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Effect of Some Fertilizers on Botanical and Chemical Characteristics of Pot Marigold Plant (*Calendula officinalis* L.)

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Abstract: The present study was carried out at the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt during the two winter successive seasons, of 2008/2009 and 2009/2010. This work was designed to investigate the effect of cattle manure (CM) and sheep manure (SM), biofertilizer (Bio, liquid inoculum containing 10^8 cells/ml of associative diasotrophs) and inorganic fertilizer (N) as single or combined treatments on morphology, yield and chemical composition of pot marigold (*Calendula officinalis, L.*) plants grown under sandy soil conditions. P and K fertilizers were used as a basal dressing for all the treatments. Results indicated that application of different fertilization treatments had a considerable effect on the different vegetative growth characteristics of *Calendula officinalis,*L. plants, compared to the unfertilized control. In both seasons, in most cases the highest values for vegetative growth characteristics (plant height, main stem length, length and diameter of median internode of main stem, number of secondary branches, number of leaves and leaf area of median leaf of main stem, diameter and stalk length of capitulum, number of capitula/ plant, number and weight of fruits/capitulum as well as fresh and dry weights of leaves, stems and roots) were determined with plants received 0.5 CM + 0.5 SM + Bio followed by that received 0.5 SM + Bio and 0.5 CM + Bio treatments, respectively. Generally, there was no significant difference between plants received 0.5 CM + 0.5 SM + Bio and that received 0.5 SM + Bio. In both seasons, plants fertilized with 0.5 CM + 0.5 SM + Bio gave the highest chlorophyll a+b, followed by that received 0.5 SM + Bio, 0.5 CM + Bio, 0.5 CM + 0.5 SM treatments in descending order. In both seasons, plants fertilized with 0.5 CM + 0.5 SM + Bio gave the highest carotenoids content (0.0.70 and 0.63 mg/g fresh matter, respectively).In both seasons, the highest total carbohydrates content was determined in leaves + stems and roots of *Calendula officinalis,* L. plants received 0.5 CM + 0.5 SM + Bio followed by that received 0.5 SM + Bio and 0.5 CM + Bio treatments, respectively. In both seasons, the highest N, P and K% of dry matter in leaves + stems and roots were recorded with plants received 0.5 CM + 0.5 SM + Bio followed by that received 0.5 SM + Bio and 0.5 CM + Bio treatments, respectively.

Key words: *Calendula officinalis* · Fertilizer · Manure · Marigold

INTRODUCTION

Calendula officinalis, L. (pot marigold, marigold and African marigold) is an herbaceous plant belonging to family Asteraceae. It is of Mediterranean origin and has been cultivated by the Egyptians, Greeks, Hindus and Arabs. Pot marigold is an important ornamental, medicinal and decorative plant. It is used in cooking; manufacturing cosmetics and phytotherapeutic products as well as a dye for fabrics [1-3].It has anti-viral, anti-HTV, anti-mutagentic, anti-inflammentory and anti-oxidant effects. It has also anti-spasmotic effect and acts as a bactericide and immunomodulatory [4-10]. Also, it is used to treat hepatic injuries, tension, difficulty falling asleep, controlling bleeding, soothing irritated tissues, remedying burns and bruises and as an emollient [11-13].

Interest of growers and consumers for organic floriculture had increased in the last years to increase both yield and quality habit of the plants and to produce safe, flavor some and chemicals free plants [14, 15]. Organic fertilizers suit best sandy and newly reclaimed desert soils of Egypt as organic material can be beneficial

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to improve organic matter and nutrients status. Biofertilizers are useful for recycling elements, reserving natural resources and reducing the need for chemical fertilizers which have adverse deleterious environmental effects on public health and national income as well as reducing the cost of fertilizers and labor [16-24].

Remarkable effects of nitrogen application on growth and flower production of *Calendula officinalis* including plant height, leaf area, number of leaves, number of seeds per flower head, seeds weight and yield, number of flowers, flower diameter, flower yield, capitulum diameter and number of seeds per capitulum have been reported by many previous studies [25-30]. Also, enhancing plant growth characteristics by using combinations between Azotobacter, phosphorus solubilizing bacteria and inorganic nitrogen was concluded by prior studies [31, 32].

Diasotrophs promote plant growth as growers benefit from them in increasing yield productivity. Maheshwari *et al*. [33] applied *Azotobacter chroococcum* to roots of palmarosa plants. The application resulted in 10.3-39.6% higher herbage yield than the control. Abdou and El-Sayed [32] on *Carum carvi*, found that the biofertilization treatment (*Azotobacter chroococcum* and *Azospirillum lipoferum*) was effective on stimulating growth traits of plant height, number of branches and stem diameter. Sakr [34] on Alexandrian senna plants, grown in sandy soil, stated that biofertilization gave the highest chlorophyll content in fresh leaves, N and P contents in the leaves, N content in the roots and total carbohydrates content in the leaves, stems, root and pods.

The positive effect of organic manures on increasing yield (flowers yield / plant, average size of flowers, average weight of fresh flowers / plant, dry flower yield /plant and dry matter yield of plant, number of capitula per plant), nitrogen and potassium nutrients as well as chlorophyll and carotenoides contents of marigold plants was recorded by previous investigations [35-38].Combining between organic and bio-fertilizers is a useful tool for optimizing plant production and quality. Sakr [34] pointed out that fertilizing Alexandrian senna plants with poultry manure + cattle manure + bio-fertilizers had a considerable effect on chlorophyll a, b and caretonoids contents in fresh leaves, macro nutrients, N, P and K contents in stems, N and P contents in pods and K content in roots. Also, cattle manure + biofertilizer treatment produced plants with higher total carbohydrates in stems and pods, N, P and K contents in stems and K content in pods and roots.

Thus, the objective of the present study is to detect the effect of two organic manures (cattle and sheep manures), biofertilizer (Liquid inoculum containing 10^8 cells/ml of associative diasotrophs: *Azotobacter chrooccum, Azospirillum brasilens, Bacillus polymyxa, Pseudomonas putida, Enterobacter agglomerans and Klebsiells pneuomoniae*) and chemical inorganic N fertilizer on morphology, yield and chemical composition of pot marigold (*Calendula officinalis*, L.) plants grown under sandy soil conditions.

MATERIALS AND METHODS

The present study was carried out at the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt during the two winter successive seasons, of 2008/2009 and 2009/2010. The seeds of local variety of pot marigold (orange flowers) were sown on October 18th and 20th, 2008 and 2009, respectively in warm seed beds. The seedlings were transplanted after 40 days from seeding in both seasons using plastic pots (30 cm diameter) filled with sandy soil brought from the Sixth of October city area, Giza governorate. Thereafter, the usual agricultural practices were followed as recommended.

The experiment layout was arranged in randomized complete block design (RCBD) with three replicates. Each block contained ten fertilization treatments that allocated randomly. Each replicate contained ten pots, each pot containing two plants.

P and K fertilizers were used as a basal dressing for the all treatments. Each pot received the following: 7g calcium super phosphate (15.5% P_2O_5) 15 days before transplanting and 2g potassium sulphate (48% K_2O) added two weeks after transplanting and repeated after four weeks.

Organic manures, cattle (CM) and sheep (SM) manures were obtained from the Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt. Each manure was added to the growing media 10 days before transplanting at the rate of 350 and 700 cm^3/pot.

Biofertilizer inoculation liquid (Bio) was secured from the Environmental Studies and Researches Unit (ESRU), Microbiology Department, Faculty of Agriculture, Cairo University. It is a mixture of the rhizobacteria from six genera including *Azotobacter chroococcum*, *Azospirillum brasilens*, *Bacillus polymyxa*, *Pseudomonas putida*, *Enterobacter agglomerans* and *klebsiells pneuomoniae*. The original liquid bacterial preparations contain

bacterial cells with densities of ca.10^8 cells/ ml. For the biofertilization treatments (alone or combined with organic or inorganic fertilizers), roots of transplants were soaked in the diluted liquid suspension, ca.10^8 cells ml^{-1}, (one liter of the original liquid bacterial preparations + 4 liters of underground water) for 30 minutes, then plants were transferred to the prepared pots. Additional boost of 25 ml diluted bacterial suspension per each pot were injected in a hole adjacent to the plants after one month from transplanting and one month later (at flowering). Biofertilizer was used as a single treatment or in combination with organic or inorganic treatments.

Nitrogenous fertilizer (N): Ammonium sulphate (20.5%N) as the source of nitrogenous fertilizer was added at the rate of 1.5 and 3g/pot after 15, 45 and 75 days from transplanting.

The experimental treatments can be summarized as the following: unfertilized control, Cattle manure (CM), Sheep manure (SM), 0.5 CM + 0.5 SM, Biofertilizer (Bio), Inorganic nitrogen (N), Bio + 0.5 N, 0.5 CM + Bio, 0.5 SM + Bio, 0.5 CM + 0.5 SM + Bio.

The mechanical and chemical characteristics of the soil used as growing media as well as the chemical analysis of the two organic fertilizers, cattle and sheep (Tables 1 and 2) were carried out at Reclamation and Development center for desert soils, Faculty of Agriculture, Cairo University, Giza, Egypt according to Jackson [39] and Page *et al.* [40].

After 95 days from transplanting during each growing season fifteen plants for each treatment, five plants / replicate, were taken at random to determine the area of leaf blade of median leaf on main stem. After 105 days from transplanting during each growing season, fifteen plants representing each treatment, five plants from each replicate were taken at random for recording the following characters: plant height, main stem length, number of secondary branches / plant, the length and diameter of the median internode on the main stem, number of inflorescences / plant, inflorescence (head flower) diameter, inflorescence stalk length, number of fruits per inflorescence representing number of achene fruits per inflorescence and fruits weight per inflorescence. Fresh leaves, leafless shoot (main stem and lateral branches), inflorescences and roots of each plant were separated from each other to determine fresh and dry weights of each of them.

Six random plants per treatment, two per replicate, through the second season (105 days after transplanting) were subjected to anatomical studies according to Sass [41]. Anatomical studies were confined to the treatments of choice i. e. the control as well as the combined treatments of biofertilizer (Bio) and cattle (CM) or sheep (SM) fertilizer ($^1/_2$ CM + $^1/_2$ SM+ Bio) which showed more beneficial effects on the studied characters. Investigated specimens included the following: the internode at the median portion of the main stem, the fully unfolded leaf at the median portion of the main stem and the main root at the basal end.

After 95 days from transplanting, pigments (chlorophylls a, b and carotenoids) contents (mg / g fresh leaves) were determined in fresh leaf samples as described by Nornai [42]. At the end of each growing season, essential oil percentage in the air dried seeds (representing one seeded achene fruitsPercentages of total carbohydrates, nitrogen (N), phosphorus (P) and potassium (K) in dried shoots and roots (oven dried at 70° C) were determined Duboies *et al.* [43], AOAC [44], Goodwine [45] and Huang and Schulte [46], respectively.

Data of morphological and yield characters were subjected to appropriate analysis of variance as reported by Snedecor and Cochran [47]. The significance of different comparisons was determined by the least significant differences (L.S.D.) at 5% probability according to Gomez and Gomez [48].

RESULTS AND DISCUSSION

Vegetative Growth Characteristics: The results recorded in the two seasons (Tables 3-8) show that the application of different fertilization treatments had a considerable effect on the different vegetative growth characteristics of *Calendula officinalis,*L. plants: plant height, main stem length, length and diameter of median internode of main stem, number of secondary branches, number of leaves and leaf area of median leaf of main stem, diameter and stalk length of capitulum, number of capitula/ plant, number and weight of fruits/capitulum as well as fresh and dry weights of leaves, stems and roots. In most cases, application of the different fertilization treatments promoted vegetative growth and resulted in significant increases in the values recorded for the different growth parameters, compared to the unfertilized control plants in most cases. Similar promotion of vegetative growth as a result of nitrogenous fertilization has been reported by prior studies [49-53]. The favorable effect of the nitrogenous fertilization treatments on the vegetative growth characteristics (compared to the control) can be explained by the important role of N in the different physiological processes within the plant, which in turn affect the plant growth. Also, nitrogen is present in the structure of protein molecules [54].

Table 1: Mechanical and chemical analysis of the soil used for sowing pot marigold plants, before treatments, during the two experimental growing seasons of 2008 / 2009 and 2009 / 2010.

Mechanical analysis						
%						
Very coarse sand	Coarse sand	Medium sand	Fine sand	Very fine sand	Silt + clay	Texture class
13.1	34.2	32.1	17.2	1.9	1.5	Sand

Chemical analysis

Humidity (%)	Bulk density (Kg / m^3)	pH	Electrical conductivity, E.C. ( )	Organic matter O.M. ( )	Organic carbon O.C. ()	Soluble anions (meq / L)			Soluble cations (meq / L)			
						HCO_3^-	Cl^-	SO_4^{--}	Na^+	K^+	Ca^{++}	Mg^{++}
1.00	1.68	7.62	0.30	0.60	-	1.00	1.00	1.08	0.85	0.13	1.20	0.90

Available macronutrients (mg / kg)			Available micronutrients (mg / kg)		
N	P	K	Cu	Fe	Mn
29.4	0.50	119	0.40	5.20	1.80

Table 2: The chemical analysis of the two organic fertilizers, cattle and sheep, applied to pot marigold plants during the two experimental growing seasons of 2008 / 2009 and 2009 / 2010

samples	Humidity %	Bulk density Kg / m^3	pH	E.C.	O.M.%	O.C.%	Soluble anions (meq / L)			Soluble cations (meq / L)				Available macronutrients(mg / kg)			Available micronutrients(mg / kg)			
							HCO_3^-	Cl^-	SO_4^-	Na^+	K^+	Ca^{++}	Mg^{++}	N	P	K	Cu	Fe	Mn	Zn
Cattle manure	13.3	332	5.53	7.29	30.80	17.90	11.50	26.0	45.79	35.85	17.44	22.00	8.00	455	7.70	1581	10.0	1200	102	136
Sheep manure	7.7	3.15	5.37	8.22	37.40	21.70	13.50	30.50	51.22	37.99	22.23	20.00	15.00	476	7.80	1479	10.9	600	396	48

Table 3: Effect of organic, bio and chemical N fertilizers on plant height and length and diameter of median internode of main stem of *Calendula officinalis*, L. plants grown in sandy soil during the two growing seasons of 2008/2009 and 2009/2010 after 105 days from transplanting

	Plant height (cm)		Main stem length (cm)		Length of internode (cm)		Diameter of internode (mm)	
Treatments	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	15.9	15.5	10.0	10.2	0.52	0.79	2.8	2.5
N	27.7	30.9	19.3	24.2	0.81	0.89	3.6	3.8
CM	26.5	29.3	17.9	22.5	0.78	0.85	3.9	4.1
SM	29.4	31.6	22.6	25.2	0.89	0.94	4.2	4.6
0.5 CM+ 0.5 SM	25.6	27.7	16.6	19.4	0.69	0.80	4.2	4.5
Bio	16.9	17.7	11.0	12.2	0.54	0.68	3.3	2.9
Bio+ 0.5 N	20.4	23.7	13.5	15.8	0.65	0.77	3.6	3.4
0.5 CM+ Bio	30.8	33.7	24.1	27.1	1.11	1.05	6.5	7.5
0.5 SM+ Bio	32.3	37.6	26.2	30.8	0.96	1.10	6.9	7.5
0.5 CM+ 0.5 SM+ Bio	36.3	40.7	28.3	33.2	1.21	1.32	7.0	7.9
LSD $_{5\%}$	2.3	2.5	1.9	2.4	0.11	0.13	0.3	0.4

Table 4: Effect of organic, bio and chemical N fertilizers on number of secondary branches, number of leaves and leaf area of median leaf of main stem of *Calendula officinalis*, L. plants grown in sandy soil during the two growing seasons of 2008/2009 and 2009/2010 after 95 days from transplanting

	Number of branches/plant		Number of leaves/plant		Leaf area of median leaf (cm^2)	
Treatments	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	2.3	2.2	11.5	12.5	70.2	65.7
N	5.7	4.4	27.1	25.0	96.2	94.5
CM	5.1	5.2	26.4	22.9	94.2	90.5
SM	5.7	4.7	29.9	25.7	112.6	100.3
0.5 CM+ 0.5 SM	4.8	5.1	23. 5	22.1	105.1	95.6
Bio	2.8	2.9	16.8	14.4	79.8	70.8
Bio+ 0.5 N	3.6	3.7	19.7	18.2	90.2	85.5
0.5 CM+ Bio	6.2	5.5	30.2	26.2	120.5	108.1
0.5 SM+ Bio	6.7	6.1	30.6	26.5	125.5	111.7
0.5 CM+ 0.5 SM+ Bio	7.9	6.4	33.4	26.9	132.7	115.9
LSD $_{5\%}$	1.1	0.6	2.8	2.2	14.1	12.5

Table 5: Effect of organic, bio and chemical N fertilizers on diameter and stalk length of capitulum of *Calendula officinalis*, L. plants grown in sandy soil during the two growing seasons of 2008/2009 and 2009/2010 after 105 days from transplanting

	Diameter of capitulum (cm)		Stalk length of capitulum (cm)	
Treatments	1st season	2nd season	1st season	2nd season
Control	1.51	1.76	1.56	1.68
N	2.14	2.05	2.11	2.14
CM	2.35	2.21	1.86	2.38
SM	2.59	2.65	1.97	2.45
0.5 CM+ 0.5 SM	2.69	2.68	2.38	2.69
Bio	1.78	1.89	1.60	1.85
Bio+ 0.5 N	1.91	1.95	1.81	1.97
0.5 CM+ Bio	2.94	2.80	2.18	2.74
0.5 SM+ Bio	3.11	2.95	2.29	2.83
0.5 CM+ 0.5 SM+ Bio	3.37	3.18	3.11	3.21
$LSD_{5\%}$	0.34	0.41	0.29	0.38

Table 6: Effect of organic, bio and chemical N fertilizers on number of capitula/plant as well as number and weight of fruits/ capitulum of *Calendula officinalis*, L. plants grown in sandy soil during the two growing seasons of 2008/2009 and 2009/2010 after 105 days from transplanting

	Number of capitula/ plant		Number of fruits/capitulum		Weight of fruits (g)/capitulum	
Treatments	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	5.0	4.2	22.1	20.1	0.22	0.29
N	6.2	6.8	25.5	26.7	0.36	0.43
CM	6.9	8.4	27.8	28.2	0.38	0.46
SM	7.5	8.7	31.2	30.4	0.34	0.48
0.5 CM+ 0.5 SM	7.8	7.5	32.9	31.7	0.41	0.50
Bio	5.4	5.3	23.4	22.3	0.24	0.30
Bio+ 0.5 N	5.6	5.9	24.6	24.5	0.29	0.37
0.5 CM+ Bio	8.0	9.8	33.1	34.8	0.50	0.55
0.5 SM+ Bio	8.9	10.4	34.5	36.9	0.50	0.58
0.5 CM+ 0.5 SM+ Bio	10.2	11.0	36.8	38.9	0.55	0.58
$LSD_{5\%}$	0.4	0.6	3.7	4.3	0.06	0.08

Table 7: Effect of organic, bio and chemical N fertilizers on fresh weights of leaves, stems and roots of *Calendula officinalis*, L. plants grown in sandy soil during the two growing seasons of 2008/2009 and 2009/2010 after 105 days from transplanting

	Fresh weight of leaves (g/plant)		Fresh weight of stems (g/plant)		Fresh weight of roots (g/plant)	
Treatments	1st season	2nd season	1st season	1st season	2nd season	2nd season
Control	22.62	21.24	19.46	19.94	17.24	18.80
N	42.30	34.22	30.20	29.62	23.80	26.38
CM	42.22	33.80	37.94	32.96	24.62	28.20
SM	43.68	41.20	45.80	37.12	33.80	30.22
0.5 CM+ 0.5 SM	44.80	40.22	44.22	35.80	29.60	28.80
Bio	25.34	23.96	20.30	23.72	21.78	21.84
Bio+ 0.5 N	27.12	27.64	26.52	25.62	23.00	24.60
0.5 CM+ Bio	46.42	43.32	47.22	39.34	33.12	31.96
0.5 SM+ Bio	50.32	48.24	48.22	45.16	36.82	33.68
0.5 CM+ 0.5 SM+ Bio	51.88	49.12	47.80	41.82	37.42	36.22
$LSD_{5\%}$	6.74	6.42	5.16	6.36	4.22	4.52

Table 8: Effect of organic, bio and chemical N fertilizers on dry weights of leaves, stems and roots of *Calendula officinalis*, L. plants grown in sandy soil during the two growing seasons of 2008/2009 and 2009/2010 after 105 days from transplanting

	Dry weight of leaves (g/plant)		Dry weight of stems (g/plant)		Dry weight of roots (g/plant)	
Treatments	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	2.30	2.08	1.98	2.28	2.20	2.10
N	5.32	4.08	4.02	4.08	3.38	3.90
CM	5.16	3.78	4.78	4.42	3.64	3.98
SM	6.02	5.56	6.82	5.56	5.68	4.96
0.5 CM+ 0.5 SM	5.86	5.06	6.20	5.12	6.64	4.32
Bio	2.82	5.52	2.42	2.92	2.98	2.92
Bio+ 0.5 N	3.20	3.02	3.26	3.34	3.32	3.40
0.5 CM+ Bio	6.54	6.42	7.18	6.02	5.52	5.34
0.5 SM+ Bio	7.30	7.48	8.14	7.10	6.30	6.20
0.5 CM+ 0.5 SM+ Bio	7.74	7.82	8.26	6.74	6.54	6.80
$LSD_{5\%}$	0.83	0.78	0.76	0.53	0.38	0.41

In most cases, plants received biofertilization only gave insignificantly higher values than those recorded with unfertilized control plants. Such results are in agreement with results obtained by Nawar [55] on *Helianthus annus*.

In both seasons, in most cases the highest values for vegetative growth characteristics were determined with plants received 0.5 CM + 0.5 SM + Bio followed by that received 0.5 SM + Bio and 0.5 CM + Bio treatments, respectively. Generally, there was no significant difference between plants received 0.5 CM + 0.5 SM + Bio and that received 0.5 SM + Bio.

In both seasons, using full dose of chemical N fertilization gave significantly higher values than that received biofertilizer + half dose of chemical N fertilization. This means that biofertilizer failed to compensate the reduction in chemical N fertilization. The favorable effect of combining biofertilizer with organic fertilization was generally clear on vegetative growth characteristics, since plants fertilized with half dose of any manure used + biofertilizer gave significantly higher values than that of the plants received the full dose of the same organic manure or that received the full dose of chemical N fertilizer in most cases. These results indicated that biofertilization may cause some reduction in the requirements of organic fertilization (approximately 50%) needed by *Calendula officinalis* L. plants. Plant growth promoting rhizobacteria (PGPR) improve plant growth and increase yield productivity, suggesting that N2-fixing bacteria may be considered as PGPR and can benefit plants growth and yield through different mechanisms of action including: a) the production of secondary metabolites such as antibiotic, hydrogen cyanide and plant hormones like substances, b) the production of siderophors, c) antagonism to soil borne root pathogens, d) phosphate solubilization and e) dinitrogen fixation [54 -58]. Such result was obtained by Mohamed [59] on periwinkle as well as Shaalan [60] on black cumin.

Chemical Composition

Leaf pigments content (Chlorophyll a+b and Carotenoids): Data presented in Table 9 revealed that in both seasons, in most cases the synthesis and accumulation of chlorophyll a+b in leaves of *Calendula officinalis,*L. plants were increased by the application of most fertilization treatments, as compared to the control (0.63 and 0.71 mg/g fresh matter, in the first and second seasons, respectively). In the first season, biofertilization only gave the lowest chlorophyll a+b content (0.60 mg/g fresh matter), whereas the unfertilized plants gave the lowest value in the second season. Such result was obtained by Hu and Zhu [61] on chilli, Jacoub [62] on *Ocimum basilicum* and Attia [37] on *Lawsonia inermis*.

In both seasons, plants fertilized with 0.5 CM + 0.5 SM + Bio gave the highest chlorophyll a+b, followed by that received 0.5 SM + Bio, 0.5 CM + Bio, 0.5 CM + 0.5 SM treatments in descending order. In the first season, plants received CM treatment gave higher chlorophyll a+b than that received SM treatment, while the opposite happened in the second season. In both seasons, plants received full dose of N gave higher chlorophyll a+b than that received half dose + biofertilization.

Data presented in Table 9 revealed that in both seasons, in most cases the synthesis and accumulation of carotenoids in leaves of *Calendula officinalis,* L. plants were increased by the application of most fertilization treatments, as compared to the control (0.16 and 0.21 mg/g fresh matter, in the first and second seasons, respectively). In both seasons, biofertilization + half dose of N gave the lowest

carotenoids content (0.13 and 0.21 mg/g fresh matter, respectively). In the second season, the plants received the previous treatment gave the same value of the unfertilized control plants. Generally, the increase in chlorophyll a+b and carotenoids contents as a result of nitrogenious fertilization coincides with the findings of Jacoub [63] on *Ocimum basilicum*, Dorgham [64] on *Dieffenbachia* and *philodendron* plants and Sakr [34] on senna plants.

In both seasons, plants fertilized with 0.5 CM + 0.5 SM + Bio gave the highest carotenoids content (0.0.70 and 0.63 mg/g fresh matter, respectively). In both seasons, in most cases, using half dose of each manure used gave higher chlorophyll a+b and carotenoids in leaves than that recorded with plants received the full dose of the any organic manure.

In both seasons, in most cases, the favourable effect of combining biofertilizer with organic fertilization was generally clear on the chlorophyll a+b and carotenoids in leaves in both seasons, since plants fertilized with half dose of any manure used + biofertilizer gave higher chlorophyll a+b and carotenoids in leaves than that of the plants received the full dose of the same organic manure. These results indicated that biofertilization may cause some reduction in the requirements of organic fertilization (approximately 50%) needed by *Calendula officinalis,* L. plants. Positive effect of organic manuring + bio-fertilizers was obtained by Sakr [34] on Alexandrian senna plants. It is worth to mention that using half dose of any manure used + biofertilizer gave higher chlorophyll a+b and carotenoids in leaves than that received the full dose of nitrgenous fertilization.

The promotion of the synthesis and accumulation of chlorophyll as a result of mineral N fertilization, organic manure or the use of N-fixing bacteria may be attributed to the role of nitrogen as an essential component in the structure of porphyrines, which are found in many metabolically active compounds, including chlorophylls. Chlorophylls are bound to and perhaps even embedded within protein molecules [54].

Total Carbohydrates in Leaves + Stems and Roots: Data tabulated in Table9 showed that fertilization of *Calendula officinalis,*L. plants with the different fertilization treatments increased the total carbohydrates% in leaves+ stems and roots, compared to that of control plants in most cases. The only exception to this general trend was observed with plants received biofertilization only that gave lower carbohydrates% in roots than that of control plants. Such results are in agreement with the findings of Dessouky [55] on *Borago officinalis* and Mohamed [59] on periwinkle.

In both seasons, the highest total carbohydrates content was determined in leaves + stems and roots of *Calendula officinalis,* L. plants received 0.5 CM + 0.5 SM + Bio followed by that received 0.5 SM + Bio and 0.5 CM + Bio treatments, respectively.In both seasons, the favorable effect of combining biofertilizer with organic fertilization was generally clear on the total carbohydrates in leaves + stems and roots, since plants fertilized with half dose of any manure used + biofertilizer gave higher total carbohydrates in leaves + stems than that of the plants received the full dose of the same organic manure. These results indicated that biofertilization may cause some reduction in the requirements of organic fertilization (approximately 50%) needed by *Calendula officinalis,* L. plants. Such result was obtained by Sakr [34] on senna plants.

It is worth to mention that using half dose of any manure used + biofertilizer gave higher total carbohydrates in leaves+ stems and roots than that received the full dose of chemical nitrgenous fertilization.

The favourable effect of the different fertilization treatments on the synthesis and accumulation of carbohydrates may be attributed to the increase in the chlorophylls content of fertilized plants and to the role played by nitrogen in the structure of porphyrine molecules (as previously mentioned) which are found in the cytochrome enzymes essential in photosynthesis. This increase in the contents of chlorophylls and cytochrome enzymes results in an increase in the rate of photosynthesis and a promotion in carbohydrate synthesis and accumulation [54].

N, P and K% of Dry Matter in Leaves+ Stems and Roots: Data presented in Tables 10 and 11 revealed that N, P and K% of dry matter in leaves + stems and roots of *Calendula officinalis*, L. plants were increased as a result of all fertilization treatments, compared to the control. The increase in the percentage of N, P and K was explained by Jain [65], who stated that raising the level of these nutrients in the root medium leads to an increase in vegetative growth and this may be accompanied by an increase in the absorption of these essential element. Such results are in agreement with Sharma [66], Jacoub [62] on *Ocimum basilicum*, Rashed [67] on *Anethum graveolens, Coriandrum sativum* and *Petroselinum sativum,*as well as Neelima and Janardhanan [68] on *Cymbopogon martinii.*

Table 9: Effect of organic, bio and chemical N fertilizers on total chlorophylls (a+b), caroteoids (mg/g fresh matter of leaves) and total carbohydrates (% dry weight) of leaves + stems as well as roots of *Calendula officinalis*, L. plants grown in sandy soil during the two growing seasons of 2008/2009 and 2009/2010

	Total chlorophylls (mg/g fresh matter)		Carotenoids (mg/g fresh matter)		Total carbohydrates (% DW leaves + stems)		Total carbohydrates (% DW roots)	
Treatments	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	0.63	0.71	0.16	0.21	52.5	51.0	51.6	52.5
N	0.76	0.88	0.24	0.29	55.3	58.1	53.6	57.9
CM	1.11	0.98	0.38	0.25	58.1	60.1	54.8	61.2
SM	1.08	1.15	0.27	0.41	58.6	61.3	57.5	62.5
0.5 CM+ 0.5 SM	1.21	1.19	0.42	0.33	58.5	62.5	58.1	61.8
Bio	0.60	0.78	0.18	0.26	52.9	53.5	50.8	51.7
Bio+ 0.5 N	0.64	0.82	0.13	0.21	54.8	53.7	52.6	54.3
0.5 CM+ Bio	1.29	1.52	0.37	0.52	59.6	62.9	59.1	62.7
0.5 SM+ Bio	1.45	1.59	0.48	0.37	60.1	64.1	61.1	62.9
0.5 CM+ 0.5 SM+ Bio	1.58	1.67	0.70	0.63	63.8	64.5	62.5	63.4

Table 10: Effect of organic, bio and chemical N fertilizers on N, P and K (% dry weight) in leaves + stems of *Calendula officinalis*, L. plants grown in sandy soil during the two growing seasons of 2008/2009 and 2009/2010

	N (% DW)		P (% DW)		K (% DW)	
Treatments	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	1.61	1.69	0.11	0.09	1.52	1.65
N	1.91	2.17	0.21	0.16	1.83	1.94
CM	1.79	2.11	0.23	0.18	1.77	2.09
SM	1.88	1.99	0.23	0.21	1.80	2.25
0.5 CM+ 0.5 SM	2.11	2.31	0.25	0.24	1.96	2.40
Bio	1.69	1.73	0.13	0.10	1.58	1.67
Bio+ 0.5 N	1.76	1.89	0.17	0.12	1.72	1.75
0.5 CM+ Bio	2.25	2.46	0.27	0.32	2.08	2.43
0.5 SM+ Bio	2.31	2.49	0.28	0.27	2.27	2.52
0.5 CM+ 0.5 SM+ Bio	2.61	2.69	0.31	0.28	2.38	2.58

Table 11: Effect of chemical, organic and bio-fertilizers on N, P and K (% dry weight) contents in roots of *Calendula officinalis*, L. plants grown in sandy soil during the two growing seasons of 2008/2009 and 2009/2010

	N (% DW)		P (% DW)		K (% DW)	
Treatments	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	1.22	1.35	0.08	0.09	1.61	1.56
N	1.52	1.81	0.13	0.17	1.75	1.78
CM	1.43	1.49	0.14	0.22	1.83	1.94
SM	1.49	1.65	0.17	0.23	1.89	2.06
0.5 CM+ 0.5 SM	1.65	1.97	0.17	0.25	1.94	2.21
Bio	1.29	1.37	0.10	0.12	1.63	1.67
Bio+ 0.5 N	1.35	1.41	0.11	0.14	1.68	1.70
0.5 CM+ Bio	1.76	2.19	0.22	0.28	1.99	2.29
0.5 SM+ Bio	1.89	2.26	0.23	0.33	2.21	2.47
0.5 CM+ 0.5 SM+ Bio	2.08	2.38	0.25	0.31	2.34	2.56

The effect of fertilization on N, P and K percentages was generally more pronounced when organic fertilization was combined with the application of biofertilization. In both seasons, the highest N, P and K% of dry matter in leaves + stems and roots were recorded with plants received 0.5 CM + 0.5 SM + Bio followed by that received 0.5 SM + Bio and 0.5 CM + Bio treatments, respectively. These results are in agreement with prior studies of Yamada and Kamata [69] who reported that soil cation exchange capacity, available N, P and K were increased as benefits of using cattle manure. In addition, Lugtenberg *et al.* [24] indicated that using microbes as biofertilizer increase the supply of mineral nutrients to the plant. Ibrahim [70] on *Ammi visnaga* and *Foeniculum vulgare* as well as Abdou and El-Sayed [50] on *Carum carvi*, L. plants revealing the positive efficiency of biofertilization on nitrogen percentage in the leaves.

In both seasons, in most cases using half dose of each manure used gave higher N, P and K% of dry matter in leaves + stems and roots than that of the plants received the full dose of any of the organic manures. Alo, the favourable effect of combining biofertilizer with organic fertilization was generally clear on the N, P and K percentages in both seasons, since plants fertilized with half dose of any manure used + biofertilizer gave higher N, P and K% of dry matter in leaves + stems and roots than that of the plants received the full dose of the same organic manure. These results indicated that biofertilization may cause some reduction in the requirements of organic fertilization (approximately 50%) needed by *Calendula officinalis,* L. plants. Also, the application of farmyard manure increased the organic matter content of the soil. Organic matter improved the soil physical condition in such a way that it increased the water holding capacity, prevented nutrients leaching and added more mineral nutrients. Ibrahim and Al- Afifi [71]. Such result was obtained by Sakr [34] on Alexandrian senna plants.

It is worth mentioning that using half dose of any organic manure + biofertilizer gave higher N, P and K% of dry matter in leaves + stems and roots than that of the plants received the full dose of chemical nitrgenous fertilization.

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