

## RESPONSE OF *Helianthus annuus* L. PLANTS TO MINERAL AND BIO-FERTILIZATION

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### ABSTRACT

This study was carried out at the experimental nursery of Horticultural Research Institute; Agricultural Research Center, Giza, during the two successive seasons of 2005 and 2006. The main objective was to study the possibility of using biofertilization (Rhizobacterein and Phosphorein) to reduce the need for mineral fertilizers and study its effect on the vegetative growth, flowering and chemical composition of *Helianthus annuus* L. (Big Smile) plants. The results indicated that using a combination of Rhizobacterein and Phosphorein promoted plant height, leaf area and total carbohydrates, compared to using each biofertilizers alone or in combination with half the full rate of chemical fertilization (full rate= 10 g Christalon/plant). Using half the amount of chemical fertilizers and replacing the other half by the combination of biofertilizers caused an increase in number of leaves, fresh and dry weights, and the leaves contained much more total chlorophylls and potassium. This treatment also caused a significant increase in number of lateral flowers. It could be concluded that treating sunflower (Big Smile) plants with 5 g Rhizobacterein and 10 g Phosphorein plus 5 g Christalon per plant produced the most vigorous vegetative growth and the largest number of lateral flowers with good characteristics.

**Keywords:** *Helianthus annuus* L., biofertilizers, flowering plants

### INTRODUCTION

Sunflower belongs to Asteraceae (Compositae) family. The old tall ornamental sunflower cultivars were used in the garden for those who, like the birds, enjoyed eating sunflower seeds. Now, there are many varieties of sunflowers that are burgundy, bi-colors of red and yellow, bright yellow and pale yellow, white, crimson, gold and bronze. And they're not all six feet tall, with many of the new varieties only two to three feet tall. Flower diameter can be from four to ten inches, and there are now many new single flowers as well as doubles and semi-doubles.

They can be used as screens and backgrounds in flowerbeds, for cutting and to attract birds and butterflies to the garden. Sunflowers, while not completely drought tolerant, will get by with less water than many ornamentals.

Big Smile, Music Box, Teddy Bear, Pacino and Solar Babies Mixture are good names to look for in the dwarf varieties. Big Smile is a bright, golden yellow sunflower. It is extra dwarf, only 2-3 feet tall, and early blooming at 55-60 days after sowing. Big Smile is also day neutral, so it can be coaxed into bloom year round either in the garden or in the greenhouse. It is an adaptable variety which will flower well at about 15 in (38 cm) in a pot or 2 ft (60cm) or more in the border.

Sunflower oil contains useful essential fatty acids and a high amount of vitamin E. Also, the oil contains vitamins A, C and D, and is high in linoleic acid. It is excellent for use in cosmetics as it will not irritate the skin.

Biologically grown agricultural products cause less risk to the environment for this reason many researchers, worked on several ornamental plants, and achieved best growth, yield and chemical constituents when bio-fertilizers were used. For example, Karuppaiah (2005) on *Tagetes patula* plants, and Bhaskaran *et al.* (2002) on *Tagetes erecta* plants, found that *Azospirillum* and *Azotobacter* inoculation with a low level of chemical nitrogen (50%) significantly increased growth, yield and bio chemical attributes (i.e. chlorophyll, total sugar, and total free amino acids) over control. Similar results were obtained by Riera-Nelson *et al.* (2003), who inoculated *Helianthus annuus* seeds with bio-fertilizers before sowing. The results showed a notable influence of inoculation on yield and soil physical properties. Sukhovitskaya *et al.* (2001) mentioned that rhizobacterin is a safe bacterial preparation for increasing yield in grain crops and reducing the need for mineral N fertilizers.

N<sub>2</sub>-fixing bacteria can benefit plant growth through the production of secondary metabolites such as antibiotics and plant hormones like substances (Gilic, 1995). Also, Swaefy *et al.* (2007) found that *Rhizobium leguminosarum* bv. *phaseoli* and *Azotobacter chroococcum* showed positive reaction for IAA, siderophores, HCN. Whereas, *Bacillus megatherium* var. *phosphaticum* showed highly positive reaction for P- solubilizers.

## **MATERIAL AND METHODS**

The research was conducted to identify the impact of bio and chemical fertilization on the growth and blooming of sunflower (*Helianthus annuus*) under the prevailing environmental conditions at the Horticultural Research Institute Agricultural Research Center, Giza, during two successive seasons (2005 and 2006).

Seeds of sunflower "Big Smile" were obtained from Ball Horticultural Company, USA. On 15<sup>th</sup> May 2005 and 2006 (in the first and second seasons, respectively), the seeds were sown directly in the field, in 100 X 90 cm plots, each including 3 rows (30 cm apart), at a spacing of 30 cm between holes. Thus, each plot contained 9 plants. Each treatment consisted of three replicates (plots), and the layout of the experiment was a randomized complete blocks design (R.C.B.D.). The mechanical and chemical analyses of the clay loamy soil were carried out before planting according to Chapman and Pratt (1961) and the characteristics are shown in Table (1).

Rhizobacterin and Phosphorein were the bio-fertilizers used in the experiment. The bio-fertilizers were obtained from the General Organization for Agricultural Equalization Fund, Agricultural Research Center, Ministry of Agriculture, Egypt. Rhizobacterin was obtained in bags containing peatmoss inoculated with a mixture of nitrogen fixing bacteria and phosphorus solubilizing bacteria (PSB), whereas in Phosphorein, the peatmoss was

inoculated with *Bacillus megatherium*. The bio-fertilizer treatments were applied to the holes just before sowing the seeds.

**Table (1): Mechanical and chemical analyses of the experimental soil**

Mechanical analysis										
Coarse sand		Fine sand			Silt			Clay		
5.3%		30.6%			38.7%			25.4%		
Chemical analysis										
		Anions (meq/L)			Cations (meq/L)			Available macro nutrients (ppm)		
E.C.	pH	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>	Ca <sup>++</sup>	Mg <sup>++</sup>	Na <sup>+</sup>	N	P	K
2.5	7.3	3.0	19	3.6	10	3.0	12	18.5	2.0	37

E.C.: Electrical conductivity m.mohs/cm<sup>2</sup>      pH: Acid value

In each seasons, the commercial chemical fertilizer Christalon (containing 20000 ppm nitrogen, 20000 ppm P, 20000 ppm K, 1000 ppm Mg, 20 ppm B and chelated elements such as Fe at 1000 ppm, Zn at 500 ppm, Mn at 500 ppm, Cu at 100 ppm, and Ca at 50 ppm) was added two and five weeks after planting, at the rate of 5 or 10 g/plant, divided into two equal doses. The high rate of Christalon (10 g/plant) was considered to be the full rate of chemical fertilization.

The plants were supplied with the following fertilization treatments:

- 1- Chemical fertilizer only (10 g Christalon/plant).
- 2- 5 g Rhizobacterein/plant.
- 3- 10 g Rhizobacterein/plant.
- 4- 5 g Phosphorein/plant.
- 5- 10 g Phosphorein/plant.
- 6- 5 g Rhizobacterein + 5 g Phosphorein/plant.
- 7- 5 g Rhizobacterein + 10 g Phosphorein/plant.
- 8- 10 g Rhizobacterein +5 g Phosphorein/plant.
- 9- 10 g Rhizobacterein + 10 g Phosphorein/plant.
- 10- 5 g Rhizobacterein + 5 g Phosphorein + 5 g Christalon/plant.
- 11- 5 g Rhizobacterein + 10 g Phosphorein + 5 g Christalon/plant.
- 12- 10 g Rhizobacterein + 5 g Phosphorein + 5 g Christalon/plant.
- 13- 10 g Rhizobacterein + 10 g Phosphorein + 5 g Christalon/plant.

Data on plant height (cm), number of leaves/plant, leaf area (cm<sup>2</sup>), fresh and dry weights of leaves, stems and roots, main flower diameter, main flower fresh and dry weight, number of lateral flowers/plant, as well as fresh and dry weights of lateral flowers, were recorded eight weeks after planting. The data were subjected to analysis of variance, and the means were compared using the "Least Significant Difference (LSD)" test at the 0.05 level, as recommended by Snedecor and Cochran (1982).

Chemical analysis of fresh leaves was conducted to determine their contents (mg/gm fresh weight) of chlorophyll (a and b) and total carotenoides, using the method described by Nornai (1982), then the total chlorophylls content was calculated. The total carbohydrates content in dry leaves was also determined, using the method recommended by Herbert *et al.* (1971),

while the N, P and K percentages were determined using the methods described by the A.O.A.C. (1980).

## RESULTS AND DISCUSSION

### Plant height

Data recorded on plant height of *Helianthus annuus* L. (Table 2) show the response of the plants to the different fertilization treatments. When Rhizobacterein or Phosphorein were used alone, plant height was increased with the increase in the rate of application from 5 to 10 g/plant. Using the combination of Rhizobacterein and Phosphorein significantly increased plant height compared to control plants (100% chemical fertilization), especially in the first season. In most cases using the combination of rhizobacterein and phosphorein fertilizer gave taller plants than those receiving 50% of the chemical fertilizer plus the combination of bio-fertilizers at different rates.

The plants inoculated with 5 g/plant Rhizobacterein plus 10g/plant Phosphorein were the tallest plants with heights of 68.00 in the first season and 60.00 cm in the second season.

**Table (2): Effect of chemical and bio-fertilization on plant height (cm) and number of leaves of sunflower plants in the 2005 and 2006 seasons.**

Fertilization treatments	Plant height (cm)		No. of Leaves	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
10 C (control)	38.67	44.00	24.33	25.33
5R	38.67	32.67	18.33	19.67
10R	41.00	40.33	19.00	20.00
5P	31.33	41.00	19.33	21.33
10P	45.33	42.67	22.33	25.33
5R+5P	52.00	48.00	25.67	25.33
5R+10P	68.00	60.00	24.67	28.67
10R+5P	56.33	42.67	27.33	25.67
10R+10P	57.67	48.33	27.67	27.33
5R+5P+50%C	45.33	47.67	25.33	27.67
5R+10P+50%C	32.33	42.33	31.33	26.33
10R+5P+50%C	42.67	46.67	37.67	31.00
10R+10P+50%C	50.00	41.67	36.67	26.33
L.S.D.	11.88	9.49	7.66	6.38

R= rhizobacterein      P= phosphorein      C= christalon

These results are in harmony with those obtained by Mostafa (2002), who found that bio-fertilizer (*Azotobacter chroococcum* and *A. vinelandii*) treatment and increased plant height of *Dimorphotica ecklonis*. Also, Kamran *et al.* (2006) found that plant height of *Helianthus annuus* was increased by plant growth promoting rhizobacteria used as a biofertilizers (*Pseudomonas*, *Azotobacter* and *Azospirillum*).

### **Number of leaves/plant**

Parameters such as number of leaves, leaf area, leaves fresh and dry weights are very important for annual plants, especially before the flowering stage, as leaves fill the space between shrubs and in borders. The data recorded on the number of leaves/plant in sunflower plants (Table 2) show that in most cases, increasing the rate of bio-fertilizers from 5 to 10 g/plant caused an increase in number of leaves/plant. Also, all the combinations between Rhizobacterein and Phosphorein fertilizers gave better results than using any of the bio-fertilizers alone.

The combination between Rhizobacterein and Phosphorein at different rates, with or without 50% of chemical fertilizers, produced more leaves/plant than those produced from plants receiving 100% of chemical fertilizers (control plants) in both seasons. The highest number of leaves/plant (37.67 and 31.00 in the first and second seasons, respectively) was found on plants fertilized with 10 g/plant Rhizobacterein and 5 g/plant Phosphorein plus 50% of the chemical fertilizer.

The obtained results are in agreement with the findings of Prabhat *et al.* (2003) on *Callistephus chinensis*, who found that application of  $\frac{3}{4}$  of the recommended dose of N and P in combination with full K + VAM + phosphobacterin was the most effective treatment in increasing number of leaves, leaf area, number of branches, flower weight, flower diameter and flowers yield.

Similar results were obtained by Shashid and Gopinath (2002) on *Calendula officinalis*. Also, Kamran *et al.* (2006) found that number of leaves on *Helianthus annuus* plants was increased by plant growth promoting rhizobacteria used as biofertilizers (*Pseudomonas*, *Azotobacter* and *Azospirillum*).

### **Leaf area**

The data recorded on the leaf area in sunflower plants (Table 3) show that, in both seasons, plants supplied with a combination of Rhizobacterein and Phosphorein fertilizers at different rates gave larger leaves than plants receiving 100% chemical fertilization (control), or plants supplied with same combination plus 50% of the chemical fertilizer. On the other hand, control plants gave larger leaves (with mean areas of 117.2 and 174.0 cm<sup>2</sup> in the first and second seasons, respectively), compared to plants receiving any of the bio- fertilizers combination plus 50% of chemical fertilizers. The largest leaf area (182.8 cm<sup>2</sup> in the first season and 191.0 cm<sup>2</sup> in the second season) was recorded from plants fertilized with Rhizobacterein at 10 g/plant and Phosphorein at 10 g/plant. Similar results were reported by Swaefy *et al.* (2007), who found that fertilization using *Bacillus megatherium* var. *phosphaticum* caused an increase in leaf area of peppermint plants, compared to fertilization with the full amount of chemical fertilizers.

**Table (3): Effect of chemical and bio-fertilization on leaf area (cm<sup>2</sup>) leaves fresh and dry weigh of sunflower plants in the 2005 and 2006 seasons.**

Fertilization treatments	Leaf area (cm <sup>2</sup> )		Leaves fresh weight (g/plant)		Leaves dry weight (g/plant)	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
10 C (control)	117.2	174.0	44.95	37.24	4.27	6.17
5R	99.6	112.7	20.23	17.24	3.06	3.11
10R	128.8	138.7	28.52	19.50	1.70	3.85
5P	92.6	85.00	22.93	27.07	3.01	2.82
10P	106.6	105.3	42.01	35.56	4.35	4.44
5R+5P	165.0	172.7	36.60	24.30	3.58	3.87
5R+10P	175.5	190.0	49.40	35.56	4.76	5.18
10R+5P	149.9	187.7	33.47	29.67	4.00	4.00
10R+10P	182.8	191.0	39.82	26.82	3.21	3.05
5R+5P+50% C	102.7	128.0	39.32	27.76	4.98	4.51
5R+10P+50% C	89.1	126.0	51.00	53.49	5.41	6.33
10R+5P+50% C	77.9	120.3	47.37	32.22	4.08	4.04
10R+10P+50% C	98.5	131.7	45.21	32.43	5.21	5.79
L.S.D.	40.55	22.45	14.86	5.82	1.03	0.96

R= rhizobacterein      P= phosphorein      C= christalon

#### **Leaves fresh and dry weights**

Fertilization of sunflower plants using a combination of 5 g/plant Rhizobacterein and 10g/plant Phosphorein plus 50% of chemical fertilizers was the most effective treatment for increasing leaves fresh and dry weights in both seasons (Table 3), giving fresh weights of 51.00 and 53.49 g/plant in the first and second seasons, and dry weights of 5.41 and 6.33 g in the two seasons, respectively.

It was clear that control plants (100% chemical fertilization) gave leaves with heavier fresh and dry weights, compared to those produced by plants inoculated with any of the bio-fertilizers alone. Also, applying 50% of chemical fertilizers to the plants inoculated with the combination of Rhizobacterein and Phosphorein at different rates gave higher leaves fresh and dry weights, compared to those obtained from plants inoculated with the combination of bio-fertilizers only.

#### **Stem fresh and dry weights**

The data presented in Table (4) show the effect of bio and chemical fertilization on the stem fresh and dry weights in sunflower 'Big Smile' plants. It is clear from the data that, in most cases, there was no significant difference between the values obtained from plants fertilized using a biofertilizer alone (either Rhizobacterein or Phosphorein), and those obtained from plants fertilized using 100% Christalon (in both seasons).

On the other hand, the combinations between Rhizobacterein and Phosphorein (with or without 50% Christalon) increased stem fresh and dry weights, compared to 100% Christalon (in both seasons). The highest stem fresh weight (86.00 and 67.44 g in the first season and second seasons,

respectively) was obtained from plants fertilized with 10 g Rhizobacterein + 5 g Phosphorein/plant, while the highest stem dry weight (8.01 and 5.79 g in the first and second seasons, respectively) was obtained from plants receiving 5 g Rhizobacterein + 10 g Phosphorein + 5 g Christalon per plant.

**Table (4): Effect of chemical and bio-fertilization on stem fresh and dry weights of sunflower plants in 2005 and 2006 seasons.**

Fertilization treatments	Stem fresh weight (g/plant)		Stem dry weight (g/plant)	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
10 C (control)	41.52	37.04	3.58	3.38
5R	32.93	37.02	4.97	4.40
10R	27.92	33.70	4.50	3.04
5P	36.43	34.66	4.47	3.02
10P	47.02	37.90	4.98	4.39
5R+5P	60.76	37.75	4.76	3.79
5R+10P	63.81	37.51	5.47	3.41
10R+5P	86.00	67.44	6.29	5.65
10R+10P	66.58	64.91	5.51	5.13
5R+5P+50%C	54.11	48.79	4.82	4.05
5R+10P+50%C	83.67	65.38	8.01	5.79
10R+5P+50%C	53.10	40.35	4.25	3.61
10R+10P+50%C	75.44	50.54	5.96	3.55
L.S.D.	16.59	10.61	1.20	0.993

R= rhizobacterein      P= phosphorein      C= christalon

#### **Root fresh and dry weights**

The data presented in Table (5) show that fertilization of sunflower plants with 5 g/plant Rhizobacterein + 10 g/plant Phosphorein + 50% Christalon was the most effective treatment for increasing root fresh and dry weights in both seasons, giving fresh weights of 22.60 and 17.45 g/plant in the first and second seasons, respectively, and dry weights of 5.06 and 4.92 g/plant in the two seasons, respectively. It was clear that control plants (fertilized with 10 g Christalon/plant) gave root fresh and dry weights which were higher than those produced from plants inoculated with any of the bio-fertilizers alone.

#### **Main flower diameter**

The data recorded on the main flower diameter in sunflower plants (Table 6) show that all the combinations of Rhizobacterein and Phosphorein gave larger flowers than those obtained from plants fertilized using any of the bio-fertilizers alone.

Plants fertilized with a combination of Rhizobacterein at the rate of 5 g/plant, Phosphorein at 10 g/plant, and Christalon at 5 g/plant (50% chemical fertilization), produced the largest main flowers, with diameters of 13.33 and 13.67 cm in the first and second seasons, respectively. In most cases, adding Christalon to any combination of Rhizobacterein and Phosphorein had no significant effect on main flower diameter. Kamran *et al.* (2006), on *Helianthus annuus* plants, found that the diameter of flowers was increased

by using plant growth promoting rhizobacteria (*Pseudomonas*, *Azotobacter* and *Azospirillum*) as biofertilizers. Also, Naik *et al.* (2005) found that the application of NPK in combination with *Azospirillum* caused the highest capitulum diameter of sunflower plants, compared with three different levels of NPK alone.

**Table (5): Effect of chemical and bio-fertilization on root fresh and dry weights of sunflower plants in 2005 and 2006 seasons.**

Fertilization treatments	Root fresh weight (g/plant)		Root dry weight (g/plant)	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
10 C (control)	18.62	10.08	3.16	2.51
5R	8.90	5.40	2.11	2.08
10R	9.45	5.90	2.09	1.98
5P	11.11	4.97	2.09	2.09
10P	12.10	7.12	2.62	2.51
5R+5P	10.34	6.07	2.68	2.14
5R+10P	13.99	9.47	2.43	1.05
10R+5P	19.39	7.68	2.98	2.35
10R+10P	17.97	9.02	3.00	2.10
5R+5P+50%C	15.60	8.22	2.83	2.58
5R+10P+50%C	22.60	17.45	5.06	4.92
10R+5P+50%C	13.44	11.72	2.11	2.51
10R+10P+50%C	21.14	9.92	4.93	1.62
L.S.D.	5.79	3.87	0.999	0.950

R= rhizobacterein      P= phosphorein      C= christalon

**Main flower fresh and dry weight**

The data obtained on main flower fresh and dry weight as affected by bio-fertilization were averaged and shown in Table (6). Raising Rhizobacterein or Phosphorein rate from 5 g/plant to 10 g/plant increased the main flower fresh and dry weight in most cases. Using 50% of Christalon with the combination between Rhizobacterein at 10 g/plant and Phosphrien at 5 or 10g/plant increased flower fresh weight as compared with using the same combination alone. The highest values for main flower fresh and dry weights (fresh weights of 32.73 and 30.00 g in first season and second seasons, respectively, and dry weights of 6.55 and 6.67 g in the two seasons, respectively) were obtained from plants fertilized with 5 g Rhizobacterein + 10 g Phosphorein/plant, followed by plants supplied with 5 g Rhizobacterein + 10 g Phosphorein + 5 g Christalon (50% chemical fertilization) per plant, which gave fresh weights of 27.72 and 28.27 g in the first and second seasons, respectively, and dry weights of 5.17 and 8.06 g in the two seasons, respectively.

**Number of lateral flowers per plant**

Data presented in Table (7) indicated that when Rhizobacterein or Phosphorein were used for fertilization of sunflower plants (separately or in combinations, without chemical fertilizers), raising the application rate from 5 g/plant to 10 g/plant increased number of lateral flowers per plant in both seasons.

**Table (6): Effect of chemical and bio-fertilization on main flower diameter, main flower fresh weight and main flower dry weight of sunflower plants in the 2005 and 2006 seasons.**

Fertilization treatments	Main flower diameter (cm)		Main flower fresh weight (g)		Main flower dry weight (g)	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
10 C (control)	10.00	12.75	26.57	25.33	3.25	6.64
5R	11.00	11.49	20.32	26.10	2.49	2.37
10R	9.40	10.5	22.41	13.67	3.08	2.08
5P	9.00	11.42	18.93	23.93	2.67	3.46
10P	10.33	11.67	23.65	24.67	3.29	6.87
5R+5P	10.33	11.17	27.00	22.86	3.04	3.16
5R+10P	13.00	13.50	32.73	30.00	6.55	6.67
10R+5P	12.67	11.00	23.63	15.17	3.01	3.42
10R+10P	12.33	12.25	22.15	17.50	3.18	4.96
5R+5P+50%C	11.67	12.67	25.77	20.90	3.35	2.06
5R+10P+50%C	13.33	13.67	27.72	28.27	5.17	8.06
10R+5P+50%C	10.33	10.50	26.40	24.33	3.63	3.27
10R+10P+50%C	12.33	12.17	23.84	22.33	3.27	3.03
L.S.D.	2.27	1.71	6.30	6.75	1.51	2.93

R= rhizobacterein      P= phosphorein      C= christalon

The data in Table (7) also show that when Rhizobacterein was added at the low rate (5 g/plant) in combination with Phosphorein at 5 or 10 g/plant, adding 5 g Christalon/plant (50% chemical fertilization) to these combinations caused significant increases in the number of lateral flowers per plant (in both seasons), compared to values recorded when these biofertilizer combination were used without Christalon. In contrast, Addition of Christalon to plants receiving Rhizobacterein at 10 g/plant + Phosphorein at 5 or 10 g/plant caused no significant increase in the number of lateral flowers, compared to values obtained from plants receiving these biofertilizer combinations without Christalon. The highest number of lateral flowers per plant (12.33 and 11.00 in the first and second seasons, respectively) was obtained with 5 g Rhizobacterein + 5 g Phosphorein + 5 g Christalon/plant. It is also worth mentioning that in the first season, this treatment (5 g Rhizobacterein + 5 g Phosphorein + 5 g Christalon/plant) was the only treatment that significantly increased the number of lateral flowers, compared to the control (100% Christalon), while in the second season, all combinations of biofertilizers and Christalon gave significantly more lateral flowers than the control.

The present results are in harmony with those obtained by Chandrikapure *et al.* (1999), who found that the highest flower yield/ha resulted from using 75% N + Azotobacter + phosphorus solubilizing bacteria (PSB), which was significantly higher than the control. Also, Shashid and Gopinath (2002) on *Calendula officinalis* recorded similar results by applying *Azotobacter* at 200 g/ha and VAM at 15.6 g/plant with different levels (100, 75 and 50%) of nitrogen and phosphorus. Eid *et al.* (2006) found that *Azospirillum* inoculation significantly increased flower yield of *Celosia argentea* when compared with un-inoculated plants.

**Table (7): Effect of chemical and bio-fertilization on No. of lateral flowers, Mean fresh and Mean dry weight of lateral flowers of sunflower plants in 2005 and 2006 seasons.**

Fertilization treatments	No. of lateral flowers/plant		Mean fresh weight of lateral flowers (g)		Mean dry weight of lateral flowers (g)	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
10 C (control)	7.67	4.67	13.81	13.83	1.36	1.15
5R	5.67	5.00	13.92	10.79	1.58	0.84
10R	7.00	6.33	13.37	9.83	1.28	0.82
5P	6.33	6.33	12.87	9.08	1.07	0.90
10P	7.00	7.00	19.27	13.97	1.87	0.88
5R+5P	4.00	5.33	14.84	14.20	1.61	1.10
5R+10P	4.67	6.00	19.97	15.81	2.67	1.09
10R+5P	6.00	6.67	4.70	7.67	0.60	0.60
10R+10P	7.00	6.67	7.73	8.53	0.91	0.86
5R+5P+50%C	12.33	11.00	10.48	11.45	1.29	0.84
5R+10P+50%C	8.33	8.33	22.47	16.33	2.68	2.05
10R+5P+50%C	8.00	8.00	9.20	8.76	1.10	0.94
10R+10P+50%C	7.67	7.80	14.77	14.43	1.92	1.14
L.S.D.	2.35	1.86	6.75	4.44	0.446	0.358

R= rhizobacterein P= phosphorein C= christalon

### Mean fresh and dry weights of lateral flowers

Regarding the effect of bio and chemical fertilizers on the lateral flowers quality parameters (including fresh and dry weights), the data presented in Table (7) indicated that using the high rate of Phosphorein alone gave better results than the low rate, while the opposite was observed with Rhizobacterein, as the low rate gave better results than the high rate in both seasons. Also, results recorded with combinations of Rhizobacterein and Phosphorein followed the same trend.

Adding 50% Christalon to the different bio-fertilizers combinations improved the lateral flowers quality in most cases (in both seasons), compared to the same bio-fertilizers combinations without chemical fertilizer. This stimulating effect was very clear with the treatment of 5 g Rhizobacterein + 10 g Phosphorein + 5 g Christalon, which gave the highest values in both seasons (with fresh weights of 22.47 and 16.33 g in the first and second seasons, respectively, and dry weights of 2.68 and 2.05 g in the two seasons, respectively). It could be explained by increasing leaves fresh weight of this treatment ( according to the results mentioned in Table 3) helped the plants in uptaking much water and nutrients and producing more assimilates which improved lateral flowers quality.

The present findings are in harmony with those reported by Rajadurai and Beulah (2000), who studied the effects of single or combined treatments of *Azospirillum*, VAM (*Glomus fasciculatum*) and NPK fertilizers on African marigold (*Tagetes erecta*). They found that increasing levels of NPK fertilizers resulted in earlier flowering. Most and heaviest flowers, as well as longest stalks, were produced with the highest fertilizers rate combined with

*Azospirillum* and VAM. Combined treatments were better than single treatments.

**Total chlorophylls and carotenoids content**

It was clear from data in Table (8) that biofertilization using 10 g Phosphorein/plant alone resulted in the highest content of total chlorophylls in leaves in both seasons. Also, the combinations of Rhizobacterein and Phosphorein (without Christalon) increased the total chlorophylls content in comparison with control plants, except with the combination of the low rate of the two biofertilizers. In most cases, adding 5 g Christalon to a combination of biofertilizers caused marked increases in total chlorophylls content, compared with the same biofertilizer combination without Christalon.

Regarding the carotenoids content, the data showed that the carotenoids content was generally lower when the Rhizobacterein and Phosphorein were used separately, compared to using combinations of the two biofertilizers. In the second season, combining bio and chemical fertilization gave higher leaf contents of carotenoids, compared to control plants in most cases.

These results are in agreement with the findings of Karuppaiah (2005) on *Tagetes patula* plants, who found that the application of *Azospirillum* and phosphobacteria in combination with FYM at 25 and 37.5 t/ha increased the chlorophyll and carotenoid contents

**Total carbohydrates**

Data presented in Table (8) revealed that, in both seasons, fertilization of sunflower plants with 5 g Rhizobacterein + 10 g Phosphorein/plant gave significantly higher total carbohydrates contents in the leaves, compared to any other treatment.

**Table (8): Effect of chemical and bio-fertilization on total chlorophylls, carotenoids and total carbohydrates contents of sunflower plants in the 2005 and 2006 seasons.**

Fertilization treatments	Total chl. (mg/L)		Carotenoids (mg/L)		Total carbohydrates (mg/L)	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
10C (Control)	2.71	2.130	0.287	0.701	23.870	25.880
5R	2.20	1.650	0.177	0.253	13.710	15.750
10R	2.70	2.050	0.044	0.680	16.540	18.580
5P	2.53	1.760	0.157	0.249	22.380	20.480
10P	3.65	2.636	0.149	0.715	21.950	23.690
5R+5P	1.42	1.021	0.172	0.224	22.350	21.380
5R+10P	3.03	2.630	0.192	0.900	38.030	36.600
10R+5P	2.81	2.264	0.230	0.739	16.780	18.780
10R+10P	2.88	2.140	0.230	0.712	33.570	32.510
5R+5P+50%C	3.17	2.630	0.110	0.854	17.490	18.480
5R+10P+50%C	3.41	2.630	0.176	0.900	20.820	19.840
10R+5P+50%C	1.99	1.021	0.167	0.224	15.390	16.380
10R+10P+50%C	2.85	2.253	0.169	0.710	26.250	23.280
L.S.D.	0.151	0.009	0.132	0.053	1.123	1.144

R= rhizobacterein

P= phosphorein

C= christalon

### Nitrogen, Phosphorus and potassium contents

Data in Table (9) indicated that, in both seasons, control plants which received 10g christalon had the highest N content compared to all the treatments. However, adding 5g christalon to the combination between Rhizobacterein and Phosphorein caused an increase in N and P contents compared to the same biofertilizers combination in most cases.

Potassium content was increased, in both seasons, by using biofertilizers either alone or in combination with chemical fertilizer, compared to control plants.

The increase in the K content as a result of the fertilization treatments is in agreement with the findings of Eid *et al.* (2006), who found that *Azospirillum* inoculation significantly increased the nutrient concentration in *Celosia argentea* plants, compared with un-inoculated plants. Also, Kamran *et al.* (2006) found that the biochemical parameters of *Helianthus annuus* plants were increased with bacterial inoculations (*Pseudomonas*, *Azotobacter* and *Azospirillum*).

**Table (9): Effect of chemical and bio-fertilization on nitrogen, phosphorus and potassium contents (% of dry weight) in the herb of sunflower plants in 2005 and 2006 seasons.**

Fertilization treatments	N (%)		P (%)		K (%)	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
10C(Control)	5.390	5.180	0.460	0.390	0.930	0.980
5R	5.070	5.013	0.330	0.310	1.250	1.810
10R	3.430	3.040	0.130	0.140	0.980	0.910
5P	3.730	3.580	0.170	0.150	1.180	1.310
10P	3.040	3.230	0.340	0.410	1.190	1.230
5R + 5P	3.690	3.330	0.170	0.300	0.800	0.910
5R + 10P	4.170	4.850	0.260	0.190	1.160	1.230
10R + 5P	3.780	3.810	0.180	0.210	1.010	1.180
10R + 10P	3.580	3.900	0.160	0.150	1.150	1.210
5R + 5P + 50%C	2.870	3.050	0.330	0.350	1.080	1.130
5R + 10P + 50%C	4.710	4.250	0.440	0.490	1.080	1.310
10R + 5P + 50%C	4.890	4.630	0.360	0.380	1.180	1.230
10R + 10P + 50%C	5.060	5.073	0.590	0.500	0.990	0.950
L.S.D	0.213	0.213	0.017	0.024	0.053	0.053

R= rhizobacterein      P= phosphorein      C= christalon

**Conclusion:** From the recorded data, sunflower growers can be advised to apply 5 g Rhizobacterein + 10 g Phosphorein/plant, in addition to 5 g Christalon/plant to get the most vigorous vegetative growth of dwarf sunflower plants during the vegetative stage, as well as a high number of lateral flowers with fresh weights similar to the main flower.

### REFERENCES

- A.O.A.C. (1995). Association of Official Analytical Chemists. Official Methods of Analysis. 13<sup>th</sup> ed., Washington, D.C.
- Bhaskaran, P.; G. Ambrose and N. Jayabalan (2002). Usefulness of bio-fertilizers in economizing nitrogenous fertilizers in *Tagetes erecta* L.. Journal of Phytological Research. 15 (2): 155-160.

- Chandrikapure, K. R.; K.T. Sadawarte; D.M. Panchbhai and B.D. Shelke (1999). Effect of bioinoculants and graded doses of nitrogen on growth and flower yield of marigold (*Tagetes erecta* L.). Orissa Journal of Horticulture. 27(2): 31-34.
- Chapman, H. D. and P. F. Pratt (1961). Methods of Soil, Plants and Water Analysis. University of California, Division of Agricultural Sciences. .PP.60-69.
- Eid, R.A.; S.A. Abo Sedera, and M. Attia (2006). Influence of nitrogen fixing bacteria incorporation with organic and/or inorganic nitrogen fertilizers on growth, flower yield and chemical composition of *Celosia argentea*. World Journal of Agricultural Sciences. 2(4): 450-458.
- Gilic, C. (1995). The enhancement of plant growth by free-living bacteria. Can. J. Microbiol., 41: 109 - 117.
- Herbert, D.; P.J. Philipps and R.E. Strange (1971): Determination of Total Carbohydrates. Methods in Microbiology. 5.b: 204-244.
- Kamran, S.; A. Shazia and H. Shahida (2006). Growth responses of *Helianthus annuus* to plant growth promoting rhizobacteria used as a biofertilizer. International Journal of Agricultural Research. 1(6): 573-581.
- Karuppaiah, P. (2005). Effect of *Azospirillum*, phosphobacteria and organic manures on growth and yield of French marigold (*Tagetes patula* L.). Plant Archives. 5 (2): 661-664.
- Mostafa, M.M. (2002). Effect of bio-fertilizer, salinity and magnetic technique on the growth of some annual plants. Alexandria Journal of Agricultural Research. 47 (2): 151-162.
- Naik, D.H.L.; B.C. Channakeshava and K.P.R. Prasanna (2005). Effect of biofertilizers on plant growth and seed yield of female parental line CMS-234A of KBSH-1 sunflower hybrid. Mysore Journal of Agricultural Sciences. 39(3): 368-373.
- Nornai, R. (1982). Formula for determination of chlorophyll pigments extracted with N.N. dimethyl formamide. Plant Physiol., 69: 1371-1381.
- Prabhat, K.; S.P.S. Raghava and R.L. Misra (2003). Effect of bio-fertilizers on growth and yield of china ester. Journal of Ornamental Horticulture, New Series. 6(2): 85-88.
- Rajadurai, K.R. and A. Beulah (2000). The effect of inorganic fertilizers, *Azospirillum* and VAM on yield characters of African marigold (*Tagetes erecta*). Journal of Ecotoxicology and Environmental Monitoring. 10 (2): 101-105.
- Riera-Nelson, M.; N. Medina-Basso and M. Bertili (2003). Use of post effects from mycorrhizae and rhizobacteria for the intensive production of crops and soil conservation. Alimentaria. 40 (431): 73-78.
- Shashid, G.R. and G. Gopinath (2002). Effect of nutrients and bio-inoculants on *Calendula*. Floriculture Research Trend in India. Proceedings of the National Symposium on Indian Floriculture in the New Millennium, LaL-Bagh, Bangalore, 25-27 February, 2002; 206-208.
- Snedecor, G. W. and W. G. Cochran (1982). Statistical Methods. The Iowa State Univ. Press, Ames, Iowa, USA, 507 pp.

- Sukhovitskaya, L.A.; S.V. Mokhova; G.V. Safronova; I.B. Chernetsova and N.V. Mel'nikova (2001). The bacterial preparation rhizobacterin: the strain, production, and results of use. *Biotekhnologiya*. 17(2): 57-63.
- Swaefy H, M.F.; W.R.A. Sakr; A.Z. Sabh and A.A. Ragab (2007). Effect of some chemical and bio-fertilizers on peppermint plants grown in sandy soil. 1. Effect on vegetative growth as well as essential oil percentage and constituents. *Annals of Agricultural Science, Ain Shams Univ., Cairo*, 52(2): 451-463.

**استجابة نباتات عباد الشمس للتسميد المعدني والحيوي**  
**هند مصطفى فهمي سويفي\* و حنان عز الدين إبراهيم أدهم\*\***  
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أجري هذا البحث بمشغل التجارب بمعهد بحوث البساتين ، مركز البحوث الزراعية، الجيزة، خلال الموسمين المتتاليين ٢٠٠٥-٢٠٠٦. وذلك بهدف دراسة إمكانية استخدام التسميد الحيوي (الريزوباكترين والفوسفورين) لتقليل الاحتياج إلى الأسمدة المعدنية ولدراسة تأثيرها على النمو الخضري والتزهير والمحتوى الكيماوي لنباتات عباد الشمس صنف متقزم (Big Smile). وقد أظهرت النتائج أن استخدام كل من الريزوباكترين والفوسفورين معا يزيد من ارتفاع النبات ومساحة الأوراق والكريبويدرات الكلية مقارنة باستخدام أي من الأسمدة الحيوية تحت الدراسة بصورة منفردة أو مع إضافة نصف كمية الأسمدة الكيماوية (الكمية الكلية المستخدمة هي ١٠ جم كريستالون/نبات). أدى إحلال نصف كمية السماد الكيماوي بمخلوط من السماد الحيوي إلى زيادة عدد الأوراق والوزن الطازج والجاف كما زاد محتوى الأوراق من الكلوروفيل الكلي واليوتاسيوم. كما أدت هذه المعاملة أيضا إلى زيادة عدد الأزهار الجانبية. يمكن أن نستخلص أن معاملة نباتات عباد الشمس صنف متقزم (Big Smile) بكل من ٥ جرام ريزوباكترين و ١٠ جرام فوسفورين بالإضافة إلى ٥ جرام كريستالون لكل نبات تنتج نباتات ذات نمو خضري قوي وأكبر عدد من الأزهار الجانبية ذات الخصائص الجيدة.