World Academy of Science, Engineering and Technology
International Journal of Agricultural and Biosystems Engineering
Vol:7, No:7, 2013

Effect of Bio-Nitrogen as a Partial Alternative to Mineral-Nitrogen Fertiliser on Growth, Nitrate and Nitrite Contents, and Yield Quality in *Brassica oleracea* L.

Saad M. Howladar, Mostafa M. Rady, and Ashraf Sh. Osman

Abstract**—**Effects of bio-nitrogen fertilizer (bio-N), as a partial alternative to mineral-nitrogen fertilizer (mineral-N), on growth, yield and yield quality of broccoli plants were investigated. Bio-N was applied at 1, 2 or 3 doses in combination with 65% of the recommended dose of mineral-N (bio-N_1, bio-N_2 or bio-N_3 + ⅔mineral-N). However, 100% of the recommended dose of mineral-N was applied as a control. Significant positive influences of the bio-N_3 + ⅔mineral-N treatment were observed on growth traits, leaf contents of nitrogen, phosphorus, potassium, nitrate and nitrite, and yield quality when compared to the other two combined treatments. In contrast, there were no significant differences in these parameters between the bio-N_3 + ⅔mineral-N and the control treatments, except for leaf contents of nitrate and nitrite. They showed lower contents in the bio-N_3 + ⅔mineral-N treatment than the control. Therefore, we recommend using bio-N as a partial alternative to mineral-N for healthy nutrition.

Keywords**—**Bio-fertilization, broccoli, growth, nitrate, nitrite, yield quality.

I. INTRODUCTION

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 [1]. 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 [2]-[4].

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.

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 [5]. 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 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 *Azospirillum* have the ability not only to fix nitrogen but also to release certain phytohormons 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 dissolution. In addition, the increase in the capacity of photosynthesis is process in [6]-[8]. 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 [9]-[11]. Inoculation of potato tuber seeds with bio-fertilizer [*Azotobacter chroococcum* (AT) + *Azospirillium brasilense* (AZ)] significantly increased growth and yield and its components [11], [12]. The application of bio-fertilizers 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 [10], [13].

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, B_1, B_2, B_5, B_6 and E), minerals (Ca, Mg, Zn and Fe) and a number of antioxidants [14], [15], which prevent the formation of cancer-causing agents [16]. It is, therefore, widely cultivated in many European and

Saad M. Howladar is with the Department of Biology, Faculty of Sciences, Al-Baha University, Al-Baha, Saudi Arabia (e-mail: showladar@hotmail.com).
Mostafa M. Rady is with the Department of Botany, Faculty of Agriculture, Fayoum University, 63514-Fayoum, Egypt (e-mail: mrady2009@yahoo.com).
Ashraf Sh. Osman is with the Department of Horticulture, Faculty of Agriculture, Fayoum University, 63514-Fayoum, Egypt (e-mail: aso00@fayoum.edu.eg).

American countries, but in Egypt it still grown in limited areas. The total cultivated area is not exactly known [17].

The objective of this study was to assess the effect of 65% of the recommended dose of mineral-nitrogen fertilizer in combination with bio-nitrogen fertilizer (*Azotobacter chroococcum* + *Azospirillium brasilense*) in 1, 2 or 3 doses on the growth, nitrate and nitrite contents as contaminated agents, and yield quality in broccoli (*Brassica oleracea* L. var. *italica*) grown under Middle East conditions.

TABLE I
PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE EXPERIMENTAL SOIL BEFORE APPLICATION OF TREATMENTS (BT) AND AT WEEK-9 AFTER APPLICATION OF BIO-FERTILIZATION (AT) IN 2010/2011 AND 2011/2012 SEASONS

Composition [% (w/w)]			pH	EC	OC[#]	N	P	K	Ca	Fe	Mn	Zn
Clay	Loam	Sand		(dS m^{-1})	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
BT, 2010/2011												
49.2	21.6	29.2	7.9	3.0	11.8	81.3	10.2	476.9	8.1	8.5	3.6	1.0
AT [soil treated with 50% recommended N dose + 3 doses of bio-fertilization (AT + AZ)[*]], 2010/2011												
49.6	21.3	29.1	7.1	2.7	14.2	108.6	11.4	513.0	10.2	9.4	4.3	1.3
BT, 2011/2012												
49.4	22.0	28.6	7.7	2.8	12.3	86.2	10.7	496.2	9.3	7.9	3.9	1.3
AT [soil treated with 50% recommended N dose + 3 doses of bio-fertilization (AT + AZ)[*]], 2011/2012												
49.6	22.3	28.1	7.0	2.4	15.3	111.6	11.7	529.6	9.9	9.8	4.5	1.7

[#]OC, organic content
[*]AT+AZ, a mixture of *Azotobacter chroococcum* and *Azospirillium brasilense*, respectively in a ratio of 1:1 (w/w)

II. MATERIALS AND METHODS

A. Treatments and Plant Material

Two field experiments were conducted, in the 2010/2011 and 2011/2012 seasons. The main characteristics of the soil (a private farm, Sonnuris district, Fayoum, Egypt) used in this research were determined [18], and are shown in Table I. During soil preparations for transplanting, all experimental areas received the complete dose of mineral-phosphorus [450 kg ha^{-1} calcium superphosphate (15.5% P_2O_5)] and mineral-potassium [300kg ha^{-1} potassium sulphate (48% K_2O)] fertilizers as recommended [19] under Egyptian conditions. They were then divided into 18m^2 (6m × 3m) plots. Prior to transplanting broccoli seedlings, 65% of the recommended dose of mineral-nitrogen fertilizer (mineral-N) in combination with 1, 2 or 3 doses of bio-nitrogen fertilizer (bio-N; *Azotobacter chroococcum* + *Azospirillium brasilense*) were applied to the plots as 3 combined treatments. In addition, the control plots were received 100% of the recommended dose of mineral-N [700kg ha^{-1} ammonium nitrate (33.5% N)] as recommended under Egyptian conditions [19]. All treatments were conducted in a randomized complete blocks with four replicates. Transplanting was conducted on 19 October 2010 and on 16 October 2011 using 5-week-old broccoli (*Brassica oleracea* L. var. *italica*) seedlings obtained from the Ministry of Agriculture Nurseries, Cairo, Egypt. Each plot contained 75 plants, spaced at 40 cm in-row and 0.6 m between rows. All other standard cultural practices were followed as recommended for commercial broccoli production.

B. Preparation of Inocula

Modified Ashby's medium was used to grow the *Azotobacter chroococcum* [20]. In addition, Dobereiner medium was used to grow the *Azospirillium brasilense* [21]. The strains (*A. chroococcum* FN 33 and *A. brasilense* FN 17) were isolated and identified in the microbiological 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, cultures were centrifuged at 1000rpm and the cell pellets were washed three times with sterile phosphate buffer (100mM, pH = 7.0). The washed cells were resuspended in the same buffer to the final concentration of about 4 × 10^8*cfu* ml^{-1}.

C. Inoculation of Bio-Nitrogen Fertilizer

Roots of broccoli seedlings were dipped in a mixture of *Azotobacter chroococcum* FN 33 and *Azospirillium brasilense* FN 17 in a ratio of 1:1 (v/v). In addition, the rhizosphere of each plant was injected once (at 4 weeks after transplanting) or twice (at 4 and repeated at 7 weeks after transplanting) in a rate of about 50ml plant $hole^{-1}$.

D. Determination of Growth Traits, Yield and Yield Quality Components

Nine-week-old broccoli plants were used to determine plant leaf number, plant leaf area, leaf dry weight (DW) $plant^{-1}$ and stem DW $plant^{-1}$. Six plants were randomly chosen from each experimental plot, cut off at the ground level and divided into leaves and stems. Leaf area $plant^{-1}$ (dm^2) was recorded using a digital leaf meter (LI-3000 Portable Area meter Produced by LI-COR Lincoln, Nebraska, USA). Leaf and stem DWs $plant^{-1}$ (in g) were estimated after drying the appropriate tissues to constant weight at 70°C using a forced air-oven.

At harvest, total yields; central and lateral heads having closed floral buds, dark green color and good compactness were weighed using all experimental plants. In addition, yield quality components; weights of central head and lateral heads

$plant^{-1}$, and number of lateral heads $plant^{-1}$ were considered using six plants that were randomly chosen from each experimental plot.

E. Determination of Nitrogen, Phosphorus and Potassium

Total leaf nitrogen (% DW) was estimated using the Microkjeldahal apparatus as described in A.O.A.C. [22]. The molybdenum-reduced molybdophosphoric blue color method [23], in sulphuric acid (with reduction to exclude arsenate), was the method used for leaf phosphorus determination (% DW). In addition, sulphomolybdic acid (molybdenum blue), diluted sulphomolybdic acid, and 8% (w/v) sodium bisulphite-H_2SO_4 solution were used as reagents. Leaf potassium content (% DW) were assessed using a Perkin-Elmer Model 52-A Flame Photometer [24].

F. Leaf Nitrate (NO_3^-) and Nitrite (NO_2^-) Determinations

Leaf samples of broccoli plants were prepared by washing in tap water, then several times in distilled water, then cut into nearly uniform-sized pieces ($2.0cm^2$) to facilitate drying at the same rate. The samples were dried in an oven at 105°C for 24 h until they were brittle and crisp. At this stage, no microorganisms could grow and care was taken to avoid any such contamination. The dried samples were ground into fine particles using a clean mortar and pestle, and sieved to obtain a < 2.0mm size-fraction. A portion (1.0g) of each sieved sample was placed in a 100ml polyethylene or glass bottle and 40ml of distilled water was added, then capped and shaken for 30min. The mixture was filtered, and the filtrate was made up to 100ml in a volumetric flask [25].

Determinations of NO_3^- content in each leaf and fruit sample solution were performed using a spectrophotometer (Model 2000; Kwf Sci-Tech Development Co. Ltd., Beijing, P.R. China) at a wavelength of 543nm. The pre-programme for NO_3^- (64 NO_3^--N) was selected and the readings were converted to NO_3^- by multiplying using a conversion factor of 4.4 [26]. The NO_3^- content of samples was calculated using the formula:

$$NO_3^- \text{ content } (\mu g\ g^{-1}) = C \times V / M$$

where, C was the concentration of NO_3^- in the sample ($\mu g\ g^{-1}$), V was the total volume of the sample solution (100ml), and M was the weight of the sample (1.0g). The data obtained were converted to mg NO_3^- g^{-1} leaf DW.

NO_2^- ion contents were determined in a similar manner except that different reagents were used. The pre-programme number for NO_2^- was 67 NO_2^--N, and the reaction time was 5 min compared to 10 min for NO_3^-. NO_2^--N contents were converted to NO_2^- by multiplying by 3.3 [26]. The NO_2^- contents of samples were calculated using the formula:

$$NO_2^- \text{ content } (\mu g\ g^{-1}) = C \times V / M$$

where, C was the concentration of NO_2^- in the sample ($\mu g\ g^{-1}$), V was the total volume of the sample solution (100 ml), and M was the weight of the sample (1.0 g) [25]. The data obtained were converted to mg NO_2^- g^{-1} leaf DW.

G. Statistical analysis

All data were subjected to ANOVA using SAS software [27], and means comparisons between the different treatments were performed using the Least Significant Differences (LSD) procedure at the $P = 0.05$ level [28].

III. Results and Discussion

A. Growth Traits as Affected by Bio- and/or Mineral-Nitrogen Fertilizer

Broccoli plants grown under the combined treatment of bio-N applied at 3 doses + 65% of the recommended dose of mineral-N (bio-N_3 + ⅔mineral-N) exhibited the highest number of leaves $plant^{-1}$, plant leaf area, leaf dry weight (DW) $plant^{-1}$ and stem DW $plant^{-1}$ when compared to the other two combined treatments (bio-N_1 or bio-N_2 + ⅔mineral-N; Table II). There were no significant differences in these parameters between the bio-N_3 + ⅔mineral-N treatment and the control (100% of the recommended dose of mineral-N). The same trend was observed over both growing seasons. These results are in agreement with those obtained in several studies [7]-[10]. In addition, Osman [11] found that inoculation of bacteria (bio-N) singly or in combination with chemical fertilizers positively affected growth characters of potato plants. This may be attributed to the increased activity and efficiency of bacteria in reduction of soil pH (Table I) by secreting organic acids i.e. acetic, propionic, fumaric and succinic [29], 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 (Table I) and in plants (Table III). Our results indicated that, bio-N is beneficial for sustainable agriculture and human healthy nutrition as a partial alternative to mineral-N fertilizer.

TABLE II
NUMBER OF LEAVES PLANT^{-1}, PLANT LEAF AREA, LEAF DRY WEIGHT (DW) PLANT^{-1}, AND STEM DW PLANT^{-1} [MEANS (N = 6) ± STANDARD DEVIATIONS] OF 9-WEK-OLD BROCCOLI PLANTS GROWN UNDER MINERAL-N OR BIO-FERTILIZATION IN 2010/2011 AND 2011/2012 SEASONS

Treatments	Parameters			
	Leaves No. plant^{-1}	Leaf area plant^{-1} (dm^2)	Leaf DW plant^{-1} (g)	Stem DW plant^{-1} (g)
2010/2011 season:				
*Control	37.5 ± 3.4a	67.4 ± 4.2a	58.7 ± 5.2a	57.4 ± 4.2a
Bio-N_1 + ½mineral-N	24.5 ± 2.6c	44.0 ± 3.1c	38.3 ± 4.1c	37.5 ± 2.6c
Bio-N_2 + ½mineral-N	31.7 ± 3.1b	56.8 ± 4.3b	49.5 ± 4.3b	48.4 ± 4.4b
Bio-N_3 + ½mineral-N	38.2 ± 3.0a	68.7 ± 5.2a	59.8 ± 4.9a	58.5 ± 4.7a
2011/2012 season:				
Control	38.7 ± 2.9a	68.5 ± 5.1a	60.1 ± 4.9a	59.9 ± 4.4a
Bio-N_1 + ½mineral-N	24.8 ± 2.4c	44.5 ± 3.2c	39.3 ± 3.2c	39.1 ± 3.3c
Bio-N_2 + ½mineral-N	32.6 ± 3.2b	57.8 ± 4.4b	50.3 ± 4.9b	50.0 ± 4.1b
Bio-N_3 + ½mineral-N	37.9 ± 4.0a	69.6 ± 4.9a	60.7 ± 4.7a	60.6 ± 4.4a

*Control = 100% of recommended mineral-N fertilizer
In a column, treatment means having a common letter(s) are not significantly different at the 5% level

B. Leaf Nitrogen, Phosphorus and Potassium Contents as Affected by Bio- and/or Mineral-Nitrogen Fertilizer

Based on leaf nitrogen (N), phosphorus (P) and potassium (K) contents (Table III), the bio-N_3 + ⅔mineral-N treatment produced broccoli plants had higher N, P and K contents than all other two combined treatments (bio-N_1 or bio-N_2 + ⅔ mineral-N). Using 100% of the recommended dose of mineral-N (control treatment) resulted in no significant differences in these nutrient contents when compared to the treatment of bio-N_3 + ⅔ mineral-N. The same trends were seen in 2010/2011 and 2011/2012. These results emphasized that the bio-N_3 + ⅔ mineral-N treatment was a great enough to reach the highest levels of N, P and K. 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 (Table I) by secreting some organic acids (e.g. acetic, propionic, fumaric and succinic) and maintaining a suitable air-moisture regime. In addition, *Azospirillum*-inoculated plants exhibited higher foliar N, P and K contents in marigold [30], and in coffee [31]. They also showed increased growth of root system that enables them to absorb more nutrients from soil [32]. Similar observations were noted by Hemavathi [33] and Shubha [34] using chrysanthemum and marigold, respectively. The increased availability of nutrients in the soil and their enhanced absorption by plant roots (Table III) due to the combined bio-N + mineral-N application resulted in increased yields and more stable soil health.

C. Leaf Nitrate and Nitrite Contents as Affected by Bio- and/or Mineral-Nitrogen Fertilizer

Broccoli plants grown in the bio-N_3+⅔ mineral-N treatment showed the lowest leaf contents of NO_3^- and NO_2^- when compared to the other two combined treatments (Table III). However, all three combined treatments (bio-N_1, bio-N_2 or bio-N_3 + ⅔ mineral-N) resulted in lower leaf contents of NO_3^- and NO_2^- than those in the control treatment. The same trends were observed over both growing seasons. The combined application is bio-N and mineral.

TABLE III
NITROGEN (N), PHOSPHORUS (P), POTASSIUM (K), NITRATE (NO_3^-) AND NITRITE (NO_2^-) CONTENTS [MEANS (N = 6) ± STANDARD DEVIATIONS] IN 9-WEEK-OLD BROCCOLI PLANTS GROWN UNDER MINERAL-N OR BIO-FERTILIZATION IN 2010/2011 AND 2011/2012 SEASONS

Treatments	Parameters				
	N (% DW)	P (% DW)	K (% DW)	NO_3^- (mg g^{-1} DW)	NO_2^- (mg g^{-1} DW)
2010/2011 season:					
*Control	3.55 ± 0.24a	0.35 ± 0.02a	2.23 ± 0.16a	2.53 ± 0.12a	0.188 ± 0.015a
Bio-N_1 + ½mineral-N	2.32 ± 0.26c	0.23 ± 0.02c	1.46 ± 0.15c	2.22 ± 0.10b	0.125 ± 0.012b
Bio-N_2 + ½mineral-N	3.00 ± 0.25b	0.30 ± 0.04b	1.88 ± 0.17b	1.64 ± 0.11c	0.095 ± 0.007c
Bio-N_3 + ½mineral-N	3.63 ± 0.29a	0.36 ± 0.03a	2.27 ± 0.18a	0.94 ± 0.08d	0.069 ± 0.004d
2011/2012 season:					
Control	3.51 ± 0.26a	0.37 ± 0.03a	2.27 ± 0.21a	2.45 ± 0.14a	0.181 ± 0.014a
Bio-N_1 + ½mineral-N	2.33 ± 0.22c	0.24 ± 0.02c	1.50 ± 0.15c	2.10 ± 0.11b	0.131 ± 0.011b
Bio-N_2 + ½mineral-N	2.95 ± 0.24b	0.31 ± 0.03b	1.91 ± 0.15b	1.50 ± 0.09c	0.098 ± 0.008c
Bio-N_3 + ½mineral-N	3.58 ± 0.28a	0.39 ± 0.04a	2.29 ± 0.21a	0.86 ± 0.07d	0.062 ± 0.004d

*Control = 100% of recommended mineral-N fertilizer
In a column, treatment means having a common letter(s) are not significantly different at the 5% level

TABLE IV
TOTAL YIELD AND ITS QUALITY [MEANS (N = 6) ± STANDARD DEVIATIONS] OF BROCCOLI PLANTS GROWN UNDER MINERAL-N OR BIO-FERTILIZATION IN 2010/2011 AND 2011/2012 SEASONS

Treatments	Parameters				
	Total yield (ton ha^{-1})		Yield quality parameters		
	Central heads	Lateral heads	Central head $plant^{-1}$ (kg)	Lateral heads $plant^{-1}$(kg)	No. of lateral heads $plant^{-1}$
2010/2011 season:					
*Control	10.17 ± 0.81a	11.19 ± 1.12a	0.27 ± 0.03a	0.30 ± 0.02a	5.82 ± 0.55a
Bio-N_1 + ½mineral-N	6.62 ± 0.62c	7.33 ± 0.76c	0.18 ± 0.02c	0.19 ± 0.02c	3.80 ± 0.32c
Bio-N_2 + ½mineral-N	8.57 ± 0.83b	9.43 ± 0.99b	0.23 ± 0.03b	0.26 ± 0.03b	4.91 ± 0.37b
Bio-N_3 + ½mineral-N	10.36 ± 0.93a	11.48 ± 0.96a	0.28 ± 0.03a	0.31 ± 0.03a	5.94 ± 0.64a
2011/2012 season:					
Control	10.48 ± 0.62a	11.57 ± 1.10a	0.29 ± 0.02a	0.31 ± 0.04a	6.05 ± 0.54a
Bio-N_1 + ½mineral-N	6.83 ± 0.52c	7.57 ± 0.74c	0.18 ± 0.02c	0.20 ± 0.01c	3.95 ± 0.41c
Bio-N_2 + ½mineral-N	8.83 ± 0.57b	9.71 ± 0.88b	0.24 ± 0.02b	0.25 ± 0.02b	5.10 ± 0.48b
Bio-N_3 + ½mineral-N	10.69 ± 0.67a	11.74 ± 0.97a	0.29 ± 0.03a	0.32 ± 0.03a	6.11 ± 0.58a

*Control = 100% of recommended mineral-N fertilizer
In a column, treatment means having a common letter(s) are not significantly different at the 5% level

N, particularly bio-N_3 + ⅔mineral-N treatment resulted in production of broccoli plants with lower contents of NO_3^- and NO_2^- for human healthy nutrition. Increased availability of N in the soil by the extensive use of mineral-N as applied in the control treatment led to an obvious increase in the contents of NO_3^- and NO_2^- in broccoli leaves. The accumulation of NO_3^- and NO_2^- ions in edible plant parts poses a problem. This was attributed to the continuous supply of NO_3^- and NO_2^- to the plants from mineral-N fertilizer [35]. In contrast, in the bio-N_3 + ⅔ mineral-N-treated plots the release of NO_3^- and NO_2^- was comparatively slow. In addition, the increase in the organic matter content in the plots treated with bio-N_3 + ⅔mineral-N (Table I) may control the release and transformation of N-fertilizer to NO_3^- and NO_2^-. The addition of bio-N to cultivated soil was effective in minimizing the NO_3^- and NO_2^- toxicity in broccoli plants. This may be attributed to the incorporation of organic material that enhanced the soil organic carbon content and had direct and indirect effects on soil properties and processes.

D. Yield and Its Quality as Affected by Bio- and/or Mineral-Nitrogen Fertilizer

No significant differences were noted in total yields of central and lateral heads ha^{-1} and their quality components (weight of central and lateral heads $plant^{-1}$ and No. of lateral heads $plant^{-1}$) between the bio-N_3 + ⅔mineral-N and control treatments (Table IV). Lower yields and their quality parameters were obtained from the bio-N_1 + ⅔mineral-N treatment than all other treatments including the control. The same trends were observed in both growing seasons. These findings may be attributed to the slow and steady supply of N by bio-N, particularly at the highest dose, which met the N requirements of plants at different stages of development. Bio-N 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 I) as a result of the bio-N_3 + ⅔mineral-N treatment were positively reflected in the nutritional status of broccoli plants (Table III) and consequently reflected in the increased growth, yields and their quality components. These results may be explained by the role of *Azospirillum* in atmospheric nitrogen fixation, better root proliferation and uptake of nutrients and water [36]. Our results are in agreement with those obtained by Osman [11] who pointed out that total yield was highly correlated with the development of vegetative growth as well as dry matter accumulation.

IV. CONCLUSIONS

Results of this study show that using the bio-nitrogen fertilizer (*Azotobacter chroococcum* and *Azospirillium brasilense*) as a partial alternative to mineral-nitrogen fertilizer enabled broccoli plants to produce higher yields with minimized levels of NO_3^- and NO_2^-. Bio-N fertilizers increased soil organic matter content and the availability of nutrients to plant roots, thus increased plant growth and yields with higher quality. Application of Bio-N fertilizer reduced the amount of synthetic chemical-N fertilizer needed for crop production, and can ameliorate or reduce the negative effects of chemical-N fertilizer on the environment. Therefore, production of broccoli plants having lower contents of NO_3^- and NO_2^- ions for human healthy nutrition is obtainable.

REFERENCES

[1] A. Hartman, "Ecophysiological aspects of growth and nitrogen fixation in Azospirillium species". Plant Soil, vol. 110, 1988, pp. 225–238.
[2] N. P. Singh, R. S. Sachan, P. C. Pandey, and P. S. Bisht, "Effect of a decade long fertilizer and manure application on soil fertility and productivity of rice-wheat system in Molisols". J. Indian Soc. Soil Sci., vol. 47, 1999, pp. 72–80.
[3] A. Bhatia, H. Pathak, and H. C. Joshi, "Use of sewage as a source of plant nutrient: potentials and problems". Fert. News, vol. 46, 2001, pp. 55–58.
[4] C. A. Palm, C. N. Gachengo, R. J. Delve, G. Cadisch, and K. E. Giller, "Organic inputs for soil fertility management in tropical agro ecosystems: application of an organic resource database". Agric. Ecosyst. Environ, vol. 83, 2001, pp. 27–42.
[5] E. C. Cocking, "Helping plants get more nitrogen from air". European Review, vol. 8, 2000, pp. 193–200.
[6] A. N. Ibrahim, and I. M. Abd El-Aziz, "Solubilization of rock phosphate by streptomyces". Agr. Talajton, vol. 26, 1977, pp. 424–434.
[7] M. Fayez, N. F. Eman, and H. E. Makbol, "The possible use of nitrogen fixing Azospirillum as biofertilizer for wheat plants". Egypt. J. Microbiol., vol. 20, 1985, pp. 199–206.
[8] M. R. Abdel-Latif, A. A. El-Bana, and A. A. Galal, "Effect of biofertilizers Microbein and Phosphorine on bacterial pod blight of guar and black cumin damping off root rot and with diseases". Proc. of the

fifth Arabian Horticulture Conference, Ismailia, Egypt, March 2001, pp. 24–28, and pp. 133–140.
[9] M. Abdel-Mouty, H. Mona Ali, A. Nisha, and A. Rizk, "Potato yield as affected by interaction between bio-and organic fertilizers". Egypt. J. Appl. Sci., vol. 16, 2002, pp. 267–276.
[10] F. M. Gadallah, and T. A. El-Masry, "Onion growth and yield as affected by bio-fertilization". Annals Agric. Sci. Moshtohor, vol. 44, 2006, pp. 987–1005.
[11] A. Sh. Osman, "Effect of partial substitution of mineral-N by biofertilization on growth, yield and yield components of potato". The 3rd Conf. Sustain. Agric. Develop., Fac. Agric., Fayoum Univ., 12–14 Nov. 2007, pp. 381–396.
[12] S. A. Ashour, A. E. Abdel-Fattah, and A. A. Tawfik, "Effect of nitrobin (biofertilizer) and different levels of nitrogen on growth and yield of potato (Solanum tuberosum, L.)". J. Agric. Sci., Mansoura Univ., vol. 22, 1997, pp. 3979–3986.
[13] A. H. Hanafy, N. F. Kheir, and N. B. Talaat, "Physiological studies on reducing the accumulation of nitrate in jew's mallow (Corchorus olitorius) and radish (Raphanus sativus L.)". Bull. Fac. Agric., Cairo Univ., vol. 48, 1997, pp. 25–64.
[14] S. Rozek, and R. Wojciechowska, "Effect of urea foliar application and different levels of nitrogen in soil on broccoli head yield and its quality in autumn growing cycle". Sodininkyste-ir Darzininkyste, vol. 24, 2005, pp. 291–301. (c.a., CAB Abst., 20063014208).
[15] R. Wojciechowska, S. Rozek, and A. Rydz, "Broccoli yield and its quality in spring growing cycle as dependent on nitrogen fertilization". Folia-Horticulturae, vol. 17, 2005, pp. 141–152. (c.a. CAB Abst. 20063029819).
[16] C. Beecher, "Cancer preventive properties of varieties of Brassica oleracea". A Review, Amer. J. Clin. Nutri., vol. 59, 1994, pp. 1166–1170.
[17] M. M. Abou El-Magd, A. M. El Bassiony, and Z. F. Fawzy, "Effect of organic manure with or without chemical fertilizers on growth, yield and quality of some varieties of broccoli plants". J. Appl. Sci. Res., vol. 2, 2006, pp. 791–798.
[18] S. A. Wilde, R. B. Corey, J. G. Lyer, and G. K. Voigt, "Soil and plant analysis for tree culture". Oxford and IBM Publishers. New Delhi. India. 3rd Ed., 1985, pp. 93–106.
[19] M. S. Tolba, "Influence of different nitrogenous and potassic fertilization levels on vegetative growth, heads yield and chemical composition of broccoli (Brassica oleracea var. italica)". Ph.D. Thesis, Fac. Agric., Fayoum Branch, Cairo Univ, Egypt, 2005.
[20] N. A. Hegazi, and S. Niemela, "A note on the estimation of Azotobacter density by membrane filter technique". J. Appl. Bacteriol., vol. 41, 1976, p. 311.
[21] J. Dobereiner, I. E. Marril, and M. Niery, "Ecological distribution of Spirillum lipoferum, Beijerinek". Can. J. Microbiol., vol. 22, 1976, pp. 1464–1473.
[22] A.O.A.C., "Association of Official Analytical Chemists. Official Methods of Analysis". 15th Ed., Washington, D.C., USA, 1995.
[23] M. L. Jackson, "Soil Chemical Analysis". New Jersey Prentice-Hall, Inc., 1967.
[24] A. I. Page, R. H. Miller, and D. R. Keeney, "Methods of Soil Analysis". Part 2: Chemical and Microbiological Properties. 2nd Ed., Amer. Soc. of Agron., Madison, Wisconsin, USA, 1982.
[25] M. Radojevic, and N. V. Bashkin, "Practical Environmental Analysis". Royal Society of Chemistry and Thomas Graham House, Cambridge, UK, 1999, pp. 180–430.
[26] La Motte, "Smart Spectro Water and Waste Water Procedure Analysis Manual". La Motte Inc., Washington, DC, USA, 2000, pp. 68–180.
[27] SAS, "SAS/STAT User's Guide". Version 6.12. SAS Institute, Cary, NC, USA, 1996. (http://www.sas.com/technologies/analytics/statistics/stat/index.html).
[28] G. W. Snedecor, and W. G. Cochran, "Statistical Methods". Oxford and J. B. H. Publ. Com. 7th ed., 1980.
[29] S. Singh, and K. K. Kapoor, "Inoculation with phosphate-solubilizing microorganisms and visicular-arbuscular mycorrhizal fungus improves dry matter yield and nutrient uptake by wheat grown in sandy soil". Biol. Ferti. Soils, vol. 28, 1999, pp. 139–144.
[30] J. Balasubramanian, "Studies on the combined effect of Azospirillium VA mycorrhizal and inorganic fertilizers on growth performance of French marigold (Tegetes putula L.)". SIH, vol. 37, 1989, p. 311.
[31] M. S. Premkumari, and A. Balasubramanian, "Effect of combined inoculation of VAM and Azospirillum on growth and nutrient uptake of coffee seedlings". Indian Coffee, vol. 57, 1993, pp. 5–11.
[32] K. J. Vessey, "Plant growth promoting rhizobacteria as biofertilizer". Plant Soil, vol. 255, 2003, pp. 571–586.
[33] M. Hemavathi, "Effect of organic manures and biofertilizers on growth and productivity of chrysanthemum (Chrysanthemum molifolium Ramat.) cv. Local yellow". M.Sc.(Agri.) Thesis, Univ. Agric. Sci., Bangalore, 1997.
[34] B. M. Shubha, "Integrated nutrient management for growth, flowering and xanthophylls Yield of marigold (Tagetes erecta L.)". M.Sc.(Agric.) Thesis, Univ. Agric. Sci., Dharwad, 2006.
[35] E. Mahmoud, N. Abd El-Kader, P. Robin, N. Akkal-Corfini, and L. Abd El-Rahman, "Effects of different organic and inorganic fertilizers on cucumber yield and some soil properties". World J. Agric. Sci., vol. 5, 2009, pp. 408–414.
[36] B. S. Kundu, and A. C. Gaur, "Effect of phosphor bacteria on the yield and phosphate Uptake of potato crop". Current Sci., vol. 49, 1980, pp. 159–160.