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# Bio, Mineral and Organic Fertilization Increase Head Yield, Enrich Mineral Content and Nutritional Value of Broccoli Heads

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**Abstract :** Two field experiments were carried in sandy soil opened field using drip irrigation system during two successive winter seasons in 2012/2013 and 2013/2014. This work aimed to investigate the effect of mineral and organic fertilizers with or without bio-fertilizers inoculation on yield, mineral content and nutritional value of broccoli heads. Five treatments of bio-fertilizers were applied using a mixture of nitrogen fixing and phosphorus solubilizing microorganisms (*Aztobacter chroococcum, Bacillus megaterium, Arbiscular mycorrhizae, Bacillus polymyxa*). Four equations of mineral and organic fertilizers were applied. Applying mixture of *Azotobacter chroococcum* and *Arbiscular mycorrhizae* recorded the highest values of total heads yield, heads mineral content, *i.e.* N, P, K, Ca and Mg and nutritional value of broccoli heads expressed as crude protein, total chlorophyll and total carotenoids contents. Fertilizer equation of 75% mineral + 25% organic of the recommended fertilizer units exhibited the highest values of total heads yield, the mineral content (P, K, Ca and Mg) and nutritional value of heads as compared with the other treatments. Fertilizer equation of 25% mineral+ 75% organic of the recommended fertilizer units achieved the highest vitamin C content of broccoli heads. The combined effect of the two mixtures of *Azotobacter chroococcum+ Arbiscular mycorrhizae* with equation of 75% mineral+ 25% organic of the recommended fertilizer units recorded the highest values of total heads yield, mineral content and nutritional value of broccoli heads except for vitamin C content. The highest vitamin C content was obtained by the equation of 25% mineral +75% organic fertilizer without bio-fertilization.
**Key words:** Broccoli; Bio-fertilizers; Mineral fertilizers; Organic fertilizers; Yield; Nutritional value; Mineral content.

## Introduction

Brassicaous vegetables represent a distinguished role of the human diet worldwide[1]. Broccoli is a high nutritional value crop belonging to Brassicacea and has been appointed as an anti-cancer source by American Cancer Society[2,3,4,5]. It is a rich source of vitamin A, vitamin $B_2$, calcium and proteins[6,7,1] as well as minerals especially K, S, P, Mg[8] and other compounds that could explain their preventive mechanisms in human health. The major protective dietary antioxidants are vitamins C, E and carotenoids[1].

**N**utritional value of broccoli plants is affected by many factors *i.e* genotype (species, cultivar), environmental conditions, soil fertility, and soil structure[9].Plant fertilization is the most important factor affecting plant quality[10]**.**The agronomical practices such as fertilization can highly influence average levels of bioactive components in brassica[11,1].

Transplanting broccoli in newly reclaimed soils faces many problems such as low soil organic matter contents, unreliable rainfall and poor soil nutrients availability coupled with poor soil management. As a result, many farmers intended to use large amounts of mineral fertilizers[12,13]**.** As known, application of mineral fertilizers have many negative effects on environment and human health[12]**.**Implementation of organic and bio-fertilizers in a fertilization system is considered a useful strategy for decreasing such negative effects. Many nitrogen fixing and phosphorus solubilizing microorganisms are widely used as bio-fertilizers. Therefore, this work was conducted to study the effect of bio, mineral and organic fertilizers supplementation on the total heads yield, mineral content and nutritional value of broccoli plants in the newly reclaimed sandy soils of Egypt.

## Materials And Methods

Two field experiments were conducted on broccoli plants (*Brassica oleracea* var *italic* plenck) at National Research Center farm, Nubaria, Beheira Governorate, Egypt during the two successive winter seasons of 2012/2013 and 2013/2014. The aim of this work was to study the effect of fertilizer sources, i.e. bio, mineral and organic on yield, mineral content and nutritional value of broccoli heads.

Physical and chemical analysis of soil samples were executed according to[14] (Table, 1).

**Table (1).Physical and chemical properties of the experimental soil.**

| | **Physical properties** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Season** | **Sand(%)** | | **Clay (%)** | | **Silt (%)** | **Texture** | | | | |
| **2012/2013** | 61.4 | | 4.9 | | 33.7 | Sandy | | | | |
| **2012/2013** | 58.4 | | 3.6 | | 38 | Sandy | | | | |
| | **Chemical properties** | | | | | | | | | |
| | | **pH** | **Cations (Meq./L)** | | | | **Anions (Meq./L)** | | | |
| **Season** | E.C.(dS/m) | | $Ca^{++}$ | $Mg^{++}$ | $Na^{++}$ | $K^{+}$ | $CO_3^{--}$ | $HCO_3^{-}$ | $Cl^{-}$ | $SO_4^{--}$ |
| **2012/2013** | 1.55 | 7.88 | 6.94 | 3.4 | 4.57 | 1.32 | Nil | 1.58 | 1.07 | 13.58 |
| **2012/2013** | 1.63 | 7.81 | 7.41 | 3.7 | 4.36 | 1.37 | Nil | 1.67 | 1.23 | 13.94 |

Ditches of 20 cm width and 20 cm depth were prepared at every irrigation line. Organic fertilizers (compost and rock phosphate), and calcium super phosphate as well as agricultural sulphur 100 Kg per faddan were spread through the ditches and coverd with sand. Drip irrigation lines were established over the ditches and soil was irrigated continuously for three days before transplanting. Compost analysis (table 2) was done according to[15]**.** Available phosphorus and available potassium have been evaluated according to[16]. Total nitrogen content was measured according to[15]**.**

**Table (2): Chemical analysis of the used compost in the two seasons of the experiment**.

| **Character** | **2013/2014** | **2012/2013** |
|---|---|---|
| **pH** | 7.50 | 7.80 |
| **E.C. (dSm-1)** | 4.30 | 3.50 |
| **Organic matter (%)** | 23.10 | 30.50 |
| **Organic carbon (%)** | 19.10 | 12.20 |
| **Total nitrogen (%)** | 1.30 | 1.17 |
| **C/N ratio** | 1:18 | 1:15 |
| **Total phosphorus (%)** | 0.75 | 0.48 |
| **Total potassium (%)** | 0.90 | 1.14 |

Rock phospahe(20.5% $P_2O_5$), was used as an organic phosphatic source. Rock phosphate were analyzed at central laboratories sector, the Egyptian mineral resources authority (EMRA), the Ministry of petroleum of Egypt as shown in Tables (3)**.**

**Table (3):Chemical analysis of the used rock phosphate**.

| **Content** | **(%)** | **Content** | **(%)** |
|---|---|---|---|
| **$SiO_2$** | 12.80 | **$Na_2O$** | 1.12 |
| **$TiO_2$** | 0.02 | **$K_2O$** | 0.05 |
| **$Al_2O_3$** | 0.35 | **$P_2O_5$** | 20.80 |
| **$Fe_2O_3$** | 1.12 | **Cl** | 1.56 |
| **MnO** | 0.07 | **$SO_3$** | 1.98 |
| **MgO** | 0.61 | **L.O.l** | 13.60 |
| **CaO** | 44.10 | | |

Broccoli transplants (45 days age) were sown 0.5 m apart on one side of the irrigation line, one transplant besides every irrigation eye. The area of the experimental plot was 11.2 $m^2$ consisted of two rows; each row was 8 m length and 0.7 m width.

The bio-fertilizer was kindly supported by Microbiology department Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt. It contains a mixture of $N_2$-fixing bacteria *(Aztobacterchroococcum* and *Bacillus polymyxa)* and phosphate dissolving bacteria such *(Bacillus megaterium)* or phosphate mobilizing bio-fertilizer (*Arbiscular mycorrhizea)*. The bio-fertilizers were prepared as mentioned by[17]**.***Mycorrhizae* was applied by dipping the roots of broccoli seedlings in liquid suspension of the *mycorrhizae* for a quarter of an hour, directly before transplanting. Other bio-fertilizaters treatments were applied by injection through irrigation water. Needed horticultural practices of growing broccoli were followed. Nitrogen source of mineral fertilizer (ammonium sulfate 21.5% N) and potassium sulfate (48% $K_2O$) at a rate of 60 $K_2O$ units/fed were applied within the growing season through irrigation system and were stopped two weeks before harvest.

1- Without: Without bio-fertilizer
2- Bio 1*: Aztobacter chroococcum+ Bacillus megaterium*
3-Bio2:*Aztobacter chroococcum+ Arbiscular mycorrhizae*
4-Bio 3: *Bacillus polymyxa+ Bacillus megaterium*
5-Bio 4:*Bacillus polymyxa+Arbiscular mycorrhizea*

Bio-fertilizer treatments were applied twice, the first was after 2 weeks and the second was after 5 weeks of transplanting.

**Mineral and organic fertilization equations:**

1- 100 % mineral of the recommended fertilizer unit (120 Unit N/fadden and 90 Unit $P_2O_5$/faddan).
2- 75% mineral + 25% organic (of the recommended fertilizer units).
3- 50% mineral + 50% organic (of the recommended fertilizer units).
4- 25% mineral + 75% organic (of the recommended fertilizer units).

**Recorded data :**

**Total head yield:**

All broccoli heads of each plot were harvested at the green mature stage. Primary head yield (main yield of the apical heads), secondary head yield (yield of the side heads) were recorded and the summation was the total heads yield**.**

**Mineral content of primary heads:**

The percentages of phosphorus and potassium in the acid digested samples of broccoli dry heads were determined**.** phosphorus was determined colorimetrically by NH4-Metavanidate method[18.] Potassium was flame-photometrically estimated[18].Calcium and magnesium were evaluated according to[18]**.**

**Nutritional value of primary heads:**

Ascorbic acid was evaluated using 2, 6 di-chlorophenol-indophenol method as described in[19]**.**

Crudeprotein content: Nitrogen content was estimated by modified Kjeldahl's methods[18]**.** Protein content was calculated by multiplying nitrogen percentage with a factor 6.25 according to [20]**.**

Total pigments content of primary heads: The total chlorophyll and total carotenoids contents of the apical head tissues were determined in representative fresh heads samples according to[21]**.** The obtained extracts were measured by spectrophotometer at the wave length of 663, 647 and 470 nm, using N,N-Dimethylformamide as a blank.

**Experimental design and Statistical analysis:**

Experimental plots were arranged in a split plot design system with three replicates. Bio-fertilizers treatments were assigned in the main plots, whereas other fertilizers treatments were allotted in the sub-plots. Data were statistically analyzed using Mstatic (M.S.) software. Comparison among different treatments means was made as illustrated by [22]**.**

## Results and Discussion

**A) Head mineral content and yield:**

**Effect of bio-fertilization:**

It is clear from Table (4) that head mineral content (P, K, Ca and Mg%) as well as primary, secondary and total heads yield were significantly affected by bio-fertilization in both seasons. The highest values of head mineral percentages, primary, secondary and total heads yield were produced by inoculated plants with Bio2 treatment (*Azotobacter+ mycrrohizae*). Whereas, the untreated plants produced the lowest values.

Enhancement of mineral content of heads, primary, secondary and total heads yields may be due to the role of *Azotobacter + mycrrohizae* in fixing nitrogen, producing some growth promoters such as GA3, IAA and cytokinines and increase nutrient dissolution[23,24]**.** The induced nutrients uptake enhancement in Bio 2 treatment may be due to many reasons such as root system efficiency improvement, rizosphere pH reduction and also the increase of nutrient dissolving and availability in the soil which encouraged photosynthetic activity of the treated plants. The mentioned photosynthesis activity encouragement produced more different metabolic substances consequently, more dry matter accumulation in plant tissues and reflected more heads yield. Many investigators reported similar results[25,26,27,28,29].

**Effect of mineral and organic sources:**

Data reported in Table (5) indicated that equation of 75% mineral + 25% organic of the recommended fertilizer units increased mineral content of primary heads (P, K, Ca and Mg), as well as primary, secondary and total yields. However, the lowest values were recorded by equation of 25% mineral + 75% organic of the recommended fertilizer units.

The increased total yields and nutrients in heads of broccoli plants could be attributed by the increase in nutrients absorption due to the combined effect of mineral fertilizers (more soluble and available to plants) and organic fertilizers (have different dynamics of nutrient availability)[30]**.** Using a fertilizer equation consisting of organic and mineral fertilizers improves the ability of soil to retain moisture and increase nutrients content of the soil solution and nutrients use efficiency from organic and mineral fertilizers[31]**.** They added that there is a negligible and parallel result between drip fertigation with 75% NPK (mineral fertilizer)+Humic substances (organic fertilizer) and drip fertigation with 100% NPK (mineral fertilizer) lonely on all macronutrients in head

tissues. Fertilizer equation of 75% mineral + 25% organic of the recommended fertilizer units increases in heads yield of broccoli plants may be due to its role in enhancing nutrients uptake, photosynthesis and biosynthesis capacities, carbohydrates and protein synthesis. Many investigators came to similar results[32,33].

**Interaction between bio-fertilizer and mineral and organic sources:**

Data presented in Table (6) indicated that application of bio-, mineral and organic fertilization treatments were significantly affected mineral content and total heads yield. The highest values of mineral content, primary, secondary and total heads yield were recorded by equation of 75% mineral + 25% organic of the recommended fertilizer units. On the other hand, the lowest values were recorded by equation of 25% mineral + 75% organic of the recommended fertilizer units without bio-fertilizer addition in both seasons.

Mineral content and total heads yield increase in broccoli heads tissues may be due to the induced plant roots absorption enhancement. This enhancement is caused by the interaction effect between bio-fertilizer with 75% mineral +25% organic [30]**.**Many investigators have obtained the same results similar to our findings[34,25].

**Table (4): Effect of bio-fertilization on head mineral content and yield of broccoli plants during 2012/2013 and 2013/2014.**

| Bio-fertilization treatments | Primary head yield (ton/fed.) | Secondary heads yield (ton/fed.) | Total heads yield (ton/fed.) | P (%) | K (%) | Ca (%) | Mg (%) |
|---|---|---|---|---|---|---|---|
| | **(2012-2013)** | | | | | | |
| **Without Bio** | 2.45 $^{E}$ | 1.24 $^{D}$ | 3.69 $^{E}$ | 2.09 $^{D}$ | 2.16 $^{D}$ | 1.57 $^{D}$ | 1.38 $^{E}$ |
| **Bio 1** | 3.53 $^{B}$ | 1.49 $^{B}$ | 5.02 $^{B}$ | 3.54 $^{B}$ | 3.76 $^{B}$ | 2.36 $^{B}$ | 3.37 $^{A}$ |
| **Bio 2** | 3.66 $^{A}$ | 1.56 $^{A}$ | 5.22 $^{A}$ | 4.18 $^{A}$ | 4.10 $^{A}$ | 2.66 $^{A}$ | 3.91 $^{A}$ |
| **Bio 3** | 3.38 $^{D}$ | 1.27 $^{D}$ | 4.65 $^{D}$ | 3.11 $^{C}$ | 3.48 $^{C}$ | 2.07 $^{C}$ | 2.87 $^{D}$ |
| **Bio 4** | 3.45 $^{C}$ | 1.41 $^{C}$ | 4.86 $^{C}$ | 3.52 $^{B}$ | 3.74 $^{B}$ | 2.20 $^{BC}$ | 3.33 $^{C}$ |
| **(2013-2014)** | | | | | | | |
| **Without Bio** | 2.13 $^{E}$ | 1.39 $^{E}$ | 3.52 $^{D}$ | 2.28 $^{E}$ | 2.28 $^{D}$ | 1.74 $^{E}$ | 1.89 $^{E}$ |
| **Bio 1** | 3.42 $^{B}$ | 1.88 $^{B}$ | 4.80 $^{B}$ | 3.90 $^{C}$ | 3.88 $^{B}$ | 2.89 $^{B}$ | 3.54 $^{B}$ |
| **Bio 2** | 3.69 $^{A}$ | 1.97 $^{A}$ | 5.16 $^{A}$ | 4.46 $^{A}$ | 3.95 $^{A}$ | 3.04 $^{A}$ | 3.98 $^{A}$ |
| **Bio 3** | 2.77 $^{D}$ | 1.52 $^{D}$ | 4.29 $^{C}$ | 3.72 $^{D}$ | 3.78 $^{C}$ | 2.45 $^{D}$ | 3.28 $^{D}$ |
| **Bio 4** | 2.98 $^{C}$ | 1.69 $^{C}$ | 4.68 $^{B}$ | 4.04 $^{B}$ | 3.84 $^{B}$ | 2.62 $^{C}$ | 3.33 $^{C}$ |

Values followed by the same letter (s) are not significantly different at 5%
Bio 1, *Aztobacterchroococcum+ Bacillus megaterium;* Bio 2,*Aztobacterchroococcum + Arbiscular mycorrhizae*
Bio 3, *Bacillus polymyxa + Bacillus megaterium;* Bio 4, *Bacillus polymyxa + Arbiscularmycorrhizea*

**Table (5): Effect of fertilizer equation on head mineral content and yield of broccoli plants during 2012/2013 and 2013/2014.**

| Fertilizer equation Treatments | Primary head yield (ton/fed.) | Secondary heads yield (ton/fed.) | Total heads Yield (ton/fed.) | P (%) | K (%) | Ca (%) | Mg (%) |
|---|---|---|---|---|---|---|---|
| | **(2012-2013)** | | | | | | |
| $^{25\%}$**M** + $^{75\%}$**O** | 2.37 $^{D}$ | 0.75 $^{D}$ | 3.12 $^{D}$ | 2.55 $^{B}$ | 2.76 $^{C}$ | 1.32 $^{C}$ | 2.40 $^{D}$ |
| $^{50\%}$**M** + $^{50\%}$**O** | 3.23 $^{C}$ | 1.31 $^{C}$ | 4.54 $^{C}$ | 2.63 $^{B}$ | 2.82 $^{C}$ | 1.69 $^{B}$ | 2.68 $^{C}$ |
| $^{75\%}$**M** + $^{25\%}$**O** | 3.82 $^{A}$ | 1.80 $^{A}$ | 5.63 $^{A}$ | 4.00 $^{A}$ | 4.29 $^{A}$ | 2.91 $^{A}$ | 3.50 $^{A}$ |
| $^{100\%}$**M** | 3.76 $^{B}$ | 1.71 $^{B}$ | 5.46 $^{B}$ | 3.98 $^{A}$ | 3.93 $^{B}$ | 2.78 $^{A}$ | 3.31 $^{B}$ |
| | **(2013-2014)** | | | | | | |
| $^{25\%}$**M** + $^{75\%}$**O** | 1.58 $^{C}$ | 0.84 $^{D}$ | 2.43 $^{D}$ | 2.51 $^{D}$ | 3.17 $^{D}$ | 1.55 $^{D}$ | 2.59 $^{D}$ |
| $^{50\%}$**M** + $^{50\%}$**O** | 2.32 $^{B}$ | 1.33 $^{C}$ | 3.65 $^{C}$ | 2.60 $^{C}$ | 3.30 $^{C}$ | 2.11 $^{C}$ | 2.99 $^{C}$ |
| $^{75\%}$**M** + $^{25\%}$**O** | 4.11 $^{A}$ | 2.32 $^{A}$ | 6.03 $^{A}$ | 4.83 $^{A}$ | 4.04 $^{A}$ | 3.33 $^{A}$ | 3.64 $^{A}$ |
| $^{100\%}$**M** | 3.99 $^{A}$ | 2.27 $^{B}$ | 5.85 $^{B}$ | 4.79 $^{B}$ | 3.68 $^{B}$ | 3.20 $^{B}$ | 3.59 $^{B}$ |

Values followed by the same letter (s) are not significantly different at 5%
M, Mineral; O, Organic

**Table (6): Effect of interaction between bio-fertilization and fertilizer equation on head mineral content and yield of broccoli plants during 2012/2013 and 2013/2014.**

| Bio-fertilization Treatments | Fertilizer equation | Primary head Yield (ton/fed.) | Secondary heads yield (ton/fed.) | Total heads Yield (ton/fed.) | P (%) | K (%) | Ca (%) | Mg (%) |
|---|---|---|---|---|---|---|---|---|
| **(2012-2013)** | | | | | | | | |
| **Without Bio** | **25% M + 75% O** | 1.92 $^{k}$ | 0.38 $^{l}$ | 2.31 $^{n}$ | 1.79 $^{g}$ | 1.67 $^{h}$ | 0.99 $^{h}$ | 1.16 $^{s}$ |
| | **50% M + 50% O** | 2.12 $^{j}$ | 1.14 $^{i}$ | 3.26 $^{l}$ | 1.85 $^{g}$ | 1.83 $^{h}$ | 1.30 $^{fgh}$ | 1.30 $^{r}$ |
| | **75% M + 25% O** | 2.73 $^{h}$ | 1.52 $^{ef}$ | 4.25 $^{j}$ | 2.35 $^{f}$ | 2.55 $^{g}$ | 1.97 $^{d}$ | 1.39 $^{q}$ |
| | **100%M** | 3.02 $^{g}$ | 1.91 $^{ab}$ | 4.93 $^{gh}$ | 2.37 $^{f}$ | 2.58 $^{g}$ | 2.05 $^{d}$ | 1.66 $^{p}$ |
| **Bio 1** | **25% M + 75% O** | **2.40 i** | **0.94 j** | **3.34 l** | **2.69 e** | **3.04 ef** | **1.48efg** | **2.83 l** |
| | **50% M + 50% O** | **3.63 e** | **1.43 fg** | **5.07 g** | **2.80 e** | **3.05 ef** | **1.77 de** | **2.92 k** |
| | **75% M + 25% O** | **4.10 ab** | **1.90 ab** | **6.00ab** | **4.36 b** | **4.69 b** | **3.24 b** | **3.96 c** |
| | **100%M** | **4.00 bc** | **1.69 cd** | **5.69 de** | **4.32 b** | **4.26 cd** | **2.97bc** | **3.76 e** |
| **Bio 2** | **25% M + 75% O** | 2.80 $^{h}$ | 1.12 $^{i}$ | 3.92 $^{k}$ | 3.38 $^{d}$ | 3.38 $^{e}$ | 1.75 $^{de}$ | 2.93 $^{k}$ |
| | **50% M + 50% O** | 3.64 e | 1.44 $^{fg}$ | 5.08 $^{g}$ | 3.43 $^{d}$ | 3.39 $^{e}$ | 2.05 $^{d}$ | 3.56 $^{g}$ |
| | **75% M + 25% O** | 4.16 a | 1.95 $^{a}$ | 6.11 $^{a}$ | 4.98 $^{a}$ | 5.04 $^{a}$ | 3.66 $^{a}$ | 4.96 $^{a}$ |
| | **100%M** | 4.05 $^{ab}$ | 1.72 $^{cd}$ | 5.76 $^{cd}$ | 4.95 $^{a}$ | 4.59 $^{bc}$ | 3.19 $^{b}$ | 4.20 $^{b}$ |
| **Bio 3** | **25% M + 75% O** | 2.35 $^{i}$ | 0.49 $^{l}$ | 2.84 $^{m}$ | 2.24 $^{f}$ | 2.72 $^{fg}$ | 1.10 $^{gh}$ | 2.37 $^{o}$ |
| | **50% M + 50% O** | 3.33 $^{f}$ | 1.20 $^{hi}$ | 4.53 $^{i}$ | 2.30 $^{f}$ | 2.79 $^{fg}$ | 1.67 $^{def}$ | 2.53 $^{n}$ |
| | **75% M + 25% O** | 4.05 $^{ab}$ | 1.78 $^{bc}$ | 5.83 $^{bcd}$ | 3.98 $^{c}$ | 4.46 $^{bc}$ | 2.76 $^{c}$ | 3.33 $^{h}$ |
| | **100%M** | 3.81 $^{d}$ | 1.60 $^{de}$ | 5.40 $^{f}$ | 3.93 $^{c}$ | 3.97 $^{d}$ | 2.74 $^{c}$ | 3.27 $^{i}$ |
| **Bio 4** | **25% M + 75% O** | 2.38 $^{i}$ | 0.80 $^{k}$ | 3.18 $^{l}$ | 2.66 $^{e}$ | 3.01 $^{f}$ | 1.26 $^{gh}$ | 2.73 $^{m}$ |
| | **50% M + 50% O** | 3.43 $^{f}$ | 1.33 $^{gh}$ | 4.76 $^{h}$ | 2.79 $^{e}$ | 3.04 $^{ef}$ | 1.68 $^{def}$ | 3.07 $^{j}$ |
| | **75% M + 25% O** | 4.07 $^{ab}$ | 1.88 $^{ab}$ | 5.95 $^{abc}$ | 4.34 $^{b}$ | 4.68 $^{b}$ | 2.94 $^{bc}$ | 3.87 $^{d}$ |
| | **100%M** | 3.91 $^{cd}$ | 1.63 $^{de}$ | 5.54 $^{ef}$ | 4.31 $^{b}$ | 4.25 $^{cd}$ | 2.93 $^{bc}$ | 3.67 $^{f}$ |

Values followed by the same letter (s) are not significantly different at 5%

Bio 1, *Aztobacterchroococcum+ Bacillus megaterium;* Bio 2,*Aztobacterchroococcum + Arbiscularmycorrhizea*

Bio 3, *Bacillus polymyxa + Bacillus megaterium;* Bio 4, *Bacillus polymyxa + Arbiscularmycorrhizea;*

M, Mineral; O, Organic

**Table (6): Continued**

| Bio-fertilization Treatments | Fertilizer equation | Primary head yield (ton/fed.) | Secondary heads yield (ton/fed.) | Total heads Yield (ton/fed.) | P (%) | K (%) | Ca (%) | Mg (%) |
|---|---|---|---|---|---|---|---|---|
| **(2013-2014)** | | | | | | | | |
| **Without Bio** | 25% M + 75% O | 1.32 $^{l}$ | 0.39 $^{l}$ | 1.72 $^{k}$ | 1.67 $^{l}$ | 1.79 $^{j}$ | 1.27 $^{m}$ | 1.71 $^{q}$ |
| | 50% M + 50% O | 1.99 $^{ij}$ | 1.20 $^{i}$ | 3.18 $^{i}$ | 1.80 $^{k}$ | 1.95 $^{i}$ | 1.78 $^{ij}$ | 1.84 $^{p}$ |
| | 75% M + 25% O | 2.10 $^{i}$ | 1.87 $^{f}$ | 3.97 $^{g}$ | 2.83 $^{fg}$ | 2.68 $^{h}$ | 1.94 $^{h}$ | 1.95 $^{o}$ |
| | 100%M | 3.11 $^{g}$ | 2.10 $^{d}$ | 5.21 $^{e}$ | 2.84 $^{fg}$ | 2.71 $^{h}$ | 1.95 $^{h}$ | 2.07 $^{n}$ |
| **Bio 1** | 25% M + 75% O | 1.71 $^{jk}$ | 1.03 $^{j}$ | 2.75 $^{j}$ | 2.72 $^{hi}$ | 3.53 $^{f}$ | 1.71 $^{j}$ | 2.84 $^{k}$ |
| | 50% M + 50% O | 2.51 $^{h}$ | 1.37 $^{g}$ | 3.88 $^{gh}$ | 2.81 $^{gh}$ | 3.68 $^{de}$ | 2.23 $^{f}$ | 3.34 $^{i}$ |
| | 75% M + 25% O | 4.93 $^{a}$ | 2.57 $^{ab}$ | 6.50 $^{a}$ | 5.07 $^{c}$ | 4.37 $^{b}$ | 3.87 $^{a}$ | 4.07 $^{c}$ |
| | 100%M | 4.52 $^{bc}$ | 2.54 $^{b}$ | 6.07 $^{bcd}$ | 5.02 $^{c}$ | 3.92 $^{c}$ | 3.74 $^{b}$ | 3.92 $^{d}$ |
| **Bio 2** | 25% M + 75% O | 2.06 $^{ij}$ | 1.24 $^{hi}$ | 3.30 $^{i}$ | 2.91 $^{ef}$ | 3.59 $^{f}$ | 1.87 $^{hi}$ | 2.85 $^{k}$ |
| | 50% M + 50% O | 2.94 $^{g}$ | 1.41 $^{g}$ | 4.35 $^{f}$ | 3.00 $^{e}$ | 3.71 $^{d}$ | 2.63 $^{e}$ | 3.86 $^{e}$ |
| | 75% M + 25% O | 5.01 $^{a}$ | 2.65 $^{a}$ | 6.66 $^{a}$ | 6.01 $^{a}$ | 4.55 $^{a}$ | 3.93 $^{a}$ | 4.68 $^{a}$ |
| | 100%M | 4.75 $^{ab}$ | 2.57 $^{ab}$ | 6.32 $^{abc}$ | 5.92 $^{a}$ | 3.94 $^{c}$ | 3.75 $^{b}$ | 4.54 $^{b}$ |
| **Bio 3** | 25% M + 75% O | 1.28 $^{l}$ | 0.54 $^{k}$ | 1.82 $^{k}$ | 2.59 $^{j}$ | 3.39 $^{g}$ | 1.37 $^{l}$ | 2.74 $^{m}$ |
| | 50% M + 50% O | 1.97 $^{ij}$ | 1.31 $^{gh}$ | 3.29 $^{i}$ | 2.70 $^{i}$ | 3.56 $^{f}$ | 1.87 $^{hi}$ | 2.96 $^{j}$ |
| | 75% M + 25% O | 4.12 $^{de}$ | 2.24 $^{c}$ | 6.37 $^{ab}$ | 4.83 $^{d}$ | 4.28 $^{b}$ | 3.43 $^{c}$ | 3.74 $^{g}$ |
| | 100%M | 3.70 $^{f}$ | 2.00 $^{e}$ | 5.70 $^{d}$ | 4.76 $^{d}$ | 3.89 $^{c}$ | 3.11 $^{d}$ | 3.68 $^{h}$ |
| **Bio 4** | 25% M + 75% O | 1.54 $^{kl}$ | 1.01 $^{j}$ | 2.55 $^{j}$ | 2.64 $^{ij}$ | 3.53 $^{f}$ | 1.51 $^{k}$ | 2.81 $^{l}$ |
| | 50% M + 50% O | 2.18 $^{hi}$ | 1.36 $^{g}$ | 3.54 $^{hi}$ | 2.70 $^{i}$ | 3.59 $^{ef}$ | 2.06 $^{g}$ | 2.98 $^{j}$ |
| | 75% M + 25% O | 4.37 $^{cd}$ | 2.28 $^{c}$ | 6.65 $^{a}$ | 5.43 $^{b}$ | 4.32 $^{b}$ | 3.46 $^{c}$ | 3.77 $^{f}$ |
| | 100%M | 3.84 $^{ef}$ | 2.13 $^{d}$ | 5.97 $^{cd}$ | 5.38 $^{b}$ | 3.90 c | 3.44 $^{c}$ | 3.76 $^{fg}$ |

Values followed by the same letter (s) are not significantly different at 5%

Bio 1, *Aztobacter chroococcum+ Bacillus megaterium;* Bio 2,*Aztobacter chroococcum + Arbiscular rmycorrhizea*

Bio 3, *Bacillus polymyxa + Bacillus megaterium;* Bio 4, *Bacillus polymyxa + Arbiscular rmycorrhizea;*

M, Mineral; O, Organic

**B) Nutritional value of broccoli heads:**

**Effect of bio-fertilization:**

It is clear from Table (7) that nutritional value of broccoli heads *i.e.* crude protein, vitamin C, total chlorophyll and total carotenoids were significantly enhanced by bio-fertilization in both seasons. The highest values of crude protein, total chlorophyll and total carotenoids were obtained by plants that inoculated with Bio 2treatment (*Azotobacter*+ *mycrrohizae*).While, no significant differences in crude protein content in broccoli heads were detected among all different bio-fertilization treatments in the second season. Whereas, the untreated plants produced the lowest values of crude protein, total chlorophyll and total carotenoids. The highest vitamin C content in broccoli heads was recorded in the non-inoculated plants following by Bio 3 treatment without significant differences in the first season when compared with that of the other bio-fertilization treatments, in both seasons. On the contrary, the lowest vitamin C content was recorded by Bio 2 treatment in both seasons.

The increase in crude protein, total chlorophyll and total carotenoids content in broccoli heads by bio-fertilization may be due to the use of beneficial micro-organisms that could colonize the roots and increase plant growth by improving supply or availability of essential nutrients to the plants[35].However, there is an inverse relationship between the increase of nutrients in plant tissue and secondary metabolites synthesis such as vitamin C[36,37]**.** Our findings agree with those reported by[38]**.**

**Effect of mineral and organic fertilizer sources:**

Data reported in Table (8) indicated that equation of 75% mineral+ 25% organic of the recommended fertilizer units increased nutritional value (crude protein, total chlorophyll and total carotenoids) of broccoli heads except with vitamin C. However, the lowest values were recorded by plants that treated with equation of 25% mineral+ 75% organic of the recommended fertilizer units. On the other hand, the highest and lowest values of vitamin C were obtained by the plants that received the fertilizer equation of 25% mineral+ 75% organic and 100% mineral of the recommended fertilizer units, respectively in each season.

Increases in nutritional value (crude protein, total chlorophyll and total carotenoids) may be caused by the increased nutrient absorption, vegetative growth and photosynthetic activity due to effect of the equation of 75% mineral+ 25% organic. Also, we should consider the high availability of N, P and K as an important factor in developing that increase. However, the lowest produced content of crude protein, total chlorophyll and total carotenoids in case of equation, 25% mineral+ 75% organic of the recommended fertilizer units treatment may be caused by the slow release of nitrogen by organic matter decay[39].These results are in accordance with those obtained from[32,36,33]**.**

**Interaction effect of bio-, mineral and organic fertilizer sources:**

Data presented in Table (9) indicated that application of bio-, mineral and organic fertilizers treatments significantly affected crude protein, vitamin C, total chlorophyll and total carotenoids. The highest values were recorded by the equation of 75% mineral+ 25% organic of the recommended units except with vitamin C. Highest vitamin C content in broccoli heads was obtained by the equation of 25% mineral+ 75% organic of the recommended fertilizer units. On the other hand, the lowest values of crude protein, total chlorophyll and total carotenoids were recorded in case of25% mineral + 75% organic of the recommended fertilizer units without any bio-fertilizers addition in both seasons.

Simultaneous application of bio, mineral and organic fertilizers led to the highest content of crude protein, total chlorophyll and total carotenoids compared to the other treatments, which may be due to the induced enhancement of soil fertility and nutrient supply and photosynthetic activity. These results were similar to[40,41].

**Table (7): Effect of bio-fertilization on nutritional value of broccoli heads during2012-2013/2013-2014.**

| Bio-fertilization treatments | Nutritional value of primary head | | | |
|---|---|---|---|---|
| | **Protein (%)** | **Vitamin C (mg/100g F.W.)** | **Chlorophyll (mg/100g F.W)** | **Carotenoids (mg/100gF.W)** |
| | **(2012-2013)** | | | |
| **without Bio** | 13.66$^{C}$ | 77.14$^{A}$ | 11.18 $^{C}$ | 4.47 $^{D}$ |
| **Bio 1** | 18.28$^{AB}$ | 68.70$^{BC}$ | 12.71 $^{B}$ | 5.09 $^{B}$ |
| **Bio 2** | 19.91$^{A}$ | 66.79$^{C}$ | 13.64 $^{A}$ | 5.45 $^{A}$ |
| **Bio 3** | 16.01$^{BC}$ | 75.56$^{A}$ | 11.70 $^{C}$ | 4.68 $^{C}$ |
| **Bio 4** | 17.98$^{AB}$ | 71.50$^{B}$ | 12.39 $^{C}$ | 4.96 $^{B}$ |
| | **(2013-2014)** | | | |
| **without Bio** | 10.15$^{B}$ | 75.54$^{A}$ | 9.67 $^{E}$ | 4.20 $^{D}$ |
| **Bio 1** | 18.03$^{A}$ | 60.20$^{D}$ | 11.92 $^{B}$ | 5.18 $^{B}$ |
| **Bio 2** | 20.71$^{A}$ | 56.84$^{E}$ | 12.86 $^{A}$ | 5.59 $^{A}$ |
| **Bio 3** | 17.02$^{A}$ | 68.28$^{B}$ | 10.63 $^{D}$ | 4.62 $^{C}$ |
| **Bio 4** | 19.96$^{A}$ | 63.33$^{C}$ | 11.52 $^{C}$ | 5.01 $^{B}$ |

Values followed by the same letter (s) are not significantly different at 5%
Bio 1, *Aztobacter chroococcum+ Bacillus megaterium;*
Bio 2,*Aztobacter chroococcum + Arbiscular mycorrhizea*
Bio 3, *Bacillus polymyxa + Bacillus megaterium;*
Bio 4, *Bacillus polymyxa + Arbiscular mycorrhizea*

**Table (8): Effect of fertilizer equation on nutritional value of broccoli heads during 2012-2013/2013-2014**

| Fertilizer equation treatments | Nutritional value in primary head | | | |
|---|---|---|---|---|
| | **Protein** (%) | **Vitamin C** (mg/100g F.W.) | **Chlorophyll**(mg/100g F.W) | **Carotenoids** (mg/100gF.W) |
| | **(2012-2013)** | | | |
| **$^{25\%}$ M +$^{75\%}$O** | 13.60$^{C}$ | 81.97$^{A}$ | 8.20 $^{D}$ | 3.28 $^{D}$ |
| **$^{50\%}$ M + $^{50\%}$ O** | 15.19$^{C}$ | 76.79$^{B}$ | 10.78 $^{C}$ | 4.31 $^{C}$ |
| **$^{75\%}$ M + $^{25\%}$ O** | 22.07$^{A}$ | 66.70$^{C}$ | 15.54 $^{A}$ | 6.22 $^{A}$ |
| **$^{100\%}$M** | 17.81$^{B}$ | 62.29$^{D}$ | 14.78 $^{B}$ | 5.91 $^{B}$ |
| | **(2013-2014)** | | | |
| **$^{25\%}$ M + $^{75\%}$ O** | 9.23$^{C}$ | 76.48$^{A}$ | 7.22 $^{D}$ | 3.14 $^{D}$ |
| **$^{50\%}$ M + $^{50\%}$ O** | 12.24$^{B}$ | 67.64$^{B}$ | 9.68 $^{C}$ | 4.21 $^{C}$ |
| **$^{75\%}$ M + $^{25\%}$ O** | 24.59$^{A}$ | 60.45$^{C}$ | 14.78 $^{A}$ | 6.43 $^{A}$ |
| **$^{100\%}$M** | 22.64$^{A}$ | 54.77$^{D}$ | 13.60 $^{B}$ | 5.91 $^{B}$ |

Values followed by the same letter (s) are not significantly different at 5%
M, Mineral; O, Organic

**Table (9): Interaction between bio-fertilization and fertilizer equation on nutritional value of broccoli heads during 2012-2013/2013-2014.**

| Bio-fertilization treatments | Fertilizer equation treatments | Nutritional value in primary head | | | |
|---|---|---|---|---|---|
| | | **Protein** (%) | **Vitamin C** (mg/100g F.W.) | **Chlorophyll**(mg/ 100g F.W**)** | **Carotenoids** (mg/100gF.W) |
| | | **(2012-2013)** | | | |
| **Without Bio** | **25% M + 75% O** | 9.74$^{i}$ | 89.38$^{a}$ | 7.68 $^{h}$ | 3.07 $^{l}$ |
| | **50% M + 50% O** | 10.47$^{hi}$ | 85.38$^{a}$ | 9.37$^{gh}$ | 3.75$^{ijk}$ |
| | **75% M + 25% O** | 16.42$^{d-g}$ | 69.33$^{d-g}$ | 12.51 $^{cde}$ | 5.01 $^{ghi}$ |
| | **100%M** | 18.00$^{def}$ | 64.47$^{f-i}$ | 15.15$^{cde}$ | 6.06$^{fgh}$ |
| **Bio 1** | **25% M + 75% O** | 16.08$^{d-g}$ | 77.23$^{bcd}$ | 8.35 $^{h}$ | 3.34$^{jkl}$ |
| | **50% M + 50% O** | 17.40$^{d-g}$ | 71.15$^{def}$ | 11.28$^{def}$ | 4.51$^{ghi}$ |
| | **75% M + 25% O** | 22.74$^{abc}$ | 65.62$^{f-i}$ | 15.88 $^{b}$ | 6.35 $^{cd}$ |
| | **100%M** | 16.90$^{d-g}$ | 60.82$^{hi}$ | 15.35$^{cde}$ | 6.14$^{efg}$ |
| **Bio 2** | **25% M + 75% O** | 15.31$^{fg}$ | 71.76$^{def}$ | 8.92 $^{h}$ | 3.57 $^{jkl}$ |
| | **50% M + 50% O** | 17.47$^{d-g}$ | 69.33$^{d-g}$ | 11.98$^{def}$ | 4.79$^{ghi}$ |
| | **75% M + 25% O** | 26.34$^{a}$ | 66.41$^{f-i}$ | 17.24 $^{a}$ | 6.90 $^{a}$ |
| | **100%M** | 20.50$^{b-e}$ | 59.68$^{i}$ | 16.41$^{bcd}$ | 6.56$^{ef}$ |
| **Bio 3** | **25% M + 75% O** | 12.88$^{ghi}$ | 87.56$^{a}$ | 7.92 $^{h}$ | 3.17 $^{kl}$ |
| | **50% M + 50% O** | 14.73$^{fgh}$ | 82.70$^{abc}$ | 10.38$^{fgh}$ | 4.15 $^{h-k}$ |
| | **75% M + 25% O** | 21.03$^{bcd}$ | 68.11$^{e-h}$ | 15.55$^{bcd}$ | 6.22 $^{de}$ |
| | **100%M** | 15.38$^{fg}$ | 63.86$^{f-i}$ | 12.94$^{efg}$ | 5.17$^{ghi}$ |
| **Bio 4** | **25% M + 75% O** | 13.98$^{f-i}$ | 83.91$^{ab}$ | 8.10 $^{h}$ | 3.24 $^{kl}$ |
| | **50% M + 50% O** | 15.88$^{efg}$ | 75.40$^{cde}$ | 10.90$^{gh}$ | 4.36$^{hij}$ |
| | **75% M + 25% O** | 23.80$^{ab}$ | 64.05$^{f-i}$ | 16.53 $^{bc}$ | 6.61$^{bc}$ |
| | **100%M** | 18.24$^{c-f}$ | 62.64$^{ghi}$ | 14.04$^{cde}$ | 5.62$^{ab}$ |

Values followed by the same letter (s) are not significantly different at 5%

Bio 1, *Aztobacter chroococcum+ Bacillus megaterium;*

Bio 2,*Aztobacter chroococcum + Arbiscular mycorrhizea*

Bio 3, *Bacillus polymyxa + Bacillus megaterium;*

Bio 4, *Bacillus polymyxa + Arbiscular mycorrhizea;*

M, Mineral; O, Organic.

**Table (9): Continued**

| Bio-fertilization treatments | Fertilizer equation treatments | Nutritional value in primary head | | | |
|---|---|---|---|---|---|
| | | **Protein** (%) | **Vitamin C** (mg/100g F.W.) | **Chlorophyll**(mg/100g F.W) | **Carotenoids** (mg/100gF.W) |
| | | **(2013-2014)** | | | |
| **without Bio** | **$^{25\%}$M + $^{75\%}$O** | 6.45$^{e}$ | 91.34$^{a}$ | 6.72$^{o}$ | 2.92$^{i}$ |
| | **$^{50\%}$M + $^{50\%}$O** | 8.31$^{de}$ | 84.63$^{b}$ | 8.17$^{kl}$ | 3.55$^{efg}$ |
| | **$^{75\%}$M + $^{25\%}$O** | 10.88$^{de}$ | 68.43$^{d}$ | 11.31$^{hi}$ | 4.92$^{d}$ |
| | **$^{100\%}$M** | 14.98$^{d}$ | 57.76$^{e-h}$ | 12.45$^{de}$ | 5.41$^{ef}$ |
| **Bio 1** | **$^{25\%}$M + $^{75\%}$O** | 8.94$^{de}$ | 70.87$^{d}$ | 7.30$^{mn}$ | 3.18$^{g}$ |
| | **$^{50\%}$M + $^{50\%}$O** | 13.81$^{de}$ | 58.67$^{e-h}$ | 10.20$^{hij}$ | 4.43$^{efg}$ |
| | **$^{75\%}$M + $^{25\%}$O** | 25.43$^{abc}$ | 57.45$^{e-h}$ | 16.85$^{b}$ | 7.33$^{b}$ |
| | **$^{100\%}$M** | 23.94$^{bc}$ | 53.79$^{hi}$ | 13.34$^{f}$ | 5.80$^{e}$ |
| **Bio 2** | **$^{25\%}$M + $^{75\%}$O** | 12.42$^{de}$ | 61.72$^{e}$ | 7.72$^{lm}$ | 3.35$^{fg}$ |
| | **$^{50\%}$M + $^{50\%}$O** | 13.94$^{de}$ | 57.45$^{e-h}$ | 10.78$^{hi}$ | 4.69$^{efg}$ |
| | **$^{75\%}$M + $^{25\%}$O** | 30.21$^{a}$ | 56.84$^{e-h}$ | 17.74$^{a}$ | 7.71$^{a}$ |
| | **$^{100\%}$M** | 26.25$^{abc}$ | 51.35$^{i}$ | 15.21$^{ef}$ | 6.61$^{d}$ |
| **Bio 3** | **$^{25\%}$M + $^{75\%}$O** | 8.83$^{de}$ | 79.41$^{c}$ | 7.15$^{o}$ | 3.11$^{h}$ |
| | **$^{50\%}$M + $^{50\%}$O** | 11.60$^{de}$ | 76.97$^{c}$ | 9.18$^{jkl}$ | 3.99$^{efg}$ |
| | **$^{75\%}$M + $^{25\%}$O** | 25.39$^{abc}$ | 60.50$^{ef}$ | 13.33$^{cd}$ | 5.80$^{c}$ |
| | **$^{100\%}$M** | 22.26$^{c}$ | 56.23$^{fgh}$ | 12.84$^{h}$ | 5.58$^{ef}$ |
| **Bio 4** | **$^{25\%}$M + $^{75\%}$O** | 9.53$^{de}$ | 79.07$^{c}$ | 7.18$^{no}$ | 3.12$^{efg}$ |
| | **$^{50\%}$M + $^{50\%}$O** | 13.52$^{de}$ | 60.50$^{ef}$ | 10.08$^{ijk}$ | 4.38$^{efg}$ |
| | **$^{75\%}$M + $^{25\%}$O** | 31.03$^{a}$ | 59.01$^{efg}$ | 14.68$^{c}$ | 6.38$^{b}$ |
| | **$^{100\%}$M** | 25.77$^{abc}$ | 54.74$^{ghi}$ | 14.15$^{g}$ | 6.15$^{ef}$ |

Values followed by the same letter (s) are not significantly different at 5%
Bio 1, *Aztobacter chroococcum+ Bacillus megaterium;*
Bio 2,*Aztobacterchroococcum + Arbiscular mycorrhizea*
Bio 3, *Bacillus polymyxa + Bacillus megaterium;*
Bio 4, *Bacillus polymyxa + Arbiscular mycorrhizea;*
M, Mineral; O, Organic.

## Conclusion

Our results indicated that the combined effect of Bio 2 with equation of 75% mineral+ 25% organic of the recommended fertilizer units resulted in higher heads yield of broccoli plants, their nutrients content (P, K, Ca and Mg) as well as the nutritional value (crude protein, total chlorophyll and total carotenoids). These results might save about 25% of the mineral fertilizer and their expenses. These results enable farmers to produce higher heads yields with high nutritional value. In addition, producing safe product for human consumption**.**

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