EFFECT OF CHEMICAL AND BIO-FERTILIZERS ON *COSMOS SULPHUREUS* CAV. PLANTS. 1 – VEGETATIVE GROWTH AND FLOWERING

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Abstract

Homogenous transplants of Cosmos were grown in plastic pots (20 cm diameter) filled with soil taken from the nursery during the seasons of 2002 and 2003. The aim of this investigation was to study the effect of chemical and biofertilizers on growth and flowering of the plants.Application of biofertilizer (mixture of *Azotobacter chroococcum, Azospirillum lipoferum, Bacillus polymixa, Bacillus megatherium* and *Pseudomonas fluorescence*) with different levels of chemical fertilizers (nitrogen, phosphorous and potassium) at the ratio of 1 : 1 : 1 and the rate of 3 or 6 gm/plant increased vegetative growth (plant height, stem diameter, fresh and dry weights of shoots as well as number of branches/plant) and flowering characters compared to chemical fertilizer treatments alone. The least vegetative growth and flowering characters were of plants supplied with chemical fertilization alone, even less than control plants.

INTRODUCTION

Cosmos sulphureus, Cav. Family Asteraceae (Compositae) is native to Mexico. It is annual or perennial herb, popular as flower – garden subject, reached 120 – 210 cm in height with much branches. Peduncles 17 – 25 cm long. Rays and disk flowers are yellow in color. It is a mistake to grow cosmos in too rich soil, as it has too vigorous growth and too few flowers, which are also late (Bailey, 1933).

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 production of growth promoting substances such as GA_3 and IAA which could stimulate plant growth, absorption of nutrients and photosynthesis process (Fayez *et al.,* 1985). In addition they have a role in the control of plant diseases (Camliel and Katan, 1993, Linderman 1994 and Abdel – Latif *et al.,* 2001).

The objective of the current study was to determine the effects of inoculation with mixed culture of N_2 – fixing bacteria (*Azotobacter chroococcum* and *Azospirillum lipoferum*), phosphorous dissolving bacteria (*Bacillus polymixa* and *Bacillus megatherium*) and a biocontrol agent (*Pseudomonas fluorescence*) under different levels of NPK fertilizers on growth and flowering of Cosmos plants. There is little information in literature regarding the effect of inoculation with biofertilizers or biocontrol agents on ornamental plants. Wange and Patil (1994) found that applying 100 kg nitrogen/ha or inoculation with *Azotobacter+ Azospirillum* mixture on *Polianthes tuberosa* significantly increased the number of flowers/stalk, number of flower stems and yield. Swarupa (1996) observed that *Azospirillum brasilense* treatment significantly increased the plant height of coffee seedlings. There was also a significant increase in stem girth by combined application of *Azospirillum brasilense* + Phosphobacteria (*Bacillus* sp.)+VAM fungi (*Gigaspora margarita*). These results indicated that application of biofertilizers could enhance the growth and vigour of coffee seedlings. Misra (1997) mentioned that all biofertilizer treatments significantly increased the vegetative growth (number of leaves, number of flowers, fresh and dry weights) of gladiolus plants. Gupta *et al.* (1999) stated that growth of *Tagetes eracta* seedlings was high after treatments with *Azotobacter* + phosphorus solubilizing bacteria (applied to soil or seedlings) in combination with 75 or 100% nitrogen application. Sheikh *et al.* (2000) on Dutch iris demonstrated that plant height, stalk length, flowering days and floret duration increased with application of N up to 60 kg N/ha, but was at par with N at 40 kg/ha. The interaction between biofertilizers (*Azotobacter* and *Azospirillum*) and N was significant for flowering days and floret size. El–Kashlan (2001) noticed that using biofertilizers increased all the vegetative growth parameters, shortened the period required to reach flowering, increased number of fruits/plant and fresh and dry weights of sepals of Roselle plants. Gad (2001) found a significant increase in plant height, number of leaves, fresh and dry weights of vegetative growth, number of branches, number of umbels / plant as a result of using biofertilizers on *Feoniculum vulgare* and *Anethum graveolens.* Kandeel *et al.* (2001) on *Feoniculum vulgare* concluded that inoculation with *Azotobacter* and *Azospirillum* in the presence of the full dose of N, P and K (300 kg ammonium sulfate+300 kg calcium superphosphate+80 kg potassium sulfate per fed) resulted in the tallest plants and the highest number of umbels/plant. Rashed (2002) on *Anethum graveolens, Coriandrum sativum* and *Petroselinum sativum* plants showed that

biofertilizer plus organic manure caused significant increase in plant height, fresh and dry weights. Mahfouz (2003) on marjoram mentioned that the highest values of vegetative growth (plant height, number of branches, fresh and dry weights of herb) were recorded at the treatment of biofertilizer plus full dose of N and P.

MATERIALS AND METHODS

A field experiment was carried out 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 study the effect of chemical and biofertilizers on the growth and flowering of Cosmos plants. Seeds were sown on 9th March in pots. On 20th April, homogenous seedlings were dipped in liquid culture of biofertilizers for one hour, then transplanted in pots (20 cm diameter) filled with clay. 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), 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. Mechanical and chemical analyses of the soil used in the study are shown in Tables B and C.

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

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.	2.81mmohs	P	37.8 ppm
Ca^{++}	14.00	Cl^-	13.00	Organic matter	0.23	K	35.1ppm
Mg^{++}	8.20						

The following data were recorded: 1- Vegetative growth (plant height (cm), number of branches/plant, stem diameter (mm) at soil surface, fresh and dry weights (gm) of shoots. 2- Flower characteristics (number of days to flower, number of inflorescences/plant, peduncle length (cm), peduncle diameter (mm), inflorescence diameter (cm), ray flower length (cm), ray flower width (mm), fresh and dry weights of inflorescence.

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

A- Vegetative growth:

As shown in Table 1 the data revealed that, generally, inoculating the plants with the mixed strains led to a significant increase in vegetative growth, especially when combined with the high dose of NPK, in both seasons. There was a gradual decrease in most characteristics of the plants received chemical fertilizers only with increasing the rate of NPK, even less than the control plants. Combined application of NPK with biofertilizers was very effective on growth than inoculation only.

1- Plant height:

Data in Table 1 revealed that in both seasons, inoculation of the plants with bio drench plus 6 gm NPK/plant resulted in the tallest plants (181.750 and 172.450

cm, respectively). While, the shortest ones resulted from NPK fertilization at 6 gm / plant (104.714 and 93.571cm, respectively). These results are in agreement with those reported by many workers. Verma *et al.* (1996) on *Dalbergia sissoo* found that combined application of *Rhizobium* and 100 gm of single superphosphate increased seedling height. Rajendran *et al.* (2000) on *Casuarina equisetifolia,* Gad (2001) on *Feoniculum vulgare* and *Anethum graveolens* and Mahfouz (2003) on marjoram concluded that inoculation with *Azotobacter* and *Azospirillum* in the presence of N, P and K resulted in the tallest plants.

2- Number of branches / plant:

From Table 1 it may be noticed that the greatest number of branches in both seasons (29.714 and 32.600 branches/plant, respectively) was found on the plants inoculated with the mixture of strains combined with NPK at 6 gm / plant. Meanwhile, the plants received NPK at 6 gm/plant only had the least number of branches (17.666 and 18.778 branches/plant respectively) in the two seasons. Many authors reported the favorable effect of biofertilizers on branching. Gad (2001) on *Feoniculum vulgare* and *Anethum graveolens,* Badran *et al.* (2002) on *Nigella sativa* , Mahfouz (2003) on marjoram and Badran *et al.* (2003) on *Pimpinella anisum* indicated that inoculation of the plants with biofertilizers in presence or absence of different doses of nitrogen and phosphorous chemical fertilizers significantly increased number of branches as compared to uninoculated plants.

3- Stem diameter:

As shown in Table 1 the data on stem diameter showed that the thickest stems in the two seasons (13.555 and 12.949 mm, respectively) were those of the plants supplied with biofertilizer (inoculation + drench) plus 6 gm NPK/plant. Whereas, the thinnest stems, in both years (8.375, and 9.625 mm, respectively) were those of plants treated with NPK at 6 gm/plant only. These results are in line with those of Verma *et al.* (1996) on *Dalbergia sissoo,* Mahmoud and Mahmoud (1999) on peach and Rajendran *et al.* (2000) on *Casuarina* who found that adding biofertilizers to the plants led to an increase in stem diameter.

4- Fresh and dry weights of shoots:

From the data shown in Table 2 it can be remarked that the heaviest fresh and dry shoots were due to the application of biofertilizers (inoculation + drench) plus 6 gm NPK/plant, in both seasons. The values were 408.318 and 323.982 gm,

respectively for fresh weight and 94.580 and 89.447 gm, respectively for dry weight. This may be due to the increment of plant height and stem diameter as already discussed (Table1). Meanwhile, the plants treated with 6 gm NPK / plant only had the least fresh and dry weights of shoots, in both seasons. The values were 119.827 and 167.565 gm, respectively for fresh weight and 29.344 and 43.675 gm, respectively for dry weight. The effect of biofertilizers on fresh and dry weights of plants was reported by many investigators. Misra (1997) on gladiolus, Gad (2001) on *Feoniculum vulgare* and *Anethum graveolens,* Rashed (2002) on *Anethum graveolens, Coriandrum sativum* and *Petroselinum sativum* plants and Mahfouz (2003) on marjoram demonstrated that inoculating the plants with biofertilizers resulted in the heaviest fresh and dry weights of them.

Table 1. Effect of chemical and biofertilizers on plant height (cm), number of branches/plant and stem diameter (mm) of *Cosmos sulphureus* Cav. plants during the two seasons of 2002 and 2003.

Treatments	Plant height (cm)		Number of branches / plant		Stem diameter (mm)	
	1st S.	2nd S	1st S.	2nd S.	1st S.	2nd S.
Control	111.187	106.750	21.750	24.889	9.000	10.429
NPK (3 gm)	109.875	104.833	19.625	22.714	9.312	10.857
NPK (6 gm)	104.714	93.571	17.666	18.778	8.375	9.625
Bio Inoc.	121.187	119.375	18.000	20.875	9.125	10.785
Inoc.+NPK (3 gm)	132.166	128.500	23.000	25.047	10.388	11.917
Inoc.+NPK (6 gm)	146.166	141.929	29.714	32.600	11.833	12.056
Inoc.+Bio drench	140.666	137.786	26.444	27.429	10.250	11.550
Inoc.+Bio drench+NPK (3 gm)	165.375	159.833	27.750	29.444	12.055	12.444
Inoc.+Bio drench+ NPK (6 gm)	181.750	172.450	28.714	30.801	13.555	12.949
L.S.D. 0.05 %	3.830	3.787	0.955	1.739	0.848	0.610

Inoc. = Inoculation S. = Season

Table 2. Effect of chemical and biofertilizers on fresh and dry weights of shoots (gm) of *Cosmos sulphureus* Cav. plants during the two seasons of 2002 and 2003.

Treatments	Fresh weight (gm)		Dry weight (gm)	
	1st S.	2nd S.	1st S.	2nd S.
Control	123.121	177.794	30.145	45.298
NPK (3 gm)	120.005	171.340	26.838	40.886
NPK (6 gm)	98.751	158.473	18.783	32.325
Bio Inoc.	119.827	167.565	29.344	43.675
Inoc. + NPK (3 gm)	171.731	191.380	41.701	60.068
Inoc. + NPK (6 gm)	288.796	297.084	72.536	79.241
Inoc. + Bio drench	173.348	186.823	40.390	48.280
Inoc. + Bio drench + NPK (3 gm)	288.710	224.170	64.541	71.746
Inoc. + Bio drench + NPK (6 gm)	408.318	323.982	94.580	89.447
L.S.D. 0.05 %	6.292	4.285	3.107	1.790

B- Flowering:

Generally, inoculation of the seedlings with biofertilizer plus supplying the plants with 3 gm NPK/plant led to the best results concerning the flowering characteristics. However, application of 6 gm NPK / plant only resulted in the least flowering traits. These results were in harmony with those of Bailey (1933) who stated that it is a mistake to grow cosmos in too rich soil, as it has too vigorous growth and too few flowers, which are also late.

1- Number of days to flower:

From data in Table 3 it can be observed that the earliest flowering occurred as a result of inoculation of the seedlings only and inoculation plus bio drench, in both seasons. Moreover, the treatment of inoculation plus 3 gm NPK/plant and control plants flowered earlier than other treatments in the second season. The latest flowering was of the plants received 6 gm NPK/plant only, in both seasons. The difference between the earliest flowering and the latest one was about 12 days in the first season and 10 days in the second one.

2- Number of inflorescences/plant:

The data in Table 3 showed that the greatest number of inflorescences/plant in both seasons (128.237 and 130.334, respectively) was formed on the plants treated with inoculation plus 6 gm NPK/plant. This treatment also increased the number of branches/plant as abovementioned in Table 1. The least number of inflorescences/plant (38.000 and 47.778, respectively) was recorded on the plants received 6 gm NPK/plant only, in both seasons.

Table 3. Effect of chemical and biofertilizers on number of days to flower and numberof inflorescences/plant of *Cosmos sulphureus* Cav. plants during the two seasons of 2002 and 2003.

Treatments	Number of days to flower		Number of inflorescences / plant	
	1st S	2nd S.	1st S.	2nd S.
Control	87.296	86.223	78.291	89.603
NPK (3 gm)	95.606	93.353	54.152	62.667
NPK (6 gm)	97.773	95.156	38.000	47.778
Bio Inoc.	84.326	85.932	94.126	89.867
Inoc. + NPK (3 gm)	87.796	86.561	99.263	106.444
Inoc. + NPK (6 gm)	89.046	89.107	128.237	130.334
Inoc. + Bio drench	83.820	85.454	56.375	68.834
Inoc. + Bio drench + NPK (3 gm)	90.756	90.097	90.920	86.667
Inoc. + Bio drench + NPK (6 gm)	90.589	90.697	112.930	117.111
L.S.D. 0.05 %	3.157	3.953	4.241	4.250

3- Peduncle length:

From Table 4 it can be noticed that inoculation + bio drench + 6 gm NPK/ plant resulted in the tallest peduncles, in both seasons (19.343 and 18.979, respectively). However, the shortest peduncles, in both seasons (14.259 and 10.399, respectively) were found on the plants supplied with 6 gm NPK/plant only.

4- Peduncle diameter:

As shown in Table 4 the data on peduncle diameter indicated that the thickest peduncles, in the two seasons (1.712 and 1.726 mm, respectively) were those of the plants treated with inoculation + 3 gm NPK/plant. Whereas, the thinnest peduncles, in both years (1.480, and 1.411 mm, respectively) were those of the plants supplied with NPK at 6 gm/plant.

5- Inflorescence diameter:

Data presented in Table 5 revealed that inoculation of the seedlings with biofertilizer plus application of NPK at 3 gm/plant resulted in the greatest inflorescence diameter, in both seasons (6.147 and 5.957 cm, respectively). This may be attributed to the increase in ray flower length and width as shown in the same Table. While, the smallest inflorescences (5.755 and 5.560 cm, respectively) were formed on the plants treated with NPK at 6 gm/plant. There was nonsignificant difference between the treatments, except the treatment of inoculation plus 3 gm NPK/plant, in both seasons.

Table 4. Effect of chemical and biofertilizers on peduncle length (cm) and peduncle diameter (mm) of *Cosmos sulphureus* Cav. plants during the two seasons of 2002 and 2003.

Treatments	Peduncle length (cm)		Peduncle diameter (mm)	
	1st S.	2nd S.	1st S.	2nd S.
Control	16.788	13.461	1.489	1.422
NPK (3 gm)	15.967	11.746	1.677	1.571
NPK (6 gm)	14.259	10.399	1.480	1.411
Bio Inoc.	16.750	13.433	1.618	1.559
Inoc. + NPK (3 gm)	17.537	15.367	1.712	1.726
Inoc. + NPK (6 gm)	18.691	16.712	1.696	1.644
Inoc. +Bio drench	16.562	14.979	1.552	1.522
Inoc. +Bio drench + NPK (3 gm)	17.278	15.640	1.625	1.590
Inoc. +Bio drench + NPK (6 gm)	19.343	18.979	1.629	1.614
L.S.D. 0.05 %	1.411	0.865	0.167	0.153

6- Ray flower length:

In Table 5 the data showed that the tallest ray flowers, in both seasons (2.871 and 2.789 cm, respectively) were recorded on the plants inoculated with the biofertilizer and supplied with NPK at 3 gm/plant. However, the shortest ray flowers (2.676 and 2.437 cm, respectively) were formed on the plants received NPK at 6 gm/plant only, in the two seasons. There was nonsignificant difference between the treatments, in the first season, except the treatment of inoculation plus 3 gm NPK/plant.

7- Ray flower width:

As shown in Table 5 the data pointed out that ray flower width followed the same trend of inflorescence diameter and ray flower length. Inoculation of the plants with biofertilizers plus application of NPK at 3 gm/plant was the most effective treatment in increasing ray flower width, in both seasons (12.624 and 11.910 mm, respectively). The narrowest ray flowers were formed on the plants treated with biofertilizer inoculation only, in both seasons (11.428 and 10.829 mm, respectively). There was nonsignificant difference between the treatments.

Table 5. Effect of chemical and biofertilizers on inflorescence characteristics of *Cosmos sulphureus* Cav. plants during the two seasons of 2002 and 2003.

Treatments	Inflorescence diameter (cm)		Ray flower length (cm)		Ray flower width (mm)	
	1st S	2nd S.	1st S.	2nd S.	1st S.	2nd S.
Control	5.850	5.658	2.722	2.577	12.253	11.212
NPK (3 gm)	5.829	5.613	2.709	2.546	12.166	11.141
NPK (6 gm)	5.755	5.560	2.676	2.437	12.415	11.524
Bio Inoc.	5.966	5.784	2.783	2.684	11.428	10.829
Inoc. + NPK (3 gm)	6.147	5.957	2.871	2.789	12.624	11.910
Inoc. + NPK (6 gm)	5.929	5.864	2.768	2.685	12.434	11.861
Inoc. +Bio drench	5.834	5.683	2.694	2.585	12.093	11.426
Inoc.+Bio drench+NPK (3 gm)	5.937	5.744	2.767	2.671	11.603	10.956
Inoc.+Bio drench+NPK (6 gm)	5.818	5.602	2.714	2.643	12.206	11.182
L.S.D. 0.05 %	0.381	0.384	0.140	0.093	1.480	1.291

8- Inflorescence fresh and dry weights:

The data on fresh and dry weights of inflorescences are shown in Table 6. They revealed that the heaviest fresh and dry weights were due to the inoculation of the plants with the mixture of biofertiliers and bio drench as well as supplying the plants with NPK at 6 gm/plant, in both seasons. The values were 0.971 and 0.931 gm, respectively for fresh weight and 0.181 and 0.174 gm, respectively for dry weight. The plants treated with NPK at 6 gm/plant only had the least fresh and dry weights of

inflorescences in the two seasons. The values were 0.790 and 0.727 gm, respectively for fresh weight and 0.145 and 0.133 gm, respectively for dry weight.

The effect of biofertilizers on flowering characteristics was reported by many investigators. Wange and Patil (1994) on *Polianthes tuberosa* and Misra (1997) on gladiolus mentioned that biofertilizer treatments significantly increased the number of flowers. Swaminathan *et al.* (1999) on tuberose stated that treatment with NPK + *Azospirillum* + phosphobacteria resulted in the highest mean spike length, number of flowers/ spike and flower weight. Sheikh *et al.* (2000) on Dutch iris demonstrated that the interaction between biofertilizers (*Azotobacter* and *Azospirillum*) and N was significant for flowering days and floret size. El – Kashlan (2001) noticed that using biofertilizers shortened the period required to reach flowering. Gad (2001) found a significant increase in number of umbels/plant as a result of using biofertilizers on *Feoniculum vulgare* and *Anethum graveolens.* Raju and Haripriya (2001) found that application of NPK + *Azospirillum* + phosphobacteria resulted in the highest flower yield of crossandra.

Table 6. Effect of chemical and biofertilizers on iInflorescence fresh and dry weights (gm) of *Cosmos sulphureus* Cav. plants during the two seasons of 2002 and 2003.

Treatments	Inflorescence fresh weight (gm)		Inflorescence dry weight (gm)	
	1st S.	2nd S.	1st S.	2nd S.
Control	0.835	0.759	0.163	0.152
NPK (3 gm)	0.864	0.812	0.150	0.141
NPK (6 gm)	0.790	0.727	0.145	0.133
Bio Inoc.	0.866	0.844	0.156	0.147
Inoc. + NPK (3 gm)	0.942	0.893	0.170	0.162
Inoc. + NPK (6 gm)	0.932	0.862	0.173	0.164
Inoc. +Bio drench	0.876	0.830	0.154	0.143
Inoc. +Bio drench + NPK (3 gm)	0.860	0.788	0.162	0.150
Inoc. +Bio drench + NPK (6 gm)	0.971	0.931	0.181	0.174
L.S.D. 0.05 %	0.137	0.079	0.016	0.010

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تأثير التسميد الكيماوى والتسميد الحيوى على نباتات الكوزموس
١- النمو الخضرى والإزهار

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زرعت شتلات الكوزموس فى أصص بلاستيك (قطر ٢٠ سم) مملوءة بتربة المشتل خلال الموسمين ٢٠٠٢ و ٢٠٠٣ بغرض دراسة تأثير التسميد الكيماوى والحيوى على النمو والإزهار . وأوضحت النتائج أن استخدام التسميد الحيوى بمخلوط من (*Azotobacter chroococcum,* *Asospirillum lipoferum, Bacillus polymixa, Bacillus megatherium* and *Pseudomonas fluorescence*) مع مستويات مختلفة من التسميد الكيماوى (نتروجين ، فوسفور ، بوتاسيوم) بنسبة ١ : ١ : ١ وبمعدل ٣ أو ٦ جم / نبات أدى إلى زيادة النمو الخضرى والزهرى . بينما كانت أقل النتائج عند استعمال التسميد الكيماوى بمفرده .