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Effect of Bio and Chemical Fertilizers on Growth and Flowering of *Petunia hybrida* Plants

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ABSTRACT

Two field experiments were carried out during two successive seasons of 2011 and 2012 to study the effect of bio- and chemical fertilizers on growth, flowering and some chemical analysis of *Petunia hybrida* (cv. Bravo White). *Petunia* plants were sprayed with *Azospirillum lipoferum* (nitrogen fixing bacteria, N.F.B.) and *Bacillus polymxa* (phosphate dissolving bacteria, P.D.B.) and their mixture in presence or absence of a complete fertilizer of 19N: $19P_2O_5$: $19K_2O$. Both bacterial inoculants and their mixture showed significantly increases in the studied vegetative growth (i.e., plant height, branches number, leaf area, dry weights of shoots and roots) and flower parameters (i.e. flowering date, number of flower/branch and flowering period) when compared with the control (full dose of NPK chemical fertilizer = 5 g plant^{-1} twice). Also the results revealed that using *Azospirillum* sp. + *Bacillus* sp. plus 5 g plant^{-1} of the chemical fertilizer produced the highest significant values of all growth, flowering parameters and chemical analysis (chlorophyll, total carbohydrates, N and P percentages) compared with the control. It was clear that biofertilizers beside its ability to improve the nutrient supply in the soil, they also increased the efficiency of added chemical fertilizer. From the obtained results it can be used the half dose of the recommended chemical fertilizer (2.5 g plant^{-1} twice) combined with each of nitrogen fixing bacteria (at 2 mL L^{-1} twice) and phosphate dissolving bacteria (at 2 mL L^{-1} twice) to grow *Petunia hybrid* cv. Bravo White with high quality, reducing the environmental from pollution and surpassed the recommended dose of the chemical fertilizer.

Key words: Biofertilization, chemical fertilizer, *Petunia hybrida*

INTRODUCTION

Increased flower production, quality of flowers and perfection in the form of plants are the important objectives to be recognized in bedding and flower production. Boodley (1975) considered quality to be a function of nutrient level. Though nitrogen, phosphorus and potassium influence the production and quality of flowers greatly, the fertilizers recommendation is very high which reflects directly on cost of production. In addition, use of synthetic fertilizers and chemicals in high ranges possess environmental pollution problem with potential hazards to flora and fauna and also on human beings. Biofertilizers are considered as an important part of environment friendly sustainable agricultural practices (Shehata and El-Khawas, 2003). Investigation took place for using biofertilizers as an alternative to chemical fertilizers or at least to minimize the levels of these chemicals in order to protect the environment from pollution, decrease the production cost and produce chemical-free product. Biofertilizers are the formulation of living microorganisms which are able to fix atmospheric nitrogen and convert insoluble phosphorus to be valuable for the plants (Mahfouz and Sharaf-Eldin, 2007). Biofertilizers are inputs containing microorganism which are capable of mobilizing nutritive elements from non-usable to usable form through biological processes (Subba Rao, 1993). The beneficial effects induced by the inoculated bacteria on plant growth and yield were attributed to one or more of the following factors.

Production of growth promoting substances such as gibberellines, cytokinins, IAA and other auxins that specially stimulate the root system and may also alter the endogeneus plant phytohormone balance (Dobbelaere *et al.*, 1999); Improvement of water and nutrients uptake, especially those of limited availability in soil such as P, N and micronutrients (Kilian *et al.*, 2000); Fixation of molecular nitrogen in rhizosphere that becomes available to inoculated plant (Skvortsova *et al.*, 1998); Production of antibiotic metabolites effective against soil borne pathogens (Kraus and Loper, 1990); Production of B-group vitamins that promote rooting capacity and affect the population of microbial community (Revillas *et al.*, 2000). Many researchers have reported the beneficial effects of inoculated rhizobacteria and free living diazotrophs on plant growth and yield. Therefore, emphasis is now focused on the use of biofertilizers like *Azotobacter*, *Azospirillum* and phosphate solubilizing bacteria.

Hybrid *Petunias* are garden standbys developed from several South American *Petunia* species. These sun and heat-loving annuals or tender perennials were among the first ornamentals to be breed for the bedding plant market in the 1950s. The common garden petunia, *Petunia hybrida*, is derived from *P. integrifolia* and *P. axillaris* that are two of many *Petunia* species endemic to South America. *Petunia* comprises annual or perennial herbs, upto 1 m tall, with erect, ascendant, decumbent or procumbent stems, rarely rooting at the nodes. The leaves are sessile or petiolate with blades elliptic, ovate or obovate, more rarely rounded or linear, membranaceous, somewhat juicy, flat and usually without marked venation. *Petunia* is cultivated in flower beds and pots and requires full sunlight to produce plants and flowers with bright attractive colors. *Petunias* are as easy to grow as they are pretty. They require amplesun and grow best in rich soil with good drainage. They bloom best with regular fertilization and will continue to flower all seasons. Bravo *Petunias* are good for beds, hanging baskets, window boxes and tubs.

In the present study, the effects of biofertilizers with nitrogen fixers, phosphate dissolving bacteria and or chemical fertilizers, separately or in different combinations on growth of *Petunia hybyrida* (cv., Bravo White) were studied aiming to reduce the used chemical fertilizers, maximizing their use efficiency and to obtain highest growth and productive parameters.

MATERIALS AND METHODS

Two field experiments were conducted at the Experimental Nursery of the Floriculture, Ornamental Plants Department, Faculty of Agriculture, Alexandria, Egypt, during the two successive seasons of 2011 and 2012.

Plant materials and fertilizers: One month old seedlings of *Petunia hybrida* (cv., Bravo White) were bought from a commercial nursery on November 2011 and 2012 in seedling trays containing peat moss:sand:perlite (1:1:1(v/v/v)). The seedling trays were kept under greenhouse conditions. After 4 weeks, the seedlings were carefully transplanted to 10 cm diameter pot (one plant pot^{-1}) filled with the same medium used for germination. After another 4 weeks, plants were repotted into 25 cm uniformly diameter pots (one plant pot^{-1}). The biofertilizers were used as a foliar spray

(Esitken *et al*., 2003; Pirlak *et al*., 2007). *Azospirillum lipoferum* (nitrogen fixing bacteria, N.F.B.) and *Bacillus polymyxa* (phosphate dissolving bacteria, P.D.B.) separately or mixed (2 mL from the solution of bacteria/liter), were applied in two doses (200 mL pot dose^{-1}) the first one after 2 weeks from the first transplanting and the second one after 3 weeks from the second repotting and at the same time of adding the chemical fertilizer. A complete chemical fertilizer of 19N:19P_2O_5:19K_2O KRISTALON (From Hydro Agri Specialition Company,Rotterdam, Holland.) (Khattab *et al*., 2001) on *Fuchsia hybrid* and Ferrante *et al*. (2006) on *Salvia splendens*. The recommended doses were divided to various level i.e., 0, 25, 50 and 100% equal to 0, 1.25, 2.50 and 5 g plant^{-1} and added different combinations of the two strains of bacteria as following:

- **T1:** Full recommended dose of the chemical fertilizer (19N:19P_2O_5:19K_2 5 g plant^{-1} (Control)
- **T2:** Inoculation with phosphate dissolving bacteria (*Bacillus polymyxa*) (P.D.B) at 2 mL L^{-1}
- **T3:** 25%of recommended dose of the chemical fertilizer (1.25 g plant^{-1})+P.D.B at 2 mL L^{-1}
- **T4:** 50% of recommended dose of the chemical fertilizer (2.5 g plant^{-1})+P.D.B at 2 mL L^{-1}
- **T5:** 100% of recommended dose of the chemical fertilizer (5 g plant^{-1})+P.D.B at 2 mL L^{-1}
- **T6:** Inoculation with nitrogen fixing bacteria (*Azospirillum lipoferum*) (N.F.B.) at 2 mL L^{-1}
- **T7:** 25% of recommended dose of the chemical fertilizer (1.25 g plant^{-1})+N.F.B at 2 mL L^{-1}
- **T8:** 50% of recommended dose of the chemical fertilizer (2.5 g plant^{-1})+N.F.B at 2 mL L^{-1}
- **T9:** 100% of recommended dose of the chemical fertilizer (5 g plant^{-1})+N.F.B at 2 mL L^{-1}
- **T10:** Inculation with mixture of nitrogen fixing bacteria and phosphate dissolving bacteria N.F.B+P.D.B at 2 mL L^{-1}
- **T11:** 25% of recommended dose of the chemical fertilizer (1.25 g plant^{-1})+N.F.B+P.D.B each at 2 mL L^{-1}
- **T12:** 50% of recommended dose of the chemical fertilizer (2.5 g plant^{-1})+N.F.B+P.D.B each at 2 mL L^{-1}
- **T13:** 100% of recommended dose of the chemical fertilizer (5 g plant^{-1}) N.F.B+P.D.B each at 2 mL L^{-1}

P.D.B: Phosphate dissolving bacteria, N.F.B: Nitrogen fixing bacteria

Experimental design and statistical analysis: The experiment was carried out in a complete randomized blocks design with thirteen treatments and three replications. Each treatment had 6 plants replicate^{-1}, so reaching a total of 234 plants (13×3×6). All obtained data were subjected to the analysis of variance according to the procedure outlined by Steel and Torrie (1982). LSD test was conducted to distinguish the differences between the means of treatments according to Snedecor and Cochran (1967).

Morphological estimation: The growth characters such as plant height (cm), branches number, leaf area (cm^2), shoot dry weight (g), root dry weight (g), flowering date (days to first flower shows its colour), flower number branch^{-1} and flowering period (days) were recorded.

Biochemical estimation: Total chlorophyll content was determined as described by Yadava (1986). While, the percentage of total carbohydrates in the dried leaves was estimated according to Dubois *et al*. (1956). In addition, leaf nitrogen percentage was determined by micro-Kjeldahl method as recommended by Bremner and Mulvany (1982), while available phosphorus was determined by the chlorostannous phosphomolybdic acid method (Jackson, 1973).

RESULTS AND DISCUSSION

Morphological characteristics

Plant height: Generally, the results of both seasons (Table 1) show that the differences between using 100% chemical NPK (control) and NFB, NFB+PDB, PDB+ 25% NPK and PDB treatments, respectively were not significant in both seasons. The tallest plants resulted from the treatment of full dose of NPK+ the two types of biofertilizer (NFB+PDB) in comparison with the other treatments in both seasons. The superiority of this treatment over the other treatments may be due to the increase of N, P and K in the root zone as these elements play an important role in the metabolic processes in plants as well as cell division and elongation, consequently increases in plant height could be obtained. These results are in harmony with the results obtained by Kandeel *et al.* (2004) on *Anethum graveolens* and *Foeniculum vulgare* Mill.

Branches number: Data in Table 1 show that the highest number of branches per plants was resulted by applying the full dose of NPK+ the mixture of the two strains of bacterias. The increment in branches number as a result of adding NPK combined with biofertilizers may be due to the important role of N, P and K elements in addition of sugars, amino acids and auxins which enhanced the differentiation of axillary buds so, fertilization increases partition in the tissues as branches. These results are in agreement with those found by Bhattacharjee (1988) on *Jasminum grandiflorum*, L. and Katiyar *et al.* (1999) on rose plants.

Leaf area: Data presented in Table 1 indicate that the maximum expansion of produced leaves was obtained by using the mixture of NFB and PDB combined with 100% chemical fertilizer in the first and second season, followed by the treatments of NFB or PDB+100% NPK as compared with control. The enhancement in leaves growth as a result of applying mineral fertilizer combined with mixture of strains of biofertilizer bactereias may be due to the production of phytohormones by the biofertilizers and improving the availability of nutrients (Martin *et al.*, 1989). Besides,

Table 1: Effect of bio and chemical fertilizers and their interactions on plant hight, No.of shoot, leaf area, shoot dry weight and root dry weigh of *Petunia hybrida* plants during the two seasons of 2011 and 2012

	Season									
	Plant hight (cm)		Branches No.		Leaf area (cm^2)		Shoot dry weight (g)		Root dry weight (g)	
Treatments	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	9.50	8.94	8.78	7.89	22.76	19.65	3.80	3.02	2.10	2.00
NFB	9.39	8.50	7.11	6.56	17.00	10.03	3.10	2.56	1.88	1.77
NFB+25%	11.44	9.94	7.67	7.22	17.93	16.95	3.21	2.68	2.40	1.99
NFB+50%	12.22	10.22	8.11	7.56	21.88	19.08	3.47	2.88	2.76	1.89
NFB+100%	12.17	11.61	10.33	8.76	28.85	23.32	4.09	3.47	3.43	2.85
PDB	8.56	7.17	7.00	7.11	18.42	19.36	2.80	2.49	1.62	1.42
PDB+25%	8.89	7.94	8.44	8.11	21.69	18.53	3.19	2.57	1.51	1.38
PDB+50%	12.06	10.61	8.33	8.00	23.85	20.54	3.37	2.87	2.38	1.58
PDB+100%	13.00	11.89	10.00	8.78	28.91	25.65	4.13	3.38	3.32	2.66
NFB+PDB	9.06	8.33	8.44	8.22	20.37	18.42	3.40	2.42	2.67	1.80
NFB+PDB+25%	12.33	11.00	9.11	9.67	22.78	19.85	3.46	2.72	2.79	1.97
NFB+PDB+50%	12.78	11.39	11.11	10.44	23.77	22.69	3.77	3.09	3.10	1.88
NFB+PDB+100%	14.22	12.06	12.33	11.44	30.51	25.86	4.75	3.67	4.21	2.88
L.S.D.(0.05)	1.48	1.10	1.06	1.12	0.87	1.43	0.24	0.18	0.28	0.24

presence of microorganisms can change form of nutrients into available ones that can be easily absorbed by plants. This may lead to an increase in photosynthesis and other biological processes that reflected on the cell width and division. These results are in agreement with those of Abbas (2003) on *Rosa hybrida* and Al-Qadasi (2004) on *Ocimum basillicum*.

Shoot dry weight: Data of the two seasons presented in Table 1, indicate that the heaviest shoot dry weight resulted from the treatments of full dose of NPK combined with the mixture of the two bacteria. The increment in dry weight as a result of either full dose of NPK and the mixture of the two biofertilizers strains may be due to that fertilization stimulate growth by increasing the availability of nutrients generally, thereby stimulating development and the size of photosynthesizing surface, consequently more dry matter could be accumulated. These results are in harmony with those obtained by Bhattacharjee (1988), Khalafalla *et al*. (1994) and Younis *et al*. (2004) on *Jasminum grandiflorum* L., Kandeel *et al*. (2004) on *Anethum graveolens* and *Foeniculum vulgare* Mill.

Root dry weight: Data presented in Table 1 show that the highest significant increases in root dry weight were found in the case of using the mixture of NFB and PDB combined with 100% chemical fertilizer in the two seasons followed by the treatments of NFB or PDB+100% NPK as compared with the other treatments. It was found also that the differences between 100% NPK all biofertilizer treatments and NFB or PDB+100% NPK were not significant in the second season. These results may be due to that presence of the microorganisms with the chemical fertilizer at a specific concentration led to activate the vegetative growth of petunia, consequently increasing root length and/or size or both, as a result the root dry weight would be increased.

Flowering date: Significant differences were observed among the different treatments of *Petunia* for flowering date (Table 2). Generally, using combination of all biofertilizers and 50 or 100%

Table 2: Effect of bio and chemical fertilizers on flowering date, No. of flower/branch and flowering period of *Petunia hybrida* plants during the two seasons of 2011 and 2012

	Season					
	Flowering date (day)		No. of flower/branch		Flowering period (days)	
Treatments	1st	2nd	1st	2nd	1st	2nd
Control	46.89	47.44	4.44	4.56	94.89	95.22
NFB	52.33	52.44	3.44	3.22	86.44	86.005
NFB+25%	51.00	50.78	4.44	4.22	91.22	92.44
NFB+50%	37.33	38.78	4.78	4.44	100.56	104.78
NFB+100%	33.33	34.78	6.11	5.22	106.89	108.11
PDB	48.67	50.00	4.00	3.78	93.44	91.89
PDB+25%	38.40	41.44	4.56	4.11	95.33	94.67
PDB+50%	35.00	37.22	5.00	4.89	103.20	101.89
PDB+100%	32.89	35.76	5.56	6.11	111.11	111.67
NFB+PDB	33.33	34.33	3.56	3.33	104.67	101.89
NFB+PDB+25%	32.33	33.56	4.22	4.11	106.44	108.00
NFB+PDB+50%	31.22	30.78	5.22	5.11	111.67	111.11
NFB+PDB+100%	29.56	29.11	6.67	6.11	113.11	113.00
L.S.D.(0.05)	1.71	1.49	0.75	0.84	1.87	1.76

chemical fertilizer gave the shortest flowering date compared with the other treatments. However, biofertilization treatments i.e., T6 (P.D.B) or T2 (N.F.B.) and T3 (N.F.B) 25% NPK gave the longest flowering date compared with the other treatments. These results are in agreement with those of Dalve *et al.* (2009) who reported that the flowering parameters were positively influenced by application the biofertilizers (*Azotobacter* sp. +*Azospirillum* sp.) in combination with nitrogen. Also, in carnation, Gupta *et al.* (2004) reported that the application of bio-fertilizers like VAM, *Azospirillum* sp., phosphate solubilizing bacterium have registered minimum number of days to first flowering. This result might be attributed to the stimulative effect of biofertilizers on vegetative growth as reported by Mahfouz and Sharaf-Eldin (2007).

Number of flowers per branch: In the first season, the number of flowers per branch was significantly increased over control with the application of different biofertilizer treatments +100% chemical fertilization and nitrogen fixing bacteria +100% chemical fertilization as shown in Table 2, followed by phosphate dissolving bacteria +100% and both P.D.B, N.F.B+50% chemical fertilization, the values reached 6.67, 6.11, 5.56 and 5.22 flowers per branch, respectively. In the second season, as a result of applying both P.D.B, N.F.B+100% chemical fertilization and P.D.B+100% NPK followed by N.F.B+100% NPK and P.D.B, N.F.B+50% chemical fertilization, respectively recorded significant values reached 6.11, 6.11, 5.11 and 4.89 flowers per branch, respectively. The treatments of full dose of NPK and ½ dose of NPK + biofertilizers forced the plants to flower profusely more than the other treatments because they improved their vegetative growth which led to increase the amount of carbohydrates in plant tissue that are important to initiate many flowering buds. Similar results were achieved by Bhattacharjee (1988), Khalafalla *et al.* (1994) and Younis *et al.* (2004) on *Jasminum grandiflorum* L. and Falahi *et al.* (2009) on chamomile.

Flowering period: Data of the two seasons in Table 2 revealed significant variations for flowering period among the different treatments. Generally, adding 100% chemical fertilizer combined with NFB (T5) or with PDB (T9) or with NFB+PDB (T12) or at 50% with NFB+PDB (T11) gave the longest significant flowering period, compared with the other treatments. The minimum flowering period was recorded in T2 where the plants were supplied with a nitrogen fixing bacteria only. The longest period may be attributed to the presence of biofertilizers especially inoculation with *Azosprillium* sp. and PSB which consequently lead to more flower initiation and flower duration. This may be due to easy uptake of nutrients and simultaneous transport of growth promoting substances like cytokinins to the axillary buds resulting in breakage of apical dominance. Ultimately, they resulted in better sink for faster mobilization of photosynthates and early transformation of plant parts from vegetative to reproductive phase. Similar findings were published by Padaganur *et al.* (2010) on tuberose and Meshram *et al.* (2008) on chrysanthemum.

Biochemical constituents

Total chlorophyll content: Generally, the total chlorophyll content of *Petunia* leaves in the two seasons was increased by the application of biofertilizers as shown in Table 3. The highest increases in chlorophyll content compared with the control treatment were obtained from treatments with the application of the two biofertilizers NFB+PDF+100% chemical fertilization dose, followed by mixture

Table 3: Effect of bio and chemical fertilizers and their interaction on chlorophyll content (SPAD unit) and (%) of total carbohydrate, nitrogen (N%) and phosphorus (P%) of *Petunia* leaves during the two seasons of 2011 and 2012

	Season							
	Total chlorophyll		Total carbohydrate (%)		N (%)		P (%)	
Treatments	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	33.43	27.94	15.42	14.69	2.11	2.14	0.257	0.247
NFB	27.10	23.69	14.58	14.31	2.07	1.98	0.190	0.177
NFB+25%	34.16	26.12	15.70	15.22	2.16	2.00	0.190	0.187
NFB+50%	36.04	29.93	16.04	15.33	2.31	2.15	0.237	0.227
NFB+100%	36.80	32.77	17.15	16.39	2.42	2.20	0.257	0.237
PDB	28.58	25.60	15.41	14.58	1.87	1.90	0.207	0.177
PDB+25%	35.20	28.27	16.57	15.86	2.07	2.06	0.217	0.227
PDB+50%	32.96	27.52	17.10	16.32	2.18	2.10	0.237	0.220
PDB+100%	37.10	30.80	17.62	16.80	2.40	2.32	0.267	0.270
NFB+PDB	29.81	24.91	15.40	14.86	2.07	2.07	0.217	0.207
NFB+PDB+25%	34.88	29.59	16.39	15.60	2.29	2.13	0.237	0.233
NFB+PDB+50%	39.51	33.89	17.47	16.87	2.37	2.22	0.267	0.247
NFB+PDB+100%	44.32	36.50	18.16	17.64	2.43	2.50	0.287	0.247
L.S.D (0.05)	1.77	2.33	0.34	0.39	0.082	0.068	0.005	0.005

of PDB and NFB+50%, NFB+100% chemical fertilizer and PDB+100% chemical fertilizer, respectively. These results may be due to supply the plants with the essential elements for chlorophyll formation such as nitrogen, magnesium and others, consequently the chlorophyll content in the leaves could be increased (Gomaa and Abou-Aly, 2001). Similar results were obtained by Hassan (2009) on Hibiscus sabdariffa.

Total carbohydrates content: Data of the two seasons, presented in Table 3, clear that the highest value of the total carbohydrates in the leaves of *Petunia* plants was obtained by addition the mixture of PDB and NFB+100% chemical fertilizer comparing with the other treatments. This result may be attributed to increase each of leaf area and leaf chlorophyll content, consequently the rate of photosynthesis process would be increased, as a result the percentage of total carbohydrate in the leaves could be also increased (Singh and Prasad, 2012). Al-Qadasi (2004) reported that inoculation with (*Azotobacter* sp.+*Azospirillum* sp.+*Bacillus* sp.) caused an increase in the total carbohydrate content of *Ocimum basilicum* herb. Hassan (2009) reported that total carbohydrates was increased as a result of applying biofertilizers alone or combined with chemical fertilizers.

Nitrogen content (%): Data of the two seasons in Table 3 show that using each of T9 (PDB+100% chemical fertilizer) or T11 (NFB+PDB+50% chemical fertilizer) or T12 (NFB+PDB+100% chemical fertilizer) gave significant increases in the nitrogen percentage in the leaves of *Petunia*, compared with the other treatments. These results were probably due to that presence of the used microorganisms led to increase the solubility and availability of the soil nitrogen, consequently increasing the uptake amount of it by roots, as a result the percentage of nitrogen in the leaves would be increased. These results are in agreement with those of Esitken *et al.* (2010) on strawberry plant. It was found by Sarawgi *et al.* (1999) that the uptake of N and P increased with application of phosphate-solubilizing bacteria.

Phosphorus content (%): Data in Table 3 show that the plants treated with mixture of the two strains +100% NPK gave the highest percentage of phosphorus in dry leaves in the first season. While, in the second season, using phosphate dissolving bacteria combined with the recommended dose of chemical fertilizer gave the highest percentage of phosphorus. These results may be due to that Phosphate-Dissolving Bacteria (PDB) secrete organic acids which lead to a transfer of fixed phosphate to soluble phosphate, also may have increased the availability and absorption of phosphorus, thus the percentage of phosphorus in *Petunia* leaves could be increased. These results are in agreement with those of Sarawgi *et al.* (1999) on *Cicer arietinum* and (Mahfouz and Sharaf-Eldin, 2007) on *Foeniculum vulgare* Mill.

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