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Evaluate Effectiveness of Bio and Mineral Fertilization on the Growth Parameters and Marketable Cut Flowers of *Matthiola incana* L.

[1]A.Rawia Eid, [2]M. Nemat Awad and [3]H.A. Hamouda

[1]Ornamental Plants and Woody Trees Department, National Research Center, Dokki, Cairo, Egypt
[2]Department of Agric Microbiology, National Research Center, Dokki, Cairo, Egypt
[3]Fertilization Technology Department, National Research Center, Dokki, Cairo, Egypt

Abstract: Biofertilizer has been identified as an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable farming. The objective of this greenhouse study was to evaluate the effects of chemical fertilizers (N and P) against two biofertilizers containing N-fixer bacteria (*Azotobacter chroococcum*) and P solubilizing bacteria (*Bacillus megaterium*) and ATP (adinosine tri-phosphate) on the growth parameters and quality of fatty acid fraction of *Matthiola incana*. The application treatments included control (no fertilizer), chemical fertilizer and two types of biofertilizer. The application of biofertilizer containing N-fixer bacteria and P solubilizer bacteria as well as mineral fertilizers significantly increased the growth parameters of *Matthiola incana*. The use of biofertilizer resulted in the highest biomass and seedling height. This greenhouse study also indicated that the biofertilizer application had similar effects when compared with chemical fertilizer treatments. Microbial inoculum not only increased the nutritional assimilation of plant (total N, P and K), but also improved the quality of oil especial linolenic acid (C18:3). The results revealed that the population of bacterial inoculation significantly increased in all the inoculated treatments, when compared to uninoculated control. The maximum population of bacterial inoculation harbored in 75% N and *Azospirillum* treated plant, which was significantly more than all other treated plants.

Key words: N-fixer bacteria, P solubilizing bacteria, ATP, *Matthiola incana*, Fatty acids, Mineral fertilization

INTRODUCTION

A group of bacteria are now referred to plant growth-promoting rhizobacteria (PGPR), which participate in many key ecosystem processes such as those involved in the biological control of plant pathogens, nutrient cycling and seedling establishment and therefore deserve particular attention for agricultural or forestry purposes [1-3]. PGPR may colonize the rhizosphere, the surface of the root, or even superficial intercellular spaces of plants [4]. It has been revealed that the effect of nitrogen fixation induced by nitrogen fixers is not only significant for legumes, but also non-legumes [5]. Moreover, some strains have multiple functions for plant growth. The beneficial effect of *Azotobacter* may derive both from its nitrogen fixation and stimulating effect on root development [5-6]. Phosphate solubilizing bacteria (PSB) may enhance mineral nutrients uptake by plants through solubilizing insoluble P from silicate in soil [7]. Some successful examples of inoculation with PGPR have been achieved both in laboratory and field trials. For example, strains of *Pseudomonas putida* and *Pseudomonas fluorescens* could increase root and shoot elongation in canola, lettuce and tomato [8-9]. It has also been reported that wheat yield increased up to 30% with *Azotobacter* inoculation and up to 43% with *Bacillus* inoculation [10]. Soil microorganisms are important components in the natural soil subecosystem because not only can they contribute to nutrient availability in the soil, but also bind soil particles into stable aggregates, which improve soil structure and reduce erosion potential [11].

In recent years, biofertilizers have emerged as an important component of the integrated nutrient supply system and hold a great promise to improve crop yields through environmentally better nutrient supplies. However, the application of microbial fertilizers in practice has not achieved constant effects. The reviewed data show statistically significant increases in yield on the

Corresponding Author: A. Rawia Eid, Department of Ornamental Plants and Woody Trees, National Research Center, Dokki, Cairo, Egypt

order of 5-30% in 60-70% of published reports [12]. Most studies of the *Azotobacter* plant association have been conducted on cereals and grasses [13] and only a few other plants familiars have been investigated so far Bashan *et al*., [14]. Although, not much information is available on the isolates from ornamental plants and their possible role in the N economy of these plants.

Numerous ornamental plants in Egypt have great potential but have not been expiated stock plant could be extended by adapting plant techniques such this plant can be increased when the cultural practices and manipulation must be identified for local Egyptian condition. Stock (*Matthiola incana* L.) is one of the most popular annual flowering crops. The Plants are 1 to 3 feet tall with attractive foliage and spikes of tiny, delicately scented flowers. Blooms usually are deeps blue, but lilac, which and pink varieties are available. Stock plant is economical to buy and grow for quick winter flowers and bloom all season. These has a wide rang of colours and heights which permit great freedom in garden design and adopt to van planting times. The flowers can be used in the flower beds and in the mixed boorders in the garden as well as for cut flowers. Seed of *Matthiola incana* contain oil rich omega-3 and linolenic acid (c18:3). Adam *et al*. [15] and Simopoulos [16] reported that omega-3 acids are receiving more and more attention as essential components of human diet. Hunter [17] reported that omega-3 fatty acids from vegetable oils could provide health benefits without any concomitant intake of cholesterol and demonstrate for the first time a beneficial effect of dietary *Matthiola incana* oil reducing cholesterol level and increasing (n-3) fatty acid levels in plasma [18].

The major objective of this experiment was to evaluate the individual effects of nitrogen levels against biofertilizers (*Azotobacter chroococcum*), adenosine tri-phosphate (ATP) as a foliar spray against phosphorus and phosphate solubilizing bacteria (*Bacillus megaterium*) on the promotion of plant growth flowering, seed yield and chemical constituents of *Matthiola incana* plants was investigated. In addition, effect of these treatments on oil quality of *Matthiola incana* L.

MATERIALS AND METHODS

Soil Analysis: Soil sample used for pot experiment was taken to determine mechanical analysis and some physico-chemical properties. The following analysis i.e. texture, pH, E.C, $CaCO_3$, total nitrogen and available phosphorus and potassium were recorded according to stander procedure [19-20].

The sandy soil used for this pot experiment mechanical analysis (sand 83.1%, silt 11.3% and clay 5.6%), physico-chemical Properties of the soil were as follows: pH 8.1, EC 2.3 dS/m, $CaCO_3$ 1.04%, organic matter content 0.18%, total N 1.08mg/100g, P 1.2mg/100g and K 7.3mg/100g.

Fertilizer and Microbial Inocula: Associative nitrogen fixing strain (*Azotobacter chroococcum*) and P solubilizing bacteria (*Bacillus megaterium*) initially isolated from the rhizosphere of *Matthiola incana* L. grown in the sandy soil and identified in the Agric. Microbiology Dep. NRC. The bacterial inoculum was prepared by transferring a loopful of 48h old culture of bacteria (nitrogen fixing or P solubilizing) to 50 ml Ashby liquid medium and LB broth, respectively. After five days of incubation in a shaking at 28°C and 200 rpm., the entire broth was transferred to one-liter capacity Erlenmeyer flask containing 500 ml. The flasks were incubated at 28°C for five days. The N-fixing bacteria and PSB were cultured under the same conditions with the density of each bacterial culture in the broth was counted using a haemocytometer. The final population sizes of N-fixing bacteria (NFB) and PSB were adjusted to $2.72x10^8$ and $2.05x10^8$ CFU ml of broth medium at the time of inoculation, respectively.

Pot Experiment: The present investigation was carried out during the two seasons of (2006/2007) (2007/2008) at the greenhouse of National Research Centre, Dokki, Cairo, Egypt. The experiment was carried out in three replicates in factorial experimental in two factorial. Detail of experimental treatments: control, ammonium nitrate (AN) at two rates 2 and 4g/pot with or without biofertilizers (NFB or PSB), NFB or PSB alone or in combination, P_2O_5 with or without biofertilizers (NFB or PSB), adenosoine tri-phosphate (ATP) at the rate of 50 ppm with or without biofertilizers (NFB or PSB) and chemical fertilizers (AN+ P_2O_5+ ATP).

In this greenhouse trial, ammonium nitrate (AN) at the rate of 0, 2.0g and 4.0g pot^{-1}, against nitrogen fixing bacteria NFB (*Azotobacter chroococcum*). Ammonium nitrate was applied at two doses at one month interval; the first one was applied after 15 days from the thinning. While NFB was applied as a pre-sowing covering agent of seeds at the rate of 10 ml^{-1} pot. Calcium superphosphate (15.5% P_2O_5) at the rate of 2.75g / pot was mixed with the

soil before planting against PSB phosphate solubilizing bacteria *(Bacillus megaterium*) at the rate of 10 ml / pot. Adenosine tri-phosphate (ATP) was sprayed twice one after month from planting and other after month from the first spray.

Matthiola seeds were sown in 30-cm pots filled with sandy soil at October. After seed germination, the seedlings were thinned to three in each pot. All the pots were randomly arranged in a greenhouse. Each pot received equal amounts of waters needed. Other agricultural processer were performed according to normal practice. Plant shoot samples were taken during the two growing seasons, at flower budding stage and fruiting stage, respectively for nutrients determination.

Biological and Chemical Analyses: Enumeration of N-fixing bacteria, (NFB) and P solubilizing bacteria (PSB) from the fresh soil sample was conducted using suspension dilution techniques on agar plates with differentiating media [for N-fixing bacteria: glucose 10.0 g, NaCl 0.2 g, $MgSO_4 7H_2O$ 0.2 g, K_2HPO_4 0.5 g, 2 drops of 1% $FeCl_3$ and 1% $MnCl_2$ solution, 1% Congo Red solution 5 ml (after pH modification), agar 20.0 g, distilled water 1.0 l, pH 7.0; for P solubilizer: glucose 10.0 g, $(NH_4)_2SO_4$ 0.5 g, NaCl 0.3 g, KCl 0.3 g, $MgSO_4 . 7H_2O$ 0.5 g, $FeSO_4 . 7H_2O$ 0.03 g, $MnSO_4 . 4H_2O$ 0.03 g, $Ca_3(PO_4)_3$ 10.0 g, agar 20.0 g, distilled water 1.0 l, pH 7.0; and expressed as CFU per gram dry soil.

After the plants were harvested, the following data were recorded at flowering stages and fruiting stage of *Matthiola incana* L. plant in both seasons: the growth parameters (plant height, no. of branches and flowers, fresh and dry weight of flowers, no. of pods, seed yield/plant and seed oil (%) under different treatments were recorded. Chlorophyll (a, b) was determined in the fresh leaf samples according to Saric *et al.* [21]. Soluble and non-soluble sugars (%) were determined using the methods described by Dubois *et al*. [22]. Macronutrients (N, P and K) concentrations in plants were determined according to Kalra and Maynard [23]. The methylesters prepared from oil samples and standard materials were analyzed by a Pye Unicam das chromatograh equipped with a dual flame ionization detector. The separation of fatty acid methyl esters was conducted with a column: SP-2310,55% cyanopropy1 pheny1 silicon (1.2×4.0mm). Column was used with temperatures program of 70 to 190°C at 8°C/min. The injector and detector temperatures were maintained at 205 and 300°C, respectively. The pressure of carrier gas (nitrogen) was 18 kg/cm^2, chart speed 0.35 cm/min. The relative percent of each compound was determined according to the peak area by Vraian 4370 integrator. The identification of the different fatty acids was known by matching their retention times (RT) with those of the authentic samples under the same conditions [24].

Data Analysis: Analysis of variance (ANOVA) was performed on microbiology experimental data and means were compared using the Duncan's multiple range test with Sigma Stat software. The significance level was $p>0.05$ unless otherwise stated. While data (means of the two growing seasons) for growth plant were subjected to statistically analysis of variance procedure. Where the means of the studied treatments were compared using L.S.D. test at 0.05of probability [25].

RESULTS AND DISCUSSION

The present results demonstrated that the population size of the inoculated rhizobacteria varied in accordance with the levels of fertilization in both seasons (Fig. 1). The low level of fertilization (2g AN, P_2O_4 and ATP) resulted in a larger community of NFB and PSB in the rhizosphere, compared to the treatments with a high level of fertilization (4g AN, P_2O_4 and ATP). According to results the propagation of *Azotobacter* spp. was seriously inhibited when the ammonium N concentration exceeded 200 mg kg^{-1}. This is in agreement with Wani, *et al*., [26] who noted that the population size of N-fixing bacteria in soil decreased significantly after N fertilizer was used. The number of indigenous phosphate solubilizing bacteria in the rhizosphere was $5.25x10^4$ / g. dry soil. The limitation of root exudates during the initial phase of bacteria development may restrict flow of energy to the bacteria and decline the proliferated of bacteria, thereafter, prevent reached an extensive cells. These limitations of root exudates may result from slower root growth rates be attributed to plants growing in nutrient-deficient soils [27].

The PSB inoculation increased the number of phosphate solubilizing bacteria (PSB) to $7.4x10^7$ cell / g. dry soils in the rhizosphere of banana plant. The numbers of PSB were stimulation greatly in the rhizosphere *Matthiola incana* L. plants with the inoculation with NFB (Fig. 1). However, inoculation with NFB allowed introduced population of PSB to maintain higher number in the rhizosphere of *Matthiola incana* plants compared to uninoculated treatments. The counts tended these after to follow a slight decline where they proliferated. ATP, generally, increased proliferated of PSB in all treatments,

Table1: Effect of nitrogen fixing bacteria (NFB) and phosphate solubilizing bacteria (PSB) combined with mineral fertilizers on growth characters of *Matthiola incana* L. plants at flowering stage (means of two seasons)

	Plant height (cm)					No. of branches/plant				
Treatments	0	P_2O_5	PSB	ATP	Mean	0	P_2O_5	PSB	ATP	Mean
Cont	18.0	28.3	43.3	49.5	34. 8	8.2	8.7	9.9	10.2	9. 3
2g AN	30.5	38.5	44.7	51.3	41. 25	11.3	14.5	16.8	19.6	15. 6
4g AN	31.8	40.9	45.8	53.4	43. 0	16.7	18.2	20.2	22.8	19. 5
Azoto	40.9	43.4	46.8	55.6	46. 7	16.8	19.4	22.5	24.3	20. 8
Mean	30. 3	37. 8	45. 2	52.5		13. 3	15. 2	17. 4	19. 2	
LSD (0.05)	A=1.53 B=2.18 AB=1.58					A=1.32 B=0.983 AB=0.384				
	No. of flowers /plant					Fresh weight of flowers (g/plant)				
Cont	43	46	48	51	47. 0	6.51	6.58	6.73	7.11	6. 73
2g AN	58	71	79	83	72. 8	6.71	8.50	8.92	11.13	8. 82
4g AN	67	78	85	90	80. 0	8.05	10.34	12.73	13.10	11. 06
Azoto	69	81	89	95	83. 5	8.17	12.53	12.61	13.70	11. 75
Mean	59. 3	69. 0	75. 3	79. 8		7. 36	9. 49	10. 25	11. 26	
LSD (0.05)	A= 2.53 B=2.11 Ab=2.85					A= 2.53 B=2.11 Ab=2.85				
	Dry weight of flowers (g /plant)					Fresh weight (g /plant)				
Cont	1.3	1.4	1.8	2.2	1. 68	45.3	53.2	115.4	155.3	92. 3
2g AN	1.5	2.4	2.8	3.0	2. 43	58.4	64.5	120.8	163.8	101. 9
4g AN	1.7	2.8	3.3	3.7	2. 88	60.5	85.6	131.9	168.7	111. 7
Azoto	1.8	3.1	3.5	4.0	3. 10	94.3	100.3	143.8	170.3	127. 2
Mean	1. 6	2. 43	2. 85	3. 23		64. 63	75. 9	128. 0	164. 5	
LSD (0.05)	A=0.13 B= 0.11 AB=0.041					A=1.31 B=1.18 AB=0.83				

Dry weight (g/plant)					
Cont	14.0	17.5	29.6	40.8	25. 5
2g AN	16.4	18.5	33.5	43.1	27. 9
4g AN	18.8	25.5	36.3	46.3	31. 7
Azoto	27.3	28.3	38.6	49.8	36. 0
Mean	19. 13	22. 45	34. 5	45. 0	
LSD (0.05)	A=0.53	B=0.81		AB=0.32	

Table 2: Effect of nitrogen fixing bacteria (NFB) and phosphate solubilizing bacteria (PSB) combined with mineral fertilizers on growth and fruiting of *Matthiola incana* L. plants at fruiting stage (means of two seasons)

	Plant height (cm)					No. of branches/plant				
Treatments	0	P2O5	PSB	ATP	Mean	0	P2O5	PSB	ATP	Mean
Cont	35.9	40.3	44.65	62.8	45. 9	6.7	7.3	7.8	8.8	7.7
2g AN	48.5	53.7	56.2	65.9	56. 1	9.3	11.2	13.5	16.3	12. 6
4g AN	50.2	57.6	58.7	75.8	60. 6	13.4	16.1	18.3	20.7	17. 1
Azoto	55.7	59.3	67.9	85.9	69. 2	14.2	17.8	20.2	21.3	18. 4
Mean	47. 6	52. 7	56. 9	72. 6		10. 9	13. 1	14. 9	16. 8	
LSD (0.05)	A=1.89	B=2.34	AB=2.10			A=1.982	B=1.03	AB=0.211		
	Dry weights (g/plant)					No. of pods/plant				
Cont	93	98	106	112	102. 3	53	59	63	67	60. 5
2g AN	96	108	120	128	113. 0	112	153	174	189	157. 0
4g AN	108	130	135	142	128. 8	143	173	183	203	175. 5
Azoto	119	138	143	150	137. 5	151	179	195	215	185. 0
Mean	104	118. 5	126	133		114. 8	141. 0	153. 8	168. 5	
LSD (0.05)	A=2.34 B=2.58 AB1.59					A=2.83 B=221 AB=3.11				
	Seed yield (g/plant)					Oil% in seeds				
Cont	4.3	4.6	5.1	5.7	4. 9	7.3	8.5	8.9	9.3	8. 5
2g AN	7.8	9.2	11.5	12.3	10. 2	17.5	18.2	19.8	20.5	19. 0
4g AN	8.9	11.4	12.0	19.3	12. 5	19.8	21.3	22.8	23.5	21. 9
Azoto	9.4	11.9	18.4	20.4	15. 0	22.8	24.2	24.7	26.8	24. 6
Mean	7. 6	9. 3	11. 8	14. 4		16. 9	18. 1	19. 1	20. 0	
LSD (0.05)	A=0.79		B=0.53	AB=0.71		A=0.48		B=0.49	AB=0.81	

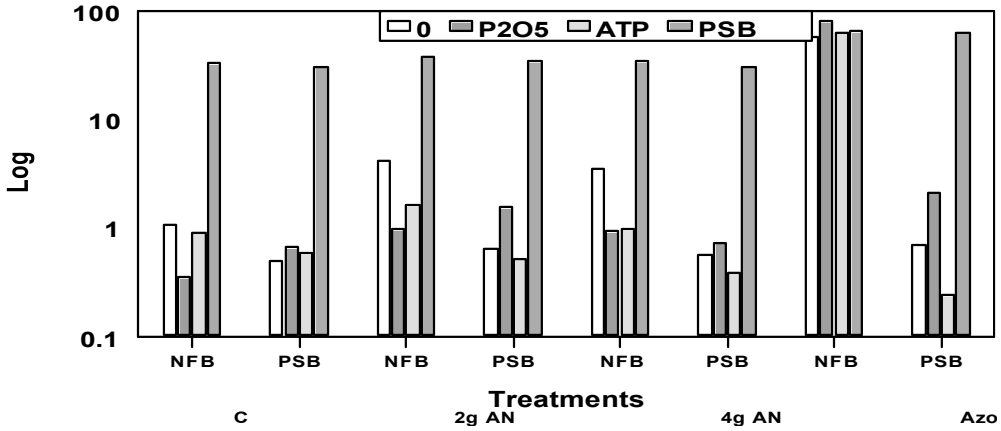

Fig. 1: The population size of introduced beneficial bacteria in the rhizosphere of *Matthiola incana* plants (NFB=nitrogen fixing bacteria, PSB=phosphate solubilizing bacteria, C=control, P_2O_5=calcium superphosphate, AN=ammonium nitrate, ATP= adenosine tri-phosphate)

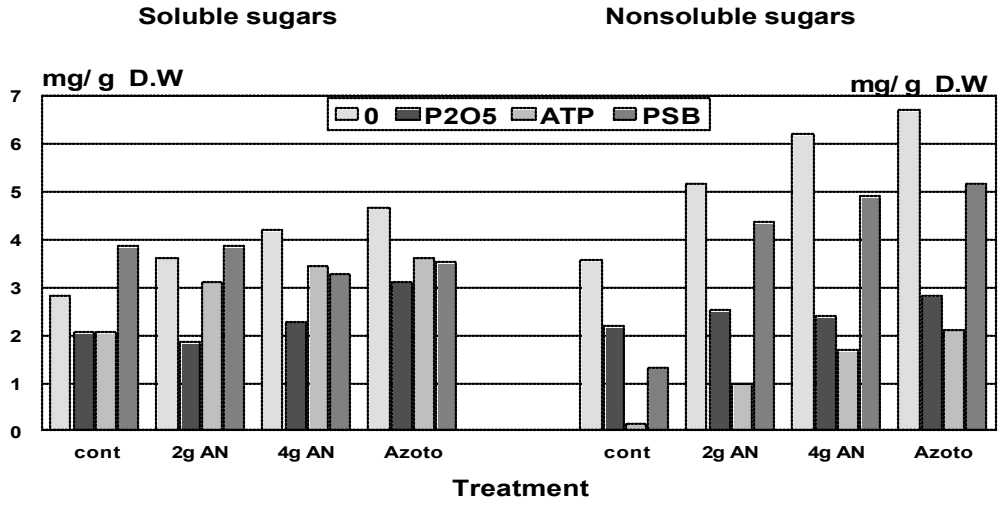

Fig. 2: The effect of nitrogen fixing bacteria (NFB) and phosphate solubilizing bacteria (PSB) combined with mineral fertilizers on soluble and nonsoluble sugars of *Matthiola* plants

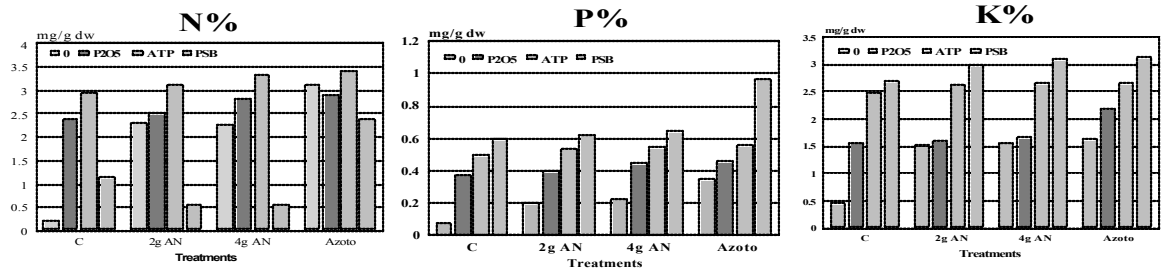

Fig. 3: The effect of nitrogen fixing bacteria (NFB) and phosphate solubilizing bacteria (PSB) combined with mineral fertilizers on N, P and K in shoot of *Matthiola incana* L. plants

both inoculated and non-inoculated, at varying multiplication rates, reaching their peak almost on the flowering stage. The counts of these bacterial strain in most of the other treatments products were significantly lower than in the ATP. Nevertheless, the counts of two strains were reasonably high at flowering stage, 10^4-10^5 CFU of *Bacillus* spp. and 10^3 to 10^4 CFU of *Azotobacter* spp. were still present (Fig. 1).

Plant Biomass Accumulation: Bacterial inoculation affected the early plant growth of *Matthiola incana* L. plants (Table 2). Plants inoculated with bacterial strains (*Bacillus* and *Azotobacter)* had a significant effect on growth of *Matthiola incana* plants in nutrient soil, while non-treated plants by comparison performed poorly under such conditions. Defreitas [28] also, demonstrated that in low fertility Asquith soil, *Pseudomonas* bacterial strains significantly enhanced early plant growth. According to Lazarovitz and Nowak [29], the bacterisation only marginally increased yields when tested under ideal climatic situations. The greatest benefits occurred when crops encountered stressful conditions for prolonged periods such as low mineral fertilizers (2gN).

After inoculation of bacterial strains combined with P fertilizer data recorded in Table 1 indicate that phosphorus treatments improved the estimated characters compared with those untreated control. They showed also considerable differences among there treatments. They superiority was far exogenous ATP spraying application. It was considered the most effective treatment for increasing growth characters i.e plant height, no of branches, fresh and dry weight/ plant and as well as no of flower and dry weight of flower. These results may be attributed to the major role of plant metabolism according to Mengel and kirkby [30]. Also, Dessouky [31] reported that nitrobeen, phosphoren and physiological phosphorus (ATP) increased the plant growth of borage plants. Chaykovskaya *et al*., [32] reported that PSB increased phosphorus accumulation in plants, yield of pea and barley. The bacterial strains were able to dissolve hard soluble organophosphates. Jumaniyazova *et al*., [33] reported that PSB *Bacillus* sp. mobilize phosphate from organic hard soluble phosphoric compounds and increased growth and yield of cotton in Calcareous soil.

The dry matter of the plants after the growth period ranged from 19.13 to 45.0 g / pot (Table 1 & 2). The control plants showed very poor growth, which may be attributed to nutrient deficiency, e.g. the lack of available P in the unfertilized soil. Moderate increases in plant biomass were observed due to the increase of nutrients either in chemical or biofertilizer form. The fertilizer effect on plant growth was much more pronounced after inoculation of *Azotobacter* and its combination with PSB. The highest no. of flower of 79.8 and seed yield 14.4 with *Azotobacter* and its combination with PSB while foliar ATP gave higher yield of flower and seed yield plant. With regards to the increase in plant biomass, PSB seemed to be more effective than *Azotobacter* at the recommended fertilization level. However, the effect of the two biofertilizers of the recommended level on plant yields was not significantly differed (p>0.05). It was noted that even plants grown on the fertilization level produced almost higher dry matter produced by plants grown on the chemical fertilizer treatment.

These results suggest that the dual inoculation of beneficial bacteria could, at least to some extent, compensate the nutrient deficiency in soils. The unexpectedly low biomass of plants grown on the chemical treatment could also be attributed to the disappearance of indigenous microbes, which may be essential to increase nutrient bioavailability and uptake in the rhizospheric soil. Stimulation of different crops by rhizobacterial inoculation has also been demonstrated by other studies both in laboratory and field trials. For example, it was reported that wheat yield increased up to 30% with *Azotobacter* inoculation and up to 43% with *Bacillus* inoculants [34]; and a 10-20% yield increases in the same crop was reported in field trials using a combination of *B. megaterium* and *A. chroococcum* [35]. Strains of *Pseudomonas* have increased root and shoot elongation in canola, lettuce and tomato [8,9].

Regarding the chlorophyll a, b, the total, soluble and non soluble sugars data presented in Table 3 and Fig. 2 indicate that increased as results as using different sources of biofertilizer in compassion with the control. The highest value was obtained from ATP application. The above mentioned results are in harmony with those obtained by Abd EL-Kawy [36] on geranium plants and Dessouky (31) on *Borage officinafis* plant. Lyons and Breidenbach [37] and Ortiz [38] mentioned that phosphorus nutrition is doubly critical because the total supply of phosphorus in most soils is low and is not readily available for the plant use.

Nutrient Acquisition: Dual inoculation with rhizobacteria seemed to be the most effective treatment combination to improve plant nutrient uptake (Fig. 3). N concentration in plants under different treatments ranged from 1.70 (control) to 2.68% (dual inoculation with rhizobacteria). Although dually inoculated plants with rhizobacteria

Table 3: The effect of nitrogen fixing bacteria (NFB) and phosphate solubilizing bacteria (PSB) combined with mineral fertilizers on chlorophyll (a, b and total) and carotinods of *Matthiola incana* L. plants (means of two seasons)

	Chlorophyll (a) mg/g FW)					Chlorophyll (b) mg/g FW)				
Treatments	0	P2O5	PSB	ATP	Mean	0	P2O5	PSB	ATP	Mean
Cont	0.63	1.15	2.35	3.25	1. 845	0.423	0.411	1.05	1.56	0. 861
2g AN	1.10	1.18	2.81	3.45	2.135	0.763	0.425	1.28	1.64	1. 027
4g AN	1.23	1.28	3.11	3.52	2. 285	0.795	0.568	1.34	1.73	1. 108
Azoto	1.32	2.13	3.18	3.83	2. 615	0.805	0.959	1.38	1.83	1. 244
Mean	1. 07	1. 44	2. 86	3. 51		0. 697	0. 591	1. 26	1. 69	
LSD (0.05)	A=0.002 B=0.001 AB=0.004					A=0.004 B=0.006 AB=0.002				
	Total chlorophyll (mg/g FW)					Carotinods				
Cont	1.0653	1.561	3.400	4.810	2. 71	0.323	0.44.3	1.211	1.422	0. 850
2g AN	1.0863	1.605	4.050	5.090	3.152	0.481	0.464	1.253	1.581	0. 945
4g AN	2.025	1.848	4.450	5.250	3.393	0.562	0.552	1.420	1.632	1. 011
Azoto	2.125	3.085	4.460	5.660	3.858	0.642	1.030	1.363	1.670	1. 176
Mean	1.767	2.025	4.115	5.205		0. 803	0.622	1. 312	1. 576	
LSD (0.05)	A=0.003 B=0.001 AB=0.005					A=0.004 B=0.003 AB=0.001				

Table 4: The effect of nitrogen fixing bacteria (NFB) and phosphate solubilizing bacteria (PSB) combined with mineral fertilizers on fatty acid fraction of fixed oil of *Matthiola incana* L. plants

Treatments		Cont	2 g AN	4 g AN	Azoto	P2 O5	PSB	ATP
Components%								
Caprylic	(8:0)	0.21	0.51	0.25	0.21	0.25	0.21	0.20
Capric	(10:0)	0.18	0.50	0.10	0.30	0.28	0.23	0.10
Lauric	(12:0)	0.13	0.70	0.11	0.62	0.15	0.65	*
Myristic	(14:0)	0.56	0.47	0.55	0.58	0.11	0.68	0.12
Palmitic	(16:0)	10.92	13.34	13.43	10.97	11.23	9.64	11.60
Arachidic	(20:0)	19.10	40.13	42.11	14.21	5.48	2.51	22.18
Oleic	(18:1)	11.23	2.25	0.06	8.89	15.56	21.80	3.85
Linoleic	(18:2)	18.80	15.34	27.24	21.65	24.20	25.9	15.10
ã-linolenic	(18:3)	16.64	19.22	14.30	35.84	39.10	38.33	45.47
Erucic	(22:1)	0.38	1.60	0.99	1.43	1.15	*	2.37
T. indentified		78.15	94.06	99.14	94.70	97.51	99.95	99.99
T. saturated		12.00	15.52	14.44	12.68	12.02	11.41	11.02
T. unsaturated		66.15	78.54	84.70	82.02	85.49	88.54	88.97
T.uns/T.s ratio		5.51	5.06	5.87	6.47	7.11	7.76	8.07

T.uns/T.S = Total unsaturated / Total saturated, * = trace

showed unexpectedly low N concentrations in the plant tissue. Inoculation also, leads to the increase of N content in the biomass of *Matthiola* plants. The inoculation with biofertilizers had a more stimulating effect on the assimilation of N than chemical fertilizers. However, PSB performed better than *Azotobacter* in stimulating P uptake, when combined with chemical fertilizer, especially at lower nutrient level. The pattern of P and K uptake by plants under different treatments was similar to N assimilation. The lowest P uptake was detected in plants grown in uninoculated and unfertilized pots. Either single treatment of chemical with bacteria inoculation resulted in an increase in P and K uptake to different degrees when compared with the control. The maximum P and K assimilation were obtained with the dual inoculation of rhizobacteria. The present results show that inoculation with microorganisms may increase the efficiency of fertilizer use at both high and low fertilization levels.

Amending soil with beneficial microbes could compensate for nutrient deficiency and maintain, at least partly, a normal plant development. The biofertilizer therefore may have a potential to decrease the input cost of agricultural production and be applied to the revegetation of low commercial value sites, such as metal tailings ponds [39]. It has been reported that *Azotobacter* not only provides nitrogen, but also produces a variety of growth-promoting substances [40], among them indole acetic acid, gibberellins and B vitamins [41]. These substances stimulate, at least to some degree, the production of root exudates. In addition, another important characteristic of *Azotobacter* associated with plant improvement is excretion of ammonia in the rhizosphere in the presence of root exudates [42]. In addition, synthesis of biocontents minerals uptake translocation and retention processes are dependent on the adenosine tri-phosphate (ATP) supply [30].

Fatty Acid Fraction: the plants inoculated with biofertilization and chemical fertilizer or combination reveal that fertilizer and ATP application resulted in relative increase in total unsaturated fatty acids but slight decreased total saturated fatty acids when compared with untreated plants (Table 4). In all the treatments the major saturated fatty acids were plamitic. It arranged from 9.64% to 13.43% with 4g ammonium nitrate.

In case of the unsaturated fatty acids, the results showed that gamma-Lino1enic acid was found as major in all treatments used. Yossef *et al.,* [43] found that gamma-linolenic acid as major component oil of *Matthiola incana* L. seeds. The maximum percentage of ATP foliar application (45.47%) followed by phosphor treatment (39.10%) which was considered as a major unsaturated fatty acid compared with the control (16.64%). The average of increment in ã-linolenic shoot per plant was (173.26% with ATP as foliar application when compared with control treatment. In addition, the data shown in Table 5 indicate that ATP application resulted in the highest value of total unsaturated fatty acids (88.97%) and the least value of total saturated ones (11.02%).

Finally, all treatments used had a marked increasing effect on the ratio of total unsaturated /total saturated (T.uns/T.s ratio) which ranged from (8.07%) at ATP foliar spraying to 5.06% at 2 g ammonium nitrate compared to the control. These results are in agreement with those obtained by Dessouky [31], he reported that (T.uns/T.s ratio) at ATP foliar spraying treatment compared to the other treatments.

CONCLUSION

In summary, the final results of the bacterial plant growth-promotion in our experiments show that NFB and PSB can play an essential role in helping annual flowering crops establish and grow in nutrient deficient treatments soil. PSB are able to mobilize more P into plants, where hard soluble phosphates are presented in soil and increased yield and growth. Moreover, low fertilization level resulted in an increase of introduced beneficial microorganisms, which indicated plants might be more dependent on N fixing bacteria and P solubilizer under a N and P-deficient condition.

In addition, the using ATP effectively improved the plant growth characters and the chemical constituents of seeds.

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