EFFECT OF CHEMICAL AND BIOFERTILIZERS ON *COSMOS SULPHUREUS* CAV. PLANTS. II – MAIN CONSTITUENTS

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Abstract

This work was conducted to determine the effect of inoculation with biofertilizers (mixture of Azotobacter chroococcum, *Azospirillum lipoferum, Bacillus polymixa, Bacillus megatherium* and *Pseudomonas fluorescence*) and two levels of chemical fertilizers NPK (nitrogen, phosphorous and potassium) at the ratio of 1 : 1 : 1 at the rate of 3 or 6 gm/plant/20 cm plastic pot on the main constituents of cosmos plants. Application of NPK at the rate of 3 g plant/20 cm plastic pot increased N content in flowers and K content in the herb, while the rate of 6 g/plant increased the carbohydrates percentage in both herb and flowers. The highest content of chlorophyll (a) and carotenoids in leaves and P in herb were found in the plants inoculated with the biofertilizers and received NPK at 6 g/plant. Meanwhile, the combined inoculation of biofertilizers and NPK at 3 g/plant resulted in the highest content of K in flowers. The highest content of chlorophyll (b) in leaves and P content in flowers resulted from inoculating the plants with the biofertilizers as well as applying them to the plants as drench plus application of NPK at 3 g/plant, however increasing the rate of NPK to 6 g/plant instead of 3 g/plant led to an increment in N content in the herb.

INTRODUCTION

Cosmos sulphureus, Cav. "Yellow Cosmos" is a plant which produces showy flowers. It belongs to family Asteraceae (Compositae). It is native to Mexico. It can easily adapt to all regions. The glowing orange-yellow flowers are extremely attractive. Tall species make excellent background plants for borders as well as providing cut flowers. Yellow cosmos is a sun-loving annual; it will not produce as many blooms if grown in the shade. It is not a heavy feeder. Excess fertilization will cause plants to produce excessive leaf growth at the expense of flower production. It tolerates heat and drought well.

There are several factors affecting the production of flowers and ornamental plants and one of the most important factors is fertilization. The intensive use of expensive mineral fertilizers in recent years resulted in environmental pollution problems. Chemical fertilizers at extremely high rates or a long period decreased the potential activity of microflora and stability of soil organic matter.

Biofertilizers are one of the most important materials required to substitute for chemical fertilizers for healthy cheap production. The microbial strains (biofertilizers) lead to nitrogen fixation (N_2-fixing bacteria) and availability of phosphorous (phosphate dissolving bacteria) as well as the synthesis of auxins, cytokinins and gibberellic acid-like substances. These growth materials are the primary substances controlling the enhanced plant growth, absorption of nutrients and photosynthesis process (Mrkovacki and Milic 2001).

Biofertilizers are microbial inoculants used for application to seed or soil to increase soil fertility with the objective of increasing the number of such microorganisms and to accelerate certain microbial processes in the rhizosphere of inoculated plants or soils. Such microbiological processes can change unavailable forms of nutrients into available forms that can be easily assimilated by plants (Subba Rao, 1981). These biofertilizers are useful for recycling elements, reserving natural resources and for protection from increasing pollution due to extensive use of mineral fertilizers. Also, they increase the amounts of nitrogen in the plants and amount of nitrogen left in the soil (El-Karamity and Hammad, 1992).

The studies on using biofertilizers for the production of flowers and ornamental plants are few. Gomaa and Abo–Aly (2001) showed that there was significant increases in carbohydrates, chlorophylls, carotenoids, total nitrogen, total phosphorus and total potassium content of anise plants due to inoculation with non–symbiotic N_2-fixers (*Azotobacter chroococcum* and *Azospirillum brasilense*) and half dose of biogas manure. Abo El–Ala (2002) on *Ocimum basilicum* and *Majorana hortensis* found that the highest total nitrogen and phosphorus contents were obtained in the case of dual inoculation with a mixture of *Azotobacter, Azospirillum* and *Bacillus* and amended with half dose of N and P fertilizers. Al–Qadasi (2004) on *Ocimum basilicum* concluded that the highest photosynthetic pigments, P, K and total carbohydrates contents in herb were recorded with the treatment of biofertilizer at 150 ml/plant plus NPK at 10.7 g/plant. While, the highest values of nitrogen content resulted from treating the plants with 10.7 g NPK/plant plus biofertilizer at 300 ml/ plant. Hamed (2004) mentioned that the highest total carbohydrates, nitrogen, phosphorus and potassium percentage in the leaves of *Salvia officinalis* was determined in the plants treated with full dose of NPK + biofertilizer.

This investigation was carried out to study the influence of biofertilizers (*Azotobacter chroococcum* and *Azospirillum lipoferum*), phosphorous dissolving bacteria (*Bacillus polymixa* and *Bacillus megatherium*) and a biocontrol agent (*Pseudomonas fluorescence*) alone or with chemical fertilizers [two levels of NPK fertilizers (3 or 6 g/plant/20 cm plastic pot)] on the main constituents of Cosmos plants.

MATERIALS AND METHODS

A field experiment was conducted in a sunny place at the nursery of the Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University, Giza, during the two successive seasons of 2002 and 2003, to investigate the effect of chemical fertilizers and biofertilizers on the main constituents of Cosmos plants. Seeds were sown on 9th March in plastic pots. On 20th April, homogenous seedlings were dipped in liquid culture of biofertilizers for one hour (inoculation) then transplanted in plastic pots (20 cm diameter) filled with sandy loam soil. Control plants and those of chemical treatments were dipped in tap water for one hour. After one month, some treatments had another dose of the biofertilizers as soil drench. The strains of biofertelizers used were *Azotobacter chroococcum, Azospirillum lipoferum, Bacillus polymixa, Bacillus megatherium* and *Pseudomonas fluorescence* obtained from Microbiology Dept. Faculty of Agriculture, Cairo University, Giza. These strains were mixed in equal parts. The plants were fertilized with NPK at the ratio of 1:1:1. Three grams of this fertilizer mixture were added to each plant of those received chemical fertilization on 3rd May. Some treatments had another dose of chemical fertilization after three weeks (6 gm). The fertilizers used were: ammonium sulphate (20% N), calcium superphosphate (15.5 % P_2O_5) and potassium sulphate (48 % K_2O). The plants were irrigated whenever required. The experiment consisted of 9 treatments, as follows: Control [without chemical or bio-fertilizers (bio)], NPK (3 gm), NPK (6 gm), inoculation, inoculation+ NPK (3 gm), inoculation + NPK (6 gm), inoculation + bio drench, inoculation+ NPK (3 gm) + bio drench and inoculation + NPK (6 gm) + bio drench. Microbial changes in the rhizosphere of cosmos plants as affected by inoculation with non-symbiotic N_2-fixers as well as phosphate dissolving bacteria are shown in Table A.

Table A. Microbial counts / gm soil.

Strains	Before adding	After adding
1- *Azotobacter chroococcum*	10^5	2×10^7
2- *Azospirillum lipoferum*	10^3	9.8×10^7
3- *Bacillus polymixa*	10^2	3.2×10^7
4- *Bacillus megatherium*	10^4	1.2×10^7
5- *Pseudomonas fluorescence*	10^3	0.8×10^7

The mechanical and chemical analyses of the soil used in the experiment are shown in Tables (B) and (C). these analyses were conducted before planting.

Table B. Mechanical analysis of the soil.

Mechanical analysis	
Sand %	55.30
Silt %	29.75
Clay %	14.93
Soil texture	Sandy loam

Table C. Chemical analysis of the soil.

Cations Meq / L		Anions Meq / L					
Na^+	9.50	HCO_3^-	4.40	pH	8.23	N	480 ppm
K^+	0.70	SO_4^{--}	25.00	E.C.	2.81 mmhos	P	37.8 ppm
Ca^{++}	14.00	Cl^-	13.00	Organic matter	0.23%	K	35.1 ppm
Mg^{++}	8.20						

The following main constituents of the plants were determined:

1. Photosynthetic pigments (in fresh leaves).
2. Total carbohydrates (in herb and flowers).
3. N, P and K in herb and flowers.

The chemical analyses were performed as follows:

- Pigments content determination was carried out in fresh leaves as mentioned by Saric *et al.* (1967).
- Total carbohydrates (Herbert *et al.*, 1971).
- Nitrogen percentage using micro-Kjeldahl method (Pregl, 1945 and piper, 1947).
- Phosphorus content (Troug and Meyer, 1939).

- Potassium content was determiined by using operation chart for Shimadzu Atomic Absorption Flame Spectrophotometer AA-646 with a boiling air-acetylene burner and recorded readout.

The layout of the experiment was a complete randomized blocks with nine treatments, each treatment contained three replicates. Each replicate consisted of ten plants, i.e. 30 plants in each treatment. The obtained data were statistically analyzed using the analysis of variance between the averages according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

1- Photosynthetic pigments:

A- Chlorophyll a :

From the data in Table 1 it may be observed that, in both seasons, the highest amount of chlorophyll a (1.09 and 0.73 mg/g FW, respectively) was found in the plants inoculated with the mixture of strains combined with NPK at 6 gm/plant, while, the application of biofertilizers (inoculation + drench) resulted in the least amount of chlorophyll a (0.57 and 0.52 mg/g FW, respectively).

B- Chlorophyll b :

As shown in Table 1 the data on chlorophyll b showed that the highest content of chlorophyll b in the two seasons (0.58 and 0.48 mg/g FW, respectively) was detected in leaves of the plants supplied with biofertilizer (inoculation + drench) plus 6 gm NPK/plant. However, Control plants had the least concentration of chlorophyll b in both years, the values were 0.27 and 0.25 mg/g FW, respectively.

C- Total chlorophylls a + b :

Data in Table 1 revealed that the highest content of total chlorophylls a+b in the first season (1.49 mg/g FW) resulted from inoculating the plants with biofertilizer plus NPK at 6 g/plant. This increment may be due to the increase in chlorophyll a while, in the second season, the greatest amount (1.18 mg/g FW) was determined in the leaves of the plants received NPK at 3 gm/plant and supplied with biofertilizers (inoculation + drench). This increase may be a result of the increment in chlorophyll b. Application of biofertilizers (inoculation + drench) resulted in the formation of the least content of total chlorophylls in both seasons. The values were 0.87 and 0.84 mg/g FW, respectively. This may be due to more N and P available to plants by using the highest dose of NPK plus biofertilizers; while application of biofertilizers only did not supply the plants sufficiently with these two elements necessary for chlorophyll synthesis.

D- Carotenoids:

From the data shown in Table 1 it can be remarked that the highest content of carotenoids was due to the inoculation of the plants with biofertilizers and applied NPK at 6 gm/plant, in both seasons. The values were 0.56 and 0.69 mg/g FW, respectively same as in chlorophyll a Meanwhile, the plants inoculated with biofertilizers only had the least amount of carotenoids, in both seasons. The value was 0.44 mg/g FW.

These results are in line with those of Bambal *et al.* (1998) who found that addition of *Azotobacter* + *Azsospirillum* + 100 % N to cauliflower as a seedlng dip resulted in the highest chlorophyll content. Gomaa and Abo–Aly (2001) showed that there were significant increases in chlorophylls and carotenoids content of anise plants due to inoculation with non–symbiotic N_2–fixers (*Azotobacter chroococcum* and *Azospirillum brasilense*) and half dose of biogas manure. Al–Qadasi (2004) on *Ocimum basilicum* concluded that the highest photosynthetic pigments content was recorded with the treatment of biofertilizer at 150 ml/plant plus NPK at 10.7 g/plant.

2- Total carbohydrates:

From Table 2 it may be noticed that the total carbohydrates content was greater in herb than in the inflorescences. Generally, application of NPK at 6 g/plant was more effective in increasing total carbohydrates content in herb and inflorescences, in both seasons. The values were 47.24 and 56.46 %, respectively in herb and 38.73 and 32.96 %, respectively in the inflorescences. Also, inoculation of the seedlings with biofertilizer plus supplying the plants with 6 gm NPK/plant led to the highest value of total carbohydrates percentage (47.38 %) in herb, in the first season same as chlorophyll a and carotenoids. Meanwhile, inoculating the plants with biofertilizers only resulted in the least amount of total carbohydrates in herb and inflorescences, in the two seasons. The values were 33.00 and 42.05 %, respectively in herb and 24.99 and 25.16 %, respectively in the inflorescences. Also the treatment of inoculation + 3 g NPK led to the least content of total carbohydrates (33.82 %) in herb, in the first season. However, control plants had the least amount of total carbohydrates (25.99 %) in the inflorescences in the second season.

Table 1. Effect of chemical and biofertilizers treatments on photosynthetic pigments content (mg/g fresh weight) in leaves of *Cosmos sulphureus* Cav. plants during the two seasons of 2002 and 2003.

Treatments	Chlorophyll a		Chlorophyll b		Total chlorophylls a + b		Carotenoids	
	1st S.	2nd S.	1st S.	2nd S.	1st S.	2nd S.	1st S.	2nd S.
Control	1.04	0.71	0.27	0.25	1.31	0.96	0.47	0.63
NPK (3 gm)	0.59	0.66	0.31	0.36	0.90	1.02	0.47	0.59
NPK (6 gm)	0.92	0.64	0.39	0.46	1.31	1.10	0.55	0.55
Bio Inoc.	0.95	0.65	0.43	0.29	1.38	0.94	0.44	0.44
Inoc.+ NPK (3 gm)	0.92	0.68	0.32	0.42	1.24	1.10	0.50	0.54
Inoc.+ NPK (6 gm)	1.09	0.73	0.40	0.36	1.49	1.09	0.56	0.69
Inoc.+ Bio drench	0.57	0.52	0.30	0.32	0.87	0.84	0.50	0.54
Inoc.+ Bio drench + NPK (3 gm)	0.78	0.70	0.58	0.48	1.36	1.18	0.45	0.60
Inoc.+ Bio drench + NPK (6 gm)	0.70	0.68	0.57	0.45	1.27	1.13	0.53	0.62
L.S.D. (0.05 %)	0.18	0.15	0.08	0.07	0.13	0.11	0.08	0.12

Inoc. = Inoculation   S. = Season

Many authors reported the favorable effect of biofertilizers on total carbohydrates. Ibrahem (2000) demonstrated that the highest total carbohydrates content in leaves of *Ammi visnaga* resulted from inoculation with *Azotobacter* + *Azospirillum* in the presence of full dose of NPK. Al–Qadasi (2004) on *Ocimum basilicum* concluded that the highest total carbohydrates content was recorded with the treatment of biofertilizer at 150 ml/plant plus NPK at 10.7 g/plant. Hamed (2004) mentioned that the highest total carbohydrates percentage in the leaves of *Salvia officinalis* was determined in the plants treated with full dose of NPK + biofertilizer.

Table 2. Effect of chemical and biofertilizers treatments on total carbohydrates percentage in herb and inflorescence of *Cosmos sulphureus* Cav. plants during the two seasons of 2002 and 2003.

Treatments	Total carbohydrate percentage			
	Herb		Inflorescences	
	1st S.	2nd S.	1st S.	2nd S.
Control	41.70	46.69	29.87	25.99
NPK (3 gm)	45.48	49.57	29.35	31.29
NPK (6 gm)	47.24	56.46	38.73	32.96
Bio Inoc.	33.00	42.05	24.99	25.16
Inoc. + NPK (3 gm)	33.82	44.99	33.52	31.48
Inoc. + NPK (6 gm)	47.38	46.64	36.13	27.63
Inoc. + Bio drench	46.01	46.74	31.44	26.35
Inoc. + Bio drench + NPK (3 gm)	45.09	43.81	34.79	29.09
Inoc. + Bio drench + NPK (6 gm)	40.31	44.17	34.61	26.51
L.S.D. 0.05 %	5.83	5.81	5.37	5.62

1st S. = First season 2nd S. = Second season

3- Nitrogen content:

The data on nitrogen content are shown in Table 3. In both seasons, the highest amount of N, in herb (2.94 and 2.73 %, respectively) was due to the inoculation of the plants with the mixture of biofertilizers and bio drench as well as supplying the plants with NPK at 6 g/plant. However, the treatment of inoculation plus 6 g NPK/plant led to the least content of nitrogen in herb, in the two seasons. The values were 1.77 and 1.55 %, respectively. Meanwhile, in both seasons, the application of NPK at 3 g/plant only resulted in the highest concentration of nitrogen in inflorescences (3.29 and 4.18 %, respectively). The least content (1.46 and 1.15 %, respectively) were determined in the plants inoculated with biofertilizers only.

Table 3. Effect of chemical and biofertilizers treatments on nitrogen content percentage in herb and inflorescence of Cosmos sulphureus Cav. plants during the two seasons of 2002 and 2003.

Treatments	Nitrogen content percentage			
	Herb		Inflorescences	
	1st S.	2nd S.	1st S.	2nd S.
Control	2.83	1.74	2.84	2.85
NPK (3 gm)	2.43	1.92	3.29	4.18
NPK (6 gm)	2.08	1.83	2.79	2.10
Bio Inoc.	1.99	1.97	1.46	1.15
Inoc. + NPK (3 gm)	2.59	2.13	2.05	3.19
Inoc. + NPK (6 gm)	1.77	1.55	1.88	2.50
Inoc. + Bio drench	2.19	1.94	2.50	3.81
Inoc. + Bio drench + NPK (3 gm)	1.92	2.61	2.51	1.42
Inoc. + Bio drench + NPK (6 gm)	2.94	2.73	2.64	2.72
L.S.D. 0.05 %	0.26	0.30	0.19	0.22

1st S. = First season 2nd S. = Second season

4- Phosphorus content:

From data in Table 4 it can be observed that in both seasons, the greatest amount of phosphorus in herb (0.526 and 0.251 %, respectively) was detected in the plants treated with inoculation plus 6 g NPK/plant, while, application of biofertilizers as drench as well as inoculation plus NPK at 6 g/plant resulted in the least content of phosphorus (0.179 and 0.120 %, respectively). However, the highest concentration of phosphorus in inflorescences was determined in the plants treated with biofertilizers (inoculation + drench) plus NPK at 3 g/plant, in the two seasons. The values were 0.467 and 0.545 %, respectively. The plants received NPK at 6 g/plant only had the least P content in inflorescence in both seasons. The values were 0.041 and 0.185 %, respectively.

Table 4. Effect of chemical and biofertilizers treatments on phosphorus content percentage in herb and inflorescence of *Cosmos sulphureus* Cav. plants during the two seasons of 2002 and 2003.

Treatments	Phosphorus content percentage			
	Herb		Inflorescences	
	1st S	2nd S.	1st S.	2nd S.
Control	0.324	0.217	0.323	0.334
NPK (3 gm)	0.225	0.219	0.068	0.207
NPK (6 gm)	0.217	0.155	0.041	0.185
Bio Inoc.	0.323	0.244	0.393	0.303
Inoc. + NPK (3 gm)	0.218	0.172	0.329	0.379
Inoc. + NPK (6 gm)	0.526	0.251	0.314	0.305
Inoc. + Bio drench	0.393	0.217	0.376	0.256
Inoc. + Bio drench + NPK (3 gm)	0.269	0.203	0.467	0.545
Inoc. + Bio drench + NPK (6 gm)	0.179	0.120	0.344	0.337
L.S.D. 0.05 %	0.116	0.026	0.101	0.062

1st S. = First season 2nd S. = Second season

5- Potassium content:

Data in Table 5 indicated that the highest content of potassium was found in the herb of the plants treated with NPK at 3 g/plant only in both seasons (1.45 and 1.46%, respectively), whereas the least percentage was detected in the plants inoculated with biofertilizers and received NPK at 6 g/plant, in the two seasons, the values were 1.14 and 1.15%, respectively. Inoculating the plants with biofertilizers and using NPK at 3 g / plant led to the highest content of K in inflorescences in both seasons, (2.06 and 2.08%, respectively). Meanwhile, in both seasons, the plants treated with NPK at 6 g/plant only had the least content of potassium in inflorescences (1.10 and 0.96 %, respectively).

Table 5. Effect of chemical and biofertilizers on potassium content percentage in herb and inflorescence of *Cosmos sulphureus* Cav. plants during the two seasons of 2002 and 2003.

Treatments	Potassium content percentage			
	Herb		Inflorescences	
	1st S	2nd S.	1st S.	2nd S.
Control	1.30	1.32	1.17	1.41
NPK (3 gm)	1.45	1.46	1.88	1.44
NPK (6 gm)	1.22	1.19	1.10	0.96
Bio Inoc.	1.36	1.34	1.39	1.21
Inoc. + NPK (3 gm)	1.28	1.18	2.06	2.08
Inoc. + NPK (6 gm)	1.14	1.15	1.41	1.55
Inoc. + Bio drench	1.26	1.32	1.90	1.68
Inoc. + Bio drench + NPK (3 gm)	1.35	1.38	1.67	1.39
Inoc. + Bio drench + NPK (6 gm)	1.26	1.34	1.49	1.41
L.S.D. 0.05 %	0.08	0.08	0.09	0.09

1st S = First season   2nd S = Second season

The effect of biofertilizers on N, P and K contents was show by some investigators. Ibrahem (2000) demonstrated that the highest N% content in leaves of *Ammi visnaga* resulted from inoculation with *Azotobacter* + *Azospirillum* in the presence of full dose of NPK. Gomaa Abo–Aly (2001) showed that there were significant increases in nitrogen, phosphorus and potassium content of anise plants due to inoculation with non–symbiotic N_2-fixers (*Azotobacter chroococcum* and *Azospirillum brasilense*) and half dose of biogas manure. Al–Qadasi (2004) on *Ocimum basilicum* concluded that the highest P and K contents in herb were recorded with the treatment of biofertilizer at 150 ml/plant plus NPK at 10.7 g/plant. While, the highest values of nitrogen content resulted from treating the plants with 10.7 g NPK/plant plus biofertilizer at 300 ml/plant. Hamed (2004) mentioned that the highest nitrogen, phosphorus and potassium percentage in the leaves of *Salvia officinalis* was determined in the plants treated with full dose of NPK + biofertilizer.

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تأثير التسميد الكيماوى والتسميد الحيوى على نباتات الكوزموس

٢- المكونات الأساسية

صفوت مصطفى كامل عبد الواحد

معهد بحوث البساتين – مركز البحوث الزراعية – الجيزة – جمهورية مصر العربية

أجرى هذا البحث خلال الموسمين ٢٠٠٢ و ٢٠٠٣ بغرض دراسة تأثير التسميد الكيماوى والحيوى على المكونات الأساسية فى نبات الكوزموس . وأوضحت النتائج الآتى:-

١. التسميد الكيماوى (نتروجين ، فوسفور ، بوتاسيوم بنسبة ١ : ١ : ١) بمعدل ٣ جم / نبات أدى إلى زيادة النتروجين فى الأزهار والبوتاسيوم فى العشب.
٢. إضافة ٦ جم سماد كيماوى/نبات أدى إلى زيادة الكربوهيدرات الكلية فى كل من الأزهار والعشب .
٣. النقع فى مخلوط السماد الحيوى (*Azotobacter chroococcum, Azospirillum lipoferum, Bacillus polymixa, Bacillus megatherium and Pseudomonas fluorescence*) مع التسميد الكيماوى بمعدل ٣ جم/نبات أدى إلى زيادة البوتاسيوم فى الأزهار، فى حين أن زيادة التسميد الكيماوى إلى ٦ جم/نبات مع نفس المخلوط من السماد الحيوى أدى إلى زيادة فى محتوى الأوراق من كلوروفيل ا والكاروتينات وأيضاً الفوسفور فى العشب .
٤. أدى النقع فى السماد الحيوى مع الإضافة إلى النباتات فى التربة مع السماد الكيماوى بمعدل ٣ جم/نبات إلى زيادة محتوى الأوراق من كلوروفيل ب ومحتوى الأزهار من الفوسفور، إلا أن زيادة السماد الكيماوى إلى ٦ جم/نبات بدلاً من ٣ جم/ نبات أدى إلى زيادة محتوى العشب من النتروجين .