysis:

All data of the two seasons were subjected to the statistical analysis according to **Snedecor and Cochran (1980)** to define the least significant difference test (L.S.D. at p = 0.05 level), which was used to verify the differences between the tested treatments.

RESULTS AND DISCUSSION

The results obtained of particle size distribution, Table (1), reveal that the studied soil is characterized by fine texture (clayey), and it attains low content of $CaCO_3$ and very low contents of gypsum and organic matter. The later may be ascribed to the low accumulated plant residues and the prevailing hot and arid climatic conditions. Also, the studied soil has relatively low values of sodicity (*i.e.,* ESP, non-alkali soil), soil pH tended to slight alkaline, ECe less than 4 dSm^{-1} and ESP, which led to classified the studied soil as non-saline and non-alkaline. Such results are emphasized by the positive effects of the progressive increments of soluble Ca^{2+} + Mg^{2+} which surpassed the soluble content of Na^{-} that reflected the signs of better soil aggregation (structure factor), bulk density, total porosity and available water range. On the other hand, the studied soil was suffering from micronutrients deficient, however, the available contents of Mn, Zn and Cu are found in inadequate amounts to the sufficient levels for plant.

I. Effect of applied bio-fertilizer combined with mineral fertilizer on Soil content of some available nutrients:

The effects of N-mineral fertilizer added as either solely or combined with bio-fertilizer (*Azotobacter chroococcum* and *Azospirillium brasilense*) to the experimental soil plots under cultivation with broccoli, furrow irrigation and efficient drainage systems, caused a pronounced ameliorated effect in each of the studied soil content of some available macronutrients (*i.e.,* N, P and K mg/kg) and micronutrients (*i.e.,* Fe, Mn, Zn and Cu mg/kg) as shown in Table (3). The data showed that a progressive significant increases in all the studied available macronutrients and micronutrients nutrients upon treating the soil with bio-fertilizer (*Azotobacter chroococcum* and *Azospirillium brasilense*) in combination with N-mineral fertilizer, particularly at the applied 75% N-mineral/fed + Three doses of (AT+AZ) as compared to the treatment of 100% N-mineral fertilizer. These findings were similar and true in both the studied two seasons of study.

The superiority of applied 75% N-mineral+ three doses of (AT+AZ)/fed is mainly attributed to its ability to release some plant promoting substances, mainly Indolic Acetic Acid (IAA), gibberellic acid and cytokinin like substances which stimulate plant growth **(Gomaa and Abou-Aly, 2001)**, beside the beneficial effects of (AT+AZ) bacteria on reducing soil pH by secreting organic acids (e.g. acetic, propionic, fumaric and succinic) which leading to change of nutrients to available forms ready for uptake by plants **(Singh and Kapoor, 1999)**. Also, the latter conditions led to enhance the microbial activity in soil, which accelerate the decomposition of organic matter and maximize soil content of nutrients, especially for those of micronutrient deficient in the soil. The present results are in agreement with those of **Shahaby (1997**) who found that nitrogen fixation bacteria increased total nitrogen content in the Nile valley and reclaimed soil.

Table (3): Influence of bio-and N-mineral fertilization on nutrients status in the studied soils in both seasons of 2010-2011 and 2011-2012.

Treatments		Macronutrients (mg/kg)			Micronutrients (mg/kg)			
		N	P	K	Fe	Mn	Zn	Cu
N-mineral fed^{-1}	**Number of doses (AT+AZ)**	**Season 2010-2011**						
100% (120 kg N)		105.45	11.05	498.12	9.10	4.21	0.98	0.83
50% (60 kg N)	one	65.47	6.85	308.83	5.64	2.61	0.61	0.51
	two	68.54	7.18	323.78	5.92	2.74	0.64	0.54
	three	79.09	8.29	**373.59**	6.83	3.16	0.74	0.62
75% (90 kg N)	one	86.47	9.28	418.42	7.64	3.54	0.82	0.70
	two	99.12	10.39	468.23	8.55	3.96	0.92	0.78
	three	108.61	11.38	513.06	9.37	4.34	1.01	0.85
L.S.D. at 0.05		**4.33**	**0.62**	**18.31**	**0.27**	**0.13**	**0.04**	**0.03**
		Season 2011-2012						
100% (120 kg N)		106.79	11.37	501.96	9.28	4.29	1.01	0.85
50% (60 kg N)	one	66.20	6.96	316.23	5.84	2.70	0.64	0.54

	two	70.48	7.29	331.29	6.12	2.83	0.67	0.56
	three	81.16	8.62	391.53	7.24	3.34	0.79	0.66
75% (90 kg N)	one	89.70	9.55	436.71	8.07	3.73	0.90	0.74
	two	101.45	10.61	481.88	8.91	4.11	0.97	0.82
	three	111.06	11.66	529.57	9.79	4.53	1.07	0.90
L.S.D. at 0.05		**4.67**	**0.59**	**20.11**	**0.24**	**0.11**	**0.05**	**0.04**

(AT+AZ): The mixture of *Azotobacter chroococcum* (AT) and *Azospirillium brasilense* (AZ) [1:1]

II. Response of vegetative growth, yield and quality of broccoli:

a) Vegetative growth characters:

Irrespective of N-mineral treatments, data shown in Table (4) reveal that there were significant gradual increases in leaf area plant^{-1}, number of leaves plant^{-1}, dry weight of leaves plant^{-1} and dry weight of stem plant^{-1} with the increase in doses of *Azotobacter chroococcum* and *Azospirillium brasilense* (AT + AZ) application from one up to three. These results are in agreement with those obtained by **Osman (2008)** who found that bacteria inoculation separately or combined with chemical fertilizers significantly improved growth characters and increased plant height and Biomass fresh weight plant^{-1} of potato compared to the untreated plants. In addition, the greatest values of the studied vegetative growth characters of broccoli plants were achieved by plants supplied with 75% recommended of N-mineral fertilizer (90kg N fed^{-1}) + three doses of (AT+AZ). Meanwhile, the lowest values of vegetative growth characters were recorded at the treatment of 50 % recommended of N-mineral fertilizer (60kg N fed^{-1}) + one dose of (AT+AZ). These findings were similar and true in both the studied two seasons of study.It was also noted that, at the same level of N-mineral fertilizer an increase in the attributes mentioned above an increase of doses of (AT+AZ), and this may be due to increased activity and efficiency of bacteria in the soil.

The differences between both 100% N-mineral fertilizer fed^{-1} and 75% N-mineral fertilizer fed^{-1} + three doses of (AT+AZ) were insignificant for all growth traits under study.

The vigor of broccoli plants growth supplied with 75% N-mineral fertilizer fed^{-1} + three doses of (AT+AZ) might be due to the more accompanied easily available essential macro and micronutrients because of its beneficial effects of (AT+AZ) bacteria on reducing soil pH by secreting organic acids (e.g. acetic, propionic, fumaric and succinic) **(Singh and Kapoor, 1999)**.

Moreover, bio-fertilizers can exert a positive effect on plant growth can be through the synthesis of phytohormones, N_2 fixation, reduction of membrane potential of the root, synthesis of some enzymes such as Amino-cyclopropane -1- Carboxylic Acid (ACC deaminase) that modulate the level of plant hormones. The noticeable increases of morphological parameters of broccoli plants by increasing the applied rates of bio-fertilizers may be confirmed by the progressively increases of the nutritional elements in rooting zone, and consequently the absorption of more nutrients **(Fayez *et al.,* 1985 and Abdel-Latif *et al.,* 2001)**.

On the other hand, these results may be due to the free living nitrogen fixing bacteria such as *Azotobacter* and *Azospirillum* have the ability not only to fix nitrogen but also to produce adequate amounts of certain phytohormons of Giberellic Acid (GA_3), Indolic Acetic Acid (IAA) and cytokinins nature which could stimulate plant growth with increasing the surface area per unit root length and enhanced the root hair branching with an eventual increase on the uptake of nutrients from the soil **(s Fayez *et al.,* 1985 and Abdel-Latif *et al.,* 2001).** The positively response of applied bio-fertilizers were studied by many authors such as **(Gomaa and Abou-Aly, 2001), (Gadallah and El-Masry, 2006) and (Osman, 2008).**

From the economical point of view, the superiority over than the other treatments was great enough to reach the level of significance, so that the treatment of 75% N-mineral/fed + Three doses of (AT+AZ) is considered the better one. These results held well in the two successive experimental seasons as well as were coincided with those reported **by Ibrahim and Abdel-Razik (1999).**

Table (4): Influence of bio-and N-mineral fertilization on some growth characters of broccoli plants in both seasons of 2010-2011 and 2011-2012.

Treatments		Leaf area plant^{-1} (cm^2)	Number of leaves plant $^{-1}$	Dry weight of leaves plant^{-1} (g)	Dry weight of stem plant^{-1} (g)
N-mineral fed^{-1}	**Number of doses (AT+AZ)**	**Season 2010-2011**			
100% (120 kg N)		6737	37.50	58.66	57.36
50% (60 kg N)	one	4131	23.02	35.96	35.17
	two	4399	24.48	38.34	37.46
	three	5054	28.14	43.99	43.02
75% (90 kg N)	one	5683	31.65	49.45	48.37
	two	6333	35.24	55.15	53.93
	three	6870	38.24	59.83	58.49
L.S.D. at 0.05		**177**	**1.45**	**2.01**	**1.95**
		Season 2011-2012			
100% (120 kg N)		6851	38.67	60.13	59.86
50% (60 kg N)	one	4201	23.69	36.90	36.72
	two	4451	24.76	39.28	39.10
	three	5144	29.04	44.81	44.53
75% (90 kg N)	one	5776	32.59	50.28	50.01
	two	6452	35.71	56.61	56.36
	three	6963	37.04	60.66	60.60
L.S.D. at 0.05		**201**	**1.67**	**1.99**	**2.02**

(AT+AZ): The mixture of *Azotobacter chroococcum* (AT) and *Azospirillium brasilense* (AZ) [1:1]

The sequence of the superiority for the applied treatments under the current experimental conditions could be arranged into an ascending order of 75% N-mineral/fed + Three doses of (AT+AZ) >100 % of N-mineral fertilizer as a control treatment > 75% N-mineral/fed + Two doses of (AT+AZ) >75 % N-mineral/fed + One dose of (AT+AZ) >50% N-mineral/fed + Three doses of (AT+AZ) >50% N-mineral/fed. + Two doses of (AT+AZ) > 50 % N-mineral/fed + One dose of (AT+AZ).

b) Nutrient and sugar contents in broccoli leaves:

Results in Table (5) show that there were significant differences in the nutrient (*i.e.,* N, P, K, Fe, Mn, Zn and Cu) and sugar contents of broccoli leaves at a period of 70 days after transplanting by using different doses of (AT +AZ) treatments in the two seasons of study. However, there was an increase in each of the studied nutrients with increasing the applied rates of N-mineral, and the same level of N-mineral fertilizer an increase in the studied nutrient and sugar contents an increase of doses of (AT + AZ).

Accordingly, the greatest values of the studied nutrient and sugar contents in tissues of broccoli leaves were produced by applying 75% N-mineral/fed combined with bio-fertilizer at three doses in the two successive seasons of study. These findings emphasized that the later treatment was great enough to reach the level of significance, so it is considered a better one from the economical point of view. On the contrary, the lowest leaf nutrient contents were 50 % N-mineral/fed + One dose of (AT+AZ), in the two seasons.

Concerning the superiority in elemental values in tissues of broccoli by increasing the bio-fertilizer, may be attributed to the more accompanied easily available essential macro and micronutrients because of its beneficial effects of (AT+AZ) bacteria on reducing soil pH by secreting organic acids (e.g. acetic, propionic, fumaric and succinic) and maintaining a suitable air-moisture regime. Also, the positive effect of *Azospirillum*-inoculated plants exhibited a higher foliar N content besides enhanced P, K and Fe content in marigold

(Balasubramanian, 1989), P, N, Fe, Cu, Zn, and Mn content in coffee **(Premkumari and Balasubramanian, 1993).**

Table (5): Influence of bio-and N-mineral fertilization on some nutrients and sugar contents of broccoli plants in both seasons of 2010-2011 and 2011-2012.

Treatments		Macronutrients %			Micronutrients (mg kg^{-1})				Sugar %
		N	P	K	Fe	Mn	Zn	Cu	
N-mineral fed^{-1}	**Number of doses (AT+AZ)**	**Season 2010-2011**							
100% (120 kg N)		3.55	0.352	2.23	110.30	71.74	37.91	11.70	6.16
50% (60 kg N)	one	2.18	0.216	1.37	68.39	44.48	23.50	7.25	3.78
	two	2.32	0.230	1.46	72.80	47.35	25.02	7.72	4.03
	three	2.66	0.264	1.67	82.73	53.81	28.43	8.78	4.62
75% (90 kg N)	one	3.00	0.297	1.88	96.18	62.56	33.05	10.20	5.20
	two	3.34	0.331	2.10	103.68	67.43	35.64	11.00	5.79
	three	3.63	0.359	2.27	110.51	72.17	37.67	11.73	6.29
L.S.D. at 0.05		**0.26**	**0.026**	**0.11**	**5.13**	**3.42**	**1.38**	**0.44**	**0.55**
		Season 2011-2012							
100% (120 kg N)		3.54	0.355	2.24	111.7	73.13	38.70	12.02	6.20
50% (60 kg N)	one	2.17	0.217	1.37	69.25	45.34	24.00	7.45	3.80
	two	2.32	0.232	1.46	73.72	48.27	25.54	7.93	4.05
	three	2.65	0.266	1.68	83.78	54.85	29.03	9.01	4.65
75% (90 kg N)	one	2.97	0.297	1.89	97.40	63.77	33.75	10.48	5.23
	two	3.31	0.333	2.11	105.00	68.74	36.38	11.30	5.81
	three	3.55	0.362	2.26	111.93	72.89	38.67	12.11	6.33
L.S.D. at 0.05		**0.24**	**0.30**	**0.09**	**5.09**	**3.30**	**1.49**	**0.39**	**0.52**

(AT+AZ): The mixture of *Azotobacter chroococcum* (AT) and *Azospirillium brasilense* (AZ) [1:1]

In addition, the nutrients uptake are favourably influenced by *Azospirillum* inoculation. *Azospirillum* has the ability for better root induction in the inoculated plants mainly due to the production of growth hormones.

As a result, such plants are capable of absorbing more and more available nutrients from the soil which, in turn, result in better establishment of seedlings and their subsequent growth **(Govindan and Purushothaman, 1984)**. Similar observations were made by **Hemavathi (1997)** in chrysanthemum and **Shubha (2006)** in marigold. Also, *Azospirillum* have the ability not only to fix nitrogen but also to produce adequate amounts of certain phytohormons of Indolic Acetic Acid (IAA) which producing Plant Growth-Promoting Rhizobacteria (PGPR) strains are believed to increase root growth and length resulting in greater root surface area which enables plants to access more nutrients from soil **(Vessey, 2003).** Application of PGPR in apricot increased the nutrient element composition of leaves *viz*., N, P, K, Ca and Mg **(Esitken *et al.*, 2003).** Several reports indicated that the inoculation of some plants with biofertilizers alone or in combination with mineral fertilizers improved plant growth, yield and chemical composition **Abdel-Mouty *et al.*, 2002; Gadallah and El-Masry (2006)** and **Osman (2008).**

c) Broccoli yield and its quality:

Results illustrated in Table (6) show that there were significant differences in the total yield of broccoli in kg fed^{-1} (*i.e.,* central heads weight and lateral heads weight fed^{-1}) as well as quality parameters of broccoli (*i.e.,* central head weight, lateral heads weight and number of lateral heads plant^{-1}) as affected by increasing the applied doses of bio-fertilizer, which are actually combined with N-mineral fertilizer, in the two seasons of study.

The greatest total yield of broccoli heads and its quality were produced by plants supplied by bio-fertilizer at three doses in combination with 75 % N-mineral fertilizer in the two successive seasons of study.On the contrary, the relatively low total yield of broccoli heads was produced by pants received one dose of (AT+AZ) fed^{-1} in combination with 50% N-mineral fertilizer, these findings held good in both experimental seasons. This was true, since the favourable conditions of soil nutrients status as a result of the positive effect of both doses number of bio-fertilizer in combination with mineral fertilizer on yield and its components may be due to increasing the activity, efficiency and availability of macro and micronutrients in rooting zone and consequently, the absorption of more nutrients which resulted a significant increase in leaf and stem dry weight plant^{-1}, number of leaves and Leaf area plant^{-1}. Thus these increments may be led to the favoured increase in yield and its components(**Abdel-Mouty *et al.*, 2002; Gadallah and El-Masry (2006) and Osman (2008).**

In addition, these results may be due to the microorganisms produce growth promoting substances resulting in more efficient absorption of nutrients, which main components of photosynthetic pigments and consequently the chlorophyll content as well as N, P and K percentages were increased **(Gomaa and Abou-Aly, 2001).** Also, the N2-fixing bacteria (*Azospirillum*) produced adequate amounts of IAA and cytokinins with increasing the surface area per unit root length and enhanced the root hair branching with an eventual increase on the uptake of nutrients from the soil **(Rodriguez and Fraga, 1999).**

On the other hand, the increase in total yield of broccoli heads and its quality may be explained by the role of *Azospirillum* though atmospheric nitrogen fixation, better root proliferation, uptake of nutrients and water, higher leaf number. Higher photosynthesis enhanced food accumulation which might have resulted in better plant growth and subsequently higher number of lateral heads plant^{-1} and hence yield plant^{-1}. Besides this, increase in of lateral heads plant^{-1} may be attributed to increased availability and uptake of phosphorus **(Kundu and Gaur, 1980).**

In here, the magnitudes of broccoli yield parameters at harvest were also behaved the same trend as mentioned before through the discussion of vegetative growth ones, as follows: there were no significant differences between both 75% N-mineral fertilizer fed^{-1} + three doses of (AT+AZ) and 100% N-mineral fertilizer fed^{-1} as a control treatment, but this two treatments > 75% N-mineral fed^{-1} + two doses of (AT+AZ) >75 % N-mineral fed^{-1} + one dose of (AT+AZ) >50% N-mineral fed^{-1} + three doses of (AT+AZ) >50% N-mineral fed^{-1} + two doses of (AT+AZ) >50 % N-mineral fed^{-1} + one dose of (AT+AZ). These results are in agreement with those obtained by **Osman (2008)** who pointed out that total yield was highly correlated with the development of vegetative growth as well as dry matter accumulation.

Table (6): Influence of bio-and N-mineral fertilization on total yield and quality of broccoli plants in both seasons of 2010-2011 and 2011-2012.

Treatments		Total yield (kg fed^{-1})		Yield quality parameters		
		Central heads	Lateral heads	Central head plant^{-1} (g)	Lateral heads plant^{-1} (g)	Number of lateral heads plant^{-1}
N-mineral fed^{-1}	**Number of doses (AT+AZ)**	**Season 2010-2011**				
100% (120 kg N)		4266	4704	270	304	5.82
50% (60 kg N)	one	2617	2883	166	187	3.57
	two	2784	3078	177	199	3.80
	three	3201	3525	202	228	4.36
75% (90 kg N)	one	3600	3963	228	257	4.91
	two	4009	4423	254	286	5.47
	three	4349	4800	276	310	5.94
L.S.D. at 0.05		**203**	**269**	**10**	**12**	**0.15**
		Season 2011-2012				
100% (120 kg N)		4401	4861	281	310	6.05
50% (60 kg N)	one	2701	2979	172	190	3.71
	two	2874	3180	183	203	3.95
	three	3301	3643	210	232	4.54
75% (90 kg N)	one	3713	4081	237	261	5.10

	two	4142	4578	265	292	5.69
	three	4487	4956	286	315	6.11
L.S.D. at 0.05		**210**	**280**	**12**	**15**	**0.17**

(AT+AZ): The mixture of *Azotobacter chroococcum* (AT) and *Azospirillium brasilense* (AZ) [1:1]

d) Some of Nutrients and sugar contents in broccoli heads:

Results given in Table (7) reflected significant differences in the contents of the studied nutrients and sugar in the tissues of broccoli heads as a result from increasing the applied doses of bio-fertilizer, which actually combined with N-mineral fertilizer. However, the greatest values of the studied nutrients and sugar in the tissues of broccoli heads were produced by plants supplied by 75% recommended dose of N-mineral fertilizer fed^{-1} + three doses of (AT+AZ) and 100% N-mineral fertilizer fed^{-1} where there were no significant differences between them in two successive seasons of study. On the other hand, the relatively low values were associated with the treatment of 50 % recommended dose of N-mineral fertilizer fed^{-1} + one dose of (AT+AZ). These results held good in the two experimental seasons. Also, the obtained results are in good agreement with that obtained by **Hassan (2009)** who found that bacteria inoculation separately or combined with chemical fertilizers significantly increased NPK percentages as a result of applying bio-fertilizers alone or combined with chemical fertilizers.

Concerning the superiority in both the studied nutrients and sugar values in tissues of broccoli heads by increasing doses of bio-fertilizer, may be attributed to the high availability, mobility of nutrients as a result of the positive effect of bio-fertilizer and are mostly found in an enough quantity which required for a good plant growth, consequently higher yield and more better quality were achieved. These findings are in accordance with those obtained by **Singh and Kapoor, (1999)**. Also, increasing such chemical constituent concentrations in leaves or heads of broccoli plants with applied doses of bio-fertilizer, which actually combined with N-mineral fertilizer, might be due to the increased plant capacity to absorb nutrient, which increased the root surface per soil volume unit. These obtained results are cope with those obtained by **(Rodriguez and Fraga, 1999, Abdel-Latif *et al.,* 2001** and **Osman, 2008).**

In addition, the applied number of doses of bio-fertilizer plays an important role on both the availability of nutrients and other biological activity in the vicinity of roots through lowering soil pH value, besides the ameliorating soil drainage and aeration, which encouraged the bio-mechanism of nutrients uptake. Such results are in harmony with those reported by **Tisdale *et al.*, (1993)** who pointed out that plant uptake of nutrients precedes best at a neutral pH value and their contents depressed by increasing its value.

Thus, the use efficiency of bio-fertilizer showed an-useful phase, since large released amounts of nutrients, which are available for uptake under the modified favorable soil media **(Isfan *et al.*, 1995)**. **Jones *et al.,* (1991)** reported that the increment of nutrient uptake is mostly dependent upon biological activity that is markedly affected by soil temperature, moisture, aeration and original soil pH. Thereby, the increments in available nutrients encouraged their uptake by plants (Tables 5 and 7) and the growth parameters (Table, 4), which were also resulted from increasing the net photosynthesis, stomatal conductance and transpiration rate when broccoli plants were subjected to the prevailing best conditions **(Naire and Khuble, 1990)**.

Table (7): Effect of the applied N-mineral fertilizer combined with bio-fertilizer on head nutrients and sugar contents for the broccoli crop in both seasons of 2010-2011 and 2011-2012.

Treatments		Macronutrients %			Micronutrients (mg kg^{-1})				Sugar %
		N	P	K	Fe	Mn	Zn	Cu	
N-mineral fed^{-1}	**Number of doses (AT+AZ)**	**Season 2010-2011**							

100% (120 kg N)		3.89	0.616	3.31	122.4	79.52	42.23	12.03	10.57
50% (60 kg N)	one	2.44	0.377	2.03	73.92	48.75	25.55	6.99	6.48
	two	2.61	0.403	2.16	79.65	51.25	26.90	7.89	6.91
	three	3.00	0.462	2.49	90.38	58.95	31.45	8.95	7.93
75% (90 kg N)	one	3.31	0.519	2.79	102.80	66.90	35.54	10.25	8.91
	two	3.68	0.579	3.12	114.50	74.72	40.35	11.58	9.94
	three	3.93	0.628	3.38	125.74	81.40	43.86	12.77	10.78
L.S.D. at 0.05		**0.28**	**0.049**	**0.17**	**5.17**	**3.78**	**2.09**	**1.03**	**0.73**
		Season 2011-2012							
100% (120 kg N)		3.98	0.634	3.30	123.8	80.74	43.72	12.52	10.72
50% (60 kg N)	one	2.46	0.389	2.03	75.52	49.25	26.67	7.64	6.57
	two	2.64	0.415	2.16	80.47	52.48	28.42	8.14	7.00
	three	3.04	0.475	2.48	92.85	60.56	32.79	9.39	8.04
75% (90 kg N)	one	3.37	0.535	2.81	103.99	67.82	36.72	10.52	9.04
	two	3.76	0.596	3.11	116.37	75.90	41.10	11.77	10.09
	three	4.00	0.647	3.37	125.28	81.15	43.59	12.85	10.67
L.S.D. at 0.05		**0.25**	**0.051**	**0.21**	**5.54**	**3.61**	**1.88**	**0.94**	**0.65**

Finally, using Italica crop cv. of broccoli with bio-fertilizer in combination with N-mineral fertilizer could be followed for producing high yield of broccoli with high quality of heads. That was true, since that interaction treatment, in general, significantly affected all vegetative growth characters of broccoli plants, probably due to the applied bio-fertilizer besides it is ability to : 1)Release some plant promoting substances, mainly IAA, gibberellic acid and cytokinin like substances which stimulate plant growth. 2) Increasing the ability to convert N_2 to NH_4^+ and thus make it available to plants. 3) Beneficial effects of (AT+AZ) bacteria on reducing soil pH by secreting organic acids (e.g. acetic, propionic, fumaric and succinic) and maintaining a suitable air-moisture regime, that finally leading to improve vegetative growth, chemical constituents and higher yield of broccoli with better quality heads . Moreover, it could by concluded that, the applied Italica crop cv. of broccoli with treatment of 75% N-mineral (90 kg N fed^{-1}) + three doses of (AT+AZ) or 100% N-mineral (120 kg N fed^{-1}) leading to improve vegetative growth, chemical constituents and higher yield, *i.e.,* central and lateral heads of broccoli as well as diminishing the environmental pollution by minimizing the harmful effect of using chemical fertilizers on human health.

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