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# Integrated Biofertilization Management and Cyanobacteria Application to Improve Growth and Flower Quality of *Matthiola Incana*

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**Abstract:** This study was designed to determine the influence of N-biofertilization on enhancing the growth and improving flowering of *Matthiola incana*. For this purpose, two pot experiments were performed over the two successive seasons 2007/2008-2008/2009. The recommended dose of N was added in 3 different forms; namely full dose of $NH_4NO_3$, full dose of nitrogen fixing bacteria (NFB) and the combination of half of the previous doses. In this respect, *Azotobacter* (*Azot*.) and *Azospirillum* (*Azosp*.) cultures were used as nitrogen fixing bacteria. The three forms of N fertilization were applied with or without adding cyanobacterial filtrates (Cyano) as a phytohormonal source. The commercial mineral fertilizer Crystalone (CRS, 19 N: 19 $P_2O_5$: 19 $K_2O$) was used (with or without Cyano) as the control. The results revealed that biofertilization with *Azot*., *Azosp*. and Cyano increased significantly plant height, number of leaves/plant, and leaf area as compared to mineral fertilization (control). Also, this treatment enhanced the flowering quality in terms of florets number and diameter, fresh and dry weights of inflorescences. Therefore, it could be suggested that *Matthiola incana* plants exhibited better growth and flower quality when biologically fertilized with $N_2$-fixing bacteria in combination with cyanobacterial filtrates.

**Key words:** biofertilizer, cyanobacteria, flower quality, growth, *Matthiola incana*,

## INTRODUCTION

*Matthiola incana* L., stock, is considered one of the most outstanding fragrant cool-season annuals. Also, it produces spikes of double flowers in shades of magenta, rose, purple, pink and white from basal rosettes of green or silvery leaves. It is mainly used for planting flowerbeds in different types of gardens, and has become an economically important floral crop [1].

The excessive use of chemical fertilizers caused several environmental problems including polluting underground water and acidification of water. In addition, it was reported that nitrate and other substances of chemical fertilizers have been linked to nitrate poisoning, cancer, deterioration of soil structure, the inhibition or killing of beneficial microorganisms and making plants more susceptible to the attack of diseases [2].

Therefore, it was suggested to replace some of the applied chemical fertilizers by some of the well known biofertilizers that are reported to be most effective as well as environment friendly. In addition, biofertilizers stimulate plant growth, improve both soil structure and conditions, restore natural soil fertility and provide protection against drought and some soil borne diseases [3].

*Azotobacter*, *Azospirillum* and *Rhizobium* are good examples of $N_2$-fixing bacteria responsible for increasing N supply of the soil as well as N availability of different crops [4]. Cyanobacteria are the most dominant photosynthetic prokaryotic microorganisms that produce a wide array of substances. These include antibiotics, algicides, toxins, pharmaceuticals and plant growth regulators[5]. Among the growth regulators; gibberellin, auxin, cytokinin, ethylene, abscisic acid and jasmonic acid have been detected in cyanobacteria[6-8]. There is accumulating evidence that cyanobacteria produce plant hormones or demonstrate plant hormone-like activity. In addition Manickavelu *et al*. [9] proved that cyanobacteria can be applied as a source of phytohormes in rice tissue culture.

The aim of this study was to evaluate the effectiveness of applying some nitrogen fixing bacteria e.g. *Azotobacter*, *Azospirillum*, *Rhizobium* as well as cyanobacteria as biofertilizers on enhancing the growth parameters and marketability of cut *Matthiola incana* flowers.

## MATERIALS AND METHODS

This study was carried out in the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, during the two successive seasons of 2007/ 2008-2008/2009. The following materials were used:

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**Seeds:** The seeds of *Matthiola incana*, were exported from BERTRAND FRERES STE D'EXPL (ETS) Company, France. Seeds were germinated in plug trays on 20th of October in both seasons. After three weeks from sowing the seedlings (approximately 10 cm tall, with 4 leaves) were transplanted to pots (20 cm, inner diameter) containing 4 kg of a mixture of clay and sand (1:1, v/v). Single seedling was planted per pot. The chemical and physical properties of the potting soil mixture were determined and are presented in Table (1)

**Chemical and Biofertilizers:** In both seasons, the potted plants were supplied with the following chemical and bio-fertilization treatments The commercial mineral fertilizer (crystalone; CRS), CRS plus cyanobacterial filtrate (cyano), complete rate of $NH_4NO_3$, complete rate of $NH_4NO_3$ plus cyano, mixture of half $NH_4NO_3$ plus half of nitrogen fixing bacteria (1/2 NFB), mixture of half $NH_4NO_3$ plus half nitrogen fixing bacteria (1/2 NFB) plus cyano, complete NFB and complete NFB plus cyano.

Crystalone (19N: $19P_2O_5$: $19K_2O$), representing the traditional fertilization applied in most nurseries, was added as a top dressing at the rate of 3 g /pot as mentioned by Hisamatsu *et al.*[1] on the same species, after 15 and 30 days from transplanting.

Gibberellic acid ($GA_3$), abscisic acid (ABA) and Indol-3-acetic acid (IAA) were determined in culture filtrate of strains (*Anabaena sp*. And *Nostoc sp*.) by Gas-liquid chromatography (Unicam Pro-GLC) according to the method described by Vogel [10], and were performed in the Central Laboratory, Faculty of Agriculture; Cairo University. Data were represented as quantity of phytohormons as g 100 $ml^{-1}$ $culture^{-1}$ filtrate.

Cyanobacterial filtrate treatment (Cyano) represented a mixture (1:1) of *Anabaena* and *Nostoc* old cultures as a source of phytohormones (Table, 2). Cyano was sprayed twice after 2 and 4 weeks from transplanting (at the same time as the CRS treatments applied).

The chemical nitrogenous fertilizer $NH_4NO_3$ (33.5% N) was added at the rate 5 g/pot, which was considered to be the complete rate of chemical N fertilization. This rate was splited into two equal doses, which were added after 2 and 4 weeks after transplanting.

The half dose of $NH_4NO_3$ (2.5 g/pot) treatment was applied at the same time as the complete rate of $NH_4NO_3$.

The biofertilization treatments consisted of a mixture of strains of the nitrogen fixing bacteria; *Azotobacter chroococcum*, *Azospirillum brasilense* and *Rhizobium sp*. The biomixture (1:1:1, v/v/v) was prepared using cultures of the three tested bacteria strains. Each strain was obtained from a culture containing $10^8$ cells /ml. The complete nitrogen fixing bacteria (NFB) treatment consisted of two doses, 10 ml each, added to the soil after 2 and 4 weeks from transplanting. The 1/2 NFB treatment was added as in the complete NFB treatment, but with half the rate per application (5 ml).

The recommended doses of phosphorus and potassium were added with all the studied treatments except crystalone and crystalone plus cyanobacteria treatments . Phorphorus was added to the soil as calcium superphosphate (15.5 % $P_2O_5$) at the rate of 6.5 g/pot before transplanting, while potassium was added as potassium sulphate (48% $K_2O$) at the rate of 5 g/pot, splited into two equal doses, which were applied after 2 and 4 weeks from transplanting.

**Recorded data:** After 60 days from transplanting, the following data were recorded:

Plant height (cm), fresh and dry weights of leaves, stem and roots (g/ plant), leaf area (of the fourth leaf from the soil surface, $cm^2$), number of leaves, number of florets, length of inflorescence (cm), fresh and dry weights of inflorescences (g/plant), and diameters of stem and floret(cm).

Fresh leaf samples were taken for chlorophyll a, b, total chlorophyll and carotenoids determination, using the method described by Nornai [11].

Dried samples of leaves and inflorescences were taken for N, P and K determination, according to Jackson [12] and Piper [13].

This experiment was designed as a completely randomized block as described by Sndecor and Cochran [14] with 3 replicates for each treatment, each replicate consisted of four plants.

## RESULTS AND DISCUSSION

**Vegetative Growth and Flowering Characters of *Matthiola Incana:*** Regarding the effect of bio-N fertilization and application of cyanobacteria filtrate on the vegetative growth characters of *Matthiola incana*, the data presented in Table (3) showed that in all cases the studied growth parameters, *e.g*. plant height, stem diameter, number of leaves and leaf area were significantly increased by the addition of full NFB + cyanobacteria filtrate, as compared to fertilization with Crystalone (control). The above mentioned results were in accordance with that reported by Mostafa [15] on the growth of *Calendula officinalis* and *Dimorphotheca ecklonis* plants and Chaitre and Patil [16] on *Callistephus chinensis*. In both seasons, the same treatment was also superior in exhibiting the highest fresh and dry weights of roots, stem and leaves of *Matthiola incana* plants, compared with the control or with the other studied treatments. This result was in accordance with that obtained by Gotmare *et al.* [17] on marigold plants.

Similar positive trends were recorded during the two successive seasons for the influence of biofertilization and cyanobacteria application on the inflorescence parameters (Table, 4), including all the studied characters, expect in two cases. The first with the spike length in the first season, when applying the complete NFB caused the highest length of spike (19.01 cm) compared to that of the mixture of NFB with Cyano (17.80 cm). The second case with the number of flower in the second season, where as this number was greater (26) when complete $NH_4NO_3$ alone or a mixture of complete NFB and Cyano was used as compared with the rest of the treatments. The same results were obtained by Eid *et al*. [18] who mentioned that using *Azotobacter* and *Azospirillum* either individually or in mixture as dual inoculants improved the flower quality and increase the flower yield of *Celosia argentea*.

An interesting observation was recorded when the cyanobacterial filtrate was added to the different types of fertilizers, as this caused a significant increase in the measurements of the majority of growth and flowering characters (Tables 3 and 4).

Regarding the influence of *Azotobacter* on plant growth, Salem[19] and Khattab *et al*. [20] on gladiolus plants suggested that the role of *Azotobacter* could be attributed to an enhancement in the synthesis of proteins, DNA and RNA and more leaves could be initiated.

**Chemical Characters of *Matthiola Incana:*** Data in Table (5) showed that through all treatments used and in both seasons (except two cases in the first season, the highest N, P, K content of leaves and inflorescences were recorded by adding a mixture of N-biofertilization and cyanobacterial filtrate as compared either with control or other treatments. The two exception cases were with the highest concentration of leaves was with complete $NH_4NO_3$ and Cyano and the highest concentration of N of the inflorescence was with the mixture of half NFB and half $NH_4NO_3$ plus Cyano as compared with the mixture of complete NFB and Cyano. The increase of N and P of the leaves and inflorescences may be attributed to a high rate of protein metabolism leading to protein synthesis, which is considered to be a possible indicator of senescence retardation after the inflorescences have been harvested. Meanwhile, the increase in K concentration in inflorescences could reflect the increase in solutes translocation towards the inflorescences [21]. The increase in N content of plants receiving biofertilization may be due to the net gain of $N_2$ fixed by *Azotobacter* and *Azospirillum*. Similar results have been reported by Mostafa [15] who found that N-biofertilization enhanced the N content of the leaves of *Calendula officinalis* plants.

**Table 1:** Physical and chemical properties of the clay sandy potting soil used for growing *Matthiola incana* plants during the 2007/2008 and 2008/2009 seasons.

| Soil properties | |
|---|---|
| Physical properties | |
| Texture (%) | Sandy clay loam |
| Coarse sand (%) | 9.90 |
| Fine sand (%) | 49.50 |
| Slit (%) | 15.50 |
| Clay (%) | 25.10 |
| $Caco_3$ (%) | 14.30 |
| CEC (meq/100g) | 19.50 |
| Field capacity(%v) | 22.20 |
| Chemical properties | |
| Organic matter (%) | 11.34 |
| PH | 7.49 |
| EC(ds/m) | 3.88 |
| N(ppm) | 30.93 |
| P (ppm) | 15.72 |
| K(ppm) | 69.90 |

**Table 1:** Continue

| | |
|---|---|
| Mg (ppm) | 27.70 |
| Fe(ppm) | 5.15 |
| Mn(ppm) | 1.25 |
| Zn(ppm) | 1.75 |
| Cu(ppm) | 0.65 |

**Table 2:** Phytohormones composition of *cyanobacterial* cultures used during this study ($g100ml^{-1}culture^{-1}$ filtrate).

| Strains | IAA * | ABA * | $GA_3$ * |
|---|---|---|---|
| *Anabaena sp* | 0.17465 | 0.5357 | 1.0325 |
| *Nostoc sp* | 0.2848 | 0.7124 | 2.1792 |

*IAA, Indole acetic acid; ABA: Abscisic acid and $GA_3$: *Gibberellic* acid.

**Table 3:** Vegetative growth characters of *Matthiola incana* as affected by bio-, chemical fertilization and cyanobacterial application during 2007/2008 and 2008/2009 seasons.

| Treatments* | First season | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Plant height (cm) | Stem diameter (cm) | Number of leaves | Leaf area ($cm^2$) | Fresh weight (g/plant) | | Dry weight (g/plant) | |
| | | | | | Shoot | Root | Shoot | Root |
| CRS | 37.33 | 0.43 | 28 | 36.89 | 21.98 | 2.42 | 3.37 | 0.87 |
| CRS+ Cyano | 41.00 | 0.63 | 31 | 39.38 | 23.48 | 3.83 | 4.44 | 1.15 |
| Complete $NH_4NO_3$ | 32.33 | 0.75 | 25 | 25.54 | 14.50 | 2.73 | 2.58 | 1.51 |
| Complete $NH_4NO_3$ + Cyano | 33.76 | 0.33 | 28 | 28.56 | 16.88 | 2.94 | 3.12 | 1.15 |
| 1/2 NFB+ 1/2 $NH_4NO_3$ | 36.00 | 0.37 | 24 | 33.50 | 18.32 | 3.04 | 2.91 | 1.42 |
| 1/2 NFB+ 1/2 $NH_4NO_3$+ Cyano | 41.67 | 0.35 | 29 | 42.10 | 26.65 | 3.38 | 4.60 | 1.70 |
| Complete NFB | 43 | 0.67 | 37 | 40.91 | 25.06 | 4.56 | 4.33 | 1.60 |
| Complete NFB + Cyano | 44 | 0.83 | 40 | 45.90 | 32.40 | 4.67 | 5.60 | 1.87 |
| L.S.D 5% | 1.34 | 0.05 | 2.01 | 2.64 | 1.47 | 0.33 | 0.55 | 0.14 |
| Treatments* | Second season | | | | | | | |
| CRS | 36.23 | 0.43 | 29 | 32.47 | 23.42 | 3.31 | 3.71 | 1.02 |
| CRS+ Cyano | 39.67 | 0.70 | 32 | 35.57 | 28.87 | 3.46 | 4.98 | 1.06 |
| Complete $NH_4NO_3$ | 33.33 | 0.85 | 30 | 24.36 | 17.96 | 2.45 | 3.17 | 0.76 |
| Complete $NH_4NO_3$ + Cyano | 34.67 | 0.37 | 26 | 29.03 | 19.15 | 4.50 | 3.03 | 1.48 |
| 1/2 NFB+ 1/2 $NH_4NO_3$ | 35.00 | 0.43 | 28 | 31.37 | 21.15 | 4.08 | 3.34 | 1.42 |
| 1/2 NFB+ 1/2 $NH_4NO_3$+ Cyano | 41.17 | 0.40 | 37 | 38.54 | 34.71 | 4.71 | 5.96 | 1.45 |
| Complete NFB | 41.83 | 0.80 | 39 | 41.76 | 38.65 | 4.92 | 6.38 | 1.85 |
| Complete NFB + Cyano | 46 | 0.90 | 42 | 43.33 | 42.77 | 5.70 | 7.05 | 2.15 |
| L.S.D 5% | 1.44 | 0.05 | 3 | 2.39 | 1.43 | 0.42 | 0.24 | 0.04 |

CRS: Crystalone; NFB: Nitrogen fixing bacteria filtrate; $NH_4NO_3$:Ammonium nitrate; Cyano: Cyanobacteria filtrates

**Table 4:** Influence of bio- , chemical fertilizers and cyanobacterial filtrate on inflorescences characters of *Matthiola incana* during the two successive seasons X`.

| Treatments* | First Season | | | | | Second Season | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Inflorescence | | | Floret | | Inflorescence | | | Floret | |
| | Length (cm) | F.W (g) | D.W (g) | Number | Diameter (cm) | Length (cm) | F.W (g) | D.W (g) | Number | Diameter (cm) |
| CRS | 15.72 | 0.99 | 4.31 | 17 | 2.36 | 11.28 | 1.00 | 3.45 | 18 | 2.48 |
| CRS+ Cyano | 16.12 | 1.12 | 3.20 | 21 | 2.42 | 12.46 | 1.10 | 3.00 | 21 | 2.39 |
| Complete $NH_4NO_3$ | 13.85 | 0.63 | 1.53 | 25 | 2.74 | 10.35 | 0.66 | 1.80 | 25 | 2,92 |
| Complete $NH_4NO_3$ + Cyano | 15.20 | 0.88 | 1.80 | 14 | 1.25 | 10.60 | 0.73 | 2.16 | 15 | 1.13 |
| 1/2 NFB+ 1/2 $NH_4NO_3$ | 14.25 | 0.83 | 2.53 | 17 | 1.56 | 11.02 | 0.86 | 2.73 | 17 | 1.80 |
| 1/2 NFB+ 1/2 $NH_4NO_3$+ Cyano | 17.71 | 1.29 | 4.51 | 16 | 1.40 | 14.81 | 1.24 | 4.04 | 16 | 1.46 |
| Complete NFB | 19.01 | 1.40 | 4.98 | 24 | 2.70 | 13.43 | 1.49 | 4.96 | 24 | 2.74 |
| Complete NFB + Cyano | 17.80 | 1.73 | 6.13 | 26 | 2.79 | 19.70 | 1.67 | 6.03 | 25 | 3.11 |
| L. S. D 5% | 0.77 | 0.18 | 0.14 | 1.53 | 0.12 | 0.54 | 0.28 | 0.21 | 1 | 0.18 |

**Table 5:** Nitrogen, phosphorus and potassium contents (mg/g dryweight) of *Matthiola incana* as influenced by different bio -, chemical fertilizers and cyanobacterial filtrate on during the first and second seasons.

| Treatments* | First season | | | Second season | | |
|---|---|---|---|---|---|---|
| | N | P | K | N | P | K |
| | | | Leaves | | | |
| CRS | 3.66 | 0.62 | 2.36 | 3.54 | 0.44 | 2.13 |
| CRS+ Cyano | 3.84 | 0.42 | 2.09 | 3.21 | 0.38 | 2.28 |
| Complete $NH_4NO_3$ | 3.42 | 0.50 | 2.33 | 2.09 | 0.36 | 1.96 |
| Complete $NH_4NO_3$ + Cyano | 4.95 | 0.82 | 2.98 | 4.54 | 0.68 | 2.43 |
| 1/2 NFB+ 1/2 $NH_4NO_3$ | 4.59 | 0.61 | 2.15 | 3.77 | 0.51 | 2.03 |
| 1/2 NFB+ 1/2 $NH_4NO_3$+ Cyano | 5.22 | 0.71 | 2.93 | 4.33 | 0.60 | 2.29 |
| Complete NFB | 5.76 | 0.79 | 2.19 | 4.35 | 0.62 | 2.31 |
| Complete NFB + Cyano | 5.84 | 0.88 | 1.75 | 4.67 | 0.71 | 2.54 |
| L.S.D 5% | 0.14 | 0.08 | 0.12 | 0.15 | 0.06 | 0.13 |
| Treatments* | | | Inflorescence | | | |
| CRS | 2.01 | 0.19 | 4.00 | 1.23 | 0.23 | 3.71 |
| CRS+ Cyano | 2.63 | 0.25 | 4.14 | 1.63 | 0.25 | 2.80 |
| Complete $NH_4NO_3$ | 2.87 | 0.31 | 3.80 | 2.40 | 0.19 | 2.34 |
| Complete $NH_4NO_3$ + Cyano | 3.05 | 0.38 | 3.91 | 2.56 | 0.22 | 3.00 |
| 1/2 NFB+ 1/2 $NH_4NO_3$ | 2.12 | 0.36 | 3.63 | 3.17 | 0.30 | 3.60 |
| 1/2 NFB+ 1/2 $NH_4NO_3$+ Cyano | 3.99 | 0.39 | 4.20 | 3.53 | 0.35 | 3.92 |
| Complete NFB | 3.02 | 0.42 | 5.03 | 3.13 | 0.50 | 4.58 |
| Complete NFB + Cyano | 3.33 | 0.52 | 5.58 | 4.20 | 0.55 | 4.65 |
| L.S.D 5% | 0.15 | 0.10 | 0.11 | 0.20 | 0.05 | 0.14 |

**Table 6:** Effect of different bio and chemical fertilizers and cyanobacterial filtrate on chlorophyll a, b and carotenoides (mg/ g fresh weight) of *Matthiola incana* plants during both seasons.

| Treatments* | First season | | | | second season | | | |
|---|---|---|---|---|---|---|---|---|
| | Chl a | Chl b | Total Chl | Carotenoids | Chl a | Chl b | Total Chl | Carotenoids |
| CRS | 2.15 | 0.61 | 2.76 | 3.49 | 2.27 | 0.77 | 3.04 | 2.32 |
| CRS+ Cyano | 2.37 | 2.02 | 4.39 | 3.39 | 2.80 | 1.27 | 4.07 | 2.92 |
| Complete $NH_4NO_3$ | 4.98 | 1.46 | 6.44 | 4.70 | 5.58 | 1.79 | 7.37 | 3.29 |
| Complete $NH_4NO_3$ + Cyano | 4.38 | 1.28 | 5.66 | 3.07 | 5.52 | 1.85 | 7.37 | 2.79 |
| 1/2 NFB+ 1/2 $NH_4NO_3$ | 3.17 | 1.10 | 4.27 | 4.71 | 3.39 | 1.35 | 4.74 | 3.29 |
| 1/2 NFB+ 1/2 $NH_4NO_3$+ Cyano | 3.65 | 1.26 | 4.91 | 3.70 | 3.89 | 1.57 | 5.46 | 3.56 |
| Complete NFB | 6.69 | 2.30 | 8.99 | 2.70 | 6.85 | 2.83 | 9.68 | 3.71 |
| Complete NFB + Cyano | 5.24 | 2.2 | 7.44 | 3.71 | 5.17 | 1.72 | 6.89 | 3.47 |
| L.S.D 5% | 0.13 | 0.09 | 0.20 | 0.10 | 0.10 | 0.09 | 0.17 | 0.13 |

The total chlorophyll contents were the greatest; 8.99 and 9.68 mg/g fresh weight in both seasons, respectively when using complete NFB as compared to the control or other treatments. The highest carotenoids contents (4.71, 3.71 mg/g fresh weight, in the first and second seasons, respectively were obtained when applying the mixture of half NFB and half $NH_4NO_3$ as compared with other treatments. Taiz and Zeiger [22] reported that leaf area and chlorophyll contents varied according to mineral status, including the N, P and K contents. Thus, the increase in leaf area, as well as in the contents of total chlorophyll and carotenoids in inoculated plants could be related to an improvement of N uptake.

The effect of bio-N fertilization on photosynthetic pigments in inoculated plants is solely recorded as an increase in the total chlorophyll content, whether in higher plants [23,24] or unicellular plants [25]. Carotenoids act as light-harvesting molecules inside the cell, allowing the efficient utilization of the light spectrum [26]. Besides, carotenoids protect the pigment-protein complexes and the chloroplast against photo oxidation [27].

**Conclusion:** Finally, it could be concluded that in the majority of the vegetative growth, the inflorescence, the total chlorophyll, carotenoides and N, P, K concentrations of *Matthiola incana* plants recorded the highest measurments when applying the mixture of NFB and Cyano compared with other different treatments used.

So, it may be recommended that using the cyanobacterial filtrate either with the chemical or bio-fertilizers could lead to the benefit results especially with the growth of the plants and flowers quality.

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