

Effect of Mineral and Bio-N Fertilization on Growth, Fruits Yield and Chemical Constituents of Pumpkin (*Cucurbita moschata* Duchesne)

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SIGNIFICANT positive influences of fertilization with $\frac{2}{3}$ of the recommended mineral-N dose + inoculation three times with a mixed bio-fertilizer (*Azotobacter chroococcum* + *Azospirillum brasilense*) were observed on growth traits, fruits yield and leaf N, P, K, NO_3^- and NO_2^- contents of pumpkin plants compared to fertilization with $\frac{2}{3}$ of the recommended mineral-N dose + inoculation once or twice with the same mixed bio-fertilizer. However, no statistical differences in the aforementioned parameters were noted between fertilization with $\frac{2}{3}$ of the recommended mineral-N dose + inoculation three times with a mixed bio-fertilizer and addition of whole recommended dose of mineral-N (control). The least significant mean values of leaf NO_3^- and NO_2^- content were attained at fertilization with $\frac{2}{3}$ of the recommended mineral-N dose + inoculation three times with a mixed bio-fertilizer. Therefore, inoculation with a mixed bio-fertilizer three times can substitute partially of mineral-N fertilizer and contribute to safety food.

Keywords: Bio-fertilization, growth, nitrate, nitrite, yield, quality, pumpkin.

Pumpkin is widely cultivated on newly-reclaimed soils in the Middle Eastern countries, including Egypt. It is consumed in different local dishes and used for some food industries such as jams, purees and cakes. Mineral fertilizers, particularly mineral-nitrogen, are an important mean of plant nutrition, growth and yield; however, they are also a potential source of environmental pollution (Hartman, 1988). An attention has therefore focused on alternative fertilizers, including bio-fertilizers. Nowadays, there is renewed interest in bio-fertilizers for nutrient supply and improve soil fertility and productivity. The integrated use of bio-fertilizers and mineral fertilizers is considered 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 (Singh *et al.*, 1999, Bhatia *et al.*, 2001 and Palm *et al.*, 2001). Bio-fertilizers are considered eco-friendly way to sustainable agriculture. They positively affect plant growth and yield, reduce negative effects of chemical fertilizers and minimize some chemical levels 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 N₂-fixation with cereals and other non-legumes by establishing N₂-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, reduction in membrane potential of roots, synthesis of some enzymes such as ACC deaminase that modulate the level of plant hormones. Free living N₂-fixing bacteria such as *Azotobacter* and *Azospirillum* have the ability not only to fix nitrogen but also to release certain phytohormones as gibberellins (GA₃), indole acetic acid (IAA) and cytokinins which could stimulate plant growth, increase the availability of nutrients for plant roots and increase the capacity of photosynthesis process (Ibrahim & Abd El-Aziz, 1977, Fayez *et al.*, 1985 and Abdel-Latif *et al.*, 2001). Several reports indicated that the inoculation of some crops with bio-fertilizers singly or in combination with mineral fertilizers improved plant growth, yield and chemical composition (Hanafy *et al.*, 1997, Gadallah & El-Masry, 2006, Osman, 2007 and Howladar *et al.*, 2013). Inoculation of potato tuber seeds with a mixed bio-fertilizer; *Azotobacter chroococcum* + *Azospirillum brasilense* significantly increased growth, tubers yield and its components (Ashour *et al.*, 1997 and Osman, 2007).

The objective of the current study was to assess the effect of fertilization with mineral-N at the recommended dose versus application $\frac{2}{3}$ of the recommended mineral-N dose + inoculation with a mixed biofertilizer (*Azotobacter chroococcum* + *Azospirillum brasilense*) once, twice and thrice on growth traits, leaf chemical constituents specially nitrate and nitrite contents as contaminated agents, fruits yield and quality of pumpkin (*Cucurbita moschata* Duchesne) grown under Egyptian conditions.

Material and Methods

Two field experiments were conducted during the summer seasons of 2012 and 2013 at the experimental Farm, Faculty of Agriculture, Fayoum University, Egypt. In order to identify some physio-chemical properties of the experimental site, soil samples preceding the initiation of each experiment and 8 weeks after application of treatments were collected and analyzed according to the procedures of Wilde *et al.* (1985) and the obtained results are shown in Table 1.

Seeds of pumpkin cultivar balady were seeded in 11 and 20 March 2012 and 2013, consecutively on rows 8m long, 2m wide and in-row spacing of 50cm. Each experiment included four treatments as follow:

- Fertilization with mineral N fertilizer at the recommended dose; 125 kg N fed^{-1} (control).
- Fertilization with $\frac{2}{3}$ of the recommended mineral N dose + inoculation once with a mixture of bio-N fertilizers (strains of *Azotobacter chroococcum* FN and *Azospirillum brasilense* FN 17 in a ratio of 1:1).
- Fertilization with $\frac{2}{3}$ of the recommended mineral N dose + inoculation twice with a mixture of bio-N fertilizers.
- Fertilization with $\frac{2}{3}$ of the recommended mineral N dose + inoculation thrice with a mixture of bio-N fertilizers.

The experimental layout was a randomized complete blocks design with four replicate. The area devoted for each experimental unit was 80 m² including 5 rows. Each two adjacent experimental units were separated by 1 m alley. Mineral N fertilizer was applied during soil preparation and ammonium nitrate (33.5 %N) was the respective form. Inoculation with mixed biofertilizer was performed after 2 weeks (once), 2 and 4 weeks (twice) and 2, 4 and 6 weeks (thrice) of seed sowing.

Preparation of bio-N fertilizer inoculant and inoculation

Soil samples of the experimental site were collected and the strains of non-symbiotic N₂-fixing bacteria; *A. chroococcum* FN 33 and *A. brasilense* FN 17 were isolated and identified by the microbiology lab., Faculty of Agriculture, Fayoum University. Modified Ashby's medium (Hegazi and Niemela, 1976) and Dobereiner medium (Dobereiner *et al.*, 1976) were used to grow *A. chroococcum* and *A. brasilense*, consecutively. At the logarithmic growth phase, 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 re-suspended in the same buffer to the final concentration of about 4 × 10⁸ cfu ml⁻¹. Isolates and inoculates were prepared immediately before inoculation. The rhizosphere of each plant was injected with about 35 ± 5 ml plant⁻¹ of the aforementioned mixture of bio-fertilizers.

During soil preparation, identical doses of 15 m³ farmyard manure, 150 kg calcium superphosphate (15.5% P₂O₅) and 100 kg elemental sulfur fed^{-1} were added while after complete earthing, 48 kg K₂O fed^{-1} at two equal portions; 20 and 40 days after seed sowing was applied. All other agro-management practices for commercial production of pumpkin were followed whenever it was necessary.

Data recorded

Growth measurements

After 8 weeks of seed sowing, five plants were randomly chosen from the outer two rows in each experimental unit, cut off at the ground level and the following measurements were recorded; plant height, number of leaves plant⁻¹, canopy dry weight plant⁻¹ after drying in a forced-air oven at 70 °C till constant weight and leaf area plant⁻¹ using a digital LI-3000 Portable area meter (LI-COR Lincoln, Nebraska, USA).

TABLE 1. Physical and chemical characteristics of the experimental soil before application (BT) and 8-weeks after application (AT) in 2012 and 2013 seasons.

Clay	Composition [% (v/w)]		pH	EC (dS m ⁻¹)	OC [#] (g kg ⁻¹)	N (mg kg ⁻¹)	P (mg kg ⁻¹)	K (mg kg ⁻¹)	Ca (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Zn (mg kg ⁻¹)
	Loam	Sand										
22.3	24.1	53.6	7.80	4.6	1.24	38.4	8.4	74.9	22.3	8.3	3.2	1.3
BT, 2012												
AT [soil treated with 65% recommended N dose + 3 doses of bio-fertilization (AT + AZ)*], 2012												
22.3	23.9	53.8	7.52	4.4	1.30	44.3	9.3	80.0	22.7	9.4	4.0	1.6
BT, 2013												
AT [soil treated with 65% recommended N dose + 3 doses of bio-fertilization (AT + AZ)*], 2013												
25.1	22.7	52.2	7.44	4.7	1.21	40.4	9.0	78.5	24.5	8.2	3.6	1.5

[#]OC = organic content.

BT and AT = before and after application

* AT+AZ = a mixture of *Azotobacter chroococcum* and *Azospirillum brasilense*, respectively in a ratio of 1:1 (w/w).

Fruits yield and quality

In each experimental unite, plants of the three inner rows were left to grow till fruits approached the marketable stage. Ten harvested fruits were utilized to measure the following parameters; average fruit weight (kg), fruit length (cm); longitudinal axe starting from peduncle to blossom end and maximum fruit diameter (cm). Total fruits yield fed^{-1} (ton) was calculated from the entire harvested fruits of the three inner rows.

Chemical constituents

After 8 weeks of seed sowing, leaf N, P and K contents were determined. Leaf N content was estimated using the Microkjeldahal apparatus as described in A.O.A.C. (1995). Leaf P content was determined as outlined by Jackson (1967). Leaf potassium content was assayed using a Perkin-Elmer Model 52-A Flame Photometer (Page *et al.*, 1982).

Leaf samples for nitrate and nitrite determinations were collected 8 weeks after seed sowing. Leaf samples were washed with tap water, rinsed numerous times in distilled water, cut into small uniform size pieces and dried in a forced-air oven at 105 C° until they became brittle and crisp to prevent contamination. The dried leaf samples were grounded and one gram of each sample was placed in a 100 ml polyethylene or glass bottle and 40 ml of distilled water was added, then capped and shaken for 30 min. The solution sample was filtered and the filtrate was made up to 100 ml in a volumetric flask (Radojevic and Bashkin, 1999). Spectrophotometer (Model 2000; Kwf Sci-Tech Development Co. Ltd., Beijing, P.R. China) at a wavelength of 543 nm was utilized to record leaf $\text{NO}_3\text{-N}$ concentration. Leaf $\text{NO}_3\text{-N}$ concentration converted to NO_3^- by multiplying in a conversion factor of 4.4 (La Motte, 2000). Leaf NO_3^- content was calculated according to the following formula .

$$\text{NO}_3^- \text{ content } (\mu\text{g g}^{-1}) = C \times V / M \quad \text{where,}$$

C = concentration of NO_3^- in leaf sample ($\mu\text{g g}^{-1}$)
V = total volume of the sample solution (100 ml)
M = weight of the sample (1.0 g)

The data obtained were converted to $\text{mg NO}_3^- \text{ g}^{-1}$ leaf D.W.

The same analytical method to determine NO_2^- content was followed except different reagents and conversion factor of 3.3 (LaMotte, 2000).

Statistical analysis

Appropriate analysis of variance on obtained results of the two experimental seasons was performed according to the design used (Snedecor and Cochran, 1980). Least significant difference test (LSD) at $p \leq 0.05$ level was utilized to verify the significant difference between treatments.

Results and Discussion

Growth measurements

Fertilization of pumpkin plants with $\frac{2}{3}$ of the recommended N- mineral fertilizer and inoculation three times with a mixed bio-fertilizer significantly resulted in taller plant height, heavier canopy dry weight, more number of leaves and bigger area of leaves plant^{-1} than those inoculated one time and/or two times (Table 2). However, no significant difference in the aforementioned vegetative traits between fertilization with the recommended N-mineral fertilizer dose (control) and the application $\frac{2}{3}$ recommended N- mineral fertilizer plus inoculation three times with a mixed bio-fertilizer. The noticeable increases of growth traits of pumpkin plants by the increase of applied bio-fertilizer dose may be related to the efficiency of non-symbiotic N_2 -fixing bacteria in the reduction of soil pH (Table 1) by secreting organic acids as acetic, propionic, fumaric and succinic (Singh and Kapoor, 1999) and consequently more solubility and availability of nutrients for plants are devoted. Also, *Azotobacter* and *Azospirillum* strains produced adequate amount of indole acetic acid, gibberellins-like substance and cytokinins which increased the surface area per unit root length and were responsible for root hair branching with an eventual increase in acquisition of nutrients from the soil (Martin *et al.*, 1989 and Jagnow *et al.*, 1991). Obtained results are in accordance with results of several investigators (Fayez *et al.*, 1985, Abdel-Latif *et al.*, 2001 and Gadallah & El-Masry, 2006). In addition, Osman (2007), on potato and Howladar *et al.* (2013), on *Brassica oleraceae*, found that single inoculation of bio-N fertilizer or in combination with chemical fertilizers positively affected growth characters.

TABLE 2. Effect of mineral and bio-N fertilization on plant height, canopy dry weight, number and area of leaves plant^{-1} of pumpkin in 2012 and 2013.

Treatments	Plant height (cm)	Canopy DW plant^{-1} (g)	No. leaves plant^{-1}	Leaf area plant^{-1} (dm^2)
2012 season				
Control (100% mineral-N)	266a *	164a	58a	191a
Bio-N ₁ + $\frac{2}{3}$ mineral-N	188c	119c	40c	110c
Bio-N ₂ + $\frac{2}{3}$ mineral-N	212b	128b	47b	152b
Bio-N ₃ + $\frac{2}{3}$ mineral-N	260a	156a	56a	182a
2013 season				
Control (100% mineral-N)	278a	174a	62a	202a
Bio-N ₁ + $\frac{2}{3}$ mineral-N	191c	123c	41c	114c
Bio-N ₂ + $\frac{2}{3}$ mineral-N	218b	133b	50b	158b
Bio-N ₃ + $\frac{2}{3}$ mineral-N	269a	165a	59a	192a

Control = recommended dose of mineral-N fertilizer

Bio-N₁, N₂ and N₃= inoculation with a mixed biofertilizer once, twice and thrice, orderly

*mean values within each column followed by a different lower-case letter(s) are significantly different using least significant difference test (LSD) at $P \leq 0.05$.

Fruits yield and quality

The statistical analysis proved that the influence of N-mineral fertilization at the recommended dose (control) and fertilization with $\frac{2}{3}$ recommended N-mineral dose plus inoculation with the a mixed bio-fertilizer at three times on fruit length and diameter, average fruit weight and total fruits yield was at par (Table 4). Nevertheless, statistical lower mean values of the aforementioned fruits yield and quality parameters were obvious when fertilization was performed at $\frac{2}{3}$ recommended N--mineral dose plus inoculation with a mixed bio-fertilize once and twice compared to the control treatment with few exceptions. These findings can be discussed on the basis that Bio-N acts as a nutrient reservoir through N_2 -fixation and N ions released slowly over the entire growth period. The slow and steady supply of N by bio-N, particularly at the three inoculants, satisfy the N requirements of plants at different growth stages and consequently leads to higher fruits yield. The favorable conditions of soil nutrients status as a result of addition $\frac{2}{3}$ mineral-N recommended dose and inoculation three times with a mixed bio-fertilizer (Table 1) aided in improvement of nutritional status of pumpkin plants (Table 3) which positively reflected on fruits yield. Our results are in agreement with those obtained by Osman (2007) and Howladar *et al.* (2013) who pointed out that total yield was highly correlated with the development of vegetative growth as well as dry matter accumulation.

TABLE 3. Effect of mineral and bio-N fertilization on fruit length and diameter, average fruit weight and total fruits yield of pumpkin in 2012 and 2013.

Treatments	Fruit length (cm)	Fruit diameter (cm)	Average fruit weight (kg)	Fruit yield fed^{-1} (ton)
	2012 season			
Control (100% mineral-N)	26.1a	17.0a	3.91a	20.1a
Bio-N ₁ + $\frac{2}{3}$ mineral-N	19.0c	13.3b	2.89c	14.6c
Bio-N ₂ + $\frac{2}{3}$ mineral-N	22.2b	15.1a	3.31b	17.6b
Bio-N ₃ + $\frac{2}{3}$ mineral-N	25.8a	16.7a	3.81a	19.6a
2013 season				
Control (100% mineral-N)	26.8a	17.2a	4.12a	21.0a
Bio-N ₁ + $\frac{2}{3}$ mineral-N	19.1c	13.3b	3.00c	15.0c
Bio-N ₂ + $\frac{2}{3}$ mineral-N	22.7b	15.3a	3.46b	18.1b
Bio-N ₃ + $\frac{2}{3}$ mineral-N	26.4a	16.8a	4.02a	20.4a

Control = recommended dose of mineral-N fertilizer

Bio-N₁, N₂ and N₃ = inoculation with a mixed biofertilizer once, twice and thrice, orderly

*mean values within each column followed by a different lower-case letter(s) are significantly different using least significant difference test (LSD) at $P \leq 0.05$.

Chemical constituents

The influence of mineral and bio- N fertilization treatments on leaf N, P and K contents was significant and the trend was identical in both years (Table 4). Similar contents of leaf N, P and K for pumpkin plants received.

TABLE 4. Effect of mineral and bio-N fertilization on nitrogen (N), phosphorus (P), potassium (K), nitrate (NO₃⁻) and nitrite (NO₂⁻) contents in leaf of pumpkin in 2012 and 2013.

Treatments	N (% DW)	P (% DW)	K (% DW)	NO ₃ ⁻ (mg g ⁻¹ DW)	NO ₂ ⁻ (mg g ⁻¹ DW)
2012 season					
Control (100% mineral-N)	30.5a []	4.63a	32.0a	2.68a	0.199a
Bio-N ₁ + 2/3 mineral-N	19.8c	3.86c	24.4c	2.21b	0.124b
Bio-N ₂ + 2/3 mineral-N	25.1b	4.31b	27.3b	1.67c	0.097c
Bio-N ₃ + 2/3 mineral-N	29.8a	4.58a	31.5a	0.96d	0.073d
2013 season					
Control (100% mineral-N)	28.8a	4.90a	33.9a	2.53a	0.188a
Bio-N ₁ + 2/3 mineral-N	19.1c	3.99c	25.2c	2.14b	0.120b
Bio-N ₂ + 2/3 mineral-N	24.1b	4.49b	28.5b	1.60c	0.093c
Bio-N ₃ + 2/3 mineral-N	28.3a	4.85a	33.2a	0.91d	0.069d

Control = recommended dose of mineral-N fertilizer

Bio-N₁, N₂ and N₃ = inoculation with a mixed biofertilizer once, twice and thrice, orderly
^{*}mean values within each column followed by a different lower-case letter(s) are significantly different using least significant difference test (LSD) at $P \leq 0.05$.

Mineral-N at the recommended dose and those received mineral-N at 2/3 of the recommended dose + three inoculants of mixed bio-fertilizers. However, the two former fertilization treatments significantly surpassed the fertilization treatments of mineral-N at 2/3 of the recommended dose + inoculation with bio-N fertilizer once or twice. The beneficial effects of non-symbiotic N₂-fixing bacteria of genera *Azotobacter chroococcum* and *Azospirillum brasilense* in production of Phytohormones, better root proliferation, improving the availability and acquisition of nutrients had been documented (Kundu & Gaur, 1980 and Vessey, 2003). Similar results on marigold and tomato plants were reported by Barakat & Said (1998) and Balasubramanian (1989), orderly.

The combined treatments of mineral-N fertilization at 2/3 of the recommended level + inoculation with a mixed bio-N fertilizer; once, twice or thrice significantly had lower mean contents of NO₃⁻ and NO₂⁻ than the control treatment. The least significant mean value of NO₃⁻ and NO₂⁻ contents was recorded when pumpkin plants were fertilized with 2/3 of the recommended mineral-N fertilizer and inoculated three times with the mixed bio-fertilizer. These results can be discussed on the basis that application of mineral-N at the recommended dose (control) possibly led to continuous and accumulated supply of NO₃⁻ and NO₂⁻ (Mahmoud *et al.*, 2009 and Howladar *et al.*, 2013). In contrast, when mineral-N was reduced and inoculation with bio-fertilizer was performed, the release of NO₃⁻ and NO₂⁻ was comparatively fewer and slow due to increasing organic matter which regulates the release and transformation of N-fertilizer to NO₃⁻ and NO₂⁻.

Conclusion

Bio-N fertilizers reduced the amount of synthetic chemical-N fertilizer needed and reduced the negative effects of chemical-N fertilizer on the environment including nitrate and nitrite accumulation in plants. Moreover, increased soil organic matter content and the availability of nutrients to plant roots, thus increased plant growth and yields.

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تأثير التسميد النتروجيني المعدني والحيوي على النمو ومحصول الثمار والمكونات الكيميائية للقرع العسلي

أشرف شوقي عثمان ومفرح سعداوى طلبة
قسم البساتين - كلية الزراعة - جامعة الفيوم - الفيوم - مصر.

لوحظت تأثيرات معنوية موجبة على النمو ومحصول الثمار ومحتوى الورقة من النتروجين والفوسفور والوتاسيوم والنترات والنترت كنتيجة لتسميد نباتات القرع العسلي بثلاثي الجرعة الموصى بها من النتروجين المعدني بالإضافة إلى ثلاثة تلقحات من السماد الحيوي المختلط المحتوي على بكتريا *Azotobacter chroococcum* and *Azospirillum brasilense* مقارنة بتسميد النباتات بثلاثي الجرعة الموصى بها من النتروجين المعدني والتلقيح بالسماد الحيوي المختلط مرة أو مرتين ، كما لم تظهر فروق إحصائية في الصفات السابقة المذكورة سابقا بين التسميد بجرعة النتروجين المعدني الموصى بها (المعاملة القياسية) والتسميد بمعدل ثلاثي الجرعة الموصى بها من النتروجين المعدني بالإضافة إلى ثلاثة تلقحات من السماد الحيوي المختلط . ايضا- اتضح ان أقل القيم معنويا لمحتوى الأوراق من النترات والنترت تحقق عند التسميد بمعدل ثلاثي النتروجين المعدني الموصى به بالإضافة إلى ثلاثة تلقحات من السماد الحيوي المختلط ، ولذلك فإن التلقيح بالسماد الحيوي المختلط ثلاث مرات يمكن ان يحل جزئيا محل السماد النتروجيني المعدني وايضا يساهم في امان الطعام.